Natasha Rekhtman
Marina K Baine
Justin A. Bishop

Quick Reference Handbook for Surgical Pathologists

Second Edition

Springer

The Art of Writing a Pathology Report:
What we Say … and What we Mean ☺

by Natasha Rekhtman, Diana Molavi, and Justin Bishop

What we Say:	What we Mean:
This is a difficult case	I have no idea what this is
This lesion is difficult to classify	I am not familiar with the new WHO classification
The differential diagnosis includes …	Your guess is as good as mine
Dr. X concurs with the diagnosis	Sure am glad somebody else here knows what this thing is
Case was shown at the quality assurance conference	We're all going down together
Invasion cannot be excluded	Probably invasive but don't feel like searching too hard … and it's time for my coffee break!
Recommend clinical correlation	Not my problem anymore!
Lesion is best seen on permanent sections	We missed it on frozen
Defer to permanents (as for thyroid frozen)	Maybe if I keep saying this … they will stop sending these?
Stains are suboptimal	Did not work at all
Stains are non-contributory	Stained the wrong block … or ordered the wrong antibody
Stains are non-evaluable	Forgot to order
Tissue with cautery artifact	Puh-leeze! Turn down that bovie!
Evaluation limited by processing artifact	Regular histotech is on vacation
Tumor approaches the margin	Positive margin but am going to dinner with the surgeon, so gotta be nice…
Tumor approaches the margin (#2)	Positive margin but am afraid of the surgeon
Focal acute appendicitis	Found a poly for you! You're welcome
An AFB stain is negative	But please don't hold me to it
Multiple step levels were examined	No, still not there. I am a pathologist, not a magician.
Representative sections submitted	One
Innumerable (as in polyps or mitotic figures)	More than 10
Rare (as in mitoses)	I didn't see any, but if I say zero there will be three on the first field when I show this case
Specimen did not survive processing	Was dropped on the floor and stepped on
Specimen was entirely submitted	Can't send me back to the bucket!
Possible lymph nodes (grossly)	Hunks of fat that I did not bother to dissect
Conservative re-excision is recommended	I forgot to ink the margins

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To Bob, Iris, Mark, Galina, and Katya.

Natasha Rekhtman

To Ian, Sophie, and Lily.

Marina K Baine

To Ashley, Riley, Avery, and Rory.

Justin A. Bishop

Preface to Second Edition

Welcome to the second edition of QRHSP! It has been over 7 years since the publication of the first edition in 2011. One of the great challenges in a book of this nature is that some of the content (e.g., IHC, molecular, grading) evolves rapidly, and some information inevitably goes out of date as soon as the book is published. Nevertheless, many general concepts and approaches covered here are fundamental and lasting, and provide a foundation on which future markers and applications can be built.

It has been immensely gratifying to know that the first edition of this book was useful to many of you, and that it has become a part of how many residents learn pathology. Although much of my clinical and academic work in the last decade has been focused on thoracic pathology and cytopathology, it still brings me great joy to work on this book. I love the process of distilling complex information into simple summaries and diagrams – which is the foundation of this book, and it has been a rewarding experience to go through this process with many talented contributors that worked with me on the current edition. I hope that this updated edition will continue to be your friend that you can count on when first learning a subject, preparing for the boards, or trying to remember that pesky IHC marker, fusion, or relevant syndrome while looking at a case.

The new edition follows largely the same framework as the first one, but now with thoroughly updated content. We have included many new markers that have entered practice in recent years (like p40, GATA3, SOX10, ERG, SF1, BAP1, and many others), added various newly described entities, like Ewing-like sarcomas with novel translocations and SDH-deficient neoplasms, and updated and expanded many IHC differentials, particularly in thoracic and ENT sections, inevitably reflecting the current focus of the main authors. Many other sections were significantly revised and updated. In particular the section on tumor genetics and cytogenetics was thoroughly updated and reorganized, such that the summaries are now organ-based, but include handy summaries and diagrams to help you remember which alterations are shared among different tumors. We also added quick summaries for a number of brand-new topics, such as the uses of IHC to detect hereditary tumor syndromes and quick reference for predictive markers and targeted therapies. Last, but not least, this edition contains significantly updated and expanded hematopathology sections. There are many new cartoon drawings which I have always found to help me remember complex information. Please check out the new cartoon for markers for carcinomas of unknown primary, many new cartoons in the glossary section, and a great new diagram for neck lymph node metastases created by Justin – it will help you think of the differential diagnosis based on which node is involved (it is a complex subject when you read it as dense paragraphs of text, but – as we hope is true for the rest of this book – it becomes very simple and easy to remember when summarized as a logical diagram).

As before, our approach was to capture the material that is difficult to keep in active memory, and for which well-organized quick references may be helpful. This book almost exclusively deals with neoplastic pathology, and is unique in that we cover not only pathology but also useful clinical information that can help you in making the diagnosis (like serologic tumor markers, patterns of metastases, syndromes). Most of the content in this book is at the level of a senior resident, but it is also useful as a quick reminder for a general pathologist. For the beginning resident, some of the differentials in IHC sections will make sense only after you have learned the entities; instead, start with the introductory material – IHC primers, potpourri of morphologic references and the glossary, and advance to other sections later in your training. As with the first edition, we did our best to vet out the content that may be too esoteric, marginally useful, or investigational. Many of these judgments are highly subjective and individual practice dependent (and I learned, increasingly difficult to apply to your favorite subspecialty area). As before, we apologize for any omissions, as some are unavoidable by the nature of this book.

I feel beyond fortunate to have had many extremely talented contributors participate in this edition, who made it possible for the update to come together. I particularly want to thank my main co-authors and co-editors Marina Baine and Justin Bishop, and other lead contributors – Jason Chang, Youran Zou, Xiaojun Wu, and Zenggang Pan. Special thanks to Marina Baine, who was the tireless lead contributor for multiple chapters. Also, many thanks to Diana Molavi, Shien Micchelli, and Laura Favazza for reviewing various portions of this edition and providing helpful feedback, and to my colleagues and trainees at MSKCC from whom I learn and get inspiration daily.

Last but not least, thanks to all of the readers for the feedback over the years. I greatly appreciate everyone who contacted me with specific suggestions and corrections, which we did our best to address in the current edition. Your feedback for this edition will be appreciated (rekhtman@mskcc.org).

Natasha Rekhtman

Acknowledgments

In addition to thanking all contributors to the current edition, many thanks to everyone who contributed to the first edition: Jennifer Broussard, Terina S. Chen, Amy S. Duffield, Tara Nikole Miller, Ross Allen Miller, and Janis M. Taube.

We would also like to thank the Springer team - Richard Hruska (Executive Editor), Barbara Lopez-Lucio (Developmental Editor), and Project Managers Rachel Taenzler, Kelita Katylin, and Prakash Jagannathan - for all their efforts with this project. Natasha also thanks Francis Bodd at Memorial Sloan Kettering Cancer Center for excellent editorial assistance.

Natasha Rekhtman, Marina Baine, Justin Bishop

Preface to First Edition

About This Book

This book is a compilation of high-yield at-a-glance summaries for various topics frequently needed in a quick reference format at the microscope (or when cramming for the boards). As recently minted pathologists, we compiled this book from the perspective of pathologists-in-training and we gathered topics which we wanted to have in quick summary format during our recent residency and fellowships. Although written with the trainees in mind, the book may also be of interest to practicing pathologists as a practical quick reference by the microscope.

The book has a unique layout in that most of the information is presented in tables and diagrams accompanied by minimal explanatory text. Our motto for this book was to boil the information down to the essentials and key elements but with just enough commentary to be accessible to a newcomer to pathology. This book is not intended as a substitute for original resources or authoritative texts, but rather its purpose is to bring under one roof compact summaries for various types of information that trainees and practicing pathologists now search for in many different sources, and give the conceptual "lay of the land" with emphasis on "must know" facts. Certainly decisions about what constitutes "must know" and "high-yield" are highly subjective, and we apologize for any omissions which are inevitable by the nature of this book.

Our other main objective was to make the format of the book as user-friendly and easy to navigate as possible, such that one can quickly find the needed information. We thank our Springer editors for agreeing to publish ths book in a non-standard format to help achieve this goal.

Content

The focus is not organ-based morphologic criteria for which there are many excellent quick-summary resources, but rather the focus is everything else that helps a pathologist make a diagnosis (and pass the boards) with emphasis on the vast and fast-growing fields of immunohistochemistry (IHC) and molecular markers.

The book starts with unique introductory "primers" – at-a-glance 1-page summaries with diagrams on the main types of marker applications and high-yield facts (such as peculiar principles of cytokeratin designation). We highlighted the rules and biological principles behind various immunostains and special stains to help residents reason through a problem rather than having to resort to memorized panels. The other part of the IHC section contains a large compilation of general and organ-based applications of IHC with numerous immunopanels. This includes the classics (such as lung adenocarcinoma versus mesothelioma) and more recent applications (such as the work-up for mismatch repair proteins).

Other sections of the book contain various quick references that are often needed at the microscope but require frequent reminders. This includes a compilation of grading systems, common prognostic systems, and other criteria that are difficult to keep committed to memory (such as size cut-points for various micro-entities like thyroid papillary microcarcinoma). Also included are summaries for tumor syndromes with a particularly practical "slide-to-syndrome" summary where we highlighted which diagnoses or features should trigger consideration of a syndrome. In tumor genetics and cytogenetics we highlighted which tumors have unique molecular characteristics that can aid in the diagnosis or are used in prognostic/predictive testing.

Another high-yield section that is not usually covered in most pathology books is a compilation of quick clinical references geared for pathologists. This section contains resources that help pathologists interpret clinical information that may be highly informative in the differential diagnosis of tumors, including a primer on metastasis (what metastatic patterns are classic vs. exceptional for certain tumors) and serologic tumor markers. We also included a brief summary of targeted therapies for which pathologists may be asked to perform predictive marker testing.

Even though the focus of the book is not organ-based morphologic criteria, we included several sections with differentials that cut across all organs. For example, this section includes at-a-glance differentials for small round blue cell tumors, and classic differentials for certain morphologic features (such as which tumors are classically associated with granulomas or have staghorn vessels). We also included an illustrated guide to microorganisms. Finally, we compiled an illustrated glossary of histopathologic descriptors with illustrations of common objects these terms are said to resemble (such as storiform or palisaded, and what Orphan Annie's eyes actually look like!). Keep this by your side as you begin to tackle the large pathology books! We are also very excited to include a handy guide for pathology web resources by Terina Chen and a user-friendly CPT coding summary by Diana Molavi.

Sources

We used a variety of sources, including standard books and mountains of primary literature. However, most importantly our "world view" of pathology this early in our careers comes primarily from our outstanding teachers at The Johns Hopkins Hospital and Memorial Sloan-Kettering Cancer Center. From them we learned the approaches and principles that come only after years of

experience but cannot be learned by reading books and papers. We were fortunate to learn pathology from these brilliant diagnosticians and generous educators, who shared their knowledge with us through sign outs, lectures and weekly unknowns during our residency at Johns Hopkins. We therefore can only take credit for organizing and presenting this stream of knowledge in a format easily accessible to a newcomer to pathology, and we give all credit for the many useful pearls and principles in this book to our teachers. On the other hand, we take full responsibility for any inaccuracies that may have inadvertently escaped our attention.

In Conclusion

It is our hope that this book will be your best friend both at the microscope and in the late night hours of studying for the boards. Because the type of information covered in this book is rapidly evolving, please be sure to check the most current sources.

Natasha Rekhtman and Justin Bishop

How This Book Came About – Part 1

I started working on this book in my second year of residency at The Johns Hopkins Hospital, although at that time I did not yet know that this was what I was doing. Like many pathologists, I am a very visual learner, and I firmly believe that a good table or diagram is worth many pages of text. Therefore I was desperately looking for resources that succinctly summarized the mountains of information I was trying to absorb, particularly in a format that was tabular or diagrammatic and was amenable to quick learning of the essentials. While there were many great resources for histologic criteria, what I felt was missing were quick references for the new and fast growing fields of immunostains and molecular markers, as well as other types of material frequently needed in pathologists' daily work but not available in a single source. I therefore started compiling these summaries and diagrams for my own use, and later started sharing them with my co-residents. After getting feedback that others were findings these summaries useful, and after I realized that creating them was an incredible motivator to learn and digest the information, I put together a small handbook which was generously printed by the Department of Pathology at Johns Hopkins as a Resident Manual in 2004 and 2007. Now in collaboration with Justin Bishop as my coeditor and main coauthor and with contributions from many former and current Hopkins residents and fellows and my current colleagues at Memorial Sloan-Kettering Cancer Center, this book has morphed into what it is today. Justin joined forces with me in the last two years, and I could not have dreamt of a more dedicated and talented collaborator, who made it possible to get this project completed.

Natasha Rekhtman

How This Book Came About – Part 2

My first interaction with this book (universally known as the "Green Book" at Hopkins) was in 2006. The more senior residents had copies of a magical book that had all the answers I was seeking as a pathology intern. Desperate for something to boil down the massive amounts of information into one resource, my fellow first-year residents and I assembled crude bootleg copies of it. At the end of that year as she left Hopkins, Natasha distributed a new edition which remains a fixture at my microscope to this day. However, as the years passed and new waves of residents entered our program, original copies of the Green Book became increasingly scarce, and the quality of copies became increasingly poor as they became 2nd and 3rd generation. My chief resident year, I was frequently confronted with a question from the junior residents: "Where can I get a copy of that Green Book?" We had heard rumors about the possibility of it being published, but no one at Hopkins knew the status of the now-mythical Green Book. Intent on getting an answer, I contacted Natasha. As luck would have it, she needed a collaborator to push the project past the finish line, and that collaborator became me. Initially a great way to study for my boards, working on the book then became a means to stay on top of the newest information as I started signing out surgical pathology. Although perhaps it was a bigger commitment than I initially realized, it was well worth the effort, and I am extremely grateful to Natasha for allowing me to be a part of this very special project.

Justin Bishop

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Common Abbreviations and Designations

**See IHC index for alternative designations of antibodies/antigens

AdenoCA – Adenocarcinoma
AFIP – Armed Forces Institute of Pathology
AJCC – American Joint Committee on Cancer
ALCL – Anaplastic large cell lymphoma
ALL – Acute lymphoblastic leukemia/lymphoma
AML – Acute myeloid leukemia
AT/RT – Atypical teratoid rhabdoid tumor
Bx – Biopsy
CA – Carcinoma
cc or CC – Clear cell
CD – Cluster of differentiation (as in CD3, CD20, etc.)
CHR – Chromogranin
CIS – Carcinoma in situ
CK – Cytokeratin(s)
CLL/SLL – Chronic lymphocytic leukemia/small lymphocytic lymphoma
CMV – Cytomegalovirus
CNS – Central nervous system
CRC – Colorectal carcinoma
CT – Computed tomography
DCIS – Ductal carcinoma in situ
DDx – Differential diagnosis
DFSP – Dermatofibrosarcoma protuberans
Diff – Differentiation
DLBCL – Diffuse large B cell lymphoma
DNA – Deoxyribonucleic acid
Dx – Diagnosis
EBV – Epstein-Barr virus
EM – Electron microscopy
ER – Estrogen receptor
ESS – Endometrial stromal sarcoma
FL – Follicular lymphoma
GBM – Glioblastoma multiforme
GCT – Germ cell tumor
GI – Gastrointestinal
GIST – Gastrointestinal stromal tumor
GU – Genitourinary
GYN – Gynecologic
HCC – Hepatocellular carcinoma
H&E – Hematoxylin and eosin
Heme – Hematopathology
HHV8 – Human herpesvirus 8
HMWCK – High molecular weight cytokeratins
HPF – High-power field (40X)
HPC – Hemangiopericytoma
HPV – Human papillomavirus
HSV – Herpes simplex virus
HTLV – Human T-lymphotropic virus
ID – Identification or identify
IHC – Immunohistochemistry
IMT – Inflammatory myofibroblastic tumor
IPMN – Intraductal papillary mucinous neoplasm
ISH – In situ hybridization
LMWCK – Low molecular weight cytokeratins
LN – Lymph node
LSIL – Low-grade squamous intraepithelial lesion
MALT – Mucosa-associated lymphoid tissue
MCL – Mantle cell lymphoma
MCN – Mucinous cystic neoplasm
MD – Moderately differentiated
ME – Myoepithelial
Met – Metastasis
MPNST – Malignant peripheral nerve sheath tumor
MRT – Malignant rhabdoid tumor
MZL – Marginal zone lymphoma
NE – Neuroendocrine
NET – Neuroendocrine tumor
NK – Natural killer
NLP-HL – Nodular lymphocyte predominant Hodgkin lymphoma
NOS – Not otherwise specified
PanNET – Pancreatic neuroendocrine tumor
PCR – Polymerase chain reaction
PD – Poorly differentiated
PEComa – Perivascular epithelioid cell tumor
PET – Positron emission tomography
PNET – Primitive neuroectodermal tumor
PR – Progesterone receptor
PTC – Papillary thyroid carcinoma
RBC – Red blood cell
RCC – Renal cell carcinoma
R-S cell – Reed-Sternberg cell
Rx – Therapy, treatment
SCCOHT – Small cell carcinoma of the ovary, hypercalcemic type
SFT – Solitary fibrous tumor
SCLC – Small cell lung carcinoma
SmCC – Small cell carcinoma
SqCC – Squamous cell carcinoma
SRBCT – Small round blue cell tumor
SYN – Synaptophysin
TB – Tuberculosis
UC – Urothelial carcinoma
undif. – Undifferentiated
vs. – Versus
WD – Well differentiated
WHO – World Health Organization

Immunohistochemistry reactivity code

+++	Overexpressed or consistently diffuse
+	Positive
+/–	Usually positive
–/+	Usually negative
–	Negative

(F+ = focally positive)

Chapter 1. Immunostains: Introduction

By Natasha Rekhtman, Marina K Baine, Youran Zou, Justin A. Bishop

Applications of Immunohistochemistry (IHC) in Anatomic Pathology

(select examples)

1. **Diagnosis of Tumors:**
   - **(a) Classification of poorly differentiated neoplasms:**
     - Carcinoma (cytokeratin+)
     - Lymphoma (CD45+)
     - Melanoma (SOX10/S100/Melan-A/HMB45+)
     - Others
   - **(b) Diagnosis of carcinoma of unknown primary:**
     - Lung (TTF-1/Napsin-A+)
     - Thyroid (PAX8/TTF-1+)
     - Prostate (PSA/NKX3.1+)
     - Colon (CDX2+)
     - Many others
   - **(c) Diagnosis of invasion:**
     - Loss of myoepithelial cells (breast cancer)
     - Loss of basal cells (prostate cancer)
     - Loss of basement membrane/collagen type IV (various carcinomas, rarely used)

2. **Assessment of Markers Reflecting Prognosis ("Prognostic" Markers):**
   - Ki67/MIB1 (general proliferation marker)
   - HER2 (adverse prognosis in breast and gastric cancer) – also predictive marker
   - CD38 (adverse prognosis in chronic lymphocytic leukemia)
   - Others

3. **Assessment of Markers Reflecting a Therapeutic Response ("Predictive" or "Theranostic" Markers)[1]:**
   - ER/PR (tamoxifen for breast cancer)
   - HER2 (Herceptin for breast cancer and gastric cancer)
   - ALK (crizotinib, recently ceritinib for lung carcinomas with ALK fusions)
   - PD-L1 (PD-1/PD-L1 inhibitory monoclonal antibodies for non-small cell lung carcinomas and other tumors)
   - Others

4. **Detection of Micrometastases:**
   - Melanoma (melanocytic markers)
   - Breast cancer (cytokeratins)

5. **Identification of Infectious Organisms[2]:**
   - Viruses (HSV, CMV, adenovirus, SV40 for *Polyomavirus*/JC virus)
   - Bacteria (*H. pylori*, anti-treponemal antibody for syphilis)
   - Other organisms (*Toxoplasma*)

1. Many predictive markers are assessed by molecular methods. Examples listed here are for markers assessed by IHC, more on this in Chapter 4.
2. IHC has recently become more widely used in identification of organisms in tissue, particularly when assessing for viral infection in transplanted organs (e.g., CMV and adenovirus in kidney transplant) or otherwise immunocompromised patients. However, in situ hybridization for some viruses is still the preferred method (EBV, HPV). For the vast majority of bacteria and fungi, the use of special stains remains to be the preferred method (GMS, AFB, etc.).

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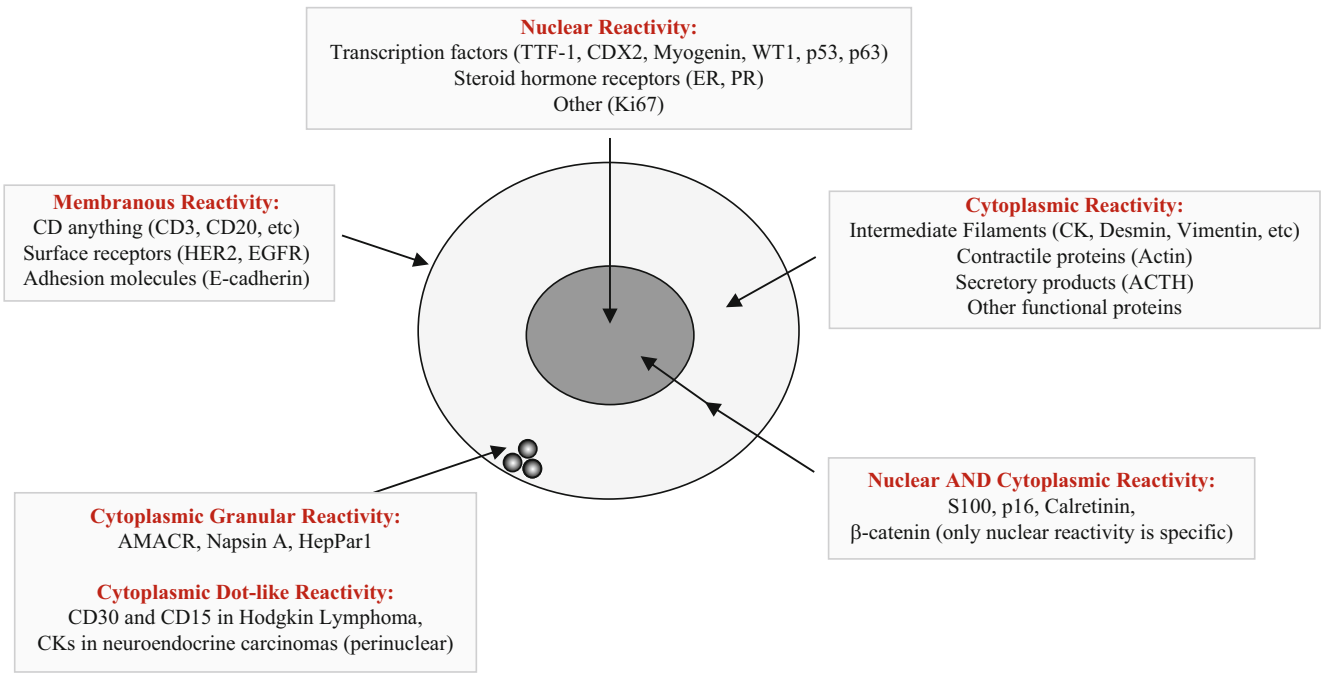
Markers of Differentiation at a Glance

Differentiation	*Markers*
Mesenchymal	Vimentin (generally not useful for diagnosis)
Epithelial	Cytokeratins, EMA *[see epithelial primer]*
Smooth muscle	Desmin, muscle-specific actin, smooth muscle actin, calponin, h-caldesmon, smooth muscle myosin heavy chain *[see muscle primer]*
Skeletal muscle	Desmin, muscle-specific actin, myogenin, MyoD *[see muscle primer]*
Myofibroblastic	Partial smooth muscle phenotype: actins (MSA, SMA) in "tram-track" distribution and calponin but not h-caldesmon *[see muscle primer]*
Myoepithelial	Polyphenotypic markers: smooth muscle (complete phenotype – smooth muscle actin, calponin, others), neural (S100), glial (GFAP), epithelial (CK), and basal/stem cell factor (p40/p63) *[see muscle primer]*
Endothelial	CD34, CD31, ERG, Fli-1, D2-40 (lymphatic), Factor VIII (outdated) *[see vascular primer]*
Lipomatous	S100 (IHC generally not used)
Melanocytic	SOX10, S100, HMB45, Melan-A/MART-1, MITF, tyrosinase *[see melanocytic primer]*
Neuroendocrine	SYN, CHR, CD56, INSM1 *[see neuroendocrine primer]*
Glial	GFAP, OLIG2 *[see neuroglial primer]*
Neuronal	Neurofilament, NeuN, SYN *[see neuroglial primer]*
Nerve sheath (Schwannian)	SOX10, S100 *[see neuroglial primer]*
Serous acinar cells	PAS (general); BCL10, trypsin, chymotrypsin, and lipase (pancreas); SOX10 and DOG-1 (salivary)
Hematopoietic	Pan-hematopoietic: CD45/LCA Pan-B cell: CD20, CD19, CD79a, PAX5 Pan-T cell: CD3, CD43 Plasma cell: CD138, κ/λ light chains (normal κ:λ ratio is 2–3:1) Myeloid: CD43, CD117/c-kit, CD34, MPO MANY others *[see hemepath section]*
Histiocytic	CD68, CD163, enzymes (lysozyme/muramidase, α1-antitrypsin) *[see hemepath section]*

Location, Location, Location! Primer on Location of Antigens

- In order to properly interpret immunoreactivity, it is important to know the expected location of the antigen of interest. Knowing the biological function of a molecule of interest can be very helpful in intuitively anticipating the site of reactivity.
- Transcription factors (TTF-1, CDX2, myogenin, PAX8, WT1, p53, p63/p40) and steroid hormone receptors (ER, PR, AR) function in the nucleus, and therefore the expected IHC signal is **nuclear**. Proliferation marker Ki67 (MIB1) is also nuclear (except for peculiar membranous/cytoplasmic reactivity in hyalinizing trabecular tumor/adenoma of thyroid).
- In contrast, cytoskeletal, contractile, and other functional proteins are **cytoplasmic**. In fact, the majority of antigens in current use are cytoplasmic. This category includes all intermediate filaments (CK, desmin, vimentin, GFAP, neurofilament), contractile proteins (actin), melanosome-associated proteins (HMB45, Melan-A), secretory products (ACTH, trypsin), and various other functional molecules.
- **Membranous** reactivity is expected for receptors (EGFR), adhesion molecules (E-cadherin), and other surface molecules. This category includes virtually all CD (cluster of differentiation) antigens, such as CD3 (T-cell marker) and CD20 (B-cell marker). Occasionally, membranous reactivity may be difficult to distinguish from cytoplasmic signal; this distinction is important for several molecules where only membranous but not cytoplasmic reactivity counts as specific (HER2, EGFR).
- Although rare, several antigens have a characteristic **combined nuclear AND cytoplasmic** reactivity. This category most notably includes **S100**, **p16**, and **calretinin** (calretinin must be BOTH cytoplasmic and nuclear to be interpreted a positive staining in mesothelioma). **β-catenin** is membranous in most normal epithelia and cytoplasmic in stromal cells. However, it is the shift to nuclear reactivity that is a characteristic feature of several tumor types associated with mutations in adenomatosis polyposis coli (*APC*) or *CTNNB1* (encodes β-catenin) genes in the WNT pathway, such as desmoid-type fibromatosis and some colon CAs.
- **Granular** reactivity usually indicates localization to cytoplasmic organelles (mitochondria, Golgi, secretory vesicles, etc.). Distinctive granular cytoplasmic reactivity is typical of AMACR/racemase (mitochondrial/peroxisomal), HepPar1 (mitochondrial), and Napsin A (lysosomal). Dot-like CD30 in ALCL and classical HL and CD15 in classical HL are attributed to Golgi staining and are seen in conjunction with typical membranous staining generating the so-called "targetoid" or "ball and chain" appearance.
- Finally, **punctate** (aka perinuclear dot-like) reactivity is typical of some antigens that aggregate in the cytoplasm, most notably CK pattern in neuroendocrine CAs, including SmCC (pan-CK) and Merkel cell CA (CK20). This occurs due to the formation of CK tangles.
- ALK stain may show different patterns of staining in different tumors depending on the translocation partner!
- Note that there are some instances in which the **lack of immunoreactivity** is what is significant. One example is the loss of E-cadherin in lobular CA of the breast. Another is the loss of SMARCB1 (INI1) and rarely SMARCA4 (BRG1) in malignant rhabdoid tumors, as well as many other recently identified INI1/BRG1-deficient tumors.
- Beware of classic **false positives** (tissue edge effect, mast cell positivity, non-specific staining of hepatocytes due to high albumin content, positive staining in entrapped benign cells like TTF-1 staining of entrapped pneumocytes in tumors metastatic to the lung) and classic **false negative** as a result of failed IHC (always check controls, particularly normal structures serving as internal positive controls). Also beware of non-specific cytoplasmic reactivity for antigens with expected nuclear localization (such as TTF-1 or ER) – this should not be accepted as positive!

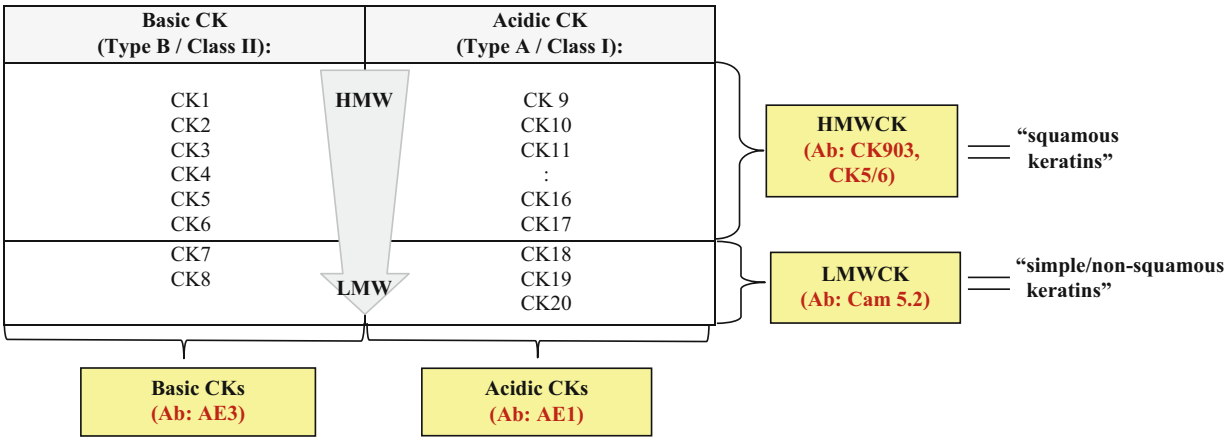


Abbreviations: *ALCL* anaplastic large cell lymphoma, *HL* Hodgkin lymphoma, *CK* cytokeratins

Primer on Cytokeratins

(*currently preferred designation for cytokeratins is “keratins”)

- Cytokeratins (CK) are cytoskeletal proteins that belong to a family of intermediate filaments (IFs). CKs are present in epithelial cells and are regarded as the most fundamental markers of epithelial differentiation. Other members of IF family are also used as markers of differentiation, including vimentin (for mesenchyme), GFAP (for glia), desmin (for muscle), and neurofilament (for neurons).
- There are 20 distinct types of CKs (plus hair- and nail-specific CKs).
- CKs were characterized by Moll et al., and the currently used CK designation system is known as the “Moll’s catalogue” [1].
- CKs are designated in a somewhat non-intuitive fashion based on their migration pattern in a two-dimensional (2D) gel electrophoresis, which separates proteins based on size and charge.
- Based on the 2D gel migration, CKs fall into two categories: basic (CK1 through 8) and acidic (CK9 through 20). Within each group, CKs are numbered in order of decreasing size, from high molecular weight (HMW) to low molecular weight (LMW), as diagramed below.



- In a cell, CKs exist as heterodimers, composed of acidic + basic subunits of a similar size. Therefore certain pairs of CKs (such as CK8/18, CK1/10) are expressed jointly.
- For diagnostic purposes, CKs are divided into LMWCK and HMWCK, as indicated on the diagram. This division corresponds to a distinct distribution of these two groups of CKs in normal tissues:
  - **HMWCK**s are expressed predominantly in squamous epithelia (and in basal cells), and they are known as “squamous keratins.” HMWCKs are large, and they are able to form a dense cytoplasmic network of filaments, accounting for resistance to mechanical stress of the surface epithelia. Large bundles of HMWCKs are known ultrastructurally (by electron microscopy) as “tonofilaments,” and these structures are the hallmark of squamous epithelia and SqCC.
  - In contrast, **LMWCKs** are loosely distributed in the cytoplasm and are unable to bundle. They are therefore characteristics of visceral organs, which experience little mechanical stress (such as the liver, kidney, and various glandular epithelia). LMWCKs are known as “non-squamous or simple keratins.” LMWCKs are expressed in all epithelial tissues with the exception of keratinizing squamous epithelium. Note that some glandular epithelia (such as breast) do co-express HMWCK in addition to LMWCK, and HMWCK can be induced in non-squamous epithelia as a result of reactive conditions (such as inflammation). So the rule of thumb “squamous epithelium = HMWCK” vs. “non-squamous epithelium = LMWCK” is not 100%.
  - A designation of “intermediate molecular weight CKs” is occasionally applied, which refers to the lighter CKs within the HMWCK group (CK 5, 6, 17). These are also known as “basal keratins” because they are expressed preferentially in basal cells.
- The above patterns of CKs are generally retained in corresponding CAs and can serve as useful diagnostic tools. As a word of caution – some CAs deviate from CK patterns of their parent epithelia. For example, high-grade SqCCs frequently co-express LMWCKs and HMWCKs. In addition, some adenoCAs are well known to co-express HMWCKs (such as CAs of the pancreas, endometrium, lung, and a subset of breast).
- To increase the yield of diagnostic IHC, expression of CKs is usually analyzed by mixtures of various CK antibodies (Abs), known as “Ab cocktails.” The commonly used Ab cocktails include:

Antibody cocktail	What it detects
AE1	All **acidic** CKs except CK 9, 12, 17, and 18
AE3	All **basic** CKs (CK1-8)
AE1/AE3 (pan-CK)	All types of CKs (except those missing in AE1)
OSCAR, PANK (MNF-116)	Broad-spectrum CK cocktail (similar to AE1/AE3)
Cam5.2	**LMWCK**s (CK 7, 8)
CK903 (K903; 34βE12)	**HMWCK**s (CK 1, 5, 10, 14)
CK5/6	**HMWCK**s (detects primarily CK5)

Summary				
Epithelium type	*Corresponding carcinoma*	*CK profile*	*Antibody reactivity*	
Squamous and **basal cells**	Squamous cell carcinoma	**HMWCK**[1]	**CK903** **CK5/6**	Pan-CK (**AE1/AE3**)
Glandular epithelia (bowel, prostate, etc.) and **visceral parenchyma** (liver, kidney, etc.)	Colon adenocarcinoma Prostate adenocarcinoma Hepatocellular carcinoma Renal cell carcinoma Etc.	**LMWCK**[2]	**Cam5.2**	
1. HMWCKs (without LMWCKs) are expressed in keratinizing squamous epithelia and in the majority of SqCCs. However, some poorly differentiated nonkeratinizing SqCCs (particularly of mucosal surfaces and visceral organs, such as the lung) do co-express LMWCKs. 2. Similarly, while the majority of adenoCAs and CAs of visceral epithelia express LMWCKs only (e.g., prostate, HCC, RCC), some adenoCAs are well known to co-express HMWCKs (e.g., pancreas, endometrium, breast, lung). Urothelial CA also co-expresses LMW and HMW CKs.				

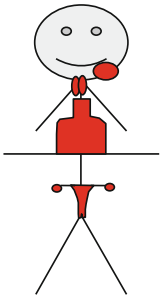
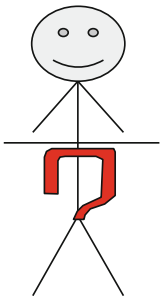
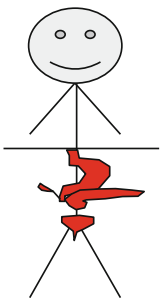
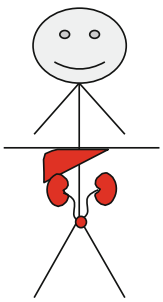
- **Practical applications:** The most common application of CKs is to identify a poorly differentiated malignancy as a carcinoma. In practical terms, expression of CKs (and to a lesser degree EMA) is what defines a poorly differentiated neoplasm as a carcinoma as opposed to sarcoma, lymphoma, melanoma, or glioma. For this "screening" purpose, CK antibodies are used as follows:
  - **AE1/AE3** (or another favorite pan-CK) is a good first-line marker: it recognizes both LMW and HMW CKs and will identify virtually all types of carcinoma, squamous and non-squamous alike. The main caveat is that there are few carcinomas, most notably HCC (see table below for complete list), which may be better recognized by Cam5.2 since they express primarily LMW keratins.
  - **Cam5.2** is therefore a good complement to AE1/AE3 as it may be better at identifying CAs expressing primarily LMW CKs. The main drawback of Cam5.2 as a screening Ab is that it may not recognize SqCC (recall that squamous epithelium has HMW but not LMW CKs!). In this sense, AE1/AE3 and Cam5.2 are complementary for screening purposes.
  - Note that **HMWCK**s have a more restricted expression profile than LMWCKs, particularly in the visceral organs where non-squamous CAs predominate. Therefore HMWCK antibodies (such as CK903) are generally not used for screening purposes but are reserved for a number of specific differentials (see table below).
  - Examples of utilization of LMW vs. HMW CKs in DDx of CAs are:
    - DDx of prostate CA (HMWCK–) vs. urothelial CA (HMWCK+)
    - DDx of Paget disease (Cam5.2+, CK903–) vs. SqCC in situ/Bowen disease (Cam5.2–, CK903+)
  - Of particular diagnostic utility are CK7 and CK20, which have a striking organ-specific distribution. These are the workhorse Abs in the work-up of CAs of unknown primary (see table below).
- In addition to CKs, EMA is sometimes used as a "CK helper" to confirm epithelial differentiation, although it is less specific and may be expressed in many other non-epithelial tumors.
- Although expression of CKs (and EMA) is held as a defining feature of CAs, beware of epithelial marker reactivity in some **non-carcinomas**, including:

1. **Tumors with true epithelial differentiation** yet NOT usual carcinomas (i.e., not tumors of surface or glandular epithelia). This includes non-seminoma germ cell tumors (yolk sac tumor, embryonal CA), trophoblastic tumors, mesothelioma, biphasic synovial sarcoma (epithelial component), and adamantinoma-like variant of Ewing sarcoma. Reactivity is for multiple CKs (and EMA) and is usually strong and diffuse.
2. Certain **sarcomas that consistently stain for epithelial markers**: epithelioid sarcoma, desmoplastic small round cell tumor, and chordoma.
3. Various neoplasms with **aberrant expression** of epithelial markers (usually negative but a fair subset frustratingly positive): Ewing sarcoma (20%), angiosarcoma and epithelioid hemangioendothelioma, leiomyosarcoma, MPNST, and even melanoma. Usually labeling is with only one epithelial marker, most commonly LMWCK Cam5.2, typically focal.
4. Gliomas and reactive astrocytes. Beware of cross-reactivity with AE1/AE3 (Cam5.2 and EMA should be negative!).

Select References: [1–6]

Epithelial Markers at a Glance		
Antibody	***Background***	***Applications***
AE1/AE3	AE1/AE3 is a **broad-spectrum CK** antibody (Ab) cocktail, which reacts with both LMWCK and HMWCK. It identifies virtually all types of epithelial neoplasms.	AE1/AE3 is the first-line epithelial marker in screening for CAs. Although AE1/AE3 reacts with virtually all epithelial neoplasms, there are few notable exceptions: • **HCC** is AE1/AE3– (Cam5.2+; CK903–, EMA–). • **RCC** is variably reactive with both AE1/AE3 and Cam5.2 (EMA is best). • **NE CAs**, including SmCC, show variable reactivity with both AE1/AE3 and Cam5.2. • **Adrenocortical neoplasms** frequently do not react with AE1/AE3 or any other epithelial markers (Cam5.2, EMA).
Cam5.2 (34βH11)	Cam 5.2 is a **LMWCK** Ab cocktail. It reacts with virtually all non-squamous epithelia; squamous cells are usually (but not always) negative for LMWCK.	• Cam5.2 is used in conjunction with AE1/AE3 to screen for CAs. In particular, Cam5.2 is useful for identification of CAs which may be missed by AE1/AE3 (see above). Cam 5.2 is also used for DDx of Paget disease (Cam5.2+, CK903–) from Bowen disease (Cam5.2–, CK903+).
CK903 (34βE12)	CK903 is a **HMWCK** Ab cocktail. It reacts with squamous, urothelial, and few glandular epithelia. It also recognizes basal and myoepithelial cells.	Because of a more restricted distribution of HMWCKs, CK903 and CK5/CK6 are not generally used as screening Abs. Instead, they have several specific applications: • DDx of urothelial CA (CK903+) vs. prostate cancer (CK903–) • DDx of mesothelioma (CK5/6+) vs. adenoCA (CK5/6–) • ID of basal cells in prostatic lesions (CK903+): present in benign glands vs. absent in invasive cancer • ID of metaplastic breast cancer (CK903+) • DDx of usual duct hyperplasia (CK903+ epithelial cells) vs. DCIS (CK903–)
CK5/CK6	CK5/CK6 is another **HMWCK** Ab. Reactivity is generally similar to CK903. Selection of CK903 versus CK5/CK6 for a particular application is usually empirically based.	
CK7 and CK20	CK7 and CK20 are LMWCKs, which show distinctive patterns of expression in various organs (see below).	CK7 and CK20 profiles are used to identify the origin of CA of unknown primary: • **CK7+**: above-the-diaphragm organs (lung, breast, thyroid) and female GYN tract (uterus, ovary) • **CK20+**: below-the-diaphragm organs (colorectum) and Merkel cell CA • **CK7+, CK20+**: peri-diaphragmatic GI organs (pancreas, biliary tree, stomach) and urothelium • **CK7- and CK20-negative:** simple visceral epithelia (except colon) – liver, kidney, prostate, and neuroendocrine cells
EMA (MUC1)	EMA labels the majority of non-squamous CAs (see below for exceptions). Strongest expression is in CAs derived from secretory epithelia (eccrine, breast, pancreas). EMA also labels several non-epithelial tissues and neoplasms (listed below). EMA is less sensitive and less specific for epithelial differentiation than CKs.	EMA is used in conjunction with CKs as a "CK helper" to ID CAs. In particular, it is helpful in identifying RCC, which is EMA+, but is variably reactive for CKs. Other uses include: • DDx of RCC (EMA+) vs. adrenocortical neoplasms (EMA–) • ID of meningioma and a few other EMA+ non-epithelial neoplasms (see table below)
CEA	CEA is expressed in some but not all CAs (see table below).	If positive, CEA supports the diagnosis of CA (as opposed to lymphoma, sarcoma, melanoma). However, negative CEA does not rule out a CA since only a fraction of CAs are reactive. Beware of the difference between polyclonal and monoclonal CEA (see Chapter 6).
Ber-EP4 and MOC31	Ber-EP4 and MOC31 are antibodies against epithelial cell adhesion molecule (EpCAM) that react with majority of adenoCAs of various sites.	Ber-EP4 and MOC31 are primarily used to differentiate lung adenoCA (Ber-EP4+ and MOC31+) from mesothelioma (Ber-EP4– and MOC31–). Favored markers in effusion cytology because they selectively label adenoCA, whereas background mesothelial cells are negative (CK would label both).
Claudin-4	Similar to Ber-EP4 – a pan-carcinoma marker.	Used to distinguish CA (+) from mesothelioma (–). Currently considered the best marker for this application. To distinguish poorly diff. CAs (+) from various sarcomas with epithelioid morphology (–) [7].
The main application of epithelial markers is to differentiate epithelial neoplasms (CAs) from non-epithelial neoplasms (lymphoma, melanoma, sarcoma, glioma). AE1/AE3, Cam5.2, and EMA are the first-line screening antibodies for this purpose. Note that in addition to CAs, epithelial marker reactivity may be seen in some non-epithelial neoplasms (e.g., synovial sarcoma); see above for details.		

CK7 and CK20 Expression Profile Diagram

CK7+ CK20– **Above-the-diaphragm** organs (lung, breast, thyroid, salivary gland) and **female GYN tract** (uterus, ovary) 	**CK7– CK20+** **Below-the-diaphragm** GI tract (colorectum) and Merkel cell carcinoma 
CK7+ CK20+ **Peri-diaphragmatic** GI organs (pancreas, biliary tree, stomach) and **bladder** 	**CK7– CK20–** **Simple visceral** epithelia (except the colon): liver, kidney, and prostate 

Abbreviations: *CK* cytokeratin, *GI* gastrointestinal, *GYN* gynecologic

CK7 and CK20 Expression Profiles

Predominant CK7/CK20 Profiles			
CK7+ CK20+	**CK7+ CK20–**	**CK7– CK20+**	**CK7– CK20–**
Pancreaticobiliary Stomach Bladder Ovary (mucinous)	Breast Lung (carcinoma + mesothelioma) Endometrium Ovary (non-mucinous) Thyroid Salivary gland Kidney (papillary RCC)	Colorectum Merkel cell carcinoma	Liver (HCC) Kidney (clear-cell RCC) Prostate Adrenal cortex

	CK7+ CK20+ (%)	**CK7+ CK20– (%)**	**CK7– CK20+ (%)**	**CK7– CK20– (%)**
Adenocarcinoma				
Breast, ductal	10	86	2	2
Breast, lobular	6	94	0	0
Cholangiocarcinoma	65	28	5	2
Colorectum[1]	8	0	82	10
Uterus	9	86	0	6
Lung	10	90	0	0
Ovary	2	98	0	0
Pancreas	64	28	5	3
Prostate	3	3	10	84
Salivary gland	0	100	0	0
Stomach	32	19	35	14
Thyroid	0	98	0	2
Squamous cell carcinoma				
Cervix	0	87	0	13
Esophagus	0	21	0	79
Head and neck	0	27	6	67
Lung	0	26	4	70
Neuroendocrine neoplasms				
GI tract, carcinoid tumor	0	13	7	80
Lung, carcinoid tumor	0	22	0	78
Lung, liver and small bowel, neuroendocrine carcinoma	0	56	0	44
Lung, small cell carcinoma	0	24	0	76
Merkel cell carcinoma	0	0	78	12
Thyroid, medullary carcinoma	0	98	0	2
Other				
Adrenocortical tumor	0	0	0	100
Epithelioid sarcoma	0	0	0	100
Germ cell tumors	0	7	0	93
Hepatocellular carcinoma	5	15	2	78
Mesothelioma	0	67	0	33
Renal cell carcinoma	0	17	3	80
Thymoma	0	0	0	100
Urothelial carcinoma	53	40	2	5

1. In contrast to the colon, rectal adenocarcinomas are frequently (~70%) CK7-positive [8], and small intestinal adenocarcinomas are typically CK7+/CK20 variable [9].

Reference: [2]

Expression of EMA, CEA, and Vimentin in Carcinomas

1. EMA is present in conjunction with cytokeratins (CKs) in the majority of carcinomas and CK-positive non-epithelial tumors (e.g., epithelioid sarcoma, synovial sarcoma, desmoplastic small round cell tumor). The tumors that show a discordant expression of CKs and EMA are listed below:

CK+/EMA–	CK–/EMA+
HCC (Cam5.2+, AE1/AE3–, CK903–) Adrenocortical neoplasms (frequently negative for all CKs) Most neuroendocrine neoplasms Embryonal carcinoma, yolk sac tumor Thyroid	Meningioma Perineurioma Plasma cell neoplasms Anaplastic large cell lymphoma Popcorn or lymphocyte-predominant (LP) cells [formerly L and H cells] in Hodgkin lymphoma RCC (sometimes)

2. CEA is variably expressed in carcinomas as detailed below. However, CEA lacks specificity, and with the exception of canalicular staining in HCC, it is not routinely used to differentiate carcinomas.

CEA-positive carcinomas	CEA-variable carcinomas	CEA-negative carcinomas
HCC (canalicular pattern with pCEA*) Colorectum Stomach Lung adenocarcinoma Pancreaticobiliary	Urothelial carcinoma Breast Cervix	Kidney Adrenal Prostate Mesothelioma Ovary (serous) Endometrium
*Polyclonal but not monoclonal CEA cross-reacts with biliary epithelium in normal and neoplastic liver		

3. Vimentin is generally considered to be a mesenchymal marker, and it has been used in the past to differentiate sarcoma (vimentin-positive) from carcinoma (vimentin-negative). It is now known that vimentin is very non-specific and is variably expressed in many carcinomas. The table below is mainly of historic interest – vimentin is now rarely used to differentiate tumors. The main current application of vimentin is to confirm tissue immunoviability when all other markers are negative.

Vimentin-positive carcinomas	Vimentin-negative carcinomas
RCC, clear-cell type Endometrium Mesothelioma Salivary gland Thyroid Sweat gland Spindle cell carcinoma of any site	RCC, chromophobe type Endocervix (adenocarcinoma) Lung carcinoma Breast Ovary Prostate Colorectum HCC

References: [4, 6]

Primer on Markers of Muscle Differentiation

	Desmin	MSA (HHF-35)	SMA (α-actin)	Calponin	h-Caldesmon	SMMHC	MyoD1*, myogenin*, α-sarcomeric actin
Skeletal muscle	+	+	–	–	–	–	+
Smooth muscle and myoepithelial cells	+	+	+	+	+	+	–
Myofibroblast	+/–	+/–	+	+/–	–	–	–

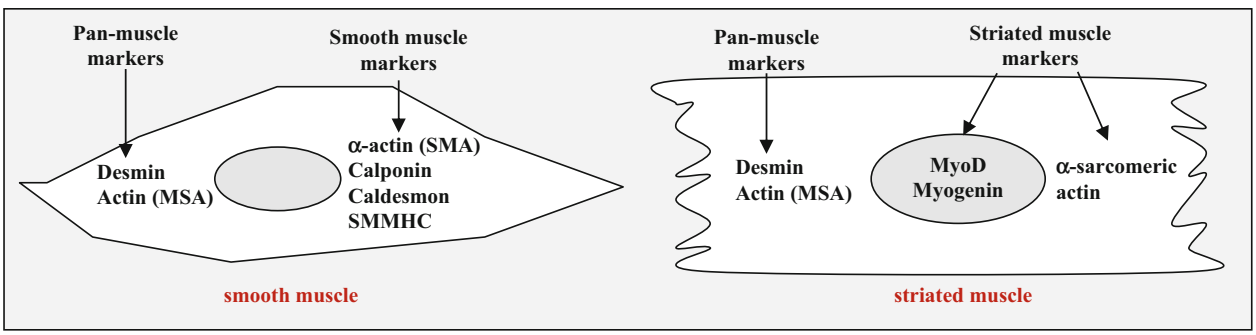
*MyoD and myogenin (both transcription factors) are nuclear; all other markers are cytoplasmic. (SMA is usually membranous with "tram-track pattern" in myofibroblasts).

- **Desmin** is a universal marker of muscle cells. It is expressed both in smooth and striated muscle cells; expression is variable in myofibroblasts. Desmin is an intermediate filament (a counterpart to cytokeratins in epithelial cells).
- **Muscle-specific actin (MSA)**, like desmin, is another pan-muscle marker. The antibody to MSA, HHF-35, recognizes the epitope common to α-skeletal, α-cardiac, and γ-smooth muscle actins.
- **Smooth muscle actin (SMA)**, aka α-actin, is a smooth muscle-specific isoform of actin; it is absent in striated muscle.
- **Calponin**, **h-Caldesmon**, and **smooth muscle myosin heavy chain (SMMHC)** are contractile apparatus-associated proteins, which are unique to smooth muscle. Note that caldesmon and SMMHC are generally absent in myofibroblasts.
- **MyoD** and **myogenin** are transcription factors (thus are nuclear), which are specific to striated muscle; they are absent in smooth muscle. **α-sarcomeric actin** is a cytoplasmic marker of striated muscle. These markers are used to differentiate rhabdomyosarcoma (positive) from leiomyosarcoma (negative). The pattern of reactivity can also be helpful, as embryonal rhabdomyosarcoma shows focal positivity, while alveolar rhabdomyosarcoma is diffusely positive for MyoD/myogenin. Usually interchangeable, but tumors of immature skeletal muscle (e.g., embryonal rhabdomyosarcoma) are sometimes MyoD+/myogenin– because MyoD is expressed earlier in rhabdomyogenesis.
- **Myoepithelial (ME) cells** show differentiation as BOTH smooth muscle and epithelial cells (they are therefore CK+). These cells are curiously polyphenotypic and express markers of various other tissues: GFAP (glial), S100 (neural), and p63/p40 (basal cell). In ME neoplasms, these various markers are expressed inconsistently, so usually the entire panel is performed. To prove ME differentiation, it is generally required that a tumor stains for at least one epithelial marker (CK or EMA) PLUS one smooth muscle marker (SMA or calponin) OR S100 [10].
- **Myofibroblasts** show differentiation as both smooth muscle cell and fibroblast. Unlike myoepithelial cells, smooth muscle differentiation is INCOMPLETE, and only some muscle markers are expressed (actin-positive, desmin-variable, caldesmon- and SMMHC-negative). This feature may be used to differentiate myofibroblastic tumors (caldesmon –) from smooth muscle tumors (caldesmon +). Oddly, myofibroblasts are sometimes CK+ (as in inflammatory myofibroblastic tumor). Another handy feature distinguishing myofibroblasts from smooth muscle cells is the pattern of actin reactivity: peripheral ("tram-track"-like) in the former vs. diffuse cytoplasmic in the latter.

Applications:

- To ID smooth or skeletal muscle differentiation in poorly differentiated neoplasms. Reactivity may be variable, so actins and desmin are best used in conjunction.
- To diagnose myoepithelial tumors (e.g., myoepithelioma) and myofibroblastic tumors (e.g., nodular fasciitis, fibromatosis).
- To diagnose invasive breast cancer: calponin, SMA, and/or SMMHC (along with p63/p40) may be used to identify myoepithelial cell layer which surrounds benign and in situ lesions and is absent in invasive carcinoma.
- Some use desmin to differentiate reactive mesothelial cells (positive) from mesothelioma (negative).

Note: Several non-myogenic tumors/tissues are unexpectedly desmin+ (but actin–). This includes desmoplastic small round cell tumor, blastemal component of Wilms tumor, mesothelial cells (benign ≫ malignant), and few others. Otherwise, desmin usually goes together with actins. On the other hand, actin is a bit more sensitive than desmin, and therefore actin+/desmin– reactivity is not unusual (such as in some leiomyosarcomas and myofibroblastic lesions).



Abbreviations: *MSA* muscle-specific actin, *SMA* smooth muscle actin, *SMMHC* smooth muscle myosin heavy chain

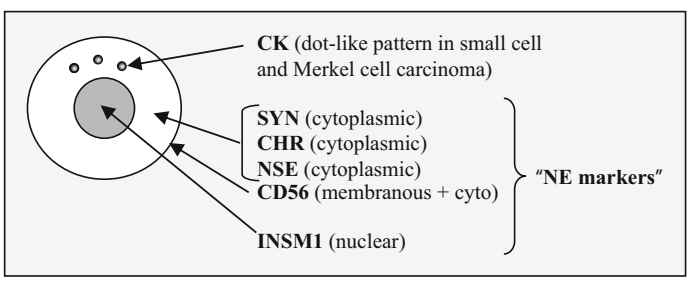
Primer on Markers of Neuroendocrine (NE) Differentiation

- The classic list of "NE markers" includes **synaptophysin** (**SYN**), **chromogranin A** (**CHR**), and **CD56** (NCAM or neural cell adhesion molecule). **CD57** (Leu-7) and **neuron-specific enolase** (**NSE**) are second-line NE markers, and are rarely used in practice. A recent addition to the NE markers is **insulinoma-associated protein 1 (INSM1)**. NE markers are usually strongly expressed in low-grade NE neoplasms (e.g., carcinoid), whereas expression may be weak/focal in high-grade NE neoplasms (e.g., SmCC).
- **SYN** and **CHR:**
  - These are the first-line markers of NE differentiation (along with CD56).
  - SYN and CHR mark neurosecretory granules and hence show granular cytoplasmic staining.
  - Overall, **SYN is more sensitive than CHR** but **CHR is more specific**. Some NE tumors will label for either CHR or SYN but not both, so **these are complementary and usually are ordered together**.
  - In addition to NE neoplasms, SYN and variably CHR are also present in neoplasms of neuronal origin (e.g., gangliocytoma) and primitive neuroectodermal neoplasms (neuroblastoma, medulloblastoma, Ewing).
  - There are few non-NE neoplasms which can be SYN+ but are always CHR– (here staining is unrelated to NE granules). These include adrenocortical neoplasms and pancreatic solid pseudopapillary neoplasm.
- Historically, **NSE** was considered to be a first-tier marker of NE neoplasms. It does have a high sensitivity but also suffers from low specificity. In addition to NE lesions, it also reacts with astrocytomas, meningioma, schwannoma, and adrenocortical neoplasms, among others. NSE is therefore no longer considered a first-line marker (think of NSE as "not-so-specific esterase").
- **CD56** is the most sensitive NE marker in every organ, but it is not entirely specific (also marks NK-cells, peripheral nerve sheath tumors, synovial sarcoma, etc.). Generally, SYN and CHR do a good enough job identifying low-grade NE neoplasms (e.g., carcinoid), and CD56 is not usually needed in this situation. However, CD56 can save the day when it comes to high-grade NE neoplasms, especially SmCC, which may be negative for all other NE markers but are usually positive for CD56.
- **INSM1** (insulinoma-associated protein 1) is a new NE marker that has thus far demonstrated high sensitivity and specificity in a number of NE tumor types in various organ systems, including the cervix (high-grade cervical NE CA), gastrointestinal tract, lung, and head and neck. At the time of writing, INSM1 promises to be more sensitive and specific compared to standard markers combined [11]**.** Stay tuned!

	SYN	CHR	CD56	NSE	INSM1
Sensitivity	++	+	++	++	++
Specificity	+	++	+/–	–	++
++ best, + intermediate, – worst					

- **Peptide hormones** may occasionally be helpful in establishing the identity of a NE CA. For example, calcitonin supports the diagnosis of medullary CA (although it can also be positive in other NE neoplasms, especially atypical carcinoid of the larynx). In fact, most NE neoplasms are capable of producing ectopic hormones (e.g., pancreatic endocrine neoplasms may produce gastrin, ACTH, PTH, etc.). In addition, some NE neoplasms are nonfunctional and will be negative for any hormones. Therefore, assigning the site of origin of an occult neuroendocrine tumor based on hormone expression is generally not recommended.

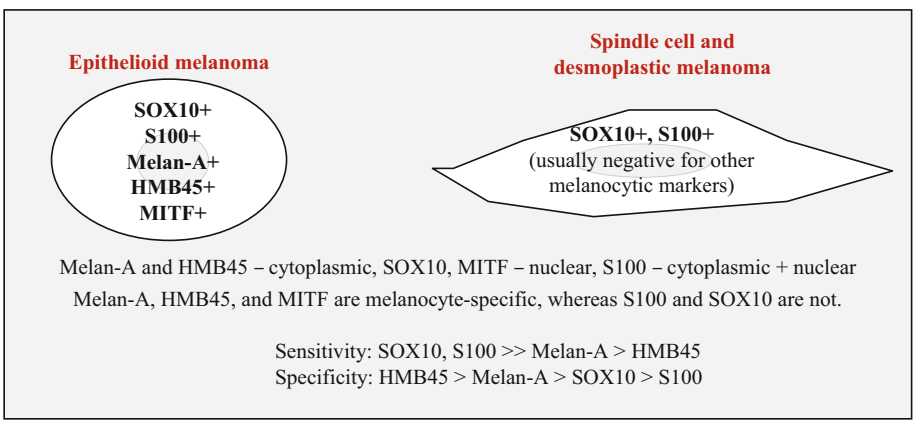
- **Cytokeratins (CK) in NE neoplasms:**
  - NE neoplasms fall into two categories: epithelial (e.g., carcinoid/NET, SmCC) and non-epithelial/neural (e.g., pheochromocytoma, paraganglioma, neuroblastoma). Epithelial NE neoplasms are CK-positive, whereas non-epithelial NE neoplasms are CK-negative.
  - Although usually at least one of CKs is positive, the reactivity is notoriously variable in NE neoplasms (particularly SmCC). Cam5.2 is considered to be a more reliable marker than AE1/AE3 in these lesions. To be safe, both Cam5.2 and AE1/AE3 should be performed if a NE tumor is in the differential. Be aware that expression can be focal and relatively unimpressive in SmCC because there is so little cytoplasm in the cells, requiring examination on high power.
  - In addition, distinctive feature of high-grade NE CAs (small cell and Merkel cell CAs) is that CK reactivity has a dot-like (punctate) perinuclear pattern. This pattern of reactivity applies to Cam5.2 and AE1/AE3 labeling of SmCC and Merkel cell CA and CK20 labeling of Merkel cell CA. Punctate reactivity is thought to be due to the formation of CK tangles in these tumors. This is a helpful diagnostic feature because punctate reactivity for CKs not only confirms that a tumor is epithelial but also suggests that it is neuroendocrine.



Abbreviations: *CHR* chromogranin A, *CK* cytokeratin, *SYN* synaptophysin, *NE* neuroendocrine, *NET* neuroendocrine tumor (carcinoid equivalent in pancreas and GI tract), *NSE* neuron-specific enolase, *INSM1* insulinoma-associated protein 1

Primer on Markers of Melanocytic Differentiation

- Key markers of melanocytic differentiation include **SOX10**, **S100, Melan-A/MART-1**, **HMB45**, and **MITF**. Also available (but rarely used) are stains for **tyrosinase** and special stains for melanin, such as Fontana Masson.
- **S100** is a calcium-binding protein, named for its solubility in 100% ammonium sulfate. S100 is a marker of nerve crest-derived tumors (melanoma, nerve sheath tumors), but it also stains many other tumors (so S100 is also known as "stains 100 things").
  - Positive in >90% of melanomas, including spindle-cell and desmoplastic types.
  - S100 has traditionally been considered the most sensitive melanocytic marker, but now SOX10 is available which has similarly high sensitivity but better specificity (S100 may still be best for desmoplastic melanomas).
  - Other S100-positive tumors include nerve sheath tumors, myoepithelial neoplasms, granular cell tumor, Langerhans cell histiocytosis, chordoma, gliomas, lipomatous tumors, and some CAs, such as the breast. It also stains dendritic cells. Therefore S100 is always used as part of a panel.
- **SOX10** (SRY-related HMG-box 10) is a nuclear transcription factor**.** This is a relatively recent marker that has emerged as the **first-line melanocytic marker** [12, 13].
  - Positive in >95% of epithelioid and spindle-cell melanomas and >80% of desmoplastic melanomas.
  - Nuclear reactivity is particularly useful for looking at melanoma in situ and/or intraepithelial component of malignant melanoma because unlike S100 it does NOT stain melanin that is present in the keratinocytes or dendritic cells.
  - Like S100, SOX10 is also a marker of nerve crest-derived tumors, and its expression has significant overlap with S100, but overall it is much more specific than S100. Like S100, SOX10 is also positive in nerve sheath tumors (schwannoma, neurofibroma, subset of MPNST), gliomas, granular cell tumor, myoepithelial and some salivary neoplasms. Unlike S100, SOX10 is negative in histiocytic and fibrohistiocytic proliferations. SOX10 is also a marker of breast CAs (enriched in triple-negatives).
- **Melan-A** (**A103**) and **MART-1** (melanoma antigen recognized by T cells) are two distinct antibodies that recognize the same antigen.
  - Positive in 80–100% of epithelioid melanomas.
  - Spindle-cell and desmoplastic melanomas are generally negative or patchy.
  - Melan-A (A103) cross-reacts with steroid hormone-producing tumors (adrenocortical neoplasms and sex cord-stromal tumors of the gonads).
- **HMB45** (human melanoma black) recognizes the gp100 protein, which is present in premelanosomes.
  - Positive in 60–90% of epithelioid melanomas.
  - Spindle-cell and desmoplastic melanomas are generally negative.
  - HMB45 is less sensitive than Melan-A and S100/SOX10 but more specific (does not react with steroid hormone-producing tumors).
  - HMB45 is expressed specifically in immature melanocytes, whereas mature melanocytes are negative. This feature can be exploited to differentiate melanoma (+) from mature nevus cells (−) both at the *primary site* (melanoma: no maturation toward the base → all cells HMB45+ vs. nevus: cells mature toward the base and become HMB45–) and in *lymph nodes* (metastatic melanoma: HMB45+ vs. intranodal nevus: HMB45–).
- **MITF** (microphthalmia transcription factor) is a nuclear regulator. It is claimed to be as sensitive as Melan-A for epithelioid melanomas, but it is also suboptimal (~40%+) for spindle-cell and desmoplastic melanomas. It has the advantage of having nuclear reactivity, which can be easier to interpret.
- In addition to melanoma, all melanoma-associated antigens are also expressed in other melanosome-containing tumors, such as clear-cell sarcoma/melanoma of soft parts, melanotic neurofibroma, melanotic schwannoma, as well as PEComas (perivascular epithelioid cell tumors) family, which includes angiomyolipoma, lymphangioleiomyomatosis, pulmonary sugar tumor, and other rare clear-cell tumors. PEComas are primarily positive for HMB45 and Melan-A but not SOX10 (S100 is positive in a subset, ~30%).
- Although practice varies, a combination of SOX10 plus Melan-A or HMB45 is a good first-line panel if melanoma is in the DDx.



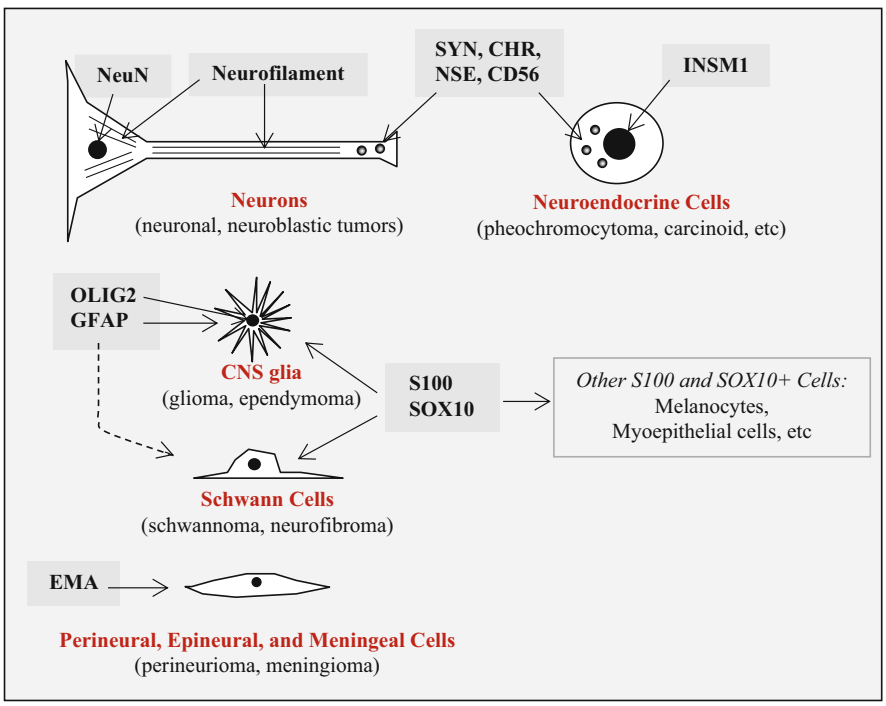
Primer on Markers of Neuroglial Differentiation

(reviewer Tajus Bale)

- **Neuronal markers** include**:**
  - **Neurofilament (NF),** which includes SMI-311 (pan-NF), SMI-32 (cell body), and SMI-31 (axons)
  - **NeuN,** neuronal nuclei (nuclear marker)
  - **Synaptophysin (SYN)** and **chromogranin A (CHR)**: react with synaptic vesicles in neurons and neurosecretory granules in neuroendocrine cells (more so SYN than CHR).
  - **Neuron-specific enolase (NSE)** and **CD56** (neural cell adhesion molecule, NCAM): react with neurons and neuroendocrine cells. Despite the name, NSE is highly non-specific but it is sensitive.

  Neuronal markers may be used to:
  i. ID of tumors with neuronal/ganglion cell differentiation (e.g., gangliocytoma) or neuroblastic differentiation (e.g., neuroblastoma, medulloblastoma). SYN is best for this purpose.
  ii. ID of brain infiltration: SMI-31 may be used to highlight normal axons to help identify the permeation of normal brain parenchyma by a glioma or meningioma.

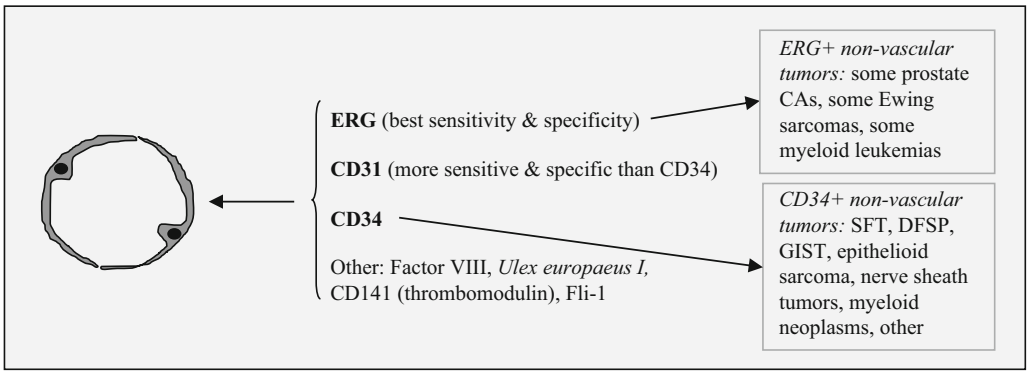
- **Glia** (astrocytes, oligodendrocytes, and ependymal cells) and corresponding neoplasms (glioma, astrocytoma, and ependymoma) are identified by **GFAP** (glial fibrillary astrocytic protein) and **OLIG2** (oligodendrocyte transcription factor; nuclear marker), although, unlike GFAP, OLIG2 is rarely expressed in ependymomas. These markers may be used to distinguish a glioma from non-glial neoplasms (lymphoma, CA, melanoma) and inflammatory conditions (e.g., multiple sclerosis). GFAP also variably reacts with Schwann cells.
- **Schwann (nerve sheath) cells** are identified primarily by **S100** and **SOX10**. These markers also react with gliomas as well as a number of other neoplasms, including melanoma. Note that neither SOX10 nor S100 can be used to distinguish neuroglial neoplasms from melanoma.
- **Neuroendocrine (NE) cells** react with SYN, CHR, INSM1, as well as NSE and CD56. Cytokeratin expression depends on the specific type of NE neoplasm: carcinoids are CK (+), whereas pheochromocytoma is CK (−). See section on NE markers for details. NE cells are S100-/SOX10-negative, but S100 and SOX10 mark supportive (sustentacular) cells in some NE neoplasms, most notably paraganglioma/pheochromocytoma.
- **Cytokeratins:** All things glial/neuronal/nerve sheath are Cam5.2 (−) and AE1/AE3 (−). However, beware of non-specific AE1/AE3 staining in normal and neoplastic brain, particularly in reactive astrocytes (Cam5.2 should be negative, though).



Abbreviations: *CHR* chromogranin, *EMA* epithelial membrane antigen, *GFAP* glial fibrillary astrocytic protein, *NSE* neuron-specific enolase, *OLIG2* oligodendrocyte transcription factor, *SYN* synaptophysin

Primer on Markers of Vascular Differentiation

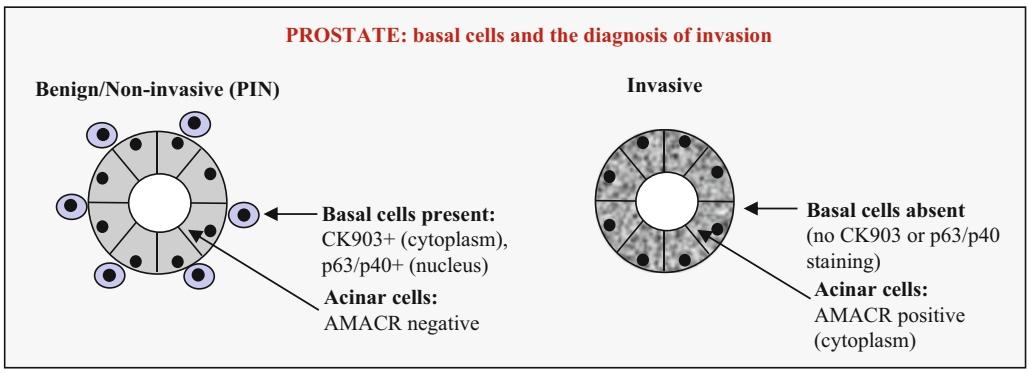
- There are several markers of vascular differentiation in current use. The most commonly used vascular markers include **CD31**, **CD34**, and the recent nuclear marker **ERG**. **ERG** is becoming a first-line vascular marker. Older (currently largely historic) vascular markers include **Factor VIII** (actual antigen is von Willebrand factor), **Fli-1**, ***Ulex europaeus I***, and **CD141** (thrombomodulin).
- These markers react with both blood vessels and lymphatics, although lymphatics are variably reactive for CD34.
- **D2-40** (Podoplanin) is a marker specific for the lymphatic endothelial cells.
- Of the three main vascular markers, CD31 is more sensitive and more specific. CD34 also reacts with MANY other soft tissue tumors (see below). ERG is much more specific in the context of sarcomas, but it is also positive in tumors with *ERG*-involved translocations (prostate CAs with *TMPRSS2-ERG* fusion, Ewing sarcoma with *EWSR1-ERG* fusion), some myeloid leukemias, and epithelioid sarcomas (clone dependent).
- Key applications of vascular markers are:
  1. To ID the vascular nature of a poorly differentiated or ambiguous neoplasm (such as angiosarcoma, epithelioid hemangioendothelioma, Kaposi sarcoma)
  2. To highlight vessels to help identify lymphovascular invasion in tumors

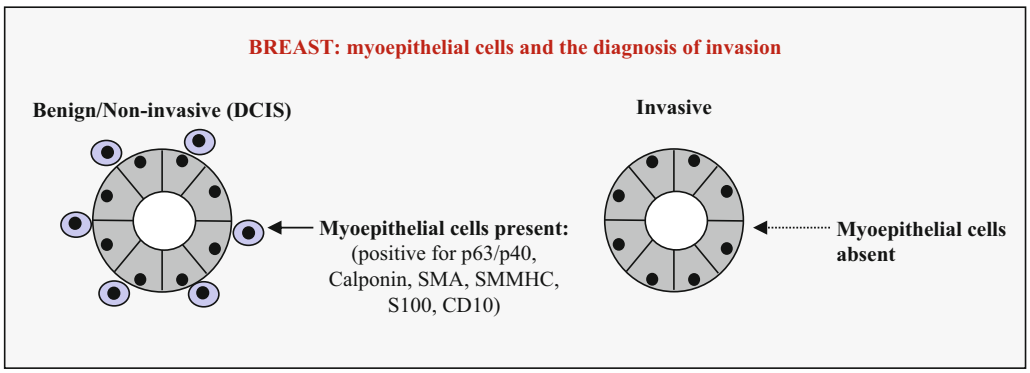


Abbreviations: *SFT* solitary fibrous tumor, *DFSP* dermatofibrosarcoma protuberans, *GIST* gastrointestinal stromal tumor

Primer on Assessment of Invasion

- Invasive carcinomas, by definition, extend beyond the surrounding basement membrane. Collagen type IV is a component of basement membrane. However, because of high background staining, this marker generally has a limited utility in the diagnosis of invasion.
- Alternative markers of invasion are basal cells and myoepithelial cells in the prostate and breast, respectively. These cells surround benign luminal cells and in situ lesions (such as PIN or DCIS) but are absent in the invasive carcinomas, as diagrammed below. (There are some exceptions – micro-glandular adenosis in breast notoriously lacks myoepithelial cells, and prostatic high grade PIN may occasionally have attenuated/partially missing basal cell layer). Included in the diagram is AMACR/racemase, which is part of a standard "prostate cancer panel" (p63/p40, CK903/34βE12, AMACR). AMACR is highly expressed in prostatic malignant acinar cells (invasive and in situ) but is usually negative in benign cells.





Abbreviations: *SMA* smooth muscle actin, *SMMHC* smooth muscle myosin heavy chain

Primer on Proliferation Markers and p53

- The main marker used to gauge proliferation rate in tumors is **Ki67/MIB1**. Ki67 is the name of both the antigen and some antibodies, whereas MIB1 is the name of the most commonly used antibody clone to Ki67.
- Believe it or not, Ki67 stands for Kiel, the city where it was discovered, and 67 is the number of the clone on a 96-well plate (so technically, the correct pronunciation is "KEE-67"). MIB1 stands for Molecular Immunology Borstel 1 – the manufacturer.
- Ki67/MIB1 is used in a variety of tumors. Common uses include classification, grading, and gauging prognosis in neuroendocrine tumors, breast carcinomas, lymphomas, and frequently in soft tissue tumors.
- **pHH3** (phosphohistone H3) is a recent proliferation marker, which detects mitotic chromatin condensation. It is therefore sensitive and specific for cells undergoing mitosis (late G2 and M). Basically, it identifies cells that we would count as having a mitotic figure (but in some cases, distinguishing mitotic from pycnotic cells can be difficult, and this marker theoretically can serve as a surrogate for mitotic counts). In contrast, Ki67 is expressed in all non-quiescent phases of the cell cycle (G1/G2/S/M) and not only cells undergoing mitosis; this explains why MIB1 rate is generally higher than either mitotic counts or pHH3. Data on utility of pHH3 is still emerging, so stay tuned.
- **p53** is not a proliferation marker, but it is used in several settings to distinguish high-grade tumors (harboring *TP53* mutations) from lower-grade tumors, which lack *TP53* mutations. Interpretation of p53 IHC as a surrogate for *TP53* mutation testing is best established in GYN tumors, as follows (but applies to other tumors as well):

  - The wild-type (i.e., normal) staining pattern is scattered variable, often weak nuclear positivity (look at surrounding normal tissue).
  - Abnormal pattern of staining may be one of the following:
    1. Diffuse and strong nuclear staining - so called "block staining" (most common pattern associated with mutations)
    2. Complete loss of staining ("null" phenotype, need internal control!)
  - p53 is a tumor suppressor, so the loss of staining is what you would expect. However, some *TP53* mutations result in defective protein degradation and lead to accumulation of nonfunctional p53, which explains the aberrant pattern 1.

Differential Diagnosis of Undifferentiated Malignant Neoplasms (Carcinoma Versus Melanoma Versus Lymphoma Versus Sarcoma Versus Others)

	Epithelial markers: CK, EMA	SOX10, S100	CD45	Others
Carcinoma	+[1]	–[5]	–	See table below for site-specific markers
Melanoma	–	+	–	HMB45, Melan-A, MITF [9]
Lymphoma	–	–	+[8]	CD3 (T cell), CD20, CD19, and CD79a (B cell)
Sarcoma	– (can be weakly +)	variable[6]	–	Vimentin[10], desmin and actin (muscle), CD34, ERG (vascular, other), etc.
Neuroendocrine (NE) neoplasms	variable[2]	variable[7]	–	NE markers (SYN, CHR, CD56, INSM1)
Mesothelioma	+	–	–	WT1, calretinin, CK5/6, D2-40, other
Germ cell tumors	variable[3]	–	–	SALL4, OCT4, PLAP, c-kit, other
Glioma	–[4]	+ (for S100, some for SOX10)	–	GFAP, OLIG2

1. In general, reactivity for epithelial markers (CK, EMA) confirms the diagnosis of a CA. However, beware of epithelial marker expression in some non-CAs (e.g., synovial sarcoma, epithelioid sarcoma, mesothelioma, non-seminomatous germ cell tumors). In addition, aberrant labeling mainly with Cam5.2 may be seen focally in some high-grade tumors, particularly melanoma and leiomyosarcoma.
2. Some NE neoplasms are CK-positive (e.g., carcinoid), whereas others are CK-negative (e.g., paraganglioma/pheochromocytoma). See section on NE markers.
3. Non-seminoma germ cell tumors (embryonal CA, yolk sac tumor) are CK-positive, whereas seminoma is CK-negative (subset can have patchy staining).
4. Glioblastomas, particularly epithelioid glioblastoma (a new WHO entity), can label for AE1/AE3 or EMA.
5. Carcinomas are generally S100- and SOX10-negative, but there are some exceptions (some salivary, breast, and adnexal CAs are positive for S100 and SOX10).
6. Sarcomas of nerve sheath and adipocytic differentiation are S100-positive.
7. Pheochromocytoma/paraganglioma and olfactory neuroblastoma show S100 reactivity in sustentacular (supportive) cells.
8. CD45 is highly specific for hematopoietic cells. However, a few hematopoietic neoplasms are notoriously negative for CD45. These include lymphoblastic lymphoma (variable), anaplastic large cell lymphoma (variable), Reed-Sternberg cells in classical Hodgkin lymphoma, plasma cell neoplasms, follicular dendritic cell sarcoma, and myeloid sarcoma (variable). Other markers are needed to identify these lesions, such as CD138/MUM1 for plasma cell neoplasm, CD30 for anaplastic large cell lymphoma and classic Hodgkin lymphoma, and CD43/myeloperoxidase/lysozyme for myeloid sarcoma.
9. Spindle-cell and desmoplastic melanomas are usually (+) for S100 and SOX10 only (HMB45 and Melan-A are generally negative or focal).
10. Vimentin is historically used to distinguish CA and glioma (vimentin-negative) from sarcoma, lymphoma, and melanoma (vimentin-positive). However, vimentin is actually expressed in many CAs (thyroid, lung, kidney, uterus, other) and is no longer considered a useful discriminating marker. Positive staining for vimentin is occasionally used to confirm that tissue is "immunoviable" when all other markers are nonreactive.

First-line panel usually includes cytokeratins (AE1/AE3 and Cam5.2), SOX10/S100, and CD45.

Carcinoma of Unknown Primary (CUP): Summary of Key Markers Used to Identify the Site of Origin

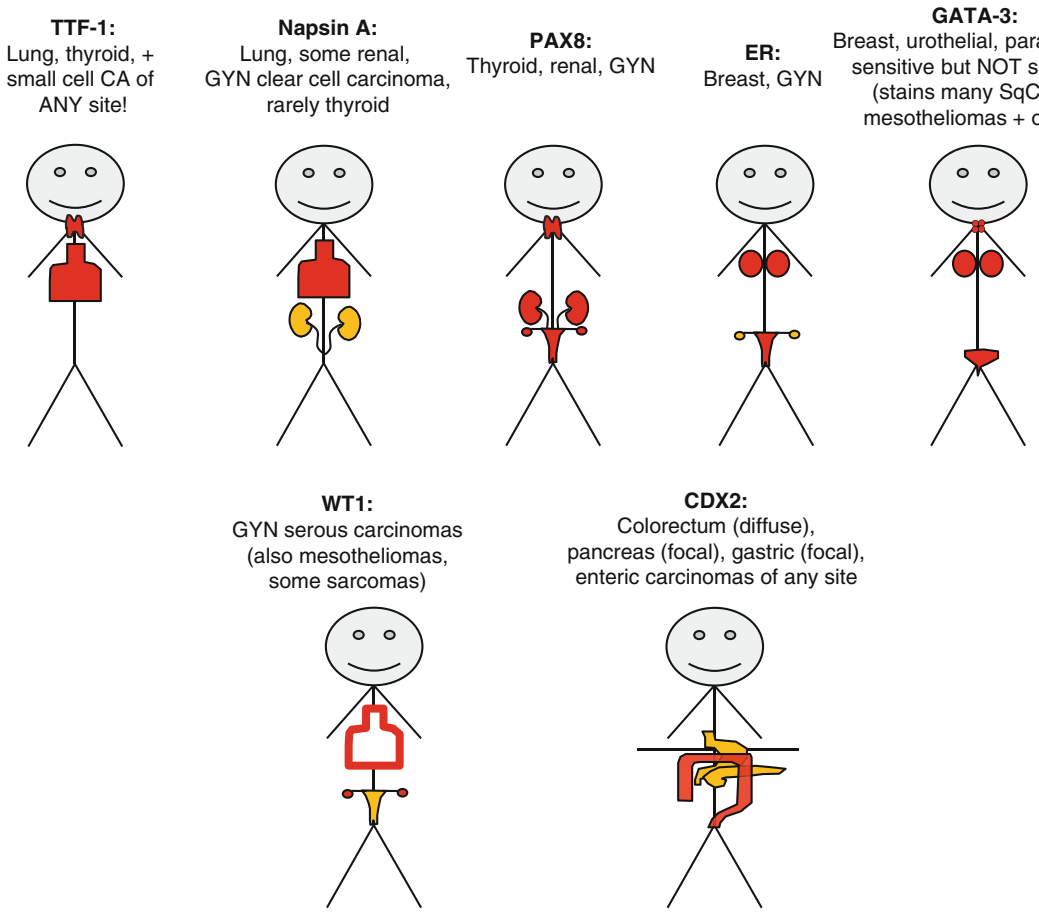
Suspected tumor type/site	Marker
Breast	GATA3 (>90%+), ER/PR (60%+), GCDFP/BRST2 (~60%+), mammaglobin (~60%+), HER2 (10–25%+, not specific for breast)
Prostate	NKX3.1 (most sensitive and specific; pitfall: male breast cancer), PSA, PSMA, PSAP, prostein (P501S)
Lung: adenoCA	TTF-1 (80%+), Napsin A (80%+), surfactant (SPA, PE10) – old/retired marker
Thyroid: papillary and follicular CA	TTF-1, thyroglobulin, PAX8
Thyroid: medullary CA	TTF-1, calcitonin, PAX8 variable
HCC	HepPar-1, Arginase, Glypican-3, albumin ISH (recent marker; considered most sensitive in poorly-diff. HCCs [14])
Pancreas	Loss of DPC4 (SMAD4) expression (in 55% of pancreatic adenoCAs, relatively specific)
Kidney	PAX8, RCC, and CD10 (last two markers have low specificity and are used infrequently) CAIX (clear-cell RCC – not specific)
Adrenal	SF1, Inhibin, Melan-A, SYN (not specific for adrenal)
GYN tract	PAX8 (pan Mullerian), ER/PR (endometrioid ~100%+, serous 50%+, cervical negative), WT1 (serous CA)
Bladder/urothelial	GATA3, Uroplakin (high specificity but low sensitivity; recent Uroplakin II more sensitive than Uroplakin III), thrombomodulin (not specific), p63/p40 (not specific for urothelium)
Intestine	CDX2, villin, SATB2, CDH17
Gallbladder, stomach	No specific markers available

Marker	Tumor type/site
TTF-1[3]	Lung (adenoCA), thyroid (papillary, follicular, medullary). SmCC of any site (TTF-1 is not specific for SmCC of lung origin!)
Napsin A	Lung (adenoCA), RCC (especially papillary), GYN clear-cell CA, rarely thyroid (weak)
ER/PR	Breast, GYN (endometrioid ≫ serous). Can label subset of other CAs (e.g., ~5% of lung CAs are ER+).
GATA3	Breast, urothelial, parathyriod, salivary gland, skin adnexal tumors (especially apocrine), gestational trophoblastic tumors, common in any SqCCs and mesotheliomas
GCDFP-15 (BRST2)	Breast (low sensitivity), salivary, skin adnexal tumors
Mammaglobin	Breast (low sensitivity), salivary, sweat glands (also stains some GYN CAs)
CDX2, villin, SATB2	Large and small intestine and any CAs with enteric phenotype (e.g., adenoCA of urinary bladder, mucinous lung adenoCA). CDX2 also heterogeneously expressed in pancreaticobiliary and gastric CAs
PAX8	Pan-renal, pan-Mullerian (uterus, ovary), thyroid (papillary/follicular ≫ medullary), thymus (thymoma and CA with polyclonal PAX8)
DPC4	Loss of expression fairly specific to pancreaticobiliary CA, though present in only 55% of cases. Few colon cancers (11%) also show loss of expression. Stain does not work well in many labs.
NKX3.1, PSA, PSMA, PSAP, prostein	Prostate markers. Usually PSA and NKX3.1 are sufficient. Few non-prostate tumors can be positive (e.g., PSAP reactivity in rectal carcinoids, PSA in salivary tumors). NKX3.1 reported as highly specific.
HepPar-1, Arginase-1, Glypican-3, albumin ISH, AFP	Hepatocellular differentiation – expressed in HCCs and rare hepatoid CAs

1. These markers are used to determine the site of origin of CA of unknown primary. None of the markers are 100% site-specific; therefore they must be interpreted in the context of morphology, clinical findings, and in conjunction with CK7/CK20 and other less specific markers (see sections on specific organs).
2. Note that the above site-specific markers apply primarily to adenoCAs. Squamous cell carcinomas of various organs generally cannot be distinguished based on their immunoprofile. Exceptions are SqCCs of the cervix, oropharynx (tonsil/base of the tongue), and anus, subsets of which are HPV-related and can be distinguished from SqCCs of other sites by positive ISH for HPV DNA/mRNA.
3. Certain clones of TTF-1 (SPT24) can label a subset of non-lung non-thyroid CAs, such as GYN, breast, colon, etc. – watch out! 8G7G3/1 clone is more specific. It is important to know which clone your lab is using (see Chapter 5)!

For details on each marker, please refer to the alphabetical index and the organ systems sections.

Key Multipurpose Markers for the Site of Origin in Carcinomas: Diagram



Differential Diagnosis of Small Round Blue Cell Tumors (SRBCT)

	CK and EMA	CD45	CD99	NE markers[1]	TTF-1	WT1	Desmin	Others
SRBCTs of childhood and adulthood								
Lymphoblastic lymphoma[2]	–	+ (few –)	+/–	–	–	–	–	TdT+, CD34+ 80% are T cell: CD3+
Myeloid sarcoma	–	+/–	+/–	+/– (CD56)	–	–	–	Myeloperoxidase, lysozyme, CD43+
Rhabdomyosarcoma, solid alveolar type	– (few F+)	–	– (few +)	+/– SYN and CD56, but CHR–	–	–[3]	+	Actin+, myogenin+ (usually diffuse), MyoD+ [15]
Wilms tumor, blastema-predominant	+	–	–	–	–	+	+ blastema	
Ewing sarcoma	– (20% F+)	–	+ (diffuse and strong membranous)	+/–[4]	–	–	–	NKX2.2 + (new marker)
***CIC*-rearranged sarcoma**	– (rare weak +)	–	–/+ (diffuse in ~20%)	–	–	+	–	ETV4 (90%+) [16]
***BCOR*-rearranged sarcoma**	– (rare weak +)	–	+/– (~50%)	–	–	–	–	BCOR+, SATB2+, TLE1+
Desmoplastic small round cell tumor	+	–	– (few +)	–	–	+	+	WT1 nuclear positivity using clones against C-terminus (such as C19), actin–
Neuroblastoma	–	–	– always	+ mainly SYN	–	–	–	PHOX2B – new marker [17]
Medulloblastoma	–	–	–	+ mainly SYN	–	–	–	NeuN, variable GFAP
Retinoblastoma	–	–	–	+ mainly SYN	–	–	–	CRX/ORX3 (new markers of photoreceptor diff.) [18]
Small cell osteosarcoma	–	–	+/–	–	–	–	–	SATB2+, osteocalcin+
Mesenchymal chondrosarcoma	–	–	+	–/+	–	–	–	SOX9+ (non-specific), S100+ in chondrocytes but not small blue cell component
SRBCTs of adulthood								
Lymphoma	–	+	–	–	–	–	–	B cell: CD20+, CD19+, CD79a+ T cell: CD3+
Small cell carcinoma	+ (dot-like)[6]	–	–	+	+/–[5]	–	–	RB1 loss of staining
Merkel cell carcinoma	+ (dot-like)[6]	–	–	+	– always	–	–	CK20+, neurofilament+, Merkel cell polyomavirus (CM2B4)+

1. NE markers include SYN, CHR, CD56, and INSM1.

2. Beware of lymphoblastic lymphoma: it may be CD45– and CD99+, thereby masquerading as Ewing sarcoma. However, unlike Ewing sarcoma, lymphoblastic lymphoma is reactive for blast markers (TdT, CD34) and either T-cell markers (CD3) or B-cell markers (CD20, CD79a).

3. Rhabdomyosarcoma often shows cytoplasmic, but not nuclear, WT1 expression [19].

4. NE marker expression in Ewing sarcoma (formerly Ewing/PNET) is variable and is largely dependent on the degree of neuroectodermal differentiation. In general, most are positive for NSE, and some are positive for synaptophysin and rarely INSM1. Chromogranin A is generally negative.

5. TTF-1 is expressed in SmCC of lung (90%) and non-lung origin (>40%). In contrast, Merkel cell CA is ALWAYS TTF-1-negative.

6. "Dot-like" perinuclear cytokeratin reactivity is typical of neuroendocrine CAs (SmCC, Merkel cell CA).

DDx also includes but is not limited to poorly differentiated synovial sarcoma (see next table) and other site-specific tumors like NUT carcinoma.

Also see SRBCT DDx section in Chapter 14

Differential Diagnosis of Spindle-Cell Tumors

Differentiation	Neoplasm	CK and EMA	S100	SOX10	SMA	Desmin	CD34	Others
Muscle	**LM**	–/rare F+	–	–	+	+	–	Caldesmon +
	LMS	–/rare F+	–	–	+	+	–	Caldesmon +
	RMS	–	–	–	–	+	–	Myogenin+, MyoD+
Nerve sheath	**Neurofibroma**	–	F+	+	–	–	+	
	Schwannoma	–	+ diffuse	+	–	–	+	
	MPNST	–/F+	+/– (50% F+)	+/– (50% F+)	–	–	+	H3K27me3– (lost in 50%) [20]
Vascular	**For example, angiosarcoma, Kaposi, EHE**	–/F+	–	–	–	–	+	CD31+, CD34+, ERG+
Myofibroblastic[1]	**Fibromatosis**	–	–	–	+	–/+	–	nuclear β-catenin+[2]
	Nodular fasciitis	–	–	–	+	–	–	
	IMT	–/rare F+	–	–	+	+/–	–	ALK+, ROS1+ (subset)
Fibrohistiocytic	**DF**	–	–	–	–	–	–	Factor XIIIa+
	DFSP	–	–	–	–	–	+	Factor XIIIa –
Adipocytic	**Dedifferentiated liposarcoma**	–	–/+	–	F+	+/–	–/+	CDK4+, MDM2+, p16
Other	**GIST**	–	–	–	–/+ 30%	–	+ 70%	c-kit+ 95%, DOG1+
	SFT	–	–	–	–	–	+	STAT6+, BCL2+, GRIA2+
	Synovial sarcoma	F+	+/– 30%	–/+ (5%)	–	–	– always	TLE1+, variable CD99
Non-sarcomas	**Spindle-cell CA**	+	–	–	–	–	–	For SqCC: CK903+, p63/p40+; various site-specific markers
	Spindle-cell melanoma	–	+ [3]	+	–	–	–	

1. Caldesmon helps to differentiate smooth muscle tumors (caldesmon +) from myofibroblastic tumors (caldesmon –). In addition, pattern of actin reactivity can be helpful: smooth muscle tumors (e.g., leiomyosarcoma) have diffuse cytoplasmic labeling, whereas myofibroblastic lesions (e.g., nodular fasciitis, IMT) have a distinctive "tram-track" pattern (due to peripheral cytoplasmic accentuation outlining the long axis of fibroblast borders as parallel tracks).

2. Nuclear β-catenin (reflecting mutations of either β-catenin or APC genes) is seen in deep/desmoid-type but not superficial fibromatosis [21].

3. Spindle-cell melanoma is usually reactive for SOX10 and S100 only; other melanocytic markers (HMB45, Melan-A, MITF) are generally negative or focal.

*For detailed IHC profiles of sarcomas, please see soft tissue section in Chapter 2.

Abbreviations: *DF* dermatofibroma, *DFSP* dermatofibrosarcoma protuberans, *EHE* epithelioid hemangioendothelioma, *GIST* gastrointestinal stromal tumor, *IMT* inflammatory myofibroblastic tumor, *LM* leiomyoma, *LMS* leiomyosarcoma, *MPNST* malignant peripheral nerve sheath tumor, *RMS* rhabdomyosarcoma, *SMA* smooth muscle actin (**α**-actin), *SFT* solitary fibrous tumor

Differential Diagnosis of Neuroendocrine (NE) and Neuroectodermal Neoplasms

	NE markers (SYN, CHR, CD56, INSM1)[1]	Epithelial markers (Cam5.2, AE1/AE3)[2]	S100	Others
Carcinoid (lung)/well-diff. neuroendocrine tumors (GI, pancreas)	+	+ (– in 20% of lung carcinoids)	– [3] (most)	Site of origin markers (see table below), hormones (variable: insulin, glucagon, somatostatin)[5]
Medullary carcinoma of the thyroid	+	+	– [3] (most)	TTF-1+, calcitonin+, CEA+ (thyroglobulin-negative, PAX8 variable)
Pheochromocytoma and paraganglioma	+	–	+ (sustentacular/ supportive cells)	PHOX2B – new marker [17]
Small cell carcinoma	+/–	+/–	–	TTF-1+[4]
Merkel cell carcinoma	+	+	–	CK20+, neurofilament+ (both punctate perinuclear), Merkel cell polyomavirus (CM2B4)+, always TTF-1–
Pituitary neoplasms	+	+	–	PIT-1, PRL, GH, ACTH, TSH, LH/FSH
Parathyroid neoplasms	+ (CHR+, SYN+)	+	–	PTH+, GATA3+
Neuroblastoma	+	–	+ [5]	PHOX2B – new marker
Olfactory neuroblastoma	+	–/+	+ (sustentacular/ supportive cells)	Calretinin+, CK may be focally+ but EMA always –
Ewing sarcoma	+/–	–/+ (20%+)	variable	CD99+, NKX2.2, PAS+, FLI1+, PAX7+ - new marker [22]

1. See section on NE markers for background.
2. Cytokeratin (CK) expression is variable in NE CAs: Cam5.2 reactivity is more consistent than AE1/AE3. Note that CKs (Cam5.2 and AE1/AE3) show punctate (dot-like) perinuclear reactivity in NE CAs. Same applies to CK20 staining in Merkel cell CA. This is a helpful diagnostic clue.
3. Some medullary thyroid CAs and carcinoid tumors show a sustentacular staining pattern with S100 (and SOX10).
4. TTF-1 is expressed in SmCC of the lung (~90%) as well as extrapulmonary sites (~40%) (see above). On the other hand, TTF-1 appears to be specific for pulmonary carcinoids (50%+) but is negative in NE tumors of GI tract and pancreas (see table below).
5. S100 reacts with Schwannian stromal cells.

Markers for the Site of Origin of Well-Differentiated NE Tumors (NETs)/Carcinoids*			
	TTF-1, OTP[1]	**CDX2**	**PAX8 (polyclonal), ISL1**
Lung (carcinoid)	+/–	–	–
Pancreas (PanNET, formerly islet cell tumors)	–	– (rare F+)	+/–
Ileum (NET, formerly carcinoid)	–	+/–	–

*NETs of unknown primary usually present as liver metastasis with DDx primarily between the pancreas and ileum, but metastatic lung carcinoids also commonly go to the liver. Lung carcinoids are usually apparent on CT, but ileum may be difficult to detect. Markers for the site of origin can be helpful, but they all have imperfect sensitivity and specificity. Octreotide scan can help pinpoint location of the primary tumor – it utilizes radiolabeled synthetic somatostatin (In-111-DTPA octreotide) that binds to somatostatin receptors on the surface of the majority of these tumors.

1. OTP (orthopedia homeobox) is a recent marker. Expressed in a subset of lung carcinoids but not in NETs of other sites. Utility is still investigational [23].

Note: Many NETs express and secrete hormones. Insulin and glucagon may be expressed not only by PanNETs but also by NE neoplasms of other sites (although the pancreas is more common). Because of the lack of specificity, these and other hormones are not generally used to assign the source of a metastatic NE neoplasm.

References: [24, 25]

Chapter 2. Immunostains: Solid Tumors

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*(*All subsections are by these authors, unless specified otherwise)*

Breast

Breast: Key Markers at a Glance	
Marker [location]	Applications
E-cadherin (CAD-E) [membranous]	E-cadherin is an adhesion molecule expressed in normal ductal and lobular cells. Loss of membranous staining is a hallmark of lobular carcinoma (in situ and invasive) – remember that lobular lesions are discohesive! The main application of E-cadherin is to differentiate ductal lesions (ADH, DCIS, ductal carcinoma) (+) from lobular ones (ALH, LCIS, lobular carcinoma) (−). p120 catenin is an E-cadherin-binding protein, which is advocated by some experts for E-cadherin-equivocal cases [membranous p120 = normal pattern/ductal lesions vs diffuse cytoplasmic p120 = abnormal pattern/lobular lesions]
ER and **PR** [nuclear]	ER and PR are present in normal and neoplastic breast epithelium. ER controls the synthesis of PR. Therefore ER+/PR– tumors are not uncommon, but the opposite should raise a suspicion of a false result. ER and PR are weakly favorable prognostic factors (expression correlates with better differentiation). Most importantly, expression of ER and PR is a strongly favorable predictive factor because these patients can be treated with tamoxifen In addition, ER and PR are used to identify metastatic breast cancer (~60%+) but note that ovarian, endometrial, and a few other non-mammary carcinomas are also positive (see antibody index for complete list).
GATA3 [nuclear]	GATA3 is a nuclear transcription factor and a relatively recent marker. It is highly sensitive – expressed in >90% of breast CAs, including ~40% of triple negatives (ER/PR/HER2–). However, GATA3 is *not* specific for breast CA. It is also expressed in urothelial CA, many SqCCs, salivary gland tumors, skin adnexal tumors, and subset of various other neoplasms (see index). Thus, it is important to always use GATA3 in conjunction with other markers in assessment of carcinomas of unknown primary site (see above).
GCDFP-15 (BRST2) [cytoplasmic]	GCDFP-15 staining in breast CA is usually patchy at best and overall only ~60% of breast CAs are positive (~10% of triple negatives). GCDFP-15 is fairly specific but may occasionally stain non-breast carcinomas, such as some lung CAs. Lobular carcinomas have more of an apocrine differentiation and stain reliably with GCDFP-15. Because it is a marker of apocrine differentiation, GCDFP-15 is also positive in salivary duct carcinoma and some skin adnexal tumors.
Mammaglobin [cytoplasmic]	Mammaglobin is positive in ~60% of breast cancer (~15% of triple-negative breast cancer) and is not as focal as GCDFP-15. Like GCDFP-15, it recognizes apocrine differentiation. Overall, mammaglobin is a bit more sensitive than GCDFP-15, but it also frequently stains GYN tumors. Given low sensitivity and imperfect specificity, mammaglobin and GCDFP-15 are always used in combination with other markers in evaluation of carcinomas of unknown primary origin.
SOX10 [nuclear]	SOX10 is a nuclear transcription factor that is primarily used in the diagnosis of tumors of melanocytic and schwannian origin (see index). It is expressed in ~40% of breast CAs and is enriched in triple-negative breast CAs (~60%+); this marker may thus be especially useful in the diagnosis of metastasis from triple-negative breast CA.
HER2 [membranous]	HER2 is a growth factor receptor, which is absent or rare in normal breast cells. Overexpression (as a result of gene amplification) is present in 10−30% of tumors. HER2 is a poor prognostic factor, though a weak one. Most importantly, HER2 is a strongly favorable predictive factor in that it predicts response to Herceptin, the anti-HER2 antibody. Note that better differentiated breast CAs are usually ER+, PR+, and HER2–, whereas the opposite is true for poorly differentiated CAs. HER2 is overexpressed in several non-mammary carcinomas, including those of the esophagus/stomach, lung, and GYN tract. It is therefore not generally used as a marker of breast origin.
Myoepithelial (ME) markers	**Calponin, p63/p40, SMA, and SMMHC** are the usual ME markers of choice (see below). ME layer is intact in benign and in situ lesions and is absent in invasive carcinomas. In addition, ME markers may be used to distinguish papilloma (intralesional ME cells present) from papillary carcinoma (intralesional ME cells absent). • False negatives: microglandular adenosis lacks myoepithelial cells (morphology and S100 positivity are key to differentiating it from non-triple-negative invasive breast cancer) • False positives: rare invasive ductal carcinomas focally retain myoepithelial cells; also be aware that surrounding reactive myofibroblasts may be positive for SMA and calponin.
Abbreviations: *ADH* atypical ductal hyperplasia, *ALH* atypical lobular hyperplasia, *DCIS* ductal carcinoma in situ, *LCIS* lobular carcinoma in situ, *SMA* smooth muscle actin, *SMMHC* smooth muscle myosin heavy chain	

Markers for Myoepithelial Cells (and what else they stain)				
	SMA (α-actin)	Calponin	SMMHC	p63/p40
Myoepithelial cells	+	+	+	+
Myofibroblasts	+	−/+	−	−
Vessels, pericytes (smooth muscle)	+	+	+	−
p63/p40 and SMMHC are superior to SMA and calponin in specificity because they do not react with myofibroblasts (less background) Other myoepithelial markers in use include S100, HMWCK, and CD10 Abbreviations: *SMA* smooth muscle actin, *SMMHC* smooth muscle myosin heavy chain				Reference: [1]

Breast: Immunoprofiles at a Glance	
Diagnosis	Immunoprofile
Breast carcinoma, general	CK7+, CK20–, GATA3 (>90%), ER/PR (~60–75%+), GCDFP-15 (~60%+), mammaglobin (~60%+), HER2 (10–30%+)
Lobular carcinoma (in situ and infiltrating)	Loss of E-cadherin membranous staining, GCDFP-15 (~100%+)
Metaplastic breast carcinoma	HMWCK (CK903 or CK5/6)+, p63/p40+, variable AE1/AE3, Cam5.2 and CK7 (most reliable epithelial marker is HMWCK), SOX10
Microglandular adenosis (MGA)	ER/PR-, S100+, absence of surrounding myoepithelial cells. Intact basement membrane (laminin/collagen IV+).

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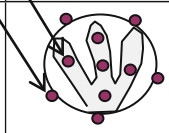
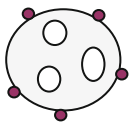
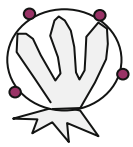
Breast: Differentials

DDx of in Situ Proliferations			
	ALH/LCIS	**ADH/DCIS**	**UDH**
E-cadherin	–	+	+
HMWCK (CK903, CK5/6)	+	–	+
ER	+	+ (diffuse)	+/– (patchy)
p120 catenin	Cytoplasmic	Membranous	Membranous

HMWCK represents a marker of "differentiation": expression is present in normal breast epithelium as well as UDH, whereas DCIS shows the loss of differentiation and concurrent loss of HMWCK expression. This may aid in the distinction of UDH from ADH/DCIS, but the gold standard is morphology in H&E. Loss of E-cadherin and the presence of mucin vacuoles (rarely used) supports LCIS.

Abbreviations: *ADH* atypical ductal hyperplasia, *ALH* atypical lobular hyperplasia, *DCIS* ductal carcinoma in situ, *UDH* usual ductal hyperplasia

References: [2, 3]

DDx of Papillary Lesions					
	Papilloma[1]	**Papillary DCIS/ intraductal papillary carcinoma**	**Encapsulated (intracystic) papillary carcinoma**[2]	**Solid papillary carcinoma**[3]	**Invasion associated with papillary DCIS**
Intralesional myoepithelial cells[4]	+	–	–	–	–
Peripheral myoepithelial cells[4]	+	+	–	+/–	– (at the site of invasion)
					
NE markers and mucicarmine	–	–	–	+ (most)/–	–

The above are the "idealized" criteria. In general, IHC is notoriously unreliable in the Dx of papillary lesions, and the distinction mainly relies on morphology.

1. Pitfalls: intralesional myoepithelial (ME) cells may be focally present in DCIS and carcinoma and absent in sclerosing papilloma
2. The entity known as "encapsulated papillary carcinoma" or "intracystic papillary carcinoma" (papillary DCIS-like lesion forming a single mass) is usually negative for peripheral ME cells, but whether this represents invasion is controversial. Most experts regard it as a variant of DCIS
3. Instead of discrete papillae, fibrovascular cores cut in cross section are seen among a solid cellular proliferation in solid papillary carcinoma. It may or may not have ME cell at its periphery. Like encapsulated papillary carcinoma, it is controversial whether it is a form of DCIS or an invasive carcinoma with a pushing border
4. ME cell markers include p63/p40, SMMHC, actin, and calponin

Abbreviations: *ME* myoepithelial, *SMMHC* smooth muscle myosin heavy chain

References: [4, 5]

DDx of Low-Grade Spindle Cell Proliferations in the Breast						
	HMWCK, p63/p40	**SMA**	**Desmin**	**ER**	**Nuclear β-catenin**	**CD34**
Metaplastic carcinoma[1,2]	+	–/+	–/+	– (usually)	–/+	–
Phyllodes tumor (stromal component)	–[3]	–	–	+/–	+/–	+
Fibromatosis	–	+	–/+	–	+	–
Nodular fasciitis	–	+	–	–	–	–
Myofibroblastoma/pseudoangiomatous stromal hyperplasia/myoid hamartoma	–	+	+	+	–	+

1. Metaplastic carcinoma is frequently AE1/AE3 and Cam5.2-negative, therefore HMWCK (CK903; CK5/6) and p63/p40 should always be used to rule it out
2. There is even a "low-grade fibromatosis-like" variant of metaplastic carcinoma (!) that can be very bland and stains with myofibroblastic markers, emphasizing that any spindle cell lesion of the breast should be worked up with epithelial markers
3. Beware that a subset of malignant phyllodes tumors may stain focally for CK and p63/p40 [6]

DDx also includes various primary sarcomas (which are uncommon relative to phyllodes tumor and metaplastic CA), most notably angiosarcoma (CD31+, CD34+, ERG+)

References: [7, 8]

DDx of Pagetoid Proliferations in the Nipple						
	LMWCK (Cam5.2, CK7), EMA	**HER2**	**Mucicarmine**	**GCDFP-15**	**Melanocytic markers**[2]	**HMWCK, p63/p40**
Paget disease of the nipple[1]	+	+ 70–100%	+ 40–70%	+ ~50%	–	–
Melanoma in situ	–	–	–	–	+	–
Bowen disease (SqCC in situ)	–	–	–	–	–	+

1. Paget disease of the nipple ALWAYS has an underlying high-grade DCIS; invasive carcinoma is present in ~50% of cases. Paget cells are CK7+/CK20–. Toker cells are the clear cells in the normal nipple epidermis, which may mimic Paget; these cells are Cam5.2 (+) but HER2 (–) and mucin (–)
2. Melanoma markers include S100, SOX10, Melan-A, and HMB45. However, S100 and SOX10 are positive in some breast CAs and corresponding Paget cells – watch out!

Also see Paget disease IHC in GYN and skin sections.

Reference: [9]

Prostate and Bladder

Prostate: Key Markers at a Glance	
Marker [compartment]	Applications
PSA and **PSAP** [cytoplasmic] – conventional prostate markers	In general, PSA (prostate-specific antigen) is more specific and PSAP (prostate-specific acid phosphatase) more sensitive. These markers may be used to identify metastatic prostate cancer (97% and 99% sensitivity, respectively) and are first-line markers for differentiating high-grade prostate cancer from urothelial carcinoma. They are highly specific for prostate but are known to cross-react with a few non-prostatic tumors. Most notably, PSAP shows reactivity with rectal carcinoid, which should not be misinterpreted as prostate cancer! In addition, PSA is positive in some salivary gland tumors (especially salivary duct carcinoma) and bladder adenoCA. See antibody index for details. Of note, immunoreactivity for PSA and PSAP decreases after androgen deprivation therapy. Reference: [10]
New-ish prostate markers: – **NKX3.1** [nuclear] – **ERG** [nuclear] – **Prostein (P501S)** [cytoplasmic, dot-like] – **PSMA** (prostate-specific membrane antigen) [membranous]	There are four newer prostate-specific markers. NKX3.1 appears to be particularly useful as it has a high sensitivity and specificity for prostate cancer. Beware that NKX3.1 can stain a subset of male breast cancers! ERG expression is a result of *TMPRSS2-ERG* rearrangement in 40–50% of prostate CA, so it is not very sensitive. PSMA has variable sensitivity (88–100%) and is NOT specific, limiting its utility. In evaluating metastases, NKX3.1 plus PSA is a commonly used first-line panel. References: [11, 12]
Basal cell markers: – **p63/p40** [nuclear] – **HMWCKs** – **CK903 (34βE12)** [cytoplasmic] – **CK5/6** [cytoplasmic]	These markers are used to distinguish invasive prostate cancer (basal cells absent) from PIN or adenosis (basal cells present). Note that basal cells are occasionally patchy or even appear to be absent in some cases of PIN and adenosis. A combination of p63/p40 with one of the HMWCKs and AMACR (present in cancer but not in benign cells, see below) in a double or triple cocktail can be of utility in such situations and is the current ISUP recommendation. Furthermore, stains must be interpreted in the context of morphology. A rare type of prostatic adenoCA can be p63/p40 positive, but they are negative for HMWCK [13]! References: [10, 14]
AMACR (racemase, P504S) [cytoplasmic]	**AMACR** is a marker that is overexpressed in prostate cancer cells, both in situ (PIN) and invasive, but is absent in benign cells. There are few exceptions: • **AMACR (+) benign lesions:** 10% adenosis, 60% nephrogenic adenoma, 25% partial atrophy • **AMACR (−) carcinomas:** 20% conventional, 65% foamy, 65% atrophic, 75% pseudohyperplastic As above, AMACR is of particular utility in diagnosis of primary prostate cancer (especially with limited tissue) when used in a cocktail with basal cell markers. AMACR is NOT specific for prostate CA and is therefore not utilized in the panels for the origin of metastasis. References: [10, 14]

Prostate: Immunoprofiles at a Glance	
Diagnosis	Immunoprofile
Prostate adenocarcinoma	Prostate-specific markers: PSA, PSAP, NKX3.1, prostein, PSMA, ERG (40–50%) Epithelial markers: CK7–/CK20–; Cam5.2+; HMWCK–
Prostate, stromal tumor of uncertain malignant potential (STUMP)	CD34+ (vs. smooth muscle neoplasms are CD34–), PR+, ER usually–, actin −/+, desmin+/−, STAT6– (vs solitary fibrous tumors are +)
Prostate, stromal sarcoma	Same as STUMP but usually negative for actin and desmin
Prostate and bladder, inflammatory myofibroblastic tumor (IMT)	Actin+, desmin+/−, CK variable (50–80%), ALK+ in a subset (2/3), CD34–

Prostate and Bladder: Differentials

Reactive Urothelial Atypia vs. Carcinoma in Situ		
	Normal urothelium or reactive urothelial atypia	**Urothelial carcinoma in situ**
p53	–	+
CK20	+ in umbrella cells only	+ in all layers
CD44	+ (normal = basal layer, atypia = all layers)	– or reduced expression
Reference: [15]		

Adenocarcinoma Involving the Bladder		
	Primary vesical adenocarcinoma	**Colorectal adenocarcinoma (metastatic or direct extension)**
β-catenin	– (no nuclear staining)	+ nuclear
CDX2, CK20, SATB2	Variable	+ (~100%)
Thrombomodulin, CK7	Variable	–
Most informative marker is β-catenin: NUCLEAR in >90% of colon primary adenoCA vs CYTOPLASMIC in >90% of bladder primary adenoCA. Otherwise, the immunoprofiles are not sufficiently specific and colon primary needs to be ruled out clinically. Reference: [15]		

Adenocarcinoma of Bladder vs. Prostate vs. Mimics			
	Nephrogenic adenoma	**Primary vesical Adenocarcinoma[1]**	**Prostate adenocarcinoma**
AMACR	+	–	+
PAX8	+	–	–
NKX3.1, PSA(P), PSMA, Prostein, ERG	– (or weak PSAP)	–	+
1. The exception is clear cell adenocarcinoma of the bladder, which has the same immunoprofile as nephrogenic adenoma. References: [16–18]			

DDx of Urothelial Carcinoma vs. High-Grade Prostate Carcinoma		
	Urothelial carcinoma	**Prostate adenocarcinoma**
HMWCK, p63/p40[1]	+	–
GATA3, thrombomodulin, uroplakin[2]	+	–
PSA(P), prostein, PSMA, NKX3.1	–	+
CK7, CK20[3]	usually 7+,20+	usually 7–,20–
1. p63/p40 is more specific (but less sensitive) for urothelial carcinoma than CK903 [19]. 2. Uroplakin III is not widely used. It has a high specificity but low sensitivity. New uroplakin II is both sensitive and specific. 3. Strongly CK7+/CK20+ profile favors bladder, whereas CK7 (–) profile is highly unusual for bladder. Any other CK7/CK20 pattern is not informative. References: [14, 20, 21]		

Kidney

Kidney: Key Markers at a Glance	
Marker [compartment]	Applications
PAX8 [nuclear]	Pan-RCC marker (stains all RCCs and some upper tract urothelial carcinomas). PAX8 has for the most part replaced a previously used marker, PAX2, which has lower sensitivity.
CAIX (Carbonic anhydrase) [membranous]	CAIX is expressed in ccRCC as a result of VHL mutations (100% inherited, 75% sporadic). VHL normally regulates the degradation of HIF (hypoxia-inducible factor). VHL mutation → ↑HIF → ↑expression of HIF-regulated genes (CAIX, VEGF). Targeted therapies for ccRCC (sunitinib) inhibit this pathway; therefore distinction of CC subtype of RCC is important. CAIX IHC in ccRCC is strong and diffuse. However, focal expression of CAIX may be seen in various tumors in areas of necrosis/ischemia as a result of ↑HIF (such as in papillary RCC). Cup-like membranous staining with CAIX, with sparing of the apical cell membrane is typical of clear cell papillary RCC (a recent diagnostic category), which has a much better prognosis than ccRCC (so important to distinguish).
RCC [cytoplasmic], **CD10** [membranous]	Older markers of RCC (prior to PAX8 and CAIX) – poor specificity, diminishing use in practice.
Epithelial markers [cytoplasmic]	Cam5.2 and AE1/AE3 are usually positive in RCCs but may be variably reactive. Most reliable epithelial marker for RCC is EMA. All RCCs are CK20–, but CK7 is type-dependent.

Kidney: Immunoprofiles at a Glance	
Diagnosis	Immunoprofile
Conventional (clear cell) RCC (ccRCC)	CAIX+ (strong, diffuse), CD10+ (sensitive but VERY non-specific – positive in other renal and many nonrenal tumors)
Papillary RCC (pRCC)	CK7+/– (positive in the lower-grade/"type 1" pRCC, but higher-grade/"type 2" pRCC frequently CK7–), AMACR+
Chromophobe RCC	Hale colloidal iron: chromophobe RCC (blue cytoplasmic staining) vs. oncocytoma (no cytoplasmic staining, though apical blush may be present). Usually CK7+ (diffuse and strong), and c-kit+ (vs ccRCC, which is CK7 and c-kit–), Ksp-cadherin+. In practice, Dx relies heavily on morphology, supported by CK7+/c-kit+. Vimentin typically (–) unlike ccRCC and most other RCCs. Reference: [22]
Oncocytoma	CK7 not diffusely + (negative or rare cells +), c-kit+, Ksp-cadherin+. Like chromophobe, also vimentin (–)
Clear cell papillary RCC	CAIX+ (cup-like, spares luminal cell surface), CK7+ (strong, diffuse). Recently recognized entity. It does not have the genetic changes of ccRCC (chromosome 3p loss), papillary RCC (trisomy 7 and 17), or MiT family translocation RCC. It has low-grade nuclei that typically show reverse polarization similar to secretory endometrium ("piano keys") [23].
Mucinous tubular and spindle cell carcinoma (MTSCC)	Rare, relatively recently identified entity. IHC profile similar to pRCC (also CK7+, AMACR+).
Tubulocystic RCC	Another recently recognized entity. Has identical IHC profile to pRCC (CK7+, AMACR+); the distinction is based primarily on morphology. Classic morphology is an admixture of tubules and cysts lined by a single layer of hobnail epithelium with high-grade nuclei (ISUP nuclear grade 3) in the background of fibrotic stroma.
Hereditary leiomyomatosis and RCC (HLRCC)-associated RCC	HLRCC is caused by germline mutations in *fumarate hydratase* (*FH*) gene. **Loss of FH** by IHC is a marker of HLRCC, although expression may be retained in some cases due to intact epitope in a nonfunctional protein. FH-deficient cells accumulate high levels of fumarate leading to high protein succination – modification of cysteine to S-(2-succinyl)cysteine (2SC). Detection of **2SC** by IHC is a recent marker of FH deficiency/HLRCC. See "Slide to Syndrome" in Chapter 12 for histologic clues.
Succinate dehydrogenase (SDH)-deficient RCC	SDHB loss (required for diagnosis; staining lost in any tumor with SDH complex abnormality), SDHA– (in rare *SDHA* deficient RCC, also SDHB–), Ksp-cadherin+. See "Slide to Syndrome" in Chapter 12 for histologic clues.
MiT family translocation RCCs (t-RCC) – TFE3 [Xp11 translocation] – TFEB [t(6;11) translocation]	TFE3 and TFEB are members of the microphthalmia transcription factor (MiT) family. TFE3 and TFEB can be detected by IHC, but confirmation by cytogenetic or molecular studies is usually necessary. PAX8+, reduced expression of epithelial markers (keratins and EMA). MiT factors regulate melanocytic and osteoclastic differentiation → t-RCCs are positive for melanocytic markers (Melan-A and HMB45) and osteoclastic protein (cathepsin K); these are more consistently expressed in t(6;11) than Xp11 t-RCC. Reference: [24]
Collecting duct carcinoma	HMWCK+, CK7+, INI+ (most)
Medullary carcinoma	INI1 (SMARCB1) loss, CK7+, OCT4+ (70%)
Angiomyolipoma (and other PEComas)	**Melanocytic markers** (HMB45+, Melan-A+, and MITF+; S100+ in 30%) and **smooth muscle markers** (SMA+, calponin+). Co-expression of melanocytic and muscle markers is nearly **pathognomonic** for angiomyolipoma. Cathepsin K+, PAX8 and epithelial markers – (vs. RCCs).
Mixed epithelial stromal tumor (MEST)	Stromal component (endometrial-type stroma): ER/PR+, desmin+, actin+, CD10+
Wilms tumor (nephroblastoma)	Epithelial component: WT1+, CK+, EMA+; blastemal component: WT1+, desmin + (at least focal)
Clear cell sarcoma of the kidney	Hallmark is the absence of any differentiation markers; only positive markers are vimentin, cyclin D1 (usually strong and diffuse), and BCL2. BCOR staining is variable.

Kidney: Differentials

DDx of Renal Tumors with Clear, Papillary or Eosinophilic Cells												
	Histologic DDx			IHC#								
	Clear cell Tumors	Papillary Tumors	Pink cell tumors*	PAX8	CAIX[1]	CK7	AMACR	c-kit	Cathepsin-K	Melanocytic markers (HMB45, Melan-A)	Other
ccRCC	√	√	√	+	+ (diffuse)	–	–	–	–	–	
pRCC	√	√	√	+	–	+/–	+	–	–	–	
ChrRCC	√	√	√	+	–	+	–	+	–	–	HCI+, vimentin–
Oncocytoma			√	+	–	–/F+	–	+	–	–	vimentin–
ccpRCC	√	√		+	+ (cup-like)	+	–	–	–	–	
t-RCC	√	√	√	+	–/F+	–	+/–	–/+	+/–	+/–	TFE3+ or TFEB +[2]
HLRCC			√	+	–	–	–	–	–	–	FH loss, 2SC+
SDH-def. RCC			√	+	–	–	–	–	–	–	SDHB loss
ACD RCC			√	+	–	–	+	–	–	–	
AML	√		√	–	–	–	–	–	+	+	SMA+, calponin+

1. CAIX can be focally positive in any tumor adjacent to necrosis.
2. TFE3 and TFEB can be detected by IHC, but confirmation by cytogenetic or molecular assays is usually needed.
See tables above for details on individual markers and entities.

* Low-grade oncocytic neoplasms (in DDx with oncocytoma) include oncocytoma, oncocytic pRCC, chrRCC, and oncocytic AML. Other than oncocytoma, other pink cell tumors are in the DDx of higher-grade eosinophilic tumors. DDx of "renal" pink cell tumors also includes urothelial carcinoma and adrenocortical neoplasms (see below).

Abbreviations: *2SC* S-(2-syccinyl)cysteine, *ACD RCC* acquired cystic kidney disease-associated RCC, *AML* angiomyolipoma, *ccRCC* clear cell renal cell carcinoma, *ccpRCC* clear cell papillary RCC, *ChrRCC* chromophobe RCC, *FH* fumarate hydratase, HCI Hale colloidal iron, *HLRCC* hereditary leiomyomatosis and RCC-associated RCC, *pRCC* papillary RCC, *SDH-def. RCC* succinate dehydrogenase-deficient RCC, t-*RCC* MiT family translocation RCCs.

References: [24–28]

DDx of Cytologically Bland Renal Tumors with Tubulo-Papillary Architecture					
	CK7	AMACR	WT1	CD57	IHC for BRAF V600E (VE1 antibody)
pRCC (type 1, solid variant)	+ diffuse	+	–	–	–
Metanephric adenoma	– or isolated cells	–	+	+	+ (reflecting mutation)
Wilms tumor (epithelial-predominant with tubular architecture)	– or isolated cells	–	+	–	–

This DDx also includes mucinous tubular and spindle cell carcinoma. Same IHC profile as pRCC.

Reference: [29]

DDx of Distal Nephron-Like Carcinomas (high-grade solid/tubular/papillary tumors with desmoplasia and inflammation)				
	PAX8	GATA3, p63/p40	CK903 (34βE12)	Other
Collecting duct carcinoma	+	–	+/–	
Medullary carcinoma	+	–	–	OCT4+, INI1 (SMARCB1) loss
High-grade papillary RCC (Type 2)	+	–	+/–	AMACR+
Urothelial carcinoma	–/+ *	+	+	Uroplakin II and III+ (II is more sensitive)

* Beware, ~20% of upper tract urothelial carcinomas are PAX8-positive!

References: [24, 29]

Adrenal

Adrenal: Immunoprofiles at a Glance	
Diagnosis	Immunoprofile
Adrenocortical neoplasms	SF1+, Melan-A+ (other melanocytic markers, S100, SOX10, and HMB45, are negative), Inhibin+ CK (AE1/AE3 and Cam5.2) usually – but may be focally +, EMA– Variable SYN (frequently +), CHR–
Pheochromocytoma	Neuroendocrine markers (CHR, SYN, NSE, CD56) +, S100+ (sustentacular), CK– SDHB loss (small subset) – syndromic GATA3+ (70%) [30] PHOX2B+ (new marker [31]) – utility to be determined.
Neuroblastoma/ ganglioneuroblastoma	Neuroendocrine markers (CHR, SYN, NSE, CD56) + especially in ganglion cells, S100+ in septae, always CD99– GATA3+ [30] PHOX2B+ (new marker [32])

Renal Cell Carcinoma vs. Adrenocortical Neoplasm		
	Renal cell carcinoma	**Adrenocortical neoplasms**
PAX8 (CD10, RCC)	+	–
EMA	+	–
CK AE1/AE3, Cam 5.2	+/–	–/+ (generally –)
Melan-A (A103), inhibin	–	+
Synaptophysin	–	+
SF1	–	+
D2–40	–	+
Reference: [33]		

Testis

Testicular Neoplasms: Key Multipurpose Markers at a Glance	
Marker [compartment]	Applications
SALL4 [nuclear]	Stem cell marker and a recent pan-germ cell tumor (GCT) marker. Primary utility is to distinguish GCTs (+) from sex cord-stromal tumors (–) and carcinomas (–). Fairly specific to GCTs but occasionally positive in somatic carcinomas (gastric, lung) and other primitive neoplasms (leukemias, malignant rhabdoid tumors) [34]
OCT4 (POU5F1) [nuclear]	Relatively recent stem cell marker, very sensitive and specific for seminoma and embryonal CA [35]
SOX2 [nuclear]	Relatively recent stem cell marker, expressed in embryonal CA.
PLAP [cytoplasmic]	Older pan-GCT marker. Positive in all GCTs, except teratoma and spermatocytic tumor (formerly known as spermatocytic seminoma). Less sensitive and less specific than other markers, so currently regarded as a second-line GCT marker.
D2–40 (Podoplanin) [membranous]	Mesothelial and lymphatic marker, which also identifies seminoma (100%+) and embryonal CA (30%+; apical staining). [36]
Epithelial markers (cytokeratins and EMA)	All non-seminoma GCTs are cytokeratin-positive (though EMA-negative), whereas seminoma (conventional and spermatocytic) are negative for all epithelial markers (some seminomas can stain with CK with perinuclear dot-like pattern)

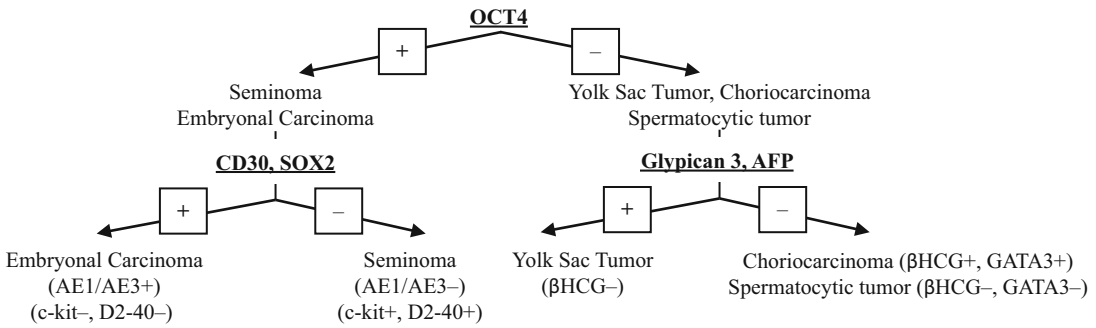
Testicular Neoplasms: Immunoprofiles at a Glance						
	Seminoma[1]	**Embryonal carcinoma**	**Yolk Sac Tumor (YST)**	**Choriocarcinoma**	**Teratoma**	**Spermatocytic tumor**
SALL4	+	+	+	+/– (70%)	+/– (50%)	+
OCT4, NANOG	+	+	–	–	–	–
c-kit (CD117)	+	–	+/– (60%)	–	–	+/–
Glypican 3	–	–	+	+ (80%)	–/+ (20%)	?
AE1/AE3	–/F+ [2]	+	+	+	+	–
EMA	–	–	–	+/– (45%)	+	–
Other	D2–40[3]	CD30, SOX2	AFP[4], CDX2[5]	GATA3[6], βHCG/hPL (ST), p63/p40 (CT), HLA-G/hPL (IT) [37].		

1. SOX17 is a recent marker of seminoma (all +); also positive in 50% of YST. Negative in embryonal CA.
2. AE1/AE3 is usually negative in seminomas, but ~1/3 of cases can have either focal or perinuclear dot-like staining.
3. D2–40 occasionally labels embryonal CA (30%) but is limited to the cell apices.
4. AFP is a sensitive but non-specific marker of YST (e.g., also positive in HCC). Rare cases of embryonal CA and teratoma may stain focally for AFP.
5. CDX2 is frequently positive in YSTs and occasionally in other GCTs – this can be an important pitfall for DDx with colorectal CA!
6. GATA3 is a sensitive pan-trophoblastic marker. It can be occasionally weakly positive in YSTs (10%).

ST syncytiotrophoblast, *CT* cytotrophoblast, *IT* intermediate trophoblast

References: [24, 38–40]

Basic Algorithm for Working Up a Germ Cell Tumor



Reference: [40]

DDx Testicular Germ Cell Tumor vs. High-Grade Metastatic Carcinoma vs. Sex Cord-Stromal Tumor			
	Germ cell tumor (GCT)	**High-grade metastatic carcinoma**	**Sex cord-stromal tumor**
SALL4, OCT4	+[1]	– (rare SALL4+)	–
EMA	–	+	–/+
CK7[2]	+/–	+/–	–
Inhibin, calretinin, SF1	–	–	+/–[3]

1. While SALL4 is a pan-GCT marker, OCT4 is negative in YST and chorioCA.
2. CK7 is most helpful in DDx of YST (consistently –) and metastatic high-grade carcinoma (frequently +). Glypican-3 is another marker that is particularly helpful with this DDx: YST+ vs CA–. CK7 is variably positive in the remaining germ cell tumors.
3. SF1 is emerging as the most sensitive and specific marker of sex cord-stromal tumors. Note that it is also positive in adrenocortical neoplasms.

References: [40, 41]

Thyroid, Parathyroid, and Sinonasal Tract

By Justin A. Bishop

Thyroid and Parathyroid Neoplasms: Immunoprofiles at a Glance					
	TTF-1	**Thyroglobulin**	**PAX8[5]**	**CKs (Cam5.2, AE1/AE3)**	**Others**
Thyroid: papillary and follicular CA[1]	+	+	+	+	
Thyroid: anaplastic CA	–	–	+/–	+ (25% negative[2])	
Thyroid: medullary CA	+	–	–/+	+	Calcitonin+, mCEA[4]+, NE markers[3]+, amyloid deposits (Congo red +)
Parathyroid neoplasms	–	–	–/+	+	PTH+, GATA3+, NE markers[3]+ Parafibromin loss in CA[6]

1. CK903, CK19, HBME-1, and galectin-3 have been reported to favor papillary CA over benign follicular lesions, but none of these markers are sensitive or specific enough for routine use.
2. Essentially any malignant spindle cell neoplasm centered in the thyroid should be presumed to be anaplastic CA, regardless of CK positivity [42].
3. Standard NE markers include SYN, CHR, CD56, and recently INSM1.
4. Proportion of calcitonin-negative versus mCEA-positive cells is reported to have an inverse relationship in medullary CA. Expression of CEA (and therefore decreased expression of calcitonin) is postulated to reflect a more aggressive disease [43].
5. PAX8 is positive in ~75% of anaplastic thyroid carcinomas (in contrast with TTF-1 and thyroglobulin, which are essentially always negative) [44, 45].
6. Parafibromin is a nuclear marker that is diffusely expressed in sporadic parathyroid adenomas but lost or only weakly/focally (+) in most parathyroid CAs (as well as adenomas of the hyperparathyroidism-jaw tumor syndrome) [46].

DDx of Poorly Differentiated Neoplasms in the Sinonasal Tract				
	Epithelial markers (CK, EMA)	**NE Markers** (SYN, CHR, CD56, INSM1)	**EBV (EBER)**	Others
Olfactory neuroblastoma (Esthesioneuroblastoma)	–/F+[1]	+ (SYN 100%)	–	S100+ (stromal/sustentacular) cells, calretinin+
Sinonasal undifferentiated carcinoma (SNUC)	+ (LMWCK)[2]	–/F+	–	p63/p40 –/+, patchy at most
NUT Carcinoma[3]	+	–	–	CD34+/–, p63/p40+, NUT+ or NUT translocation confirms the diagnosis
SMARCB1-deficient sinonasal carcinoma	+	–/F+	–	SMARCB1 (INI1)-negative
Lymphoepithelial carcinoma/nasopharyngeal carcinoma (NPC)[4]	+ (HMWCK)[2]	–	+[5]	p63/p40+
NK/T-cell lymphoma, nasal type	–	– (CD56 +[6])	+	CD45+, CD2+, cytoplasmic CD3ε + (pan-T-cell markers such as CD3 usually absent), cytotoxic proteins (e.g., perforin)+

1. Olfactory neuroblastoma can be focally CK+, but EMA should be negative [47].
2. SNUC shows no squamous differentiation by H&E and is typically HMWCK (CK903, CK5/6)–negative.
3. NUT carcinoma was formerly known as "NUT midline carcinoma" but has now been encountered in many non-midline sites. The sinonasal tract is a preferred site for this tumor.
4. NPC arises, of course, in nasopharynx but may secondarily involve sinonasal tract. Lymphoepithelial carcinoma is an identical tumor that arises in the sinonasal tract. It is a type of SqCC (p40+, HMWCK+).
5. It has been reported that a subset of NPCs is EBV negative but HPV positive [48, 49], but these often represent carcinomas extending from the oropharynx, where HPV is a well-recognized causative agent [50].
6. NK/T-cell lymphoma is CD56+ (CD56 is a marker of both NE cells and NK cells).

DDx also includes neoplasms not unique to these sites, including basaloid SqCC (p63/p40, HMWCK+), melanoma (S100, SOX10, HMB45, Melan-A+), SmCC (NE markers+, TTF-1+/–), alveolar rhabdomyosarcoma (desmin, myogenin+), and Ewing sarcoma (CD99, NKX2.2+).

References: [51–54]

DDx of Basaloid Carcinomas of the Head and Neck						
	HMWCK	**CK7**	**p63/p40**	**ME markers other than p63/p40**	**NE markers**	**Others**
Basaloid squamous cell carcinoma	+	–	+ (diffuse)	–	–	
HPV-related oropharyngeal squamous cell carcinoma[1]	+	–	+ (diffuse)	–	–	High-risk HPV+, p16+[2]
Adenoid cystic carcinoma (particularly solid pattern)	+	+	+ (peripheral)	+	–	MYB+, c-kit+, SOX10+
Basal cell adenocarcinoma (of salivary gland origin)	+/–	+	+ (patchy)	+ (patchy)	–	LEF1 +/–, nuclear β-catenin +/–
Small cell carcinoma	–	+/–	–/F+	–	+	TTF-1+/–
Adamantinoma-like Ewing sarcoma[3]	+	–	+ (diffuse)	–	+/–	CD99+, NKX2.2+

1. Some HPV-related oropharyngeal SqCCs are morphologically identical to basaloid SqCC. The distinction is critical; however, because while basaloid SqCC carries a poor prognosis, HPV-related oropharyngeal SqCCs respond very well to therapy. HPV testing is the only way to definitively distinguish the two entities.
2. p16 is sensitive, but not specific (particularly outside the oropharynx), for the diagnosis of HPV-related SqCC.
3. The adamantinoma-like variant of Ewing sarcoma is a newly recognized variant with some features of typical Ewing sarcoma (including *EWSR1-FLI1* fusions) that exhibits overt squamous differentiation. It appears to have a predilection for the head and neck (salivary, thyroid, sinonasal tract).

Abbreviations: *ME* myoepithelial

References: [55–61]

Salivary Glands

By Justin A. Bishop

Salivary Gland Neoplasms: Key Markers at a Glance	
Marker [compartment]	Applications
Androgen receptor (AR) [nuclear] and **GCDFP-15** [cytoplasmic]	Positive in any tumor with apocrine differentiation, including 100% of salivary duct carcinomas. Also positive in apocrine variants of certain tumors (e.g., epithelial-myoepithelial CA, intraductal CA).
β-catenin [nuclear]	Positive in a subset of basal cell adenomas (~80%) and adenoCAs (30–70%), typically in the basal/myoepithelial cell component, reflecting mutations in *CTNNB1* gene [60, 62].
c-kit (CD117) [membranous and cytoplasmic]	Positive in ductal cell component of various biphasic tumors (e.g., AdCC, epithelial-myoepithelial CA) as well as normal ducts. Diffusely positive in some AdCCs. Not specific for any salivary gland tumor.
DOG1 [membranous]	Ductal and acinar marker. Most commonly used in diagnosing acinic cell CA, but not entirely specific [63].
GATA3 [nuclear]	Positive in many salivary gland tumors (~50% overall). Strong and diffuse in "breast-like" tumors – salivary duct CA and secretory CA (~100% for both) [64].
LEF1 [nuclear]	Somewhat sensitive and specific for basal cell adenoma/adenoCA, similar to nuclear β-catenin [56, 65].
Mammaglobin [cytoplasmic]	Classically positive in secretory CA (~90%) but not specific. For example, staining also seen in most polymorphous adenoCAs [66].
MYB [nuclear]	Relatively sensitive, but not specific, marker for adenoid cystic CA. Also positive in a subset of epithelial-myoepithelial CA, basal cell adenoma/adenoCA and basaloid SqCC. Does not correlate with *MYB* translocation [67–69].
p63 and **p40** [nuclear]	Basal, myoepithelial, and squamoid markers. Not very specific in the salivary glands. Characteristically positive in all cells in clear cell CA, intermediate and squamoid cells of mucoepidermoid CA, and basal/myoepithelial cell component of biphasic tumors (e.g., basal cell adenoma/adenoCA, adenoid cystic CA, pleomorphic adenoma). Usually interchangeable, except for polymorphous adenoCA (p63+/p40–).
PLAG1[nuclear]	Sensitive but not specific marker for PA and carcinomas ex-PA. Usually, but not always, correlates with *PLAG1* abnormalities by FISH. Not terribly useful in practice [70].
S100 [nuclear and cytoplasmic] and **SOX10** [nuclear]	Both markers variably stain ducts and myoepithelial cells in the biphasic tumor group. SOX10 also stains normal acini and acinic cell CAs. Both markers diffusely stain normal intercalated ducts as well as tumors that arise from them (e.g., low-grade intraductal CA, secretory CA, polymorphous adenoCA, canalicular adenoma, and others). Consistently negative in clear cell CA. SOX10 is promising but not as widely studied or used as S100 (yet) [71, 72].

Salivary Gland Neoplasms: Immunoprofiles, Special Stains, and Genetics at a Glance		
Tumor	Immunostaining (and Special Stain) Pattern	Molecular alteration
Acinic cell carcinoma	PAS+, PASD+, DOG1+, SOX10+, ME markers–	
Adenoid cystic carcinoma (AdCC)	MYB+, biphasic staining pattern, with epithelial component: CK, EMA, CEA, c-kit and myoepithelial component: ME markers+	*MYB-NFIB* or *MYBL1-NFIB* (~60–70%)
Basal cell adenoma, basal cell adenocarcinoma	LEF1+, nuclear β-catenin +/–, biphasic staining pattern, with epithelial component: CK, EMA, CEA, c-kit and basal/myoepithelial component: ME markers+ (can be focal)	
Clear cell carcinoma (previously hyalinizing clear cell carcinoma)	p63/p40+, CK5/6+, mucicarmine +/–, SOX10–	*EWSR1-ATF1* 80%)
Epithelial-myoepithelial carcinoma	Biphasic staining pattern, with epithelial component: CK, EMA, CEA, c-kit and myoepithelial component: ME markers+	
Low-grade intraductal carcinoma (previously low-grade cribriform cystadenocarcinoma, low-grade salivary duct carcinoma)	S100+, mammaglobin+, ME cell markers+ on cells surrounding tumor nests	*NCOA4-RET* (~50%)
Mucoepidermoid carcinoma	Mucicarmine+ (goblet cells), p63/p40+ (intermediate cells); other ME markers–, DOG1–, S100/SOX10–	*CRTC1-MAML2* or *CRTC3-MAML2* (~70–80%)
Myoepithelioma, myoepithelial carcinoma	ME markers+	
Oncocytoma, oncocytic carcinoma	p63/p40+ (basal pattern), PTAH+ (not in routine use)	
Pleomorphic adenoma (benign mixed tumor) and carcinoma ex-pleomorphic adenoma	PLAG1+, biphasic staining pattern, with epithelial component: CK, EMA, CEA, c-kit+ and myoepithelial component: ME markers+	*PLAG1* and *HMGA2* fusions (~60–90%)
Polymorphous adenocarcinoma (previously polymorphous low-grade adenocarcinoma)	S100+/SOX10+, p63+, but p40–, other ME markers–	*PRKD1* fusions/mutations or *PRKD2/PRKD3* fusions (~75%)
Salivary duct carcinoma	Androgen receptor+, GCDFP-15+, GATA3+, HER2 amplification –/+ (not useful diagnostically)	
Secretory carcinoma (previously mammary analogue secretory carcinoma/MASC)	S100+/SOX10+, mammaglobin+, GATA3+	*ETV6-NTRK3* (~97%), *ETV6-RET* (~3%)

H&E histology is still king in salivary gland pathology. As you will notice, there is no "magic bullet" marker or combination of markers that is specific for any salivary gland tumor diagnosis. As two examples, many tumors have a mixed ductal/myoepithelial cell pattern, and androgen receptor positivity is not limited to salivary duct CA. The molecular alterations are specific but are not practical to perform routinely and, for the most part, are not present in all examples of each tumor. To avoid pitfalls, allow immunostains to support your histologic impression.

ME (myoepithelial markers): p63/p40, calponin, SMA, GFAP, S100, SOX10. You'll notice there are a lot of them. This is because in salivary ME tumors, not all are positive, and it is unpredictable which markers will stain. Generally speaking, you want to see CK and at least 2–3 ME markers positive to regard as myoepithelial.

Quick DDx guide by pattern (use IHC and FISH accordingly):

- **Blue (basaloid) neoplasms**: adenoid cystic CA, basal cell adenoma/adenoCA, cellular PA, polymorphous adenoCA
- **Pink (oncocytic) neoplasms**: acinic cell CA, mucoepidermoid CA, oncocytoma/oncocytic CA, myoepithelioma/myoepithelial CA, low-grade intraductal CA, PA, salivary duct CA, clear cell CA (not always clear!)
- **Clear cell neoplasms**: clear cell CA, acinic cell CA, mucoepidermoid CA, oncocytoma/oncocytic CA, epithelial-myoepithelial CA, myoepithelioma/myoepithelial CA, metastases (especially RCC)

References: [56, 60, 63, 73–75]

Pancreas

Pancreas: Immunoprofiles at a Glance	
Adenocarcinoma	Gene mutation and loss of expression of **DPC4 (aka SMAD4)** is found in 55% of pancreatic CA (loss of expression supports the diagnosis of metastases from pancreatic primary, but positive staining does not rule it out). Negative nuclear AND cytoplasmic staining counts as negative. A few other neoplasms show a loss of DPC4, although much less frequently: the gallbladder 19%, colon 11%, and 0% ovary, appendix, small bowel, stomach, endocervix [74, 76]. Finicky antibody which does not work in many labs. CK7+, CK20+ (usually), CA19.9+, CEA+. No site-specific marker currently available.
Pancreatic neuroendocrine tumor (PanNET)	SYN+, CHR+, CD56+, INSM1. PAX8+ & ISL1+ (~80%). In metastatic setting, the latter markers recently shown to differentiate PanNET (+) from ileal NET (–) and lung carcinoid (–; only rarely +).
Acinar cell carcinoma (ACC)	Acinar markers (trypsin, lipase, chymotrypsin), recent marker: BCL10 [77]
Solid pseudopapillary neoplasm (SPN)	Several recent specific markers: LEF1+ [78], TFE3 + [79], CD99+ in a perinuclear dot-like pattern [80]; also nuclear β-catenin+ and CD10+. SYN can be positive but CHR always negative. PR+/ER–
Pancreatoblastoma	Acinar markers (trypsin, lipase, chymotrypsin), neuroendocrine markers (CHR, NSE, SYN), and ductal markers (CK, CEA), each corresponding to different tumor components; nuclear β-catenin (most +)
Mucinous cystic neoplasm	ER+/PR+/ Inhibin+ (ovarian stroma); other positive markers: CEA, CA19–9, CK7, CD10, and MUC5AC
Intraductal papillary mucinous neoplasm (IPMN)	Variable expression of different "MUCs" based on histologic subtype (gastric vs intestinal vs pancreatobiliary), usually not used in practice. Note that IPMN and IPMN-associated carcinomas have intact DPC4 [81, 82].
IgG4-related pancreatitis (formerly lymphoplasmacytic sclerosing pancreatitis)	Infiltration by IgG4+ plasma cells (>10/HPF in biopsies, >50/HPF in resections). Recent criteria also include >40% IgG4/IgG ratio [83–85] (note that this is different from "type 2" autoimmune pancreatitis, which is instead characterized by granulocytic epithelial lesions/GELs). HPF = high-power field

DDx of "Cellular" Pancreatic Neoplasms with Acinar/Solid Growth Pattern (i.e., high ratio of tumor cells to stroma and lack of desmoplasia – distinct from typical ductal adenocarcinoma)			
	Pancreatic neuroendocrine tumor (formerly islet cell tumor)	**Acinar cell carcinoma** (and pancreatoblastoma**)	**Solid pseudopapillary neoplasm**
Cytokeratins (AE1/AE3, Cam5.2)	+	+	– (30% F+)
NE Markers (SYN, CHR, INSM1)	+	–/+*	–/+ (CHR always negative)
Acinar markers (trypsin, chymotrypsin, lipase, PAS/D)	–	+ (trypsin/chymotrypsin most sensitive)	–
BCL10	–	+	–
CD10	– (25% F+)	–	+
Nuclear β-catenin	–	–/+ (25% +)	+
LEF1	–	–	+
TFE3	–	?	+
CD99	–	–	+ (perinuclear dot-like)
E-cadherin	+	+	–
PR	+ (ER always negative)	–	+ (ER always negative)

* Focal neuroendocrine component is present in up to 25% of acinar cell carcinomas.
** Acinar cell carcinoma and pancreatoblastoma have similar immunoprofile of acinar component (entities distinguished by the presence of squamoid nests and younger age of the latter). References: [77, 80, 86, 87]

Tubular Gastrointestinal Tract

Markers for Adenocarcinomas of the Tubular GI Tract at a Glance	
Marker [compartment]	Site of origin
CK7 [cytoplasmic]	Positive in most gastroesophageal adenoCAs (CK20, if present, is typically only focal). Some rectal adenoCAs are also positive.
CK20 [cytoplasmic]	Positive in most colorectal CAs (CRC), urachal, and a subset of small bowel CAs.
CDX2 [nuclear]	Predominantly a marker of lower GI tract, but also positive in gastroesophageal CAs and some pancreaticobiliary (usually focal and weak); may be lost in some high-grade CRCs.
SATB2 [nuclear]	Sensitive and specific marker of CRC that is positive in ~50% of small bowel adenoCAs; retained in poorly differentiated CAs including medullary carcinoma of the large intestine [88]; only rarely seen in other GI tumors.
CDH17 [membranous]	Recent marker that is similar to CDX2 in that it is predominantly positive in adenoCAs of the lower GI tract but also marks gastroesophageal and some pancreaticobiliary adenoCAs [86]. Unlike CDX2 but similar to SATB2, it is frequently retained in poorly differentiated tumors (including medullary carcinoma of the large intestine [88]) and is therefore a useful marker in evaluating carcinoma of unknown primary.
Reference: [89]	

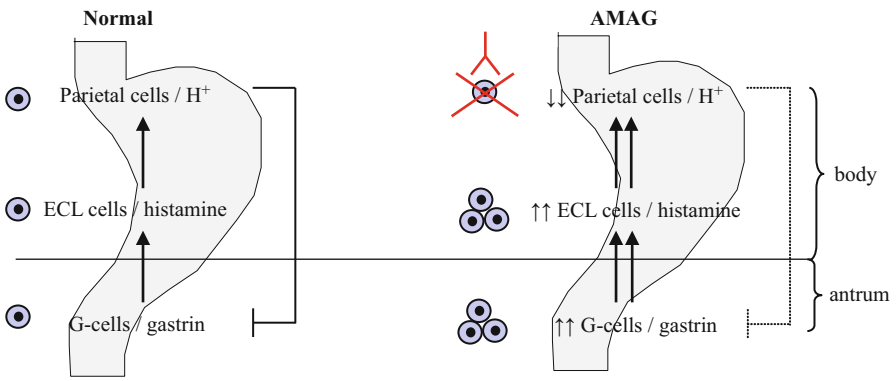
DDx Low-Grade Spindle Cell Neoplasm of the Tubular GI Tract						
	c-kit, DOG-1	**CD34**	**β-catenin (nuclear)**	**Desmin**	**SMA**	**Others**
GIST*	+	+/–	–	– (rare+)	–/+ (20–30%+)	
Schwannoma	– (rare c-kit+)	– (rare+)	–	–	–	S100, SOX10
Leiomyoma	–	–	–	+	+	Caldesmon
SFT	–	+	+/–	–	– (rare+)	STAT6, BCL2
IMT	–	–	–	–/+	+	ALK (~50%)
Inflammatory fibroid polyp	–	+	–	–	+	
Desmoid tumor	–/+ (some c-kit+)	–	+/–	–/+	+/–	

* A small subset of predominantly pediatric GISTs lacks the characteristic *c-KIT* or *PDGFRA* mutations and is characterized by succinate dehydrogenase B (SDHB) deficiency. They are characteristically gastric, display epithelioid morphology, and may be part of the Carney-Stratakis syndrome. >90% of these tumors are still positive for c-kit and DOG-1, but they lack SDHB staining by IHC. As they do not respond to the traditional c-kit targeted therapy (Gleevec), their identification by IHC +/– molecular analysis is crucial for therapy selection.

Abbreviations: *GIST* gastrointestinal stromal tumor, *SFT* solitary fibrous tumor, *SMA* smooth muscle actin, *IMT* inflammatory myofibroblastic tumor

References: [90, 91]

Diagnosis of Autoimmune Metaplastic Atrophic Gastritis (AMAG)/"Pernicious Anemia"



Quick background on ***A**utoimmune **M**etaplastic **A**trophic **G**astritis* (AMAG):

- Acid is produced by parietal cell, which are located in the gastric body. Acid release is stimulated by gastrin (product of G cells, located in the antrum) through an intermediary action on histamine (product of ECL cells, located in the body).
- AMAG is caused by autoantibodies to parietal cells, which secrete intrinsic factor (IF) in addition to acid. As a result, parietal cells are wiped out, and the body begins to look histologically like the antrum (gets "antralized"), except there are no G cells.
- Hypochlorhydria ($\downarrow H^+$) causes compensatory increase in antral G cells and hypergastrinemia. High gastrin stimulates ECL cells to grow, which manifests as nodular and linear ECL cell hyperplasia in the body.

Immunostains aid in the diagnosis of AMAG as follows:

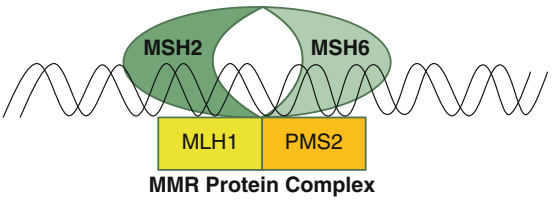
- **Chromogranin** (CHR) is a marker of NE cells; it marks both ECL cells in the body and G cells in the antrum. Normally only rare ECL and G cells are present. In contrast, these cells are abundant in the setting of AMAG, which are highlighted by CHR.
- **Gastrin** identifies gastrin-secreting G cells, which are located exclusively the antrum and not in the body (even when the body is "antralized" as a result of AMAG). IHC for gastrin serves two purposes in the diagnosis of AMAG:
  1. Gastrin highlights G cell hyperplasia in the antrum (same as CHR).
  2. Gastrin should be negative in the body: "antralized" body looks identical to the antrum minus the G cells. Negative gastrin stain confirms that the biopsy is indeed taken from the body rather than inadvertently from the antrum.

Abbreviations: *CRC* colorectal carcinoma

Immunohistochemical Testing of DNA Mismatch Repair (MMR) Proteins

By Marina K Baine & Ashlie L. Burkart

- Loss of expression of DNA mismatch repair (MMR) proteins can occur by two mechanisms: one is promoter hypermethylation, which silences gene expression and occurs in sporadic colorectal carcinoma (CRC) (10–15% of CRC) [92], and second is germline mutation, which occurs in hereditary cases (aka Lynch syndrome/HNPCC) (~3% of CRC) [93]. Testing for the loss of protein expression is done by IHC as summarized below.
- The consequence of defective MMR is instability of microsatellites (small repetitive sequences of DNA). Testing for microsatellite instability (MSI) is done by PCR. In tumors with defective MMR, there is instability in ≥2 out of 5 microsatellite markers (called "MSI-high"). PCR testing is not discussed further here.
- The advantage of IHC is that it tells which MMR protein is defective. There are four main DNA MMR proteins- **MLH1, PMS2, MSH2, MSH6**. If all four proteins are utilized, IHC identifies ~95% of tumors with defective MMR. The specificity of IHC is virtually 100% [93]. However, the majority of the staining patterns require confirmation by genetic analysis (see flow chart below).



There are six (five major) possible patterns of NUCLEAR IHC staining for the 4 DNA MMR proteins. Understanding these patterns requires a basic understanding of how these proteins work in pairs:
- MLH1 and PMS2 are partners, and if MLH1 (the dominant partner) is lost, PMS2 is degraded. On the other hand, the loss of PMS2 does not affect MLH1.
- Same applies to MSH2 and MSH6, where MSH2 is the dominant partner: loss of MSH2 leads to the loss of MSH6, but not vice versa.

Interpretation of IHC for DNA MMR Proteins

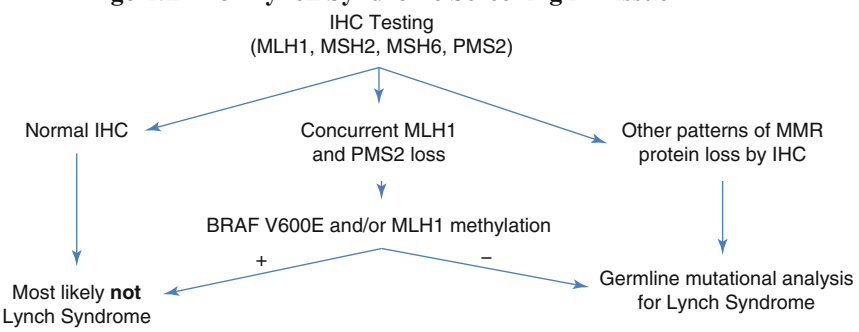
MLH1	PMS2	MSH2	MSH6	IHC Interpretation	Implication for germline mutation (Lynch syndrome) vs. sporadic loss
+	+	+	+	**No defects**	No evidence of defective MMR as assessed by IHC
–	– or +	+	+	**Defective MLH1**	~90% sporadic loss (MLH1 promoter hypermethylation or BRAFV600E mutation)[1]; ~10% germline gene mutation (most commonly MLH1, or very rarely PMS2)
+	–	+	+	**Defective PMS2**	Germline >> sporadic (PMS2, or very rarely MLH1)
+	+	–	– or +	**Defective MSH2**	Germline >> sporadic[2]
+	+	+	–	**Defective MSH6**	Germline >> sporadic (MSH6, or very rarely MSH2)[3]
–	–	–	–	**Null Pattern** (none of the MMR proteins expressed)	Extremely rare; most reported cases are due to MSH2 germline mutation in combination with MLH1 hypermethylation

\+ Retained nuclear expression by invasive adenoCA (expression can be patchy or faint)
– Complete loss of nuclear staining by invasive adenoCA (patchy loss does not count)
1. Unlike in CRC, sporadic loss of MLH1 in endometrial CAs is almost never associated with *BRAF* mutations (0.1%) [94], and are commonly due to MLH1 promoter hypermethylation associated with loss of both MLH1 and PMS2 protein expression by IHC [95].
2. Recent data shows that the loss of MSH2 can be caused either by inherited mutation in MSH2 gene OR inherited deletion of 3′ end of *EPCAM gene* leading to inactivation of adjacent MSH2 gene through promoter methylation. In rare cases, the loss of both MSH2 and MSH6 staining by IHC may be due to a mutation in the MSH6 gene. However, when only MSH2 is lost by IHC, the primary mechanism is either an MSH2 mutation or an *EPCAM* deletion.
3. Some sporadic CRCs that have been treated with chemotherapy can demonstrate isolated loss of MSH6 immunostaining; in those scenarios repeat the test in pre-treatment material. Also, microsatellite loci are present within *MSH6* gene; therefore MSH6 staining may be lost in cases with other MMR abnormalities (such as cases with MLH1 promoter hypermethylation).

Note: It is very important to have a positive internal control on the slide to make sure the stain worked properly (such as normal colon epithelial cells, stromal cells or lymphocytes). References: [89, 96]

- Applications:
  - The primary application of IHC for MMR proteins is to screen for Lynch syndrome (to identify patients at higher risk for additional colonic and extracolonic tumors and with at-risk family members).
  - Other applications include evaluation of prognosis and chemosensitivity in sporadic CRC. MMR deficient/MSI-high CRC (1) have a better prognosis by stage, (2) may not have the same response to 5-FU chemotherapy, and (3) may have improved outcome with irinotecan and PD-1/PD-L1 targeted immunotherapy [93, 97, 98].
  - Testing of adenomas is controversial. IHC may be performed on adenomas of patients with a clinical concern for Lynch syndrome, but normal staining does not exclude Lynch syndrome as the test is less sensitive in adenomas compared to CRC [99–102].
  - Initially, IHC has been used to test CRC. Presently, endometrial CAs are also routinely screened for Lynch syndrome by IHC. Although no current guidelines exist for screening other known Lynch syndrome-associated malignancies (e.g., upper GU tract urothelial CA), some of these tumors are also being similarly screened.
  - MMR deficient/MSI-high colorectal (and endometrial) CAs have distinct pathologic features (see Chapter 12 for further details), which may be used to help decide whether a tumor needs to undergo testing for Lynch syndrome by IHC and/or other methods.

Algorithm for Lynch Syndrome Screening in Tissue



Abbreviations: *HNPCC* hereditary nonpolyposis colorectal cancer, *CRC* colorectal carcinoma, MMR mismatch repair, *MSI* microsatellite instability

Liver

HCC vs. Cholangiocarcinoma vs. Metastatic Adenocarcinoma			
	Hepatocellular carcinoma (HCC)	**Cholangiocarcinoma**	**Metastatic adenocarcinoma**
HepPar-1	+ 95% (fairly specific)	–	– (rare +)
Glypican-3	+	–	– (rare +)
Arginase-1	+	–	–
Albumin RNA ISH*	+	– (+ in intrahepatic cholangio)	–
AFP	+/– (~50%+)	– (rare +)	variable
mCEA	–	+	+
pCEA	+ 80% (canalicular, highly specific)	+ (cytoplasmic)	+ (cytoplasmic)
CD10 and villin	+ (canalicular)	–	site-dependent
CK7	– (small subset +)	+	site-dependent
CK20	– (small subset +)	+ (usually)	site-dependent
CK AE1/AE3 (pan-CK)	– (but up to 30%+)	+	+
Cam5.2 (LMWCK)	+	+	+
CK903 (HMWCK)	–	+	site-dependent
Markers of glandular epithelium: **EMA, MOC-31, Mucicarmine**	–	+	+
Other	Fibrolamellar carcinoma is CK7+, CD68+, Glypican-3 frequently –, and AFP–	CK19+ (70–100%) There are no good markers which are specific for cholangiocarcinoma (cannot differentiate metastasis by immunostains).	

*Albumin ISH is highly sensitive for HCC, and is considered to be the most sensitive marker for poorly-differentiated HCC [103]. It is also highly specific for HCC, but is also positive in intrahepatic cholangiocarcinoma; the latter is usually negative for other hepatocellular markers (Hep-Par1, Glypican-3, Arginase-1). Another recent marker of both HCC and intra-hepatic cholangiocarcioma is CRP (C-reactive protein). Note that all hepatocellular markers (HepPar1, Glypican-1, Arginase-1, Albumin ISH) also label rare hepatoid carcinomas of the stomach and other sites [104].

References: [20, 86, 105–107]

HCC vs. Benign Hepatocellular Nodule		
	HCC	**Benign hepatocellular nodules** (adenoma, focal nodular hyperplasia, regenerative nodule in cirrhosis)
Reticulin	Thickened hepatocyte plates (> 3-cell thick)	Normal thickness of hepatocyte plates (1–2 cell thick)
CD34, Factor VIII	+ ("sinusoidal capillarization")	– (can be patchy in FNH and adenoma)
Glypican-3, HSP70	+	– (some cirrhotic nodules may be +)
Clusterin	+ (enhanced canalicular)	+ (cytoplasmic)

Note: Sensitivity is higher if >1 supportive marker is positive.

References: [86, 108–110]

Hepatocellular Adenomas vs. Focal Nodular Hyperplasia					
	Adenoma				**FNH**
	HNF-α inactivated (35–40%)	**β-catenin activated*** (15–19%)	**Inflammatory** (30–35%)	**Unclassified** (10%)	
LFABP1	–	+	+	+	+
Nuclear β-catenin	–	+/– (usually focal; one nucleus is enough)	–**	–	–
Glutamine synthetase	–	+ (strong diffuse)	– (some patchy centrilobular)**	–	+ ("maplike")
SAA, CRP	–	–	+	–	–

* Hepatocellular adenomas have a small but definite risk of malignant transformation: β-catenin-activated adenomas are considered high risk for transformation to HCC, making this DDx of particular relevance on biopsy [111].

** 10% of inflammatory adenomas are β-catenin activated (+ nuclear IHC) and diffusely glutamine synthetase-positive. Those with nuclear β-catenin positivity are more likely to undergo malignant transformation.

Abbreviations: *SAA* serum amyloid alpha, *CRP* C-reactive protein, *LFABP1* liver fatty acid binding protein 1, *FNH* focal nodular hyperplasia

Reference: [112]

Lung

By Natasha Rekhtman

Common Markers for Lung Carcinomas at a Glance	
TTF-1 (thyroid transcription factor 1)	Positive in ~80% of lung adenoCAs; thus, negative TTF-1 does not exclude (or argue against) a lung primary. Critical to know which clone is used: there are two widely used clones. SPT24 clone is more sensitive but less specific and is the clone that causes non-specific problems with TTF-1 in unexpected locations. 8G7G3/1 is much more specific and is recommended in thoracic pathology literature [113]. TTF-1 also stains thyroid (expected), but even 8G7G3/1 stains some GYN carcinomas – watch out! [114, 115]
Napsin A (novel aspartic proteinases of the pepsin family)	New-ish marker of lung adenoCA. Similar sensitivity as TTF-1, but some adenoCAs can stain for one of the two markers, so sensitivity (and specificity) is increased if both markers are used. Do not use polyclonal Napsin A [116] Other Napsin A-positive tumors are RCC (papillary, clear cell), clear cell carcinoma of GYN tract, and minority of thyroid carcinoma (esp. when oncocytic).
p40/p63	Markers of SqCC and basal cells. p40 (ΔNp63) is a recent marker, which is a variant of p63. Unlike the conventional p63 antibody (4A4), p40 does not cross-react with lung adenoCA (and various other unexpected tumors, such as lymphomas). Conversely, p63 is positive in ~30% of lung adenoCAs (usually focally, but sometimes diffusely). p40 does occasionally label adenoCA, usually as focal scattered cells [117].

Lung: Immunoprofiles at a Glance								
	TTF-1	**Napsin A**	**CK7**	**CK20**	**p63/p40**	**CK5/6, CK903**	**NE markers[1]**	**Other**
Non-mucinous adenocarcinoma	~80%+	~80%+	+	–	p40–p63–/+	–/+	–	
Mucinous adenocarcinoma[2]	–/+ (10–20%+)	–/+ (10–20%+)	+	+/–	p40–p63–/+	–/+	–	May have focal CDX2
Squamous cell carcinoma (SqCC)	–	–	–/+	–	+	+	–	
Small cell carcinoma (SmCC)	90% +[3]	–	–/+	–	–	–	+	Punctate Cam5.2 and AE1/AE3 Diffuse p40, CK903 or CK5/6 exclude SmCC
Carcinoid	50% +[3]	–	+/–	–	–	–	+	
Mesothelioma	–	–	+	–	–	+	–	WT1, Calretinin, D2–40

All above entities are AE1/AE3+ and Cam5.2+, although reactivity is variable in NE tumors (e.g., carcinoid, SmCC).

1. Standard NE markers include SYN, CHR, CD56, and recently INSM1. Expression of these markers in SmCC can be weak/focal – look closely.
2. Metastatic pancreatic and upper GI adenoCA may be difficult to distinguish from primary lung mucinous adenoCA by either morphology or immunoprofile (both may be CK7+/CK20+ and TTF-1–). DPC4 may be helpful: it is deleted in 55% of carcinomas of the pancreatic but not lung origin.
3. TTF-1 is not specific for SmCC of the lung because >40% of extrapulmonary SmCCs are also TTF-1-positive. In contrast, TTF-1 is positive in pulmonary but usually not non-pulmonary carcinoids/NETs.

Common differentials and panels for primary lung carcinoma include:

- AdenoCA (TTF-1/Napsin A+/–, p63 variable, p40–) vs. SqCC (TTF-1–, p63/p40+) [86, 87]
- SmCC (TTF-1+/–, NE marker+, p63/p40–, HMWCK–) vs. basaloid SqCC (consistently TTF-1–, NE marker–, p63/p40+, HMWCK+)

Reference: [118]

DDx of Neuroendocrine Neoplasms of the Lung				
	Typical carcinoid	**Atypical carcinoid**	**Large cell neuroendocrine carcinoma**	**Small cell carcinoma**
TTF-1	+/– (~30%+)		50%+	90%+
Cytokeratins	+/– (~80%+)		+	+ (dot-like)
CHR, SYN, CD56, INSM1	+ strong diffuse		+ diffuse to focal	+ frequently weak focal[2]
Ki67[1]	<2% (mean 1%)	<20% (mean 10%)	> > 20% (mean 50%)	> > 20% (mean 70%)

1. Ki67 rate is very helpful in evaluating small specimens, particularly if crushed. Carcinoids can have a crush artifact and may be overinterpreted as SmCC in the absence of Ki67. Always do Ki67 for crushed neuroendocrine neoplasms.
2. NE marker expression is frequently weak/focal in small cell carcinoma; 20% of cases are negative for both SYN and CHR, and stain only for CD56 (data on INSM1 still emerging). Carcinoids are consistently diffusely positive for NE markers, which can be used as a soft feature to distinguish these tumors.

Reference: [118]

Lung: Common Differentials

DDx Primary Lung vs. Breast Carcinoma		
	Lung AdenoCA	**Breast primary**
ER	–/F+ (~20%; 6F11 & SP1 clones >1D5 clone)	+ (60–70%)
PR	–	
GCDFP-15 (BRST2)	–/F+ (~5%+)	
Mammaglobin	–/F+ (very rare)	
GATA-3	–/F+ (~5%+)*	~90% +
TTF-1	~80% +	Rare (usually with SPT24 clone; extremely rare with 8G7G3/1 clone)
Napsin A		–
Bottom line – lung adenoCAs quite commonly stain for ER (particularly with 6F11 & SP1 clones), but PR is usually negative. Conversely, TTF-1 staining of breast carcinomas (with more specific clone 8G7G3/1) is extremely uncommon. So ER focal +/PR–/TTF-1+ carcinoma = lung. Napsin A+ can further support this. * GATA3 is frequently positive in SqCC of the lung; but adenoCAs are only rarely positive, and usually weak/focal (unlike breast). References: [119–123]		

DDx Primary Lung CA vs. GYN Carcinoma		
	Lung adenoCA	**GYN primary**
PAX8	–	+ (>90%; pan-GYN marker)
WT1	–	+ (in serious CA)
TTF-1	~80% +	–/+ (~10%, even with 8G7G3/1), watch out!!
Napsin A	~80% +	+ (in clear cell CA)
References: [114, 115]		

DDx Primary Lung Mucinous Carcinoma vs. Metastatic Mucinous Carcinoma		
	Lung ("Invasive Mucinous AdenoCA/IMA")	**Pancreas, upper GI**
CK7, CK20	7+, 20+/–	7+, 20+/–
CDX2	F+/–	F+/–
TTF-1, Napsin A	–/+ (~15% F+)	– (watch out for entrapped pneumocytes!)
DPC4 (SMAD4)*	Retained	Lost in ~50%
Primary lung IMA is morphologically indistinguishable from metastatic pancreatic/upper GI carcinomas. Other than TTF-1/Napsin A, which are positive in a minority of IMAs, other markers are not helpful. So, order TTF-1 +/– Napsin A. If positive → primary lung IMA. If negative (as in most cases) – the diagnosis must be deferred to clinicoradiologic correlation: – Single mass/nodule, and no evidence of abdominal disease – favor lung primary – If known pancreas primary – EXTREMELY high probably of metastasis, even if lung lesion is solitary – Multiple consolidative nodules and bilateral disease does not indicate metastasis for mucinous CAs – this is a common presentation for advanced IMAs! * DPC4 loss is reported as specific to pancreas, but antibody does not work in most labs. IMAs grow over underlying pneumocytes, which are TTF-1/Napsin A positive and can be misinterpreted as positive staining of tumor. Watch out! Abbreviations: *IMA* invasive mucinous adenoCA (former mucinous bronchioloalveolar carcinoma)		

DDx of Lung SqCC vs. Metastatic Urothelial Carcinoma (UC)		
	Lung SqCC	**Urothelial carcinoma**
CK7/CK20	7+/20– (usually)	7+/20+ (usually, but can be 20–)
GATA3	–/+ (~30%+)	+ (almost all)
Uroplakin II (recent Ab – more sensitive than Uroplakin III)	– (need more data)	+/– (>70%)
Uroplakin III	–	–/+ (15-50%)
Unless CK20 and uroplakins are positive, impossible to distinguish lung primary from metastatic UC. Also, negative GATA3 argues against UC since it is a sensitive urothelial marker. Conversely, positive GATA3 is not helpful as lung SqCCs are frequently GATA3-positive. Similarly, metastatic SqCC from elsewhere cannot be distinguished from primary lung SqCC, with exception of HPV-related SqCC (tonsillar, anogenital) – HPV is consistently negative in lung SqCC. Reference: [124]		

Carcinoid Tumorlet vs. Minute Meningothelial-Like Nodule		
	Carcinoid tumorlet	**Minute meningothelial-like nodule ("chemodectoma-like body")**
CHR, SYN, INSM1	+	–
CK	+	–
EMA	+	+
PR	–	+
CD56	+	+
IHC generally not needed in practice – both are benign lesions and usually are readily distinguishable morphologically. Reference: [125]		

Mesothelioma and Thymic Epithelial Neoplasms

By Natasha Rekhtman

Mesothelioma vs. Adenocarcinoma				
			Mesothelioma[1]	**Adenocarcinoma**
Immunohistochemistry	**Mesothelial markers**	**Calretinin**	+	–
		WT1	+	–
		CK5/6	+	–
		D2–40	+	–
		Rarely used: thrombomodulin (CD141), HBME-1, N-cadherin	+/–	–/+
	Adenocarcinoma markers	**TTF-1, Napsin A** (in the pleura)	– (always)	+/–
		BerEP4	–	+/–
		mCEA	–/+	+/–
		CD15 (Leu-M1)	–	+
		2nd line markers: B72.3, BG8, MOC-31	–/+	+/–
	Epithelial markers	**EMA**	–/+ (membranous)	+/– (cytoplasmic)
		Claudin-4	– (always)	+
		Cytokeratins (AE1/AE3, Cam5.2)[2]	+ perinuclear accentuation	+ Peripheral (membrane) accentuation
Special stains[3]		**Mucicarmine**	–	+/–
		PAS → PAS/Diastase	+ → –	+ → +
		AB → AB/hyaluronidase	+ → –	+ → +
EM		Length of microvilli	long (length to width ratio > 10:1)	short

A standard panel includes mesothelial markers (WT1, Calretinin, D2–40, CK5/6) versus adenoCA markers (Claudin-4, BerEP4 +/– mucicarmine). Recently, Claudin-4 has emerged as a first-line epithelial marker; strong membranous staining is highly specific for carcinoma [126]. In the pleura, always include TTF-1 (+/– Napsin A) as adenoCA markers. Beware that peritoneal mesotheliomas can be PAX8-positive! [127]

1. This immunoprofile applies primarily to epithelioid mesothelioma. In contrast, sarcomatoid mesothelioma is usually less reactive or negative for mesothelial markers (cytokeratin positivity is consistently retained).
2. Both mesothelioma and adenoCA are CK7+/CK20–.
3. Special stains distinguish mucin (mucicarmine+, PAS+) produced by some adenoCA from hyaluronic acid (AB+/hyaluronidase sensitive) and glycogen (PAS+/diastase sensitive) produced by mesothelioma.

References: [20, 128–130]

DDx Malignant vs. Reactive Mesothelial Proliferations		
	Mesothelioma	**Reactive mesothelial proliferations**
BAP1[1]	Loss (~40%; epithelioid>sarcomatoid)	No loss (nuclear stain retained)
p16/CDKN2A/9p21 FISH[1, 2]	Loss of 9p21 locus (~60% overall; sarcomatoid>epithelioid)	No loss by FISH
p53, EMA, CD146 (not used in practice)	+	–
Desmin (not used in practice)	–	+
GLUT1 (not used in practice)	+	–

1. BAP1 IHC and p16 FISH are recent breakthrough markers that help distinguish mesothelioma (loss of either marker) from reactive mesothelial proliferations (both markers retained). The loss of either marker is 100% specific for mesothelioma, but combined sensitivity is only ~80%. It is most efficient to do BAP1 IHC first, and if retained – proceed to p16 FISH. Remember that if the loss is not documented – mesothelioma is still not excluded [129, 131]. These markers also work for peritoneal mesothelioma, though the loss of p16/CDKN2A is much less common (25%). BAP1 is lost in ~50% [132].
2. p16 IHC does not work for this application – requires FISH for p16/CDKN2A/9p21 locus. IHC for MTAP (methylthioadenosine phosphorylase) – another gene on 9p21 locus – is emerging as a promising surrogate for "p16" FISH [133]. Stay tuned.

DDx of Thymic Epithelial Neoplasms					
	TdT, CD99, CD1a (markers of immature/thymic T lymphocytes)	**CD5** (dual marker of T cells and malignant thymic epithelium)	**c-kit (CD117)**	**PAX8**[#]	**Cytokeratins**
Thymoma	+ (lymphocytes)	+ (lymphocytes only)	–	+ (>90%)	+ (epithelium)
Thymic carcinoma	–	+ (epithelium and lymphocytes)	+ (epithelium)	+ (80%)	+ (epithelium)
Non-thymic carcinoma	–	–	–/+	–/+	+

PAX8 was recently identified as a marker of thymic epithelium (but primarily applies to polyclonal PAX8 antibody). It can be used to distinguish thymic carcinoma (PAX8+) from lung carcinoma (PAX8–) and metastasis (except for renal, mullerian and thyroid carcinomas which are also PAX8+).

References: [134–136]

Soft Tissue

By Youran Zou & Jason C. Chang

Selected Mesenchymal Tumors: Immunoprofiles at a Glance – 1		
Diagnosis	*Immunophenotype*	*Genetic markers*
Angiosarcoma	Vascular markers (ERG/CD31/CD34)+, watch out – can stain for keratins (especially LMWCK)!	*c-MYC* amplification in a significant subset of postradiation or lymphedema-associated angiosarcoma
Alveolar soft part sarcoma (ASPS)	Nuclear TFE3+; desmin (focal in 50%), actin (10%), S100 (25%); PAS+/diastase-resistant rhomboid cytoplasmic crystals (pathognomonic by EM)	*TFE3-ASPL* fusion
Angiomatoid fibrous histiocytoma (AFH)	Desmin+/–, CD68+/–, CD99+/–, EMA+/– (all around 40–50%) (the combination of EMA and desmin positivity should prompt consideration for this entity)	*EWSR1-CREB1* fusion (90%) *EWSR1-ATF1* fusion *FUS-ATF1* fusion
***BCOR*-rearranged sarcoma**	BCOR+, SATB2+/–, cyclin D1+/–, TLE1+/–, CD99 +/– (~50%) [137]	*BCOR-CCNB3* fusion *BCOR-MAML3* fusion
Clear cell sarcoma of soft tissue (CCS)/ Melanoma of soft parts	Stains like melanoma (S100+, HMB45+, Melan-A+); HMB45/Melan-A usually negative in CCS of the GI tract	*EWSR1-ATF1* fusion (90%) *EWSR1-CREB1* fusion
Chordoma	CK+, EMA+, S100+, Brachyury+ [138]; loss of INI1 in subset of aggressive ones	
***CIC*-rearranged sarcoma**	WT1+, variable CD99 (diffuse in only 20% of cases), ETV4+ (recent marker; negative in other SRBCTs)	*CIC-DUX4* fusion *CIC-FOXO4* fusion (rare)
Dermatofibroma (DF)/benign fibrous histiocytoma (BFH)	CD34– (may show weak positivity at periphery of the lesion), factor XIIIa+, D2–40+	*PRKCB* and *PRKCD* rearrangement (usually not used for diagnosis)
Dermatofibrosarcoma protuberans (DFSP)	CD34+, factor XIIIa–, D2–40–	*COL1A1-PDGFB* fusion
Desmoplastic small round cell tumor (DSRCT)	Polyphenotypic marker expression: WT1+ (C-terminus), CK+, EMA+, desmin+ (dot-like), actin–,SYN–, CHR–, CD99 variable	*EWSR1-WT1* fusion
Epithelioid hemangioendothelioma (EHE)	Vascular markers (ERG/CD31/CD34)+, CAMTA1+, watch out – can stain for keratins (especially LMWCK)! [139]	*WWTR1-CAMTA1* fusion *YAP1-TFE3* fusion in distinct subset (with different morphology)
Epithelioid sarcoma	CK+ (strong), EMA+, CD34+ (50%) (CK and CD34 co-expression is nearly **unique** to epithelioid sarcoma plus epithelioid angiosarcomas and NUT carcinomas) Loss of INI1 (hSNF5/BAF47/SMARCB1) expression in >90% [140] Watch out – antibodies to N-terminus of ERG and FLI1 can cause diagnostic confusion for vascular tumor; use antibodies to C-terminus instead [141]	Biallelic *SMARCB1* inactivation (due to 22q11 deletion)
Ewing sarcoma/PNET	CD99+ (strong and diffuse membranous), NKX2.2+ (recent marker), focal CK+ in 20%, NE marker +/–, desmin very rarely+; FLI1/ERG positivity is NOT specific	Fusion of *EWSR1* (rarely *FUS*) with *ETS* family genes *(FLI*~90%, *ERG~5%*, *ETV1*, others).
Extraskeletal myxoid chondrosarcoma (EMC)	S100 and synaptophysin+/–, CK–, EMA–, INI1 loss in a subset of cases	*NR4A3* rearrangement
Fibromatosis	Nuclear β-catenin+ (positive in desmoid-type but not in superficial fibromatoses), actin+	*CTNNB1* or *APC* mutations in desmoid-type fibromatosis
Gastrointestinal stromal tumor (GIST)	c-kit (CD117) + (>95%, diffuse staining), DOG1 (>95%), CD34+ (70%), actin+ (30%) ~10% SDHB-IHC negative/loss (distinct subset; SDHB is a useful marker to screen for mutations involving SDH complex)	*KIT* or *PDGFRA* mutations; rarely *SDH*, *BRAF*, *NF1* mutations
Glomus tumor	SMA+, caldesmon+, desmin–, pericellular type IV collagen	*MIR143-NOTCH* fusion in subset
Granular cell tumor	SOX10+, S100+, CD68+, inhibin+, PAS+	
Inflammatory myofibroblastic tumor (IMT)	Actin* (80%), desmin (40%), ALK+ in *ALK*-rearranged cases (50%), watch out – can stain for keratins (especially LMWCK)! *actins have distinctive "tram track" pattern (peripheral cytoplasmic accentuation) in IMT, whereas actins in smooth muscle tumors have diffuse cytoplasmic distribution	*ALK* (50%), *ROS1*, *RET, NTRK* rearrangement
Kaposi sarcoma	Vascular markers (ERG/CD31/CD34)+, HHV8+	
Leiomyoma/leiomyosarcoma (LMS)	Desmin, actin, caldesmon+, most soft tissue (non-uterine) leiomyosarcomas are ER– (vs ER+ in uterine ones); watch out – can stain for keratins (especially LMWCK)!	
Liposarcoma, well-diff and de-diff	MDM2 and/or CDK4+; a small subset can be positive for STAT6 (potential diagnostic pitfall since de-diff can mimic SFT; staining due to proximity of *STAT6* to 12q14–15 amplicon and may also be amplified)	*12q14–15* amplification (*MDM2 and CDK4* are in this region)
Low-grade fibromyxoid sarcoma (Evans tumor)	MUC4+ (vs soft tissue perineurioma, intramuscular myxoma: MUC4–) [142]	*FUS-CREB3L2* fusion (>90%) *FUS-CREB3L1* fusion

Soft Tissue – 2

Selected Mesenchymal Tumors: Immunoprofiles at a Glance – 2		
Malignant rhabdoid tumor (renal, extrarenal, cranial = AT/RT)	Vast majority have loss of INI1/BAF47/SMARCB1 (due to 22q11 deletion); few INI1-retained cases have a loss of BRG1/SMARCA4. Peculiar polyphenotypic marker expression: (EMA+, CK variable, neurofilament+, SYN+/−). Consistently negative for tight junction marker Claudin-4 [143].	Biallelic *SMARCB1* inactivation
Mesenchymal chondrosarcoma	SOX9+, CD99+/−, S100 (+ in chondrocytes); rarely positive for muscle markers	*HEY1-NCOA2* fusion
Malignant peripheral nerve sheath tumor (MPNST)	S100/SOX10 < 50%+, mostly focal or patchy (vs strongly and diffusely positive in cellular schwannoma and spindle cell melanoma), H3K27me3 (histone 3) loss in ~50%. INI1 loss in ~50% of epithelioid MPNST, which is a distinct variant with strong S100/SOX10 expression.	*NF1* mutation PRC2 complex inactivation through *EED* or *SUZ12* loss
Myoepithelial tumor	Polyphenotypic: variable staining combination of epithelial markers (EMA/CK/p63), myoepithelial markers (S100/SOX10/GFAP), muscle markers (SMA/calponin). INI1 loss in subset of myoepithelial carcinomas.	*EWSR1* rearrangement in ~50% of cases
Nerve sheath tumors (neurofibroma, schwannoma)	SOX10+, S100+ (diffuse in schwannoma, patchy in neurofibroma) CD34+ favors neurofibroma Neurofilament stains entrapped axons within neurofibroma	
Nerve sheath tumor (perineurioma)	EMA+ (can be very focal and weak, exam under high magnification), CD34+, GLUT1+, S100/SOX10–	
Nodular fasciitis	SMA+ (tram track pattern), calponin+; desmin–, caldesmon– (vs. LMS: caldesmon +)	*MYH9-USP6* fusion
Ossifying fibromyxoid tumor (OFMT)	S100+ (~80%), desmin+ (50%), CK/SMA–	*PHF1* rearrangement in subset of cases
Perivascular clear cell tumor (PEComa)	Smooth muscle (SMA+, desmin+/−) and melanocytic differentiation (HMB45+, Melan-A+, MITF+, S100 −/+), cathepsin-K+ (not very specific, stains many other tumors), TFE3+ (subset) [144]	*TFE3* rearrangement (20%) or *TSC1/2* mutations [144, 145]
Pseudomyogenic hemangioendothelioma (Epithelioid sarcoma-like hemangioendothelioma)	CK AE1/AE3+, ERG+, FLI1+, FOSB+, CD31+/−, CD34– (unusual for vascular tumors) EMA−, INI1 retained	*SERPINE1-FOSB* fusion
Rhabdomyosarcoma (RMS)	Desmin+, myogenin+ (diffuse in alveolar, focal to patchy in embryonal and spindle cell/sclerosing), MyoD1+	Alveolar RMS– *PAX3-FOXO1* fusion (60%) *PAX7-FOXO1* fusion (20%)
Solitary fibrous tumor (SFT)	STAT6+, CD34+ (in contrast to SS), BCL2+ (rarely used)	*NAB2–STAT6 fusion*
Synovial sarcoma (SS)	CK/EMA +/− (strong in epithelial component, often very focal or neg in spindle/round cell component), usually CD99+, S100 focally + in 30%, almost all CD34–, TLE1+ (sensitive markers but not very specific), INI1 may show mosaic staining pattern (weak and partial loss) [146, 147]	*SS18-SSX1/2* fusion

Abbreviations: *SRBCT* small round blue cell tumors

References: [148, 149]

Central Nervous System

By Marina K Baine & Tejus A. Bale

Please see "Primer on Markers of Neuroglial Differentiation" in Chapter 1 for marker overview.

Central Nervous System (CNS) Tumors: Staining Profiles at a Glance	
Gliomas (astrocytoma, oligodendroglioma, ependymoma)	GFAP+, OLIG2+ (negative in ependymoma) See below for DDx of astrocytoma vs. oligodendroglioma.
Meningioma	EMA+ (but CK–), vimentin+, PR + (>50%), SSTR+, S100 variable • **Fibrous (fibroblastic) meningioma**: S100+ (80%), not as diffuse as in schwannomas • **Secretory meningioma**: CK+ (>50%), cytoplasmic inclusions are CEA+ and PAS+
Neuronal/glioneuronal/neurocytic neoplasms	Generally neuronal cells are SYN+, CHR+ (feature of dysplastic/abnormal neurons), Neurofilament+, MAP2+. NeuN works best for normal neurons, but it is not as reliable for tumors. • **Gangliocytoma:** SYN+, CHR+, neurofilament+, MAP2+ • **Ganglioglioma**: neurons (SYN/CHR/neurofilament/MAP2+, CD34+) and glia (GFAP+) • **Central neurocytoma:** SYN+, usually NeuN+, may be GFAP+, CHR– • **Dysembryoplastic neuroepithelial tumor (DNT):** glia (S100+ and OLIG2+) & neuronal (GFAP+, NeuN focally+, SYN–)

Embryonal neoplasms	All SYN+
	• **Medulloblastoma**: often both neuronal (at least focal SYN+ and NeuN+) and/or glial (GFAP+)
	  • *SHH-activated*: GAB1+, YAP1 +, cytoplasmic filamin A+
	  • *WNT-activated*: β-catenin + (nuclear), cytoplasmic filamin A+
	  • *Non-WNT, non-SHH*: all negative (GAB1, YAP1, filamin A, β-catenin [cytoplasmic])
	• **Embryonal tumor with multilayered rosettes (ETMR), C19MC-altered:**
	  • Encompasses ETANTR, ependymoblastoma, and most medulloepitheliomas
	  • Primitive neuroepithelial component, rosettes, and tubular structures: nestin and vimentin++
	  • Neuropil-like areas (including neoplastic neurons): SYN and NeuN+ & GFAP+/–
	  • LIN28A + in all ETMRs (but not specific)
	Reference: [150]

Atypical teratoid/rhabdoid tumor (AT/RT)	INI1 (SMARCB1/hSNF5/BAF47) – loss of nuclear expression (due to 22q11 deletion), sensitive and specific for AT/RT; Positive markers (polyphenotypic): EMA, neurofilament, GFAP, CK, vimentin, SMA, SYN
Pituitary neoplasms	Pituitary adenoma: SYN+, CHR+, PIT1+ (except corticotroph [Tpit+] and gonadotroph [SF1+]), hormones (ACTH, GH, PRL, etc.) Pituicytoma, granular cell tumors, spindle cell oncocytoma: TTF1+
Craniopharyngioma	Adamantinomatous: β-catenin + (nuclear, consistent with underlying mutations in *CTNNB1*) Papillary: BRAF V600E IHC + (consistent with underlying mutation)
Pineal neoplasms	SYN+, CRX+
Choroid plexus tumors	CK+ (mostly CK7), vimentin+, EMA–, variably S100+ (may be focal in carcinoma), transthyretin +

Reticulin in CNS at a glance	**Negative:** all gliomas (except PXA), meningioma, SFT. **Pericellular**: HPC, fibrosarcoma, schwannoma, PXA, lymphoma, sarcomatous areas of gliosarcoma. **Nested**: hemangioblastoma, pituitary (destruction of normal acinar architecture in adenoma compared to normal)

Abbreviations: *ETANTR* embryonal tumor with abundant neuropil and true rosettes, *SFT* solitary fibrous tumor, *PXA* pleomorphic xanthoastrocytoma

CNS: Differentials

Astrocytoma vs. Oligodendroglioma		
	Astrocytoma	**Oligodendroglioma**
GFAP	+	Mostly +
p53[1]	+ strong, diffuse in subset depending on grade [151]	Scattered
ATRX[1]	– (lost)	+ (retained)
1p/19q deletion[2]	–	+
IDH1 R132H[3]	+ (most cases)	+ (most cases)

1. ATRX loss and p53 expression may be used to distinguish astrocytoma (ATRX– and p53+) from oligodendroglioma (ATRX+ and p53 wild type/scattered).
2. 1p/19q deletion is specific for oligodendroglioma: cytogenetic/molecular detection of this deletion is necessary to render this diagnosis.
3. The antibody specific for the IDH1 variant p.R132H is utilized to detect IDH-mutated gliomas. This mutation accounts of approximately 90% of all IDH mutations. Molecular confirmation is necessary in cases with negative IHC, in order to detect the remaining 10% of IDH mutations. True IDH-wild-type gliomas have been shown to be clinically and molecularly similar to glioblastoma, WHO Grade IV, despite low-grade histology [152].

References: [150, 153]

DDx of Vascular Nested Tumors in CNS			
	Hemangioblastoma	**Metastatic renal cell carcinoma**	**Angiomatous meningioma**
Inhibin	+	–	–
CAIX	+ (because of VHL mutations/↑HIF-see RCC section on CAIX)	+	–
PAX8	–	+	–
EMA	–	+	+
SSTR2A	–	–	+
Reticulin (nested and pericellular)	+	–	–

References: [154–160]

DDx of Dural-Based Spindle Cell Tumors			
	Meningioma (fibrous)	**Solitary fibrous tumor**	**Melanocytoma**
EMA, PR, SSTR2A	+	–	–
STAT6, CD34, BCL2	–	+	–
Melanocytic markers	–	–	+

DDx of Myxoid and Chondromyxoid Lesions of the CNS/Coverings					
	Chordoma	**Myxoid chondrosarcoma**	**Myxopapillary ependymoma**	**Chordoid meningioma**	**Chordoid glioma**
Cytokeratin	+	–	–	–	+/–
EMA	+	–	– (most)	+	+/–
GFAP	–	–	+	–	+
S100	+	+/–	+/–	–	+ (most)
D2–40	–	+	+/–	+/–	–
Brachyury	+	–	–	–	–
Typical location	Sacrum, clivus (extradural)	Bone and soft tissue	Filum terminale (intradural)	Dural	Always the third ventricle

Also exclude metastatic mucinous adenocarcinoma! References: [150, 161–163]

Gynecologic Tract

Gynecologic Tract: Key Markers at a Glance	
Marker [localization]	Applications
ER/PR [nuclear]	ER/PR expression is highest in endometrioid CA (>90% of cases) and is lower in serous CA (50% of cases). HPV-related cervical adenoCAs are negative, which may be used to differentiate cervical from endometrial primary.
WT1 [nuclear]	WT1 (Wilms tumor 1 protein) is expressed in serous CA of the ovary and serves as a good marker for the ovary as a source of a CA of unknown primary. Note that WT1 is also a marker of mesothelial cells and mesotheliomas. Other markers may be needed to distinguish WT1+ serious CA (Claudin-4/PAX8+) vs mesothelioma (Claudin-4/PAX8–). Watch out – some peritoneal mesotheliomas can be PAX8-positive!
PAX8 [nuclear]	Pan-Mullerian (endometrial, ovarian, and endocervical) marker. Also expressed in renal and thyroid CAs. Useful in the differential of ER-/PR-positive tumors, e.g., breast (PAX8–) vs. GYN tract (PAX8+).
p53 [nuclear]	p53 is a tumor suppressor protein. The wild-type (i.e., normal) staining pattern is scattered variable, often weak nuclear positivity (look at surrounding normal tissue). Abnormal pattern of staining may be one of the following: (1) diffuse and strong nuclear staining (due to defective protein degradation of mutated p53); (2) complete loss of staining ("Null" phenotype, need internal control!); (3) cytoplasmic staining with variable nuclear staining. Abnormal staining pattern usually (though not 100%) indicates underlying mutation and is seen in high-grade serous CA of the ovary and endometrium, serous tubal intraepithelial carcinoma (STIC), and a subset of high-grade endometrioid CAs [164].
p16 [nuclear and cytoplasmic]	Positivity of p16 is defined as block-like (full thickness), strong, and diffuse nuclear and cytoplasmic staining. Anything short of that (patchy/weak staining) is negative! Similar to p53, when describing result of p16 in your report, it is best to specify intensity and extent of staining (i.e., weak vs strong, diffuse vs patchy) rather than saying generically "positive" or "negative." In the GYN tract p16 is used in two different settings: One use of p16 is as a surrogate marker of HR-HPV infection (as in other organs). As such, p16 is used to distinguish HSIL (p16+) from atypical squamous metaplasia (p16–), as well as HPV-associated cervical adenoCA (p16+) from endometrioid CA (p16– or focal). Note that the majority of LSILs are associated with HR-HPV, and some have block-like p16 (reflecting HPV integration). However, most LSILs have only weak and/or focal p16, which can aid in distinguishing them from HSIL. Second use of p16 is unrelated to HPV. Analogously to p53, diffuse overexpression of p16 is a feature of high-grade serous CA (p16 strong, diffuse) and may be used in the DDx from endometrioid CA (p16 negative or focal).
Napsin A [cytoplasmic], **HNF-1β** [nuclear]	In the GYN tract, Napsin A and HNF-1β are used to identify clear cell CA (ovarian and endometrial) and are of particular utility in distinguishing this entity from high-grade (FIGO grade 3, nuclear grade 3) endometrioid CA and/or serous CA, which are negative for these markers.
Abbreviations: *HR-HPV* high-risk HPV, *SIL* squamous intraepithelial lesion, *LSIL* low-grade SIL, *HSIL* high-grade SIL	

Gynecologic Tract: Immunoprofiles at a Glance (all carcinomas of GYN tract are CK7+ and CK20–)	
Diagnosis	Immunoprofile
Uterus, endometrioid carcinoma	PAX8+, ER+/PR+(>90%), p16 (negative or patchy), p53 abnormal pattern in subset of grade 3
Uterus, serous carcinoma	PAX8+, ER/PR (50%+), p53 (diffuse – in nearly every cell or null – completely absent), p16 diffuse/strong+, very high Ki67 Unlike ovarian serous CA, WT1 usually (–) in serous CA of endometrial origin.
Ovary, serous carcinoma	PAX8+, WT1+, ER/PR (50%+), p53 abnormal pattern in high-grade ones. [vs. breast ca: also ER/PR+ but PAX8–, WT1–, GCDFP-15 (2/3+)] and [vs. mesothelioma: also WT1+ but PAX8– (but not always!), calretinin+] References: [165–167]
Ovary, mucinous neoplasms	PAX8+ (~50%, often weak and patchy), CK7+, CK20+/–, CDX2+/– [if PAX8 is negative, distinction from GI tract metastasis depends a lot on other parameters rather than IHC, such as size, unilateral vs bilateral and surface involvement, etc.]
Ovarian or endometrial, clear cell carcinoma	PAX8+, ER/PR/WT1 usually–, p53 wild type, HNF-1β+, Napsin A+
Ovary, small cell carcinoma, hypercalcemic type (SCCOHT)	CK+, WT1/calretinin/EMA+/–, loss of BRG1 (SMARCA4) – sensitive and quite specific for SCCOHT (also seen in dedifferentiated endometrioid adenoCA) Reference: [168]
Ovary, sex cord-stromal tumors (granulosa cell tumor, Sertoli/Leydig cell tumors, Thecoma, Fibroma)	Inhibin+, calretinin+, SF1+, FOXL2+, Melan-A (A103) + (steroid cell tumors), CD10+ Epithelial markers: CK usually (–) but can be positive, EMA consistently (–). Therefore EMA is used more often than CK to exclude epithelial tumors. References: [169–172]
Cervix, adenocarcinoma	HR-HPV DNA and RNA ISH+ and p16+ (diffuse), ER/PR-negative or weak
Endometriosis	PAX8+, ER/PR+ (both glands and stroma), CD10+ (stroma)

Ovarian Germ Cell Tumors at a Glance								
	SALL4	**PLAP**	**c-kit**	**OCT4**	**D2–40**	**Cam 5.2, AE1/AE3**	**EMA**	**Others**
Dysgerminoma (counterpart to testicular seminoma)	+	+	+	+	+	–/F+ (dot-like)	–	
Embryonal carcinoma	+	+	–	+	–/+	+	–	CD30+, SOX2+
Yolk sac tumor	+	+	–	–	–	+	–	AFP+ (not specific), Glypican-3+
Choriocarcinoma	+/–	+/–	–	–	–	+	+ (50%)	GATA3, βHCG/hPL (ST), p63/p40 (CT), HLA-G/hPL (IT)
Abbreviations: *ST* syncytiotrophoblast, *CT* cytotrophoblast, *IT* intermediate trophoblast								

Gynecologic Tract: Differentials

DDx of Dysplastic vs. Reactive Squamous Intraepithelial Lesions (SIL) of the Cervix		
	High-grade squamous intraepithelial lesion (HSIL)	**Atypical immature metaplasia (and normal squamous mucosa)**
p16 and HPV ISH	+	–
Ki67	+++ (extending up to the surface)	rare + (parabasal cells only or only rare cells above the parabasal area)
P16 positivity (block-like strong and diffuse nuclear and cytoplasmic stain) indicates that HR-HPV is present. Neither presence of block-like p16 nor HPV ISH can be used to distinguish LSIL from HSIL: while all HSILs are caused by HR-HPV, 80% of LSILs are also caused by high-risk (rather than low risk) HPV and 30% have block-like p16. Same panel may be used to distinguish adenoCA in situ (p16+/HPV ISH+, high Ki67) from benign mimics, such as microglandular hyperplasia and tubal metaplasia (p16 negative or patchy, low Ki67). Abbreviations: *HR-HPV* high-risk HPV, *LSIL* low-grade squamous intraepithelial lesion, *HSIL* high-grade squamous intraepithelial lesion		

DDx of Uterine Carcinomas			
	Endometrioid carcinoma	**Serous carcinoma**	**Clear cell carcinoma**
ER/PR	+ (>90%)	+/– (50%)	–/+
Ki67	Variable based on grade	High	Intermediate (variable, can be low)
p53	Wild-type pattern in most cases	Abnormal pattern	Wild-type pattern
p16	– or patchy	+ (diffuse and strong)	– or patchy
HNF-1β, Napsin A	– (rare Napsin A+)	–	+
WT1	–	+ in the ovary, but –/+ in endometrium	–
Note that in serous CA, expression of p16 is unrelated to HPV. Here overexpression of p16 is used analogously to p53, in that it is an indicator of a highly proliferative malignancy. PAX8 is positive in all of the above CAs. P53 may show abnormal staining pattern in about 15% of high-grade endometrioid CAs. References: [173–176]			

Endometrial vs. Endocervical Adenocarcinoma		
	Endometrioid carcinoma	**Endocervical adenocarcinoma, usual type**
p16 and HPV in situ hybridization	– or patchy	+ (diffuse and strong)
ER/PR	+	– (or low)
CEA (low specificity)	–	+
Vimentin (low specificity)	+	–
PAX8 is positive in both		

DDx of Serous Tubal Intraepithelial Carcinoma (STIC)		
	Serous tubal intraepithelial carcinoma (STIC)	**Stratified benign tubal epithelium**
p53	Abnormal pattern	Wild-type pattern
Ki67	High	Low
Reference: [177]		

DDx of Endocervical Adenocarcinoma Types					
	Usual type	**Gastric type***	**Clear cell type**	**Endometrioid**	**Serous**
ER/PR	–	–	–	+	–
p16	+ (strong and diffuse)	– or patchy	+ (focal to diffuse)	– or patchy	+ (strong and diffuse)
p53 pattern	wild type	abnormal (~50%)	most wild type	wild type	abnormal
HNF-1β, Napsin A	–	–	+	–	–
* Other markers of gastric-type cervical adenoCA include MUC6 and HIK-1083. References: [178, 179]					

DDx of Ovarian Tumors with Tubular/Trabecular/Cribriform Pattern				
	Sertoli cell tumor /granulosa cell tumor	**Endometrioid carcinoma**	**Carcinoid tumor**	**Struma ovarii**
Sex cord-stromal markers (FOXL2, SF1, inhibin, calretinin, Melan-A)	+ (variable)	–	–	–
EMA	–	+	variable	+
Neuroendocrine markers (SYN, CHR)	– (variable)	–	+	–
TTF-1, PAX8, Thyroglobulin	–	– (PAX8+)	–	+
Reference: [172]				

Gynecologic Tract: Differentials – 2

DDx of Pagetoid Proliferations in the Vulva

	Vulvar Paget disease (Primary)	Urothelial carcinoma (secondary Paget disease)	Anorectal adenocarcinoma (secondary Paget disease)
GATA3	+	+	– (most)
GCDFP-15 (BRST2)	+	–	–
p63/p40, HMWCK	–	+	–
CK7	+	+	–/+
CK20	–	+	+
CDX2, SATB2	–	– (most)	+
SOX10 (other melanocytic markers)	–	–	–

In the vulva, these are more commonly primary than secondary (extension from urothelial or anorectal CA). Also see Paget disease IHC in breast and skin sections.
As at other sites, DDx also includes melanoma in situ (melanocytic marker +) and SqCC in situ (p63/p40+, HMWCK+).

DDx of Uterine Spindle Cell Neoplasms

	Endometrial Stromal Tumors				Uterine smooth muscle tumors (leiomyoma and leiomyosarcoma)	Uterine tumor resembling ovarian sex cord tumors (UTROSCT)
	LGESS[3]	HGESS with *YWHAE* rearrangement		HGESS with *BCOR* alterations[4]		
		LG component	HG component			
SMA and desmin	–/+	–	–	–/+ (<50%)	+	+/–
h-caldesmon[1]	– (rare +)	–	–	– (rare +)	+	–
CD10	+	+	–	+	–/+	+/– (focal)
ER/PR	+	+	–	–/+	+	+
BCOR	–	–	+ (100%)	+ (50%)	– (rare +)	–
Cyclin D1	–	–	+	+	–/+ (rare + in LMS)	–
Sex cord-stromal markers (FOXL2, SF1, inhibin, calretinin)	– (rare F+)	–	–	–	–	+ (calretinin is most sensitive)
Others	Nuclear β-catenin (>50%), IFITM1 +[5]	c-kit – DOG1 –	c-kit + DOG1 –			
Molecular findings	t(7;17) *JAZF1-SUZ12 (JJAZ1)* fusion, other. See molecular chapter	t(10;17) *YWHAE-NUTM2 (FAM22)* fusion[2]		t(X;22) *ZC3H7B-BCOR* fusion, *BCOR* ITD	Variable	Variable

1. Caldesmon is the most sensitive and specific marker for smooth muscle differentiation.
2. *YWHAE-NUTM2* fusion is specific to HGESS among GYN tumors but also occurs in clear cell sarcoma of the kidney and round cell sarcomas of the soft tissue.
3. Staining with smooth muscle and sex cord-stromal markers in LGESS is variable and depends on the presence and extent of corresponding variant histologic differentiation, which can be a diagnostic pitfall.
4. *BCOR*-rearranged ESS is a morphologic mimicker of myxoid leiomyosarcoma. Above IHC markers and/or the characteristic chromosomal translocation are necessary to make the distinction.
5. IFITM1 is a new marker that has recently been shown to be superior to CD10 in DDx of LGESS from other (non-ESS) uterine tumors [180].

Other entities on the DDx include IMT (SMA+, desmin+, and ALK1+/–) and PEComas (+ melanocytic [HMB45, Melan-A, MITF] and smooth muscle [SMA, desmin] markers, with subset TFE3+); see other sections for complete immunoprofiles for these.
Abbreviations: *HGESS* high-grade endometrial stromal sarcoma, *LGESS* low-grade endometrial stromal sarcoma, *LMS* leiomyosarcoma, *ITD* internal tandem duplication
References: [181–186]

DDx of Gestational Trophoblastic Tumors[1]

	Tumors of implantation site IT		Tumors of chorionic-type IT		Tumor of mixed-type IT
	Exaggerated placental site	**Placental site trophoblastic tumor**	**Placental site nodule**	**Epithelioid trophoblastic tumor**	**Choriocarcinoma**
hPL, Mel-CAM (CD146)	+++		–/+		+ (IT and ST)
p63/p40	–		+++		+ (CT)
Ki67	<1%	>8%	<8%	>10%	>50%
Others			Cyclin E–	Cyclin E++	SALL4+, βHCG+ (ST)

1. All types of trophoblastic cells (ST, CT, IT) are + for LMWCKs and GATA3 (pan-trophoblast markers).
Abbreviations: *IT* intermediate trophoblast, *ST* syncytiotrophoblast, *CT* cytotrophoblast
Reference: [187]

DDx of Hydatidiform Moles[1]

	Complete mole	Partial mole	Hydropic abortus
Cytogenetics	XX or XY (both paternal)	XXY or XXX (2:1 paternal:maternal)	Normal or variable
p57(KIP2)[2] – paternally imprinted gene, transcribed entirely from a maternal allele	Loss of expression	Intact expression	Intact expression

1. Hydatidiform moles are lesions of trophoblastic tissue (CK+, Inhibin+, HLA-G+, GATA3+).
2. *p57* is a paternally imprinted gene, which is normally transcribed entirely from a maternal allele. Complete moles contain paternal DNA only and therefore show the loss of expression of p57 (in villous stroma and villous cytotrophoblast), whereas maternal tissue (decidua) and intermediate trophoblast (IT) islands retain expression and serve as internal positive controls (retained expression in IT is surprising because these are fetal cells, but this is proposed to be due to incomplete imprinting in this cell type). In contrast, p57 expression is intact in partial moles and in a hydropic abortus.
References: [188, 189]

Skin

By Youran Zou
(Prior edition by Janis Taube, Natasha Rekhtman, Justin A. Bishop)

For overview of melanocytic markers, refer to "Primer on Markers of Melanocytic Differentiation" in Chapter 1.

DDx of Melanocytic Lesions		
	Melanoma	**Intradermal nevus**
HMB45*	Superficial and deep cells	Superficial cells only (deep/mature nevocytes are negative)
Ki67	Increased	– (rare cells +)

* HMB45 is a marker of immature melanocytes. In benign nevus, there is diminution of HMB45 from the surface (immature, HMB45-positive cells) toward the base (mature, HMB45-negative cells). In contrast, no maturation is seen in melanoma (both superficial and deep cells are HMB45+).
S100 and Melan-A react with both immature and mature melanocytes. Therefore, these markers are uniformly positive in both nevus and melanoma and cannot be used to differentiate the two lesions.

Reference: [190]

DDx of Cutaneous Storiform Spindle Cell Lesions		
	Dermatofibroma (DF)	**Dermatofibrosarcoma protuberans (DFSP)**
Factor XIIIa	+	–
CD34	– (may show weak positivity at periphery)	+ (usually strong and diffuse)
D2–40 (podoplanin)	+	–

Reference: [191]

DDx of Cutaneous Basaloid Lesions		
	Basal cell carcinoma (BCC)	**Trichoepithelioma/trichoblastoma**
CK20	– (no scattered Merkel cells)	+ (scattered Merkel cells)
CD10	+ (usually in the tumor cells)	+ (usually in the stroma)
BCL2	+ (strong and diffuse)	+ (periphery, not diffuse)

Reference: [192]

DDx of Cutaneous High-Grade Neuroendocrine Carcinomas		
	Merkel cell carcinoma (MCC)	**Metastatic small cell carcinoma**
CK20	+ (dot-like perinuclear pattern)	–
TTF-1	–	+ (most)
Neurofilament	+ (100%)	–
NE markers (SYN, CHR, CD56, INSM1)	+	+
Cytokeratins (AE1/AE3, Cam5.2)	+	+
Merkel cell polyomavirus (CM2B4)*	+	–
RB	+ (retained)**	– (lost)

* Viral antigen can be detected by IHC [193].
** There are reports of rare MC polyoma virus-negative MCCs, which also harbor mutated/lost RB; those probably represent lung-type small cell carcinomas of the skin [194].

DDx of Pleomorphic Spindle Cell Neoplasms of the Skin				
	AFX*	**True sarcoma (e.g., PDS/UPS, LMS)**	**Spindle cell or sarcomatoid SqCC**	**Sarcomatoid or desmoplastic melanoma**
HMWCK, p63/p40	–	–	+	–
Melanoma markers (especially S100 and SOX10)	–	–	–	+
Actin	+/–	+ in LMS; –/+ in PDS/UPS	–/+	–
Desmin	–	+ in LMS; –/+ in PDS/UPS	–	–

* AFX and PDS/UPS usually occur in sun-exposed areas, especially of the head and neck. Distinction relies on evaluation of infiltrative border, tumor necrosis, lymphovascular invasion, perineural invasion, and/or involvement of subcutaneous tissue, not IHC.
Abbreviations: *AFX* atypical fibroxanthoma, *LMS* leiomyosarcoma, *SqCC* squamous cell carcinoma, *PDS* pleomorphic dermal sarcoma, *UPS* undifferentiated pleomorphic sarcoma

References: [195–197]

Skin – 2

DDx of Pagetoid Proliferations in the Skin			
Squamous cell carcinoma in situ (Bowen disease)	**Melanoma in situ**	**Sebaceous carcinoma**	**Paget disease**
HMWCK (CK903 or CK5/6)+, p63/ p40+	Melanoma markers: S100/ SOX10+ (may also be positive in mammary Paget disease), Melan-A+, HMB45+, MITF+	BerEP4+, EMA+, CK7+, adipophilin+ Mismatch repair protein loss in a subset of tumors	LMWCK (Cam5.2 or CK7) +, see table below for further workup

DDx of Different Types of Paget Disease				
	GCDFP-15[1]	**HER2**	**CK7**	**CK20, CDX2**
Mammary Paget disease - MPD (almost always secondary, involving the nipple and surrounding the skin)	+/– (~50%+)	+	+	–
Primary extra-mammary Paget disease - EMPD (no underlying carcinoma)	+/– (~30%+)	–/+	+	–
Secondary EMPD (with an underlying carcinoma, such as rectal, urothelial, or sebaceous)	–	–	variable[2]	variable[2]

Note: For EMPD, common sites include vulvar, perianal, and scrotal skin, axilla, and the eyelid. Mammary Paget disease nearly always has an underlying DCIS; invasive carcinoma is present in ~50% of cases. Similarly, anal Paget is usually secondary, whereas Paget disease of the vulva or scrotum is usually primary.

1. GCDFP-15 is a marker of breast epithelium as well as apocrine cells in general; hence both mammary and extra-mammary Paget may be (+). A new breast marker, GATA3, is positive in primary EMPD but also in secondary EMPD of urothelial origin.
2. CK7/CK20 pattern of Paget cells reflects an underlying neoplasm: urothelial CA (CK7+/CK20+) vs. anorectal CA (CK7 variable/CK20+/CDX2+) vs. cervical CA (CK7+/CK20–).

Also see Paget disease IHC in breast and GYN sections. References: [9, 198, 199]

Chapter 3. Immunostains: Hematopoietic System

Update by Xiaojun Wu & Zenggang Pan

(Prior edition by Amy Duffield, Justin A. Bishop, Tara Miller, Ross Miller, Natasha Rekhtman)

Hematopoietic Markers at a Glance	
Pan-hematopoietic	CD45/LCA*
Pan-B cell	CD19, CD20, *CD22*, CD79a, PAX5, BOB1, OCT2. CD79a is the widest pan-B-cell marker (see diagram on B cell development) **Note: CD20 may be lost after treatment with rituximab (anti-CD20 antibody)**
Naïve B cell	CD5+/–, CD23+ (origin of a subset of CLL) CD19, CD20, *CD22*, CD79a, PAX5 Note: CD5+ B cells can be increased in children and patients with rheumatologic disorders, non-clonal
Mantle cell	Normal mantle cells: CD5–/+, CD23+; neoplastic mantle cells (MCL): CD5+, CD23– CD19, CD20, *CD22*, CD79a, PAX5, BCL2
Germinal center (GC) cell	CD10, BCL6, GCET1, HGAL, LMO2 (GC cells are the origin of FL, many DLBCLs, and Burkitt lymphoma) CD19, CD20, *CD22*, CD79a, PAX5 Note: Normal GC B cells are BCL2– (reactivity seen in GC due to scattered T cells). In follicular lymphomas, BCL2 is aberrantly expressed in GC B cells due to *BCL2-IGH* translocation t(14;18)
Plasma cell	CD138, Ig κ or λ light chain, CD38 (bright), MUM1, CD79a. Normal κ/λ ratio is 2–3:1 Normal plasma cells are often positive for CD19 and CD43 but negative for CD45 and CD56 Neoplastic plasma cells may aberrantly express CD20, CD56, CD117, cyclin D1, and CD10 **Note: CD38 may be lost after treatment with daratumumab (anti-CD38 antibody)**
Follicular dendritic cell	CD21, CD23, CD35, clusterin, D2-40, fascin, CXCL13
Pan-T cell	CD2, CD3, CD5, CD7, CD43; mature T cells express either CD4 or CD8 (see diagram on T cell development)
Follicular T helper cell	CD3, CD4, CD57, PD1, BCL6, CD10, CXCL13
T-large granular lymphocyte	CD3, CD8, CD57, CD27, CD28, cytotoxic protein (perforin, granzyme B, TIA-1)
T-regulatory cell	CD3, CD4, CD25, FOXP3
Gamma-delta T cell	CD3+, CD4–, CD8–, CD5–
NK cell	Positive for *cytoplasmic CD3ε* (origin of NK/T-cell lymphoma, nasal type), CD2, CD56, and cytotoxic proteins (perforin, granzyme B, TIA-1); negative for pan-T cell markers (surface CD3, CD5)
Activated B or T cell	CD30, CD25, *CD27*, *CD28*, *HLA-DR* Note: Large CD30-positive cells may be immunoblasts (i.e., activated B cells) and are not necessarily Reed-Sternberg cells **Note: CD30 can be lost after treatment with brentuximab (anti-CD30 antibody)**
Myeloid cell	MPO, CD43, *CD11b*, CD13, CD15, CD33; CD34 and/or CD117 are positive in myeloid progenitor cells (myeloblasts)
Neutrophil	CD10, CD16, CD15, *CD11b*, CD13
Eosinophil	Positive for dim CD13, CD15, CD33, bright *HLA-DR*; negative for CD10 and CD16
Basophil	Positive for dim CD45 (blast gate), dim CD13, CD33, CD38, CD123; negative for *HLA-DR*, CD10, *CD16*
Plasmacytoid dendritic cell	Positive for BDCA2, CD2AP, CD4, CD123, CD68; neoplastic PDCs positive for CD56, TCL1, TdT; negative for lysozyme, MPO
Monocyte (histiocyte)	CD68, CD163, lysozyme, CD4, *CD11b*, *CD11c*, *CD14*, CD15, *CD64*, *HLA-DR*, CD33
Megakaryocyte	Factor VIII, CD41 (GPIIb), CD42b (GP1b alpha), CD61 (GPIIIa)
Erythrocyte	Hemoglobin A, spectrin, glycophorin (CD235a), transferrin (CD71), E-cadherin
Mast cell	CD117, calretinin, mast cell tryptase, Giemsa (special stain). Neoplastic mast cells are commonly CD2+, CD25+, and CD123+

*Several hematopoietic entities are commonly CD45 negative: R-S cells in classic Hodgkin lymphoma, B and T lymphoblastic lymphoma (variable), ALCL (variable), plasma cell neoplasms, follicular dendritic cell sarcoma, and granulocytic sarcoma (variable). If a hematopoietic neoplasm is suspected in CD45-negative cases, CD43 should be applied, as it is also positive in many hematologic neoplasms.

The *italicized markers* may only be available by flow cytometry

Abbreviations: *ALCL* anaplastic large cell lymphoma, *CLL* chronic lymphocytic leukemia, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *MCL* mantle cell lymphoma

Markers for Identification of Blasts							
	CD34	**TdT**	**CD10**	**CD117**	**HLA-DR**	**Lineage-specific markers[3]**	**sIg**
Myeloblast[1]	+/–	– (rarely + in M0)	– (rarely +)	+/–	+/–	Myeloperoxidase (MPO)	–
Monoblast	–/+ (30%)	–	–	+/–	+	≥ 2 of the following: nonspecific esterase, CD11c, CD14, CD64, lysozyme	–
B lymphoblast[2]	+ (may be –)	+	+ (rarely –)	–	+ (rarely –)	Strong CD19 with ≥1 of CD79a, cCD22, CD10 or weak CD19 with ≥2 of CD79a, cCD22, CD10	– (or dim +)
T lymphoblast	+/–	+	+/–	– (very rarely +)	– (rarely +)	Strong cytoplasmic or surface CD3 (often cytoplasmic only)	–

1. CD34 and/or CD117 are typically present in acute myeloid leukemia (AML), although some forms of AML may be entirely negative for CD34 and CD117, notably AML with monocytic differentiation. Most AMLs express HLA-DR, but some myeloid leukemias (i.e., acute promyelocytic leukemia and AML with *NPM1* and/or *FLT3* mutations) are HLA-DR negative.
2. DDx: Burkitt lymphoma has a mature B-cell phenotype (sIg+ i.e. κ or λ light chain+, CD20+, CD10+, BCL2–) and is negative for blast markers (CD34, TdT).
3. See Chapter 9 for lineage-specific markers.

Reference: [1]

Cytochemistry for Identification of Blasts and Burkitt lymphoma*		
Type of blast	**Stain**	**Comment**
Myeloblast	Myeloperoxidase (MPO), Sudan Black B (SBB) Chloroacetate esterase (Leder stain)	Flow cytometry is more sensitive than cytochemistry in the detection of MPO
Monoblast	Nonspecific esterase (α-naphthyl acetate and butyrate)	Reactivity is inhibited by sodium fluoride (NaF)
Lymphoblast, erythroblast, megakaryoblast	PAS	Erythroblasts show chunky globular staining Burkitt lymphoma is PAS-negative
Burkitt lymphoma (mature B-cell phenotype)	Oil Red O	Oil Red O highlights lipid vacuoles

*These methods have largely fallen out of use due to advance in flow cytometry and IHC.

Reference: [1]

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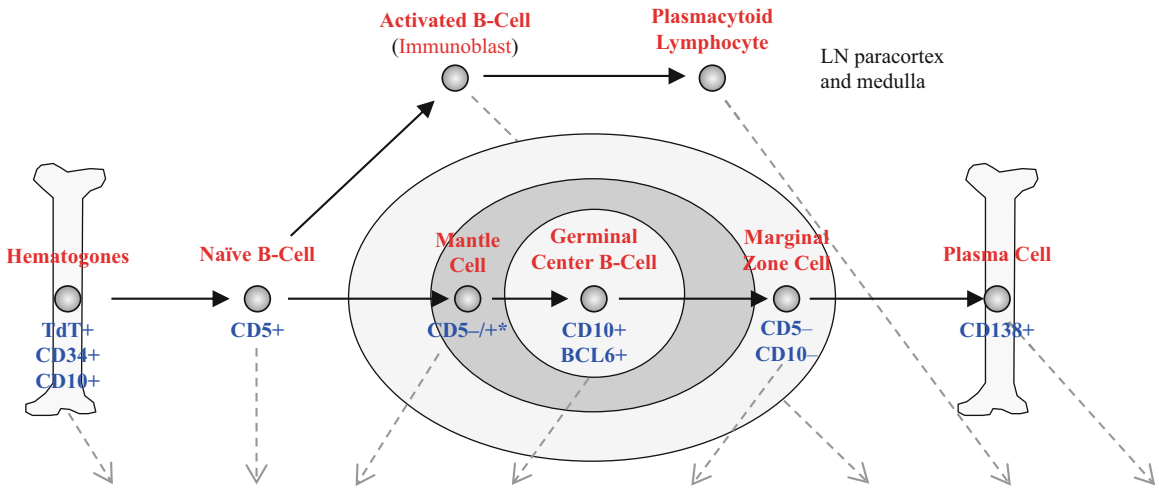
N. Rekhtman et al., *Quick Reference Handbook for Surgical Pathologists*, https://doi.org/10.1007/978-3-319-97508-5_3

Stages of Lymphocyte Differentiation and Corresponding Lymphomas

Normal stages of B cell development and corresponding lymphomas



Cell of origin	Progenitor/ precursor B cell	Naïve B cell	Mantle cell*	Germinal center (centroblast → centrocyte)	Marginal zone cell (memory B cell)	Activated B cell (immunoblast)	Plasmacytoid lymphocyte	Plasma cell
Corresponding lymphoma/ leukemia	**B-ALL**	**CLL/SLL** (some) [5+10–23+]	**MCL*** [5+10–23–]	**FL, Burkitt**, many **DLBCL** (GCB type), most **DHL** [5–10+BCL6+]	**MZL** [5–10–]	some **DLBCL** (non-GCB type)	**LPL**	**Myeloma**
CD45	–/dim	+	+	+	+	+	+	–
TdT	+	–	–	–	–	–	–	–
CD34	+/–	–	–	–	–	–	–	–
CD19, PAX5	+	+	+	+	+	+	+/–	–
CD20	–/dim	+, dim	+	+	+	+	+/–	–/+
CD79a	+	+	+	+	+	+	+	+
CD5	–	+	+	–	–	–	–	–
CD23	–	+	–	–	–	–	–	–
CD10	–/+	–	–	+	–	–	–	–
BCL6	–	–	–	+	–	–	–	–
CD138	–	–	–	–	–	–	+	+
cIg	–/μ chains	–/weak +					+ (IgM)	+ (IgG, A)
sIg	–	+ (IgD, M)		+ (IgM, G, A)			+/– (IgM)	–

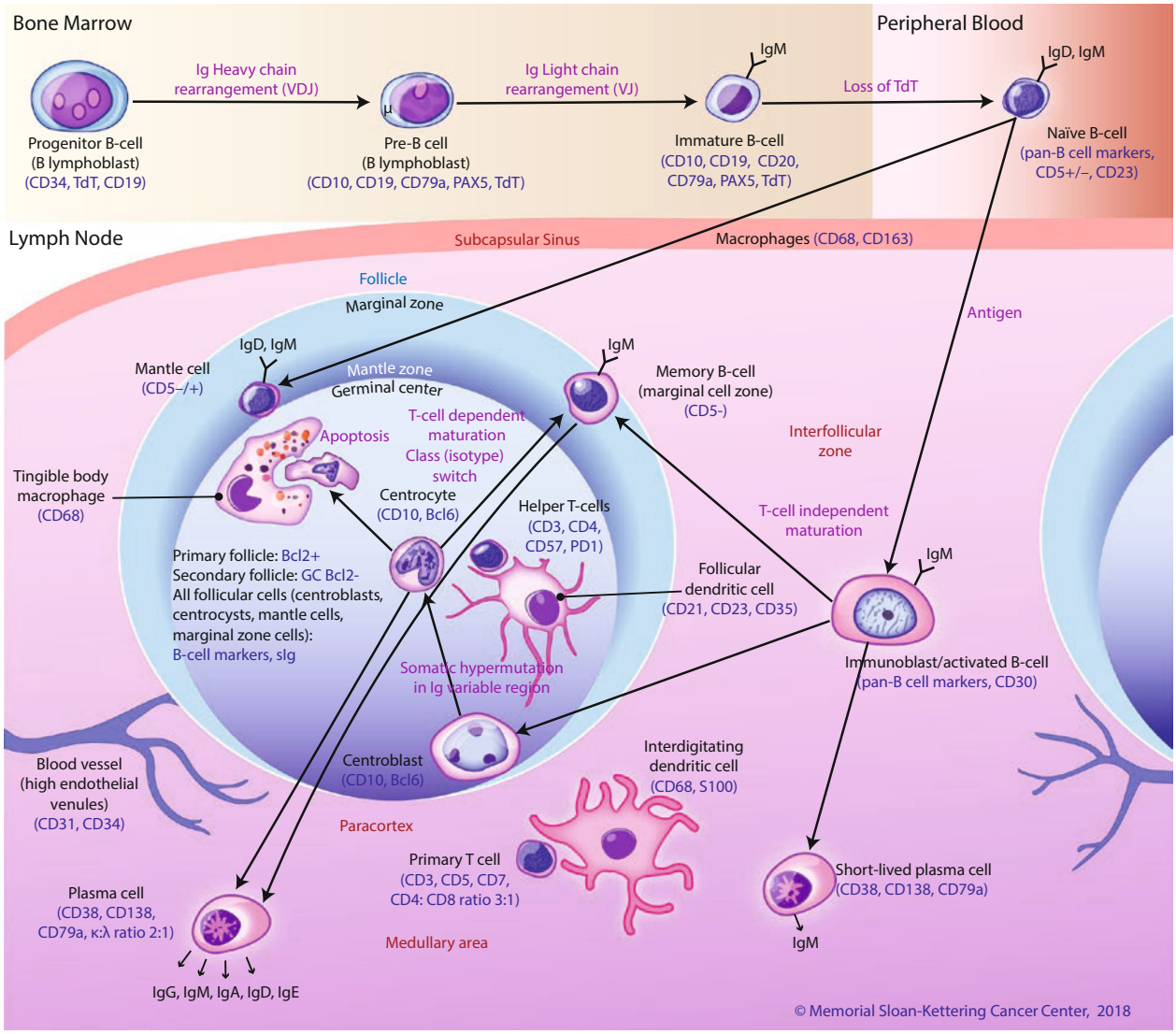
The table refers to staining of lymphomas/leukemias, which mostly (but not always) corresponds to that of normal counterparts. Some additional markers represent aberrant expression, not seen in normal cells (e.g. Cyclin D1 in MCL, BCL2 in FL).
* CD5/CD23 profile of normal vs neoplastic mantle cells differs: normal (CD5–/+, CD23+) vs MCL (CD5+, CD23–).
Abbreviations: *B-ALL* B-cell acute lymphoblastic lymphoma/leukemia, *CLL/SLL* chronic lymphocytic leukemia/small lymphocytic lymphoma, *DHL* double-hit lymphoma, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *MCL* mantle cell lymphoma, *MZL* marginal zone lymphoma, *LPL* lymphoplasmacytic lymphoma

Normal stages of T-cell development and corresponding lymphomas

	Thymus				Peripheral blood & organs (such as skin)
Cell of origin	**Pro-T cell**	**Pre-T cell**	**Cortical thymocyte**	**Medullary T cell**	**Mature T cell**
Corresponding lymphoma	**T lymphoblastic lymphoma**				**Peripheral T-cell lymphoma**
CD45	dim/+	dim/+	dim/+	dim/+	+
TdT[1]	+	+	+/–	–/+	–
CD34	–/+	+/–	–	–	–
CD1a	–	–	+	–	–
CD2	–	+	+	+	+[2]
CD5, CD7	+	+	+	+	+[2]
Cytoplasmic CD3	+	+	+	+	+
Surface CD3	–	–	–	+	+[2]
CD4, CD8	Double negative	Double negative	Double positive	Single positive	Single positive[2]

1. CD99 expression is often seen in TdT-positive neoplasms; an immunostain for CD99 may aid in the identification of T lymphoblasts.
2. Mature T cells are positive for either CD4 or CD8 (except gamma/delta T cells which are double-negative). In contrast, mature T-cell lymphomas may be aberrantly double-positive or double-negative for CD4/CD8, and commonly loose one or more pan-T cell markers (CD2, cCD3, CD5, CD7).

Normal Stages of B-Cell Development, Immunostains, and Corresponding Lymphomas



Update by Natasha Rekhtman and Xiaojun Wu. Original diagram by Tara Miller, Ross Miller, Amy Duffield, and Natasha Rekhtman.

References: [1–4]

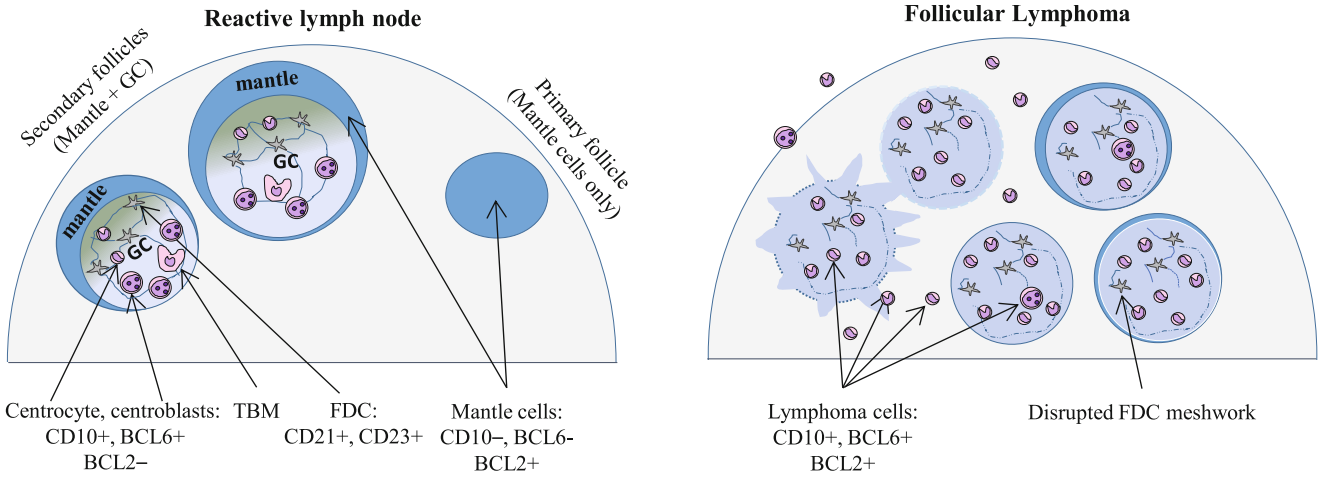
B-cell lymphomas (typical positive staining pattern)	**"Pre"-germinal center**	**Germinal center**	**"Post"-germinal center**
All (+) for CD45 except some lymphoblastic leukemia/ lymphomas, classic Hodgkin lymphomas, and plasma cell neoplasms All (+) for pan-B-cell markers except classic Hodgkin lymphoma (weak or negative pan-B markers), plasma cell neoplasms (CD79a + only), and lymphoblastic leukemia/ lymphomas (CD20−/+)	B lymphoblastic leukemia/ lymphoma CD34, TdT, CD10, HLA-DR Mantle cell lymphoma* CD5, cyclin D1, FMC7, SOX11 *CD5/CD23 expression on normal vs neoplastic mantle cells differs (see prior page). A subset of MCLs are thought to be of post-germinal center type.	Follicular lymphoma CD10, BCL6, BCL2 Diffuse large B-cell lymphoma GC: CD10+ or CD10-/ BCL6+/MUM1- Non-GC: CD10-/BCL6- or CD10-/BCL6+/MUM1+ Burkitt lymphoma CD10, BCL6, Ki67 near 100% Hodgkin lymphoma Classic types: CD15, CD30 NLPHL: pan-B-cell markers	Marginal zone lymphoma No specific markers CLL/SLL (some are pre-GC origin) CD5, CD23 Lymphoplasmacytic lymphoma CD20, CD138, IgM+ Plasma cell myeloma CD38, CD138, monotypic κ or λ

Note on diagnostic utility of Ig gene molecular studies:

- Heavy chain rearrangement used as a marker of clonality to distinguish low-grade lymphoma from reactive proliferations
- Somatic hypermutation is used as a marker of stage of transition through GC

Pan-B-cell markers = CD19, CD20, CD22, CD79a, PAX5
GC = germinal center
sIg = surface immunoglobulin

Reactive Hyperplasia versus Follicular Lymphoma



	Reactive Follicular Hyperplasia	Follicular Lymphoma
IHC		
CD20	In primary and secondary follicles	Diffusely, in follicles and increased interfollicular positivity
CD3	Paracortical and interfollicular T cells	Decreased, highlights T cells that encircle neoplastic follicle
BCL2 in GC B cells[1,2]	Negative	Positive (90%)
Germinal center markers (CD10, BCL6)	Restricted to germinal centers	Germinal centers and interfollicular zones
FDC (CD21, CD23)	Preserved	FDC meshwork disrupted
Ki67 staining	Very high; polarized pattern	Lower (except in high-grade tumors)
Special studies		
κ or λ light chain restriction by flow cytometry	Polyclonal[3]	Monoclonal restriction
t(14;18)	Absent	Present
IGH rearrangement	Polyclonal	Clonal
Morphologic clues		
General lymph node architecture	Preserved with patent sinuses	Effaced, +/– extracapsular extension
Follicles	Variable in size, spaced apart, mostly in the cortex	More uniform, packed, in cortex and medulla ("cracking" around follicles)
Germinal centers	Polarized (dark zone away from the capsule)	Loss of polarity, loss of TBM
Mantles	Prominent and polarized (thicker closest to capsule)	Loss or attenuation
Interfollicular areas	Preserved	Compressed, infiltrated by centrocytes

1. Do not mistake primary (resting) follicles for FL based on BCL2 expression: primary follicles are composed entirely of mantle cells (BCL2+), but these follicles do not have germinal center cells and are negative for CD10 and BCL6. In addition, primary follicles are IgD-positive, whereas FL is IgD-negative.
2. BCL2 will highlight scattered normal germinal center T cells with intensity similar to that seen in T cells outside of follicles. If stronger BCL2 staining is seen in a germinal center, especially in an otherwise unremarkable lymph node, consider in situ follicular neoplasia.
3. Occasionally florid follicular hyperplasia may show light chain restriction by flow cytometry, particularly in children and adolescents.

Abbreviations: *FDC* follicular dendritic cell, *FL* follicular lymphoma, *GC* germinal center, *TBM* tingible body macrophage

B-Cell Lymphomas

Low-Grade B-Cell Lymphomas (LGBCL) (all positive for CD45, pan-B markers CD19/20/22/79a, and sIg)								
	CD10 BCL6	**BCL2**	**CD5**	**CD23**	**CD43**[7]	**Other IHC markers**	**Flow**	**Molecular in clinical use**
CLL/SLL[1]	–	+	+	+	+/–	LEF1+, CD200+; CD38+ and ZAP-70+: adverse prognosis	Small FMC7– B cells, LC dim, CD20 dim	13q14 del, 11q22-23 del, trisomy 12, *TP53* del, 6q del
MCL[2]	–	+	+	–	+/–	BCL1 (cyclin D1)+[3], SOX11+[4] Blastoid variant is also cyclin D1+	Small FMC7+ B cells, LC bright, CD20 bright	t(11;14) *CCND1-IGH*
FL	+[5]	+[6] (90%)	–	–	– (rare +)	GCET1+, HGAL+, LMO2+[3]	Small-medium-sized B cells, LC restricted, CD10+	t(14;18) *IGH-BCL2*
MZL	–	+/–	–	–	–/+ (30%)	No specific marker CD138+ (plasmacytic cells)	LC restricted small B cells and plasma cells; Often mixture of neoplastic and non-neoplastic B cells	See Chapter 8
LPL	–	+/–	–	–	–/+ (20%)	CD138+ (plasmacytic cells), IgM+, sIg (κ or λ)	LC restricted small B cells and plasma cells, increased mast cells in the background	*MYD88* mutation
HCL	–	+	–	–		CD25+/–, CD123+, annexin A1+, cyclin D1+/–, SOX11–/+ (weak)	CD20 bright, CD22 bright, CD103+, CD11c+, CD25+; very few monocytes in PB/BM	*BRAF V600E* mutation

1. In CLL/SLL, two prognostic subgroups are recognized:
   (a) *Pre-GC (naïve) B-cell type* – unmutated IgV_H, CD38+, ZAP-70+, associated with 17p (10%) or 11q (25%) deletions, poor prognosis
   (b) *Post-GC center type* – mutated IgV_H, CD38–, ZAP-70–, associated with isolated 13q deletion, good prognosis
2. MCL is usually an aggressive lymphoma; however, it is generally included in the DDx of LGBCLs because it is composed of small to medium sized lymphoid cells.
3. Cyclin D1 IHC is not specific for MCL. It is also expressed in plasma cell neoplasms (10–20%) with *CCND1* rearrangement and overexpressed in HCL (~100%) and proliferation centers of CLL/SLL (20–30%) without *CCND1* rearrangement.
4. SOX11 is a new marker of MCL, particularly for cyclin D1-negative cases. It is also positive in HCL, Burkitt lymphoma, and ALL [5–7].
5. CD10 and BCL6 are germinal center markers, positive in FL, Burkitt lymphoma, and some DLBCL. However, some FLs can lose their expression, and a few MCL and MZL can show aberrant expression. Additional GC markers GCET1, HGAL, and LMO2 are of diagnostic value in providing evidence of GC origin [8, 9].
6. BCL2 may be negative in a subset of FL, particularly in high grade, pediatric, and primary cutaneous.
7. CD43 is a T-cell marker but may be aberrantly expressed in various LGBCLs (very rare in FL).

Reference: [1]

Hodgkin Lymphoma (HL)			
		Classic HL (NS, MC, LR, LD)	**Nodular lymphocyte predominant HL**
Neoplastic cells	**Designation**	Reed-Sternberg/Hodgkin (R-S/H) cells and variants	Lymphocyte predominant (LP) cells or "popcorn cells" [formerly L and H cells]
	CD45	–	+
	Pan-B-cell markers	Weak PAX5+, BOB.1, OCT2 variable, typically CD20–	Strongly+ for PAX5, BOB1, OCT2, and CD20
	CD15, CD30, fascin	+ (occasionally R-S cells are CD15–)	–
	EBV (EBER by ISH)	~10–75% + (esp. MC-CHL ~75% +)	–
Background cells		• T cells and mixed inflammatory cells, i.e., eosinophils, plasma cells, and histiocytes (varies with the subtype) • If bands of fibrosis, prominent aggregates of R-S cells, and patchy necrosis – consider the "syncytial variant" of NS-HL	• Abundant small B cells (CD20+) • T cells (CD3+, CD57+, PD1+) surround LP cells forming "T-cell rosettes" • Expanded nodular dendritic cell meshwork (CD21+ and CD23+)
Differential diagnosis		• AITL: prominent vascular proliferation, no true R-S/H cells (CD30+ cells are immunoblasts and lack CD15) • ALCL: neoplastic ("hallmark") cells are CD30+, CD15–, ALK+ (subset), and T-cell and cytotoxic markers positive	• THRLBCL: few small B cells in the background, lacks nodular architecture; T-cell "rosettes" are not present. It may be difficult to distinguish THRLBCL from NLPHL on core biopsies. Neoplastic cells in both THRLBCL and NLPHL show variable EMA+

Abbreviations: *AITL* angioimmunoblastic T-cell lymphoma, *ALCL* anaplastic large cell lymphoma, *ALL* acute lymphoblastic leukemia, *CLL/SLL* chronic lymphocytic leukemia/small lymphocytic lymphoma, *FL* follicular lymphoma, *HCL* hairy cell leukemia, *HL* Hodgkin lymphoma, *ISH* in situ hybridization, *LC* light chain, *LD* lymphocyte depleted, *LPL* lymphoplasmacytic lymphoma, *LR* lymphocyte rich, *MC* mixed cellularity, *MCL* mantle cell lymphoma, *MZL* marginal zone lymphoma, *NLPHL* nodular lymphocyte predominant Hodgkin lymphoma, *NS* nodular sclerosis, *R-S/H* Reed-Sternberg/Hodgkin, *THRLBCL* T-cell/histiocyte-rich large B-cell lymphoma

Plasma Cell-Rich and Plasmablastic Neoplasms

DDx of Plasma Cell-Rich Neoplasms						
		Plasma cell neoplasms (myeloma, plasmacytoma)	**Lymphoplasmacytic lymphoma (LPL)[1]**	**Nodal marginal zone lymphoma (NMZL)[1]**	**Extranodal marginal zone lymphoma, MALT type**	**Splenic marginal zone lymphoma**
CD20, PAX5, CD45		– (5%+)	+	+	+	+
CD79		+	+	+	+	+
CD38, CD138, MUM1		+	+ in plasma cells	+/– in plasma cells	+ in plasma cells	–/+ in plasma cells
CD43		+	+/–	+/–	+/–	–
CD56/CD117/cyclin D1		–/+[1]	–	–	–	–
Cytoplasmic immunoglobulins		+	+ (patchy); usually IgM	+/–	+/–	+/–
Bone marrow	**Involvement**	Usually	Usually	Can be	Rarely (2–20%)	Almost always
	Pattern of involvement	Diffuse and/or interstitial	Nodular, diffuse, and/or interstitial	Interstitial or nodular, rarely intrasinusoidal	Varies	Nodular (surrounds reactive follicles) and intrasinusoidal
	Plasma cells	Clusters/sheets	Distinct clusters	Varies	Varies	Varies
Extramedullary/extra-skeletal sites (visceral organs, lymph nodes, soft tissue)		Rare In sheets, space occupying	+/–, LN may have retained architecture, increased mast cells Lymphocytes, plasma cells, plasmacytoid lymphocytes	+, lymph node as primary site Para-follicle small centrocyte-like and monocytoid B cell	+, primary mucosal sites Lymphoepithelial lesion and monocytoid B-cell proliferation	+, spleen as primary site Prominent white pulp with expanded marginal zone
Cytogenetic and molecular		Complex karyotype	*MYD88* mutation (>90% cases) *CXCR4* mutation (30%) *ARID1A* mutation (17%)	Gain of chromosome 3 and 18 and loss of 6q23-24	t(11;18): gastric and pulmonary t(3;14): thyroid, ocular adnexa, orbit, and skin t(14;18): orbit, ocular adnexa, and salivary gland	Heterozygous deletion in 7q (30%) *NOTCH2* mutation (10–25%) Absence of rearrangements seen in MALT lymphoma

1. LPL and MZL may be very difficult to differentiate; in this case, a diagnosis of small B-cell neoplasm with plasmacytic differentiation is appropriate.

Note: LPL, NMZL, MALT lymphoma, and splenic MZL lymphoma commonly contain neoplastic B cells and clonally related plasma cells; the B cells express B-cell markers but no plasma cell markers, whereas the plasma cells typically express plasma cell markers with no B-cell markers. Occasionally other low-grade B-cell lymphomas such as FL and CLL/SLL can show plasmacytic differentiation.

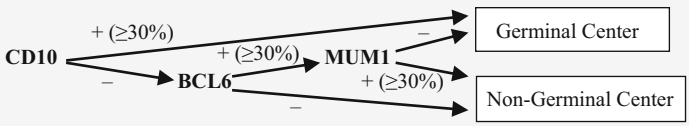
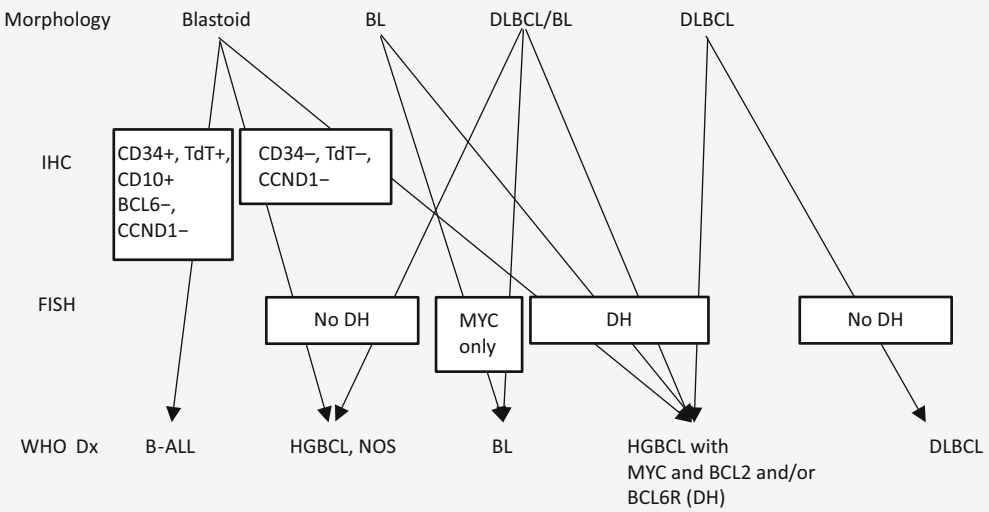
References: [1–3]

DDx of Plasmablastic Neoplasms					
	Clinical	**Site**	**EBER**	**HHV8**	Other IHC
Plasmablastic lymphoma	Adult **Immunodeficiency** 1. HIV+, 2. elderly, and 3. iatrogenic immunosuppression	Extranodal, most common in the head and neck (oral cavity) and GI tract	+	–	CD20–, CD138+, CD30+/–, BOB1+, OCT2+, light chain restricted, high Ki-67
Plasmablastic myeloma	History of **myeloma**, paraproteinemia, lytic bone lesions	Bone marrow or extramedullary sites	–	–	CD20–, CD138+, CD56+/–, light chain restricted
Primary effusion lymphoma	**HIV**+, nearly exclusively male	Serous effusion with no tumor masses	+	+	Pan-B-cell markers–, surface light chain–, CD30+, CD45+, CD138+, EMA+, BOB1+, OCT2+
ALK-positive large B-cell lymphoma	More in young man, no association with immunosuppression	Lymph node with diffuse or sinusoidal involvement	–	–	EMA+, CD138+, MUM1+, pan-B-cell markers–, CD30–, cytoplasmic light chain restricted, BOB1+, OCT2+ **ALK+** (cytoplasmic granular pattern)
HHV8+ diffuse large B-cell lymphoma	**HIV**+, usually associated with multicentric Castleman disease	Lymph node, spleen, and extranodal sites	–	+	CD20+/–, CD138–, lambda light chain restricted

References: [1, 10, 11]

Abbreviations: *ALK* anaplastic lymphoma kinase, *EBER* Epstein-Barr virus-encoded RNA, *HHV*8 human herpes virus 8, *HIV* human immunodeficiency virus

Immunoprofiles of Selected Hematopoietic Disorders

Immunoprofiles of Selected Hematopoietic Disorders: B-Cell and Plasma Cell Neoplasms	
Diagnosis	Immunoprofile
B lymphoblastic leukemia/lymphoma	CD19+, CD22+, PAX5+, CD79a+, TdT+ CD10 usually + but may be –, CD20 variable, CD34 variable, CD45 dim or – Note: expression of sIg (dim), CD13, or CD33 may be seen on flow cytometric analysis *Typical panel: CD45, CD3, CD20, CD19 (or CD79a, CD22 or PAX5), CD10, MPO, CD34, TdT, Ki67*
Burkitt lymphoma t(8;14)(*MYC-IGH*) t(8;22)(*MYC-IGL*) t(2;8)(*MYC-IGK*)	CD45+, pan-B-cell markers (CD19/20/22/79a/PAX5)+, CD10+, BCL6+, Ki67 near 100%, sIg+, BCL2–; c-MYC+ with *MYC* rearrangement+; Oil Red O+ Negative blast markers (TdT–, CD34–) *Typical panel: CD3,CD20,CD10, BCL2, BCL6, CD34, TdT, cyclin D1, c-MYC, Ki67*
Diffuse large B-cell lymphoma (DLBCL), NOS	CD45+, pan-B-cell markers (CD19/CD20/CD22/CD79a/PAX5)+ CD20 is negative in DLBCL treated with rituximab (anti-CD20 antibody), but CD19, CD22, CD79a, and PAX5 remain (+) Variable expression of CD10, BCL2, and BCL6 Usually sIg+, Ki67 typically >40–50% Based on recent gene expression profiling, two types of DLBCL have been recognized: 1. Germinal center B-cell-like DLBCL (better prognosis) 2. Non-germinal center (activated) B-cell-like DLBCL (worse prognosis) A 3-marker Han's algorithm to distinguish these two subtypes is commonly used [12, 13]:  An updated, 5-marker algorithm that incorporates GCET1 and FOXP1 has shown higher accuracy [14] Note: DLBCLs rarely express CD5, but if CD5 is positive in a high-grade mature B-cell neoplasm, then the specimen should be stained for cyclin D1 to rule out blastoid variant of mantle cell lymphoma *Typical panel: CD3,CD20,CD10, BCL6, BCL2,CD5, Ki67, MUM1 (if treated with rituximab, add CD79a, PAX5, or CD19), cyclin D1* *LBCL can be diagnosed on small biopsies using an abbreviated panel of CD3, CD20, and Ki67*
High-grade B-cell lymphoma with/without rearrangement of *MYC* and *BCL2* and/or *BCL6*	Cases carry rearrangements of *MYC* and *BCL2* and/or *BCL6*: aka double-hit (DH) lymphoma [1, 15]  *Typical panel, similar to DLBCL, BL, and blastoid MCL, requires FISH for MYC, BCL2, and BCL6 rearrangements* Abbreviations: *BL* Burkitt lymphoma, *DHL* double-hit lymphoma
Blastoid variant of mantle cell lymphoma t(11;14) (*CCND1-IGH*)	CD45+, pan-B-cell markers (CD19/CD20/CD22/CD79a/PAX5)+, CD5+ (rarely–), cyclin D1+, SOX11+, CD10+/–, sIg+ Note: The blastoid variant of mantle cell lymphoma may be leukemic. If the disease involves the peripheral blood, it typically demonstrates a dimorphic population of circulating tumor cells (small mature lymphocytes and blast-like cells) *Typical panel: CD3,CD20,CD10, BCL6, BCL2,CD5, cyclin D1, Ki67, CD34, TdT*

Immunoprofiles of Selected Hematopoietic Disorders – Continued

Immunoprofiles of Selected Hematopoietic Disorders: B-Cell and Plasma Cell Neoplasms	
Diagnosis	Immunoprofile
Primary mediastinal (thymic) large B-cell lymphoma	CD45+, pan-B-cell markers (CD19/CD20/CD22/CD79a/PAX5)+ CD30+ (>80%; weak and heterogeneous), MUM1+ (75%), CD23+ (70%), MAL+ Variable expression of CD10, CD15, BCL6, and BCL2 Surface Ig – (best evaluated by flow cytometry) *Typical panel: CD3,CD20,CD10, BCL2, BCL6, MUM1, CD30, CD15, CD23, Ki67, PAX5, EBER-ISH*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma	CD45+, pan-B-cell markers (CD19/20/22/79a/PAX5)+ BCL6 +/–, CD10–; EBER–; usually CD30+, CD15+ Note: Typically has overlapping morphologic features of classic Hodgkin lymphoma and DLBCL (particularly primary mediastinal large B-cell lymphoma); most frequently presents as a mediastinal mass *Typical panel: CD3,CD20,CD10, BCL6, BCL2, MUM1, CD30, CD15, CD23, Ki67, EBER-ISH*
Plasmablastic lymphoma	CD138+, CD38+, MUM1+, CD45–, CD20–, PAX5–, cytoplasmic immunoglobulin commonly with restricted expression CD30+/–, CD79a+/–, CD56– (usually), Ki67 >90% (usually). EBER +(60–75%), HHV8– Note: Typically adult patient with immunodeficiency, due to HIV, immune senescence, or iatrogenic immunosuppression. Most frequently at an extranodal site *Typical panel: CD138, CD20, MUM1, Ki67, HHV8, ALK, κ, λ, EBER-ISH*
Plasma cell neoplasms (MGUS vs. plasma cell myeloma, depending on % of plasma cells in the bone marrow, clinical presentation, laboratory findings)	CD138+, CD38+, MUM1+, EMA+, IG κ or λ light chain restriction; CD79a+ but often negative for other pan-B markers (CD19, 20, 22, 20), cyclin D1 +/–, CD43 –/+; CD45 shows occasional patchy staining CD56, CD117, CD10, or CD20 may be aberrantly expressed in neoplastic plasma cells If frequent mitotic figures, apoptotic cells and prominent nucleoli – rule out plasmablastic lymphoma (EBER+) *Typical panel: CD138, κ, λ, CD56, CD117, and cyclin D1*

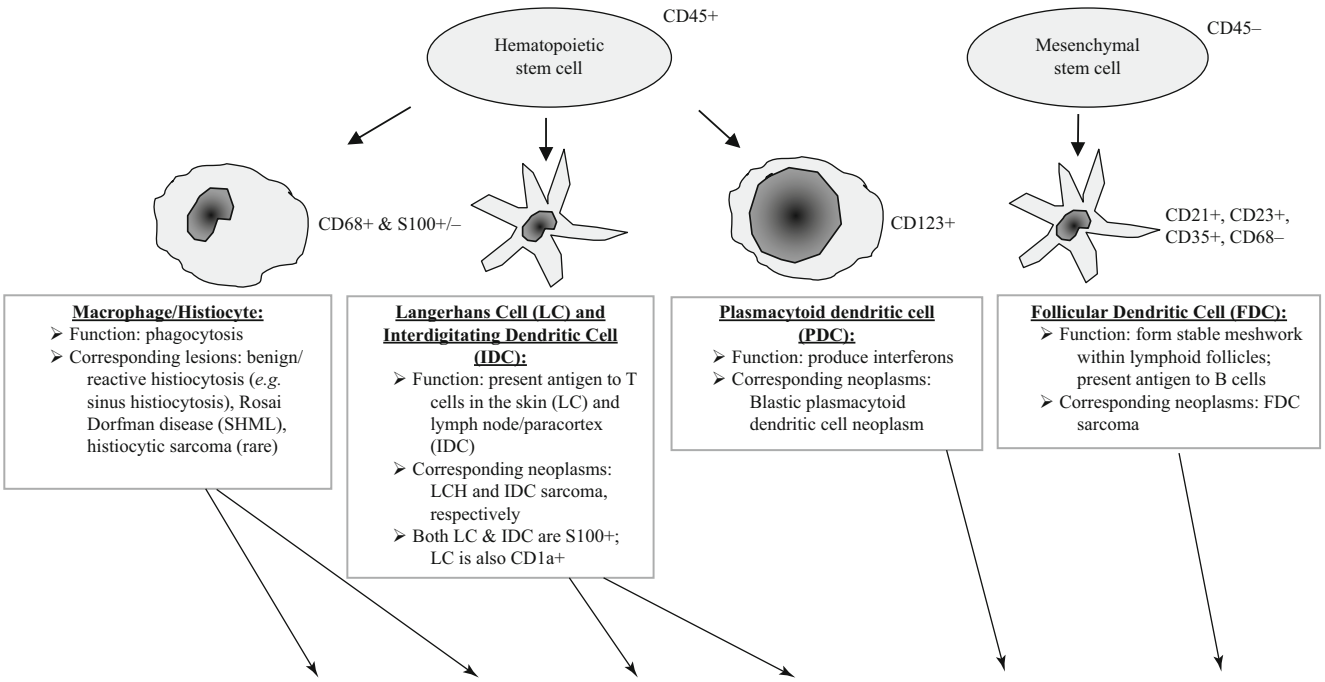
Immunoprofiles of Selected Hematopoietic Disorders: T-Cell Neoplasms	
Diagnosis	Immunoprofile
Precursor T lymphoblastic lymphoma/leukemia	TdT+, cytoplasmic CD3 usually +, CD7 usually + Often CD4/CD8 double positive or double negative Variable expression of CD2, surface CD3, CD5, CD34, CD10, CD99, CD1a, and CD45 Occasionally positive for CD79a, CD33, CD13, CD117, CD56 *Typical panel: CD3, CD20, CD4, CD8, CD7, CD34, TdT, CD1a, CD99,CD10, Ki67*
T prolymphocytic lymphoma	Pan-T-cell antigen (CD3, CD2, CD5, CD7), CD52+, and TCL1+; negative for TdT and CD1a; 65% CD4+, 25% CD4+/CD8+, 15% CD8+ *Typical panel: CD3, CD20, CD4, CD5, CD7, CD8, TCL1, CD52, TdT, CD1a*
Anaplastic large cell lymphoma (ALCL) (systemic lymphoma) 1. ALK+ ALCL: t(2;5)(*NMP-ALK*) 2. ALK– ALCL (a) *DUSP22* rearranged (b) *TP63* rearranged (c) Triple negative	CD45 and CD45RO are variable, usually CD43+ Always CD30+ (intense membranous and perinuclear "target-like" pattern) but almost entirely CD15-negative At least some T-cell antigens are + (CD2, CD4 or CD5), but CD3 and CD8 are frequently absent. Cytotoxic proteins (TIA-1, granzyme, perforin) are often +, CD25+, EMA+/– ALK+ 60–85% (ALK+ patients are younger and do better than ALK– patients) *TP63* rearranged ALCL commonly positive for p63 with inferior prognosis *DUSP22* rearranged ALCL could show MUM1+ with loss of cytotoxic markers, e.g., TIA-1, and good prognosis Primary skin ALCL, a separate entity, is ALK– but has a good prognosis; this diagnosis requires clinical correlation Provisional entity: breast implant-associated ALCL, mostly excellent outcomes *Typical panel: CD3,CD4,CD5,CD7,CD8,CD20,CD30,CD15, EMA, ALK, TIA-1*
Peripheral T-cell lymphoma, NOS	CD3+, CD4>CD8 (rarely double positive), loss or dim expression of CD5, CD4, CD7, and/or CD8 CD30+/–, CD56+/–, typically T-cell receptor βF1+ (T-cell receptor β chain), high proliferation index (Ki67) CD10–, EBV– A subset could express T follicular phenotype: CD10, BCL6, CD57, CXCL13, and PD1 *Typical panel: CD3, CD20, CD4, CD5, CD7, CD8, CD30, Ki67, follicular helper T-cell markers (CD10, BCL6, CD57, and PD1), and cytotoxic markers (TIA-1, granzyme B, and perforin)*
Angioimmunoblastic T-cell lymphoma	Neoplastic T cells: CD3+, CD4+; loss of CD5 or CD7 in some cases; at least a subset of the T cells are positive for some follicular helper T-cell markers (CD10, BCL6, CD57, PD1, and CXCL13) Background B cells and plasma cells are polyclonal, and immunoblasts are CD20+, CD30+, and CD15- Usually EBV+ (EBER in situ hybridization preferred) in immunoblasts Expanded dendritic cell meshwork: CD21+, CD23+ *Typical panel: CD20, CD3, CD4, CD5, CD7, CD8, CD10, CD30, CD15, CD21 (or CD23), CD57, BCL6, PD1, CXCL13, EBER-ISH*

Immunoprofiles of Selected Hematopoietic Disorders – Continued

Immunoprofiles of Selected Hematopoietic Disorders: T-Cell Neoplasms	
Diagnosis	Immunoprofile
Hepatosplenic T-cell lymphoma Isochromosome 7q	CD3+, CD56 +/–, CD8+/–, TIA-1+, granzyme B–, CD4–, CD5–, EBV– Usually T-cell receptor βF1- (tumor cells are most often of the γδ T-cell receptor type and do not express T-cell receptor αβ) although some are αβ type Spleen: diffuse involvement of red pulp and sinuses Bone marrow: characteristic sinusoidal distribution of tumor cells *Typical panel: CD3, CD20, CD4, CD5, CD7, CD8, CD56, TIA-1, granzyme B, βF1, EBER-ISH*
Cutaneous T-cell lymphoma (mycosis fungoides)	T-cell antigens (CD3+, CD2+) with abnormal CD4/CD8 ratio (usually CD4>>CD8) and aberrant loss of some T-cell markers (CD7 and/or CD5) Expression of CD30 is associated with histologic transformation Loss of CD26 is seen on flow cytometric analysis *Typical panel: CD3,CD20,CD4,CD8,CD5,CD7*
Adult T-cell leukemia/lymphoma	Mostly CD4+/CD25+ T cell with loss of CD7, FOXP3+, PCR for HTLV-1 positive in tumor tissue *Typical panel: CD3, CD20, CD4, CD5, CD7, CD8, CD25, FOXP3, TIA-1, granzyme B, HTLV-1 by molecular study*
Enteropathy-associated T-cell lymphoma	Associated with celiac disease, occurs more common in western countries, less in Asian >90% in the small intestine; polymorphic infiltrating lymphocytes are largely pleomorphic with frequent angioinvasion. Adjacent mucosa frequently shows changes of histomorphologic features of celiac disease *Distinct phenotype: CD3+, CD7+, CD103+, CD4–, CD8–, CD5–, and cytotoxic+* *Typical panel:* CD3, CD20, CD5, CD7, CD4, CD8, β-F1, PAX5, TIA1, CD56, EBER-ISH
Monomorphic epitheliotropic intestinal T-cell lymphoma	No clear association with celiac disease; occurs mostly in Asian and Hispanic population Most often present in the small intestine, diffuse infiltrate of medium size and monotonous lymphocytes with prominent epitheliotropism (formerly type II EATL) *Distinct phenotype: CD3+, CD8+, CD56+, CD5–, TIA1+, 20% with aberrant CD20 expression* *Typical panel: CD3, CD20, CD5, CD7, CD4, CD8, β-F1, PAX5, TIA1, CD56, EBER-ISH*
Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract	Chronic and relapsing disease, usually involving multiple sites in GI tract, particularly in the small intestine and colon, presenting as mucosal thickening Mature T-cell phenotype: CD3+, CD8+, TIA1+, granzyme B–, CD2+, CD5+, CD7 variable, CD56–, Ki67 low (<10%), TCR αβ+. TCR rearrangement shows clonal population *Typical panel: CD3, CD2, CD5, CD7, CD4, CD8, CD56, TIA1, granzyme B, CD20*

Immunoprofiles of Select Hematopoietic Disorders: Others	
Extranodal NK/T-cell lymphoma, nasal type	NK-cell (CD56+, surface CD3–) or T-cell (CD56–, CD3+) phenotype; cytoplasmic CD3ε+; cytotoxic proteins+ (perforin, TIA-1, and granzyme B), EBV+, CD45+, CD43+ Negative for CD4, CD5, CD8, CD57. Occasionally positive for CD7 and CD30 *Typical panel:CD45, CD3, CD20, CD30, CD4, CD5, CD7, CD8, CD56, CD57, TIA-1, EBER-ISH*
Myeloid sarcoma (granulocytic sarcoma, chloroma)	Most myeloid sarcomas are CD43+. Blast markers (CD34, CD117, TdT), CD99, and CD45 are variable *Myeloid (granulocytic) sarcoma*: CD13, CD33, CD34, CD15, MPO, and CD117 *Monoblastic chloroma*: CD14, CD64, CD11b, CD68, and lysozyme (typically negative for the blast markers CD34 and CD117) *Typical panel: CD45, CD43, MPO, lysozyme, CD34, CD117, CD123, Ki67, CD4, CD56; also CD3 and CD20 (to rule out lymphoma)*
Blastic plasmacytoid dendritic cell neoplasm (BPDCN)	Positive for CD4, CD56, CD123, BDCA2(CD303), CD2AP, CD43, TCL1, and CD68 (cytoplasmic dotted pattern) Could be positive for CD2, TdT, CD7, CD33, and S100 But negative for MPO, CD3, CD19, CD20, CD11c, CD13, CD14, lysozyme, cytotoxic markers (granzyme B, TIA-1, etc.), CD21, CD34, CD117, and EBER-ISH
Paroxysmal nocturnal hemoglobinuria (PNH)	Deficiency of glycophosphatidylinositol (GPI)-anchored proteins is best detected by flow cytometry using peripheral blood. Red blood cells: loss of CD59 (preferred) and CD55 Monocytes: loss of CD14 and CD55; failure to bind FLAER* Granulocyte: loss of CD16 and CD24; failure to bind FLAER* *Fluorescent aerolysin (FLAER) is a protein that binds specifically to the GPI anchor. Absence of FLAER binding to WBCs is the most sensitive measure of PNH and can be detected using flow cytometry.

Histiocytic and Dendritic Cell Lesions



	Macrophage/ histiocyte lesions[1]	Rosai-Dorfman disease (SHML)	Langerhans cell histiocytosis	Interdigitating dendritic cell sarcoma	Blastic plasmacytoid dendritic cell neoplasm (BPDCN)[3]	Follicular dendritic cell sarcoma
CD45	+	+	+ (weak)	+ (weak)	– (CD45RA+)	–[2]
CD68, CD163, CD4, Lysozyme (pan-histiocyte markers)	+	+	CD68+ CD163+ CD4+ Lysozyme (low)	– (variable)	CD68+ CD163– CD4+ Lysozyme –	– (usually)
S100	– (or focally +)	+	+	+	–	– (variable)
CD1a, langerin	–	–	+	–	–	–
CD21, CD23, CD35, D2-40, clusterin, fascin, CXCL13 (FDC markers)	–	–	–	+ fascin only (all other FDC markers –)	–	+ (all FDC markers)
CD56, CD123, TdT	–	–	–	–	CD56+, CD123+ TdT+/–[4]	–

1. The group includes both benign proliferations (histiocytosis) and histiocytic neoplasms (histiocytic sarcoma). Ki67 may be helpful in determining whether a histiocytic proliferation is benign or malignant since the proliferation index can be high in true histiocytic malignancies.

2. All above entities, except FDC sarcoma, are CD45+ at least weakly/focally. FDC sarcoma is CD45 (–) because it is non-hematopoietic in origin (see diagram). Dendritic cells (interdigitating and follicular) were formerly known as reticulum cells (no relation to reticular fibers).

3. BPDCN (formerly blastic natural killer cell lymphoma) is an extremely rare neoplasm of plasmacytoid dendritic cells; this entity is included with AML and related precursor lesions in the 2016 WHO.

4. CD56 and TdT are specific for neoplastic PDCs, whereas CD123 is positive in both normal/reactive and neoplastic PDCs.

Note: True histiocytic malignancies must be differentiated from hemophagocytic syndromes as well as neoplasms that are rich in histiocytes, including T-cell/histiocyte-rich large B-cell lymphoma, lymphoepithelioid peripheral T-cell lymphoma (Lennert lymphoma), histiocyte-rich classic Hodgkin lymphoma, and the lymphohistiocytic pattern of ALCL.

Typical panel: CD45, CD43, CD68, CD163, S100, CD1a, CD21, CD23, CD20, CD3, CD4, CD56, CD30, Ki67, S100

Diagram based on reference [16]

Check for updates

Chapter 4. Predictive Markers

By Marina K Baine, Xiaojun Wu, Caleb Ho, Justin A. Bishop, Natasha Rekhtman

Semiquantitative Assessment of Predictive Markers by IHC

By Marina K Baine & Natasha Rekhtman

Estrogen and Progesterone Receptor Expression Interpretation in Breast Carcinomas
(only nuclear staining is scored)

Criteria	*Interpretation*
≥1% staining of any intensity	Positive
<1% staining with appropriately staining internal control tissue	Negative

- Also report % of tumor cells staining, staining intensity (3+/strong, 2+/medium, or 1+/weak), internal and external controls (positive, negative, or not present), and whether standard assay conditions were met/not met. Internal control tissue not staining or specimen handling did not conform to guideline requirements (e.g., fixation <6 h or >72 h, fixation solution other than 10% buffered formalin) → reported as uninterpretable.
- Hormone receptor-positive cancers are treated with Tamoxifen or other analogous hormonal agents.

Reference: [1]

HER2 (c-*erb*B-2) Dako HercepTest Interpretation in Breast Carcinomas
(only membranous staining is scored)

Staining intensity	*Staining pattern*	*Proportion of positive tumor cells*	*Score and interpretation*
Any	Any	≤10%	0 = negative
Weak	Partial membranous	>10%	1+ = negative
Weak to moderate	Complete membranous		2+ = weakly positive (equivocal)*
Strong			3+ = strongly positive

* Two unusual scenarios that should be interpreted as 2+ equivocal are 1) basolateral staining in micropapillary variant of breast carcinoma and 2) strong complete membranous staining in ≤10% of tumor cells.

- Cases that are negative by IHC (0 & 1+) are reported out as "negative," and strongly positive cases (3+) are reported out as "positive." Weakly positive (2+) or equivocal IHC should be further analyzed for *HER2* gene amplification by FISH (or CISH) on the same specimen, or repeat IHC and/or ISH must be done on a new specimen if available.
- Dual-probe FISH criteria are:
  - *HER2* amplified: FISH ratio (*HER2* gene signals to chromosome 17 signals) ≥2.0 and >4 *HER2* gene copies/nucleus
  - *HER2* non-amplified: FISH ratio <2.0 and <4.0 *HER2* gene copies/nucleus
  - All other combinations of FISH ratio and HER2 copy number (~5% overall prevalence) must be further worked up taking into consideration concomitant IHC results on the same specimen
- HER2-positive cancers are treated with trastuzumab (Herceptin), a monoclonal antibody directed against HER2.

See Human EGFR2 Testing in Breast Cancer: ASCO/CAP Clinical Practice Guideline Focused Update 2018 for details [2]

References: [3, 4]

HER2 (c-*erb*B-2) Dako HercepTest Interpretation in Gastroesophageal Adenocarcinoma

Staining intensity	*Staining pattern*	*Proportion of positive tumor cells*		*Score and interpretation*
		% cells (resection)	*# of cells (biopsy)*	
None or weak	Non-membranous	<10%	<5 cohesive cells	0 = negative
Weak	Non- or partially membranous	≥10%	≥5 cohesive cells	1+ = negative
Weak to moderate	Complete or basolateral membranous			2+ = equivocal
Strong	Complete or basolateral membranous			3+ = positive

- Similar to breast cancer, all equivocal cases (2+) by IHC should be further analyzed for *HER2* gene amplification by FISH. Criteria for FISH interpretation are as described in the table for breast cancer above.
- HER2-positive gastric and GE junction adenocarcinomas are treated with trastuzumab (Herceptin).

References: [5, 6]

PD-L1 IHC 22C3 PharmDx Assay Interpretation in Non-Small Cell Lung Carcinoma
(only membranous staining of viable tumor cells is scored)

Criteria[1]	*Score and interpretation*
<1%	TPS[2] <1%, no PD-L1 expression
1–49%, any intensity partial or complete membrane staining	TPS 1–49%, PD-L1 expression
≥50%, any intensity partial or complete membrane staining	TPS ≥50%, high PD-L1 expression[3]

1. For heterogeneous PD-L1 expression, average PD-L1 expression across the entire examined tissue is reported.
2. TPS = Tumor Proportion Score = percentage of PD-L1- positive tumor cells relative to all viable tumor cells.
3. TPS ≥ 50% qualifies for first- line Keytruda (Pembrolizumab, anti-PD-1 immunotherapy), while TPS 1–49% qualifies for second- line Keytruda (as of 2018).

Note: Stay tuned – other PD-L1 assays and other predictive assays for immunotherapies are being developed.

Reference: [7]

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N. Rekhtman et al., *Quick Reference Handbook for Surgical Pathologists*, https://doi.org/10.1007/978-3-319-97508-5_4

Predictive Biomarkers and Targeted Therapies: Solid Tumors (Selected Examples)

By Marina K Baine, Justin A. Bishop, Natasha Rekhtman

For details on specific molecular alterations, please see Chapter 10.

Tissue type	Tumor type	Biomarker and method **(bolded if involves IHC)**	Drug (italicized if biomarker predicts resistance)	Comments
Brain	**Pleomorphic xanthoastrocytoma (PXA)**	*BRAF* V600E	Vemurafenib, dabrafenib	Treatment with BRAF inhibitors improves outcome. References: [8, 9]
	High-grade glioma	*MGMT* promoter methylation	Temozolomide, alkylating agents, radiation therapy	
Skin	**Melanoma**	*BRAF* V600E	Vemurafenib, dabrafenib	Mutation occurs primarily in cutaneous melanoma.
		KIT, PDGFRA mutations	Imatinib (Gleevec), sunitinib, dasatinib	Mutations occur primarily in acral and mucosal melanoma.
Soft tissue	**GIST**	*KIT*, *PDGFRA* mutations	Imatinib (Gleevec), sunitinib	
		SDH gene (A, B, C, and D) mutations	*Imatinib (Gleevec)*	SDH-deficient GISTs (primarily with mutations in *SDHB*) are resistant to Gleevec.
	DFSP	*PDGFRB* rearrangement	Imatinib (Gleevec)	
	IMT	*ALK* rearrangement (50%)	Crizotinib	t(1;2) gene rearrangement, primarily involving *TPM3-ALK*, predicts responsiveness to crizotinib. **ALK IHC sensitive for fusions**.
Head and neck	**Oropharyngeal/ tonsillar SqCC**	HR-HPV and **p16**	Chemotherapy/radiation therapy	HR-HPV+ oropharyngeal SqCC is associated with favorable prognosis and superior chemo- and radiosensitivity. All oropharyngeal SqCCs should thus be tested for HR-HPV by **p16 IHC** (**70% nuclear and cytoplasmic staining cutoff**); additional viral-specific testing is optional. References:[10–12]
	Salivary duct carcinoma	*HER2* amplification	Trastuzumab	Approximately 30% of salivary duct carcinomas demonstrate *HER2* amplification/**overexpression by IHC**, which predicts sensitivity to trastuzumab (similar to breast CA). Reference:[13]
Thyroid	**Papillary carcinoma**	*BRAF* V600E	Vemurafenib	Decreased ability to take up RAI. Response to vemurafenib in RAI-refractory progressive disease. Reference: [14]
Lung (selected alterations)	**Adenocarcinoma**	*EGFR* mutations	1st gen: gefitinib, erlotinib; 2nd gen: afatinib; 3rd gen: osimertinib	Acquired mutations in *EGFR* (e.g., T790 M, exon 20) confer resistance to first- and/or second-generation EGFR inhibitors.
		ALK rearrangement	First gen, crizotinib; second gen, alectinib, ceritinib	**ALK (D5F3 clone) IHC is a companion diagnostic**; treatment can be initiated based on positive IHC result.
		ROS1 rearrangement	Crizotinib	Preferred testing method is FISH or NGS, but **IHC (D4D6 clone) is a sensitive and cost-effective screening method** that requires confirmation of positive or indeterminate cases.
	NSCLC (AdenoCA + SqCC)	*BRAF* V600E	Dabrafenib + trametinib	2018 approval
		MET exon 14 skipping mutations	Crizotinib	
		PD-L1	PD-1/PD-L1 (MABs) – immune checkpoint inhibitors	High tumor PD-L1 expression (> 50%) predicts response to pembrolizumab (anti-PD1 MAB; FDA-approved for first-line treatment); but lower PD-L1 (1–49%) is approved for second-line treatment.
Breast	**Adenocarcinoma, ductal and lobular**	**ER, PR**	Tamoxifen (ER/PR+), anastrozole (ER+/PR-)	Hormone-positive breast cancer demonstrates good response to hormone therapy.
		HER2 amplification (IHC +/– FISH)	Trastuzumab	Response to trastuzumab. Cases with equivocal IHC are triaged for FISH.
Tubular GI tract	**Gastroesophageal adenocarcinoma**	**HER2** amplification (IHC +/– FISH)	Trastuzumab	HER2 amplification is more common with intestinal histology. Cases with equivocal IHC are triaged for FISH. Reference: [15]
	Colorectal adenocarcinoma	*BRAF* V600E	*EGFR-targeted agents*	Resistance to EGFR TKIs
		MMR proteins – (IHC + MSI PCR)	*5-FU* Irinotecan, PD-1 inhibitors (MABs)	Resistance to 5-FU Improved response to irinotecan and PD-1 MABs.
		KRAS, NRAS, HRAS mutations	*EGFR-targeted agents*	Predict resistance to EGFR TKIs
		CDX2 loss	Adjuvant chemotherapy	CDX2 loss helps identify high risk stage II/III patients who may benefit from adjuvant chemotherapy. Reference: [16]

Note: In the era of the rise of molecular diagnostics, NGS is becoming the technique of choice for assessing the genomic alterations in tumors. The vast majority of the markers listed in the table above can be assessed by NGS, and the prevalence of this technique is expected to rise as the availability/accessibility improves and the cost drops.

Abbreviations: *DFSP* dermatofibrosarcoma protuberans, *SqCC* squamous cell carcinoma, *HR-HPV* high-risk human papillomavirus, *IMT* inflammatory myofibroblastic tumor, *MAB* monoclonal antibody, *NGS* next-generation sequencing, *RAI* radioactive iodine, *TKIs* tyrosine kinase inhibitors

Predictive Biomarkers and Targeted Therapies: Hematopoietic System (Selected Examples)

By Xiaojun Wu & Caleb Ho

Class of neoplasm	Type	Biomarker	Drug	Comments
Myeloproliferative neoplasms	**CML**	*BCR-ABL1*	Imatinib (Gleevec), sunitinib, dasatinib	In TKI therapy era, the most important prognostic indicator is response to treatment at the hematologic, cytogenetic, and molecular levels. Resistance to TKI could develop.
	Post PV myelofibrosis and PMF, overt fibrotic stage	*JAK2* mutation	Ruxolitinib	Development of overt myelofibrosis and blast phase disease is associated with poor prognosis and disease progression. JAK inhibitor improves symptoms but has no clear disease-modifying activity.
Myeloid and lymphoid neoplasms with eosinophilia and gene rearrangement	**Myeloid and lymphoid neoplasm with *PDGFRA*, *PDGFRB* rearrangement**	Rearrangement of *PDGFRA*, *PDGFRB*	Imatinib (Gleevec)	Good prognosis due to response to TKIs. TKI resistance can develop; alternative TKIs may be effective.
Mastocytosis	**Systemic mastocytosis**	*KIT* mutations	Imatinib (Gleevec) midostaurin	Patients with *KIT* D816V mutation are resistant to imatinib but could respond to midostaurin.
AML with related myeloid precursor neoplasms	**AML with *PML-RARA* (APL)**	*PML-RARA*	Tretinoin and arsenic trioxide	Promote differentiation of promyelocytic blasts to mature neutrophils, leading to complete responses.
	AML	*FLT3* ITD or TKD mutation	Midostaurin, sunitinib, sorafenib, etc.	Midostaurin was FDA-approved for *FLT3*-mutant AML in 2017.
		IDH1/2 mutation	Enasidenib	Enasidenib was FDA-approved for *IDH2*-mutant AML in 2017.
Precursor lymphoid neoplasms	**B-ALL/LBL, *BCR-ABL1***	*BCR-ABL1*	Imatinib, dasatinib	The addition of TKIs significantly improves the previously very poor outcome in these patients.
	B-ALL/LBL, *BCR-ABL1* like	Translocation involving *CRLF2*, *JAK2*, *PDGFRB*, etc.	Imatinib, dasatinib, ruxolitinib	Very poor prognosis when treated with conventional chemotherapy. Some patients show dramatic responses to TKIs and JAK inhibitor.
B-cell neoplasms, CD20 positive	**CLL/SLL, MZL, MCL, LPL, B-PLL, FL, DLBCL, B-ALL/LBL, etc.**	Nonspecific	Rituximab (anti-CD20 MAB) with/without chemotherapy	**CD20 reactivity can be lost after rituximab treatment**.
Mature B-cell neoplasms	**Hairy cell leukemia**	*BRAF* V600E mutation	Pentostatin, cladribine	HCL with *BRAF* V600E is uniquely sensitive to purine analogues. Patients often achieve complete and durable remission. HCL with *IGHV4–34* may not have similar treatment effectiveness.
Mature T-cell neoplasms	**ALK+ ALCL** **ALK– ALCL**	*NPM1-ALK* rearrangement	Standard chemotherapy plus brentuximab vedotin (anti-CD30 MAB) Crizotinib, ceritinib	As second-line agent, brentuximab leads to complete responses in half of patients with refractory and relapsed disease. There is only limited information on ALK inhibitors. **CD30 reactivity can be lost after brentuximab treatment**.

Abbreviations: *AML* acute myeloid leukemia, *ALCL* anaplastic large-cell lymphoma, *ALL/LBL* acute lymphoblastic leukemia/lymphoma, APL acute promyelocytic leukemia, *B-PLL* B-cell prolymphocytic leukemia, *CLL/SLL* chronic lymphocytic leukemia/small lymphocytic lymphoma, *CML* chronic myeloid leukemia, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *HCL* hairy cell leukemia, *LPL* lymphoplasmacytic lymphoma, *MAB* monoclonal antibody, *MCL* mantle cell lymphoma, *MZL* marginal zone lymphoma, *PMF* primary myelofibrosis, *TKIs* tyrosine kinase inhibitors

Chapter 5. Immunostains: Antibody Index – Solid Tumors

By Marina K Baine, Justin A. Bishop, Natasha Rekhtman

Common Multipurpose Immunostains at a Glance

ALK:
- ***Solid tumors*:** IMT, lung adenoCAs with *EML4-ALK* rearrangement;
- ***Heme*:** ALCL, rare ALK+ DLBCL, ALK + histiocytosis of infancy.

Calretinin: mesothelioma, adenomatoid tumor, cardiac myxoma, sex cord-stromal tumors, adrenocortical neoplasms

CD5:
- ***Solid tumors*:** thymic CA;
- ***Heme*:** CLL/SLL, mantle cell lymphoma, T-cell lymphomas and leukemia (but aberrant loss of CD5 is common), and others.

CD10 (CALLA):
- ***Solid tumors*:** HCC (canalicular pattern), RCC, pancreatic solid-pseudopapillary neoplasm, sex-cord stromal tumors, and endometrial stromal sarcoma;
- ***Heme*:** most B-lymphoblastic leukemia/lymphomas, some T-lymphoblastic lymphomas (B-ALL > T-ALL), follicular lymphoma, Burkitt lymphoma, some DLBCL, and neoplastic T lymphocytes in angioimmunoblastic T-cell lymphoma.

CD30 (Ki1):
- ***Solid tumors*:** embryonal CA;
- ***Heme*:** RS cells in classic Hodgkin lymphoma, anaplastic large cell (Ki1) lymphoma, subset of DLBCL (often EBV-related), and mycosis fungoides (associated with transformation).

CD34:
- Many soft tissue tumors
  - vascular tumors (angiosarcoma, Kaposi, etc.)
  - DFSP (+) vs. dermatofibroma (−)
  - GIST (70%+) vs. fibromatosis (−) vs. leiomyoma/leiomyosarcoma (−)
  - SFT (strong/diffuse +, also STAT6+) vs. synovial sarcoma (always –)
  - nerve sheath tumors (schwannoma, neurofibroma, MPNST)
  - epithelioid sarcoma (50%+)
  - adipocytic tumors
- Others
  - primitive leukemias (including myeloid, B and T cell – more common in B than T-ALL)
  - HCC ("sinusoidal capillarization") vs. benign hepatocellular nodules (−)
- **CD34-negative tumors**: carcinomas (except NUT carcinoma – about 50%+), melanoma, and mature lymphoma.

CD99/O13:
- ***Solid tumors*:** Ewing sarcoma (strong diffuse membranous staining – but relatively nonspecific marker as expressed in many other soft tissue tumors), sex cord-stromal tumors, solid pseudopapillary neoplasm of pancreas (punctate reactivity), immature T cells in thymoma, and always negative in neuroblastoma;
- ***Heme*:** B- and T-cell lymphoblastic leukemia/lymphoma (mature lymphomas are mostly negative).

C-kit/CD117:
- ***Solid tumors*:** GIST (95%), seminoma (membranous), thymic CA, some salivary neoplasms, melanoma (~30%), and others;
- ***Heme*:** blasts in acute myeloid leukemia and mast cell lesions.

D2-40: mesothelioma, hemangioblastoma, dermatofibroma, skin adnexal tumors, adrenocortical neoplasms, lymphatic endothelium and related endotheliomas, seminoma, embryonal CA (~30%+), nerve sheath tumors, and follicular dendritic cells and tumors

ER/PR: breast, uterus, and ovary (endometrioid CA ≫ serous CA), some skin adnexal tumors, cystic neoplasms with ovarian-type stroma (e.g., MEST/mixed epithelial stromal tumor of the kidney), meningioma (PR+), pancreatic solid-pseudopapillary neoplasm (PR+), and pancreatic NETs (PR+)

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ERG: vascular neoplasms (e.g., angiosarcoma, Kaposi sarcoma, epithelioid and pseudomyogenic hemangioendothelioma, etc.), tumors with *ERG* fusions (prostate adenoCAs with *TMPRSS2-ERG*, Ewing sarcoma with *EWSR1-ERG*), some epithelioid sarcomas (clone dependent), and some myeloid leukemias

GATA3: breast, urothelial, salivary gland, skin adnexal tumors (especially apocrine), trophoblastic tumors, chorioCA, many SqCCs (of any site), most mesotheliomas, pheochromocytomas/paragangliomas, neuroblastoma, subset of various other CAs (like pancreatic), and subset of peripheral T cell lymphomas. So, **GATA be careful**!

Glypican-3: HCC and hepatoblastoma, yolk sac tumor, and some chorioCAs

HMB-45: melanoma (non-desmoplastic), other melanosome-containing tumors: e.g., clear cell sarcoma/melanoma of soft parts, melanotic schwannoma, and PEComa-family tumors (e.g., angiomyolipoma, clear cell "sugar" tumor of the lung, lymphangioleiomyomatosis, and rare clear cell tumors of other sites)

Inhibin: adrenocortical neoplasms, sex cord-stromal tumors, trophoblastic tumors, hemangioblastoma, and granular cell tumor

Melan-A (A103 clone): melanoma (non-desmoplastic), other melanosome-containing tumors (same as for HMB-45 above), and steroid-rich tumors (adrenocortical neoplasms and sex cord-stromal tumors)

Napsin A: lung adenoCA, papillary RCC, and GYN clear cell CA

p63/p40: SqCC (any site), urothelial CA, myoepithelial, and trophoblastic neoplasms; also used to document the loss of basal/myoepithelial cells in prostate and breast CA. p40 (ΔNp63) is positive in all tumors where p63 expression is expected (SqCC, urothelial, trophoblastic, etc.), but it is much more specific. Unlike p63, p40 does not stain lung adenoCA (only rare cases are focal), lymphomas, and sarcomas. The only tumor that is consistently p63+/p40– is salivary polymorphous adenoCA.

PAX8: pan-RCC, thyroid, pan-Mullerian, and thymic tumors (only with polyclonal antibody)

S100: marker of neural crest-derived tumors (nerve sheath and melanocytic tumors) but also stains many other tumors – so S100 is also known as "Stains 100 things":
- ***Nerve sheath/glia***: schwannoma (diffuse), neurofibroma (focal), MPNST (focal), and gliomas;
- ***Melanocytes*****:** melanoma (including desmoplastic) and nevi;
- ***Soft tissue***: clear cell sarcoma, synovial sarcoma (30%), chordoma, chondrosarcoma, and lipomatous tumors;
- ***Histiocytes***: Langerhans cell histiocytosis, Rosai-Dorfman disease, and interdigitating dentritic cell sarcoma;
- ***Myoepithelial cells*****:** myoepithelioma, myoepithelial CA;
- *Others:* granular cell tumor, sustentacular cells in pheochromocytoma/paraganglioma, and olfactory neuroblastoma;
- Note: S100 is generally negative in CAs except some salivary and breast CAs (30%).

SF1: sex cord-stromal tumors, adrenocortical neoplasms, and some pituitary adenomas (gonadotrophs)

SOX10: like S100, primarily a marker of neural crest-derived tumors but also stains other tumors, many overlapping with S100:
- *Like S100,* positive in nerve sheath tumors, gliomas, melanomas, granular cell tumor, myoepithelial and some salivary tumors
- *Unlike S100,* negative in histiocytic and fibrohistiocytic proliferations, as well as synovial sarcoma and biphenotypic sinonasal sarcoma
- SOX10 is also a marker of breast CAs (enriched in triple-negatives)

SWI/SNF complex-related tumors – where loss by IHC is relevant to the diagnosis:
- **SMARCB1 (INI1/BAF47/hSNF5)**: pediatric malignant rhabdoid tumors (MRTs; renal, extrarenal, AT/RT), renal medullary CA, epithelioid sarcoma; subset of epithelioid MPNST, myoepithelial CAs (of soft tissue), extraskeletal myxoid chondrosarcoma, CAs with rhabdoid features (like sinonasal), and others
- **SMARCA4 (BRG1)**: INI1-proficient MRTs (very rare), SCCOHT (small cell CA of the ovary, hypercalcemic type), and various CAs with rhabdoid/undifferentiated features (esp. endometrial)
- **SMARCA2** (**BRM**): typically co-deficient with SMARCA4 in MRTs and SCCOHT

TFE3: Xp11-translocation RCC, alveolar soft part sarcoma, granular cell tumor, subset of renal angiomyolipomas, and other PEComas

TTF-1: lung (75% of non-mucinous adenoCAs), thyroid (all types except anaplastic CA), SmCC of the lung (~90%+), SmCC of various extrapulmonary sites. Watch out – SPT24 clone labels a subset of unexpected tumors, usually weakly/focally (GYN, breast, colon CAs, and many gliomas); 8G7G1 clone is more specific.

WT1: mesothelioma, serous ovarian CA, Wilms tumor, intraabdominal desmoplastic small round cell tumor (clone-dependent), and CIC-DUX sarcomas

Immunostains Where Antibody Clone Matters, Sometimes a Lot

By Justin A. Bishop and Natasha Rekhtman

Antigen	Antibody	Epitope (if pertinent)	Comment
TTF-1	8G7G3/1		– Less sensitive, more specific – Generally recommended in thoracic literature
	SPT24		– More sensitive, less specific – Stains a variety of non-lung CAs and many GBMs References: [1–3]
	SP141		New clone, little is known. Seems to be similar to SPT24 for sensitivity but even more nonspecific (e.g., stains 10% of mesotheliomas, mostly sarcomatoid). Reference: [4]
PAX8	Polyclonal	Binds N-terminal 212 amino acids. Includes 128 amino acids of DNA-binding domain common to all PAX transcription factors	Stains: – Endocrine neoplasms (PanNETs, GI NETs)- cross reaction with PAX6 – B-cell lymphoma-cross reaction with PAX5 – Thymomas/thymic CAs – positive for polyclonal but not monoclonal PAX8 References: [5, 6]
	PAX8R1	Binds C-terminal amino acids 318-426	Similarly sensitive for thyroid, renal, and GYN tract CAs but more specific
Napsin A	Monoclonal (TMU-Ad02, IP64)		Similarly sensitive, more specific
	Polyclonal		More nonspecific staining, particularly in mucinous CAs of any site Reference: [7]
Ki67	MIB1		– Standardized for use in breast CA and NETs – MIB1 antibody only is strongly membranous positive in hyalinizing trabecular adenoma of thyroid.
	Ki67 (30-9) and others		– Significant variability in staining in breast CA has been documented, though the significance of the variability is unclear. References: [8, 9]
ER	1D5		All 3 antibodies are endorsed for the use in breast predictive testing by CAP/ASCO [10], where their performance is comparable. Outside of breast, though, 6F11 and SP1 are less specific for breast CAs than 1D5. Both 6F11 and even more so SP1 stain some non-breast CAs: 6F11 stains 5–20% lung adenoCA, and SP1 stains up to 30%. 1D5 is most specific but may also stain non-breast CAs – use as part of a panel and not as a single marker. References: [11–13]
	6F11		
	SP1		
WT1	WT49, 6F-H2	C-terminus	Only this clone stains desmoplastic small round cell tumors (DSRCT). References: [14, 15]
	Polyclonal	N-terminus	
ERG	EPR3864	C-terminus	Similarly sensitive but more specific for vascular tumors
	9FY	N-terminus	Much more likely to stain epithelioid sarcoma (40–60%) References: [16, 17]
ALK	ALK1		Less sensitive. Okay for ALCL and IMT but not for lung cancer fusion testing.
	D5F3		More sensitive. Can be used for all of the above. Companion diagnostic for ALK fusion testing in lung cancer. Reference: [18]
CEA	mCEA (monoclonal)		Much more specific for medullary CA and C cells in the thyroid.
	pCEA (polyclonal)		Canalicular staining in HCC (not seen with monoclonal)

Alphabetical Antibody Index: Solid Tumors

By Marina K Baine, Justin A. Bishop, Natasha Rekhtman

Antibody or antigen (other names)	Cellular localization	Normal tissues stained and functional information if pertinent	What this marker is used to identify and differential diagnosis
2SC (S-(2-succino)cysteine)	Cytoplasmic	not found in normal cells/tissues; marker of aberrant protein succination detected in fumarate hydratase deficient cells/tissues	– Hereditary leiomyomatosis and RCC-associated RCC – HLRCC (+); in conjunction with FH (fumurate hydratase) loss.
4A4 → see p63			
34ßE12 → see Cytokeratins			
α1-antitrypsin (AAT-1)	Cytoplasmic	Hepatocytes, histiocytes	– Globules of AAT (which accumulate in the liver in AAT deficiency); – HCC (HepPar-1 is more specific); – Histiocytic lesions (CD68 is more specific).
A103 → see Melan-A			
Actins: **α-actin** (Smooth muscle actin, SMA)	Cytoplasmic	Smooth muscle, myoepithelial cells, myofibroblasts	– Smooth muscle differentiation (skeletal muscle is negative); – Myoepithelial cells/differentiation (e.g., myoepithelial CA, layer present in benign, or in situ breast lesions/absent in invasive breast CA).
Actins: **Muscle-specific actin** (MSA; actin, HHF-35)	Cytoplasmic	Smooth, skeletal, and cardiac muscle, myoepithelial cells	– Smooth and skeletal muscle differentiation (less sensitive for smooth muscle than α-actin).
Adipophilin (ARP)	Membranous staining of cytoplasmic vesicles	Lipid vesicles within sebaceous cells	– Sebaceous CA and sebaceous variants of CAs (e.g., salivary gland); – ccRCC may stain, but in a nonspecific, granular cytoplasmic pattern. References: [19, 20]
AE1/AE3 → see Cytokeratins			
AFP (Alpha fetoprotein)	Cytoplasmic	Fetal liver	– Yolk sac tumor (not specific, also present in some embryonal CA); – HCC except fibrolamellar variant (not specific, HepPar-1, arginase-1, albumin RNA ISH are better markers); – Hepatoblastoma (particularly helpful as a serum marker, where it is usually very high).
ALK (Anaplastic lymphoma kinase; p80) Also see heme index	Nuclear, cytoplasmic, membranous	Few neuronal cells	– Inflammatory myofibroblastic tumor (~60%, cytoplasmic); – Lung adenoCA with *EML4-ALK* rearrangement; – ALCL (~70%); other lymphomas are (−), except rare DLBCL are (+); – Expression is a result of t(2;5)/*NMP-ALK* and other *ALK* translocations. Most commonly ALK is both nuclear and cytoplasmic, but location depends on the type of translocation.
AMACR (Alpha-methylacyl-CoA reductase; p504S; racemase)	Cytoplasmic	Prostatic CA and PIN (normal prostate is negative)	– Prostate cancer (+) vs. adenosis and other benign mimics (−). See Chapter 2 for false-positives and false-negatives; – Not specific for the prostate; also (+) in other CAs such as the lung, breast, papillary RCC, and clear cell adenoCA of bladder.
Androgen receptor (AR)	Nuclear	Prostate, normal apocrine glands	– Salivary duct CA; – Juvenile nasopharyngeal angiofibroma; – Prostate adenoCA (not diagnostically useful); – Subset of triple-negative breast CA (20–50%) (not diagnostically useful); – Not very specific; positive in any tumor with apocrine differentiation.
Arginase-1	Cytoplasmic	Normal hepatocytes	– Sensitive and specific marker of hepatocellular differentiation and HCC [21].
ATRX (Alpha-thalassemia/ mental retardation syndrome X linked)	Nuclear	Brain/CNS	– Used in subclassification of gliomas in conjunction with p53 and 1p/19q codeletion: • Astrocytoma (ATRX–, p53+, no 1p/19q codeletion) vs. • Oligodendroglioma (ATRX+, p53–, 1p19q codeleted).
BAP1 (BRCA-associated protein 1)	Nuclear	All normal cells	– DDx mesothelioma (lost) vs. benign mesothelial proliferations (retained) [22]; – May be used to help ID patients with germline *BAP1* mutations (BAP1 hereditary cancer predisposition syndrome), in whom germline testing should be pursued in the correct clinical setting [23].
B72.3	Cytoplasmic, membranous	Secretory endometrium	– AdenoCA (+) vs. mesothelioma (−) – a second-line marker.

Alphabetical Antibody Index: Solid Tumors – Continued

β-catenin	Only nuclear staining is significant	Cytoplasm of most cells, where it binds to APC (adenomatous polyposis coli) protein. This interaction regulates degradation of β-catenin. If interaction is disrupted (as a result of mutation in either APC or β-catenin) → β-catenin accumulates and can be detected by IHC in the nucleus. Endothelial cells serve as a good internal positive control for staining	– Colon CA, pancreatic solid-pseudopapillary neoplasm; craniopharyngioma; pancreatoblastoma; hepatoblastoma; sinonasal glomangiopericytoma; – Familial adenomatous polyposis (FAP)-associated tumors (e.g., deep [desmoid] fibromatosis, nasal angiofibroma, fundic gland polyps, cribriform-morular variant of papillary thyroid CA); – DDx tubular adenoma (+) vs. dysplasia-associated lesion or mass (DALM) (−).
β-HCG → see Human chorionic gonadotropin			
BAF47 → see INI1			
BCL1 → see Cyclin D1			
BCL2 (B-cell lymphoma 2) Also see heme index	Membranous and cytoplasmic	Inhibits apoptosis. Normally present in mantle cells and turns OFF in a germinal center	– Synovial sarcoma and some CD34+ tumors (SFT, GIST), but specificity is low; – FL (+) vs. reactive follicles (−).
BCL10 Also see heme index	Cytoplasmic	Pancreas	– Pancreatic acinar cell CA; – MALT lymphoma.
BCOR (B-cell CLL/lymphoma 6 (BCL6)-interacting co-repressor)	Nuclear	Transcription repressor required for germinal center formation, not expressed in normal tissue	– *BCOR*-rearranged sarcomas; – High-grade ESS (+) vs. low-grade ESS or stromal nodule (−); – Clear cell sarcoma of the kidney.
Ber-EP4 (Anti-EpCAM antibody)	Membranous	Epithelial cells	– AdenoCA in general (similar to EMA); – DDx adenoCA (+) vs. mesothelioma (−);
BG8	Cytoplasmic	RBC	– AdenoCA (+) vs. mesothelioma (−) – a second-line marker.
Brachyury	Nuclear	Transcription factor involved in notochord development. Expressed in some normal spermatogonia	– Chordoma; – Hemangioblastoma.
BRG1 (Brahma-related gene 1; SMARCA4)	Nuclear	Enzymatic domain of the SWI/SNF chromatin remodeling complex; expressed in epithelia of multiple organs and in the B germinal centers of the spleen and tonsil (remodeling and proliferating cell types)	– Loss of expression in: • Malignant rhabdoid tumors (renal, extrarenal) and subset of AT/RT with retained INI1; • Small cell CA of the ovary, hypercalcemic type (SCCOHT) [24]; • Various undiff./de-diff. CAs (endometrium, pancreas, lung) [25–29].
BRST2 → see GCDFP-15			
BSAP (B-cell-specific activator protein) → see PAX-5			
CAIX (Carbonic anhydrase)	Membranous	Ischemic tissues (expression related to hypoxia inducible factor)	– ccRCC (among renal tumors, diffuse CAIX is very sensitive and specific for ccRCC, but focal expression may be seen in various tumors in areas of necrosis/ischemia such as in papillary RCC); – Clear cell papillary RCC (cup-like staining with sparing of apical membrane).
CA-125	Luminal	Many cell types	– Serum marker for monitoring ovarian cancer, but is not specific for ovary by IHC (many other tumors are positive).
CA 19-9 (Carbohydrate antigen 19-9)	Cytoplasmic	Many cell types	– Serum marker for monitoring pancreatic and GI cancers, but is not specific for these sites by IHC (many other tumors are positive).
Calcitonin	Cytoplasmic and extracellular	C cells of the thyroid	– Medullary CA of the thyroid (not entirely specific, can be positive in other NE tumors).
Caldesmon (h-Caldesmon)	Cytoplasmic	Smooth muscle and myoepithelial cells, negative in myofibroblasts	– Leiomyosarcoma (+) vs. myofibroblastic lesions, such as fibromatosis (−).
CALLA (common acute lymphoblastic leukemia antigen) → see CD10			
Calponin	Cytoplasmic	Smooth muscle and myoepithelial cells, variable in myofibroblasts	– Same as α-actin but variable in myofibroblasts.

Alphabetical Antibody Index: Solid Tumors – Continued

Calretinin (CRT)	Cytoplasmic and nuclear	Mesothelium, sex-cord stromal cells, some neural and epithelial cells	– Mesothelioma; – Adrenocortical tumors; – Sex cord-stromal tumors; – Adenomatoid tumor; – Olfactory neuroblastoma; – Cardiac myxoma.
Cam5.2 → see Cytokeratins			
CAMTA1 (Calmodulin-binding transcription activator 1)	Nuclear	Normal brain	– Epithelioid hemangioendothelioma [30].
Cathepsin K	Cytoplasmic	Osteoclasts	– MiT family translocation RCCs (TFE3 [Xp11 translocation] and TFEB [t(6;11) translocation]); – PEComas; – Chordoma.
CD1a Also see heme index	Membranous	Thymocytes (immature T cells), Langerhans cells	– Admixed thymocytes in thymoma (CD1a, TdT, CD99+); – Langerhans cell histiocytosis; – Some T-cell lymphoblastic lymphomas.
CD5 Also see heme index	Membranous	T cells and subset of B cells (naïve B cells)	– Thymic CA (+ in epithelial cells) vs. thymoma (−); – Thyroid CASTLE (+ in epithelial cells) vs. other thyroid CAs (−); – CLL/SLL, MCL (including blastoid); – Aberrant loss in some T-cell lymphomas.
CD10 (CALLA, common acute lymphoblastic leukemia antigen) Also see heme index	Membranous	Liver canaliculi, myoepithelial cells, endometrial stroma, precursor B and T cells, germinal center B cells, granulocytes	– HCC (+ canalicular pattern) vs. cholangioCA (−); – RCC; – Pancreatic solid pseudopapillary neoplasm; – Sex cord-stromal tumors; – Endometrial stromal sarcoma; – Myoepithelial cells (p63/p40 or SMMHC are better markers); – DDx of atypical fibroxanthoma (+) vs. sarcoma, sarcomatoid CA, and melanoma of the skin (−); – CD10 (+) lymphomas: B-ALL > T-ALL, FL, Burkitt lymphoma, some DLBCL; neoplastic T lymphocytes in angioimmunoblastic T-cell lymphoma.
CD15 Also see heme index	Membranous and golgi (paranuclear dot-like)	Endothelial cells and some CAs; monocytes, myelocytes, granulocytes	– AdenoCA (+) vs. mesothelioma (−); – Sebaceous cells (second-line marker, adipophilin is better); – R-S cells in classic HL (most +).
CD30 (Ki-1) Also see heme index	Membranous and golgi (paranuclear dot-like)	Activated B (immunoblasts) and T lymphocytes, plasma cells, some non-heme cells	– Embryonal CA (+) vs. other germ cell tumors (−); – R-S cells in classic Hodgkin lymphoma, ALCL, subset of DLBCL (often EBV-related) and mycosis fungoides (associated with transformation).
CD31	Cytoplasmic and membranous	Endothelial cells, megakaryocytes, macrophages, and others	– Endothelial differentiation (e.g., angiosarcoma, Kaposi sarcoma); more sensitive and specific than CD34.
CD34	Cytoplasmic and membranous	Endothelial cells, fibroblasts, and hematopoietic blasts (stem cells)	– Many soft tissue tumors: • Vascular tumors (angiosarcoma, Kaposi, etc.); • DFSP (+) vs. dermatofibroma (−); • GIST (70%+) vs. fibromatosis (−) vs. leiomyosarcoma (−); • SFT (strong/diffuse +) vs. synovial sarcoma (always –) nerve sheath tumors (schwannoma, neurofibroma, MPNST); • Epithelioid sarcoma (50%+); • Adipocytic tumors. – Others: • Primitive leukemias (including myeloid, B and T cell – more common in B- than T-ALL); • HCC ("sinusoidal capillarization") vs. benign hepatocellular nodules (−). – **CD34-negative tumors**: CA (except NUT CAs – about 50%+), melanoma, lymphoma (except ALL/lymphoblastic lymphoma). Reference: [31]
CD44	Membranous	Normal urothelium (basal layer), also considered a cancer stem cell marker	– Normal urothelium (+ in basal layer) vs. reactive urothelium (+ in all layers) vs. CIS (− or reduced).
CD56 (NCAM, neural cell adhesion molecule) Also see heme index	Membranous	Neuroendocrine cells, Schwann cells, NK cells	– Neuroendocrine neoplasms. Particularly useful to identify SmCC, which may be nonreactive for other neuroendocrine markers (SYN, CHR); – Nasal-type NK/T-cell lymphoma and neoplastic plasma cells.

Alphabetical Antibody Index: Solid Tumors – Continued

CD57 Also see heme index	Membranous	Neuroendocrine cells, Schwann cells, NK-like T cells	– Metanephric adenoma of the kidney; – Neuroendocrine neoplasms and some nerve sheath tumors; – T-cell large granular cell leukemia and T-cell "rosettes" surrounding LP cells of NLP-HL.
CD68 Also see heme index	Cytoplasmic, membranous	Lysosomal marker in histiocytes/macrophages/monocytes, granulocytes, and others	– Histiocytic differentiation; – Fibrolamellar HCC; – Myeloid sarcoma (AML with monocytic differentiation).
CD99 (MIC2, O13)	Membranous (more specific reactivity) and cytoplasmic	Immature T cells including cortical thymocytes, various epithelial cells, endothelial cells	– Ewing sarcoma (strong diffuse membranous staining), but many other sarcomas, particularly small round cell tumors of childhood, are also (+). Neuroblastoma is always (−); – Sex cord-stromal tumors; – Solid pseudopapillary neoplasm of pancreas (punctate reactivity) [32]; – Thymoma: admixed immature T cells (CD99+, TdT+); – B- and T-cell lymphoblastic leukemia/lymphoma; mature lymphomas are mostly (−).
CD117 → see c-kit			
CD138 Also see heme index	Membranous	Squamous epithelium and plasma cells	– Plasma cell differentiation; – Many CAs (such as SqCC).
CD141 → see Thrombomodulin			
CD146 **(MelCAM)**	Membranous	Intermediate trophoblast, smooth muscle, vascular endothelial cells	– Tumors of implantation site intermediate trophoblast: exaggerated placental site and placental site trophoblastic tumor; – ChorioCA; – Melanoma; – Mesothelioma (+) vs. reactive mesothelial proliferation (−): not used in practice.
CD163 Also see heme index	Membranous	Member of scavenger receptor cysteine-rich superfamily restricted to the monocyte/macrophage line	– Histiocytic differentiation.
CDH17 (Cadherin 17)	Membranous	Tubular GI tract	– Recent marker of adenoCAs of the lower GI tract but also marks gastroesophageal and some pancreaticobiliary adenoCAs [33]; – Retained in poorly differentiated adenoCAs [34], making it particularly useful for the diagnosis of CA of unknown primary.
CDK4 (Cyclin-dependent kinase 4)	Nuclear	Dividing cells	– Well-diff. and de-diff. liposarcoma (CDK4+, MDM2+) vs. benign lipomatous tumors and other sarcomas (−) [35].
CDX2	Nuclear	Intestine (from duodenum to rectum)	– Strong/diffuse expression in intestinal CAs; – Variable/focal expression in gastric and pancreaticobiliary CAs; – Variable in CAs with enteric phenotype (e.g., mucinous ovarian CA, intestinal sinonasal adenoCA, and adenoCA of urinary bladder); – Subset of neuroendocrine tumors (carcinoids) of GI tract; – Yolk sac tumor (40%) [36].
CEA (Carcinoembryonic antigen)	Cytoplasmic	Fetal tissues and glandular epithelium (strongest in mucin-secreting glandular tissues)	– AdenoCA (mCEA+) vs. mesothelioma (mCEA–); – HCC (canalicular pCEA) vs. cholangioCA and metastatic adenoCA (cytoplasmic pCEA); – AdenoCA in general (e.g., lung, colon, pancreas); – Medullary thyroid CA (mCEA).
c-erB → see HER2			
Chromogranin A (CHR)	Cytoplasmic (granular)	Neurosecretory granules in neuroendocrine tissues and neurons	– Neuroendocrine differentiation (pheochromocytoma, carcinoid/NET, SmCC, Merkel cell CA, etc.).
Chymotrypsin → see Trypsin			
CK5/6 → see Cytokeratins			
CK903 (34ßE12, K903) → see Cytokeratins			
c-kit (CD117, stem cell factor receptor)	Cytoplasmic and membranous	Interstitial cells of Cajal (origin of GIST), germ cells, hematopoietic progenitor cells, mast cells	– GIST (95%+, diffuse staining) vs. leiomyoma and schwannoma (−); – Seminoma (membranous); – Thymic CA (+) vs. thymoma (−); – Salivary gland tumors: luminal epithelium in tumors with epithelial and myoepithelial components; – Melanoma (30–40%+; predominantly uveal, acral and mucosal); – Sclerosing mesenteritis; – PEComas; – Chromophobe RCC and oncocytoma; – Blasts in acute myeloid leukemia (some); – Mast cell lesions.

Alphabetical Antibody Index: Solid Tumors – Continued

Claudin-4	Membranous	All epithelial cells	– DDx CA (+) vs. mesothelioma (−). Currently considered the best marker for this application; – DDx poorly diff. CAs (+) vs. various sarcomas with epithelioid morphology (−).
Clusterin	Cytoplasmic	Follicular dendritic cells	– Follicular dendritic cell tumors; – HCC (enhanced canalicular staining) vs. benign hepatocellular nodules such as adenoma, FNH, and cirrhotic nodule (cytoplasmic); – Also tenosynovial giant cell tumors, pancreatic NE tumors, and many others.
CYTOKERATINS			
AE1/AE3 (Pan-cytokeratin cocktail)	Cytoplasmic	Most epithelial cells	– Used in conjunction with Cam5.2 to screen for CA (see "Epithelial Markers" section for details): IDs all CAs except HCC, RCC, adrenocortical CA, and some high-grade neuroendocrine CAs.
Cam5.2	Cytoplasmic	Low-molecular weight keratins present in simple (non-squamous) epithelia	– Used in conjunction with AE1/AE3 to screen for CAs but is usually negative in SqCC; – Particularly useful to ID CAs that are negative for AE1/AE3, most notably HCC (negative for AE1/AE3, CK903, and EMA) and some undiff. CAs; – Paget disease (+) vs. Bowen disease/SqCC in situ (−).
CK7	Cytoplasmic	A specific LMW cytokeratin	– CK7 and 20 are used in combination to narrow the differential of CA of unknown primary: • CK7 is generally positive in above-the-diaphragm CAs (lung, breast, thyroid) and GYN organs. • CK20 is generally positive in below-the-diaphragm CAs (colon CA), and in Merkel cell CA. • CK7 and CK20 are co-expressed in peri-diaphragmatic organs (pancreas, stomach) and the bladder. • Negative for both are simple visceral organs (liver, kidney, prostate). (See 7/20 diagram in Chap. 1 for details). – CK7: Barrett mucosa (+) vs. intestinal metaplasia in gastric cardia (−); – CK20: urothelial CIS (+ in all layers) vs. reactive urothelium (+ in umbrella cell layer only).
CK20	Cytoplasmic	A specific LMW cytokeratin	
CK903 (34ßE12, K903)	Cytoplasmic	High molecular weight keratin present in stratified epithelia (squamous, urothelial, respiratory) plus myoepithelial and basal cells	– Urothelial (+) vs. prostate (−) CA; – Prostatic basal cells (loss of staining indicates CA); – UDH (+) and ALH/LCIS (+) vs. DCIS (−); – Metaplastic breast cancer (+).
CK5/6	Cytoplasmic	Two specific HMW keratins	– SqCC (+) and mesothelioma (+) vs. adenoCA (−); – Prostatic basal cells and metaplastic CA (similar to CK903).
CRP (C-reactive protein)	Cytoplasmic	Synthesized in the liver	– Together with SAA, aids in the DDx of hepatic adenomas: inflammatory adenoma (+) vs. other adenoma subtypes and FNH (−).
CRX (Cone-rod homeobox-containing gene)	Nuclear	Transcription factor; preferentially expressed in retinal photoreceptor cells	– Retinoblastoma (sensitive but not entirely specific). Also positive in ~ 40% of medulloblastomas and focally positive in some pineal tumors [37].
Cyclin D1 (BCL1) Also see heme index	Nuclear	Dividing cells	– Sarcomas (visceral and soft tissue): clear cell sarcoma of the kidney, *BCOR*-rearranged sarcoma, high-grade endometrial stromal sarcoma (>70%); – Mantle cell lymphoma, including blastoid variant.
Cyclin E	Nuclear	Dividing cells	– Primary utility: placental site nodule (−) vs. epithelioid trophoblastic tumor (+).
Desmin (DES)	Cytoplasmic	Intermediate filament in smooth, striated, and cardiac muscle	– Smooth and skeletal muscle differentiation in tumors; – Reactive mesothelial cells (+) vs. mesothelioma (−). Low specificity.
D2-40 (Poloplanin)	Membranous	Marker of mesothelial cells, germ cells, lymphatic endothelial cells, FDCs	– Mesothelioma (+) vs. adenoCA (−); – Hemangioblastoma (+) vs. RCC (−); – Dermatofibroma (+) vs. DFSP (−); – Primary skin adnexal tumors (+) vs. metastatic adenoCA (−); – Adrenocortical neoplasms (+) vs. RCC (−); – Lymphatic endothelium and related endotheliomas (+); – Seminoma (100%+) and embryonal CA (30%+, apical staining only); – Nerve sheath tumors: schwannoma and MPNST; – Follicular dendritic cell tumors. References: [38–40]
DPC-4, clone B8 (Deleted in pancreatic carcinoma, SMAD4)	Nuclear and cytoplasmic	Most normal tissues	– Pancreatic CA – 55% of invasive cancer exhibits loss of expression. Both nuclear and cytoplasmic staining must be negative to count. Loss of expression is fairly specific to pancreas but is also seen in a subset of colon CA.

Alphabetical Antibody Index: Solid Tumors – Continued

DOG1 (Discovered on GIST-1)	Membranous and cytoplasmic	Interstitial cells of Cajal	– GIST (reportedly better sensitivity and specificity than c-kit); – Stains ~1/3 of c-kit-negative GISTs; – Acinic cell CA of the salivary gland. Reference: [41]
E-cadherin (CAD-E)	Membranous	Breast – normal ductal and lobular cell (functions as adhesion molecule)	– In situ and invasive lobular CA (−) vs. ductal lesions (+); – Pancreas: NET (+) and acinar cell CA (+) vs. solid pseudopapillary neoplasm (−); – Loss of expression also seen in gastric signet-ring CA and undifferentiated pancreas CA (note that neoplasms with the loss of E-cadherin are discohesive, which are consistent with its role as adhesion molecule).
EMA (Epithelial membrane antigen, MUC1)	Cytoplasmic or membranous	Epithelial, perineurial, meningothelial cells	– CAs in general, synovial sarcoma, epithelioid sarcoma, chordoma (used in conjunction with CKs); – EMA(+)/CK(−) tumors: meningioma, perineurioma, plasma cell neoplasms, ALCL, LP cells in NLP-HL; – Mesothelioma (strong membranous staining) vs. adenoCA (cytoplasmic staining) – rarely used in practice.
Estrogen receptor (ER) and **Progesterone receptor (PR)**	Nuclear	Breast, ovary, endometrium	– Breast CA (~60%); – Tumors of the uterus and ovary (endometrioid > serious); cervical tumors are ER/PR-negative; – (+) in few non-mammary/non-GYN tumors: ~5% of lung adenoCAs, skin adnexal tumors, cystic neoplasms with ovarian-type stroma (e.g., mixed epithelial stromal tumor), meningioma (PR+), pancreatic solid pseudopapillary neoplasm (PR+), PanNET (PR+).
ERG (ETS-related gene)	Nuclear	Endothelial cells	– Sensitive and specific marker of vascular neoplasms (e.g., angiosarcoma, Kaposi sarcoma, epithelioid, and pseudomyogenic hemangioendothelioma); – Tumors with *ERG* fusions (prostate adenoCA with *TMPRSS2-ERG* – ~50% of prostate CA; Ewing sarcoma with *EWSR1-ERG* fusion); – Some epithelioid sarcomas (clone-dependent); – Some myeloid leukemias.
ETV4 (ETS translocation variant 4, PEA3)	Nuclear	Transcription factor, low-level expression in normal adult tissue	– Round cell sarcoma with *CIC* rearrangement.
Factor VIII (Factor VIII-related antigen, vWF) Also see heme index	Cytoplasmic	Endothelial cells, megakaryocytes, platelets	– Endothelial differentiation – specific but not sensitive; – AML with megakaryocytic differentiation (formerly FAB M7).
Factor XIIIa	Cytoplasmic	Histiocytes, fibrohistiocytic cells, other	– Dermatofibroma (+) vs. DFSP (−); – Histiocytic differentiation (a pan-histiocytic marker, similar to CD68); – Sinonasal glomangiopericytoma (often with peculiar nuclear localization).
FH (Fumarate hydratase)	Cytoplasmic	Krebs cycle enzyme, expressed in all normal epithelial cells and other cell types	– HLRCC-associated RCC (loss by IHC); in conjunction with strong +2SC (S-(2-succino)cysteine) staining by IHC; – May also be of utility in cutaneous leiomyomas to screen for HLRCC syndrome [42, 43] (FH loss in uterine leiomyomas can be both syndromic and sporadic, so the role in screening for HLRCC is more limited).
Filamin A (FLNA; actin-binding protein 280, ABP-280)	Cytoplasmic	Cytoskeletal protein; selectively expressed in smooth muscle, myoepithelial, squamous epithelial and subsets of lymphoid cells	– Used in conjunction with GAB1, YAP1, and β-catenin IHC to predict molecular subtype of medulloblastoma (SHH activated: GAB1+, YAP1+, filamin A+ vs. WNT activated: GAB1–, YAP1+, nuc β-catenin+, filamin A+ vs. non-SHH/WNT: negative for all). References: [44, 45]
Fli-1 (Friend leukemia integration 1)	Nuclear	Endothelial cells, many other cell types	– Endothelial differentiation; – Ewing sarcoma with *EWSR1-FLI1* translocation – not specific, present in many tumors.
FOXL2 (Forkhead Box L2)	Nuclear	Ovarian granulosa cells	– Ovarian sex cord-stromal tumors and some testicular granulosa cell tumors; – Pituitary adenoma (87% of gonadotrophs and 73% null cell) [46].
GAB1 (GRB2-associated binding protein)	Cytoplasmic	Near ubiquitous expression; most prominent in the brain and testes	– Used in conjunction with filamin A, YAP1, and β-catenin IHC to predict molecular subtype of medulloblastoma (see filamin A above for details).
Gastrin	Cytoplasmic	G cells	– G-cell hyperplasia in autoimmune metaplastic atrophic gastritis; – Gastric antrum (+) vs. “antralized” body (−).

Alphabetical Antibody Index: Solid Tumors – Continued

GATA3 (Transcription factor named for binding to "GATA" nucleotide motif in gene promoters) Also see heme index	Nuclear	Breast, bladder, placental trophoblast	– Breast CA (>90%); – Urothelial CA (almost all); – Salivary gland and skin adnexal tumors (especially apocrine); – Parathyroid neoplasms; – Trophoblastic tumors, esp. chorioCA; – Many SqCCs (of any site) and mesotheliomas; – Pheochromocytomas/paragangliomas and neuroblastomas.
GCDFP-15 (Gross cystic disease fluid protein-15, BRST2)	Cytoplasmic	Apocrine cells of the breast and sweat glands	– Breast CA (~60%+, only 10% of triple negative). Staining is notoriously focal. Stains lobular CA best; – Other tumors with apocrine differentiation: tumors of salivary gland (especially salivary duct CA) and skin adenxa.
GFAP (Glial fibrillary astrocytic protein)	Cytoplasmic	Glial, myoepithelial and Schwann cells	– Gliomas (astrocytoma, ependymoma; oligodendroglioma may be focal), myoepithelial neoplasms, some schwannomas.
GLUT1 (Glucose transporter 1)	Membranous	RBCs and many tissues	– Mesothelioma (+) vs. reactive mesothelium (−) – not used in practice; – Thymic CA (+) vs. thymoma (−), not specific; – Juvenile hemangioma (+) vs. other benign vascular lesions (−); – Perineurioma.
Glutamine synthetase (GS)	Cytoplasmic	Produced in the liver	– DDx of hepatic adenomas (strong and diffuse in β-catenin activated type, which has the highest malignant potential) and FNH ("map-like").
Glypican-3 (GPC3)	Cytoplasmic, membranous, and canalicular	Embryonic liver, placenta (syncytiotrophoblasts)	– HCC and hepatoblastoma; – Yolk sac tumor and some chorioCAs [47]; – HCC (+) vs. benign hepatic nodules (−).
GRIA2	Cytoplasmic	CNS, epithelium of skin, upper respiratory tract, GI tract, breast, and bladder; not expressed in normal fibroblasts	– Solitary fibrous tumor (new marker); can also stain DFSP and occasional myoepitheliomas [48].
H3K27me3 (Histone H3 trimethylated at lysine 27)	Nuclear	Epigenetic marker of gene silencing	– Complete loss in ~50% of MPNSTs; not entirely specific; – H3K27M is a different antibody from H3K27me3, which is used to detected mutations in this gene in brain tumors (see Chapter 10).
HBME-1	Cytoplasmic and membranous	Epithelial and mesothelial cells	– Mesothelioma (+) vs. adenoCA – not specific; – Thyroid CA (+/−) vs. benign follicular lesions (−/+) – not specific.
HepPar-1 (Hepatocyte paraffin 1; OCH1E5)	Granular cytoplasmic	Mitochondria in normal hepatocytes, small intestinal epithelia	– Hepatocellular differentiation: HCC (90%+), hepatoblastoma, and CAs with hepatoid phenotype (gastric, other).
HER2 (Her2Neu)	Membranous and cytoplasmic	Growth factor receptor which is only weakly expressed in normal epithelial cells	– To evaluate breast CAs: overexpression is a poor prognostic sign but can be treated with Herceptin (only membranous reactivity counts); – Also used to evaluate stomach and GE junction adenoCAs for treatment selection and prognostication; – Generally not used to ID metastatic breast cancer because HER2 is overexpressed in several non-mammary CAs, such as those in the lung and GYN tract.
HHF-35 → see Actins			
HLA-G (Human leukocyte antigen G; MHC-G, major histocompatibility complex, class I, G)	Membranous	Extravillous and intermediate trophoblast cells in placenta	– Sensitive and specific for intermediate trophoblast (IT) in all gestational trophoblastic tissues, including tumors (negative in cytotrophoblasts and syncytiotrophoblasts); – DDx chorioCA (+ in IT) vs. other germ cell tumors or gestational trophoblastic diseases (−); ancillary marker (not used as fist line).
HMB45 (Human melanoma, black)	Cytoplasmic	Immature melanocytes (negative in mature melanocytes such as those present at the base of normal nevi)	– Melanoma (epithelioid > spindle cell) and other melanosome-containing tumors, including clear cell sarcoma/melanoma of soft parts, melanotic schwannoma, PEComa-family tumors (angiomyolipoma, clear cell "sugar" tumor of the lung, lymphangioleiomyomatosis, and rare clear cell tumors of other sites); – Melanoma (+) vs. nevus (−). Nevus shows progressive diminution of HMB45 as cells mature toward the base; – Metastatic melanoma (+) vs. benign nevus inclusion (−) in a lymph node.
hMLH1, hMSH2, hMSH6 → see under MLH1 (Human *mutL* homolog 1 and human *mutS* homolog 1 and 6) – genes encoding mismatch repair proteins MLH1, MSH2, MSH6			

Alphabetical Antibody Index: Solid Tumors – Continued

HNF-1β (Hepatocyte nuclear factor)	Nuclear	Hepatocytes	– Clear cell CA of the GYN tract and bladder.
hSNF5 → see INI1			
HSP70 (Heat shock protein 70)	Nuclear	Ubiquitous; stress induced	– HCC (+) vs. benign hepatocellular nodules such as adenoma or FNH (−) (same pattern as with glypican-3).
Human chorionic gonadotropin (hCG beta chain; βHCG)	Cytoplasmic	Syncytiotrophoblasts	– ChorioCA (stains syncytiotrophoblasts); – Detects syncytiotrophoblastic giant cells in seminoma (better prognosis than chorioCA) and some CAs – those are associated with elevated serum HCG.
Human placental lactogen (hPL)	Cytoplasmic	Trophoblasts (syncytiotrophoblasts and intermediate trophoblasts)	– ChorioCA (stains syncytiotrophoblasts and intermediate trophoblasts), tumors of intermediate trophoblasts (placental site tumors), moles.
IDH1 R132H (Isocytrate dehydrogenase 1, IDH1)	Nuclear	Mutated form of IDH1 specific for high-grade gliomas; not expressed in normal tissue	– Used in diagnosis, classification, and prognostic stratification of high-grade gliomas.
IgG4	Cytoplasmic	Subset of plasma cells	– Increased number of IgG4+ plasma cells in a spectrum of fibrosclerosing diseases including autoimmune pancreatitis, chronic sclerosing sialadenitis (Kuttner tumor), and sclerosing mesenteritis (though criteria for what qualifies as "increased" are not uniform).
Inhibin (INH)	Cytoplasmic	Granulosa cells, Sertoli cells, adrenal cortical cells, trophoblasts, and others	– Adrenocortical neoplasms; – Sex cord-stromal tumors (granulosa cell, Sertoli and Leydig, fibrothecomas); – Trophoblastic tumors; – Hemangioblastoma; – Granular cell tumor.
INI1 (hSNF5/BAF47/SMARCB1)	Nuclear	Expressed in normal tissues (product of tumor suppressor gene on 22q11.2)	– Loss of expression (due to gene deletions) in AT/RT, rhabdoid tumor of the kidney (and other sites), epithelioid sarcoma, medullary CA of the kidney, epithelioid MPNST (50%), subset of myoepithelial CAs of soft tissue, and others [49].
INSM1 (Insulinoma-associated protein 1)	Nuclear	Neural and neuroendocrine tissues – plays a critical role in embryonic development of these tissues	– New marker that has thus far been shown to be highly sensitive and specific for neuroendocrine differentiation; – (+) in neuroendocrine neoplasms of various origins: cervix, GI tract, lung, head and neck, etc.
ISL1 (Insulin gene enhancer protein ISL1, Islet 1)	Nuclear	Pancreatic islet cells	– DDx of PanNETs (+) vs. non-pancreatic NETs (−) [50].
K903 → see Cytokeratins			
Ki-1 → see CD30			
Ki67 (Named for Kiel – the city where discovered – and 67 the number of the clone on 96-well plate) (MIB-1 – most common anti-Ki67 antibody)	Nuclear	Any proliferating cell	– To gauge mitotic activity for prognosis and classification of some tumors; – See primer on proliferation markers in Chapter 1.
KP1 → see CD68			
Ksp-cadherin (Kidney specific cadherin; cadherin 16, CDH16)	Membranous	Primarily in the distal nephron cells of the kidney	– DDx chromophobe RCC (+) vs. oncocytoma (−); – Also + in SDH-deficient RCC.
Langerin	Membranous and cytoplasmic	Langerhans cells	– Langerhans cell histiocytosis [51].
LEF1 (Lymphoid enhancer-binding factor 1) Also see heme index	Nuclear	Transcription factor; T cells and immature pro-B cells but not in normal mature B cells; also expressed in squamous epithelial cells of the oral mucosa	– Basal cell adenoma and basal cell adenoCA of the salivary gland; – CLL/SLL.
Leu7 → see CD57			
LeuM1 → see CD15			
LFABP (Liver fatty acid-binding protein)	Cytoplasmic	Liver	– DDx of hepatic adenomas: negative in HNF-α inactivated subtype and positive in all other subtypes of adenoma and in FNH.
LIN28A	Cytoplasmic	Marker of human embryonic stem cells, highly expressed in the testes	– New marker found in all embryonal tumors with multilayered rosettes (ETMRs), irrespective of morphology; – Not specific. Also + in a subset of AT/RT (~25%, usually focal and/or weak). References: [52, 53]

Alphabetical Antibody Index: Solid Tumors – Continued

Mammaglobin	Cytoplasmic	Breast epithelium, sweat glands	– Tumors with apocrine differentiation: breast CA (~60%+), sweat gland tumors, and salivary gland tumors (similar to GCDFP-15); – Also frequently labels GYN tumors (ovary, endometrium, cervix); – A bit more sensitive than GCDFP-15 for ID of metastatic breast cancer. References: [54, 55]
MAP2 (Microtubule-associated protein 2)	Cytoplasmic	Brain	– Glioneuronal tumors: gangliocytoma, ganglioglioma.
MART-1 (Melanoma antigen recognized by T cells, N2-7C10 clone)	Cytoplasmic	Melanocytes	– Melanoma (mainly epithelioid), more sensitive than HMB45. Recognizes same protein as Melan-A antibody.
MDM2 (Murine double minute 2)	Nuclear	Oncoprotein, not expressed at high levels in normal cells	– DDx well-diff. liposarcoma (+) vs. lipoma (−). FISH more sensitive than IHC [56, 57]; – De-diff. liposarcoma (+) vs. poorly differentiated sarcomas (−); – Low-grade osteosarcoma (+) vs. benign fibro-osseous lesions (−) [58].
Melan-A (A103 clone)	Cytoplasmic	Melanocytes	– Melanoma (epithelioid > spindle cells) and other melanosome-containing tumors (same as for HMB-45 above). More sensitive than HMB45; – Steroid cell tumors (adrenocortical, Sertoli/Leydig and granulosa cell tumors).
MelCAM (melanoma cell adhesion molecule) → see CD146			
Mesothelin	Membranous	Mesothelial cells	– Many tumors (including mesothelioma, serous ovarian CA, and pancreatic CA), not site specific. A target for immunotherapy, so is an investigational predictive marker.
MIB1 → see Ki67 (Molecular immunology borstel 1)			
MITF (Microphthalmia transcription factor)	Nuclear	Melanocytes	– Melanoma and melanocytic tumors, also angiomyolipoma and other PEComas (but can also stain macrophages).
MLH1, MSH2, MSH6, PMS2 (*mutL* homolog 1, *mutS* homolog 2 and 6, postmeiotic segregation increased 2)	Nuclear	DNA mismatch repair (MMR) proteins are present in most normal cells. Mutation and consequent loss of expression lead to microsatellite instability (MSI)	– Loss of MMR proteins is due to germline mutations in MMR genes in Lynch syndrome or due to promoter hypermethylation in 10–15% of sporadic CRCs; – Because of different distribution of affected genes in Lynch syndrome vs. sporadic tumors, the loss of MSH2, MSH6, or PMS2 is nearly diagnostic of Lynch syndrome (germline mutation), whereas the loss of MLH1 is more commonly sporadic; – Tumors with defective MMR/MSI high have distinctive histology and clinical behavior; – See "IHC testing of DNA MMR Proteins" section in Chapter 2 and "Lynch Syndrome" section in Chapter 12.
MOC31 (Anti-EpCAM antibody: different epitope from BerEP4)	Membranous	Most epithelial cells	– AdenoCA in general; – Lung adenoCA (+) vs. mesothelioma (−).
MUC1→ see EMA			
MUC4	Cytoplasmic	Transmembrane glycoprotein expressed on the surface of some glandular epithelial cells	– Epithelial marker which in soft tissue tumors is a reliable marker of: • Low-grade fibromyxoid sarcoma [59]; • Subset of sclerosing epithelioid fibrosarcomas (with *FUS* gene rearrangement) [60]; – DDx of epithelioid mesothelioma (−) vs. lung adenoCA or SqCC (+) [61]; – Positive in both IPMN and PanIN (not used in practice).
MUC18 → see CD146			
Muramidase → see Lysozyme			
Muscle-specific actin (MSA) → see Actins			
MYB	Nuclear	Colon, rectum, hematopoietic cells	– Sensitive but not specific marker of adenoid cystic CA (IHC has no correlation with *MYB* translocation).
Myogenin (MGN) and **MyoD1**	Nuclear	Transcription factors in regenerating, but not normal, skeletal muscle	– Skeletal muscle differentiation (rhabdomyosarcoma+) but negative in rhabdomyoma as is normal mature skeletal muscle.

Alphabetical Antibody Index: Solid Tumors – Continued

NANOG (Homeobox protein NANOG)	Nuclear	Germline stem cells within the developing testis	– DDx of testicular GCTs: seminoma and embryonal CA (+) vs. other GCTs and spermatocytic tumor (–).
Napsin A (novel aspartic proteinase of the pepsin family)	Cytoplasmic (granular)	Pneumocytes and renal tubular cells	– Lung adenoCA. Also positive in some RCCs (clear cell and papillary), clear cell CA of GYN tract (~100%), focal in small % of thyroid CAs. Similar sensitivity for lung adenoCA as TTF1.
NCAM (neural cell adhesion molecule) → see CD56			
NeuN	Nuclear	Neurons	– Neuronal/ganglion cell tumors; – At least focally positive in medulloblastoma; – Better marker of normal neurons, not as reliable in tumors.
Neurofilament Sm311 (pan-NF), Sm32 (cell body), Sm31 (axons)	Cytoplasmic	Neurons	– Neuronal/ganglion cell tumors (gangliocytoma, ganglioglioma), neuroblastic tumors (neuroblastoma, medulloblastoma, Merkel cell CA), and some neuroendocrine tumors (pheochromocytoma). SYN is best for this purpose; – DDx Merkel cell CA (+) vs. SmCC (–); – Demyelinating disorders: highlights relative axonal preservation; – Brain infiltration: Sm31 may be used to highlight normal axons to help ID permeation of normal brain parenchyma by a glioma or meningioma.
Neuron-specific enolase (NSE)	Cytoplasmic	Neuroectodermal and neuroendocrine cells	– Neural and neuroendocrine differentiation but not specific (not the same as nonspecific esterase, an enzyme assay for heme path). – Sensitive for neuroblastoma. Much less specific than synaptophysin and chromogranin, and rarely used in practice.
NKX2.2	Nuclear	Highest in the brain; GI and GU tissues	– New marker, used in DDx of soft tissue and sinonasal Ewing sarcoma (+) vs. other small blue round cell tumors (–); – Highly specific for soft tissue Ewing sarcoma in combination with CD99. References: [62, 63]
NKX3.1	Nuclear	Almost exclusively expressed in the prostate	– New marker, currently considered to be the most sensitive and specific marker of prostate adenoCA.
NUT (Nuclear protein in the Testis)	Nuclear	Germ cells in the testis and ovary	– NUT CA (most often seen in thymus and sinonasal tract) – diffuse and strong staining with distinctive "speckled" pattern; – Some germ cell tumors (generally weak and non-speckled staining).
O13 → see CD99			
OCT4 (OCT3/4)	Nuclear	Not expressed in normal, differentiated cells	– Seminoma/dysgerminoma and embryonal CA; – Kidney: medullary CA (+ in most) vs. collecting duct CA (–).
OLIG2 (Oligodendrocyte transcription factor)	Nuclear	Glial cells and motor neurons	– Universally expressed in diffuse gliomas (astrocytoma, oligodendroglioma, and oligoastrocytomas) [64].
p16	Nuclear and cytoplasmic	Cell with inactivated pRb (due to cell's high-risk HPV or other means)	– Surrogate marker for HPV-driven SqCCs: tonsil/oropharynx, cervix, anus; – But many non-HPV CAs are p16+ (like lung SqCCs); thus, testing for HPV is typically needed to confirm a metastasis from HPV-related CAs, although non-HPV SqCC of head and neck are typically p16-negative; – Only strong, diffuse nuclear, and cytoplasmic positivity ("block-like staining") counts when used as a surrogate for HPV; – DDx of serous CA of GYN tract (robustly +) vs. endometrioid CA (–) – analogous to p53 and unrelated to HPV; – DDx of cervical HSIL (+) vs. immature metaplasia (–); – DDx of endocervical adenoCA (+/diffuse) vs. endometrial CA (– or patchy); – Loss of p16/CDKN2A by FISH (not IHC) is specific for mesothelioma vs. benign mesothelial proliferation (intact).
p40 (ΔNp63)	Nuclear	A variant of p63	– p40 is a protein in p63 family. It has the same sensitivity as p63 but much higher specificity (e.g., p40 only rarely labels lung adenoCAs and is consistently negative in lymphomas, unlike p63) [65]; – Positive in all other tumors where p63 expression is expected (SqCC of any site, urothelial, myoepithelial, basal cell) vs. negative in tumors with aberrant p63 (lung adenoCA, lymphomas, sarcomas); – The only tumor that is consistently p63+/p40– is salivary polymorphous adenoCA; – DDx of lung SqCC (+diffuse) vs. AdenoCA (–, rarely focal). Same sensitivity but much higher specificity than p63; – For other applications see p63.

Alphabetical Antibody Index: Solid Tumors – Continued

p53	Nuclear	Tumor suppressor gene, not expressed at high levels in normal cells	– Aberrant p53 expression (reflecting *TP53* mutations) serves as a diagnostic marker in several tumors (see Primer on p53 in Chapter 1): • DDx of serous CA of GYN tract (robustly + or completely lost) vs. endometrioid CA ("wild-type" pattern = focal weak); • DDx of urothelial CA in situ (+) vs. reactive urothelium (−); • DDx of astrocytoma (subset +) vs. oligodendroglioma (−). – Aberrant p53 also used as a negative prognostic marker in several tumors (gliomas, lymphomas).
p57	Nuclear	Trophoblasts	– *p57* gene is paternally imprinted and is normally transcribed entirely from a maternal allele (absent in complete mole); – DDx of complete mole (−) vs. partial mole or hydropic fetus (+).
p63 (4A4)	Nuclear	Stem cell factor in basal-type cells. Expressed in basal cells of stratified epithelia (squamous, urothelial, respiratory), myoepithelial cells, thymic epithelial cells, trophoblasts	– Marker of SqCC (any site), basal cells, urothelial, myoepithelial, thymic, and trophoblastic cells and neoplasms. However, it frequently stains unrelated tumors, like lung adenoCAs (~30%) and lymphomas (~50%). Antibody to p63-related protein – p40 (ΔNp63) is much more specific than p63, but similarly sensitive. Thus, p40 has now completely replaced p63 in some practices; – Uses of p63 and p40 include: • Dx of invasion: highlights the loss of breast myoepithelial cells and prostate basal cells; • DDx of lung SqCC (+) vs. AdenoCA (usually negative but can be focal to diffuse with p63; p40 is much more specific) [65]; • DDx of metaplastic (spindle cell) CA of breast (p63/p40+, HMWCK+) vs. other spindle cell lesions (−).
p80 → see ALK			
p120 (p120 catenin)	Membranous and cytoplasmic	E-cadherin-binding protein in breast epithelium	– Lobular CA in cases with equivocal E-cadherin: ductal CA (membranous p120) vs. lobular CA (strong cytoplasmic p120). If E-cadherin is absent, the cytoplasmic pool of p120 increases.
P501S → see Prostein			
P504S → see Racemase			
Parafibromin	Nuclear	Protein product of tumor suppressor gene *HRPT2*, expressed in normal tissues	– *Loss* of expression seen in parathyroid CA and parathyroid adenomas of the hyperparathyroidism-jaw tumor syndrome (intact in sporadic parathyroid adenomas). Reference: [66]
Parvalbumin	Cytoplasmic	Distal tubule and collecting duct cells of fetal and adult kidney	– DDx of chromophobe RCC (+) vs. other RCC types (−); – Positive in some oncocytomas.
PAX8 (Paired box gene 8)	Nuclear	Normal thyroid follicles, renal epithelial cells (all segments of renal tubules), tissue of Mullerian origin, lymphocytes, thymic epithelium	– Pan-RCC and other renal cortical neoplasm marker; also labels some upper tract urothelial CAs; – Thyroid CAs (follicular origin; most anaplastic CAs; usually negative in medullary) [67, 68]; – Pan-Mullerian marker (endometrial, ovarian, and endocervical); – Thymic epithelial neoplasms (thymoma, thymic CA) – with polyclonal antibody [69]; – Pancreatic NETs (with polyclonal antibody); – Can be positive in some peritoneal mesotheliomas.
PD-L1 (Programmed death-ligand 1, CD274, B7-H1)	Membranous	Antigen-presenting cells (macrophages, dendritic cells, etc.) and all tumor types	– Predictive marker only; – High expression in tumor (+/− tumor immune infiltrate) is predictive of better response to PD-1 and PD-L1 targeted agents (see Chapter 4).
PE10 → see Surfactant protein A			
PHOX2B (Paired-like homeobox 2b)	Nuclear	Adrenal gland	– Pheochromocytoma/ paraganglioma and neuroblastoma; – DDx of neuroblastoma (+) vs. other small round blue cell tumors. References: [70, 71]
PIT-1 (Pituitary-specific positive transcription factor 1, GH factor 1)	Nuclear	Transcription factor regulating PRL-GH-TSH pathway	– Pituitary adenomas (PRL, GH, and TSH producing). Reference: [72]
Placental alkaline phosphatase (PLAP)	Cytoplasmic	Placenta	– GCTs (does not stain spermatocytic tumor or teratoma).
PLAG1 (Pleomorphic adenoma gene 1)	Nuclear	Unknown	– Pleomorphic adenoma (PA) and CA ex-PA (IHC usually corresponds to *PLAG1* FISH); sensitive but not specific [73].
Podoplanin → see D2-40			
POU5F1 → see OCT4			

Alphabetical Antibody Index: Solid Tumors – Continued

PMS2 (postmeiotic segregation increased 2) → see under MLH1			
Progesterone receptor (PR) → see Estrogen receptor			
Prostein (P501S)	Cytoplasmic (perinuclear dots pattern)	Prostatic epithelium	– Prostate CA. One of the newer prostate markers, with high sensitivity and specificity, comparable to that of NKX3.1 [74] Use varies by institution.
PSA (Prostate-specific antigen)	Cytoplasmic	Prostatic epithelium but also salivary gland	– Prostate CA (sensitivity 80% in metastatic setting); – PSA reactivity is also present in benign and neoplastic salivary gland duct epithelium (pleomorphic adenoma, mucoepidermoid CA), periurethral glands of women, anal glands of men, and glandular urothelium (cystitis glandularis, urachal remnants, urothelial adenoCA) [75]; – PSA is more specific but less sensitive than PSAP.
PSAP or **PAP** (Prostate acid phosphatase)	Cytoplasmic	Prostatic epithelium	– Prostate CA; – (+) in carcinoids and some bladder adenoCAs. Be careful not to mistake rectal carcinoid for prostate CA!
PSMA (Prostate-specific membrane antigen)	Cytoplasmic, membranous	Prostatic epithelium, urothelium	– Prostate CA – in contrast to PSA and PSAP, expression does not decrease with tumor grade; – One of the less frequently utilized prostate markers.
Racemase → see AMACR			
RCC (Renal cell carcinoma marker, gp200/RTA)	Cytoplasmic	Proximal renal tubules	– RCC (poor sensitivity and specificity). Virtually retired marker after PAX8 became available.
S100 (Solubility in 100% ammonium sulfate)	Nuclear and cytoplasmic	Schwann cells/glia, melanocytes, histiocytes, dendritic and Langerhans cells, myoepithelial cells, and other mesenchymal cells	– Primarily a marker of neural crest-derived tumors (nerve sheath and melanocytic tumors) but also stains many other tumors; – *Nerve sheath/glia*: schwannoma (diffuse), neurofibroma (focal), MPNST (focal), gliomas; – *Melanocytes*: melanoma (including desmoplastic), nevi; – *Soft tissue*: clear cell sarcoma, synovial sarcoma (30%), chordoma, chondrosarcoma, lipomatous tumors; – *Histiocytes*: Langerhans cell histiocytosis, Rosai-Dorfman, histiocytic sarcoma, benign histiocytoses; – *Myoepithelial cells*: myoepithelioma, myoepithelial CA; – *Other*: granular cell tumor, sustentacular cells in pheochromocytoma/paraganglioma, and olfactory neuroblastoma; – Note: S100 is generally negative in CAs except some salivary and breast CAs (30%); – Not used to screen lymph nodes for metastatic melanoma because normal dendritic cells are (+).
SAA (Serum amyloid alpha)	Cytoplasmic	Hepatocytes	– Together with CRP, aids in the DDx of hepatic adenomas: inflammatory adenoma (+) vs. other adenoma subtypes and FNH (–).
SALL4 (SAL-like protein 4)	Nuclear	Embryonic stem cells	– pan-GCT marker, including spermatocytic tumor; – Some leukemias.
SATB2 (Special AT-rich sequence-binding protein 2)	Nuclear	Osteoblasts, lower GI tract	– Novel marker of osteoblastic differentiation [76]: all skeletal and most extraskeletal osteosarcomas, osteoblastomas, osteoid osteomas, and fibrous dysplasia, most giant cell tumors, etc.; – Colorectal adenoCA; like CDH17, it is preserved in poorly differentiated CRCs, making it particularly useful in evaluation of CAs of unknown primary; – Also positive in *BCOR-CCNB3* (Ewing-like) sarcoma.
SDHB (Succinate dehydrogenase B)	Cytoplasmic (granular/ mitochondrial pattern)	Ubiquitously expressed in all normal eukaryotic cells	– SDH-deficient GIST – predominantly pediatric, usually with epithelioid morphology, lacking c-kit and PDGFRA mutations (Carney-Stratakis syndrome; Carney triad); – SDH-deficient RCC; – Subset of pheochromocytomas (familial SDH-related pheochromocytoma/ paraganglioma syndromes).
SF1 (Steroidogenic factor 1)	Nuclear	Transcription factor regulating gonadotroph pathway	– Sex cord-stromal tumors; – Adrenocortical neoplasms; – Some pituitary adenomas (gonadotrophic LH or FSH-producing) [72].
Sm311, Sm32, Sm31 → see neurofilament			
SMAD4 → see DPC4			
SMARCA4 → see BRG1			

Alphabetical Antibody Index: Solid Tumors – Continued

Smooth muscle actin (SMA) → see **α-actin**			
Smooth muscle myosin heavy chain (SMMHC)	Cytoplasmic	Myoepithelial cells, blood vessels, myofibroblasts	– Myoepithelial layer in the breast to rule out invasive breast cancer.
Smoothelin	Cytoplasmic	Terminally differentiated smooth muscle	– Bladder muscularis propria (strong) vs. muscularis mucosae (negative or weak) in assessing depth of invasion in bladder CA.
SOX2 (Sex determining region of Y chromosome-related high-mobility group box 2)	Nuclear	Fetal CNS tissue	– Embryonal CA; – Immature elements in CNS teratomas [77]; – SqCCs of any site.
SOX9 (Sex-determining region of Y chromosome-related high-mobility group box 9)	Nuclear	Normal cartilage – acts as master regulator of chondrogenesis	– Cartilaginous differentiation (mesenchymal chondrosarcoma) but not specific.
SOX10 (Sex-determining region of Y chromosome-related high-mobility group box 10)	Nuclear	Melanocytes, Schwann cells, myoepithelial cells	– Similar to S100, SOX10 is primarily a marker of neural crest-derived tumors but also stains other tumors (but not as widely as S100, so it is more specific): • *Like S100,* positive in nerve sheath tumors, gliomas, melanomas, granular cell tumor, sustentacular cells of pheochromocytoma/paraganglioma, myoepithelial and some salivary gland neoplasms; • *Unlike S100,* negative in histiocytic and fibrohistiocytic proliferations, as well as synovial sarcoma and biphenotypic sinonasal sarcoma; – SOX10 is also a marker of breast CAs (enriched in triple-negatives). Reference: [78]
SOX17 (Sex-determining region of Y chromosome-related high-mobility group box 17)	Nuclear	Variety of epithelial cells, with highest expression in the female urogenital tract	– DDx of testicular GCTs: seminomas (almost all +), yolk sac tumors (50%+) vs. other GCTs and spermatocytic tumor (−).
SSTR2A (Somatostatin receptor type 2A, SST2A, SSTR2)	Membranous and cytoplasmic	Predominantly brain	– NETs of various sites; – Pheochromocytoma (particularly SDH-deficient) [79]; – Meningioma (monoclonal antibody UMB1 more sensitive than EMA) [80].
STAT6 (Signal transducer and activator of transcription 6)	Nuclear	Transcription factor, reactivity in normal tissue uncommon	– Solitary fibrous tumor; – Small subset of de-diff. liposarcomas (watch out!) [81].
Surfactant protein A (SPA, PE10)	Cytoplasmic	Pneumocytes	– Marker of pneumocytes and lung adenoCA (rarely if ever used – napsin A is a much more sensitive marker for this role).
Synaptophysin (SYN; secretogranin)	Cytoplasmic	Neuroendocrine cells, neuronal cells, neuromuscular junction, Merkel cells	– Neuroendocrine neoplasms (e.g., carcinoids/NETs, pheochromocytoma, SmCC, Merkel cell CA, and medullary CA of thyroid), primitive neuroectodermal tumors (neuroblastoma, medulloblastoma, some Ewing sarcomas), and neuronal tumors (ganglioglioma); – Adrenocortical tumors and pancreatic solid-pseudopapillary neoplasm (SYN +/ CHR–).
TdT (Terminal deoxytransferase) Also see heme index	Nuclear	Immature B and T lymphocytes	– Thymoma (admixed immature T cells are TdT, CD1a, CD99+); – Precursor B and T leukemia/lymphoma (+) vs. lymphoma of mature cells, including Burkitt lymphoma (−).
TFE3 (Transcription factor E3)	Nuclear	Transcription factor, reactivity in normal tissue extremely rare	– Xp11 translocation RCC; – Alveolar soft part sarcoma; – Granular cell tumor; – Subset of renal angiomyolipomas and other PEComas [82].
TFEB (Transcription factor EB)	Nuclear	Transcription factor, reactivity in normal tissue extremely rare	– t(6;11) translocation RCC.
Thyroglobulin (TGB)	Cytoplasmic	Thyroid follicles	– Well-differentiated CAs of thyroid follicular origin (papillary and follicular); medullary and anaplastic CAs are negative; – Struma ovarii.
Thrombomodulin (CD141)	Cytoplasmic and membranous	Endothelial (cytoplasmic) and mesothelial (membranous) cells	– Urothelial CA, mesothelioma (second line), some vascular tumors; – Older marker, virtually retired in practice.
TLE1 (Transducin-like enhancer protein)	Nuclear	Transcription factor whose gene was discovered to be upregulated in synovial sarcoma	– Synovial sarcoma, but not specific (can be focal in schwannoma, SFT); – *BCOR*-rearranged sarcoma (+/−).

Alphabetical Antibody Index: Solid Tumors – Continued

Tpit (TBX19)	Nuclear	Present only in the two POMC-expressing lineages in pituitary (corticotrophs and melanotrophs)	– Corticotroph-producing pituitary adenomas, in which PIT1 may be negative.
TTF-1 (Thyroid transcription factor 1)	Nuclear	Developmental transcription factor in the lung and thyroid	– Thyroid CA (+ in follicular, papillary, and medullary); anaplastic is –; – Lung adenoCA (80%+) vs. extrapulmonary adenoCA, SqCC, mesothelioma (–); – Lung carcinoid (50+, usually weak) vs. extrapulmonary NETs (–); – SmCC of the lung (90%) and other sites – does not distinguish the site of origin for SmCC [24, 25]; – HCC (+) for cytoplasmic TTF1 (not useful clinically); – Note: watch out for TTF1 labeling in a subset of unexpected (non-lung non-thyroid) tumors, particularly CAs of GYN tract. This usually occurs with less specific SPT24 clone (but rarely with more specific clone 8G7G3/1 as well).
Trypsin and **chymotrypsin**	Cytoplasmic	Pancreatic acinar cells	– Pancreatic acinar cell CA and pancreatoblastoma.
Uroplakin II and III	Cytoplasmic	Urothelium	– Urothelial CA. Both highly specific. Standard marker – uroplakin III – has low sensitivity, but recent uroplakin II is more sensitive.
Villin	Cytoplasmic ("brush border" pattern)	Enterocytes	– Colorectal CA and CAs with enteric differentiation (see under CDX2); – HCC (+ canalicular staining, similar to CD10).
Vimentin	Cytoplasmic	Most mesenchymal cells including fibroblasts, endothelium, smooth muscle	– Sarcoma, lymphoma, and melanoma (+) vs. CA and glioma (–/+) – historical use; – ccRCC (+) vs. chromophobe RCC and oncocytoma (–); – Bladder muscularis mucosa (+) vs. muscularis propria (–) – used in conjunction with smoothelin, which has the opposite pattern; – Because of wide reactivity, currently used mainly to confirm "immunoviability" of tissue.
vWF (von Willebrand factor) → see Factor VIII			
WT1 (Wilms tumor 1)	Nuclear	Tumor suppressor gene in developing nephrons, nephrogenic rests, and adult glomerular podocytes. Also stains normal and neoplastic mesothelium	– Mesothelioma; – Ovarian serous CA (80%+), usually negative in endometrial serous CA; – Wilms tumor; – Desmoplastic small round cell tumor (clone-dependent); – *CIC*-rearranged sarcomas [83].
YAP1 (Yes-associated protein; YAP, YAP65)	Nuclear	Transcriptional regulator of cell proliferation and apoptotic gene suppressor	– Used in conjunction with filamin A, GAB1, and β-catenin IHC to predict molecular subtype of medulloblastoma (see filamin A above for details).

Abbreviations: *adenoCA* adenocarcinoma, *ALCL* anaplastic large cell lymphoma, *ALL* acute lymphocytic leukemia, *AML* acute myeloid leukemia, *AT/RT* atypical teratoid/rhabdoid tumor, *ccRCC* clear cell renal cell carcinoma, *CA* carcinoma, *CASTLE* carcinoma showing thymus-like differentiation of the thyroid, *CHR* chromogranin, *CLL/SLL* chronic lymphocytic leukemia/small lymphocytic lymphoma, *DLBCL* diffuse large B-cell lymphoma, *ESS* endometrial stromal sarcoma, *FL* follicular lymphoma, *FNH* focal nodular hyperplasia, *GBM* glioblastoma multiforme, *GCT* germ cell tumor, *GIST* gastrointestinal stromal tumor, *HCC* hepatocellular carcinoma, *HL* Hodgkin lymphoma, *HLRCC* hereditary leiomyomatosis and renal cell carcinoma, *ID* identify/identification, *IMT* inflammatory myofibroblastic tumor, *ISH* in situ hybridization, *LP* lymphocyte predominant, *MALT* mucosa-associated lymphoid tissue, *MCL* mantle cell lymphoma, *MRT* malignant rhabdoid tumor, *MZL* marginal zone lymphoma, *MPNST* malignant peripheral nerve sheath tumor, *NE* neuroendocrine, *NET* neuroendocrine tumor, *NLP* nodular lymphocyte predominant, *PanNET* pancreatic neuroendocrine tumor, *PEComa* perivascular epithelioid cell tumor, *PNET* primitive neuroectodermal tumor, *RCC* renal cell carcinoma, *R-S* Reed-Sternberg, *SCCOHT* small cell carcinoma of the ovary hypercalcemic type, *SFT* solitary fibrous tumor, *SYN* synaptophysin

Chapter 6. Immunostains: Antibody Index – Hematopoietic System

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(Prior edition by Amy Duffield, Justin A. Bishop, Natasha Rekhtman)

Antibody or antigen (other names)	Cellular localization	Normal tissues stained and functional information if pertinent	What this marker is used to identify and differential diagnosis
4A4 → see p63			
ALK (Anaplastic Lymphoma Kinase) (alt name p80) Also see solid tumor index	Nuclear, cytoplasmic, membranous	Few neuronal cells	– ALCL (~70%); – Expression is a result of t(2;5)/*NPM1-ALK* and other *ALK* translocations Most commonly ALK is both nuclear and cytoplasmic, but location depends on the type of translocation; – ALK-positive large B-cell lymphoma, t(2;17) with *CLTC-ALK* rearrangement; – ALK-positive histiocytosis of infancy [1].
Annexin A1	Membranous and/or cytoplasmic	T lymphocytes, myeloid cells, and macrophages	– Hairy cell leukemia.
BCL1 → see cyclin D1			
BCL2 (B-cell lymphoma-2) Also see solid tumor index	Membranous and cytoplasmic	Inhibits apoptosis. Normally present in mantle cells and reactive T cells. Turns OFF in germinal centers (normal secondary follicles are BCL2-negative)	– FL (+) vs. reactive follicles (−). In FL, BCL2 expression is maintained due to t(14;18)/*BCL2-IGH*. BCL2 is NOT specific for FL (also + in CLL/SLL, MCL, MZL); – BCL2-negative lymphomas: Burkitt lymphoma, primary cutaneous follicle center lymphoma, and pediatric follicular lymphoma.
BCL6 (B-cell lymphoma-6)	Nuclear	Germinal center B cells	– Lymphomas of follicular origin (FL, Burkitt lymphoma, some DLBCL, LP cells in NLPHL), neoplastic T lymphocytes in AITL.
BDCA2 (blood dendritic cell antigen2) (alt name CD303)	Cytoplasmic	Plasmacytoid dendritic cells	– PDC nodule in CMML as well as PDC reactive proliferation in other situations such as Kikuchi disease; – BPDCN.
BOB.1 (B-cell-specific octamer binding protein 1)	Nuclear and cytoplasmic	Transcription factor in B cells	– RS cells in CHL (− or weak) vs. NLPHL (+); – B-cell lymphomas [2]; – B-cell neoplasms with plasmacytic/plasmablastic differentiation.
BSAP (B-cell-specific activator protein) → see PAX5			
CALLA (common acute lymphoblastic leukemia antigen) → see CD10			
Calretinin (CRT) Also see solid tumor index	Cytoplasmic and nuclear	Mast cells	– Cutaneous mastocytosis.
CD1a	Membranous	Thymocytes (immature T cells), Langerhans cells	– Langerhans cell histiocytosis; – Some T-ALL/LBL; – Admixed thymocytes (CD1a, TdT, CD99+) in thymoma.
CD2	Membranous	Pan-T-cell marker, NK cells	– T and NK cell lymphomas and leukemias; – Systemic mastocytosis.
CD2AP (*CD2*-associated protein)	Cytoplasmic	Plasmacytoid dendritic cells	– BPDCN; – PDC nodules in CMML as well as reactive PDC proliferation, such as Kikuchi disease.
cCD3 (flow marker)	Cytoplasmic	Pan-T-cell marker	– Most lineage-specific marker for T-cell differentiation; NK cells; – Flow cytometric analysis of cytoplasmic CD3 expression is determined by permeabilizing the cells prior to incubation with the antibody.
CD3	Membranous cytoplasmic	Pan-T-cell marker	– T-cell lymphomas and leukemias (best pan-T-cell IHC marker); often lost in ALCL.
CD4 and **CD8**	Membranous	CD4: helper T cells, monocytes CD8: cytotoxic and suppressor T cells, NK-like T cells CD4+/CD8+: thymus T cells	– T-cell lymphomas and leukemias (majority of peripheral T-cell lymphomas are CD4+); – CD4 is also (+) in monocytic/histiocytic lesions (e.g., monocytic AML) and BPDCN; – Large populations of double-negative or double-positive T cells are typically neoplastic except in the thymus where these phenotypes are normally seen. A small number of circulating CD4/CD8 double-negative T cells is normal.

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CD5	Membranous	T cells and subset of B cells (naïve B cells)	– CD5+ small B-cell lymphomas: CLL/SLL and MCL; – CD5+ high-grade B-cell lymphomas: blastoid MCL, B-PLL, occasional DLBCL (de novo or transformed); – T-cell lymphomas and leukemia: aberrant loss of pan-T antigens, particularly CD7 and CD5, is a common signature in peripheral T-cell lymphomas, such as MF and HSTCL; – Thymic carcinoma (+ in epithelial cells) vs. thymoma (–).
CD7	Membranous	T cells; NK cells	– T-ALL/LBL (near 100% + and often very brightly expressed); – MF and other mature T-cell lymphomas (aberrant loss of CD7) vs. reactive T-cell proliferations (+); – Aberrant expression in AML is used for flow cytometric monitoring of residual disease; expression of CD7 on myeloid blasts can occasionally be seen in recovering marrow.
CD9 (flow marker)	Membranous	Hematopoietic and non-hematopoietic cells	– B-ALL/LBL; – Subset of APL [3, 4].
CD10 (alt name CALLA) Also see solid tumor index	Membranous	Precursor B and T cells, germinal center B cells, granulocytes	– CD10 (+) lymphomas: B- and T-ALL/LBL (B > T), FL, Burkitt lymphoma, some DLBCLs; neoplastic cells in AITL.
CD11b (flow marker)	Membranous	Monocytes, granulocytes; NK cells	– AML with monocytic differentiation; – AML with/without maturation; – NK-cell tumors.
CD11c (flow marker)	Membranous	Myeloid and lymphoid cells	– Hairy cell leukemia; – Myeloid leukemia with monocytic differentiation.
CD13, ***CD14*** (flow marker), **CD33**	Membranous	Myeloid cells (CD13, CD33) and monocytes (CD14, CD33)	– AML with/without monocytic differentiation; – Rare cases of T- and B-ALL/LBL.
CD15 (alt name LeuM1) Also see solid tumor index	Membranous and golgi (perinuclear dot-like)	Monocytes, myelocytes, granulocytes	– RS cells in CHL (occasionally negative in CHL but always negative in NLPHL); – AML with differentiation, especially granulocytic.
CD19, **CD20** (alt name L26), **CD22**	Cytoplasmic and membranous	Pan-B-cell markers	– Mature B-cell lymphomas (+), but plasma cell neoplasm (–); – B-ALL/LBL almost always expresses CD19, but CD22 and CD20 are variable; – CD20 reactivity is lost in DLBCL after rituximab treatment (anti-CD20 antibody), but CD19 and CD79a remain (+).
CD21, CD35	Membranous	Follicular dendritic cells (FDC)	– FDC network in lymphomas (FL, MCL, NLPHL, AITL); – FDC sarcoma.
CD23	Membranous	B cells, follicular dendritic cells (FDC)	– DDx of CD5+/CD10- small B-cell lymphomas: SLL/CLL (CD23+) vs. MCL (CD23-); – FDC network in lymphomas (FL, MCL, NLPHL, AITL); – FDC sarcoma.
CD25 (alt name IL2 receptor)	Membranous and cytoplasmic	Activated T and B cells	– Hairy cell leukemia; – Adult T-cell leukemia/lymphoma (HTLV1-related); – Most ALCLs; – Neoplastic mast cells.
CD27 (flow marker)	Membranous	Medullary thymocytes, T and B lymphocytes, plasma cells, and NK cells	– Plasma cell myeloma (+ in 64%); – T-cell large granular lymphocytic leukemia (negative); – Adult T-cell leukemia/lymphoma.
CD28 (flow marker)	Membranous	Thymocytes, CD4 T lymphocytes, and CD8+ cytotoxic T lymphocytes	– Plasma cell myeloma; – T-cell large granular lymphocytic leukemia (negative).
CD30 (alt name Ki-1) Also see solid tumor index	Membranous and golgi (perinuclear dot-like)	Activated B (immunoblasts) and T lymphocytes, plasma cells	– RS cells in CHL (+) vs. NLPHL (–); – PMLBCL; – ALCL (strong staining with characteristic “target-like” membrane and golgi pattern); – MF (+/– focally; suggests transformation); – Reactive immunoblasts; – Subset of DLBCLs and PTCLs; – CD30 reactivity can be lost after brentuximab treatment (anti-CD30 antibody).
CD31 Also see solid tumor index	Cytoplasmic and membranous	Megakaryocytes, macrophages	– Megakaryocytes in general (normal megakaryocytes and abnormal megakaryocytes in MPN, MDS, and AML).
CD34 Also see solid tumor index	Cytoplasmic and membranous	Hematopoietic blasts (stem cells)	– Primitive leukemias (including AML, B- and T-ALL/LBL, more common in B- than T-ALL/LBL); – CD34-negative: mature hematolymphoid neoplasms and AML with monocytic, erythroid, or megakaryocytic differentiation.
CD38	Membranous	Immature lymphocytes, plasma cells	– Plasma cell differentiation; – Poor prognosis in CLL/SLL.

CD41, CD42b, CD61	Membranous	Megakaryocytes, platelets	– AML with megakaryocytic differentiation.
CD43	Membranous	T cells, plasma cells, and myelocytes	– Classification of small B-cell lymphomas: aberrant expression in CLL/SLL, MCL, MZL, but not FL; – Normal and malignant T cells, myeloid cells – more sensitive than CD45.
CD45 (alt name LCA or CLA)	Cytoplasmic, membranous	Pan-leukocyte marker (lymphocytes, myeloid cells, and histiocytes) but absent on plasma cells and nucleated erythroid precursors	– Screening for hematopoietic origin in an unknown malignancy (part of a standard first-line panel); – Positive in most hematopoietic neoplasms, except myelomas, RS cells in CHL, some T- and B-ALL/LBL, some ALCL, some myeloid sarcomas, and FDC sarcoma.
CD56 (alt name NCAM) Also see solid tumor index	Membranous	NK cells	– Extranodal NK/T-cell lymphoma, nasal type, and some T-cell lymphomas [subcutaneous panniculitis-like T-cell lymphoma, hepatosplenic T-cell lymphoma, monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL), primary cutaneous gamma-delta T-cell lymphoma]; – BPDCN; – Neoplastic plasma cells (70–80%); – Aberrant expression in acute myeloid leukemia.
CD57 Also see solid tumor index	Membranous	Neuroendocrine cells, Schwann cells, NK-like T cells	– NLPHL (CD57+ T lymphocytes surround LP cells forming "rosettes") vs. T-cell/histiocyte-rich LBCL or CHL (rosettes of CD57 cells absent); – T-cell large granular lymphocytic leukemia.
CD58 (flow marker)	Membranous	Ligand for CD2, present in hematopoietic and non-hematopoietic cells, expression decreased in B lymphocytes as they mature	– Expression in over 99% of B-ALL, useful for MRD detection in B-ALL/LBL [5].
CD65 (flow marker)	Membranous	Myeloid cell and monocyte	– More commonly seen as a myeloid marker co-expressed with CD15 in B-ALL/LBL, *KMT2A*-rearranged.
CD68	Cytoplasmic, membranous	Lysosomal marker in histiocytes/macrophages/ monocytes, granulocytes, others	– Histiocytic differentiation; – AML with monocytic differentiation; – BPDCN: weak cytoplasmic staining, dot pattern.
CD71 (alt name transferrin receptor) (flow marker)	Membranous	Erythroid cells (not specific)	– AML with erythroid (M6) or megakaryocytic (M7) differentiation; – CD10+ B-cell lymphomas (by flow), aggressive (+) vs. indolent (−).
CD79a	Cytoplasmic	B cells and plasma cells (broader than CD20)	– B-cell neoplasms, including B-ALL/LBL and plasma cell myelomas (myelomas may be negative for other pan-B markers); – Also (+) in some cases of T-ALL/LBL.
CD81 (flow marker)	Membranous	Regulates CD19 expression	– Expressed in ~40% of plasma cell myeloma cases and associated with poor prognosis [6].
CD99 (alt name MIC2, O13) Also see solid tumor index	Membranous (more specific reactivity) and cytoplasmic	Immature and mature T cells including cortical thymocytes	– B- and T- ALL/LBL; – A small subset of mature B- and T-cell lymphomas [7]; – Immature T cells/thymocytes (CD99+, TdT+) admixed with epithelial cells in thymoma.
CD103 (flow marker)	Membranous	Intestinal epithelial T lymphocytes	– Hairy cell leukemia; – Enteropathy-associated T-cell lymphoma.
CD117 → see c-kit			
CD123 (alt name IL3R)	Membranous	Myeloid and lymphoid progenitor cell, basophil, eosinophil	– AML; – BPDCN; – Hairy cell leukemia; – ALL/LBL; – Systemic mastocytosis.
CD138 Also see solid tumor index	Membranous	Plasma cells	– Plasma cell differentiation.
CD163	Membranous	Member of scavenger receptor cysteine-rich superfamily restricted to the monocyte/ macrophage lineage	– Histiocytic differentiation.
CD200	Membranous and cytoplasmic	T cell, B cell, thymocyte, dendritic cell, endothelial cell, and neuron	– CLL/SLL, but negative in other CD5+ B-cell lymphomas, such as MCL; – Plasma cell myeloma [8]; – Hairy cell leukemia; – B-ALL/LBL, AML; – CHL and PMLBCL but not in NLPHL and rarely in DLBCL; – AITL (neoplastic T cells).
CD207 → see langerin			

CD235a → see glycophorin			
CD279 → see PD-1			
CD303 → BDCA2			
c-kit (alt name CD117) Also see solid tumor index	Cytoplasmic and membranous	Hematopoietic progenitor cells, mast cells	– Mast cell lesions; – Blasts in acute myeloid leukemia.
CLA (common leukocyte antigen) → see CD45			
Clusterin Also see solid tumor index	Cytoplasmic	Follicular dendritic cells	– FDC sarcoma.
c-MYC	Nuclear	Protein involved in cell cycle progression, apoptosis, and cellular progression	– Aggressive B-cell lymphoma, e.g., Burkitt lymphoma, DHL, a subset of de novo DLBCL, and large B-cell lymphoma transformed from low-grade B-cell lymphomas.
CXCL13 (C-X-C motif chemokine ligand 13)	Cytoplasmic	Mature B lymphocyte, follicular helper T cells, and FDC	– FDC sarcoma [9]; – AITL [10].
Cyclin D1 (alt name BCL1) Also see solid tumor index	Nuclear	Dividing cells	– MCL and blastoid MCL – t(11;14)/*CCND1-IGH*. Endothelial cells are normally cyclin D1 (+), which may be used as an internal control in lymphoma work-up; – Plasma cell neoplasm (subset, usually with lymphoplasmacytic morphology and *CCND1-IGH* rearrangement); – Hairy cell leukemia: variably positive in most cases; – Proliferation center of SLL.
D2–40 (alt name podoplanin) Also see solid tumor index	Membranous	FDCs	– FDC sarcoma.
EBV (Epstein-Barr virus) Also see solid tumor index — **EBER** (EBV-encoded early RNA)	Nuclear	EBV-infected cells	– Most sensitive markers for EBV. Can identify all EBV-related tumors. Detected by in situ hybridization.
EBV (Epstein-Barr virus) Also see solid tumor index — **LMP-1** (late membrane protein)	Membranous	EBV-infected cells	– Less sensitive than EBER. Identifies PTLD and AIDS-related lymphomas, variable in NPC, CHL, and Burkitt lymphoma.
EBV (Epstein-Barr virus) Also see solid tumor index — **EBNA** (EBV nuclear antigen)	Nuclear	EBV-infected cells	– Least sensitive EBV marker. Identify PTLD and AIDS-related lymphomas only.
E-cadherin (CAD-E) Also see solid tumor index	Membranous	Erythroblast	– Erythroblast, AML with erythroid differentiation.
EMA (epithelial membrane antigen) (alt name MUC1) Also see solid tumor index	Cytoplasmic or membranous	Plasma cells	– Plasma cell neoplasms; – ALCL; – LP cells in NLPHL (>50%).
Factor VIII Also see solid tumor index	Cytoplasmic	Megakaryocytes, platelets, related with vWF	– AML with megakaryocytic differentiation.
Factor XIIIa Also see solid tumor index	Cytoplasmic	Histiocytes	– Histiocytic differentiation (a pan-histiocytic marker, similar to CD68).
Fascin	Cytoplasmic	Many cell types	– RS cells in CHL; – FDC sarcoma.
FMC-7 (flow marker)	Membranous	B cells	– CD5+ lymphomas: MCL (+) vs. CLL/SLL (−). Expression is opposite of CD23.
GATA-3 Also see solid tumor index	Nuclear	T lymphocyte	– A subset of PTCL, NOS, predicting poor prognosis.
GCET1 (germinal center B-cell-expressed transcript 1)	Nuclear	Germinal center B cells	– Lymphomas of follicular origin (FL, Burkitt lymphoma, some DLBCL, LP cells in NLPHL).
GCET2 (germinal center B-cell-expressed transcript 2) → see HGAL			
Glycophorin (alt name CD235a)	Membranous	RBC and erythroid precursor	– Erythroid lineage cells and RBCs.
Granzyme B	Cytoplasmic	Cytotoxic proteins in CD8+ T cells and NK cells	– T- and NK-cell lymphomas; – Differential expression can be seen in: • Hepatosplenic T-cell lymphoma: TIA1+, granzyme B−/+, perforin+; • Indolent T-cell lymphoproliferative disorder of the GI tract: TIA1+, granzyme B−; • Primary cutaneous acral CD8+ T-cell lymphoma: TIA1+ golgi dot-like, granzyme B−/+ and perforin−/+.
Hemoglobin A	Cytoplasmic	RBCs and erythroid precursors	– Erythroid lineage cells and RBCs.

HGAL (human germinal center-associated lymphoma) (alt name GCET2)	Nuclear	Germinal center B cells	– Same expression and use as GCET1.
HLA-DR	Membranous	Antigen-presenting cells	– Most myeloid leukemias (+) vs. APL (–).
IgG4	Cytoplasmic	Subset of plasma cells	– Increased in IgG4-related sclerosing diseases, including type I autoimmune pancreatitis, chronic sclerosing sialadenitis (Küttner tumor), and sclerosing mesenteritis (though criteria for what qualifies as "increased" are not uniform).
IL3R → see CD123			
IL12 receptor → see CD25			
Immunoglobulin kappa and **lambda light chains**	Surface, cytoplasmic	B cells (surface) Plasma cells (cytoplasmic)	– Restricted kappa or lambda staining indicates a monoclonal population of B or plasma cells; double-surface negative B cells are neoplastic (typically seen in PMLBCL and occasional CLL/SLL cases); – Surface staining in B-cell neoplasms is best assessed by flow cytometry; cytoplasmic staining in plasma cells can be assessed by IHC or flow cytometry.
IRF4 (interferon regulatory factor 4) → see MUM-1			
Ki-1 → see CD30			
Ki67 (alt name MIB-1) Also see solid tumor index	Nuclear	Any proliferating cell	– To gauge mitotic activity for prognosis; primarily used in B-cell lymphomas; – Burkitt lymphoma (100% positivity).
L26 → see CD20			
Langerin (alt name CD207)	Membranous and cytoplasmic	Langerhans cells	– Langerhans cell histiocytosis [11].
LMP-1 (late membrane protein) → see EBV			
LCA (leukocyte common antigen) → see CD45			
Leu7 → see CD57			
LeuM1 → see CD15			
LEF1 (lymphoid enhancer binding factor 1)	Nuclear	Pan-T cells, aberrantly expressed in B cells	– CLL; – DLBCL; – Loss of expression in T cells.
LMO2 (LIM domain only 2)	Nuclear	Germinal center B cells	– Same expression and use as GCET1.
Lysozyme (alt name muramidase)	Cytoplasmic	Monocyte/macrophage/histiocyte, salivary gland	– Histiocytic differentiation; – Myeloid sarcomas with monocytic differentiation.
MAL (myelin and lymphocyte)	Granular cytoplasmic accentuation in the golgi area	Differential expression in T-cell development, thymocytes, non-hematopoietic tissue	– PMLBCL (70%) [12].
Mast cell tryptase (MCT)	Cytoplasmic	Mast cells	– Systemic mastocytosis.
MIB-1 → see Ki67			
MIC2 → see CD99			
MUM1 (alt name IRF4) (multiple myeloma 1) Also see solid tumor index	Nuclear and cytoplasmic	Various hematolymphoid cells (particularly plasma cells)	– Plasma cell neoplasms; – Subtype of DLBCLs.
Muramidase → see lysozyme			
Myeloperoxidase (MPO)	Cytoplasmic	Enzyme granules in myeloid cells	– AML and myeloid sarcoma (chloroma).
NCAM (neural cell adhesion molecule) → see CD56			
O13 → see CD99			
OCT2 (octamer transcription factor-2)	Nuclear	Transcription factor in B cells	– RS cells in CHL (– or weak) vs. NLPHL (+); – B-cell lymphomas [2]; – B-cell neoplasms with plasmacytic/plasmablastic differentiation.
p63 (alt name 4A4) also see solid tumor index	Nuclear	Thymic epithelial cells	– Lymphomas such as ALK-negative ALCL with *TP63* rearrangement; – Various other lymphomas are frequently positive for p63 (4A4) but always negative for p40 [13]; – Thymoma.
p80 → see ALK			

PAX5 (paired box gene 5) Also see solid tumor index	Nuclear	B-cell-specific transcription factor (plasma cells are negative)	– B-cell differentiation including lymphoblasts (used as a novel pan-B-cell marker); – RS cells in CHL (weak) vs. NLPHL (+) [2].
Perforin	Cytoplasmic	Cytotoxic proteins in CD8+ T cells and NK cells	– Same expression and use as granzyme B and TIA1.
PD1 (program cell death protein 1) (alt name CD279)	Membranous	Germinal center T cell	– AITL; – PTCL, NOS, with follicular T helper phenotype; – Primary cutaneous CD4+ small/medium T-cell LPD; – NLPHL (highlights T lymphocytes that form rosettes around LP cells).
PU.1	Nuclear	Transcription factor regulating lymphoid and myeloid cell development	– NLPHL but not in CHL.
S100 (solubility in 100% ammonium sulfate) Also see solid tumor index	Nuclear and cytoplasmic	Interdigitating dendritic cells and Langerhans cells	– Rosai-Dorfman disease; – Langerhans cell histiocytosis/Langerhans cell sarcoma; – Interdigitating cell sarcoma; – Subset of BPDCNs.
SOX11 (SRY-box 11)	Nuclear	CNS, colon, cardiac muscle, testis	– Mantle cell lymphoma, especially cyclin D1-negative MCL; – HCL; – Burkitt lymphoma; – B and T lymphoblastic leukemia/lymphoma [14].
Spectrin	Membranous	RBCs and precursors	– Erythroid lineage cells and AML with erythroid differentiation.
TCL1 (T-cell leukemia/ lymphoma 1)	Nuclear	B cells up to germinal stage, PDC	– T-PLL; – BPDCN; – CLL (poor prognosis).
TdT (terminal deoxynucleotidyl transferase)	Nuclear	Hematogone, cortical thymocytes	– Immature thymic T cells (thymocytes) in normal thymic tissue and thymomas; – Acute leukemia (T and B lymphoblastic, myeloid, and undifferentiated); – BPDCN; – Indolent T lymphoblastic proliferation.
TIA-1 (T-cell intracellular antigen 1)	Cytoplasmic	Cytotoxic proteins in CD8+ T cells and NK cells	– Same expression and use as granzyme B and perforin.
Transferrin → see CD71			
Vimentin Also see solid tumor index	Cytoplasmic	Lymphoid tissue	– Lymphoma – Historical use.
vWF (von Willebrand factor)	Cytoplasmic	Megakaryocytes, platelets, carrier protein for factor VIII	– AML with megakaryocytic differentiation.
ZAP-70 (zeta chain-associated protein kinase 70) (flow marker)	Membranous	Part of T-cell receptor T cells and NK cells	– CLL, as prognostic marker; – T cells and NK cells.

Abbreviations: *AIDS* acquired immunodeficiency syndrome, *AITL* angioimmunoblastic T-cell lymphoma, *ALCL* anaplastic large cell lymphoma, *ALL/LBL* acute lymphoblastic leukemia/lymphoma, *AML* acute myeloid leukemia, *APL* acute promyelocytic leukemia, *BPDCN* blastic plasmacytoid dendritic cell neoplasm, *B-PLL* B-cell prolymphocytic leukemia, *CHL* classic Hodgkin lymphoma, *CLL/SLL* chronic lymphocytic leukemia/small lymphocytic lymphoma, *CMML* chronic myelomonocytic leukemia, *CNS* central nerve system, *DLBCL* diffuse large B-cell lymphoma, *EBV* Epstein-Barr virus, *FDC* follicular dendritic cell, *FL* follicular lymphoma, *GI* gastrointestinal, *GIST* gastrointestinal stromal tumor, *HL* Hodgkin lymphoma, *HSTCL* hepatosplenic T-cell lymphoma, *LP* lymphocyte predominant, *LPD* lymphoproliferative disorder, *MCL* mantle cell lymphoma, *MDS* myelodysplastic syndrome, *MF* mycosis fungoides, *MPN* myeloproliferative neoplasm, *MZL* marginal zone lymphoma, *NK* nature killer, *NLPHL* nodular lymphocyte predominant Hodgkin lymphoma, *NPC* nasopharyngeal carcinoma, *PDC* plasmacytoid dendritic cell, *PMLBCL* primary mediastinal large B-cell lymphoma, *PTCL* peripheral T-cell lymphoma, *NOS* not otherwise specified, *PTLD* posttransplant lymphoproliferative disorder, *RBC* red blood cells, *RS* Reed-Sternberg, *T-PLL* T-cell prolymphocytic leukemia

Chapter 7. Special Stains

By Natasha Rekhtman, Marina K Baine, Justin A. Bishop

Quick Primer on Mucins

- Mucins (aka mucoproteins or mucopolysaccharides) are large glycoproteins which are the chief components of mucus. Note that these are biochemically distinct from lipids and do not react with a lipid stain Oil Red O.
- Mucins are secreted by epithelial cells for protection and lubrication such as in the mucosal surfaces of the GI and respiratory tracts. This type of mucin is known as "epithelial mucin," and it may be produced in abundance (or focally) by some adenocarcinomas.
- Stromal tissues also contain mucopolysaccharides, which impart resilience to connective tissue. Stromal mucins are biochemically distinct from epithelial mucins in that they consist chiefly of hyaluronic acid. In contrast, hyaluronic acid is absent from epithelial mucins. By convention, stromal mucins are referred to as "myxoid" material. Many types of sarcoma secrete myxoid substances (such as myxoid chondrosarcoma), and various tissues may undergo myxoid change as a degenerative process (such as heart valves). Epithelial mucin and stromal myxoid substances are usually readily distinguishable by H&E – mucin is thick and stringy, whereas myxoid material is not. But in some situations, they may look similar, and then special stains can be used to distinguish the two, although this is rarely used for diagnostic purposes. See table and diagram below.
- Epithelial mucins come in two varieties, acidic and neutral:
  - **Acid mucins** are present in goblet cells and esophageal submucosal glands. They are **Alcian Blue (AB)-positive** (**blue color**). Most adenocarcinomas produce acid mucins.
  - **Neutral mucins** are present in gastric foveolar cells, duodenal Brunner glands, and prostate glands. They are **PAS-positive** (**pink color**). Unlike acid mucins, neutral mucins do not react with mucicarmine, AB, or colloidal iron.
- Acid mucins are further subdivided into two groups (not of major diagnostic importance):
  - **Sialomucins** are the simplest form; they are present in small and large bowel.
  - **Sulfomucins** are the more complex sulfated forms, which are present only in the large bowel.

	Composition	**Location**	**Mucicarmine** (pink)	**PAS** (pink)	**AB**[1] (blue)	**PAS/AB**[3]	**Hale colloidal iron**
Epithelial mucin	Acid mucins, sialated (sialomucins)	Small and large bowel, salivary glands	+	+	+ (pH 2.5 only)	+ Blue	+
	Acid mucins, sulfated (sulfomucins)	Large bowel (goblet cells)	+	+	+ (pH 2.5 and 0.5)	+ Blue	+
	Neutral mucin	Gastric foveolar cells, pyloric and Brunner glands, prostate	–	+	–	+ Magenta/pink	–
Stromal mucin[2] (myxoid material)	Hyaluronic acid (among others)	Myxoid sarcomas Mesothelioma Skin in lupus and granuloma annulare	–	–	+ (removed by hyaluronidase digestion)	–	+

1. The pH of AB can be adjusted to specifically recognize sialomucins (react at pH 2.5, but not 0.5) vs. sulfomucins (react at either pH). Only standard pH (2.5) is used for routine applications, wherein all types of mucin are recognized.
2. Note that stromal mucins are detected by AB and Hale colloidal iron only, whereas mucicarmine and PAS are negative! In addition, sensitivity to digestion with hyaluronidase distinguishes stromal mucins (hyaluronidase-sensitive) from epithelial mucins (hyaluronidase-resistant).
3. Because of distinct biochemical composition, various types of mucin can be distinguished by special stains: PAS/Alcian Blue (PAS/AB) is the most versatile. It stains neutral mucin pink/magenta and acid mucin (goblet cells) blue or purple if combined with neutral mucins.

- Distinguishing the types of mucin is utilized in the following differentials:
  1. Distinction of mesothelioma (hyaluronic acid-rich; AB+/hyaluronidase-sensitive) from adenocarcinoma (epithelial mucin-rich; AB+/hyaluronidase-resistant) – rarely used in current practice.
  2. Diagnosis of intestinal metaplasia in Barrett esophagus or stomach (see table below for details): native gastric epithelium has neutral mucin (PAS-positive/pink), whereas metaplastic intestinal epithelium has acid mucin (AB-positive/blue). Furthermore, some experts have suggested that sulfomucins portend a poorer prognosis than sialomucins in these metaplasias, but this is controversial.
  3. Diagnosis of gastric foveolar metaplasia in peptic duodenitis: confirmed by magenta staining with PAS of surface cells.
  4. Distinction of mucinous carcinoma (mucicarmine+) from myxoid sarcoma (mucicarmine–). This application is mainly of historic interest because immunostains can easily resolve this differential.

Abbreviations: *PAS* periodic acid Schiff, *AB* Alcian Blue

References: [1–3]

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Special Stains at a Glance

Carbohydrates: Glycogen and Mucosubstances	
Stain [color]	*Background and key applications*
Mucicarmine [Deep rose to red]	• Stains **epithelial mucin** (stromal myxoid substances, as in myxoid sarcomas, are mucicarmine–) • Used to ID intracytoplasmic mucin; this is a rapid and cheap method to diagnose adenocarcinoma • Highlights *Cryptococcus* cell wall • Also used to ID other mucin-producing tumors (e.g., mucoepidermoid carcinoma)
Periodic acid Schiff, PAS [Pink]	• Stains **glycogen** and **mucin**; also stains basement membranes and fungi • Used to ID glycogen-rich tumors: acinar carcinoma, pancreatic serous cystadenoma • PAS historically used for DDx of Ewing sarcoma and rhabdomyosarcoma (PAS+) versus lymphoma (PAS –) • Alveolar soft part sarcoma has PAS+ intracytoplasmic crystals • In practice, diagnosis for the above relies on IHC and FISH
PAS with diastase digestion, PAS/D	• Diastase (D) enzyme digests glycogen yielding a negative PAS reaction; in contrast, mucin is resistant to digestion, and PAS remains positive • Used to differentiate glycogen (PAS/$D^{Sensitive}$) from mucin (PAS/$D^{Resistant}$) • Used in routine medical liver biopsy to identify α1-antitrypsin globules (PAS/$D^{Resistant}$)
Alcian Blue, AB [Blue, dah!]	• Stains **acid mucin** (goblet cells) and stromal mucins (myxoid sarcomas) • Staining properties are pH-dependent (see above)
PAS/Alcian Blue, PAS/AB	• "**Pan-mucin**" stain: reacts with both neutral (PAS+) and acid (AB+) mucins. Commonly used for GI biopsies • Used to ID intestinal metaplasia with goblet cells (*AB+/deep blue*) in the esophagus (Barrett mucosa) and stomach • Used to ID gastric mucin cell metaplasia (*PAS+/pink*) in small bowel (seen in chronic peptic duodenitis and IBD) • Aids in the diagnosis of adenocarcinoma: PAS/AB may be used in conjunction with or in place of mucicarmine to demonstrate intracytoplasmic mucin
AB with hyaluronidase digestion	• Can be used to differentiate adenocarcinoma (AB+/hyaluronidase digest resistant) from hyaluronic acid-rich mesothelioma (AB+/ hyaluronidase digest sensitive), although diagnosis usually relies on IHC
Hale colloidal iron [Light blue]	• Mucin stain with iron as a reagent (this is *not* a stain for iron!) • Used to distinguish renal chromophobe carcinoma (positive) from oncocytoma (negative) • Also used to identify intradermal mucin (as in granuloma annulare)

Connective Tissue	
Masson trichrome ("Three colors")	• Can be used to differentiate collagen [blue] from smooth muscle [red] • Also used for evaluation of collagen fibrosis in bone marrow biopsies for MF • Routine stain in evaluation of the medical liver and kidney (degree of fibrosis)
Movat pentachrome ("Five colors")	• Primarily used to evaluate lung disease: – Loose collagen (mucopolysaccharide-rich) is blue-green: indicates a subacute process (e.g., myxoid plugs in organizing pneumonia or fibroblast foci in usual interstitial pneumonia) – Dense collagen/fibrosis is yellow: indicates chronicity (e.g., areas of established fibrosis in usual interstitial pneumonia) • Can aid in the evaluation of vessels and pleura (stain elastic fibers)
Reticulin	• Reticulin pattern can aid in DDx of certain neoplasms: – Meningioma (reticulin-negative) vs. HPC (reticulin-positive) (see neuropath section) – Gliosarcoma (+ reticulin in sarcomatous component and in glial) – Loss of reticulin network differentiates well-diff HCC from hepatic adenoma (see liver section) and normal pituitary from adenoma – Lymphomas – fine reticulin network • Used to evaluate bone marrow (to r/o reticulin fibrosis in MPN/MF)
Elastic (VVG or Verhoeff-Van Gieson)	• Used to assess the invasion of elastica in the visceral pleura in staging of pulmonary carcinomas • Also used to evaluate vessels (wall integrity, venous tumor invasion)

Other Common Applications	
Fat (lipids)	Oil Red O [red], Sudan Black B [black]; tissue must be fresh or frozen, not fixed!
Melanin	Fontana-Masson [black]
Calcium	Von Kossa [black]
Iron	Prussian blue [blue]
Amyloid	• Congo red (dense salmon pink in direct light; apple green birefringence in polarized light), crystal violet, Sirius Red, Thioflavin T (amyloid fluoresces in UV light) • Also used are immunostains for Ig κ and λ light chains (primary amyloidosis), amyloid A protein (secondary amyloidosis), β_2 microglobulin (dialysis-associated amyloidosis), transthyretin (hereditary amyloidosis), others

Special Stains at a Glance: Microorganisms

General	
Fungi	• **GMS (Grocott methenamine silver)** [black] – Demonstrates all fungi, including *Pneumocystis* – Also stains *Actinomyces*, *Nocardia*, and some encapsulated bacteria • **PAS and PAS/LG** (PAS/light green is preferred for dermatophytes) [red] • **Mucicarmine (and AB):** capsule of *Cryptococcus* [red (and blue)] • **Fontana-Masson:** melanin pigment in *Cryptococcus* and pigmented filamentous fungi [black]
Bacteria	• **Brown and Brenn (or Brown and Hopps modification)**: demonstrates Gram – [red] and Gram + [blue] bacteria • **Gram-Weigert**: demonstrates Gram + bacteria [blue to purple] and PJP [blue to purple] but not Gram – bacteria! • **Warthin Starry:** demonstrates spirochetes (*Treponema*), *Helicobacter*, *Chlamydia*, and *Legionella* [black]
Acid-fast organisms	• **Kinyoun** (a variant of **Ziehl-Neelsen**): routine AFB stain [red]; detects all mycobacteria, including MAI • **Auramine-rhodamine** (fluorescent stain): more sensitive than Kinyoun • **Fite:** demonstrates delicate acid-fast organisms (*Nocardia*, *M. leprae*) [red] • **Feulgen:** stains microbial DNA [magenta] • **Gram-Weigert:** acid-fast organisms are Gram +, but sensitivity is low

Select Microorganisms	
Pneumocystis	GMS is the best [black]**;** Gram-Weigert [blue to purple], Giemsa [intracystic trophozoites are purple]
Nocardia	Fite [bright red], Brown-Hopps [blue], Gram-Weigert [blue], GMS [black]
Histoplasma	GMS [black], Giemsa [reddish blue]
Cryptococcus	GMS [black], mucicarmine [capsule red], Alcian Blue [capsule blue], PAS [cell wall red], Fontana-Masson [cell wall black – great for detection of capsule-negative crypto], India ink (on CSF samples; historic use)
Spirochetes	Warthin Starry [black], Dieterle [dark brown-black]
Actinomyces	Brown-Hopps [blue], Gram-Weigert [blue], GMS [black]
Helicobacter pylori	Diff-Quik [dark blue], Giemsa [dark blue], Warthin-Starry [black]; now routinely diagnosed by IHC
Tropheryma whippelii (Whipple disease)	PAS highlights filamentous organisms [pink] within macrophages (diastase resistant); AFB negative; also, an immunostain for *Tropheryma whippelii* is available (DDx: MAI is chunky on PAS, AFB+)
Leishmania	Giemsa [reddish blue], GMS-negative, PAS-negative
Entamoeba	PAS [pink cytoplasm]

Alphabetical Index of Special Stains

Update by Marina K Baine and Natasha Rekhtman (prior edition by Jennifer Broussard, Natasha Rekhtman, Justin A. Bishop)

Stain	What this stain is used to identify and comment	How it stains
Acid-fast bacteria (AFB, Kinyoun, Ziehl-Neelsen)	• Acid-fast bacilli (mycobacteria) include *M. tuberculosis* (MTB), *M. leprae*, and atypical/non-tuberculous mycobacteria (*M. avium-intracellulare* complex – MAC or MAI – and other rare mycobacteria) • AFB detects MTB and MAI. *M. leprae* requires Fite • Other organisms detected by AFB: *Cryptosporidium*, *Isospora*, and hooklets of cysticerci • Kinyoun = modified Ziehl-Neelsen • Note: "acid-fast" refers to the organism's ability to retain the red dye in the presence of acid (meaning it is "color-fast" or "holds on tight to color"). Some delicate organisms (*Nocardia*, *M. leprae*) are "weakly acid fast": they get decolorized by strong acids in standard AFB stains (Kinyoun) but are able to retain the dye when treated with weaker acids in modified acid-fast stain (Fite)	Acid-fast bacilli: bright red Background: blue
Alcian Blue (AB)	• **Acid mucin** (goblet cells) and stromal mucins (myxoid sarcomas) • Staining properties are pH-dependent (see mucin primer above for details)	Acid mucins/mucosubstances: blue Nuclei: reddish pink
Alcian Blue with hyaluronidase digestion	• Hyaluronidase digests hyaluronic acid but not mucin and can be used to differentiate adenocarcinoma with mucin (AB+/hyaluronidase digest ^{resistant}) from hyaluronic acid-rich mesothelioma (AB+/ hyaluronidase digest ^{sensitive}); although diagnosis usually relies on IHC	Digested areas: unstained Undigested areas: blue Nuclei: reddish pink
Auramine-rhodamine	• Acid-fast bacteria. Supposed to have higher sensitivity than AFB, but you have to use a fluorescent microscope	Acid-fast organisms: reddish yellow fluorescence Background: black
Bilirubin (Hall bilirubin stain)	• Bilirubin (the principal bile pigment and a normal product of red cell degradation) • Excessive amounts of bile pigment in the liver may be found in cases of hepatic or extrahepatic biliary obstruction	Bile pigment: green Muscle and cell cytoplasm: yellow Collagen: red
Brown and Hopps (Brown and Brenn)	• Gram-negative and Gram-positive bacteria in tissue	Gram-positive bacteria: blue Gram-negative bacteria: red Nuclei: red Background: yellow
Congo red (CR)	• Amyloid deposits in tissue sections	Amyloid: red to pink (direct light); apple green (polarized light) Nuclei: blue Need to cut sections slightly thicker (8µm) for optimal birefringence
Copper	• Copper deposits left in the liver, such as in Wilson disease	Copper deposits: bright red to orange
Cresyl violet	• Nerve cells and glia	Nerve cell nucleus: pale blue Nissl bodies: dark blue Astrocytes: pale blue Oligodendroglia: very dark blue
Crystal violet	• Carbohydrates • May be used to highlight amyloid	Amyloid: blue-purple All other tissue elements: blue
Dieterle	• Spirochetes, *Legionella*, and other bacteria • Melanin granules, chromatin, formalin pigment, and some foreign material also stain	Spirochetes, *Legionella*, etc.: black to dark brown; Background: pale yellow to tan
Diff-Quik (Diff-Quik®, Rapid Romanowsky)	• *H. pylori* • A modified Giemsa stain • Routine stain for air-dried smears in cytopathology	*H. pylori*: dark blue Nuclei: blue Cytoplasm: pink
Elastic Van Gieson (EVG) → see Verhoeff-Van Gieson		
Feulgen	• DNA in tissue • Often used for ploidy studies • Also used to identify AFB	Nuclei: magenta Background: green
Fite	• *M. tuberculosis* (MTB) plus delicate acid-fast organisms – *Mycobacterium leprae* (leprosy) and *Nocardia* • Uses a weaker acid than other AFB stains • More user-friendly than AFB because the background tissue architecture is visible	Acid-fast bacilli: red Background: blue
Fontana-Masson	• Argentaffin granules and melanin • *Cryptococcus* and other melanin-producing fungi. Great for detection of capsule-deficient cryptococci	Melanin, argentaffin cells: black Nuclei: red
Giemsa	• *H. pylori* • Also differentiates hematopoietic cells and is used in blood smears	*Helicobacter* bacteria: dark blue Cell nuclei: blue Connective tissues: pink Red blood cells: salmon pink Starch and cellulose: sky blue

Alphabetical Index of Special Stains – Continued

Giemsa– Bone Marrow	• Differentiation of cells present in hematopoietic tissue • Also used for the demonstration of some microorganisms	Nuclear chromatin: dark blue Cytoplasm of lymphocytes and monocytes: pale blue Neutrophil granules: purple Eosinophil granules: pink/orange Basophil granules: purple/black Nucleoli: blue Erythrocytes: pink Connective tissue: pink to light purple Mast cell granules: dark purple
Gram-Weigert	• Gram-positive bacteria • *Pneumocystis jirovecii* (formerly *P. carinii* or PCP) • Does not stain Gram-negative bacteria, so often ordered with Brown and Hopps	Gram-positive bacteria: blue-purple Background: pink
Grocott (or Gomori) methenamine silver (GMS)	• Fungi, including *Pneumocystis jirovecii* (formerly *P. carinii* or PCP) • *Actinomyces*, *Nocardia*, and some encapsulated bacteria • Also stains elastic fibers, suture material, calcifications, and others	Fungi: black Background: green
Hale colloidal iron	• A mucin stain with iron as a reagent (This is *not* a stain for iron!) • Used to ID intradermal mucin (as in granuloma annulare) • Chromophobe RCC (+) vs. oncocytoma (–)	Cytoplasm of chromophobe RCC, stromal mucins: light blue Nuclei: red
Hall bilirubin stain → see bilirubin		
Iron	• Ferric iron in tissue sections • Small amounts of iron are found normally in the spleen and bone marrow. Excessive amounts are present in hemochromatosis (deposits in the liver and pancreas) and hemosiderosis (deposits in the liver, spleen, and lymph nodes) • Used in routine bone marrow aspirate evaluation to identify ring sideroblasts	Iron (hemosiderin): blue Nuclei: red Background: pink
Kinyoun → see acid-fast bacteria		
Leder	• Neutrophils, mast cells, and their precursors	Cytoplasm of neutrophilic myeloid cells and mast cells: red Nuclei: blue Erythrocytes: pale pink to colorless
Luxol fast blue	• Myelinated fibers	Myelinated fibers: blue Neutrophils: pink Nerve cells: purple
Masson trichrome ("three colors")	• Collagen (+) vs. smooth muscle (–) in tumors • Collagen in diseases such as cirrhosis • Reinke crystals in Leydig cell tumor • Routine stain for liver and kidney biopsies to evaluate extent of fibrosis	Nuclei: black Cytoplasm, muscle, erythrocytes: red Collagen: blue
Melanin bleach	• When melanin pigment is present in large amounts, cell detail may be obscured • Also the ability to be bleached serves as an identifying factor for melanin	If the pigment is melanin, it will not be present on the slide that was bleached
Miller elastic	• Elastic fibers	Elastic fibers, nuclei, and mast cells: blue-black Muscle: yellow Collagen: red
Movat pentachrome ("Five colors")	• Connective tissue stain that demonstrates many entities: nuclei, elastin, collagen, ground substance, mucin, muscle, and fibrin • Can aid in evaluation of vessels and pleura (stains elastic fibers) • Primarily used to evaluate lung disease: – Loose collagen (mucopolysaccharide-rich) is blue-green: indicates a subacute process (e.g., organizing fibroplasia in organizing pneumonia) – Dense collagen/fibrosis is yellow: Indicates chronicity (e.g., usual interstitial pneumonia)	Nuclei: black Elastic fibers: black Collagen (established): yellow Ground substance/mucin/subacute fibroblastic tissue: blue to green Muscle: red Fibrinoid: intense red
Mucicarmine	• Epithelial mucin (stromal myxoid substances, as in myxoid sarcomas, are mucicarmine–) • Demonstration of intracytoplasmic mucin is a rapid and cheap method to diagnose adenocarcinoma and other mucin-producing tumors (e.g., mucoepidermoid carcinoma) • (see mucin primer above for details)	Mucin: deep rose Nuclei: black Other tissue elements: yellow
Oil Red O	• Fat or lipids • Requires *fresh* (*unfixed*) tissue • Fat occurring in an abnormal place, such as fat emboli that may develop after either a bone fracture or an injury that crushes a fatty body area or fat accumulation in the liver (steatosis) • Certain tumors (e.g., liposarcoma, sebaceous carcinoma, lipid vacuoles in Burkitt lymphoma) • Hemangioblastoma (+) vs. RCC (–)	Fat: red Nuclei: blue

Alphabetical Index of Special Stains – Continued

Periodic acid Schiff (PAS)	• Glycogen, neutral mucin, basement membranes, and fungi • A routine stain for kidney biopsies • Can be difficult to read because of high background • Has applications in tumor diagnosis: – Glycogen-rich tumors: acinar carcinoma, pancreatic serous cystadenoma – Alveolar soft part sarcoma – contains PAS+ intracytoplasmic crystals – Ewing sarcoma and rhabdomyosarcoma (+) versus lymphoma (PAS–) this is rarely utilized diagnostically as a result of advances in immunohistochemistry	Mucin, glycogen, fungus: pink Nuclei: blue
PAS/AB (periodic acid Schiff/ Alcian Blue)	• Both acid (AB) and neutral (PAS) mucins • Commonly used for GI biopsies with many applications: – Intestinal metaplasia with goblet cells (*AB+/deep blue*) in the esophagus (Barrett mucosa) and stomach – Gastric mucin cell metaplasia (*PAS+/pink*) in the small bowel (seen in chronic peptic duodenitis and inflammatory bowel disease) – Adenocarcinoma at any site: PAS/AB may be used in conjunction with or in place of mucicarmine to demonstrate intracytoplasmic mucin	Acid mucosubstances (AB+): blue Neutral polysaccharides (PAS+): pink
PAS/diastase (PAS/D)	• Fungus • Diastase enzyme digests glycogen yielding a negative PAS reaction, whereas mucin is resistant to digestion and PAS remains positive • Used to differentiate glycogen ($PAS/D^{Sensitive}$) from mucin ($PAS/D^{Resistant}$) • Used to identify α1-antitrypsin globules ($PAS/D^{Resistant}$) in routine liver biopsy	Glycogen: pink by PAS and absent by PAS/D Mucin: pink by PAS and PAS/D Note: If slide is overdigested, the tissue must be recut. Overdigestion has the appearance of lace; there is no tissue left.
PAS light green	• Fungus • Does not stain mucin or basement membranes	Fungus: pink Background: green
PAS/methenamine silver (PAS/MS)	• Basement membrane • Especially suitable for demonstrating fine glomerular basement membranes in thin sections	Basement membrane: black Nuclei: blue Background: pink
PTAH (Phosphotungstic acid-hematoxylin)	• Muscle cross-striations and fibrin • Nemaline rods, present in some skeletal muscle diseases, may also be demonstrated by this method • Truly oncocytic neoplasms, wherein PTAH stains mitochondria	Cross-striations, fibrin, glial fibers: blue Neurons: pink Mitochondria: blue Nuclei: blue Collagen: red-brown Elastic fibers: purplish
Reticulin	• A silver impregnation technique that demonstrates reticulin fibers, which provide structural support to the parenchyma in many organs in the body and are abundant in the liver, spleen, and kidney • The reticulin framework is characteristically lost in some tumors (e.g., HCC, pituitary adenoma) • Used in routine bone marrow biopsy to evaluate for reticulin fibrosis (increased in advanced myeloproliferative disorders, myelofibrosis, and metastatic disease to the marrow) • Reticulin fibers also form characteristic patterns in relationship to certain tumor cells (positive in sarcoma and lymphoma, negative in carcinoma and glioma)	Reticular fibers: black Nuclei: red
Sirius Red	• Amyloid	Amyloid: rose red Nuclei: blue Background: pale pink
Sudan Black	• Fat • Like Oil Red O, tissue must be fresh	Fat: blue-black Nuclei: red
Thioflavin S	• Amyloid • A fluorescent stain	Amyloid: fluorescent green Background: black
Toluidine blue	• Mast cells. Their cytoplasm contains metachromatic granules composed of heparin and histamine	Mast cells: violet Background: blue
Verhoeff-Van Gieson (VVG or elastic Van Gieson, EVG)	• Useful in evaluation of elastic fibers in vascular diseases • Used to confirm invasion through elastic fibers in the visceral pleura for lung cancer staging (upstage to pT2a) • Used to diagnose bronchiolitis obliterans (in chronic GVHD and chronic lung transplant rejection) • Can aid in detection of venous tumor invasion [4]	Elastic fibers and nuclei: black Collagen: red Other tissue elements: yellow
Von Kossa	• Deposits of calcium in any area of the body • May discriminate between urate crystals and true calcium, which appear similar histologically, to make the diagnosis of chondrocalcinosis (pseudogout)	Calcium salts: black Nuclei: red Cytoplasm: pink
Warthin Starry	• Spirochetes, *H. pylori*, *B. henselae* (cat scratch), and *Legionella*	Bacteria: black Nuclei and red blood cells: brown Background: pale yellow
Ziehl-Neelsen→ see acid-fast bacteria		

Chapter 8. Grading (and Classification) Systems Quick Reference: Solid Tumors

By Marina K Baine, Justin A. Bishop*, Diana Weedman Molavi, Youran Zou, Tejus A. Bale, Natasha Rekhtman**
*(*All subsections are by these authors, unless specified otherwise)*

Grading of Adenocarcinoma, NOS[a]	
	Fraction of tumor composed of glands
Well differentiated (Grade 1)	>95%
Moderately differentiated (Grade 2)	50–95%
Poorly differentiated (Grade 3)	<50%

- Applies mainly to adenocarcinomas of the gastrointestinal tract (esophagus, stomach, bowel, anus).
- Undifferentiated CA applies to carcinomas that are so poorly differentiated that they cannot be identified as adenoCA vs. SqCC vs. others. Small cell and large cell neuroendocrine carcinomas are always high grade.
- Grade is usually assigned based on the least differentiated area.

[a]Note that this is a "rule of thumb," and more detailed grading systems for individual organs (incorporating other features such as cytologic pleomorphism, necrosis, mitoses, etc.) are either available or being developed. However, the loss of glandular architecture is a general hallmark of poor differentiation in adenocarcinomas. Exception is micropapillary pattern, which in carcinomas of virtually all sites is a high-grade pattern.

References: [1, 2]

Grading of Squamous Cell Carcinoma (SqCC), NOS		
	Nuclear pleomorphism and mitoses (including atypical mitoses)	Keratinization and intercellular bridges
Well differentiated (Grade 1)	Absent	Abundant
Moderately differentiated (Grade 2)	Intermediate	Intermediate
Poorly differentiated (Grade 3)	Abundant	Nearly absent

- Applies to SqCC of any site: the head and neck, lung, abdominal organs (esophagus, bladder), skin, etc.
- There is no widely accepted quantitative definition of grading in SqCC. As a "rule of thumb," WD SqCC are said to closely resemble normal squamous epithelium, whereas PD SqCC are those in which squamous origin can be barely discerned.
- It is generally emphasized that the grade should be assigned based on nuclear features rather than degree of keratinization, although the two almost always go together. Nevertheless, the degree of keratinization is usually expressed by designating a SqCC as "keratinizing" vs. "nonkeratinizing" separately from the grade.
- HPV-related SqCC of the oropharynx should not be graded [3].
- As for adenocarcinoma, grade is assigned based on the least differentiated area.
- In contrast to adenocarcinoma, grade does not appear to be a strong predictive factor in SqCC, particularly of the head and neck.

References: [4, 5]

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N. Rekhtman et al., *Quick Reference Handbook for Surgical Pathologists*, https://doi.org/10.1007/978-3-319-97508-5_8

Breast

Elston[a] Grading of Infiltrating Breast Cancer		
Parameter	**Point score**	**Final score** (add point scores in rows 1, 2, and 3) **and corresponding grade**
1. Tubule formation (% composed of tubules)		**3–5 points → Grade I** (well differentiated) **6–7 points → Grade II** (moderately differentiated) **8–9 points → Grade III** (poorly differentiated)
• ≥75%	1	
• 10–75%	2	
• <10%	3	
2. Nuclear pleomorphism[1]		
• Mild	1	
• Moderate	2	
• Severe	3	
3. Mitoses[2] (per mm²)		
• ≤3	1	
• 4–7	2	
• ≥8	3	

1. Nuclei are evaluated at the periphery of the tumor or in the area with highest nuclear grade:
   Mild pleomorphism: uniform nuclei, size similar to normal duct cells.
   Moderate and severe pleomorphism: increasing severity of nuclear enlargement, size and shape variability, clumping (vesicular) of chromatin, and prominence of nucleoli.
2. Mitotic count should be performed in the most mitotically active part of carcinoma, which is usually at the periphery of the tumor.
   Mitotic count should be done in 10 HPFs (40X objective, i.e., 400X field). The actual size of 400X field is microscope-dependent and should be measured with stage micrometer. For a table with conversion between field size, mitotic count, and point score, see Reference [6].

Note: Lobular carcinoma is always given 3 points for lack of tubule formation. It is still usually Elston grade I or II, as nuclei generally get 1–2 points and mitotic count gets 1 point. An exception to this is pleomorphic lobular carcinoma, which by definition has marked nuclear atypia (nuclear score of 3), making it at least Elston grade II. Ductal carcinoma is more commonly Elston grade II or III. Tubular cancer is by definition grade I.

[a]"Elston grade" is mercifully short for "Elston-Ellis modification of Scarff-Bloom-Richardson" grading system (or Nottingham combined histological grade).

References: [1, 6, 7]

Van Nuys Nuclear Grading of Ductal Carcinoma In Situ (DCIS)			
	Nuclear size	**Mitoses per 10 HPF**	**Nuclear pleomorphism[a]**
Grade 1	<1.5 RBC or normal duct cell	Rare	Mild
Grade 2	1–2 RBC	Sparse	Moderate
Grade 3	>2.5 RBC	Frequent	Severe

[a]Nuclear pleomorphism is graded as described above for invasive lesions.

Notes:
1. The most recent WHO grading scheme is based on nuclear grade alone, without incorporating necrosis. Similarly, CAP recommends using the same nuclear grading scheme and reporting the presence or absence of necrosis, instead of combining the features of both. When present, necrosis should be described as focal (punctate) or central ("comedo"), the latter of which is generally reserved for grade 3 lesions.
2. Architectural histopathologic features are not taken into account for grading purposes but should be reported as they have prognostic significance.
3. LCIS is not generally graded.

Reference: [1, 6]

Grading of Phyllodes Tumor					
	Stromal cellularity	**Stromal overgrowth (4X field is all stroma)**	**Stromal pleomorphism**	**Infiltrative border**	**Mitoses/10 HPF**
Benign	Mild	–	None to minimal	–	<5
Borderline/low-grade malignant	Moderate	– (or very focal)	Mild to moderate	F+	5–9
Malignant[a]	Marked	+	Marked	+	>10

[a]Malignant heterologous elements may be present in a malignant phyllodes tumor but never seen in benign or borderline lesions.

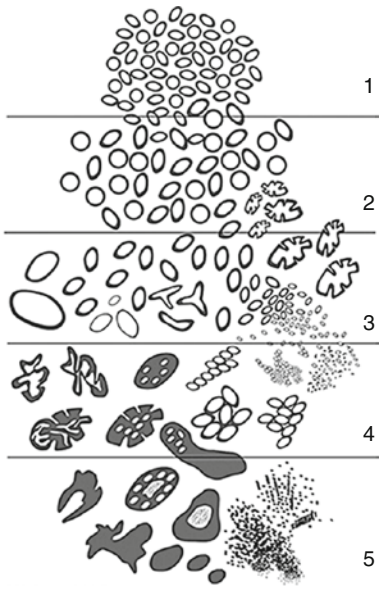
References: [1, 8]

Genitourinary Tract: Prostate

Gleason Grading of Prostate Cancer

The Gleason system is a five-tier system based entirely on architectural pattern; nuclear features are not factored in. The grade is reported as a sum of the most prevalent (primary) and second most prevalent (secondary) pattern to obtain a "combined Gleason grade" or "Gleason score." For example, a tumor with primary Gleason pattern 3 and secondary Gleason pattern 4 is reported as Gleason score 3 + 4 = 7. Note that in this example, 3 and 4 are "Gleason patterns," and 7 is a "Gleason score."

Gleason pattern 1	• Non-infiltrative nodule • Round to oval back-to-back glands • Exceedingly rare diagnosis, usually seen on TURP specimens
Gleason pattern 2	• Fairly well-circumscribed nodule, but minimal infiltration is allowed • Glands are more loosely arranged and not as uniform as those in pattern 1 • Exceedingly rare diagnosis, usually but not always in transition zone
Gleason pattern 3	• Clearly infiltrative pattern (unlike patterns 1 and 2) • Glands vary in size and shape • All glands are distinct, such that one can draw a mental circle around each gland • Microcystic, atrophic pattern, branching, and pseudohyperplastic glands are now recognized as pattern 3 • PIN-like ductal adenocarcinoma
Gleason pattern 4	• Glands are no longer separate as seen in patterns 1–3 (**one cannot draw a mental circle around each gland**): glands are fused, poorly defined, cribriform, or glomeruloid • All cribriform glands are now considered pattern 4 • Ductal adenocarcinoma (except PIN-like variant, which is graded as pattern 3, and ductal adenocarcinoma with necrosis, which is graded as pattern 5)
Gleason pattern 5	• Cells in solid nests and sheets, rosettes, cords, or single cells with virtually no glandular differentiation • Nests of tumor with central "comedonecrosis" are also classified as pattern 5
Not graded	• Small cell prostate carcinoma • Adenocarcinoma with Paneth cell-like differentiation (by criteria, would be graded 5 + 5 but behaves like 3 + 3)



Weinzerl | Visual Media
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Because clinical decisions are based primarily on the total Gleason score, several modifications to the traditional Gleason grading have been proposed to better convey the severity of disease:

1. "5% cutoff rule": If lower-grade pattern occupies <5% of the tumor, it can be ignored. For example, a 4 + 3 = 7 in traditional Gleason grading should be diagnosed as 4 + 4 = 8, if pattern 3 comprises <5% of the tumor. The highest-grade pattern is included in the score **regardless** of its quantity.
2. When three Gleason patterns (e.g., 3, 4, 5) are present, the Gleason score is derived by adding the most prevalent and the highest grades. This is true on biopsies regardless of the amount of the highest-grade pattern (5 in this case). On resections, however, this applies only when the highest-grade pattern comprises >5% of the tumor.
3. Gleason patterns 1 and 2 are essentially historical, in that they are no longer assigned on biopsy and exceedingly rarely on resection. In fact, the current WHO urges against assigning Gleason scores 2–5 on biopsy due to low reproducibility, poor correlation with final grade on resection, and potentially misleading prognosis.

Illustration from: *Am J Surg Pathol* 2016, 40(2): 244–252; Epstein JI; The 2014 ISUP Consensus Conference on Gleason Grading of Prostatic Carcinoma; with permission from © Wolters Kluwer Health 2016

Abbreviations: *PIN* prostatic intraepithelial neoplasia, *TURP* transurethral resection of the prostate

Reference: [9]

Prostate Cancer Prognostic Grade Groups[a]

Grade group	Gleason score (patterns)
1	≤6
2	7 (3 + 4)
3	7 (4 + 3)
4	8 (4 + 4; 3 + 5; 5 + 3)
5	9–10

[a]Based on the most recent multi-institutional data, prostate cancer has been assigned into grade groups (1–5), each associated with a unique prognosis, and thus a distinct therapeutic approach. The WHO recommends to report both Gleason grade and grade group in a surgical pathology report, which is now done by most GU pathologists. Reference: [9]

Grading of Prostatic Intraepithelial Neoplasia (PIN)[a]

	Nuclear cytology
LGPIN (low-grade prostatic intraepithelial neoplasia)	Enlarged, marked size variation; Normal chromatin pattern; Rare prominent nucleoli (<10% of cells)
HGPIN (high-grade prostatic intraepithelial neoplasia)	Enlarged, mild to moderate size variation; hyperchromasia and chromatin clumping; prominent nucleoli

[a]Although not part of grading criteria, integrity of the basal cell layer is another helpful distinguishing feature of LG versus HG PIN. While it is intact in LGPIN, it is often disrupted or attenuated in HGPIN.

Reference: [10]

Genitourinary Tract: Kidney and Bladder

WHO (2014)/ISUP Grading of Renal Cell Carcinoma		
	Nucleoli (400X magnification)	**Nucleoli (100X magnification)**
Grade 1	Absent or inconspicuous	Absent
Grade 2	Conspicuous and eosinophilic	Visible but not prominent
Grade 3	Prominent	Conspicuous and eosinophilic
Grade 4[a]	Prominent	Prominent

[a]Rather than nucleolar prominence, Grade 4 is defined by marked nuclear pleomorphism, multinucleated giant cells, and/or rhabdoid and/or sarcomatoid differentiation.

- Tumors are graded by the worst area, however focal
- Grading is applied to clear cell and papillary RCC
- Collecting duct carcinoma is ISUP grades 3–4
- Chromophobe RCC is generally not graded
- Oncocytoma is benign and therefore not graded

Reference: [10]

Nephroblastoma (Wilms Tumor): Criteria for Anaplasia (unfavorable histology)
1. Nucleomegaly (at least 3X enlargement).
2. Nuclear hyperchromasia.
3. Atypical mitoses (large and multipolar).
Note: Anaplasia predicts resistance to chemotherapy and inherent aggressiveness, the latter of which has been reflected in more recent data [11]. Reference: [10]

The WHO (2004)/ISUP Consensus Classification of Non invasive (*In Situ*) Papillary Urothelial Neoplasms (see http://pathology.jhu.edu/tutorials/bladder/ for tutorial)					
	Urothelial thickness	**Cellular disorganization: loss of polarity, crowding**	**Pleomorphism**[4]	**Mitoses**	**Fusion and branching of papillae (soft feature)**
Papilloma	Normal (<7 layers)	Absent (perfectly orderly, identical to normal)	Absent	Absent	None
PUNLMP[1]	Increased	Absent (perfectly orderly, identical to normal)	Absent	Rare, basal	Rare
LGPUC[2]	Increased	Minimal	Mild	Occasional, at any level	Occasional
HGPUC[3]	Increased	Prominent	Moderate to severe	Frequent, at any level	Frequent

1. **PUNLMP** (papillary urothelial neoplasm of low malignant potential): cells may be uniformly enlarged, but they are identical to each other in all fields and are perfectly oriented (orderly).
2. **LGPUC** (low-grade papillary urothelial carcinoma): overall low-power appearance is orderly, but there is distinctive variation of architectural and/or cytological features.
3. **HGPUC** (high-grade papillary urothelial carcinoma): distinctive pleomorphism and loss of polarity/crowding. Necrosis and cellular discohesion, when present, are specific to HGPUC.
4. Pleomorphism refers to nuclear enlargement, hyperchromasia, variation in size and shape, and prominence of nucleoli. Nuclear grooves, a feature of normal urothelium, are preserved in PUNLMP but are lost in carcinomas (low-grade and high-grade).

"**5% rule**": Grade is assigned based on the highest-grade area, unless it is <5% of the tumor (presence of a small higher-grade area may be mentioned in a note).

References: [10, 12]

The WHO (2004)/ISUP Consensus Classification of Flat *In Situ* Urothelial Neoplasms (see http://pathology.jhu.edu/tutorials/bladder/ for tutorial)	
Dysplasia	Some features of CIS are present but fall short of the threshold for CIS (cytology similar to LGPUC). Uncommon diagnosis.
Carcinoma in situ (CIS)	Nucleomegaly (nuclei are **5X** the size of stromal lymphocytes vs. normal urothelium is 2–3X), pleomorphism, 1–2 irregular nucleoli, nuclear crowding, loss of polarity (cytology similar to HGPUC).
References: [10, 12]	

Abbreviations: *PUC* papillary urothelial carcinoma (*LG* low grade, *HG* high grade), *ISUP* International Society of Urological Pathology

Head and Neck

by Justin A. Bishop

Grading of Thyroid Carcinomas	
Well differentiated[1]	Papillary carcinoma[2] Follicular carcinoma[3] • Minimally invasive • Encapsulated angioinvasive • Widely invasive
"Moderately differentiated"	None[4]
Poorly differentiated	Insular, solid, or trabecular architecture + no papillary nuclear features + one of these three: Convoluted nuclei, elevated mitoses (≥3/10 HPF), or necrosis[5]
Anaplastic (undifferentiated)	Minimal or no thyroid differentiation. Includes squamoid, pleomorphic/giant-cell, and spindled variants

1. Medullary carcinoma has a significantly worse prognosis than papillary or follicular carcinoma and is not graded. As a result, when you hear the term "well-differentiated thyroid cancer," it refers to just the papillary and follicular types.
2. Tall cell, columnar cell, hobnail, and diffuse sclerosing variants have a worse prognosis and should be mentioned in the report.
3. In widely invasive follicular carcinoma, there is typically no capsule to evaluate because the cancer has pretty much blown past it as invasive nodules in the parenchyma. The term "minimally invasive" should be limited to cases that have capsular invasion only [13]. For encapsulated angioinvasive follicular carcinomas, the number of foci of vascular invasion should be reported (if <4, the prognosis is good).
4. Some regard the high-risk variants of papillary CA as well as widely invasive follicular CA as "moderately differentiated" thyroid carcinoma [14]. We do not use this designation at our institutions, and it is not recognized in modern classification schemes.
5. The criteria listed above are from the 2006 Turin proposal [15] which were encoded into the 2017 WHO classification [16]. However, at some institutions the criteria are less strict, requiring only elevated mitoses (>4/10 HPF) *or* necrosis [17]. Regardless of what criteria are used to diagnose poorly differentiated carcinoma, the presence of the high-grade features (elevated mitoses or necrosis) in a follicular or papillary carcinoma should be mentioned.

Grading of Salivary Gland Carcinomas[1]			
Low-grade	**Intermediate-grade**	**High-grade**	**Variable grade**
Acinic cell carcinoma Polymorphous adenocarcinoma Basal cell adenocarcinoma Epithelial-myoepithelial carcinoma Secretory carcinoma Clear cell carcinoma	Adenoid cystic carcinoma[2] Myoepithelial carcinoma	Salivary duct carcinoma Neuroendocrine carcinomas Large cell undifferentiated carcinoma Lymphoepithelial carcinoma Primary squamous cell carcinoma	Mucoepidermoid carcinoma (see table below) Adenocarcinoma, NOS[3] Carcinoma ex-pleomorphic adenoma[4] Intraductal carcinoma[5]

1. Most salivary gland carcinomas have a default grade for typical examples, but tumors should be "upgraded" if they show more aggressive histologic features (e.g., basal cell adenocarcinoma with a highly infiltrative pattern, necrosis, and marked pleomorphism would be regarded as high-grade). Moreover, virtually all types of low- or intermediate-grade carcinoma may rarely exhibit high-grade transformation ("dedifferentiation") into a high-grade adenocarcinoma NOS or large cell undifferentiated carcinoma.
2. Although classic adenoid cystic carcinoma is generally considered an intermediate-grade carcinoma, tumors with solid areas (especially >30%) behave worse (more like high-grade). The approximate percentage of solid pattern should be noted.
3. Adenocarcinoma, NOS, is graded low, intermediate, or high-grade based on cytological features, presence/absence of necrosis, and degree of invasiveness.
4. The type of carcinoma arising in the mixed tumor should be graded as it would if it had arisen de novo.
5. Intraductal carcinoma is the salivary analogue to breast ductal carcinoma in situ. It should be graded as low, intermediate, or high-grade based on cellular features and necrosis but has an excellent prognosis in its pure form (i.e., no invasive component).

References: [5, 18]

Mucoepidermoid Carcinoma, AFIP Grading System		
Histopathological feature	**Point value**	**Total point score** (add points in point value column) **and corresponding tumor grade**
Cystic component <20%	2	0–4 → **Low-grade** 5–6 → **Intermediate-grade** >7 → **High-grade**
Neural invasion	2	
Necrosis	3	
>4 mitoses per 10 HPF	3	
Anaplasia	4	

This is the most widely used grading scheme, but the WHO classification does not endorse any specific grading system.

References: [5, 18]

Evaluation of Autoimmune Sialiadenitis (Sjögren Syndrome) in Labial Biopsy		
Grade	**Amount of inflammation** (lymphocytes, plasma cells, histiocytes)	**Likelihood of Sjögren Syndrome**
0	Absent	Nondiagnostic
1	Slight infiltrate	Nondiagnostic
2	Moderate infiltrate (less than 1 focus[a] per 4 mm^2)	Nondiagnostic
3	One focus per 4 mm^2	Suggestive
4	More than one focus per 4 mm^2	Diagnostic

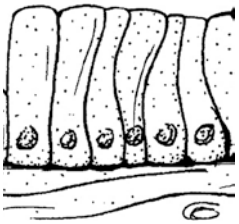
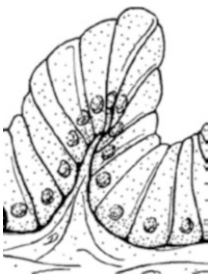
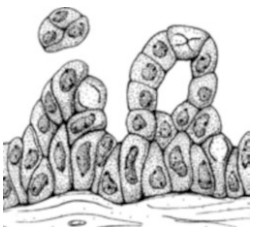
[a]"Focus" is defined as an aggregate containing at least 50 lymphocytes, plasma cells, or macrophages.

There is a lack of standardization among oral pathologists' grading for Sjögren syndrome. Another common approach is the American College of Rheumatology recommendation which consists of focus score = (number of foci*4)/(area of glandular tissue present in mm2), with a focus score ≥1 being supportive of Sjögren syndrome if other required criteria are met [19].

References: [20, 21]

Pancreas and Biliary Tree

Grading of Pancreatic Intraepithelial Neoplasia (PanIN) and Pancreatic Cystic Mucinous Neoplasms (IPMN and MCN)

Terminology for PanIN, IPMN, and MCN			Cytology	Architecture	Illustration
WHO 2010		**Revised 2015**			
PanIN-1A	IPMN/MCN – Low-grade dysplasia	**Low-grade PanIN, IPMN, or MCN**	Small bland cuboidal basally located nuclei	Flat	 PanIN-1A
PanIN-1B	IPMN/MCN – Low-grade dysplasia	**Low-grade PanIN, IPMN, or MCN**	Small bland cuboidal basally located nuclei	Papillary	 PanIN-1B
PanIN-2	IPMN/MCN – Moderate dysplasia	**Low-grade PanIN, IPMN, or MCN**	– Moderate pleomorphism (↑N/C ratio, prominent nucleoli) – Stratified nuclei some rising to luminal surface	Usually papillary	 PanIN-2
PanIN-3	IPMN/MCN – Severe dysplasia	**High-grade PanIN, IPMN, or MCN**	– Severe pleomorphism (as in carcinoma) – Loss of nuclear polarity – Dystrophic goblet cells (goblet cells with flipped polarity – nuclei oriented toward the lumen and mucinous cytoplasm toward the basement membrane) – Atypical mitoses	Papillary or micropapillary, luminal budding, fusion of micropapillae, cribriforming + necrosis (Even with bland cytology, complex architecture supports PanIN-3)	 PanIN-3

The currently recommended reporting terminology is based on the revised 2015 classification of the neoplastic precursor lesions. The former terminology (based on WHO 2010 classification) may still be included in reports as a supplementation and indicated in parentheses pending the new WHO classification revision. Low-grade PanINs do not need to be reported, especially in the absence of invasive carcinoma, due to the lack of proven clinical significance of these lesions.

Rule of thumb: Dysplasia in pancreatic ducts is graded a step above of how you would grade dysplasia in a colon adenoma, such that low-grade dysplasia (typical adenoma) in the colon = moderate dysplasia in the pancreas.

The key distinguishing features of PanIN vs. IPMN vs. MCN are:
- PanIN – usually <5 mm, radiologically occult
- IPMN – grossly visible (usually >1 cm), associated with pancreatic duct (main or branch), mucin extrusion at the papilla
- MCN – almost exclusively women, not connected to pancreatic duct, associated with ovarian-type stroma

References: https://pathology.jhu.edu/pc/professionals/DuctLesions.php [22–27]

Illustration adapted from Cornish TC and Hruban RH. Surg Pathol Clin 2011[28]; with permission from © Surg Pathol Clin

Abbreviations: *PanIN* pancreatic intraepithelial neoplasia, *IPMN* intraductal papillary mucinous neoplasm, *MCN* mucinous cystic neoplasm

Esophagus

Grading of Dysplasia in Barrett Mucosa				
	Architectural atypia[a]	**Cytologic atypia**[b]	**Surface maturation**	**Inflammation**
NFD (reactive)	None[1]	None	Present	Variable
IFD	None to minimal	Mild	Present	Frequent
LGD	Minimal	Moderate	Absent	Minimal
HGD	Prominent	Severe (loss of nuclear polarity)	Absent	Minimal

[a]**Architectural atypia** = glandular crowding and complexity (budding, branching, contour irregularity, papillary projections into the lumen)
[b]**Cytologic atypia** = ↑N/C ratio, hyperchromasia, ↑nucleoli, stratified nuclei, loss of mucin

1. In cases of regenerative changes, particularly in the setting of marked inflammation, variable degrees of architectural atypia may be seen, but in the presence of surface maturation, preserved N/C ratio, and lack of significant cytologic atypia (other than prominent nucleoli), this should not be interpreted as dysplasia.

Abbreviations: *HGD* high-grade dysplasia, *IFD* indefinite for dysplasia, *LGD* low-grade dysplasia, *NFD* negative for dysplasia

References: [29, 30]

Liver Biopsy

Grading and Staging of Chronic Viral Hepatitis	
Grade = Lymphocytic inflammation and "necrosis[a]" (indicates "activity") • Portal inflammation • Periportal inflammation/necrosis (= interface activity = piecemeal necrosis[a]) • Lobular inflammation/necrosis	increasing severity ↓
Stage = Fibrosis (indicates "chronicity") • Portal fibrosis • Bridging fibrosis (early → established) • Cirrhosis	increasing severity ↓

[a]Note that "necrosis" does not manifest as necrotic debris in the setting of viral hepatitis but rather as replacement of hepatic parenchyma by lymphocytes.

There are multiple scoring systems in use to quantify the above parameters. These are nicely reviewed (with diagrams) in Reference [31].

Neuroendocrine Neoplasms

Pulmonary Neuroendocrine Neoplasms, WHO 2015			
	Mitoses per 2 mm²	**Necrosis**	**Ki67 (Mib1)[a]**
Typical carcinoid (=low grade; G1)	<2	Absent	<2%
Atypical carcinoid (=intermediate grade; G2)	2–10	Focal	<20% (mean 10%)
Neuroendocrine carcinoma (small-cell and large-cell type) (=high grade; G3)	>10	Extensive	20–100% (mean for small cell >80%)

For consistent reporting, the grading criteria require assessment of mitotic index within a 2 mm² area. The number of HPFs (high-power fields) per 2 mm² varies among microscopes and has to be individually calculated.
[a]Ki67 is not part of the WHO 2014 criteria, but it is very helpful in small crushed biopsies where distinction of carcinoid tumors and small cell carcinoma can be difficult.
Carcinoid tumorlet is defined by size of ≤0.5 cm.

References: [32, 33]

Pancreatic (WHO 2017) and GI (WHO 2010) Neuroendocrine Neoplasms			
	Mitoses per 10 HPF	**Ki67**	**Morphology**
Well differentiated (= NETs): NET, grade 1 NET, grade 2 NET, grade 3 (for pancreas only)[1]	 <2 2–20 >20	 <3% 3–20% >20%	Look like carcinoid of any site
Poorly differentiated (= NECs): NEC, small cell type, grade 3 NEC, large cell type, grade 3	>20	>20%	Look like small cell or large cell neuroendocrine carcinomas of any site
MANEC (GI), MiNEN (pancreas)[2]	NA	NA	Mixed neuroendocrine and carcinomatous neoplastic components (at least 30% each)

1. Well-differentiated grade 3 category is currently unique to the pancreatic NETs (PanNETs). This separates NETs with elevated proliferation rate from neuroendocrine carcinomas (NECs) that are morphologically, genetically, and prognostically distinct. Although not currently in use for the remainder of the GI tract or the lung, these changes are likely forthcoming in these organ systems.

2. Mixed neuroendocrine-nonneuroendocrine neoplasm (MiNEN) category has replaced mixed adenoneuroendocrine carcinoma (MANEC) in the pancreatic WHO criteria to account for well-differentiated entities in this category and entities with components other than adenocarcinoma (e.g., squamous cell carcinoma, acinar cell carcinoma).

Notes:

- Grading requires a mitotic count in at least 50 HPF (with 1 HPF = 0.2 mm², 10 HPF = 2 mm², and a Ki67 index as a percentage of at least 500 cells counted in "hot spots." Get your coffee ready!!
- If the mitotic rate and Ki67 index differ, use the higher of the two.
- Even though NECs are included as grade 3, in reality they are NOT graded. They are by definition and always high grade.
- "Micro" neuroendocrine proliferations are considered benign and include pancreatic neuroendocrine microadenoma (≤0.5 cm) and gastric carcinoid (ECL cell) tumorlet (≤0.5 cm).
- For pancreatic NETs, additional feature associated with prognosis is the type of hypersecretory syndrome:
  - Insulinoma – better prognosis (may be related to earlier detection due to symptoms)
  - Glucagonoma – worse prognosis
- For GI NETs, additional prognostic features include:
  - Anatomic location: bad (colon, esophagus), good (appendix, rectum), intermediate (small bowel, stomach)
  - Clinical setting (for gastric NETs): tumors arising in the setting of hypergastrinemia (Zollinger-Ellison syndrome/MEN1 or autoimmune metaplastic atrophic gastritis/pernicious anemia) have excellent prognosis, whereas sporadic tumors are aggressive
  - Size and depth of invasion which are a part of the staging system

References: [24, 34]

Abbreviations: *NE* neuroendocrine, *NET* neuroendocrine tumor, *NEC* neuroendocrine carcinoma

Neuroblastoma

Neuroblastoma: Revised Shimada Grading System (Not Graded if Metastatic or Posttreatment)			
Designation	Histology		Prognosis
Ganglioneuroma, maturing	**Stroma-rich**[1,2] (Schwannian stroma >50%)	No microscopic nodules of NB cells	FH
Ganglioneuroblastoma, intermixed		Microscopic nodules of NB cells present	FH
Ganglioneuroblastoma, nodular		Macroscopic (gross) nodules of NB cells present	UH/FH
Undifferentiated neuroblastoma	**Stroma-poor** (Schwannian stroma <50%)	No ganglion cells; No neuropil	Always UH (any age)
Poorly differentiated neuroblastoma		<5% ganglion cells; Neuropil present	UH if age >1.5 yrs or MKI[3] >4% Otherwise FH
Differentiating neuroblastoma		>5% ganglion cells; Neuropil present	UH if any of the following: – Age >5 yrs or – Age 1.5–5 yrs plus MKI >2% or – Age <1.5 yrs plus MKI >4% Otherwise FH

1. Schwannian stroma consists of spindle cells, which resemble schwannoma or neurofibroma. In contrast, neuropil consists of fibrillary processes similar to the kind seen in ependymoma.
2. Stroma-rich neuroblastomas generally have >50% ganglion cells, but this feature is not a criterion in grading of ganglioneuroblastoma.
3. MKI (mitosis-karyorrhexis index): percentage of mitotic and karyorrhectic cells based on a 5000-cell count (2% is 100 of 5000 cells, and 4% is 200 of 5000 cells). A 900-cell count is sometimes mercifully applied (2% is 19 of 900 cells, and 4% is 36 of 900 cells).
   Sample sign-out: "Neuroblastoma, stroma poor, differentiating, low MKI."

Abbreviations: *FH* favorable histology, *MKI* mitosis-karyorrhexis index, *NB* neuroblast, *UH* unfavorable histology

Reference: [35]

Olfactory Neuroblastoma, Hyams Grading System				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Architecture	Lobular	Lobular	Variable	Variable
Mitotic activity	Absent	Present	Prominent	Marked
Nuclear pleomorphism	Absent	Moderate	Prominent	Marked
Necrosis	Absent	Absent	+/– Present	Common
Fibrillary matrix	Prominent	Present	Minimal	Absent
Rosette type	Homer Wright	Homer Wright	Flexner-Wintersteiner	Flexner-Wintersteiner

The four-tiered system may be simplified into low grade (Hyams grades 1 and 2) and high grade (Hyams grade 3 and 4).

Reference: [5]

Sarcoma Grading (Not for the Faint of Heart!)

by Youran Zou & Justin A. Bishop

There are two main systems, the NCI system and the French Federation of Cancer Centers (FNCLCC or "French") system.

French Grading System for Soft Tissue Sarcomas		
Parameter	Point score	
1. Tissue differentiation (how closely the tumor resembles the tissue from which it arose) **see table below** • Tumors closely resembling normal mesenchymal tissue (i.e., difficult to distinguish from a benign tumor), e.g., well-differentiated leiomyosarcoma • Tumors of a definite histologic type, e.g., myxoid liposarcoma • Tumors that are embryonal, poorly differentiated, or of uncertain histologic type	1 2 3	
2. Mitoses • 0–9/10 HPF • 10–19/10 HPF • ≥ 20/10 HPF	1 2 3	
3. Tumor necrosis • No necrosis at all • <50% • ≥50%	0 1 2	
	Final score (combined point score) and corresponding grade	
	2–3 points = Grade 1	Low-grade
	4–5 points = Grade 2	High-grade
	6–8 points = Grade 3	
• A high-power field is = 0.1744 mm^2. • Sectioning the tumor at least 1 section/2 cm is recommended. Reference:[36]		

For the most commonly encountered sarcomas (assuming you know what type it is!), the differentiation score can simply be looked up in this table:

Histology-Specific Tumor Differentiation Scores	
Sarcoma	**Score**
Adipocytic	
Myxoid liposarcoma	2
High-grade myxoid (round cell) liposarcoma	3
Pleomorphic liposarcoma	3
Dedifferentiated liposarcoma	3
Fibrous/Fibrohistiocytic	
Well-differentiated fibrosarcoma	1
Conventional fibrosarcoma	2
Poorly differentiated Fibrosarcoma	3
Myxofibrosarcoma	2
Undifferentiated (spindle cell and pleomorphic) sarcoma	3
Smooth muscle	
Well-differentiated leiomyosarcoma	1
Conventional leiomyosarcoma	2
Poorly differentiated/pleomorphic leiomyosarcoma	3
Others/unknown	
Synovial sarcoma	3
Ewing sarcoma	3
Mesenchymal chondrosarcoma	3
Extraskeletal osteosarcoma	3
Extrarenal rhabdoid tumor	3
References: [36, 37]	

But unfortunately it's not that simple. In practice, some of the sarcomas are high-grade or low-grade by definition. Also, the French Federation of Cancer Centers Sarcoma Group "doesn't recommend" grading a few of the common sarcomas. This is really confusing, since most of the "differentiation charts" include these sarcomas that they don't recommend grading.

So basically, for these tumors, you *can* go through the fun process of grading them by counting up the points, but it would be a waste of time because (1) for some sarcomas, you will always get to a certain grade (i.e., they are either high-grade or low-grade by definition) or (2) applying a grade would be misleading because the actual prognosis doesn't match it.

Sarcomas for Which Grading Is Generally Not Recommended or Not Necessary	
Sarcoma	**Reason**
Alveolar and embryonal rhabdomyosarcoma (except for botryoid and spindle cell variants)	Grade 3 by definition
Ewing sarcoma	Grade 3 by definition
Angiosarcoma	Grade 3 by definition
Desmoplastic small round cell tumor	Grade 3 by definition
Extrarenal rhabdoid tumor	Grade 3 by definition
Extraskeletal osteosarcoma	Grade 3 by definition
Mesenchymal chondrosarcoma	Grade 3 by definition
Infantile fibrosarcoma	Grade 1 by definition (has a good prognosis, but if grade strictly applied, would be high)
DFSP	Tumors of intermediate malignancy that are low-grade by definition
Well-differentiated liposarcoma	
MPNST and dedifferentiated liposarcoma	Grading is controversial
Extraskeletal myxoid chondrosarcoma	Grade does not predict outcome. Would be low-grade based on histology but meets late in 40% of cases.
Alveolar soft part sarcoma	Considered by many experts to be "ungradable" but usually managed as high-grade sarcomas. Would often meet histologic criteria for low-grade but often metastasize long term (within 10–20 years).
Clear cell sarcoma	
Epithelioid sarcoma	
"Low-grade" fibromyxoid sarcoma	
Abbreviations: *DFSP* dermatofibrosarcoma protuberans, *MPNST* malignant peripheral nerve sheath tumor References: [37–42]	

Central Nervous System

by Marina K Baine & Tejus A. Bale

Tips and Tricks: WHO Grading of CNS Tumors[1]	
Grade I	Most sellar tumors None of the oligodendrogliomas
Grade II	You're on your own
Grade III	All tumors with "anaplastic" in the name: Anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic pleomorphic xanthoastrocytoma, anaplastic ependymoma, anaplastic ganglioglioma, and anaplastic (malignant) meningioma
Grade IV [2]	All embryonal tumors: Medulloblastoma (all subtypes), embryonal tumor with multilayered rosettes C19MC-altered, medulloepithelioma, CNS embryonal tumor NOS, atypical teratoid/rhabdoid tumor, CNS embryonal tumor with rhabdoid features Most tumors with "blastoma" in the name[3] (e.g., glioblastoma, pineoblastoma, medulloblastoma, etc.)

1. Grading of CNS tumors relies on histologic features and tumor classification.
2. The remaining **two** Grade IV entities are diffuse midline glioma, H3 K27 M-mutant (predominantly pediatric), and malignant peripheral nerve sheath tumor (MPNST), which can be WHO grade IV but has its own clinically unvalidated and marginally reproducible grading system.
3. Exceptions are hemangioblastoma (WHO grade I) and myofibroblastoma, which is a rare (in CNS) benign mesenchymal neoplasm.

Astrocytic and Oligodendroglial Neoplasms: WHO Grading System

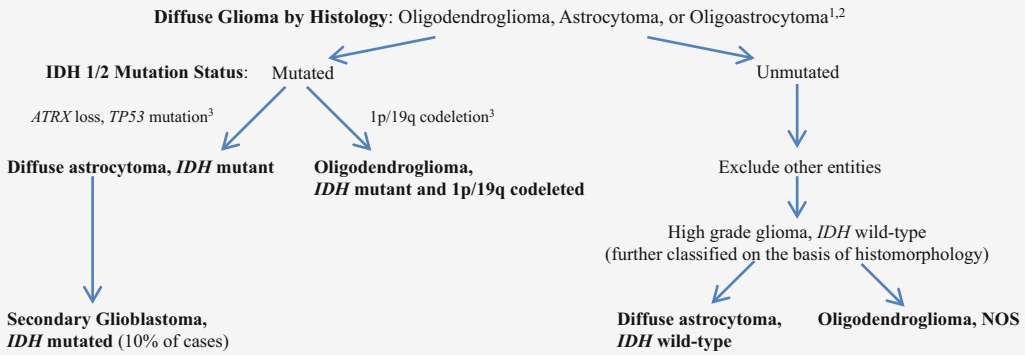
WHO grade	Histology					Other features
	Atypia	Cellularity	Ki67[1]	Mitoses	Necrosis +/– MVP	
I [2]	Variable but generally minimal	Variable but generally low	Variable but generally <4%	Minimal mitotic activity	–	"Rosenthal fibers" Possibility of cure after complete resection
II [3]	↑	↑	<4% astrocytic <5% oligodendroglial	Minimal-rare mitotic activity (see below)	–	Infiltrative and often recur despite low proliferative activity Some progress to grades III and IV
III	↑↑	↑↑	5–10% astrocytic 6–10% oligodendroglial	Readily identifiable mitoses; in a small biopsy **even one is enough!**	– or + [1]	Most patients require adjuvant chemotherapy and radiation High rate of recurrence and/or progression
IV	↑↑/↑↑↑	↑↑↑	>10%	Brisk mitotic activity	+	Widespread infiltration with high incidence of craniospinal spread Rapid pre- and postoperative disease evolution with fatal outcome

1. There are no definitive criteria for proliferative index assessment for oligodendroglioma grading, but generally a cutoff of 5% is used to distinguish WHO grades II and III. If a tumor is truly an oligodendroglioma, 1p19q co-deleted, then even mitosis, necrosis, and MVP still only make it to anaplastic oligodendroglioma, grade III. The same histologic features in an astrocytoma would amount to GBM, WHO grade IV.
2. WHO grade I tumors are a unique category composed of heterogeneous group of tumors with a known benign course.
3. Pleomorphic xanthoastrocytoma (PXA) (grade II) is an exception, often with MVP and/or necrosis, and defined by nuclear pleomorphism, but consistent with its grade, it has low proliferative activity (<5 mitoses/10 HPF, and Ki67 < 1%).

Abbreviations: *MVP* microvascular proliferation

Reference: [43]

High-Grade Gliomas: WHO Classification



1. Genotype trumps morphology!

2. If genetic testing is not done or is inconclusive, the diagnosis is made based on histomorphology with the NOS designation (i.e., diffuse astrocytoma, NOS, oligodendroglioma, NOS or glioblastoma, NOS).
3. ATRX loss and *TP53* mutation are characteristic of IDH-mutated diffuse astrocytoma, but NOT required for the diagnosis. 1p/19q codeletion, however, MUST be present for the diagnosis of oligodendroglioma. When two molecularly distinct populations are seen in a single tumor, oligoastrocytoma may be diagnosed (rare reports); or in the absence of diagnostic molecular testing, oligoastrocytoma, NOS, may be diagnosed (provisional diagnosis).

Reference: [43]

Central Nervous System: 2

Ependymal Tumors: WHO Grading System[1]

Tumor type	WHO grade	Histology			
		Cytology	Mitotic activity	Cellularity	Palisading necrosis and/or MVP
Subependymoma	**I**	Unique slow-growing mitotically inactive tumors with a generally benign course			
Myxopapillary ependymoma					
Ependymoma[2]	**II**	Bland	Low	↑ – ↑↑	–/+ [3]
Ependymoma, *RELA* fusion-positive[4]					
Anaplastic ependymoma	**III**	High N:C ratio	Brisk	↑↑↑	–

1. Among grades II and III ependymomas, the grade does not appear to correlate with tumor aggressiveness or survival. It is therefore rarely used for treatment stratification. Stay tuned for the likely extinction of histologic grading of ependymomas!
2. Including papillary, clear cell, and tanycytic variants.
3. Classic ependymoma may have areas of geographic necrosis, but palisading necrosis and MVP are only focal.
4. Ependymoma with *RELA* fusion is histologically indistinct from other ependymomas and is graded based on above histopathologic features. Regardless of grade, it carries the worst prognosis.

Abbreviations: *N:C ratio* nuclear-to-cytoplasmic ratio

Reference: [43]

Meningioma: WHO Grading System

Grade I	Lack of higher-grade features
Grade II (atypical)[1]	Any 1 of the 3 criteria: 1. ≥4 mitoses/10 HPF or 2. Brain invasion 3. At least three of the following features: • Sheet-like growth (i.e., loss of lobular architecture) with uninterrupted patternless growth • Prominent nucleoli • Hypercellularity • Small cells with high N:C ratio • Foci of spontaneous necrosis
Grade III (anaplastic/malignant)[2]	Frankly malignant cytology (like that of a carcinoma, melanoma, or undifferentiated pleomorphic sarcoma) and/or ≥20 mitoses/10 HPF (0.16 mm^2)

1. Clear cell and chordoid meningioma are always grade II. An alternative grading approach combines hypercellularity with ≥5 mitoses/10 HPF.
2. Papillary and rhabdoid meningioma are always grade III.

Note: Bone invasion does not raise the grade.

Reference: [43]

Gynecologic Tract

Endometrioid Adenocarcinoma: FIGO Grading	
	% solid growth
Grade 1 (well differentiated)	<5%
Grade 2 (moderately differentiated)	6–50%
Grade 3 (poorly differentiated)	>50%

- Squamoid areas are not counted as solid growth.
- Presence of severe nuclear atypia (grade 3 nuclei) raises the grade by one.

Reference: [44]

Smooth Muscle Neoplasms of the Uterus			
	Mitoses per 10 HPF	Atypia	Coagulative necrosis
Leiomyoma or cellular leiomyoma (increased cellularity)	<5	–	–
Atypical leiomyoma (aka symplastic, pleomorphic, or bizarre)	<10	+	–
Mitotically active leiomyoma	≥5	–	–
Leiomyosarcoma (diagnosis requires at least 2 of 3 features)[1]	>10	+	+

1. When only one of three features is present, the diagnosis of STUMP is made. In a STUMP, however, no more than 15 mitoses per 10 HPF are permissible, but mitotically active STUMP may display some focal atypia.

References: [44–47]

Epithelioid Smooth Muscle Neoplasms of the Uterus			
	Mitoses per 10 HPF	Atypia	Coagulative necrosis
Epithelioid leiomyoma	<5	Minimal	–
Epithelioid STUMP	<5	Moderate to severe	–
Epithelioid leiomyosarcoma	>5[1]	Moderate to severe	+[1]

1. Presence of either >5 mitoses/10 HPF or necrosis qualifies for the diagnosis of leiomyosarcoma.

Reference: [47]

Endometrial Stromal Sarcomas			
	Mitotic activity (mitoses per 10 HPF)	Atypia	Coagulative necrosis
Low-grade	Low (usually <5)	None to minimal	–/+
High-grade	High (>10)	Mild to moderate	+
Undifferentiated	Very high (usually >20)	Severe[1]	+

1. Undifferentiated stromal sarcomas typically display marked pleomorphism, bearing no resemblance to endometrial stroma.

Reference: [46]

Grading of Immature Ovarian Teratomas	
	Fields occupied by immature neuroepithelial elements
Grade I	<1 LPF (4X objective)
Grade II	1–3 LPF
Grade III	>3 LPF

Rule of thumb: grade I, if immature areas are hard to find; grade III, easy to find.

Reference: [46]

Abbreviations: *FIGO* International Federation of Gynecology and Obstetrics, *STUMP* smooth muscle tumor of uncertain malignant potential

Gynecologic Tract: 2

Dating of Endometrium[a] *By Diana Weedman Molavi*	
Proliferative endometrium cannot be dated. The first secretory change occurs, on average, on day 16 or so of a 28-day cycle. This change is the appearance of clear secretory vacuoles at the base of the epithelial cells, below the nuclei. When you see just a few of these in a generally proliferative endometrium, it is called interval endometrium. Beyond that day, specific histologic criteria are:	
From day 16 to day 20, the glands are the most helpful feature.	
Day 16	Subnuclear vacuoles, pseudostratified nuclei
Day 17	Subnuclear vacuoles but with an orderly row of nuclei
Day 18	Vacuoles above and below nuclei (the "piano key" look)
Day 19	Vacuoles diminishing, only above nuclei; orderly row of nuclei, no mitoses
Day 20	Peak secretions in lumen and ragged luminal border, vacuoles rare
From day 21 to 28, the glands stay pretty much the same – they are exhausted and appear low columnar with orderly nuclei, no mitoses, and ragged luminal edges. They may also have degenerative apical vacuoles – tricky to discern from day 19 to 20. After day 21, the stroma is the key.	
Day 21	Stromal edema begins, secretion continues
Day 22	Peak stromal edema with naked nuclei
Day 23	Spiral arteries become prominent
Day 24	Periarteriolar cuffing with predecidua (stromal cells around the arteries begin to get plump pink cytoplasm, creating a pink halo around the vessels)
Day 25	Predecidual change under the surface epithelium
Day 26	Decidual islands coalesce; polys begin to infiltrate stroma
Day 27	Lots of polys in a solid sheet of decidua, with focal necrosis and hemorrhage
Day 28	Prominent necrosis, hemorrhage, clumping, and breakup
[a]Note: The reliability of endometrial dating is controversial.	

Grading and Staging of Infections in the Placenta		
Stage (Reflects Duration)		
	Chorioamnionitis (Maternal neutrophils involving the membranes)	**Funisitis** (Fetal neutrophils migrating from fetal vessels into the umbilical cord and/or chorionic plate)
Stage I	**Subchorionitis** (neutrophils line up beneath the chorion) and **chorionitis** (neutrophils involve the chorion)	**Umbilical phlebitis** (neutrophils in the wall of umbilical vein) or **chorionic vasculitis** (neutrophils in the wall of vessels located in the chorionic plate)
Stage II	**Chorioamnionitis**: neutrophils extend into the amnion	**Umbilical arteritis:** neutrophils in the wall of umbilical arteries
Stage III	**Necrotizing chorioamnionitis:** above plus reactive amnion or necrosis or amniotic basement membrane thickening or band-like inflammation	**Necrotizing funisitis**: neutrophils extend into Wharton jelly and form microabscesses or band-like inflammation
Grade (Reflects Severity)		
Grade I	Mild to moderate	
Grade II	Severe (such as subchorionic microabscesses)	
Note that chorioamnionitis represents a maternal response to infection, whereas funisitis fetal response to infection. Funisitis usually develops later than chorioamnionitis.		
References: [48, 49]		

Transplant Pathology

Grading of Cellular Lung Allograft Rejection, (2007 Update) International Society for Heart and Lung Transplantation (ISHLT) system [reported as, e.g., ISHLT A_0B_x]	
Grade of rejection	**Histologic features**
Acute rejection	
Grade A0 (no rejection)	
Grade A1 (minimal rejection)	Infrequent, scattered perivascular lymphocytes forming a ring 2–3 cells thick
Grade A2 (mild rejection)	More frequent perivascular lymphocytes readily seen at low power (4X objective), cuffing the vessels and expanding the perivascular interstitium
Grade A3 (moderate rejection)	Lymphocytes extend into alveolar septa and airspaces
Grade A4 (severe rejection)	Diffuse interstitial lymphoid infiltrate with diffuse alveolar damage, hemorrhage, and/or necrosis
Bronchial/bronchiolar inflammation	
Grade B0 (no airway inflammation)	
Grade B1R (low grade)	Mononuclear cells within the submucosa of bronchioles without evidence of epithelial damage or intraepithelial infiltration (combines former B1 and B2 categories)
Grade B2R (high grade)	Mononuclear cells are increased in number, are larger, and accompanied by more eosinophils and plasmacytoid cells (but not many neutrophils, which would make you think infection). Also there is epithelial damage (e.g., necrosis, metaplasia) and intraepithelial lymphocytes.
Grade Bx (ungradable)	No evaluable bronchial tissue
Chronic rejection (obliterative bronchiolitis)	
Grade C0	Bronchiolar obliteration absent
Grade C1	Bronchiolar obliteration via fibrosis present. Often subtle and/or focal (a trichrome stain can be helpful).
Chronic vascular rejection	
Grade D	Thickening of arteries and veins, similar to the coronary artery disease seen in transplanted hearts. Not applicable to transbronchial biopsies.
At least five pieces of alveolated lung parenchyma each containing bronchioles and >100 air sacs are defined as sufficient to rule out rejection by ISHLT criteria. Reference: [50]	

Staging of Antibody-Mediated Rejection (AMR) of Lung Transplant International Society for Heart and Lung Transplantation (ISHLT) System	
Criteria	**Description of criteria**
Donor-specific antibodies (DSAs)	High serum antibody titer
Pathology	Neutrophilic capillaritis[1] and/or margination[2] Acute lung injury with or without diffuse alveolar damage and endothelialitis
C4d IHC[3]	>50% capillary staining

Note: There are three stages of AMR, which are defined based on the number of met criteria: definite (all three criteria), probable (any two of three), and possible (any one of three).

1. Neutrophilic capillaritis can be patchy or diffuse and is defined as a dense neutrophilic septal infiltrate with neutrophilic karyorrhexis and fibrin with or without microvascular fibrin thrombi, alveolar hemorrhage, and neutrophil spillover into adjacent airspaces.
2. Neutrophilic margination is characterized by both septal and interstitial neutrophilic capillary infiltration in the absence of karyorrhexis and fibrin.
3. Aside from characteristic pathologic findings described above, additional histopathologic indications for C4d IHC include high-grade acute or cellular rejection (≥A3, B2R, or C1) and persistent/recurrent ACR (any A grade or grade B1R). Furthermore, in the absence of any histologic findings, clinical graft dysfunction or newly detected DSA positivity warrants C4d IHC evaluation.

References: [51, 52]

Grading of Acute Graft-versus-Host Disease (GVHD) in Intestinal (Usually Rectal) Biopsy	
Grade I	Rare apoptotic cells (approximately >3 per crypt; normal is ≤1 per crypt)
Grade II	Loss of individual crypts
Grade III	Loss of two or more contiguous crypts
Grade IV	Complete loss of crypts; mucosal ulceration (neuroendocrine cells are relatively spared from the damages of GVHD, and they may appear as little nests)
Chemotherapy-related changes may be indistinguishable (best not to biopsy <20 days post-BMT). Mycophenolate mofetil immunosuppression therapy, among other etiologies, may also mimic acute GVHD histologically. References: [53, 54]	

Transplant Pathology: 2

Grading of Acute Graft-versus-Host Disease (GVHD) in Skin Biopsy	
Grade I	Vacuolization of the basal layer
Grade II	Above + dyskeratotic/necrotic keratinocytes
Grade III	Above + subepidermal clefting
Grade IV	Above + necrosis and separation of epidermis

Drug reaction looks virtually indistinguishable from GVHD and must be ruled out on clinical grounds. A soft feature that favors GVHD is dyskeratotic cells on hair follicles. Lymphocytes are either absent or minimal in GVHD (unlike drug reaction). Eosinophils favor drug reaction.

With advents of immunosuppression, finding GVHD >grade I is very rare. Thus, this grading system is largely of historical value.

Reference: [55]

Criteria for Acute Cellular Liver Allograft Rejection	
Criteria	**Description**
1. **Portal inflammation**	Lymphocytes with admixed neutrophils and eosinophils involving portal tracts
2. **Ductulitis**	Lymphocytes involving bile ducts with evidence of bile duct damage
3. **Endothelialitis**	Subendothelial and perivenular lymphocytes involving portal and/or hepatic venules

The diagnosis of rejection requires at least two of the three above criteria. The severity of rejection is further qualified as “mild, moderate, or severe” based on intensity of inflammation and the number of involved structures.

Similar to lung transplant, evaluation for acute antibody-mediated rejection (AMR) of liver transplants involves both clinical (elevated serum DSAs), C4d IHC, and histologic criteria, the latter of which are meticulously scored based on degree and extent of portal and periportal endothelial injury (hypertrophy, endotheliitis, and dilation). Other causes, such as obstructive cholangiopathy or reperfusion injury, must be excluded.

Criteria for evaluation of chronic AMR are not as well-defined, making this entity particularly difficult to diagnose.

References: [56, 57]

The Good the Bad and the Ugly:

Prognostic Features in Neoplasms with Difficult-to-Predict Behavior

Adrenocortical Neoplasms

The only definitive criteria for malignancy are distant metastasis and/or local invasion, but various histologic criteria have been devised to predict an aggressive phenotype (Weiss criteria for classical adrenocortical tumors and Lin-Weiss-Bisceglia criteria for oncocytic neoplasms are listed below).

- **Weiss criteria** for histologic assessment of malignancy in adrenocortical neoplasms: [58, 59]
  1. >5 mitoses per 50 HPF*
  2. Atypical mitoses*
  3. Venous invasion*
  4. Sinusoidal invasion
  5. Capsular invasion
  6. Nuclear pleomorphism (equivalent to renal ISUP nuclear grades III and IV)
  7. Clear cells representing <25% of tumor cells (>75% eosinophilic cells)
  8. Diffuse architecture (>33% of tumor)
  9. Necrosis

Malignant tumors have ≥3 of the above criteria, whereas benign tumors have <2. In addition, the *asterisked criteria are found exclusively in malignant tumors. Weiss criteria cannot be applied to oncocytic adrenal tumors because high nuclear grade, <25% clear cells and diffuse architecture are intrinsic features of these tumors regardless of their behavior. Modified criteria have therefore been proposed based on several case series (see Lin-Weiss-Bisceglia criteria below).

- **Lin-Weiss-Bisceglia criteria** for histologic assessment of malignancy in **oncocytic** adrenocortical neoplasms: [60, 61]

Major criteria
1. >5 mitoses per 50 HPF
2. Atypical mitoses
3. Venous invasion

Minor criteria
1. Weight >200 g and/or size >10 cm
2. Necrosis (microscopic)
3. Capsular invasion
4. Sinusoidal invasion

The presence of one major criterion indicates malignancy. In the absence of major criteria, the presence of any of the minor criteria indicates borderline malignant potential. Lack of any of the above features is consistent with benignity.

- Other criteria: Ki67 >5–20% [16]. It is recommended that Ki67 should be scored in a hot spot area within 500–2000 cells, similar to GI neuroendocrine neoplasms [62]. Furthermore, Ki67 has been determined to be an important prognostic marker [63, 64] and should therefore be reported for all adrenocortical carcinomas.
- Helsinki score (3 x mitotic rate/50 HPF + 5 x presence of necrosis + Ki67 index) recently developed, and appears to be superior to the Weiss criteria for predicting malignant behavior [65, 66]. Given its simplicity and accuracy, it is likely to replace Weiss criteria in the future. Stay tuned!

Note: According to some reports, the criteria for malignancy in pediatric tumors should have a higher threshold [16], and additional parameters have impact on prognosis [67, 68].

Pheochromocytoma and Paraganglioma

Ten percent of cases are considered to be malignant. The only definitive criterion for malignancy is distant metastasis. Local invasiveness is not an unequivocal malignant feature. Aggressive behavior is impossible to predict based on any histologic features, but the following two sets of criteria incorporate features that have been associated with malignancy:

PASS (Pheochromocytoma of the adrenal gland scaled score) [69]

- Extension into adjacent adipose tissue [2 points]
- Confluent tumor necrosis (may occur in benign) [2 points]
- Expanded large nests (>3 times the normal "Zellballen" size) and diffuse growth [2 points]
- Increased cellularity [2 points]
- Increased mitoses (>3 mitoses per 10 HPF) [2 points]
- Atypical mitotic figs. [2 points]
- Tumor cell spindling (including focal) [2 points]
- Vascular invasion [1 point]
- Capsular invasion [1 point]
- Profound nuclear atypia with macronucleoli [1 point] and hyperchromasia [1 point] (may occur in benign)

Applies to pheochromocytoma only due to distinctive histologic features. All tumors with subsequent malignant behavior had a PASS ≥4 at initial diagnosis. However, not all tumors with PASS ≥4 subsequently metastasized.

GAPP (Grading system for adrenal Pheochromocytoma and Paraganglioma) [70]

- Histological pattern:
  - Zellballen [0 points]
  - Large, irregular cell nests [1 point]
  - Pseudorosette [1 point]
- Cellularity:
  - Low (<150 cells/U[a]) [0 points]
  - Moderate (150–250 cells/U) [1 point]
  - High (>250 cells/U) [3 points]
- Confluent tumor necrosis [2 points, if present]
- Vascular or capsular invasion [1 point, if present]
- Ki67 (%):
  - <1 [0 points]
  - 1–3 [1 point]
  - >3 [2 points]
- Catecholamine type:
  - Norepinephrine type [1 point]
  - All others [0 points]

The majority of tumors with GAPP <3 (well differentiated) have benign behavior (<4% metastatic rate and 100% 5-year survival). Moderately differentiated (GAPP 3–6) and poorly differentiated (GAPP 7–10) tumors are associated with intermediate and high risk of metastasis and 5-year mortality, respectively.

Other factors: Tumor size >5 cm; *SDHB* mutation (lack of SDHB IHC staining)
Both scoring systems are included in the new WHO classification (2017) for reference, but more recent data has demonstrated **GAPP** to be **superior** to PASS.

[a]U, number of cells in a 10 mm^2 area observed at high power (X400)

References: [16, 69, 70]

The Good the Bad and the Ugly: 2

Parathyroid Neoplasms

The only definitive criteria for malignancy are distant metastasis and/or local invasion. Features that have been associated with malignant behavior include:

- Thick fibrous bands (present in 90% of carcinomas but low specificity)
- Thick capsule
- Infiltrative growth (with adherence to the thyroid and/or soft tissue extension)
- Capsular invasion[a] (present in 2/3 of carcinomas)
- Vascular invasion[a]
- Perineural invasion (pathognomic but present in only 5% of the cases)
- Tumor necrosis
- >5 mitoses per 50 HPF or Ki67 >6%
- Atypical mitotic figures
- Diffuse, marked pleomorphism with macronucleoli (may occur in benign)
- Spindling of tumor cells
- Large size (mean size 3 cm, mean weight 12 g)
- Complete loss of parafibromin immunoexpression (also seen in adenomas of the hyperparathyroidism-jaw tumor syndrome) [71]

[a]Vascular and capsular invasion are assessed using the same criteria as those applied to thyroid follicular carcinoma: vascular invasion should be present within or beyond the tumor capsule, and capsular invasion should be completely penetrating.

Reference: [72]

Gastrointestinal Stromal Tumor (GIST), AFIP Risk Stratification Scheme

Tumor parameters		*Risk of poor outcome by site*			
Size (cm)	**Mitotic rate**	**Stomach**	**Jejunum/ileum**	**Duodenum**	**Rectum**
≤2	≤5 per 5 mm^2	None	None	None	None
>2–5		Very low	Low	Low	Low
>5–10		Low	Moderate	High	High
>10		Moderate	High		
≤2	>5 per 5 mm^2	None	High	Insufficient data	High
>2–5		Moderate		High	
>5–10		High			
>10					

Key poor prognostic factors in GIST are large tumor size, extragastric location, and high mitotic rate. NIH consensus criteria and AFIP criteria for risk stratification of disease recurrence after surgical resection of GIST incorporate these three main prognostic features. Tumor rupture has also been associated with high risk of relapse (80–100%), irrespective of other risk factors [73], and was subsequently incorporated into the modified NIH consensus scheme. Of these three main existing schemes, the AFIP system is favored by the National Comprehensive Cancer Network, the College of American Pathologists, and the European Society for Medical Oncology and was adopted by the AJCC staging manual for clinical practice guidelines [74].

Reference: [75]

Solitary Fibrous Tumor (SFT)

Proposed histologic criteria for malignancy in pleural SFT include [76]:

- High cellularity (crowded, overlapping nuclei)
- >4 mitoses per 10 HPF
- Pleomorphism
- Hemorrhage
- Necrosis

Resectability is the single most important indicator of clinical outcome (regardless of "histologic malignancy"). Size >10 cm also predicts worse outcome. These criteria have also been applied to extrapleural SFT [77].

Reference: [78]

Sertoli and Leydig Cell Tumors[1]

- Size >5 cm
- >5 mitoses per 10 HPF[2]
- Necrosis
- Moderate to severe nuclear pleomorphism/cytologic atypia
- Vascular invasion
- Infiltrative borders/extraprostatic extension

1. Generally, all features are present concurrently in the malignant Sertoli cell tumors. The vast majority of malignant Leydig cell tumors display ≥2 of the above features. Overall, 5% of neoplasms are malignant.
2. The mitotic criteria for malignant Leydig cell tumors are >3 mitoses per 10 HPF.

Reference: [10]

The Good the Bad and the Ugly: 3

PEComas (Perivascular Epithelioid Cell Tumors)[1]		
	Folpe criteria (2005) [79]	**Modified Folpe criteria (2015)** [80]
Benign	Absence of the features listed below	≤1 of the following features: – Invasive edge – Size 5–9 cm – Mitotic rate 2–3/50 HPF – Vascular invasion
Uncertain malignant potential	1 of the following features: – Nuclear pleomorphism/multinucleated giant cells[2] – Size >5cm[3]	1 of the following features: – Marked atypia – Size ≥10 cm – Mitotic count ≥4/HPF
Malignant	≥2 of the following features: – >5 cm – Infiltrative – High nuclear grade and cellularity – Mitotic rate ≥1/50 HPF – Necrosis – Vascular invasion	Any necrosis or ≥2 of the above features

1. PEComas include a variety of tumors with special names: angiomyolipoma (kidney and other sites); clear cell "sugar" tumor (lung); lymphangioleiomyomatosis or LAM (lung); several unusual visceral, intra-abdominal, and soft tissue/bone tumors (clear cell myomelanocytic tumor of the falciform ligament/ligamentum teres, abdominopelvic sarcoma of perivascular epithelioid cells, etc.), plus "PEComa" with no special name, soft tissue/bone, visceral, GYN tract, skin.

   Criteria for predicting malignant potential have been modified over the years. While the original Folpe criteria were designed on the basis of both soft tissue and GYN PEComas, the later criteria (Schoolmeester and modified Folpe) were established based on GYN tumors only. Nonetheless, it is currently recommended to apply **modified Folpe criteria** to categorize the tumors as above and to apply the more stringent Schoolmeester criteria [81] to determine which of the **malignant** tumors are likely to **recur early** [80].

2. "Symplastic" PEComa suggested to be likely benign, but this is uncertain due to few reported cases.

3. It is essential to thoroughly sample large tumors.

References: [79–82]

Chapter 9. Grading (and Classification) Quick Reference: Hematopoietic System

By Xiaojun Wu & Zenggang Pan (Prior edition by Amy Duffield)

Follicular Lymphoma, WHO Grading System	
Grade	**Number of centroblasts per HPF**[1]
Grade 1[a]	0–5
Grade 2[a]	6–15
Grade 3[b]	>15
3A	Residual centrocytes present
3B	Centroblasts form solid sheets with no residual centrocytes
Pattern	**Proportion of follicular pattern**[2]
Follicular	>75%
Follicular and diffuse	25–75%
Focally follicular/predominantly diffuse	<25%
Diffuse	0

1. *HPF*, high-power field (40 × objective, 0.159 mm^2). Number should be based on the average of 10-field count.
2. The relative proportions of follicular and diffuse areas should be provided in the pathology report.
[a]Grades 1 and 2 are both clinically indolent. Distinction between the two is not encouraged, and low-grade FL is best reported as "Grade 1–2 of 3."
[b]Grade 3 (A) lymphomas with a diffuse pattern are reported as "1. Diffuse large B-cell lymphoma (%). 2. Follicular lymphoma, grade 3 (A) (%)."

Reference: [1]

Plasma Cell Neoplasms and Related Entities, WHO 2016 Update	
Plasma cell myeloma (PCM)	Requires both: 1. Clonal bone marrow plasmacytosis (typically ≥10%, but a minimal % is not designated in the setting of symptomatic myeloma) or biopsy-proven myeloma 2. One or more of the following myeloma-defining events: (a) Evidence of end-organ damage (CRAB)[1] (b) ≥1 of the following biomarkers of malignancy (i) Clonal plasma cells ≥60% (ii) An involved-to-uninvolved serum-free light-chain ratio ≥100 (iii) >1 focal lesions on MRI
Smoldering plasma cell myeloma	Requires both: 1. Serum monoclonal protein (IgA or IgG) >3 g/100 ml *and/or* clonal bone marrow plasmacytosis 10–60% 2. Absence of myeloma-defining events or amyloidosis
Solitary plasmacytoma (bone and extraosseous)	Requires all three (+ item #4 for solitary plasmacytoma of bone): 1. Biopsy-proven solitary clonal plasma cell lesion 2. No evidence of bone marrow clonal plasmacytosis (random iliac crest biopsy) 3. Absence of myeloma-defining events 4. Normal skeletal survey and spine/pelvis MRI or CT (except for the primary solitary bone lesion) Note: Approximately 20% of patients have a small M protein Extraosseus plasmacytoma must be distinguished from a lymphoma with prominent plasmacytic differentiation
Solitary plasmacytoma with minimal bone marrow involvement	Similar to solitary plasmacytoma; however, in addition, there is bone marrow clonal plasmacytosis <10% in a random iliac crest biopsy (usually identified by flow cytometry)
Monoclonal gammopathy of undetermined significance (MGUS) (IgM MGUS, non-IgM MGUS, and light-chain [LC] MGUS)	Requires all three: 1. IgM or non IgM MGUS: serum monoclonal protein <3g/100 ml or abnormal FLC ratio (<0.26 or >1.65); LC MGUS: increase of involved LC with complete loss of HC expression in serum/urine LC M protein < 0.5g/24hrs 2. <10% clonal bone marrow plasmacytosis in non-IgM MGUS and LC MGUS or <10% clonal lymphoplasmacytic infiltration in IgM MGUS 3. Absence of end-organ damage[1] and bone lytic lesions, hyperviscosity, lymphadenopathy, hepatosplenomegaly, and amyloidosis Note: Since clinical and laboratory data are frequently not available with the biopsy, <10% clonal bone marrow plasmacytosis is typically signed out as "plasma cell neoplasm." If progression occurs, IgM MGUS tends to progress to LPL/WM, other B cell neoplasms, or primary amyloidosis. Non-IgM MGUS progresses to PCM, solitary plasmacytoma, or amyloidosis. LC MGUS progresses to LC-smoldering PCM or LC-PCM.

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Primary amyloidosis	Requires all four: 1. Presence of amyloid-related compromised organ function[2] 2. Amyloid deposition in tissue, Congo red+ with birefringence in polarized light 3. Evidence that the amyloid is light-chain restricted (except for rare disease caused by heavy chains) 4. Evidence of an underlying plasma cell or lymphoplasmacytic neoplasm (e.g., M protein, clonal plasma cells in bone marrow) Note: Deposition of eosinophilic, amorphous monoclonal immunoglobulin that compromises organ function[2] can be non-amyloid and Congo red negative, as seen in light-chain and heavy-chain deposition disease.
Waldenstrom macroglobulinemia	Requires both: 1. IgM monoclonal gammopathy of any concentration 2. Lymphoplasmacytic lymphoma with bone marrow involvement Note: The residual disease after treatment may be virtually all plasma cells, mimicking myelomatous involvement.
Plasma cell neoplasm (PCN) with associated paraneoplastic syndrome: POEMS	POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin changes) (a) Mandatory criteria: polyneuropathy and monoclonal plasma cell proliferative disorder (could be osteosclerotic myeloma) (b) Major criteria (≥1 required): Castleman disease, osteosclerotic bone lesions, and/or VEGF elevation (c) Minor criteria (≥1 required): organomegaly, endocrinopathy, skin changes, papilledema, thrombocytosis, and/or extravascular volume overload
PCN with associated paraneoplastic syndrome: TEMPI syndrome	TEMPI (telangiectasia, erythrocytosis with elevated erythropoietin levels, monoclonal gammopathy, perinephric-fluid collections, and intrapulmonary shunting)

1. End-organ damage "CRAB" (hypercalcemia, renal insufficiency, anemia, and/or bone lesions (≥1 osteolytic lesion on skeletal radiography, CT, or PET/CT) that can be attributed to the plasma cell proliferative disorder).
2. For example, renal, liver, heart, GI, or peripheral nerve involvement.

Abbreviations: *FLC* serum free light chain, *LC* light chain, *LPL* lymphoplasmacytic lymphoma, *MGUS* monoclonal gammopathy of undetermined significance, *PCM* plasma cell myeloma, *WM* Waldenström macroglobulinemia

Reference: [1]

Myeloid Neoplasms

Myelodysplastic Syndromes (MDS), WHO 2016 Update				
MDS class	Ring sideroblasts	% Blasts	Key features	Risk group[5]
MDS with single lineage dysplasia (MDS-SLD)[1]	<15% or <5% if *SF3B1* mutation is present	<5% in BM <1% in PB No Auer rods	Unilineage dysplasia[2] in ≥10% of the cells Uni- or bicytopenia[3] (see note below) (if pancytopenia, see MDS unclassifiable)	Low-risk group
MDS-SLD with ring sideroblasts (MDS-RS-SLD)	≥15% or ≥5% if *SF3B1* mutation is present	Same as above	Above features with ring sideroblasts	
MDS associated with isolated del(5q) [or with one other cytogenetic abnormality except for monosomy 7 or del(7q)]	None or any		Anemia (often macrocytic) Normal or increased platelet count Normal or increased small hypolobated megakaryocytes Often in middle-aged women	
MDS with multiple lineage dysplasia (MDS-MLD)	<15% or <5% if *SF3B1* mutation is present	Same as above	Dysplasia[2] in two or more lineages One or more cytopenia[3] (see note below)	Intermediate-risk group
MDS-MLD with ring sideroblasts (MDS-RS-MLD)	≥15% or ≥5% if *SF3B1* mutation is present	Same as above	Above features with ring sideroblasts	
MDS with excess blasts (EB)-1 MDS-EB-1	None or any	5–9% in BM 2–4% in PB No Auer rods	Cytopenia(s)[3] Variable degree of dysplasia[2]	High-risk group
MDS-EB-2	None or any	10–19% in BM 5–19% in PB or Auer rods present	Same as above	
MDS, unclassifiable	None or any	(a) <5% in BM <1% in PB no Auer rods (b) <5% in BM <1% in PB no Auer rods (c) <5% in BM 1% in PB no Auer rods	(a) Persistent cytopenia(s)[3], no unequivocal dysplasia[2] but cytogenetic abnormalities (presumptive evidence in MDS) (b) Pancytopenia[3] in low-risk group MDS (c) 1% blasts in blood[4] in low- to intermediate-risk group MDS	Heterogeneous clinical behavior

1. Includes refractory anemia, refractory neutropenia, and refractory thrombocytopenia [thrombocytosis can be seen in MDS with isolated del(5q) or with inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2)].

2. Characteristics of dysplasia:
   - *Erythroid dysplasia* (*dyserythropoiesis*): multinucleation, nuclear budding, intranuclear bridging, karyorrhexis, megaloblastoid change (nuclear to cytoplasmic asynchrony, i.e., nucleus too big or immature for the degree of cytoplasmic maturity), ring sideroblasts, cytoplasmic vacuolization.
     - Ring sideroblasts (RS) are defined as red cell precursors with sideroblastic granules encircling ≥5 granules and ≥1/3 circumference of a nucleus (iron stain).
     - Ring sideroblasts may be seen in many myelodysplastic syndromes including high-grade disease.
     - The presence of RS does not necessarily imply MDS. Low percentage of RS can be seen in alcoholism, malnutrition, drug toxicity, lead toxicity, etc [4].
   - *Myeloid dysplasia* (*dysgranulopoiesis*): cytoplasmic hypogranulation, nuclear hypolobation (pelgeroid or pseudo-Pelger-Huët cells), and small size
   - *Megakaryocytic dysplasia*: hypolobated megakaryocytes and micromegakaryocytes; megakaryocytes with multiple widely separated nuclei ("pawn ball" nuclei).

3. Cytopenias: Hemoglobin <10 g/dL; platelet count <100 × 10^9/L; or absolute neutrophil count <1.8 × 10^9/L.

4. 1% blast in PB must be recorded on ≥2 separate occasions.

5. The comprehensive risk stratification scheme for MDS includes degree of cytopenia, blast percentage, cytogenetics, and mutational status.

Abbreviations: *BM* bone marrow, *MDS* myelodysplastic syndrome, *PB* peripheral blood

References: [1–5]

Myeloproliferative Disorders, WHO 2016 Update[1]				
	Peripheral blood	Marrow	Key features	Clinical
Chronic myeloid leukemia, *BCR-ABL1* positive (CML) Chronic phase: CP Accelerated phase: AP Blast phase: BP	CP: leukocytosis; <2% blasts AP: 1. persistent or increasing leukocytosis or thrombocytosis, thrombocytopenia, or splenomegaly; OR 2. ≥20% basophils OR 3. 10–19% blasts OR 4. evidence of clonal evolution OR 5. response-to-TKI criteria[2] BP: ≥20% blasts or extramedullary blast proliferation	CP: <5% blasts AP: 10–19% blasts BP: ≥20% blasts	1. Hypercellular marrow 2. Markedly increased myeloid:erythroid ratio 3. Small megakaryocytes with hypolobated nuclei 4. Increased reticulin fibrosis **Philadelphia chromosome t(9;22) is required for diagnosis**	Very good prognosis if disease is responsive to tyrosine kinase inhibitors
Polycythemia vera (PV) Polycythemic phase: PCV Post-polycythemic myelofibrosis: pPV-MF	Polycythemic phase (PCV): 1. Hemoglobin >16.5 g/dL in men; >16 g/dL in women (but serum erythropoietin not elevated) 2. Increased RBC mass (>25% above mean) pPV-MF (spent phase): anemia, leukoerythroblastosis	PCV: <5% blasts pPV-MF: >10% blasts suggests transformation to accelerated phase	PCV: Hypercellular marrow with proliferation of erythroid, granulocytic, and megakaryocytic lineages (panmyelosis); loose aggregates of atypical variably sized and hyperlobated megakaryocytes; marrow iron storage depleted pPV-MF[3]: Marked fibrosis +/− osteosclerosis ***JAK2 mutation is required for diagnosis***	Median survival >10 years. Most patients die of thrombosis or hemorrhage 3–7% progress to AML
Primary myelofibrosis (PMF) Prefibrotic stage Fibrotic stage	Anemia, leukoerythroblastosis May also see leukocytosis or thrombocytosis early in disease	Prefibrotic: <5% blasts Fibrotic: <10% blasts; 10–19% blasts suggests accelerated phase	Prefibrotic: Hypercellular marrow with atypical megakaryocytes (small to giant pleomorphic, cloud-like, bulbous, and/or hyperchromatic nuclei) Fibrotic[3]: Significant marrow fibrosis, extramedullary hematopoiesis Majority have *JAK2*, *CALR*, or *MPL* mutation	Frequent splenomegaly ~5–30% progress to AML
Essential thrombocythemia (ET) Thrombocythemic: ET Post-ET myelofibrosis: post-ET MF	ET: Sustained platelet count of ≥450 × 10^9/L Post-ET MF: anemia, leukoerythroblastosis	<5% blasts	ET: Normocellular or moderately hypercellular bone marrow; proliferation of megakaryocytic lineage; uniform large megakaryocytes with deeply lobulated and hyperlobated nuclei; no increased fibrosis Post-ET MF[3] (relatively rare): Increased marrow fibrosis Majority have *JAK2*, *CALR*, or *MPL* mutation	Indolent; mortality often due to thrombosis or hemorrhage <5% progress to MDS or AML
Chronic neutrophilic leukemia	1. WBC ≥25 K/uL 2. Neutrophils + bands ≥80% 3. Neutrophil precursors <10%; rare myeloblasts 4. Monocytes <1 K/uL 5. No significant dysplasia	<5% blasts	Hypercellular marrow Persistent nonreactive neutrophilia with normal maturation *CSF3R* mutation	Slowly progressive
Chronic eosinophilic leukemia	Eosinophilia ≥1.5 × 10^9/L Blasts ≥2% but <20%	Blasts ≥5% – <20%	Hypercellular marrow due to eosinophilic proliferation with increased myeloblasts; dysplasia in other cell lineages and occasional marrow fibrosis No evidence of *BCR-ABL* or t(5:12) No rearrangements of *PDGFRs*, *FGFR1*, *PCM1-JAK2*, inv(16), t(16;16), or t(8;21)[4]	Good prognosis Middle-aged women
Myeloproliferative neoplasm, unclassifiable	Peripheral cytosis	MPN with features that do not meet the clinical and/or morphologic criteria for a specific MPN subtype: 1. Very early stage disease, characteristic features not fully developed 2. Advance-staged of marrow fibrosis, without prior history or histology, cannot be further characterized 3. Convincing MPN with coexisting neoplastic or inflammatory disorder obscuring diagnostic clinical and morphologic features		Varies

1. In the WHO 2016 update, mastocytosis is listed separately from MPN.
2. Response-to-TKI criteria (provisional): (1) Hematologic resistance to first generation TKIs; (2) any grade of resistance to two sequential TKIs; (3) occurrence of two or more *BCR-ABL1* mutations.
3. It may be impossible to differentiate the fibrotic stages of PV, PMF, and ET based on bone marrow histomorphology. These cases are best signed out descriptively.
4. Myeloid and lymphoid neoplasms with eosinophilia and abnormalities in *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM1-JAK2* are considered separately in the WHO 2016.

Reference: [1]

Myelodysplastic/Myeloproliferative Disorders, WHO 2016 Update[1]				
	Peripheral blood	Marrow	Key features	Clinical
Chronic myelomonocytic leukemia (CMML) Blast count (myeloblast, monoblast, and promonocyte): CMML-0: <2% in PB and <5% in BM; no Auer rods CMML-1: 2–4% in PB and 5–9% in BM; no Auer rods CMML-2: 5–19% in PB and 10–19% in BM or Auer rods +	1. Persistent peripheral blood monocytosis (≥1 K/uL and >10%) 2. Blasts <20% 3. Dysplasia in ≥1 myeloid lineages	Blasts <20%	1. Hypercellular marrow[1,2] 2. Dysplasia in ≥1 myeloid lineages or (a) Acquired clonal cytogenetic or molecular evidence (b) Persistent and nonreactive monocytosis ≥3 months	Median survival time ~1–2.5 years 15–30% progress to AML Prognosis depends on blood and marrow blast count, karyotype, WBC count, and hematopoietic function
Atypical chronic myeloid leukemia, *BCR-ABL1* negative	1. Peripheral leukocytosis with neutrophil precursors ≥10% 2. Dysgranulopoiesis 3. No basophilia or monocytosis 4. <20% blasts	Blasts <20%	1. Hypercellular marrow with granulocytic hyperplasia and dysplasia, +/− dysplasia in erythroid and megakaryocytic lineages[1,2] 2. <20% blasts	Median survival ~1–2.5 years 30–40% progress to AML, remaining patients die of marrow failure
Juvenile myelomonocytic leukemia	1. Peripheral monocytosis ≥1 K/uL 2. Blasts <20%	Blasts <20%	1. Hypercellular marrow with proliferation of granulocytic and monocytic lineages and erythroid and megakaryocytic abnormality 2. Splenomegaly 3. Elevated HbF 4. No *BCR-ABL1*, whereas mutations involve the *RAS* pathway genes, *PTPN11*, *NF1*, and *CBL*	Children 0–14 years old Prognosis varies depending on genetic background
Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis	1. Anemia 2. Persistent thrombocytosis, platelets ≥450 K/uL 3. <1% blasts	≥15% ring sideroblasts <5% blasts	1. Dyserythropoiesis 2. *SF3B1* mutation+/−, often concurrent with *JAK2 V617F* mutation 3. No *BCR/ABL1*[2,3] 4. No history of MPN, MDS (except for MDS-RS), or other MDS/MPN	Median survival time ~6–10+ years
Myelodysplastic/myeloproliferative neoplasm, unclassifiable	Myeloid neoplasm with mixed myeloproliferative and myelodysplastic features *at onset*; not meeting the WHO criteria for any other MDS/MPN, MDS, or MPN			Prognosis is variable

1. Do not meet WHO criteria for CML, PV, PMF, and ET
2. No rearrangement involving *PDGFR*-alpha, *PDGFR*-beta, *FGFR1*, and *PCM1-JAK2*
3. No t(3;3)(q21.3;q26.2), inv(3)(q21.3;q26.2), or del(5q)

Reference: [1]

Myeloproliferative Neoplasm vs. Myelodysplastic Disorder		
	Myeloproliferative neoplasm	**Myelodysplastic disorder**
Effective hematopoiesis	Yes	No
CBC count	High (leukocytosis, neutrophilia, monocytosis, eosinophilia, erythrocytosis, thrombocytosis)	Low (leukopenia, anemia, thrombocytopenia)
Bone marrow cellularity	High	High; could be low in hypoplastic MDS
Cell morphology	Normal myeloid and erythroid; megakaryocytes mostly have abnormal morphology	Dysplastic changes in one or multiple lineages
Marrow fibrosis	Often present	Usually absent or very mild, except for MDS with myelofibrosis
Organomegaly	Often present	Absent, except for MDS with myelofibrosis
Cytogenetic and molecular findings	Usually normal karyotype except CML; MPN-associated mutations, including *JAK2*, *CALR*, *MPL*, and *CSF3R* common	MDS-associated chromosomal abnormalities common
Risk of transformation to acute leukemia	Yes	Yes, higher than MPN
Common differential diagnosis	Reactive, e.g., dehydration and high altitude sports, infection, medication such as G-CSF	Nutritional deficiency, medication, systemic disease (autoimmune disease), congenital disease

Acute Myeloid Leukemia, WHO Classification 2016 Update		
Classification	Definition and key features	Clinical
AML with recurrent genetic abnormalities (balanced translocations)		
AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*	May present as myeloid sarcoma. Cases with t(8;21)(q22;q22.1) and <20% bone marrow blasts should be diagnosed as AML Morphology: large blasts with abundant basophilic cytoplasm with numerous azurophilic granules, may have pseudo-Chediak-Higashi granules, occasional Auer rods, and perinuclear clearing (hof) (see glossary) *Unique flow: high intensity of CD34+ blasts with aberrant expression of* CD56 and *B lymphoid markers, CD19*, *PAX5*, and *cCD79a*	High rate of complete remission and favorable long-term outcome
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	Cases with inv(16)(p13.1q22) or t(16;16)(p13.1;q22) and <20% bone marrow blasts should be diagnosed as AML Morphology: monocytic blasts, immature cells with both eosinophilic and basophilic granules (see glossary) *Flow: high CD34+ blasts commonly show coexpression of CD2*	Higher frequency in younger patients High rate of complete remission and favorable long-term outcome
Acute promyelocytic leukemia (APL) with *PML-RARA*	Cases with *PML-RARA* and <20% bone marrow blasts should be diagnosed as AML Morphology: (1) hypergranular blasts with cytoplasmic bundles of Auer rods and atypical promyelocytes with dustlike granules; (2) hypogranular/microgranular blasts with bilobed nuclei and paucity or absence of granules (see glossary) *Flow: low or absent CD34 and HLA-DR*, *MPO strongly positive. A subset of hypogranular cases has CD34 and CD2 coexpression*	Both APLs are frequently associated with coagulopathy (DIC) Sensitive to treatment with tretinoin and arsenic trioxide; better prognosis
AML with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*	≥20% of marrow nucleated cells and/or peripheral blood are blasts Morphology: blasts are promonocytes and monoblasts *Flow: see M5 for monocytic blast immunophenotype*	More common in children Intermediate to poor prognosis among various *KMT2A* translocations
AML with t(6;9)(p23;q34.1); *DEK-NUP214*	≥20% of marrow nucleated cells and/or peripheral blood are blasts Morphology: often basophilia and multilineage dysplasia	Poor prognosis High frequency of *FLT3-ITD*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.1); *RBM15-MKL1*	≥20% of marrow nucleated cells and/or peripheral blood are blasts after the correlation with fibrous marrow biopsy Morphology: megakaryoblasts (medium to large) with basophilic, agranular cytoplasm forming distinct blebs or pseudopods; micromegakaryocytes are common; dense marrow fibrosis *Flow: see M7 for megakaryoblast immunophenotype*	Rare, restricted to infant and young children (≤3 yo) without trisomy 21; female predominance Presents with marked organomegaly
AML with *BCR-ABL1*	≥20% of marrow nucleated cells and/or peripheral blood are blasts Excluding BP of CML, mixed phenotype acute leukemia, therapy-related myeloid neoplasm, or other AML with recurrent genetic abnormalities Morphology: nonspecific but classic marrow findings of blast transformation of CML, e.g., dwarf megakaryocytes and markedly increased M/E ratio, are absent *Flow: CD34+ blasts; aberrant expression of CD7, CD19, and TdT is more common*	Rare, primarily in adult males Aggressive disease Improved survival with TKI followed by BM transplant
AML with recurrent genetic abnormalities (gene mutations)		
AML with mutated *NPM1*	≥20% of marrow nucleated cells and/or peripheral blood are blasts Morphology: frequently myelomonocytic and monocytic features (irregular and cleaved nuclei) May have cuplike intranuclear cytoplasm invagination and background multilineage dysplasia	Favorable prognosis if normal karyotype and no *FLT3-ITD*
AML with biallelic mutation of *CEBPA*	≥20% of marrow nucleated cells and/or peripheral blood are blasts Morphology: nonspecific, can have background multilineage dysplasia	If young patients, consider germline mutation with predisposition to AML Favorable prognosis
AML with mutated *RUNX1*	≥20% of marrow nucleated cells and/or peripheral blood are blasts Excluding AML with MDS-related changes, therapy-related myeloid neoplasm, and other AML with recurrent genetic abnormalities	May be associated with germline mutation of *RUNX1* (check family history for thrombocytopenia, etc.)
AML NOS (myeloid)		
AML, minimally differentiated (*FAB: M0*)[1]	Marrow packed with blasts. Normal hematopoiesis is usually absent Blasts: medium size with round nuclei, agranular cytoplasm, and no Auer rods; i.e., no morphologic evidence of myeloid differentiation	Infants and older adults; marrow failure; +/– leukocytosis; poor prognosis
AML without maturation (*FAB: M1*)	Marrow packed with blasts, >90% of which have not matured past myeloblast stage Blasts: some morphologic features of myeloid differentiation (scattered granules and Auer rods)	Adults; marrow failure; +/– leukocytosis; poor prognosis
AML with maturation (*FAB: M2*)	Marrow packed with blasts. At least 10% of myeloid cells have matured past myeloblast stage Monocytic cells comprise <20% of non-erythroid cells Blasts: +/– azurophilic granules and frequent Auer rods	All age groups; marrow failure with variable WBC count; moderate prognosis
AML NOS (myelo/monocytic)		
Acute myelomonocytic leukemia (AMML) (*FAB: M4*)	≥20% of marrow nucleated cells and/or peripheral blood are blasts (monoblasts + promonocytes) Monocytes/monocyte precursors and neutrophils/neutrophilic precursors each constitute >20% of marrow cells. The peripheral blood may contain high numbers of monocytic cells *Flow typically shows more than one blast population: (A) CD34+ myeloblasts and (B) CD34 –/+ monocytic blasts (see below M5 for immunophenotype)*	Older adults; bone marrow failure with variable white count; moderate prognosis

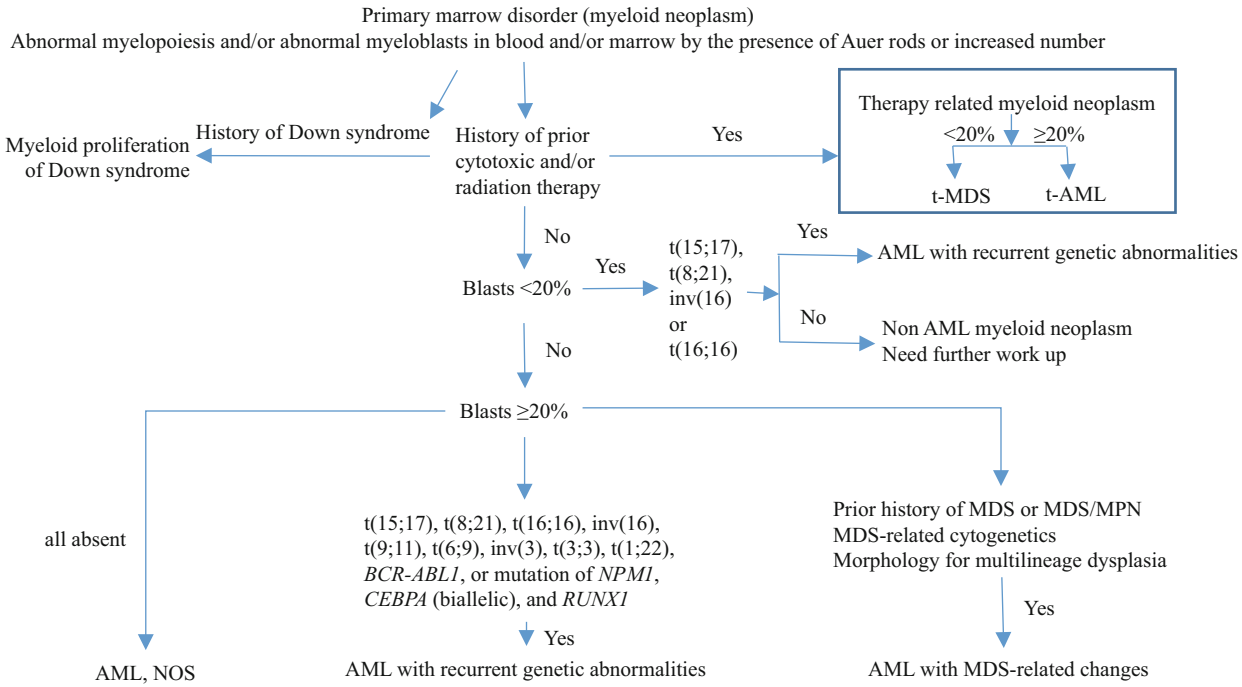
Acute monocytic/ monoblastic leukemia (*FAB: M5*)	≥20% of marrow nucleated cells and/or peripheral blood are blasts (monoblasts + promonocytes), and >80% of non-erythroid cells are of the monocyte lineage (monoblasts + promonocytes + monocytes) *Flow: CD34−/+, CD117+/−, HLA-DR+, CD13+, CD33bright, CD4, CD11b+, CD15+, CD14+, CD64+, MPO +/− may have CD7 or CD56*	Monoblastic: young people; monocytic: adults; soft tissue infiltration and bleeding disorders; moderate prognosis
AML NOS		
Acute erythroid leukemia[2] (*FAB: M6*)	Pure erythroid leukemia (M6b): >80% of bone marrow cells are erythroid, with >30% proerythroblasts. No significant myeloblast population. Erythroblasts: deeply basophilic cytoplasm, prominent nucleoli, cytoplasmic vacuoles *Flow: CD34-, CD117+/−, HLA-DR-, CD71+*	Rare (<5% AML); poor prognosis
Acute megakaryoblastic leukemia (*FAB: M7*)	≥20% of marrow nucleated cells and/or peripheral blood are blasts and ≥50% of blasts are megakaryocytic lineage. Associated with marrow fibrosis. Megakaryoblasts: large with cytoplasmic blebs/pseudopods and zones of basophilic cytoplasm Differential diagnosis: – Acute panmyelosis with myelofibrosis: acute onset, severe constitutional symptoms, no splenomegaly, marked marrow fibrosis, CD34+ blasts lack megakaryocytic markers – AML with myelofibrosis: blasts lack megakaryocytic markers – High-grade MDS (MDS-EB2) with fibrosis: less abrupt onset, <20% blasts, often has chromosomal abnormalities associated with MDS, del5q, del7q, −5, or −7 – Myeloid leukemia associated with Down syndrome: typically children <5 years old, associated with mutations in *GATA1*, very favorable prognosis *Flow: CD34-, HLA-DR-,CD45-, CD13+/−, CD33+/−, MPO-,CD7+/−, megakaryocytic markers (CD41, CD61, and /or CD42b)*	Adults and children, bone marrow failure, marrow often shows dysplasia, poor prognosis Associated with mediastinal germ cell tumors in young males, often with i(12p)
Acute basophilic leukemia	≥20% of marrow nucleated cells and/or peripheral blood are blasts with a primary differentiation to basophils (with coarse basophilic granules) *Flow: CD34−/+, HLA-DR+/−, CD13+, CD33+, CD123, CD11b, CD117-*	Very rare (<1% AML), skin involvement, organomegaly, lytic lesions, and symptoms related to hyperhistaminaemia
Acute panmyelosis with myelofibrosis	≥20% of marrow nucleated cells and/or peripheral blood are blasts. Hypercellular marrow with panmyelosis (all three lineages). Variable degree of dysplasia, marked marrow fibrosis, CD34+ blasts which usually account for 20–25% of marrow cellularity. No significant component of megakaryoblasts	Acute onset, severe constitutional symptoms, no splenomegaly, rapid and progressive clinical evolution
Other		
AML with myelodysplasia-related changes	Requires all three: 1. ≥20% blood or marrow blasts 2. Any of the following: (a) History of MDS or MDS/MPN (b) MDS-related cytogenetic abnormalities (c) Multilineage dysplasia[3] 3. Do not qualify for t-MN and AML with recurrent genetic abnormalities	Mainly elderly patients; often severe pancytopenia; cases with 20–29% blasts may be slowly progressive; poor prognosis with a lower rate of CR than other AML subtypes
Myeloid sarcoma	Tumor mass of myeloid blasts with or without maturation effaces extramedullary tissue architecture; many with myelomonocytic differentiation. Equivalent of a diagnosis of AML	May occur precede or coincide with AML
Therapy-related myeloid neoplasms (t-MN) Including t-AML, t-MDS, and t-MDS/ MPN	Subset 1: • 5–10 years after exposure to alkylating agents and/or ionizing radiation • Present with MDS • Often involving chromosome 5 and/or 7, complex karyotype, and mutations or loss of *TP53* Subset 2: • 1–5 years after exposure to topoisomerase II inhibitors • Present with overt acute leukemia without a preceding MDS phase • Often associated with recurrent balanced translocations	Subset 1 generally has a poorer prognosis than subset 2
Myeloid proliferations associated with Down syndrome	Transient abnormal myelopoiesis (TAM) associated with Down syndrome: 1. Newborns with Down syndrome, 3–7 days old 2. Thrombocytopenia, leukocytosis with circulating megakaryoblasts 3. Hepatosplenomegaly 4. Blood and marrow morphology of acute megakaryoblastic leukemia 5. Trisomy 21, *GATA1* mutation 6. CD34+, CD117+, CD13+, CD33+, CD41+, CD42+, CD61+, often CD7+, CD56+, MPO- Myeloid leukemia associated with Down syndrome: 1. Children with Down syndrome, 1–5 year old 2. 20–30% with prior TAM 3. Many with preleukemic MDS 4. Blood and marrow findings of AML; may have dysplasia in three lineages 5. Trisomy 21, *GATA1* mutation, and commonly trisomy 8 6. Blast immunophenotype similar to TAM but CD34- in 50% of cases	TAM has a high rate of spontaneous remission Myeloid leukemia associated with Down syndrome has better prognosis than AML in children without Down syndrome

1. The FAB classification (e.g., "M7") is no longer in use and should not be incorporated into the pathologic diagnosis.
2. The previous erythroid/myeloid type acute erythroid leukemia (erythroleukemia, M6a): ≥50% erythroid precursors in the marrow and ≥20% myeloid blasts in the non-erythroid population is now classified as MDS with excess blasts and erythroid predominance.
3. The multilineage dysplasia must be present in ≥50% of the cells in at least two lineages. Multilineage dysplasia alone is insufficient for a diagnosis of AML-MRC in a de novo AML with mutated *NPM1* or biallelic mutation of *CEBPA.*

Please note: Promonocytes are blast equivalents in AML with monocytic differentiation. Promyelocytes are blast equivalents in APL.

Reference: [1]

Diagnostic Approach to AML



Abbreviations: *AML* acute myeloid leukemia, *AML, NOS* acute myeloid leukemia, not otherwise specified, *AMML* acute myelomonocytic leukemia, *BM* bone marrow, *CML* chronic myeloid leukemia, *DIC* disseminated intravascular coagulation, *EB* excess blasts, *ET* essential thrombocythemia, *FAB* French-American-British, *FL* follicular lymphoma, *MDS* myelodysplastic syndrome, *MLD* multiple lineage dysplasia, *MPO* myeloperoxidase, *PB* peripheral blood, *PCV* polycythemia vera, *PMF* primary myelofibrosis, *RS* ring sideroblasts, *SLD* single lineage dysplasia, *t-MDS* therapy-related myelodysplastic syndrome, *t-AML* therapy-related acute myeloid leukemia, *WBC* white blood cell

Chapter 10. Tumor Genetics and Cytogenetics: Solid Tumors

By Jason C. Chang, Justin A. Bishop, Tejus A. Bale, Sounak Gupta, Natasha Rekhtman

General Principles

By Natasha Rekhtman & Jason C. Chang

For complete list see http://AtlasGeneticsOncology.org

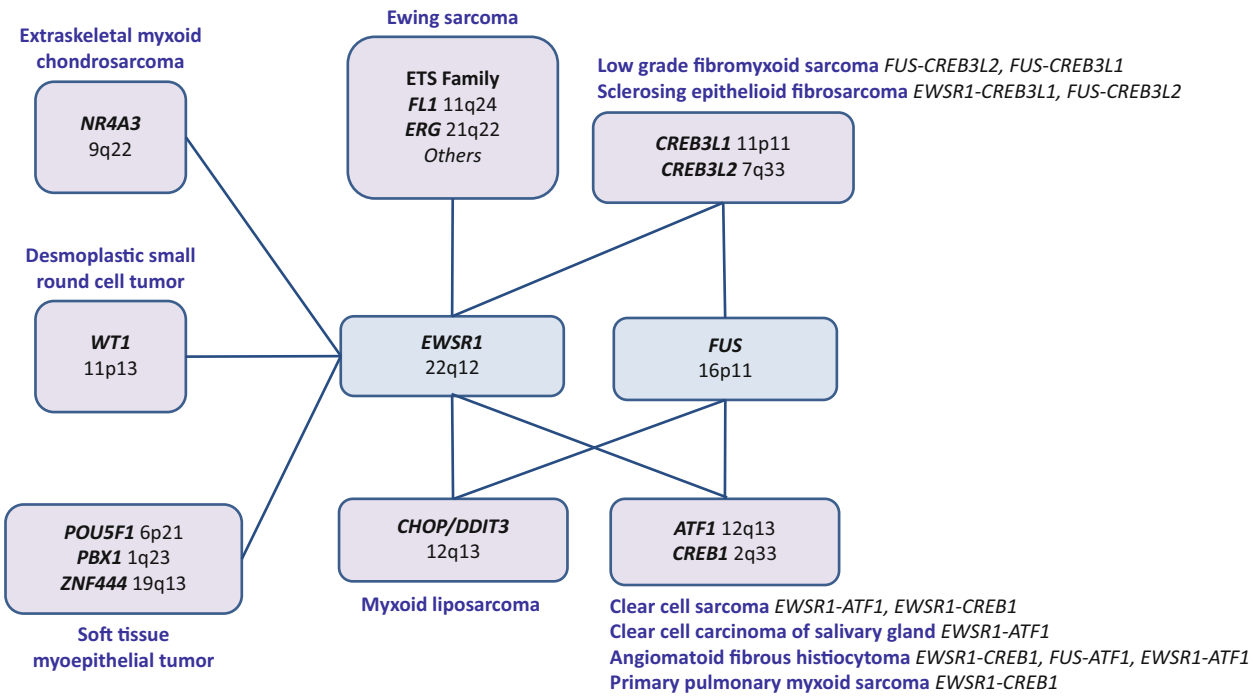
Recurrent chromosomal translocations have traditionally been associated with leukemias/lymphomas and sarcomas. Translocations cause either formation of chimeric proteins (such as BCR-ABL) or abnormal protein expression (such as overexpression of c-Myc as a result of translocation into Ig promoter sequences in Burkitt lymphoma). In contrast, carcinomas generally have complex karyotypes with no recurrent translocations. Instead, carcinomas typically have activating mutations in proto-oncogenes (e.g., *KRAS*) or inactivation of tumor suppressor genes (e.g., *TP53*). In recent years this paradigm has shifted, and an increasing number of carcinomas are being recognized as having recurrent translocations. Notable examples are salivary carcinomas, pediatric renal cell carcinoma, thyroid carcinoma, and some lung adenocarcinomas.

Translocations are traditionally identified by conventional cytogenetics (karyotyping/chromosomal banding analysis), although FISH and sequencing-based assays are becoming increasingly utilized. In contrast, small deletions and point mutations typical of carcinomas generally cannot be visualized by cytogenetics and require nucleic acid-based methods. In some instances immunohistochemistry (IHC) can be used to identify aberrant protein expression resulting from a translocation or mutation (such as IHC for BCL2 in follicular lymphoma or ALK in lung adenocarcinoma). Instances where IHC can be applied as a surrogate for a molecular test are of particular relevance to anatomic pathologists, and these are ***highlighted*** in the tables below.

An interesting rule of thumb is that sarcomas with characteristic translocations are morphologically UNIFORM (rather than highly pleomorphic) and lack atypical mitoses, whereas truly pleomorphic undifferentiated sarcomas typically have complex cytogenetics with multiple nonrecurrent alterations analogous to carcinomas.

Also note a trend for some genes (like *EWSR1*) to appear with multiple translocation partners in different tumors. Even more curiously, there are rare examples of identical translocations in histogenetically unrelated tumors (e.g., *ETV6-NTRK3* infantile fibrosarcoma, congenital mesoblastic nephroma, secretory breast and salivary carcinoma, or *EWSR1-ATF1* in clear cell sarcoma/melanoma of soft parts, salivary clear cell carcinoma, angiomatoid fibrous histiocytoma). See diagram and table below for more detailed list.

By convention, translocations are designated in numerical order for chromosomes [such as t(11;22)] and in 5′-3′ order for chimeric gene products [*EWSR1-WT1*], which confusingly may not necessarily be in the same order [*EWSR1* gene is on chromosome 22, and *WT1* is on 11].



Recurrent gene fusions involving *EWSR1* and *FUS*.
Reference: [1]

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N. Rekhtman et al., *Quick Reference Handbook for Surgical Pathologists*, https://doi.org/10.1007/978-3-319-97508-5_10

Multipurpose Alterations at a Glance

(Selected genes with mutations or translocations involved in multiple tumors or syndromes)

Gene or protein	*Tumor associations*
ALK **-** chr. 2	**ALK+ ALCL** *NPM1-ALK* (80%); *TPM3-ALK* (10%) **Inflammatory myofibroblastic tumor** various translocations of *ALK* **Lung adenocarcinoma** *EML4-ALK* **Neuroblastoma** point mutations in *ALK*
BCL2 **-** chr. 18	Translocations: **FL**; **DLBCL**
BCR-ABL (Philadelphia chromosome) *ABL* **-** chr. 9 *BCR* **-** chr. 22	**CML** Ph p210 > p230> > p190 **B-ALL/LBL** Ph p190> > p210* **De novo AML with BCR/ABL** Ph p210> > p190* *The presence of p210 in a patient with acute leukemia should prompt consideration of CML in blast crisis
BCOR	**Round cell sarcomas** with *BCOR* genetic abnormalities **Clear cell sarcoma of kidney** **Endometrial stromal sarcoma**
BRAF	Mutations: **Papillary thyroid CA, melanoma, colorectal carcinoma, hairy cell leukemia, histiocytic lesions,** many others Fusions: **Spitzoid melanoma, pilocytic astrocytoma, acinar cell carcinoma of pancreas**
ERG	**Ewing sarcoma** with *EWSR1-ERG* **Prostate carcinoma** *TMPRSS2-ERG*
ETV6 **(*TEL*) -** chr.12	**B-ALL/LBL** *ETV6-RUNX1(AML1)* **Infantile fibrosarcoma** **Congenital mesoblastic nephroma** **Secretory carcinoma of the breast** **Secretory carcinoma of salivary glands** Identical translocation: t(12;15) *ETV6-NTRK3*
EWSR1 **-** chr. 22q12 [*see diagram*]	**Ewing sarcoma** *EWSR1-FLI1*, *EWSR1-ERG*, other **Extraskeletal myxoid chondrosarcoma** *EWSR1-NR4A3* **Desmoplastic small round cell tumor** *EWSR1-WT1* **Myoepithelial tumor of soft tissue** *EWSR1-ZNF444*, *EWSR1-POU5F1*, *EWSR1-PBX1* **Myxoid liposarcoma** *EWSR1-DDIT3(CHOP)* **Clear cell sarcoma of soft tissue** *EWSR1-ATF1* **Clear cell carcinoma of salivary gland** *EWSR1-ATF1* **Angiomatoid fibrous histiocytoma** *EWSR1-CREB1*, *EWSR1-ATF1* **Primary pulmonary myxoid sarcoma** *EWSR1-CREB1* **Sclerosing epithelioid fibrosarcoma** *EWSR1-CREB3L1* *EWSR1* (or *FUS*)-*ATF1* (or *CREBx*) translocations
FUS (*TLS*) **-** chr. 16p11 [*see diagram*]	**Angiomatoid fibrous histiocytoma** *FUS-ATF1* **Low-grade fibromyxoid sarcoma** (Evans tumor) *FUS-CREB3L1/2* **Sclerosing epithelioid fibrosarcoma** *FUS-CREB3L2* **Myxoid liposarcoma** *FUS-DDIT3(CHOP)*
IGK **(Igκ) -** chr. 2 ***IGL*** **(Igλ) -** chr. 22 ***IGH*** **(Ig heavy chain) -** chr. 14	Translocations: **B-cell lymphomas** (follicular, mantle cell, lymphoplasmacytic, Burkitt) and **myeloma**
KIT	Mutations: **GIST, melanoma, mastocytosis**
KRAS	Mutations: **Pancreas, colon, lung, ovary (mucinous),** many other tumors
MET	Mutations: **Papillary RCC** (hereditary and occasionally sporadic), **lung carcinoma**
MLL (*KMT2A*) **-** chr. 11q23	Translocations: **AML** (M5), **t-AML** post topoisomerase II inhibitor therapy, **B-ALL/LBL**
MYC **(c-Myc) -** chr.8	Translocations: **Burkitt lymphoma**, **PBL**, **DLBCL**, **DHL**
MYCN **(n-Myc) -** chr. 2	Amplification: **Neuroblastoma**
PDGFR	Mutations: **GIST** Fusions: **Myeloid and lymphoid neoplasms** with eosinophilia and abnormalities of *PDGFRA* or *PDGFRB*
RET	Activating mutations: **Thyroid (medullary CA),** MEN2a, MEN2b Inactivating mutations: **Hirschsprung disease** Fusions: **Lung adenocarcinoma, papillary thyroid CA, some salivary CAs**
SMARCA4 (BRG1)	Deletions/mutations: some **rhabdoid tumors, small cell carcinoma of the ovary hypercalcemic type,** other
SMARCB1 **-** 22q11 (INI1/hSNF5/BAF47)	Deletions/losses: **Rhabdoid tumors** (renal and extrarenal rhabdoid tumors, atypical teratoid/rhabdoid tumor of the brain), **epithelioid sarcoma**, **myoepithelial carcinoma of soft tissue**, **medullary carcinoma** of the kidney, others
TFE3 **-** chr. Xp11.2	**Alveolar soft part sarcoma** **RCC with Xp11.2** Identical translocation: (X;17) *ASPL-TFE3* **PEComas** *TFE3* rearrangements or amplification
TRA and ***TRD*** **(TCR α** and **δ) -** chr. 14 ***TRB*** and ***TRG*** **(TCR β** and **γ)** chr. 7q (β); 7p (γ)	Translocations: **T-cell leukemia/lymphoma**
TP53 **-** chr. 17p	Mutated in many sporadic tumors and in **Li-Fraumeni syndrome**
VHL **-** chr. 3p	Inactivated in sporadic and hereditary **clear cell RCC**, **von Hippel-Lindau syndrome**
WT1 **-** chr. 11p13	**Wilms tumor** 11p13 mutation or deletion **Desmoplastic small round cell tumor** *EWSR1-WT1*

Abbreviations: *ALCL* anaplastic large cell lymphoma, *ALL/LBL* acute lymphoblastic leukemia/lymphoblastic lymphoma, *AML* acute myeloid leukemia, *CML* chronic myeloid leukemia, *DHL* double-hit lymphoma, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *PBL* plasmablastic lymphoma, *RCC* renal cell carcinoma

Cytogenetic and Genetic Alterations in Solid Tumors: Quick Reference

(focusing on somatic alterations with relevance to targeted therapies or tumor diagnosis)
Also see Tumor Syndromes (Chapter 12) for genetic alterations in inherited tumor syndromes and Predictive Markers (Chapter 4) for more on targeted therapies.

Soft Tissue

By Jason C. Chang

Tumor type	*Chromosome*	*Gene/protein*	*Prevalence*	*Comment*
Alveolar soft part sarcoma	t(X;17) (unbalanced)	*ASPL-TFE3* Xp11.2 = site of TFE3	>90%	Same translocation in MiT Family RCC. ***TFE3 (+) by IHC.***
Angiomatoid fibrous histiocytoma (AFH)	t(2;22)	*EWSR1-CREB1*	90%	
	t(12;16)	*FUS-ATF1*	Rare	
	t(12;22)	*EWSR1-ATF1*	Rare	
Round cell sarcomas with *BCOR* genetic abnormalities	inv(X)	*BCOR-CCNB3*	~30%	***BCOR (+) by IHC*** – new marker. References: [2, 3]
	t(X;4)	*BCOR-MAML3*	<10%	
		BCOR ITD	~60%	
Clear cell sarcoma (Melanoma of soft parts)	t(12;22)	*EWSR1-ATF1*	90%	
	t(2;22)	*EWSR1-CREB1*	<10%	
Clear cell sarcoma of GI tract	t(12;22)	*EWSR1-ATF1*	50%	
	t(2;22)	*EWSR1-CREB1*	25%	
***CIC*-rearranged sarcoma**	t(4;19)	*CIC-DUX4*	>90%	***ETV4 (+) by IHC[a].*** Other rare variants: *CIC-DUX10, CIC-FOXO4.* Reference: [4]
Dermatofibrosarcoma protuberans (DFSP)	t(17;22)	*COL1A1-PDGFB*	>90%	Same translocation present in giant cell fibroblastoma (regarded as a pediatric variant of DFSP).
Desmoplastic small round cell tumor (DSRCT)	t(11;22)	*EWSR1-WT1*	99%	***WT1 (+) by IHC.***
Epithelioid hemangioendothelioma (EHE)	t(1;3)	*WWTR1-CAMTA1*	>90%	***CAMTA (+) by IHC*** [5].
	t(X;11)	*YAP1-TFE3*	<10%	
Extraskeletal myxoid chondrosarcoma	t(9;22)	*EWSR1-NR4A3*	70%	
	t(9;17)	*TAF15-NR4A3*	20%	
Ewing sarcoma	t(11;22)	*EWSR1-FLI1*	90%	***NKX2.2 (+) by IHC[a].*** *FLI1* (or ERG) and *EWSR1* have variant translocation breakpoints. Most common breakpoint (type 1) is associated with a favorable prognosis. References: [6, 7]
	t(21;22)	*EWSR1-ERG*	5%	
	Various	*EWSR1-others*		
Gastrointestinal stromal tumor (GIST)	n/a (mutations are not detectable via cytogenetic analyses)	*KIT* mutation	~80%	***95% CD117/c-kit (+) by IHC***. *KIT* wild-type GISTs are often epithelioid and may have *PDGFRA* mutations. Different mutations confer different responses to Gleevec; see targeted therapies section (Chapter 4). A distinct set harbors mutations in SDH complex genes.
		PDGFRA mutation	~5–7%	
Inflammatory myofibroblastic tumor (IMT)	Various	*ALK* fusions rare *ROS1*, *RET* fusions	50%	*ALK* gene also rearranged in ALCL via t(2;5) [8] ***ALK (+) by IHC in 50% of cases.***
Infantile fibrosarcoma	t(12;15)	*ETV6-NTRK3*	90%	Same translocation in congenital mesoblastic nephroma and secretory CA of the breast and salivary glands.
Liposarcoma, myxoid, and round cell	t(12;16)	*FUS-DDIT3*	>90%	
	t(12;22)	*EWSR1-DDIT3*	Rare	
Liposarcoma, well differentiated and dedifferentiated	Ring chromosome 12	*HMGA2, MDM2, CDK4* amplification	80%	***MDM2 (+) by IHC*** (lipoma is MDM2-negative).
Low-grade fibromyxoid sarcoma (LGFMS, Evans tumor)	t(7;16)	*FUS-CREB3L2*	>90%	***MUC4 (+) by IHC[a].*** Same translocation found in hyalinizing spindle cell tumor with giant rosettes; now considered a variant of the same entity [9].
	t(11,16)	*FUS-CREB3L1*	Rare	
Myoepithelial tumor of soft tissue (and some visceral organs)	t(19;22) t(6;22) t(1;22)	*EWSR1-ZNF444* *EWSR1-POU5F1* *EWSR1-PBX1*	45% have *EWSR1* rearrangements [8]	Often seen in children. These molecular alterations are not seen in myoepithelial tumors of the salivary glands. Can display loss of INI1. References: [10, 11]
Mesenchymal chondrosarcoma	t(8;8)	*HEY1-NCOA2*	~80%	
Nodular fasciitis	t(17;22)	*MYH9-USP6*	90%	Identical fusion is seen in approximately 70% of primary aneurysmal bone cysts [12].
PEComa	Xp11 rearrangements or amplification	*TFE3*	~20%	***TFE3 (+) by IHC*** (though positivity is not specific for a gene alteration) [13].
Rhabdomyosarcoma, alveolar	t(2;13)	*PAX3-FOXO1*	60%	Unfavorable prognosis.
	t(1;13)	*PAX7-FOXO1*	20%	Favorable prognosis.
Rhabdomyosarcoma, embryonal	Loss of 11p15 No recurrent translocations			11p15 also mutated in Beckwith-Wiedemann syndrome; Recent studies suggest that "fusion-negative" ARMS are clinically/molecularly indistinguishable from ERMS [14].
Sclerosing epithelioid fibrosarcoma (SEF)	t(11;22)	*EWSR1-CREB3L1*	Pure SEF	***MUC4 (+) by IHC* in 80% of cases[a]** [15].
	t(7;16)	*FUS-CREB3L2*	Hybrid SEF-LGFMS	
Solitary fibrous tumor (SFT)	inv(12)	*NAB2-STAT6*	>90%	***STAT6(+) by IHC.***
Synovial sarcoma (SS)	t(X;18)	*SS18(SYT)-SSX1*	60%	Monophasic or biphasic SS.
	t(X;18)	*SS18(SYT)-SSX2*	35%	Monophasic SS; better prognosis in localized tumors.

[a]Indicates IHC markers (ETV4, NKX2.2, MUC4) whose expression is NOT a direct transcript from a fusion but which are specifically expressed downstream of fusions. These can thus be considered surrogate markers for their respective associated fusions.

Salivary Tumors

By Justin A. Bishop

Tumor type	*Gene alteration*	*Prevalence*	*Comment*
Adenoid cystic carcinoma (AdCC)	*MYB-NFIB* t(6;9)	~60%	– Among salivary gland tumors, not entirely sensitive but 100% specific for AdCC. – Same translocation found in some AdCC of other organs (e.g., breast, prostate) and dermal cylindromas. – ***MYB by IHC is sensitive but not specific.*** References: [16–19]
	MYBL1-NFIB t(8;9)	~10%	
Basal cell adenoma, basal cell adenocarcinoma	*CTNNB1* mutation	50–80% adenomas 5–10% carcinomas	– Not diagnostically useful. – Basal cell adenocarcinoma genetically more complex than basal cell adenoma. References: [20, 21]
Clear cell carcinoma (formerly hyalinizing clear cell carcinoma)	*EWSR1-ATF1* t(12;22)	~80%	– Among salivary gland tumors, 100% specific for clear cell CA. – Same translocation as in clear cell sarcoma, angiomatoid fibrous histiocytoma, myoepithelioma of soft tissue, others. – Remember that demonstrating *EWSR1* rearrangement by itself is not entirely specific; some myoepithelial carcinomas and rare salivary sarcomas may also harbor it. References: [22, 23]
Epithelial-myoepithelial carcinoma	*HMGA2* rearrangements	~25%	– Recent study found that about 50% of epithelial-myoepithelial CAs have molecular signature of pleomorphic adenoma (unclear whether that is because they are related tumors or are epithelial-myoepithelial CAs ex-PA). – *HRAS* mutations seen in other salivary gland tumors and therefore not diagnostically helpful. – A subset of intermediate grade "epithelial-myoepithelial CAs" or "hybrid" epithelial myoepithelial/AdCC harbor *MYB* translocations; these are probably just tubular variants of AdCC. References: [24, 25]
	PLAG1 rearrangements	~25%	
	HRAS mutations	~20%	
Low-grade intraductal carcinoma (formerly low-grade cribriform cystadenocarcinoma, low-grade salivary duct carcinoma)	*RET* rearrangements	~50%	– Partners include *NCOA4* and others not yet identified. – Not particularly useful diagnostically. – High-grade intraductal CAs have a salivary duct CA-like genetic profile. Reference: [26]
Mucoepidermoid carcinoma (MEC)	*CRTC1-MAML2* t(11;19)	~60%	– Originally described more in low-intermediate-grade tumors with better prognosis [27] but now recognized that many fusion-negative "MECs" are something else [28]. – 100% specific among salivary tumors [15,16]. Previously described in some Warthin tumors which are now recognized as "Warthin-like" MEC [29]. Also described in 50% of clear cell hidradenoma of the skin [30].
	CRTC3-MAML2 t(11;15)	~10%	
Myoepithelial carcinoma	*EWSR1*-X	~5%	– ~30–40% of the uncommon clear cell variant of myoepithelial CA (usually high-grade) harbor *EWSR1* translocation with an unknown partner. – Genetically different from soft tissue myoepithelial tumors.
Pleomorphic adenoma (benign mixed tumor) and carcinoma ex-pleomorphic adenoma	*PLAG1* rearrangements	50–60%	– *PLAG1* partners include *CTNNB1*, *LIFR*, *FGFR1*; *HMGA2* partners include *NFIB*, *FHIT*, and *WIF1*. – Rearrangements not very diagnostically useful, because they do not distinguish benign (PA) from malignant (carcinoma ex-PA). – ***PLAG1 IHC is sensitive but not specific for PA or CA ex PA; HMGA2 is specific but not sensitive. Ultimately neither very useful diagnostically.*** References: [31–34]
	HMGA2 rearrangements	20–30%	
Polymorphous adenocarcinoma (formerly polymorphous low-grade adenocarcinoma)	*PRKD1* mutations	50–60%	– Classic polymorphous adenocarcinoma usually harbors *PRKD1* point mutations; the cribriform adenocarcinoma variant usually harbors translocations in *PRKD1/2/3* (partners include *ARID1A* and *DDX3X*). Tumors with mixed features can have either alteration. – Because somewhat complex (i.e., multiple genes, mutations, or translocations), not often utilized diagnostically. References: [35, 36]
	PRKD1, *2*, or *3* rearrangements	20–30%	
Salivary duct carcinoma	*TP53* mutation	50–60%	– Most complex genetic profiles of any salivary gland CA (SqCC-like). – Most salivary duct CAs ex PA harbor *HGMA2* or *PLAG1* rearrangements. – Sequencing not useful diagnostically, though may be helpful for selecting targeted therapies. – ***HER2 immunohistochemistry may be used to screen for ERBB2 amplification.*** References: [24, 37–39]
	HRAS mutation	~25%	
	PIK3CA mutation	~25%	
	PTEN loss	~35%	
	ERBB2 amplification	~35%	
	HMGA2 rearrangements	~25%	
	PLAG1 rearrangements	~25%	
Secretory carcinoma (formerly mammary analogue secretory carcinoma, MASC)	*ETV6-NTRK3* t(12;15)	~98%	– *ETV6* rearrangement is 100% sensitive and 100% specific. – *ETV6-NTRK3* also in congenital mesoblastic nephroma, infantile fibrosarcoma, some ALK-negative IMTs, some PTCs, others. – *ETV6-RET* tumors recently described (not eligible for TRK-inhibitor therapy). References: [40, 41]
	ETV6-RET t(10;12)	~2%	

Thyroid
By Justin A. Bishop

Tumor type	*Chromosome*	*Gene/protein*	*Prevalence*	*Comment*
Papillary carcinoma	10q11.2 translocation or inversion	*RET* fusions	20% (highest in children and postradiation)	– Screening for these alterations is now a standard ancillary tool in FNA of thyroid nodules with atypical FNA results. – Only *BRAF* V600E mutations seem to be entirely specific for carcinoma. – Radiation-induced PTC enriched in *RET*, *NTRK1*, and *NTRK3* fusions. – *ALK* fusion can be detected by ALK IHC or FISH (similar to lung adenocarcinoma). – *RAS* mutations are usually seen in encapsulated, invasive follicular variants of PTC. In addition, many tumors previously reported as PTC with *RAS* mutations are now regarded as "non-invasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP)."
	1q23 or 15q25 translocations	*NTRK1* or *NTRK3* fusions	5–10%	
		BRAF V600E mutations	30–50% (highest in tall cell variant)	
		TERT promoter mutations	5–15%	
		HRAS/KRAS/NRAS mutations	30–50% of encapsulated follicular variants	
	2p23 translocations	*ALK* fusions	5%	
Follicular carcinoma	t(2;3)	*PAX8-PPARγ1* fusion	30–40%	– Translocation is not specific for follicular carcinoma (has been seen in follicular adenomas and occasionally follicular variants of PTC).
		HRAS/KRAS/NRAS mutations	40–50%	
		TERT promoter mutations	10–30%	
Medullary carcinoma		*RET*-activating mutations	40–60% in somatic cases	– Mutation present in syndromic (MEN2) and sporadic CA. There are no *RET/PTC* rearrangements typical of PTC.
Anaplastic carcinoma		*TP53* mutations	70%	– ***Strong immunoreactivity for p53 supports the diagnosis of anaplastic carcinoma vs. mimickers*** (e.g., squamous metaplasia in PTC).
		CTNNB1 (β-catenin) mutations	5–10%	
		PIK3CA mutations	5–25%	
		PTEN alterations	10–15%	
		HRAS/KRAS/NRAS mutations	20–40%	
		BRAF V600E mutations	20%	
		TERT promoter mutations	30–70%	
	2p23 translocations	*ALK* fusions	5%	

Brain
By Tejus A. Bale
(focused on molecularly defined entities in 2016 WHO Classification of CNS Tumors)

Tumor type	*Gene alterations*	*Prevalence*	*Comment*
Oligodendroglioma, IDH-mutant[b], 1p19q co-deleted	Mutations in *IDH1/2* [a] AND 1p/19q co-deletion [a] Also: *TERT* promoter and *CIC* mutations	100%[a]	– Whole arm deletions of chr 1p and 19q result from an unbalanced translocation between chromosomes 1 and 19, with loss of a derivative chromosome.
Astrocytoma, IDH-mutant[b]	Mutations in *IDH1/2* [a], Also: mutations in *ATRX*, *TP53*	100%[a]	– IHC profile: ***IDH1 (R132H) positive[b]***, ***ATRX loss***, ***p53 positive.***
Glioblastoma, IDH-mutant[b]	Mutations in *IDH1/2* [a], Also: mutations in *ATRX*, *TP53*, amplification of *MYC*, *CCND2*, *PDGFRA*	100%[a]	– These "secondary GBMs" are thought to arise out of anaplastic astrocytoma, IDH-mutant, and therefore share many of the same key alterations and IHC profile (see Chapter 2).
Glioblastoma, IDH wild type	*EGFR* amplification	40%	– Genetic profile also seen in lower-grade IDH wild-type gliomas, which have been shown to clinically behave similarly to GBM [42]. – Characterized by high intratumoral heterogeneity.
	Loss of *CDKN2A* or *RB1*	~80%	
	Loss of *PTEN*	15–40%	
	TERT promoter mutation	~80%	
Diffuse midline glioma, H3 K27M mutant[a]	p.K27M mutation in histone genes *H3F3A*, *HIST1H3B/C*	100%[a]	– Includes tumor formerly known as diffuse intrinsic pontine glioma (DIPG). – ***K27M-mutant H3 can be detected by IHC.***
Angiocentric glioma	*MYB* (6q23) rearrangements	~100%	– Most commonly *MYB-QKI* rearrangement [43].
Embryonal tumor with multilayered rosettes	*C19MC* alterations[a]	100%[a]	– Amplification and fusions of chr 19q13.42. – ***LIN28A positive on IHC (not specific).***
Atypical teratoid/ rhabdoid tumor (AT/RT)	22q11.2 deletion or mutation: loss of *SMARCB1* (INI1/hSNF5)	75%	– Same gene mutated in renal/extrarenal rhabdoid tumors. 75% have detectable. – Deletion or mutations, almost all show ***loss of INI1 expression by IHC.***
Medulloblastoma	WNT activated (*CTNNB1* mutation, monosomy 6)	10%	– Molecular groups defined by transcriptome analysis (gold standard), but sequencing, copy number analysis, and IHC are routinely employed for subtyping. Genetic definitions are in addition to histologic subtype. Four subgroups recognized: WNT, SHH, and two others; these have different prognostic significance and may alter therapy [44].
	SHH activated (mutations in *PTCH1*, *SUFU*, when *TP53* wt vs. *SHH*/ *GLI2*/ *MYCN* amplification)	~30%	
	Non-WNT, non-SHH (*MYC* amplification, chr 17 copy number alterations)	~60%	
Ependymoma, RELA fusion-positive[a]	Chromosome 11q13.1 chromothripsis; *C11or f95-RELA* is the most common fusion product	100%[a]	– Comprises 70% of pediatric supratentorial ependymomas; the most aggressive of supratentorial ependymoma molecular groups.
Pilocytic astrocytoma	7q34 duplication: *KIAA1549-BRAF* fusion	>70% (mostly infratentorial)	– Common CNS tumor in neurofibromatosis 1.
(Many)	*BRAF* V600E		– Found in many possibly related tumors: ganglioglioma, pleomorphic xanthoastrocytoma, epithelioid GBM, pilocytic astrocytomas (mostly supratentorial), and others.

Note: Under the 2016 WHO, genetic features trump histology for tumor classification, and grading remains contingent on histology.
[a]Genetic alteration defines tumor type, therefore "prevalence" = 100%.
[b]Mutations in IDH1 and IDH2 are the "first hits" in oligodendrogliomas and astrocytomas. The most common IDH variant, IDH1 p.R132H, can be detected by IHC.

Genitourinary Tumors

By Sounak Gupta & Jason C. Chang

Also see table in Syndromes (Chapter 12) for germline mutations associated with RCC: Birt-Hogg-Dube syndrome, HLRCC, SDH-deficient RCC, etc.

Tumor type	*Chromosome*	*Gene/protein*	*Prevalence*	*Comment*
Adult renal tumors				
Clear cell RCC (ccRCC)	3p deletion (*VHL* locus)		deletion/mutation/ methylation in 90% of sporadic ccRCC	– *VHL* mutations occur in BOTH sporadic and inherited forms of ccRCC. – Germline mutations in *VHL* von Hippel-Lindau syndrome. – ↑CAIX in RCC is a consequence of VHL gene inactivation. ***Diffuse IHC for CAIX is a hallmark of ccRCC.***
	n/a	*VHL* gene inactivation by mutation or promoter methylation		
Papillary RCC (pRCC), **type 1**	Trisomy 7 and 17	n/a	~80%	
	n/a	*MET* mutations (gain of function)		– *MET* mutations are present in both inherited and some sporadic forms of pRCC.
Papillary RCC, type 2	No recurrent patterns	n/a		
Chromophobe RCC	Multiple chromosomal losses	n/a		
MiT family translocation RCC	t(X;17)	*ASPSCR1-TFE3* (balanced)		– ***TFE3*** (+) ***by IHC.*** – Distinct features: young age, papillary architecture, clear cells, psammoma bodies. Identical translocation (*ASPSCR1-TFE3*) also seen in alveolar soft parts sarcoma (unbalanced).
	t(X;1)	*PRCC-TFE3* *PSF-TFE3*		
	inv(X)	*NonO-TFE3*		
	t(6;11)	*MALAT1-TFEB*		– ***TFEB (+) by IHC.*** [45] – Distinct features: young age, clear and eosinophilic cells in nests and tubules.
Renal medullary carcinoma	*SMARCB1* on 22q11.2	*SMARCB1* (INI1) loss	>90%	– Mostly reported in African Americans and associated with sickle cell trait/disease.
Metanephric adenoma	n/a	*BRAF* V600E mutation	90%	
Angiomyolipoma (AML)	9p34	*TSC1* (hamartin) loss of function	> 90% have biallelic loss of either *TSC1* or *TSC2*	– *TSC1* and *TSC2* alterations can occur in BOTH inherited and sporadic forms of AML. Most cases are sporadic. – Germline *TSC1* or *TSC2* (tuberous sclerosis).
	16p13	*TSC2* (tuberin) loss of function		
Pediatric renal tumors				
Wilms tumor	11p13 deletion/ mutation	*WT1*	15%	– Same mutation in syndromic Wilms tumors (WAGR; Denys-Drash).
	11p15 imprinting	"WT2" locus	3%	– Imprinting in Wilms tumors associated with Beckwith-Wiedemann syndrome.
Congenital mesoblastic nephroma, cellular type	t(12;15)	*ETV6(TEL)-NTRK3*	90%	– Same translocation as infantile fibrosarcoma and secretory CAs of the breast and salivary gland.
Malignant rhabdoid tumor of the kidney	22q11.2 deletion or mutation	*SMARCB1* (INI1) loss of function	>70%	– Same gene mutated in AT/RT. – ***Loss of INI1 expression by IHC. Loss of SMARCA4* (BRG1) *in a minority of INI1-proficient MRTs.***
Clear cell sarcoma of kidney	t(10;17)	*YWHAE-NUTM2 (FAM22)*	~10%	– *YWHAE* translocation and *BCOR* ITD are mutually exclusive. Both abnormalities have also been identified in a subset of endometrial stromal sarcomas [46].
	n/a	*BCOR* internal tandem duplication (ITD)	80%	
Adrenal tumors				
Neuroblastoma	–1p or deletion 1p32–36	Gene unknown	30%	– Bad prognosis
	Double minutes	*MYCN* amplification	30%	– Bad prognosis
	+17q		50%	– Bad prognosis
	Hyperdiploidy		40%	– Good prognosis
	Molecular alterations in high-risk neuroblastomas include *MYCN* amplification, mutations of *ALK* and *ATRX*, and rearrangements involving *TERT*. References: [47, 48]			

Other Solid Tumors with Recurrent Mutations or Fusions – Highly Selected *By Jason C. Chang & Natasha Rekhtman*			
Tumor type	*Alteration*	*Prevalence*	*Comments*
Lung adenocarcinoma (selected alterations)	*EGFR* mutations	15% (West) 30% (Asia)	– Clinicopathologic associations: younger age, never-smokers, women, Asian ethnicity, lepidic/papillary histology (but any histology can have *EGFR* mutations and should be tested). – Targeted therapies: First gen-gefitinib, erlotinib; second gen-afatinib; third gen-osimertinib.
	ALK rearrangement	5%	– Clinicopathologic associations: younger age, never-smokers, signet ring cells and cribriform/ mucinous histology. **ALK IHC is a companion diagnostic**, and treatment can be initiated based on positive IHC result. – Targeted therapies: First gen-crizotinib; second gen-alectinib, ceritinib.
	ROS1 rearrangement	1%	– Similar patient and histologic features as ALK. **IHC for ROS1 is sensitive but not entirely specific** – all positives require a confirmation by FISH or molecular assays. – Targeted therapies: Crizotinib.
	KRAS mutations	30% (West) 10% (Asia)	– Most common alteration in lung adenoCA (in Western patients). Not targetable but is mutually exclusive with other driver alterations.
Lung, non-small cell CA (AdenoCA + SqCC)	*BRAF* V600E mutation	1%	– Recent approval of targeted therapies (dabrafenib + trametinib).
	MET exon 14 skipping mutations	3–5%	– Associated with sarcomatoid histology. – Targeted therapies: Crizotinib.
Colorectal adenocarcinoma (CRC)	*BRAF* V600E mutation	8–15%	– Predicts resistance to EGFR TKIs. – Associated with microsatellite instability due to hypermethylation of *MLH1* gene promoter.
	KRAS, *NRAS*, *HRAS* mutations	40–50%	– Predicts resistance to EGFR TKIs.
	APC (adenomatous polyposis coli) inactivation	~80% (of sporadic CRC)	– Germline mutation in *APC* → Familial adenomatous polyposis (FAP) syndrome (see Tumor Syndromes section). – Somatic inactivation of *APC* through truncating mutation or loss of heterozygosity (LOH). – APC inhibits oncogene β-catenin. Mutation of APC → ***nuclear shift of β-catenin (detected by IHC).***
Melanoma, skin, and mucosal	*BRAF* V600E mutation	40–50%	– Predominantly found in cutaneous melanomas. – Targeted therapies: Vemurafenib, dabrafenib.
	KIT mutations	5–20%	– More common in acral and mucosal sites [49]. – Unlike in GISTs, most *KIT* alterations are deletions or insertions. – Targeted therapies: Imatinib (Gleevec), sunitinib, dasatinib.
Melanoma, ocular	*GNAQ or GNA11 mutations*	80%	– Characteristic of uveal melanomas but rarely seen in mucosal melanomas. – No targeted therapy yet.
GIST	*KIT* mutations	80–85%	– Targeted therapies: Imatinib (Gleevec), sunitinib.
	PDGFRA mutations	5%	– Targeted therapies: Imatinib (Gleevec), sunitinib.
	SDH gene (A, B, C, and D) mutations	7%	– SDH-deficient GISTs (primarily mutations in *SDHB*) – imatinib (Gleevec) insensitive.
NUT carcinoma	t(15;19) *BRD4-NUT*	67%	– Invariably lethal CA arising adjacent to respiratory tract in children and young adults. ***NUT IHC is very sensitive and specific*** [50].
	t(9;15) *BRD3-NUT*	33%	
Prostate cancer	21q22.2–3 deletion *TMPRSS2-ERG*	50%	– Specific for prostate cancer. Associated with low Gleason scores [51]. – ***ERG IHC helpful for confirming prostate origin, but only helpful in 50% of cases*** (ERG is also a vascular marker) [52].
Breast, secretory carcinoma	t(12;15) *ETV6-NTRK3*	>90%	– Same translocation as infantile fibrosarcoma, congenital mesoblastic nephroma, and salivary secretory carcinoma. – Targeted therapy: larotrectinib.
Fibrolamellar hepatocellular carcinoma	Intrachromosomal deletion on chromosome 19p13.12 *DNAJB1-PRKACA*	>90%	
Endometrial stromal sarcoma	t(7;17) *JAZF1-SUZ12 (JJAZ1)*	50%	
	6p21 (*PHF1*) translocations	Uncommon	– Many variants: *JAZF1-PHF1*, *MEAF6-PHF1*, *EPC1/PHF1*.
	t(10;17) *YWHAE-NUTM2 (FAM22)*	Uncommon	– Typically high-grade morphology; ***BCOR IHC is a sensitive marker*** (for all three alterations) [53, 54, 55].
	t(X;22) *ZC3H7B-BCOR*	Uncommon	
	BCOR ITD	Uncommon	

Abbreviations: *ITD* internal tandem duplication, *TKI* tyrosine kinase inhibitors

Chapter 11. Tumor Genetics and Cytogenetics: Hematopoietic System

By Zenggang Pan, Xiaojun Wu & Caleb Ho (prior edition by Amy Duffield)

B-Cell Leukemia/Lymphoma					
Tumor type	*Chromosomal abnormality*	*Gene/protein*	*Prevalence*	*Prognosis (if relevant)*	*Comment*
Follicular lymphoma (FL)	t(14;18)(q32;q21)	*IGH-BCL2*	~90%		t(14;18) also present in some DLBCLs. Typically negative in pediatric FL, primary cutaneous follicle center lymphoma, and Burkitt lymphoma. ***BCL2 can be detected by IHC***
	3q27 translocation	*BCL6* translocation	5–15%		
	1p36 alteration	*TNFRSF14* alterations	Unclear		*TNFRSF14* alterations associated with pediatric FL and FL with diffuse growth pattern
Mantle cell lymphoma	t(11;14)(q13;q32)	*CCND1-IGH*	~100%		*CCND1* = cyclin D1 or BCL1. ***Cyclin D1 can be detected by IHC.*** Rare cases with *CCND2* translocation (cyclin D1 IHC negative but SOX11 positive)
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT)	t(11;18)(q21;q21)	*API2-MALT1*	30–40%		The lung and stomach; resistant to antibiotic therapy in the stomach
	t(14;18)(q32;q21)	*IGH-MALT1*	16–25%		Ocular adnexa/orbit and salivary gland
	t(3;14)(p14.1;q32)	*FOXP1-IGH*	10–50%		Thyroid, ocular adnexa/orbit, and the skin
	trisomy 3				Also seen in ~20% of splenic MZL
Splenic marginal zone lymphoma	7q deletion		~40% (−7q)		Not seen in extranodal or nodal MZL
	3q gain		10–20%		
	t(2;7)(p12;q21)	*IGH-CDK6*	Rare		
Chronic lymphocytic leukemia/small lymphocytic lymphoma	del 13q14.3	microRNA genes	50%	Good	Additional prognostic markers: Good prognosis: mutated IgV_H; CD38-; ZAP70– Poor prognosis: unmutated IgV_H; CD38+; ZAP70+
	trisomy 12		20%	Fair	
	del 11q22–23	*ATM* and *BIRC3*	20%	Poor	
	del 17p13	*TP53*	5–10%	Poor	
	del 6q		Rare	Poor	
Burkitt lymphoma (BL)	t(8;14)(q24;q32)	*MYC-IGH*	85%		Concurrent *BCL2* and *BCL6* translocations should not be present ***MYC can be detected by IHC***
	t(8;22)(q24;q11)	*MYC-IGL*	Rare		
	t(2;8)(p12;q24)	*MYC-IGK*	Rare		
Burkitt-like lymphoma with 11q aberration	Proximal gain and telomeric loss of 11q		Rare in B cell lymphomas		<40 years old, usual morphologic and clinical features of BL. Complex karyotype, aberrancy in 11q, and lack of *MYC* rearrangement
Diffuse large B-cell lymphoma (DLBCL), NOS	t(14;18)(q32;q21)	*IGH-BCL2*	20–30%		
	3q27 translocation	*BCL6* translocation	Up to 30%		
	8q24 translocation	*MYC* translocation	8–14%		
Large B-cell lymphoma with *IRF4* rearrangement	Rearrangement of 6p25.3	*IRF4-IGH* (most cases)	Rare	Good	More in children and young adults. Predominantly involves Waldeyer ring or head and neck lymph nodes
High-grade B-cell lymphoma (HGBCL) with *MYC* and *BCL2* and/or *BCL6* rearrangements	Rearrangement of 8q24 and 18q21 and/or 3q27	*MYC* rearrangement and *BCL2* rearrangement and/or *BCL6* rearrangement	Uncommon in B cell lymphomas	Very poor	Diagnosis requires presence of *MYC* and *BCL2* and/or *BCL6* rearrangements. Cases with morphologic features of DLBCL or BL, but with the above rearrangements, are classified into this category ***BCL2 and MYC can be detected by IHC***
B-ALL/lymphoblastic lymphoma	t(9;22)(q34.1;q11.2)	*BCR-ABL* (Ph), mostly p190 fusion	25% adults, 2–4% peds	Poor	Ph chromosome also seen in CML (mostly p210 fusion). Can respond to tyrosine kinase inhibitors
	t(v;11q23.3)	*KMT2A (MLL)*–rearranged	Most common in infants and adults	Poor	The *KMT2A* locus is most commonly rearranged with *AF4* and can also rearrange with *AF9* t(9;11). Blasts are often CD19+/CD10-/CD15+/CD24- *KMT2A* rearrangements are also seen in AML
	t(5;14)(q31.1;q32.1)	*IGH-IL3*	<1% in children and adults	Unknown (too rare)	Reactive peripheral eosinophilia. Typical lymphoblast morphology, usually CD19+ and CD10+
	t(1;19)(q23;p13.3)	*TCF3-PBX1*	6% in children	Good*	Blasts often CD19+/CD10+/CD9+/CD34- and cytoplasmic mu heavy chain+
	Hypodiploidy (<46 chromosomes)		1–5%	Poor	No unique morphology or immunophenotype. Most treatment protocols require ≤43 chromosomes
	t(12;21)(p13.2;q22.1)	*TEL(ETV6)-AML1(RUNX1)*	25% in children	Good	Blasts are often CD19+/CD10+/CD34+/CD9-/CD20-
	Hyperdiploidy (>50 but <66 chromosomes)		25% in children	Good	No unique morphology or immunophenotype
	BCL-ABL1-like	Rearrangement of non-*BCR-ABL* tyrosine kinase, *CRLF2*, etc.	10–25%, mostly adults	Poor	Various tyrosine kinase-type chromosomal rearrangements such as *CRLF2* rearrangements. Can respond to tyrosine kinase inhibitors
	iAMP21	Amplification of a portion of chr 21	2%, mostly children	Good*	No unique morphology or immunophenotype Can be detected by a probe for *RUNX1*
Plasma cell myeloma	t(11;14)	*CCND1-IGH*	16%	Standard	Usually shows complex karyotypes (multiple chromosome gains and losses). Myeloma with t(11;14) may show lymphoid appearance and CD20 expression

* The poor prognosis formerly seen in B-ALL with t(1;19) and B-ALL with iAMP21 has now been overcome with modern intensive therapy.

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T-Cell Leukemia/Lymphoma					
Tumor type	*Chromosomal abnormality*	*Gene/protein*	*Prevalence*	*Prognosis*	*Comment*
Anaplastic large cell lymphoma (ALCL), ALK-positive	t(2;5)(p23;q35)	*NPM-ALK*	85%	Good	*ALK* translocations are also present in IMT and lung adenocarcinomas. Primary cutaneous ALCL – no *ALK* translocation. ***ALK can be detected by IHC***
	Other 2p23 translocations	Other (most frequently *TPM3)-ALK*	15%		
Anaplastic large cell lymphoma (ALCL), ALK-negative	Rearrangement of 6q25.3	*DUSP22* rearrangement	30% of ALK(-) ALCL	Good	More commonly to have pan-T-cell markers (CD2, CD3), less likely to have cytotoxic marker expression. Can be detected by FISH probes for *DUSP22-IRF4*
	Rearrangement of 3q28	*TP63* rearrangement	8% of ALK(-) ALCL	Poor	***p63 can be detected by IHC***
T-ALL/LBL (various translocations involving *TCR* – chr 7 and chr 14) Activating mutation of *NOTCH1* (50%)	t(1;14)	*ΔTAL1* or *TAL1-TCRα/δ*	20–30%		
	t(8;14)	*MYC-TCRα/δ*	6%		
	t(10;14)	*HOX11 (TLX1)-TCRα/δ*	7–30%		
	t(5;14)	*HOX11L2(TLX3)-TCRα/δ*	10–20%		
	del(9p)	loss of *CDKN2A*	30%		
Hepatosplenic T-cell lymphoma	Isochromosome 7q		50–80%		
T-cell prolymphocytic leukemia	inv(14)(q11q32)	*TRA/TRD-TCL1A/B*	80%		Abnormalities of chromosome 14. ***TCL-1 can be detected by IHC***
	t(14;14)(q11;q32)	*TRA/TRD-TCL1A/B*	10%		
	t(X;14)(q28;q11)	*MTCP1-TRA/TRD*	Uncommon		

Selected Lymphoid Neoplasms where Gene Mutations are of Important Diagnostic Value			
Tumor type	*Mutation*	*Prevalence*	*Comment*
Lymphoplasmacytic lymphoma	*MYD88* L265P	>90%	Associated with improved overall survival than cases with wild-type *MYD88*
	CXCR4 mutations	30%	Associated with more symptomatic and active disease
	Del 6q21–22	40–60%	Associated with an adverse prognosis
Hairy cell leukemia	*BRAF* V600E	~100%	Vemurafenib, an inhibitor of BRAF, can be used to treat HCL cases that are resistant to other therapies. ***Mutated BRAF V600E can be detected by IHC with VE1 antibody***
T-large granular lymphocytic leukemia	*STAT3* mutation	Up to 50%	Associated with a greater need for treatment, methotrexate responsiveness, and pure red cell aplasia
	STAT5b mutation	<5%	Associated with more aggressive clinical course
Angioimmunoblastic T-cell lymphoma/PTCL with TFH phenotype	*IDH/RHOA/DNMT3A/ TET2* mutations	20–80%	

Myeloid Leukemia and Myelodysplastic Syndrome						
Chronic myeloid leukemia (CML), *BCR-ABL1* positive		t(9;22)(q34;q11)	*BCR-ABL* (Philadelphia chromosome, Ph); *ABL* (chr 9) is the tyrosine kinase	100%	Very good	p210 fusion is most common. Variant splice forms, p230 and p190, are present in a small subset of CML. p190 is also seen in B-ALL/LBL. Targeted therapy with imatinib (Gleevec) and other tyrosine kinase inhibitors
Myeloid and lymphoid neoplasms with eosinophilia and *PDGFRA* rearrangement		Cryptic del(4)(q12) and other translocations involving 4q12	*FIP1L1-PDGFRA* and other translocations involving *PDGFRA*		Improved with TKI	Present in MPN, AML or T-ALL/LBL with eosinophilia. Targeted therapy with TKI, *e.g.*, Gleevec
Myeloid and lymphoid neoplasms with eosinophilia and *PDGFRB* rearrangement		t(5;12)(q32;p13.2) and other translocations involving 5q32	*ETV6(TEL)-PDGFRB* and other translocations involving *PDGFRB*		Improved with TKI	Clinical picture is more often CMML with eosinophilia, less often similar to typical CML. Targeted therapy with Gleevec
Myeloid and lymphoid neoplasms with *FGFR1* rearrangement		Rearrangement of 8p11.2	*FGFR1* with various partners		Poor	May have T- or B-ALL/LBL associated with prominent tissue eosinophilia
Myeloid and lymphoid neoplasms with *PCM1-JAK2*		t(8;9)(p22;p24.1)	*PCM1-JAK2*	Rare	Variable	Provisional WHO entity. Variant translocations involving *JAK2* and other genes, *e.g.*, *ETV6* and *BCR*
AML with *RUNX1-RUNX1T1* (subset of former FAB M2)		t(8;21)(q22;q22.1)	*AML1(RUNX1)-ETO (RUNX1T1)* (**e**ight **t**wenty **o**ne)	~5% of AML	Good	May present as granulocytic sarcoma
AML with *PML-RARA*/acute promyelocytic leukemia (formerly FAB M3)		t(15;17)(q24.1;q21.2) and rare variant translocations	*PML-RARA* and variant translocations of *RARA* with *NPM1*, *ZBTB16, STAT5B*, and others	5–8% of AML	Very good	t(15;17) responds to all-trans retinoic acid (ATRA). Some variant translocations are ATRA-sensitive (*NPM1*) but some (*ZBTB16*, *STAT5B)* are ATRA-resistant
AML with *CBFB-MYH11* (formerly FAB M4 $_{Eo}$)		inv(16)(p13.1q22) or t(16;16)(p13.1;q22)	*CBFB-MYH11*	5–8% of AML	Good	Associated with increased abnormal eosinophils and granulocytic sarcoma
AML with *KMT2A-MLLT3* (subset of former FAB M4, M5)		t(9;11)(p21.3;q23.3)	*KMT2A* (*MLL*)-*MLLT3*	2% adults, 9–12% peds	Poor	Commonly blasts with monocytic/monoblastic differentiation *KMT2A* translocation also seen in AML s/p topoisomerase II inhibitors (t-AML) and infants with B-ALL/LBL
AML with *RBM15-MKL1* (formerly FAB M7)		t(1;22)(p13.3;q13.1)	*RBM15-MKL1*	<1%	Fair	Commonly megakaryoblastic phenotype Most commonly seen in infants and children, not associated with Down syndrome
AML with *DEK-NUP214*		t(6;9)(p23;q34.1)	*DEK-NUP214*	1–2%	Poor	Associated with monocytic features, basophilia, and multilineage dysplasia
AML with *GATA2, MECOM*		inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)	*GATA2, MECOM*	1–2%	Very poor	Normal or elevated platelet counts, 7q deletion, CD7 expression, and small mono- or binucleated megakaryocytes
Therapy-related AML	Alkylating agents (cyclophosphamide) and radiation therapy	Same as MDS: −7, del(7q), −5, and del(5q), among others		70–80%	Poor	5–10-year lag: presents with treatment-related MDS (marrow failure and cytopenias). Definition is currently based on history of therapy exposure rather than cytogenetic changes or gene mutations
	Topoisomerase II inhibitors (doxorubicin)	11q23.3 translocations	e.g., *KMT2A (MLL)* translocation	20–30%	Poor	1–5-year lag: presents as overt acute leukemia with no MDS phase. Definition is currently based on history of therapy exposure rather than cytogenetic changes or gene mutations
Myelodysplastic syndrome (MDS)		Isolated del(5q) ("5q- syndrome")	bands q31-q33 are invariably deleted	10% overall	Good	Middle-aged women, severe macrocytic anemia, increased platelets, monolobated micromegakaryocytes May have one other cytogenetic abnormality [aside from −7, del(7q)]
		Isolated del(20q)		5–8%	Good	Involvement of erythroid and megakaryocytic lineages
		−7, del(7q), −5, del(5q)			Poor	The gene(s) deleted in del(5q) are thought to be distinct from gene(s) deleted in "isolated 5q- syndrome." Often seen as part of a complex karyotype
		del(17p)	*TP53* deletion		Poor	MDS and AML with pseudo-Pelger-Huet cells and small vacuolated PMNs associated with therapy-related MDS
		None	*SF3B1* mutation	15–32%	Good	Associated with MDS with ring sideroblasts (MDS-RS) (>80% cases have *SF3B1* mutation)

Selected Myeloid Neoplasms where Gene Mutations are of Important Diagnostic Value			
Tumor type	*Mutation*	*Prevalence*	*Comment*
AML with gene mutations	*FLT3* internal tandem duplications or point mutations in tyrosine kinase domain	1/3 of AMLs with normal karyotype	Not a distinct subtype of AML. Associated with an adverse prognosis in cytogenetically normal AML.
	NPM1 mutation	27–35% adults, 2–8% peds	Good prognosis in absence of *FLT3*-ITD; blasts can show cuplike nuclei. OK for marrow to have multilineage dysplasia
	Biallelic *CEBPA* mutation	4–9% of children and young adults	Biallelic mutations with improved prognosis; single allelic mutation with no prognostic significance. OK for marrow to have multilineage dysplasia
	RUNX1 mutation	4–16%	Provisional WHO entity. Higher resistant disease rate and possible genetic predisposition
MPN and **MDS/MPN**	*JAK2 V617F*		Polycythemia vera (95–97%) Primary myelofibrosis (55–60%) Essential thrombocythemia (50–60%) MDS/MPN-RS-T (50%)
	JAK2 exon 12 mutation		Polycythemia vera (2–3%)
	CALR exon 9 mutations		Pathogenic mutations are usually frameshift insertions/deletions (indels). In-frame indel can be germline polymorphism. Polycythemia vera (rare)
			Primary myelofibrosis (25%) Essential thrombocythemia (25%)
	MPL exon 10 mutations		Polycythemia vera (rare) Primary myelofibrosis (5–10%) Essential thrombocythemia (3–5%)
	SF3B1 mutation		MDS/MPN-RS-T (66.7%–86.5%). Also seen in MDS with ring sideroblasts
	CSF3R mutation		Chronic neutrophilic leukemia (80%)
	ASXL1 (35%–40%), *TET2* (50%–60%), *SRSF2* (40%–50%), *RUNX1* (15%), *NRAS* (11%), and *CBL* (10%)		Most cases of chronic myelomonocytic leukemia (CMML) have mutation in at least one of the following: *TET2*, *SRSF2*, *SETBP1*, and *ASXL1*. The combination of *ASXL1*, *TET2*, and *SRSF2* is highly suggestive of this diagnosis
	SETBP1 and *ETNK1* mutations		Atypical chronic myeloid leukemia (aCML), *BCR-ABL1*-negative
Mastocytosis	*KIT* point mutations (often p. D816V)	Up to 90%	Common in systemic mastocytosis. Less common in isolated cutaneous mastocytosis *KIT* D816V is resistant to imatinib therapy

Abbreviations: *ALCL* anaplastic large cell lymphoma, *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *CML* chronic myeloid leukemia, *CMML* chronic myelomonocytic leukemia, *FAB* French-American-British, *MDS* myelodysplastic syndrome, *MDS/MPN-RS-T* myelodysplastic syndrome/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis, *MPN* myeloproliferative neoplasm, *MDS-RS* myelodysplastic syndrome with ring sideroblasts, *MZL* marginal zone lymphoma, *TKI* tyrosine kinase inhibitor

Reference [1]

Chapter 12. Tumor Syndromes

By Marina K Baine, Justin A. Bishop, Natasha Rekhtman
(All subsubsections are by these authors, unless specified otherwise)

Where Is This Syndrome Listed?		
Syndromes (alphabetical)	**Table where listed**	**Page**
Ataxia-telangiectasia	Other	139
BAP1 hereditary cancer predisposition syndrome	Other	139
Beckwith-Wiedemann	Syndromes associated with renal neoplasms	136
Birt-Hogg-Dube	Syndromes associated with renal neoplasms	136
Bloom syndrome	Other	139
Carney complex or carney syndrome	Other	139
Carney triad	Syndromes associated with skin tumors	139
Carney-Stratakis syndrome	Syndromes associated with skin tumors	139
Cowden disease (multiple hamartoma syndrome)	Syndromes associated with breast Cancer	138
Cronkhite-Canada	Syndromes associated with GI polyps and neoplasms	138
Denys-Drash	Syndromes associated with renal neoplasms	136
DICER1 syndrome	Other	139
Familial adenomatous polyposis (FAP)	Syndromes associated with GI polyps and neoplasms	137
Familial atypical multiple mole melanoma syndrome (FAMMM or B-K mole syndrome)	Syndromes associated with skin tumors	138
Fanconi anemia	Other	139
Gardner (FAP variant)	Syndromes associated with GI polyps and neoplasms	137
Gorlin syndrome (nevoid basal-cell carcinoma syndrome, NBCCS)	Syndromes associated with breast Cancer	138
Hereditary breast and ovarian cancer	Syndromes associated with breast Cancer	138
Hereditary diffuse gastric cancer syndrome	Syndromes associated with GI polyps and neoplasms	138
Hereditary leiomyomatosis and renal cell carcinoma (HLRCC)	Syndromes associated with renal neoplasms	136
Hereditary nonpolyposis colorectal cancer (Lynch syndrome/HNPCC)	Syndromes associated with GI polyps and neoplasms	137
Hereditary papillary renal cell cancer	Syndromes associated with renal neoplasms	136
Hyperparathyroidism-jaw tumor syndrome (HPT-JT)	Syndromes associated with skin tumors	139
Juvenile polyposis (JP)	Syndromes associated with GI polyps and neoplasms	138
Li-Fraumeni	Other	139
Maffucci syndrome	Syndromes associated with tumors of bone	136
Mahvash disease (aka PanNET syndrome or glucagon cell hyperplasia and neoplasia)	Syndromes associated with skin tumors	139
Mazabraud syndrome	Syndromes associated with tumors of bone	136
McCune-Albright syndrome	Syndromes associated with tumors of bone	136
MEN1	Multiple endocrine neoplasia (MEN) syndromes	135
MEN2A	Multiple endocrine neoplasia (MEN) syndromes	135
MEN2B	Multiple endocrine neoplasia (MEN) syndromes	135
MEN4	Multiple endocrine neoplasia (MEN) syndromes	135
Muir-Torre (Lynch/HNPCC variant)	Syndromes associated with GI polyps and neoplasms	137
MYH-associated polyposis (MAP)	Syndromes associated with GI polyps and neoplasms	137
Neurofibromatosis type 1 (NF1)	Neurocutaneous syndromes	135
Neurofibromatosis type 2 (NF2)	Neurocutaneous syndromes	135
Ollier disease	Syndromes associated with tumors of bone	136
Peutz-Jeghers syndrome (PJS)	Syndromes associated with GI polyps and neoplasms	138
Rendu-Osler-Weber syndrome or hereditary hemorrhagic telangiectasia	Other	139
Retinoblastoma	Other	139
Rhabdoid tumor predisposition syndrome	Other	139
Ruvalcaba-Myhre-Smith (Bannayan-Riley-Ruvalcaba)	Syndromes associated with GI polyps and neoplasms	138
SDH-related pheochromocytoma/paraganglioma syndromes	Syndromes associated with skin tumors	139
Sturge-Weber	Neurocutaneous syndromes	135
Succinate dehydrogenase (SDH)-deficient renal cell carcinoma	Syndromes associated with renal neoplasms	136
Tuberous sclerosis	Neurocutaneous syndromes	135
Turcot (either FAP variant or Lynch/HNPCC variant)	Syndromes associated with GI polyps and neoplasms	137
Von Hippel-Lindau (VHL)	Syndromes associated with renal neoplasms	136
WAGR	Syndromes associated with renal neoplasms	136

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Tumor Syndromes: Introduction

Quick Overview of Tumor Syndromes

By Natasha Rekhtman

A general rule of thumb for inherited tumor syndromes is that virtually all inherited mutations are *inactivating* mutations in *tumor suppressor genes*. Notice in the tables below that nearly all genes involved in inherited tumor syndromes are tumor suppressors (*TP53*, *RB1*, *APC*, *VHL*). The second allele is inactivated somatically later in life, which serves as a trigger for tumorigenesis. This follows a famous "two-hit model of oncogenesis," for which retinoblastoma serves as a paradigm. Since only one mutant allele needs to be inherited for disease to develop, the mode of inheritance is *autosomal dominant*. A possible explanation for this principle is that if dominant mutations were to manifest in utero (as would occur with recessive inheritance of two mutated tumor suppressors or with inheritance of activating mutations of oncogenes), they would be lethal.

Note an interesting contrast of inherited tumor syndromes with sporadic tumors. Sporadic tumors may be associated either with inactivation of tumor suppressors (*TP53*) or activation of oncogenes (*RAS*, *MYC*, *KIT*, *EGFR*), whereas the latter molecules are not involved in inherited tumor syndromes. A notable exception to this rule is MEN2 syndrome, which is caused by inheritance of activating mutations in the *RET* oncogene.

Other notable exceptions to the rule of autosomal dominant inheritance of mutations in tumor suppressors are syndromes caused by inherited defects in DNA repair (ataxia-telangiectasia, Bloom syndrome, MYH-associated polyposis, xeroderma pigmentosa, and Fanconi anemia) – these have an autosomal recessive mode of inheritance. An exception to this exception is Lynch syndrome (HNPCC) – inherited syndrome due to mutation of DNA mismatch repair genes, which has autosomal dominant transmission.

General Principles and Clues to Hereditary Tumor Syndromes
By Marina K Baine

Clinicopathologic features	*Examples*	*Notes*
Early age of onset	Young age at diagnosis of carcinoma of any organ	Specific, but not sensitive
Multifocal disease (synchronous or metachronous)		Specific, but not sensitive ≥2 histogenetically and phenotypically distinctive tumors are defining or at least suspicious for hereditary etiology Must distinguish from metastatic disease or therapy-induced secondary malignancy: • Histologic features of primary disease (e.g., involvement of muscle and/or autonomic nerves by primary GIST) • Location relative to prior treatment (of particular utility with prior radiation)
• **Many of the same thing**	• Multiple neurofibromas in NF1, CRCs in FAP	
• **Multiple of different things**	• CRC + EMC = HNPCC; lobular breast CA + diffuse gastric CA = hereditary gastric cancer syndrome (*CDH1* mutation)	
Concurrent precursor lesions	• Oligo- or unicryptic TAs in FAP • C-cell hyperplasia in MEN2 • Diffuse Cajal cell hyperplasia in familial GIST syndromes	Single-allele errors (haploinsufficiency) leading to microscopic proliferations
Unusual host or site for tumor type		Variable sensitivity and specificity
• **Age**	• CRC diagnosis at age < 50 → think HNPCC or FAP	
• **Site**	• Visceral neurofibroma → think NF1 vs. cutaneous neurofibromas → think sporadic	
Genotype-specific histology – Tumor type or specific histologic features within a tumor type linked to a syndrome: (few examples, see "slide to syndrome" for detailed list)	• Plexiform neurofibroma → NF1 • PTC, cribriform-morular variant → APC • Epithelioid gastric GIST, multinodular → germline *SDH* mutation • Eosinophilic RCC with flocculent cytoplasm → germline *SDH* mutation • Papillary RCC with "viral-like inclusion" nuclei and loss of FH by IHC = HLRCC-RCC → germline *FH* mutation	In more frequent hereditary cancers the features are *suggestive* but <u>not</u> specific (e.g., triple negative breast carcinomas with medullary features in BRCA1)

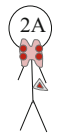
Abbreviations: *APC* adenomatous polyposis coli, *CRC* colorectal carcinoma, *EMC* endometrial carcinoma, *FAP* familial adenomatous polyposis, *FH* fumarate hydratase, *GIST* gastrointestinal stromal tumor, *HLRCC-RCC* hereditary leiomyomatosis and renal cell carcinoma-associated renal cell carcinoma, *HNPCC* hereditary nonpolyposis colorectal cancer, *NF1* neurofibromatosis type 1, *MEN2* multiple endocrine neoplasia type 2, *PTC* papillary thyroid carcinoma, *RCC* renal cell carcinoma, *SDH* succinate dehydrogenase, *TA* tubular adenoma

Reference: [1]

At-a-Glance Summaries of Tumor Syndromes

Includes inherited tumor syndromes, tumors associated with germline mutations and some apparently non-familiar tumor syndromes (for somatic alterations in tumors see Chapter 10)

For complete list, see http://AtlasGeneticsOncology.org

Multiple Endocrine Neoplasia (MEN) Syndromes					
Syndrome (other names)	*Inheritance*	*Gene [Protein]*	*Chromosome*	*Pathology and key clinical features*	*Diagram*
MEN1 (Wermer syndrome)	AD	*MEN1* [menin]	11q13	Pituitary adenoma or hyperplasia (~2/3) Parathyroid hyperplasia (90%) Pancreatic NETs (~2/3), duodenal gastrin-producing NETs [both are a cause of hypergastrinemia/Zollinger-Ellison syndrome]	
MEN2A (Sipple syndrome)	AD	*RET*	10q11	Medullary thyroid carcinoma (100%) and C-cell hyperplasia Parathyroid hyperplasia (50%) Pheochromocytoma (50%)	
MEN2B (Gorlin syndrome – Not to be confused with nevoid basal-cell carcinoma syndrome, also bearing Gorlin's eponym)	AD	*RET*	10q11	Medullary thyroid carcinoma (85%) and C-cell hyperplasia Pheochromocytoma (50%) Diffuse ganglioneuromatosis of the GI tract (typically colon) (100%) Marfanoid body habitus	
MEN4	AD	*CDKN1B* (p27, aka $p27^{Kip1}$)	12p13	Similar phenotype to MEN1 but no *MEN1* mutation: NE hyperplasia/tumors in the parathyroid, pituitary, and pancreas (most common) NE tumors of other sites (cervix, bronchus, stomach, etc.)	

Neurocutaneous Syndromes				
Neurofibromatosis type 1 (von Recklinghausen disease or peripheral neurofibromatosis)	AD or sporadic	*NF1* [neurofibromin] (p21/ras pathway)	17q11.2	Multiple **neurofibromas** (NF): Plexiform NF – Nearly pathognomonic for NF1; diffuse NF in 10%, MPNST in 10% **Optic nerve gliomas** (pilocytic astrocytoma) **Other tumors**: Ampullary somatostatinoma, duodenal gangliocytic paraganglioma, GIST (5–25%), pheochromocytoma, juvenile xanthogranuloma, other **Non-tumor:** Cafe au lait spots, Lisch nodules (pigmented iris hamartomas), skeletal lesions (spinal deformities and bone cysts)
Neurofibromatosis type 2 (central or acoustic neurofibromatosis)	AD or sporadic	*NF2* [merlin] (cytoskeletal defect)	22q12	**Bilateral acoustic schwannomas** **Meningiomas** (may be multiple) Spinal cord **ependymomas** Cafe au lait spots, no Lisch nodules
Tuberous sclerosis (Bourneville disease)	AD	*TSC1* [hamartin] *TSC2* [tuberin]	9p34 16p13	**PEComas** (perivascular epithelioid cell tumors): Renal angiomyolipoma, pulmonary lymphangioleiomyomatosis (LAM)* and sugar tumor, other **CNS:** Cortical tubers, subependymal giant cell astrocytoma (SEGA), white matter heterotopias **Cardiac rhabdomyoma** **Skin:** Angiofibroma (aka adenoma sebaceum), periungual fibroma, connective tissue nevi (*peau chagrin* or shagreen patches), hypopigmented (ash leaf) patches **Eosinophilic solid and cystic RCC** *LAM occurs in >30% of patients, virtually always women
Sturge-Weber (fourth phacomatosis, encephalotrigeminal angiomatosis)	Not familial	Unknown		**Port-wine stain/nevus flammeus** (dilated vessels) in the distribution of trigeminal nerve Angiomatosis of the ipsilateral leptomeninges **Pheochromocytoma**

Syndromes Associated with Renal Neoplasms				
Syndrome (other names)	*Inheritance*	*Gene Protein*	*Chromosome*	*Pathology and main clinical features*
Von Hippel-Lindau	AD	*VHL* [pVHL] (role in ubiquitination)	3p25	**RCC**, clear cell (multiple bilateral) **Cysts** of kidney, pancreas, and liver **Hemangioblastomas:** Cerebellum (Lindau tumor), spinal cord, retinal (von Hippel tumor) **Pheochromocytomas** (clear cell change, CAIX, and tyrosine hydroxylase +) Pancreatic neuroendocrine tumor/islet cell tumor (clear cell variant, CAIX+) Papillary cystadenoma of epididymis and broad ligament Endolymphatic sac tumor of the ear (Heffner tumor)
Birt-Hogg-Dube	AD	*BHD* [folliculin]	17p11.2	**Renal tumors:** Multiple renal cell carcinomas of various types (clear cell, chromophobe, papillary); oncocytomas; **hybrid oncocytic tumors** (**chromophobe/oncocytoma**) – The latter highly specific for this syndrome **Skin:** Facial fibrofolliculomas and skin tags **Lung:** Cysts/spontaneous pneumothorax
Beckwith-Wiedemann	Sporadic or AD (15%)	Duplication of paternal allele	11p15	"**Overgrowth** syndrome:" organomegaly, macroglossia Increased childhood **neoplasia**: Wilms tumor (<5%), hepatoblastoma, pancreatoblastoma, neuroblastoma
WAGR	Not familial	Deletion of *WT1* gene	11p13	Wilms tumor (>30%), aniridia, genitourinary abnormalities, mental retardation
Denys-Drash	Not familial	*WT1* point mutation	11p13	Wilms tumor (>90%), gonadoblastoma, diffuse mesangial sclerosis
Hereditary papillary renal cell cancer (PRCC)	AD	*MET* (acts via hepatocyte growth factor)	7q34	Multiple bilateral pRCC (type 1)
Hereditary leiomyomatosis and renal cell carcinoma (HLRCC)	AD	*Fumarate hydratase*	1q42–43	pRCC (type 2) and leiomyomas (cutaneous and uterine) with distinct cytologic features (see "slide to syndrome")
Succinate dehydrogenase-deficient renal cell carcinoma (SDH-RCC)	AD	*SDHB* *SDHC* *SDHD* (rare) *SDHA* (rare)	1p15.33 1q23.3 11q23.1 5p15.33	Solid, tubular, or nested **RCC** with distinctive histologic features (see "slide to syndrome") 30% of patients have other SDH-deficient tumors (pheochromocytoma/paraganglioma and GIST and rarely pituitary adenoma, as part of Carney-Stratakis syndrome; see below) Mutations of *SDH* genes most commonly involve the *SDHB* gene (and less commonly *SDHA*, *SDHC*, or *SDHD*). **SDHB is lost by IHC in all cases.**
Also see BAP1 hereditary cancer predisposition syndrome below (associated with ccRCC)				References: [2, 3]

Syndromes Associated with Tumors of Bone				
McCune-Albright syndrome	Not familial	Mosaicism for a mutation in the *GNAS1* gene	20q13	Bone: **Fibrous dysplasia** (polyostotic) Skin: Cafe au lait spots Endocrine abnormalities: Precocious puberty, thyrotoxicosis, pituitary gigantism, and Cushing syndrome
Mazabraud syndrome	Not familial	Activating *GNAS1* mutations	20q13	**Fibrous dysplasia** **Soft tissue myxoma**
Ollier disease	Not familial	*PTH1R* mutations may be involved in some cases	3p21–22	**Multiple enchondromas (enchondromatosis)** Increased risk of chondrosarcoma
Maffucci syndrome	Not familial	*PTH1R* mutations may be involved in some cases	3p21–22	**Multiple enchondromas (enchondromatosis)** PLUS **Soft tissue hemangiomas** Increased risk of chondrosarcoma and angiosarcoma
				Reference: [4]

Syndromes Associated with GI Polyps and Neoplasms *(Prior edition by Ashlie Burkart and Natasha Rekhtman)*				
Syndrome (other names)	***Inheritance***	***Gene Protein***	***Chromosome***	***Pathology and main clinical features***
Familial adenomatous polyposis (FAP)	AD	*APC* (adenomatous polyposis coli) Normal APC degrades β-catenin (a proto-oncogene) mutation/ inactivation of APC causes β-catenin to accumulate in the nucleus resulting in activated transcription.	5q21	**Intestine**: Early onset of 100s to 1000s of adenomas. Virtually 100% will develop colorectal carcinoma if not treated with total colectomy. Small intestinal adenomas (particularly of proximal duodenum and periampullary), periampullary adenocarcinoma is the major cause of death following colectomy; fundic gland polyps (25–40% of fundic gland polyps have dysplasia although these polyps are biologically inert). **Soft tissue tumors (Gardner)**: Fibromatosis (desmoid tumor), osteomas, nuchal fibroma, and Gardner fibroma **Skin lesions (Gardner)**: Epidermoid cysts, pilomatrixomas **Dental abnormalities (Gardner)**: Unerupted teeth, supernumerary teeth **Brain tumors (Turcot)**: Medulloblastomas **Other**: Thyroid cancer (1–2% of young women with FAP) strongly suggestive of FAP is the **cribriform-morular variant of papillary thyroid CA (CMV-PTC)**, which often precedes polyposis; juvenile nasopharyngeal angiofibromas (adolescent males, 25x risk). **"Attenuated FAP:"** far fewer adenomas (~ 30 adenomas) and cancer develops ~ 10 years later *In both syndromic and sporadic settings, many FAP-associated tumors (tubular adenoma, fibromatosis, JNA, fundic gland polyps, CMV-PTC) can be identified by IHC for nuclear β-catenin.*
Hereditary nonpolyposis colorectal cancer (Lynch syndrome/HNPCC*)	AD	*hMLH1* (~50%) *hMSH2* (40%) *hMSH6* (10%) *PMS2* (<5%) [above genes encode DNA mismatch repair (MMR) proteins] *EPCAM* (1–3%)	3p21 2p22 2p16.3 7p22.1	**Lynch syndrome-associated tumors**: Colorectum (~80% lifetime risk), endometrium (~60% lifetime risk), ovary (~12% lifetime risk), stomach, pancreatobiliary, urothelial carcinoma of upper urinary tract (particularly with inverted growth), small bowel, brain, skin (sebaceous adenomas and carcinomas and keratoacanthomas) Whereas Lynch syndrome is caused by inherited mutations in MMR genes, these genes are also inactivated in 10–15% of sporadic colorectal cancer, primarily via promoter hypermethylation of *hMLH1*. *Therefore, by IHC the loss of MSH2, MSH6, or PMS2 is nearly diagnostic of Lynch syndrome (germline mutation), whereas the loss of MLH1 is more commonly sporadic.* * Lynch/HNPCC terms are frequently used interchangeably BUT by strict definition HNPCC refers to patients meeting the clinical definition of inherited colorectal carcinoma ("Amsterdam criteria") vs. Lynch syndrome is reserved only for patients with confirmed germline mutation in the mismatch repair pathway. # the loss of MSH2 can be caused either by inherited mutation in *MSH2* gene OR inherited deletion of 3′ end of *EPCAM* gene leading to inactivation of adjacent *MSH2* gene through methylation induction of its promoter. See below for clinicopathologic clues to Lynch syndrome/HNPCC. References: [5, 6]
Gardner (FAP variant)	AD	*APC*	5q21	Manifestations of FAP (see FAP) plus skin and soft tissue lesions: fibromatosis, nuchal fibroma, osteomas, pilomatrixomas, epidermoid cysts
Turcot (either FAP variant or Lynch/HNPCC variant)	AD	*PMS2*		CNS tumors and polyposis. Two types: (1) medulloblastoma and FAP (2/3 cases) (2) glioblastoma and Lynch syndrome/HNPCC (1/3 cases)
Muir-Torre (Lynch/HNPCC variant)	AD	*MSH2* and *MLH1*		**Lynch syndrome/HNPCC-related tumors** (see Lynch) and **skin tumors** (sebaceous adenomas and carcinomas and keratoacanthomas)
MYH-associated polyposis (MAP)	AR	*MYH* Two mutations account for ~85%: Y165C and G382D		Phenotypically similar to attenuated FAP (~10–100 polyps and extracolonic features). *MYH* is a DNA repair "caretaker" gene. Its inactivation can result in accumulation of mutations in *APC*, which is why it is so phenotypically similar to FAP. Mainly affects European populations. Think of this disease if you have a patient with attenuated FAP-like disease but no evidence of AD transmission.

Syndromes Associated with GI Polyps and Neoplasms – Continued				
Hereditary diffuse gastric cancer syndrome	AD	*CDH1* (E-cadherin gene)		Diffuse gastric cancer, lobular breast cancer – Loss of E-cadherin demonstrated by IHC (similar to sporadic tumors, where loss of expression is due to promoter hypermethylation).
Peutz-Jeghers syndrome (PJS)	AD	*STK11/LKB1*	19p13	**GI polyps**: Hamartomatous polyps with arborizing smooth muscle. Most occur in the small intestine although may also occur in stomach and colon. Sporadic PJ polyps are rare. **Overall risk of malignancy**: Lifetime risk 93%. **GI malignancies**: Colon (39%), pancreas (36%), stomach (29%), small intestine (13%) **Tumors of reproductive organs**: Ovary (21%); cervix (10%); uterus (9%); testes (29%). These particular tumors are highly associated with PJS: Minimal deviation adenocarcinoma (AKA adenoma malignum) of uterine cervix, sex cord tumor with annular tubules (SCTAT), large cell calcifying Sertoli cell tumor. **Other malignancies:** Breast (54%), lung (15%) **Mucocutaneous lesions:** Pigmented macules (esp. lips) Reference: [7]
Juvenile polyposis (JP)	AD	(1) *SMAD4/DPC4* (2) *BMPR1A* (3) *PTEN*		**GI:** Multiple juvenile polyps involving colon (juvenile polyposis coli) (defined as >3–5 polyps or >1 polyp and family history) or juvenile polyps involving the entire GI tract (generalized juvenile polyposis syndrome). Increased risk of colorectal carcinoma.
Cronkhite-Canada	Not familial	Unknown		**GI:** Numerous polyps, usually of the stomach +/– small intestine and colon. The polypoid and non-polypoid mucosa is hyperplastic polyp-like in the stomach and cystically dilated and edematous in the remainder of the bowel and presents in older adults. Sometimes associated with colorectal adenocarcinoma. **Ectodermal changes**: Alopecia, macular hyperpigmentation of skin, nail dystrophy
Ruvalcaba-Myhre-Smith (Bannayan-Riley-Ruvalcaba)	AD	*PTEN* (same as Cowden)	10q23	**GI:** Hamartomatous polyps (often Peutz-Jeghers-like) **Soft tissue lesions:** Lipomas, hemangiomas **Other**: Macrocephaly, dark penile freckles (males)
Another syndrome with GI polyps is Cowden disease (see below) References: [8, 9]				

Syndromes Associated with Breast Cancer				
Hereditary breast and ovarian cancer	AD	*BRCA1* (40–50% of hereditary breast cancer)	17q21	**Breast cancer** (>70%); enriched for medullary carcinoma **Ovarian cancer** (30–60%): Serous carcinoma and tubal intraepithelial carcinoma (TIC) – Entire tube MUST be submitted; greater risk than BRCA2
		BRCA2 (20–30% of hereditary breast cancer)	13q	**Breast cancer** (>60%) **Ovarian cancer** Other tumors: Male breast cancer, prostate cancer, pancreatic cancer
Cowden disease (multiple hamartoma syndrome)	AD	*PTEN*	10q	**Multiple neoplasms and hamartomas** of endo-, ecto-, and mesodermal origin **Breast cancer:** >50% lifetime risk (often bilateral) **Skin:** Facial **trichilemmomas**; café au lait spots, vitiligo, epidermoid cysts **GI:** Polyps of any type in ~1/3 of patients (hamartomatous, hyperplastic, adenomatous, or inflammatory) **Soft tissue:** Hemangiomas, lymphangiomas, lipomas, neurofibromas, leiomyoma **Other tumors**: Thyroid, RCC, Merkel cell carcinoma, lymphoma, melanoma, meningioma
Increased breast cancer also seen in ataxia-telangiectasia syndrome (11% breast cancer risk by age 50), Li-Fraumeni syndrome, Peutz-Jeghers syndrome, and hereditary diffuse gastric cancer syndrome (lobular)				

Syndromes Associated with Skin Tumors				
Familial atypical multiple mole melanoma syndrome (FAMMM syndrome or B-K mole syndrome)	AD	*CDKN2A* [p16]	9p21	100+ nevi, atypical (dysplastic) nevi, increased risk of melanoma Pancreatic adenocarcinoma (12–20-fold increased risk)
Gorlin syndrome (nevoid basal-cell carcinoma syndrome, NBCCS)	AD or sporadic	*PTCH* (patched gene)	9q22.3–q31	**Two or more basal-cell carcinomas** before age 20 **Odontogenic keratocyst** of the jaw **Ovarian fibroma** (multinodular, bilateral, calcified) **Medulloblastoma** Macrocephaly and other congenital malformations Skeletal abnormalities
References: [3, 4, 10]				

Syndromes Associated with Endocrine/Neuroendocrine Tumors (Other than MENs)					
Syndrome (other names)		*Inheritance*	*Gene Protein*	*Chromosome*	*Pathology and main clinical features*
Mahvash disease (aka PanNET syndrome or glucagon cell hyperplasia and neoplasia)		AR	*GCGR* [glucagon receptor]	17q25	Pancreatic islet glucagon cell hyperplasia and micro- and macroglucagonomas
Hyperparathyroidism-jaw tumor syndrome (HPT-JT)		AD	*CDC73* [parafibromin]	1q25-q31	**Parathyroid carcinoma** Jaw tumors (usually ossifying fibromas)
Familial SDHB-related paraganglioma/ pheochromocytoma syndromes	**Carney-Stratakis syndrome**	AD	*SDHB* (6–8%), *SDHD* (5–6%), *SDHC* (1–2%), *SDHA* (1%) *SDHAF2* (rare)	1p15.33 11q23.1 1q23.3 5p15.33	**Paraganglioma/pheochromocytoma** **GIST**s (epithelioid, gastric, children and young adults; see "slide to syndrome;" usually indolent course even with nodal metastases) May also include SDH-deficient **RCC** (see "slide to syndrome") and **pituitary adenomas** (rare)
	Carney triad	Nonfamilial	*SDHC* promoter hypermethylation		**Paraganglioma/pheochromocytoma** **GIST** (same as above) **Pulmonary chondroma** Predominantly young females
Other syndromes with associated endocrine/NE tumors include Von Hippel-Lindau syndrome, Carney complex, DICER1 syndrome, and NF1 (see other tables). Reference: [11]					

Other					
Li-Fraumeni – Syndrome of multiple sarcomas and carcinomas		AD	*TP53* [p53]	17p13	Multiple primary tumors at young age: Sarcoma, carcinoma (breast, colon, pancreas, adrenal cortex), leukemia, melanoma, glioma
Inherited defects in DNA repair	**Ataxia-telangiectasia**	AR	*ATM* (ataxia-telangiectasia mutated)	11q22–23	**100-fold increased risk of various malignancies:** Acute lymphoblastic leukemia in children, solid tumors in adults Progressive ataxia, ocular, and cutaneous telangiectasia, thymic hypoplasia, variable immunodeficiency (IgA). Sensitivity to ionizing radiation.
	Bloom syndrome	AR	*BLM* [BLM DNA helicase]	15	Predisposition to wide range of **cancers**, esp. leukemias. Various developmental defects
	Fanconi anemia	AR	Several candidate genes identified		Predisposition to **leukemias** and **solid tumors** (HCC in 10%) Hypoplasia of the bone marrow (anemia), kidney, spleen, and bone (thumbs and radii)
Carney complex or **Carney syndrome**		AD	*PRKAR1A* [protein kinase A, type 1 regulatory subunit]	17q22–24 and 2p16	"Familial multiple neoplasia and lentiginosis syndrome" (formerly termed LAMB syndrome – Lentigines, **a**trial myxomas, **m**ucocutaneous myxomas, and **b**lue nevi) **Myxoid lesions**: Cardiac myxoma, skin angiomyxoma, myxoid fibroadenoma of the breast **Pigmented and calcifying lesions**: Spotty skin pigmentation, epithelioid blue nevus, pigmented nodular adrenocortical hyperplasia, psammomatous melanotic schwannoma, large cell calcifying Sertoli cell tumor **Endocrine hyperactivity**: GH +/– PRL producing pituitary adenomas (acromegaly +/– hyperprolactinemia)
Retinoblastoma		40% inherited (AD)	*RB*	13q14	**Bilateral retinoblastomas** **Pineoblastoma** Increased risk of **osteosarcoma** and other sarcomas
Rendu-Osler-Weber syndrome or **hereditary hemorrhagic telangiectasia**		AD	(1) *ACVRL1* (2) *ENG* Both involved in TGF-beta pathway	(1) 12q11–14 (2) 9q33–34	**Aneurysmal telangiectasias** involving multiple organs, including the skin and mucosal surfaces of the oral cavity, GI tract, respiratory tract, urinary tract, and visceral organs. Complicated by bleeding.
DICER1 syndrome		AD	*DICER1* [dicer]	14q32.13	**Pleuropulmonary blastoma** Cystic nephroma Sertoli-Leydig cell tumor of the ovary Multinodular goiter Other rare tumors (gynandroblastoma, pituitary blastoma, etc.) Presentation in childhood, adolescence, or early adulthood
BAP1 hereditary cancer predisposition syndrome		AD	*BAP1*	3p21.31-p21.2	**Uveal and skin melanoma** **Mesothelioma** **ccRCC** Various other neoplasms
Rhabdoid tumor predisposition syndrome		AD	(1) *SMARCB1* (INI1) (2) *SMARCA4* (BRG1)	(1) 22q11.2 (2) 19p13.2	**Malignant rhabdoid tumors (renal and extrarenal), cranial rhabdoid tumors (AT/RT)** **SCCOHT** (small cell carcinoma of the ovary, hypercalcemic type) Typical presentation in infants and children with synchronous aggressive tumors

Abbreviations: *AD* autosomal dominant, *AR* autosomal recessive, *MPNST* malignant peripheral nerve sheath tumor, *PanNET* pancreatic neuroendocrine tumor

IHC as a Surrogate for Germline Testing in Hereditary Cancer Syndromes

Uses of IHC with established or emerging applications in identifying hereditary cancer syndromes

by Marina K Baine

Syndrome	Mutated gene(s)	Tumor type application	IHC	Comment
Carney complex	*PRKAR1A* (chromosome 17q22–24, >70% cases)	Cardiac myxomas; Melanotic schwannomas	**Loss of PRKAR1A staining** [positive membranous and cytoplasmic staining in normal tissue]	Germline testing is recommended in PRKAR1A-mutated cardiac myxomas; sporadic (somatic) mutations can also occur. IHC screening awaits further validation.
Familial adenomatous polyposis (FAP)	*APC* *CTNNB1*	Cribriform-morular variant of PTC (CMV-PTC)	**Nuclear and cytoplastmic β-catenin accumulation** [membranous in normal tissue]	CMV-PTC strongly associated with FAP and present in ~40% of cases. Rarely sporadic, often precedes polyposis. IHC for nuclear β-catenin to screen CMV-PTC patients for FAP can help guide genetic testing.
Succinate dehydrogenase (SDH)-deficient neoplasia: SDH-deficient neoplasia (in general)	*SDHx*	Pheochromocytomas/ paragangliomas; GIST; RCC.	**Loss of SDHB staining** [granular cytoplasmic (i.e., mitochondrial) staining in normal tissue]	Inactivating mutations in *SDH* genes (*SDHA*, *SDHAF1*, *SDHB*, *SDHC*, or *SDHD*) are highly specific for syndromic disease. Any *SDHx* mutation is associated with loss of SDHB staining by IHC. *SDHA*-mutated tumors also lack SDHA staining.
SDH-related hereditary paraganglioma (PG)/ pheochromocytoma (Pheo) syndrome (HPGL/PCC)				*SDHB* mutations in PG/Pheo are associated with high metastatic rate (screening by IHC is recommended [12]).
SDH-deficient GIST (Carney-Stratakis syndrome)				SDH-deficient GISTs are gastric and occur in children and young adults: 1. Carney-Stratakis syndrome (GISTs + PG/Pheo): Germline mutation with autosomal dominant inheritance, no sex predilection; 2. Carney triad (PG/Pheo, GISTs, and pulmonary chondromas): Nonheritable, strong female predilection.
SDH-deficient RCC				All SDH-deficient RCC are associated with germline mutations in *SDHx*, with ~30% patients also developing SDH-deficient GISTs and/or PG/Pheo.
SDH-deficient pituitary adenoma				SDH-deficient pituitary adenomas are very rare; they are generally large and are more likely to produce prolactin. May be seen in association with Carney-Stratakis syndrome. Screening by IHC is not recommended due to very low incidence.
Hereditary leiomyomatosis and renal cell cancer (HLRCC)	*FH* (fumarate hydratase) (chromosome 1q42.3–43)	Renal cell carcinoma	**Loss of FH staining** (high specificity, moderate sensitivity) **2SC** [S-(2-succino)-cysteine] **overexpression** (moderate specificity, high sensitivity)	HLRCC is highly aggressive. Loss of FH staining or 2SC overexpression by IHC in RCC is highly specific for *FH* germline mutations. IHC for FH and 2SC in cutaneous and uterine leiomyomas is not helpful for evaluation of HLRCC because only a small proportion of *FH*-mutated cases are associated with germline mutations.
Medullary thyroid cancer and multiple endocrine neoplasia 2 (MEN2)	*RET*	Medullary thyroid carcinoma	**Positive NRAS (Q61R) (clone SP174) staining to rule out** MEN2 syndromic cases	Germline *RET* (98% of MEN2 and familial cases) and somatic *RAS* mutations (10–45% of sporadic MTC) are mutually exclusive. IHC with an antibody (clone SP174) detects p.Q61R in *NRAS*, *KRAS* and *HRAS*. IHC has not been formally validated for this purpose but may be a useful tool in ruling out MEN2.
Hyperparathyroidism-jaw tumor syndrome (HPT-JT)	*CDC73* (parafibromin)	Parathyroid carcinoma	**Loss of nuclear parafibromin staining**	Inactivating germline mutations in *CDC73* gene encoding the parafibromin protein. Loss of nuclear parafibromin by IHC in >70% of HPT-JT-associated parathyroid carcinomas and may be used to confirm definitive parathyroid malignancy and guide germline *CDC73* mutation testing.
Mahvash disease (aka pancreatic neuroendocrine tumor syndrome or glucagon cell hyperplasia and neoplasia)	*GCGR* (glucagon receptor)	Pancreatic neuroendocrine tumor (PanNET)	**Glucagon positivity in multiple tumors**	Multiple glucagon-secreting PanNETs and pancreatic α-cell hyperplasia NOT associated with glucagonoma syndrome. See above for details. Multiple PanNETs positive for glucagon by IHC = highly suggestive of a germline *GCGR* mutation.

Lynch syndrome/HNPCC is the prototypic example of IHC utility in detection of syndromic conditions and is described in detail in the table above as well as in Chapter 2.

Abbreviations: *PG* paraganglioma, *Pheo* pheochromocytoma

References: [12–14]

"Slide to Syndrome"

Selected Tumors or Histologic Features in Tumors that Should Make You Think of a Syndrome or Clinical Condition

(This table does not cover tumors associated with obvious conditions like cirrhosis/HCC or environmental exposure associations like asbestos/mesothelioma, etc.)

Tumor	Syndrome(s) or condition	% of cases associated with syndrome/condition	Comment
Genitourinary:			
Hybrid chromophobe RCC-oncocytoma	Birt-Hogg-Dube	Almost 100%	
HLRCC-associated RCC *(histologic clues in the comment)*	HLRCC		Papillary architecture with macronucleoli surrounded by clear halo (viral inclusion-like); same peculiar nuclei also seen in leiomyomas associated with HLRCC [15]
SDH-deficient RCC *(histologic clues in the comment)*	SDH deficiency-associated neoplasia	>95%	Eosinophilic cells with flocculent cytoplasm and at least focal cytoplasmic inclusions and/or vacuoles; NE-like nuclei; nested, solid, or tubular architecture
Eosinophilic solid and cystic RCC *(histologic clues in the comment)*	Tuberous sclerosis	<100% (can be sporadic)	Solid + cystic; hobnailing; granular eosinophilic cytoplasm; CK20+/CK7-
Acquired cystic disease-associated RCC	End-stage renal disease	Almost 100%	Intratumoral calcium oxalate crystals
Renal medullary carcinoma	Sickle cell trait	Almost 100%	
Wilms tumor	WAGR, Denys-Drash, BWS	10–15% overall	Nephrogenic rests (intralobar) → high risk of contralateral Wilms tumor
Inverted papillary urothelial carcinoma of renal pelvis	HNPCC	30%	
Angiomyolipoma	Tuberous sclerosis	20%	
Adrenal rest tumors of the testis	Congenital adrenal hyperplasia	100%	Mimics of leydig cell tumor but are bilateral/ multifocal, associated with dense fibrosis and do not have Reinke crystals
Papillary cystadenoma of epididymis	VHL	33% of males	Identical morphology and IHC to clear cell papillary RCC: CAIX (basolateral, "cuplike"), PAX8+ and AMACR– [16]
Gynecologic:			
Adenoma malignum (minimal deviation adenocarcinoma) of the cervix	PJ	5%	*STK11* gene mutation
Adnexal papillary cystadenoma of probable mesonephric origin (APMO)	VHL	100% so far	Female counterpart of epididymal papillary cystadenoma
Gonadoblastoma	Dysgenetic gonad (Turner syndrome)	>90%	
Ovarian fibroma	Meig syndrome (ascites, right hydrothorax), NBCCS	Rare	
Sex cord-stromal (either sex):			
Large cell calcifying sertoli cell tumor	Carney complex > PJ	40%	
SCTAT	PJ	30–40%	
Skin:			
Angiofibroma (adenoma sebaceum, fibrous papule)	Tuberous sclerosis, MEN1	High if multiple	
Angiokeratoma, corporis diffusum type	Fabry and other storage diseases	>90%	
Basal cell carcinoma (multiple tumors at young age, particularly children)	NBCCS, xeroderma pigmentosum	Majority are syndromic	Basal cell carcinomas at older age are usually non-syndromic
Fibrofolliculoma	Birt-Hogg-Dube	Almost 100% when multiple	
Sebaceous adenoma/carcinoma	Muir-Torre (HNPCC variant)	40% above the chin, 80% elsewhere	Associated with *MSH2* and occasionally *MLH1* mutations
Trichilemmoma, multiple facial	Cowden	Almost 100%	
Thyroid:			
PTC, cribriform-morular variant	FAP	80%	Nuclear β-catenin+
Medullary carcinoma (especially with background C-cell hyperplasia)	MEN2A, MEN2B; inherited endocrinopathy (isolated site)	25% overall	
Head and neck:			
Endolymphatic sac tumor	VHL	15%	Another clear cell papillary tumor often seen in VHL
OKC, especially multiple	NBCCS	High if multiple	
CNS and nerve sheath:			
Hemangioblastoma	VHL	25%	
Medulloblastoma	Turcot (FAP variant), NBCCS	10%	
Subependymal giant cell astrocytoma	Tuberous sclerosis	>90%	
Neurofibroma, plexiform, and diffuse types	NF1	>90% plexiform, 10% diffuse	Plexiform schwannoma is *not* associated with NF1
Neurofibroma, visceral (esp. GI tract)	NF1	44% in the abdomen/pelvis 20% in the thorax [17]	Cutaneous *not* associated with NF1. See below
Psammomatous melanotic schwannoma	Carney complex	>50%	

"Slide to Syndrome" – continued

Thoracic:			
Lymphangioleiomyomatosis (LAM) of the lung	Tuberous sclerosis	>50%	Syndromic LAM is more common than sporadic LAM
Mediastinal carcinoid tumor	MEN1	25%	% syndromic is much lower for pulmonary carcinoids
Pleuropulmonary blastoma – PPB (in kids/infants)	DICER1 syndrome	65%	Reference: [18]
Breast:			
Breast + ovarian (serous) carcinoma	*BRCA1* mutation	>50% if both	
Breast carcinoma in males	*BRCA2* mutation	5–40%	
Lobular carcinoma + diffuse gastric cancer	*CDH1* mutation	>70% if both	Hereditary diffuse gastric cancer syndrome (HDGCS)
Medullary carcinoma	*BRCA1* mutation	10%	
Heart:			
Fibroma	NBCCS	5%	
Myxoma	Carney complex	<5%	
Rhabdomyoma	Tuberous sclerosis	50%	
Gastrointestinal:			
Clear cell PanNET and clear cell serous cystadenoma of the pancreas	VHL	Not well defined but appears high	
Colorectal adenocarcinoma (see below for histologic and clinical features suggesting Lynch syndrome/HNPCC)	Lynch syndrome/HNPCC With polyposis syndromes - FAP, PJS, JP, MAP	~5% syndromic (vast majority are sporadic)	
SDH-deficient GIST *(histologic clues in the comment)*	SDH deficiency-associated neoplasia	~8% overall Almost 100% in patients <20 years old	Epithelioid morphology, multinodular or plexiform architecture, exclusively gastric location, predominantly young age [12]
Gangliocytic paraganglioma (duodenum)	NF1	Rare	
Ganglioneuromatous polyposis	Cowden, JP, NF1, FAP	Almost 100% if multiple (but not well defined)	Solitary polyp has no association with syndrome
Diffuse ganglioneuromatosis	MEN2B, NF1	Almost 100%	Almost invariably present in patients with MEN2B
Neurofibroma (of the GI tract)	NF1	Almost 100%	GI neurofibromas outside the setting of NF1 are extremely rare
Peutz-Jeghers polyp (PJP)	PJ	Almost 100%	Sporadic PJPs are rare [19]
Gastrin-secreting PanNETs, gastrinoma of duodenum	MEN1	20–25%	Most are functional resulting in Zollinger-Ellison syndrome (peptic ulcers and thickened gastric folds)
Pancreatic endocrine microadenomatosis	MEN1, Mahvash disease, rarely VHL	>95%	References: [20, 21]
Glucagonomas (multiple micro and/or macro)	Mahvash disease	100%	
Somatostatinoma (duodenum)	NF1	50%	
Endocrine/neuroendocrine:			
Adrenocortical carcinoma in children	BWS, MEN1 and 4, Li-Fraumeni	50–80% overall	
Congenital adrenal cytomegaly	BWS	% not known	Marked pleomorphism but without mitoses
Parathyroid microadenomatosis	MEN1, MEN2A, MEN2B, HPT-JT	10% if single adenoma Nearly 100% if multiple	
Pheochromocytoma/paraganglioma	MEN2A, MEN2B, VHL, NF1, Sturge-weber, *SDHx*-deficient familial paraganglioma-pheochromocytoma, carney triad, isolated familial pheochromocytoma	10–30%	
Pituitary adenoma	MEN1 and 4, Carney complex, McCune-Albright syndrome	5%	
Pituitary blastoma	DICER1 syndrome	100%	Pathognomonic of DICER1 syndrome; occurs by 2 years of age
Primary pigmented nodular adrenocortical disease	Carney complex	>90%	
Well-differentiated neuroendocrine tumors (lung carcinoid, gastro-entero-pancreatic NETs)	MEN1 and MEN4 (pancreatic, gastric, duodenal, mediastinal, lung NET/carcinoids), VHL (clear cell PanNET), NF1 (pancreatic and duodenal somatostatinoma), Mahvash disease (pancreatic islet glucagon cell hyperplasia and micro- and macroglucagonomas)	Site dependent	MEN1 association highest for mediastinum followed by pancreas and low for lung and ileum

Abbreviations: *BRCA* breast cancer susceptibility protein, *BWS* Beckwith-Wiedemann syndrome, *CRC* colorectal carcinoma, *GIST* gastrointestinal stromal tumor, *FAP* familial adenomatous polyposis, *HLRCC* hereditary leiomyomatosis and renal cell carcinoma, *HNPCC* hereditary nonpolyposis colorectal cancer, *HPT-JT* hyperparathyroidism-jaw tumor syndrome, *JP* juvenile polyposis, *MAP* MYH-associated polyposis, *MEN* multiple endocrine neoplasia, *MSI* microsatellite instability, *NBCCS* nevoid basal-cell carcinoma (Gorlin) syndrome, *NET* neuroendocrine tumor, *NF* neurofibromatosis, *OKC* odontogenic keratocyst, *PanNET* pancreatic neuroendocrine tumor, *PJP* Peutz-Jeghers polyp, *PJS* Peutz-Jeghers syndrome, *SCTAT* sex cord tumor with annular tubules, *VHL* Von Hippel-Lindau, *WAGR* Wilms tumor, aniridia, genitourinary abnormalities, mental retardation syndrome

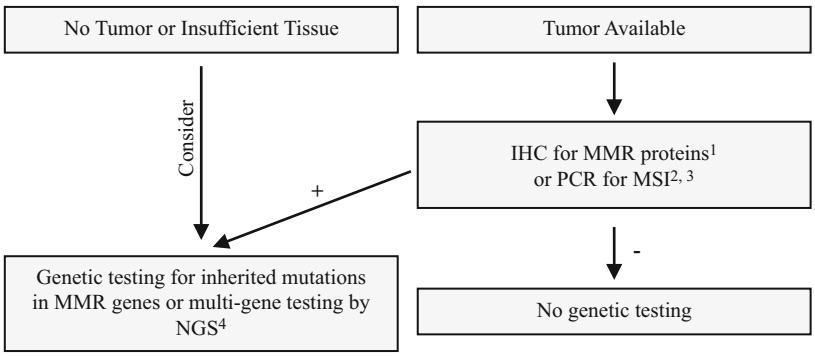
Lynch Syndrome/HNPCC

Clinicopathologic Predictors and Testing Algorithms (in Evolution)

By Marina K Baine (Prior edition by Justin A. Bishop, Ashlie L. Burkart, Natasha Rekhtman)

#Universal Screening of All CRC/EMC

[testing for inherited mutations in mismatch repair (MMR) genes - *MSH2, MLH1, MSH6, PMS2* or *EPCAM*]



(1) Histologic features associated with MMR deficient/MSI-high tumors are summarized below, but these are not factored in for universal testing of all-comers [22–24]. See section on interpretation of IHC for MMR proteins in Chapter 2.

(2) MSI is tested by PCR and is reported as MSI-high (instability in >2 or >30% of microsatellite markers), MSI-low (instability in 1 or <30% of microsatellite markers), or microsatellite stable (MSS).

(3) Approximately 15% of sporadic MMR-deficient/MSI-high CRCs are due to *MLH1* promoter hypermethylation, over 50% of which harbor *BRAF* V600E mutations, whereas this mutation virtually excludes Lynch syndrome. Therefore, *BRAF* is now routinely included in testing algorithms for patients with CRC. *BRAF* testing is NOT utilized in the screening of endometrial carcinomas, since *BRAF* mutations are exceedingly rare in *MLH1* hypermethylated endometrial cancers.

(4) Multigene testing by NGS may be the preferred testing route in patients with strong family history or those diagnosed before the age of 50 [25].

The 2017 NCCN Guidelines recommend universal testing of all patients with CRC or EMC according to above algorithm. At a minimum, those of age <70 or >70 and meeting Bethesda criteria (see below) should be tested. This is supported by occasional presentation of low penetrance mutations (e.g. MSH6 and PMS2) later in life [481]. Although the universal approach is more sensitive, an older selective screening approach based on clinical criteria (Amsterdam II or Bethesda; see below) is also supported by NCCN Guidelines.

References: [27, 28]

Amsterdam ("3–2–1") Criteria (Revised in 1999 to include extracolonic cancers)
≥3 relatives with CRC (or other HNPCC-related tumors*), at least 1 of which is a first-degree relative
≥2 consecutive generations affected
1 or more family member with CRC at age <50
References: [29, 30]

Revised Bethesda Guideline (2003) Only one needs to be met:
Patients diagnosed with CRC before age 50
Patients with 2 HNPCC-related cancers, including synchronous and metachronous CRC or associated extracolonic cancers*, regardless of age
Patients with CRC with MSI-high morphology** before age 60
Patients with CRC with ≥2 relatives also with HNPCC-related tumors, regardless of age
Patients with CRC with ≥1 first-degree relative also with CRC or other HNPCC-related tumors, one of which must have been diagnosed before age 50 (or, if a colorectal adenoma, before age 40)
Reference: [31]

*HNPCC/Lynch syndrome-associated tumors include carcinomas of the colorectum, endometrium, ovary, stomach, pancreatobiliary, urinary tract (esp. inverted urothelial carcinoma of the upper tract), small bowel, brain (glioblastoma), and sebaceous skin neoplasms

**__Pathologic features of CRC associated with Lynch syndrome include__ tumor-infiltrating lymphocytes, Crohn-like reaction^, extracellular mucin (>10%)^, signet ring cell differentiation^, medullary growth pattern^, poorly differentiated/undifferentiated, heterogeneous histology, pushing border, right-sided location [22]

**__Pathologic features of endometrial carcinoma associated with Lynch syndrome include__ (recent data – not part of the revised Bethesda criteria as of mid-2018) tumor-infiltrating lymphocytes, tumor heterogeneity including dedifferentiated/undifferentiated histology, and lower uterine segment location [31]

^Criteria for "MSI-high histology" per the revised Bethesda Guidelines

Abbreviations: *CRC* colorectal carcinoma, *EMC* endometrial carcinoma, *HNPCC* hereditary non-polyposis colorectal cancer, *MMR* mismatch repair, *MSI* microsatellite instability, *NGS* next-generation sequencing

Chapter 13. Quick Clinical References for Pathologists

By Natasha Rekhtman, Marina K Baine, Justin A. Bishop

(All subsections are by these authors, unless specified otherwise)

Metastases: A Quick Reference

General Principles

By Natasha Rekhtman

Carcinomas generally metastasize via lymphatics (i.e., initial spread is to the lymph nodes). Notable exceptions are renal cell carcinoma, follicular carcinoma of the thyroid, and choriocarcinoma, which disseminate hematogenously. In contrast to carcinomas, **sarcomas** generally metastasize hematogenously to organs such as the liver and lung, bypassing the lymph nodes. Lymph node metastases are rare or in some cases (such as Ewing sarcoma) never occur. Exceptions are clear cell sarcoma/melanoma of soft parts, epithelioid sarcoma, alveolar rhabdomyosarcoma, synovial sarcoma, and angiosarcoma – these sarcomas are an exception in that they frequently metastasize to the lymph nodes.

Some metastases follow a predictable route of dissemination based on the circulatory map. As such, tumors in organs drained by portal circulation (bowel, pancreas) metastasize to the liver first. In contrast, tumors in organs drained by the inferior vena cava (such as kidney, rectum) metastasize first to the lung. However, usual circulatory flow can be disrupted due to obstruction by the tumor or as a result of surgery and/or radiation; therefore tumor spread not conforming to normal circulatory map is not unusual. In addition, certain tumors show proclivity for metastasis to unusual sites not based on vascular drainage. Notable examples are lobular breast carcinoma and renal cell carcinoma, which tend to metastasize to unusual sites, such as GI tract. In addition, certain sites such as the bone (not a particularly vascular organ) appear to be the preferential target of metastasis.

Tumors known to have late metastases (may present as metastases many years after primary diagnosis)

- Renal cell carcinoma
- Salivary gland carcinomas (particularly adenoid cystic carcinoma)
- Breast carcinoma
- Endometrioid carcinoma
- Carcinoid tumors
- Melanoma
- Granulosa cell tumor of the ovary
- Sarcomas (endometrial stromal sarcoma, alveolar soft part sarcoma, extraskeletal myxoid chondrosarcoma, synovial sarcoma)

Organs where metastases are more common than primary tumors

- Liver
- Lung, pleura (for multiple nodules, for solitary – primaries are more common than metastases)
- Heart
- Bone (in adult)
- Brain
- Adrenal (adrenocortical adenomas are common, but for malignant tumors – metastases are more common than primary)

Most common destinations of distant metastases

- The lung, liver, bone, and brain

Metastases in children

- Clear cell sarcoma of the kidney (in infants)
- Rhabdoid tumor
- Wilms tumor
- Neuroblastoma

Carcinoma of unknown primary (CUP) = carcinoma initially presenting with metastasis, where the primary site is occult (inapparent clinicoradiologically). The site of origin may be eventually identified by morphology/IHC or may be found only at autopsy. Most common sites (if discovered) are:

- Pancreaticobiliary
- Lung
- Gastric

Patterns of lung metastasis and differentials The lung is THE most common site of distant metastases because (1) the entire systemic circulation from the right heart goes through the lung, (2) the lung is the first capillary bed encountered by lymphatic drainage (thoracic duct → subclavian → right heart → lung), and (3) the lung has the densest capillary bed in the body.

Pattern of mets in the lung	Classic primary	Clinical DDx	Comment
Multiple nodules	Any	Granulomas, OP, abscesses, infarcts	Most common pattern of metastasis
Solitary nodule	Sarcoma, melanoma, germ cell tumor, colorectal, endometrial	Granuloma (usually PET+), OP, lung primary	
Lymphangitic (massive involvement of the lymphatics)	Breast, stomach, pancreas, lung, prostate	Pulmonary edema, infectious, ILD	Interstitial (reticular) pattern on CT + thickened septae and bronchovascular structures. Ominous clinically
Consolidative/"pneumonic-type" (spreading through air spaces)	Invasive mucinous adenocarcinoma (formerly mucinous BAC), pancreaticobiliary metastases	Pneumonia	
Pleural seeding	Lung, breast, ovary, thymoma	Mesothelioma	
Miliary (innumerable tiny nodules, analogous to miliary pattern of TB)	Thyroid, RCC	TB, carcinoid tumorlets, minute meningothelioid nodules	Usually associated with highly vascular neoplasms

Abbreviations: *OP* organizing pneumonia, *ILD* interstitial lung disease, *BAC* bronchioloalveolar carcinoma. References: [1, 2]

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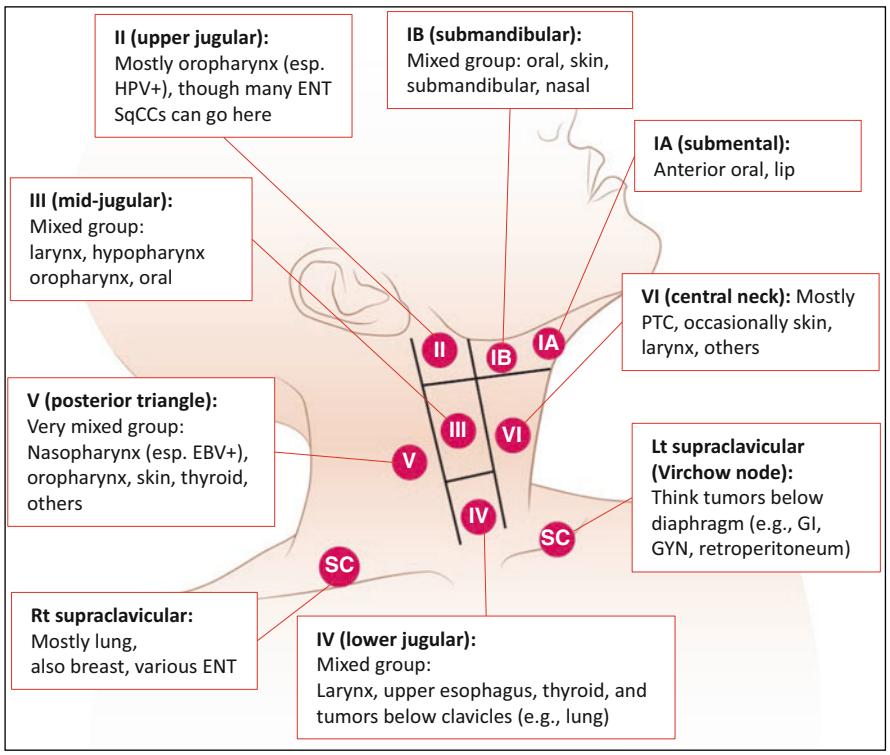
Metastases "From → To": At-a-Glance Summary

Metastasis FROM	TO – lymph nodes (LN)	TO – distant sites
Adrenocortical	Regional LNs (aortic, retroperitoneal)	Liver, lung, peritoneal and pleural surfaces, bone
Anus	Above the dentate line: inferior mesenteric LNs Below the dentate line: superficial inguinal LNs	Liver, lung
Bladder	Regional LNs (hypogastric, obturator, iliac, perivesical pelvic, sacral, presacral)	Retroperitoneal lymph nodes, lung, bone, and liver
Breast	Axillary, internal mammary, supraclavicular LNs	Ductal carcinoma: lungs, bone, liver, brain, adrenal Lobular carcinoma: tendency to metastasize to unusual sites (GI tract, GYN tract, bone marrow, endocrine organs, meninges, mesothelial surfaces/ produces effusions)
Colon	Regional LNs	Liver, peritoneum, lung, ovaries
Carcinoid (NET) tumor of the GI tract	Regional LNs	Mets to the liver first (typical presentation of ileal carcinoid is massive liver metastasis in otherwise healthy patient with unknown primary; an unlikely scenario for carcinoma). Can also metastasize to the bone, skin, or almost any organ
Carcinoid tumor of the lung	Regional LNs (hilar)	Bone – common site of extra-thoracic mets Late mets possible (need 10+ year follow-up)
GYN tract		
Uterus	Pelvic and periaortic LNs	Lung, vagina, peritoneal surfaces, and omentum for serous and clear cell carcinoma
Cervix	Pelvic, inguinal LNs	Lung
Ovaries	Regional LNs (iliac, obturator, para-aortic, inguinal, pelvic, retroperitoneal)	Intra-peritoneal spread typical. Mets to the lung, pleura
Liver, HCC	Regional LNs (hilar, hepatoduodenal ligament, inferior phrenic, caval)	Lung, bone, adrenal
Liver, cholangiocarcinoma	Regional LNs (porta hepatis)	Lung, bone, adrenal, peritoneal surfaces
Lung, non-small cell carcinoma	Regional LNs (hilar), supraclavicular	Adrenal (the lung is the most common origin of adrenal metastasis), bone, brain
Lung, small cell carcinoma	Regional LNs (hilar), supraclavicular. Classic presentation – massive hilar adenopathy (with or without obvious lung primary)	Extra-thoracic metastasis at presentation in majority of pts Brain mets in >50% (therefore prophylactic cranial radiation recommended). Other common sites – liver, adrenal, bone, bone marrow
Melanoma	Regional LNs depending on primary site	Any site is possible. Common source of unknown primary. Common sites include the soft tissue, skin, liver, brain, bone, and GI tract. May form solitary metastasis in the lung (DDx primary non-small cell carcinoma)
Neuroendocrine tumors (lung, pancreas, GI)	Regional LNs	Liver is #1 for all sites. Bone and brain are uncommon for pancreas and GI, but are common for lung carcinoids
Pancreas	Regional LNs surrounding the pancreas	Liver, peritoneal cavity, lung (metastases may colonize alveolar walls, mimicking mucinous adenocarcinoma)
Parathyroid	Regional LNs (cervical or mediastinal)	Lung, liver, bone
Pheochromocytoma	Regional LNs	Predilection for bone mets (always need bone scan), liver
Prostate	Regional: true pelvis (LNs below the bifurcation of the common iliac arteries: pelvic, NOS, hypogastric, obturator, internal and external iliac, sacral) Distant (staged as M1): para-aortic, common iliac, inguinal, cervical/supraclavicular	Hematogenous spread (via spinal cord venous plexus) to AXIAL skeleton – spine, femur, pelvis, ribs Non-bony distant metastases are uncommon (particularly in the absence of skeletal metastasis). Common sites include the lung, liver, and adrenal gland
Renal cell carcinoma	LN mets are rare (mets usually hematogenous)	Hematogenous mets to the lung, bone, liver, brain, adrenal, other Proclivity for mets to unusual sites (small bowel, thyroid, soft tissue, scapula) and late mets (10+yrs)
Salivary gland	Regional LNs in an orderly fashion, first nodes within/adjacent to the gland, then to cervical nodes	Lung. Adenoid cystic CA – late metastases characteristic
Stomach	Regional LNs (perigastric greater curvature, lesser curvature, and pancreatic/splenic areas)	Liver, peritoneal surfaces, and distant lymph nodes Classic sites include supraclavicular node (Virchow), periumbilical (Sister Mary Joseph nodule), ovary (Krukenberg tumor)
Testis	Lymphatic to retroperitoneal (para-aortic) LNs, later mediastinal and left supraclavicular (not inguinal)	Lung (most common), liver, brain, bone Note: Lymphatic route is typical of seminoma vs. hematogenous route is typical of non-seminoma germ cell tumors (particularly choriocarcinoma)
Thyroid	Papillary: Mets via lymphatics (to regional LNs, especially central neck level VI). Surprisingly, lymph node mets are of limited prognostic significance	Follicular: Hematogenous mets to distant sites (lung and bone)
Thyroid, medullary	Spread similar to papillary thyroid carcinoma, though much more prognostically significant	Distant mets common (miliary pattern of metastasis typical in the lung)

Metastases "From → To": (Continued)

Pediatric tumors:		
Neuroblastoma	Lymphatic spread to LNs	Common sites: liver, bone, lymph nodes, ovary Rare sites: lung Blue-gray skin mets ("blueberry muffin" babies)
Wilms tumor	Lymphatic spread to LNs	Mets to the **l**ymph nodes, **l**iver, **l**ung ("the three L's"). Mets to the bone or brain – rare
Clear cell sarcoma of the kidney		Proclivity for bone mets (i.e., "bone metastasizing renal tumor of childhood"); also mets to usual sites (e.g., brain, soft tissue) – Wilms tumor almost never mets to these sites
Sarcomas:		
Alveolar soft parts sarcoma		Lung, brain, bone
Ewing sarcoma	NEVER mets to LNs	Lung, bone
Myxoid liposarcoma		Mets to other soft tissue sites (and lung)

Metastases "To ← From": At-a-Glance Summary

Metastasis to	From
Lymph nodes:	
Neck: Cervical (levels I–IV) and supraclavicular. See diagram	 Cervical nodes: mets from ENT sites Supraclavicular LN (SCLN): mets from ENT sites in only <50% of cases. The lung, breast, and ENT metastasize to both right and left SCLNs; abdominal tumors metastasize to Lt> > Rt (Lt SCLN = "Virchow node")
Intra-parotid	SqCC, melanoma, and Merkel cell CA (all of the overlying facial skin), primary salivary gland
Axillary	Breast, lung, melanoma
Intra-thoracic	Adult – lung, breast Pediatric – germ cell tumor
Intra-abdominal	Adult – GI tract, GYN, pancreaticobiliary Pediatric – Wilms tumor, germ cell tumor, neuroblastoma
Superficial inguinal	Lower extremity, vulvar/penile
Deep inguinal	Anorectal, GYN, genitourinary, melanoma
Iliac	Prostate
Paraumbilical (Sister Mary Joseph)	Visceral organs (stomach is classic site)

Metastases "To ← From": (Continued)

Metastasis to	From
Adrenal	Lung, breast, kidney, stomach, pancreas (adrenocortical adenoma "CT-incidentaloma" is much more common than mets)
Bone	Most common mets to large bones are from the breast, lung, thyroid, kidney, prostate (mnemonic = "BLT and Kosher Pickle") ***Osteolytic***: Lung, thyroid, kidney ***Osteoblastic***: Prostate ***Either***: Breast Children: primary bone tumors > metastasis vs. adults: metastasis > primary bone tumors
Brain	Lung, breast, melanoma, RCC, colon, thyroid
Heart	Lung, melanoma, breast, RCC
Liver	Overall 90% mets, 10% primary – In non-cirrhotic liver: 98% mets – In cirrhotic liver: 75% mets, 25% primary (HCC) Mets in adult: colorectum, pancreas, stomach, breast, lung, kidney, melanoma (prostate unusual) Mets in children: neuroblastoma, Wilms tumor, rhabdomyosarcoma, germ cell tumor
Lung	Most common destination of metastases. Overall mets>primary tumors Sites of origin: any carcinoma (frequency largely reflects population incidence – breast, colon, etc.), melanoma, sarcoma, germ cell tumors
Meninges	Melanoma, breast, leukemia in pediatric
Pleura	Mets > > primary Most common lung, breast; mets from any organ are possible
Skin	Adult – lung, breast, colon, melanoma, oral cavity Pediatric – leukemia, rhabdomyosarcoma, neuroblastoma
Soft tissue	Lung, breast, RCC, aerodigestive tract, melanoma
Thyroid	RCC, lung, GI tract, breast
References: [3–5]	

Malignant effusions	Most common sites of origin
Pleural	Lung, breast (in female), lymphoma
Peritoneal	Female: #1 GYN tract (ovary, endometrium, cervix), peritoneal serous, #2 GI Male: #1 GI (colon, rectum, stomach)

Serologic Tumor Markers: Common Associations

Serum marker	Main association(s)	Comment
αFP	HCC, yolk sac tumor	Diagnosis of HCC unlikely in the setting of normal α-FP (except fibrolamellar variant)
βHCG	Choriocarcinoma, trophoblastic tumors, various tumors with admixed syncytiotrophoblasts	
CA125	Ovary	Non-specific, used for monitoring (e.g., following treatment)
CA19.9	Pancreas	Non-specific
CA27.29	Breast	Non-specific
Calcitonin	Medullary thyroid carcinoma	
Catecholamines (serum and urine)	Pheochromocytoma, neuroblastoma	Most sensitive for pheochromocytoma is plasma metanephrines/normetanephrines; most sensitive for neuroblastoma is urinary vanillylmandelic acid/homovanillic acid
CEA	Colon, pancreas, medullary thyroid carcinoma	Non-specific
Chromogranin A	NE neoplasms, NE differentiation in prostate cancer after androgen-deprivation Rx (unresponsive to hormone Rx)	
Hypercalcemia	Lung SqCC (PTH-like hormone secretion), ovarian small cell carcinoma hypercalcemic type, ATLL; parathyroid; extensive bone lysis (myeloma, metastatic breast cancer)	
Lipase	Acinar cell carcinoma	Widespread subcutaneous fat necrosis, polyarthritis, and eosinophilia ("Schmid triad")
Hormones		
Insulin	PanNET, SFT	Symptoms of hypoglycemia (anxiety, tremor)
Glucagon	PanNET	Necrolytic migratory erythema, diabetes
Gastrin	PanNET	Gastric ulcers
Somatostatin	PanNET	Diabetes, $\downarrow H^+$, steatorrhea
Pancreatic polypeptide (PP)	PanNET	Asymptomatic
Vasoactive intestinal peptide (VIP)	PanNET	Watery diarrhea, $\downarrow H^+$, $\downarrow K^+$
Serotonin	Small bowel NET/carcinoid (uncommon for NETs of the lung, thymus, pancreas)	Carcinoid syndrome (flushing, diarrhea). Usually develops with midgut carcinoid metastatic to the liver (presumably due to overwhelmed clearance)
ACTH, cortisol	ACTH due to pituitary adenoma Ectopic ACTH due to SmCC, thymic carcinoid Cortisol due to adrenocortical tumors Unrelated to tumor (exogenous steroids)	Cushing syndrome (central obesity, glucose intolerance, hypertension, striae, hirsutism)
Prolactin	Pituitary adenoma	Galactorrhea, hypogonadism, infertility
Growth hormone	Pituitary adenoma	Acromegaly
TSH	Pituitary adenoma	Asymptomatic or hyperthyroidism
LH, FSH	Pituitary adenoma	Asymptomatic (most); some may present with precocious puberty, supraphysiological testosterone, or large testicles
ADH (vasopressin)	Pulmonary SmCC, intracranial tumors	Symptoms of siADH/$\downarrow Na^+$ – fluid overload, neurologic
Estrogen	Granulosa cell tumor, thecoma	Precocious puberty, endometrial hyperplasia/bleeding
Androgens in women	Sertoli-Leydig and steroid cell tumors (↑testosterone, nl or low 5DHEA) Adrenocortical tumors (↑5DHEA)	Virilization
PSA, total	Prostate cancer, BPH	Normal <4, equivocal = 4–10, high = 10 (though normal levels increase with age, obesity, etc.). May be negative in some high-grade carcinomas, equivocal in BPH
PSA, free	Prostate cancer << BPH	The lower the value, the higher the risk, but rarely used in isolation (see PSA ratio below)
PSA ratio (free: Total)	Prostate cancer > > BPH	Most useful in patients with PSA from 4 to 10: low ratios (<0.10) are associated with higher risk of prostate cancer (49–65%, depending on age); more specific for carcinoma than PSA
Thyroglobulin	Thyroid cancer	Used to monitor recurrence (requires total thyroidectomy or I* ablation); 15–20% have anti-thyroglobulin antibodies that interfere with test

Abbreviations: *α-FP* alpha fetoprotein, *βHCG* beta human chorionic gonadotropin, *ACTH* adrenocorticotropic hormone, *ADH* antidiuretic hormone, *ATLL* adult T-cell leukemia/lymphoma, *BPH* benign prostatic hyperplasia, *CEA* carcinoembryonic antigen, *DHEA* dehydroepiandrosterone, *FSH* follicle-stimulating hormone, H^+ hydrogen, K^+ potassium, *I** radioactive iodine, *LH* luteinizing hormone, Na^+ sodium, *NET* neuroendocrine tumor, *PanNET* pancreatic neuroendocrine tumor, *PSA* prostate specific antigen, *PTH* parathyroid hormone, *SFT* solitary fibrous tumor, *siADH* syndrome of inappropriate antidiuretic hormone, *SmCC* small cell carcinoma, *TSH* thyroid-stimulating hormone

Reference: [6]

Paraneoplastic Syndromes

By Marina K Baine and Natasha Rekhtman

Clinical syndrome	Associated tumor(s)	Mechanism
Cushing	SCLC, well-diff NET (particularly thymic carcinoid – ~50% have paraneoplastic Cushing, other sites – rare)	ACTH hypersecretion
SiADH (hyponatremia)	SmCC – pulmonary and extrapulmonary (accounts for the vast majority of cases)	ADH hypersecretion
Carcinoid syndrome (flushing, etc.)	Intestinal NET/carcinoid (unusual for the lung, thymic, or pancreatic NETs), usually develops with midgut NET/carcinoid metastatic to the liver (due to secreted mediators that bypass clearance in the liver or direct secretion into the hepatic vein)	Serotonin, kallikrein (ultimately converted to bradykinin)
Hypercalcemia	Solid tumors with squamous histology, esp. lung SqCC, breast cancer, Hodgkin and non-Hodgkin lymphoma, ATLL	PTHrP (squamous tumors), osteolytic metastases, 1,25-dihydroxyvitamin D (lymphomas), ectopic PTH
Hypoglycemia	PanNET (insulinoma), adult nesidioblastosis, SFT	Insulin hypersecretion
Acanthosis nigricans	Gastric adenoCA	TGF-α
Dermatomyositis	Adenocarcinomas of the cervix, lung, ovaries, pancreas, bladder, and stomach (70%); nasopharyngeal carcinoma (12%, particularly in Southeast Asian patients)	Immunologic
Necrolytic migratory erythema (NME)	PanNET (glucagonoma)	Glucagon hypersecretion
Amyloidosis	Multiple myeloma or Waldenstrom macroglobulinemia (lymphoplasmacytic lymphoma)	Aberrant immunoglobulin light chains
Lambert-Eaton syndrome	60% of SCLC, SqCC, other	Immunologic
Polycythemia	RCC (prototypic), HCC, hemangioblastoma, pheochromocytoma, uterine leiomyomas	Erythropoietin
Myasthenia gravis, pure red cell aplasia	Thymoma	Immunologic
Trousseau syndrome (migratory superficial thrombophlebitis)	Pancreatic, gastric, and lung adenocarcinoma	Unknown
Acromegaly (rare)	NETs (particularly well-differentiated) of multiple organ sites (lung and pancreatic>>duodenal>thymic), including those associated with MEN1 syndrome; hypothalamic tumors (hamartomas, choristomas, gliomas, and gangliocytomas)	Ectopic GHRH
Oncogenic osteomalacia	Phosphaturic mesenchymal tumors of the mixed connective tissue type (PMTMCT), hemangiopericytoma; rarely osteosarcoma and fibrosarcoma	FGF23 and other phosphaturic proteins

Abbreviations: *ACTH* adrenocorticotropic hormone, *ADH* antidiuretic hormone, ATLL adult T-cell leukemia/lymphoma, *FGF* fibroblast growth factor, *GHRH* growth hormone-releasing hormone, *NET* neuroendocrine tumor, *PanNET* pancreatic NET, *PTH* parathyroid hormone, *PTHrP* parathyroid hormone-related protein, *SCLC* small cell lung carcinoma, *SFT* solitary fibrous tumor, *SmCC* small cell carcinoma, *TGF* transforming growth factor

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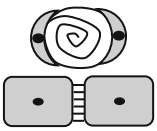
Chapter 14. Potpourri of Quick Morphologic References

By Natasha Rekhtman, Youran Zou, Marina K Baine, Justin A. Bishop

Tumor Differentials 101

Differentials 101: Generic Tumor Types[1]
(Main tumor types seen in ALMOST any organ)

Tumor type	Key features	Key immunostains
Carcinoma	The hallmarks of epithelial cells are cohesiveness (cells stick together), distinct cell borders, and usually abundant cytoplasm (cells resembling this description are called "epithelioid"). Main types of carcinoma are listed below:	Epithelial markers (CK, EMA) +
	Squamous cell carcinoma (SqCC)[2] The two hallmark features are as follows: 1. **Keratinization** – manifesting as keratin pearls/squamous eddies or isolated cells with glassy salmon-pink cytoplasm/dyskeratotic cells: 2. **Intercellular bridges** – desmosomes seen in the prickle cell layer of the epidermis: These two features may not be appreciable in nonkeratinizing or basaloid SqCC 	
	Adenocarcinoma[2] • Easy! All you need is gland (or papillae, micropapillae) formation, however focal. Don't be fooled by neuroendocrine or neuroblastic rosettes though, which can mimic glands. Also, some tumors can become discohesive and mimic true glands (e.g., acantholytic SqCC). • Intracellular mucin is another hint. Detection of mucin may be aided by a mucicarmine stain. 	
	Papillary carcinoma • Papillary carcinomas may be squamous, urothelial, or glandular, depending on the covering epithelium. Fibrovascular cores are a defining feature. • Note that papillary morphology applies to both in situ and invasive lesions (curiously, in situ lesions do not usually invade as papillary carcinomas – e.g., IPMN usually invades as a colloid CA, etc.). • DDx: ovarian serous CA, lung, kidney, thyroid. • Beware – not all that is papillary is a carcinoma (e.g., mesothelioma, myxopapillary ependymoma, papillary meningioma). • **Micropapillary CA** is a variant of papillary CA, which is defined by the absence of fibrovascular cores in the papillae (also typical are clear halos/retraction artifact around micropapillae). DDx includes the ovary (micropapillary serous CA), bladder, breast, lung, and salivary gland. Behavior is typically aggressive. Lymphovascular invasion is nearly universal. 	
Neuroendocrine (NE) neoplasm	This umbrella category encompasses NE neoplasms that are either low grade (e.g., carcinoid) or high grade (e.g., small cell CA, Merkel cell CA). Both types share a set of defining "NE features": • NE cytology: overall nuclear uniformity/monotony (even when high grade), stippled evenly distributed "salt and pepper" chromatin; absence of prominent nucleoli is key (although there are exceptions); low-grade lesions may have scattered cells showing "endocrine atypia," which manifests as large bizarre nuclei with smudged/hyperchromatic chromatin. • NE architecture: nests, trabeculae, ribbons, rosettes (subtle in high-grade lesions).	NE markers (SYN, CHR, CD56, INSM1); CK expression is type-dependent
Small cell carcinoma	Unless otherwise specified, this term implies small cell NE carcinoma, formerly known as "oat cell carcinoma" (note that there are small cell variants of melanoma and some carcinomas). • Despite the name, small size (<3 lymphocytes) is not the only defining feature. • Other key defining features are nuclear molding, very high N/C ratio, lots of mitoses, apoptotic bodies, and geographic necrosis. • Crush artifact with DNA streaming and DNA deposition in vessels ("Azzopardi phenomenon") are characteristic. • NE nature is evidenced by uniform distribution of chromatin (despite high grade), inconspicuous nucleoli, and occasional presence of trabeculae and rosettes.	NE markers (sometimes focal), CK+ (frequently focal), TTF1 often positive (regardless of organ of origin)
Melanoma	• Can look like anything: epithelioid, spindle cell, small cell, pink cell, clear cell, etc. (remember – melanoma is a "great mimicker" in pathology!) • Prominent cherry-red nucleoli and nuclear pseudoinclusions are characteristic. • Presence of melanin pigment is diagnostic, but make sure to distinguish it from hemosiderin and tattoo pigment; also some melanomas are amelanotic.	S100+, SOX10+, Melan-A+, HMB45+, MITF
Lymphoma	• Sheets of cells, ranging from normal lymphocyte-like (e.g., chronic lymphocytic leukemia) to large epithelioid cells (e.g., diffuse large B-cell lymphoma). • Large-cell lymphoma may be histologically indistinguishable from poorly differentiated carcinoma or melanoma. • General signature of lymphomas is cellular discohesion (cells fall apart) and clefted (indented) nuclei. • On high power (especially cytology), look for lymphoglandular bodies (cytoplasmic fragments).	CD45+, many other markers
Sarcoma	• Most commonly composed of spindle or stellate cells but can also look epithelioid, small round blue cell, clear/oncocytic, or highly pleomorphic. • Look for specific features of muscle, vascular, neural, or adipocytic differentiation (see below).	Highly variable, vimentin+ (but not specific)

1. In general, these generic tumors have similar morphology irrespective of the organ of origin, and the origin of metastasis cannot be determined without immunostains or clinical history. However, carcinomas of some organs do have a distinctive morphology. Here are a few notable examples:
   - Colon: tall pseudostratified nuclei and "dirty, garland necrosis"
   - Prostate (acinar type): low-grade and usually monomorphic nuclei with prominent nucleoli forming acini
   - Breast: relatively bland cytology, nests and glands (ductal), or solid sheet/single-file plasmacytoid cells (lobular)
   - Endometrioid: complex cribriform structures with pseudostratified columnar cells (some resemblance to colon), variable squamous differentiation
   - Endocervical: resembles endometrioid with prominent apoptotic bodies and apical mitoses
   - Clear cell RCC: nests of cells with clear or eosinophilic cytoplasm, blood-filled follicles, and complete vascular network surrounding each nest
2. Some tumors can have BOTH glandular and squamous differentiation (known in some organs as adenosquamous CAs); adenoCAs that commonly have a squamous component include pancreatic, endometrioid, lung (this is uncommon in the prostate, colon, breast)

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Differentials 101: Tumors by Cell Type	
Tumor type	*Differential*
Epithelioid tumors	Defined as round, plump cells with some cytoplasm (may be of different quality which generates other patterns as follows) resembling epithelium. DDx includes "carcinoma-melanoma-lymphoma-sarcoma" (the "Big 4"): 1. **Carcinoma**: look for evidence of glandular or squamous differentiation, however focal. Associated CIS/dysplasia seals the deal. 2. **Melanoma**: look for melanin pigment, nuclear pseudoinclusions, and cherry-red nucleoli. Associated melanoma in situ clinches the diagnosis. 3. **Lymphoma**: DLBCL, ALCL, plasmablastic lymphoma, etc. (these can even stain for cytokeratin and ALCL stains for EMA! Always keep these in DDx when encountering a poorly differentiated epithelioid tumor). 4. **Sarcoma**: think of epithelioid sarcoma, epithelioid hemangioendothelioma and angiosarcoma, myoepithelial tumor, epithelioid GIST, and others. **Other**: histiocytic neoplasms, germ cell tumors, epithelioid mesothelioma. Diagnosis usually requires immunostains. Typical initial panel includes CK for carcinoma, SOX10/S100 for melanoma, and CD45 for lymphoma (although some lymphomas may be negative for CD45).
Pink cell tumors (oncocytic/ eosinophilic cytoplasm)	Defined as epithelioid cells with abundant pink cytoplasm. DDx has significant overlap with epithelioid pattern Rhabdoid falls under this category but is specifically coined for cells with dense eosinophilic cytoplasmic inclusions that push the nucleus aside. 1. **Many carcinomas**: most notably HCC (look for bile pigment and lipid vacuoles), RCC (chromophobe or high-grade clear cell, look for nested pattern and prominent vascularity), adrenocortical CAs, thyroid Hurthle cell CA, parathyroid. 2. **Neuroendocrine tumors**: such as paraganglioma and oncocytic carcinoid/NETs 3. **Melanoma**: commonly has abundant pink cytoplasm (always think of melanoma in "pink cell tumor" DDx**.** 4. **Mesenchymal**: ASPS, PEComa, CCS, pleomorphic rhabdomyosarcoma, rhabdoid tumors, epithelioid angiomyolipoma. 5. **Other**: oncocytomas of various organs, granular cell tumor Cytoplasmic granularity in pink cell tumors may be due to the following: • Mitochondria – oncocytic neoplasms of the salivary gland or kidney, Hurthle cell neoplasms of the thyroid • Lysosomes – granular cell tumor • Zymogen granules – acinic cell CA of the salivary gland, acinar cell CA of the pancreas • NE granules – carcinoid tumor
Clear cell (CC) tumors	Defined as epithelioid cells with clear cytoplasm (glycogen, lipid, etc.). Again DDx has significant overlap with epithelioid pattern. First think carcinoma, but certain types of soft tissue tumors (e.g., CCS, PEComa) and very rarely melanoma and lymphoma can be clear. Ironically, many tumors with "clear cell" in their name can be very eosinophilic and have entirely oncocytic pattern rather than clear cell pattern and vice versa. The first-line differential is ccRCC (most common), adrenocortical CA, and ccHCC (uncommon). Complete list of CC neoplasms is vast (almost any carcinoma can have clear cell change, at least focally). Classic examples are: • *Head and neck*: oncocytic and Hurthle cell neoplasms (these are particularly prone to clear cell change), parathyroid, salivary gland neoplasms (e.g., CC carcinoma of salivary gland, myoepithelial tumors, oncocytic tumors, acinic cell, and mucoepidermoid CAs) • *Lung*: CC sugar tumor (PEComa), CC SqCC, or less commonly adenoCA • *GYN tract*: ovarian CC CA • *Soft tissue*: CCS, PEComa
Spindle cell tumors	Defined as cells with a long axis (like a spindle) in contrast to the round appearance of epithelioid cells. Remember: spindle cell melanoma and carcinoma are more common than sarcoma in general! Sarcoma diagnosis always comes after exclusion of carcinoma and melanoma (also mesothelioma in some locations)! Cytologic clues to differentiation in mesenchymal spindle cell neoplasms: • **Smooth muscle**: "cigar"-shaped (blunt-ended) nucleus with bubbly eosinophilic cytoplasm (fascicles intersect at right angles) • **Skeletal muscle**: pink cytoplasmic inclusions with cross-striations and "strap cells" (rhabdomyoblasts) • **Fibroblast/myofibroblast**: bipolar or stellate nucleus with pointy ends and wispy scant lightly eosinophilic cytoplasm myofibroblasts have more plump appearance, small nucleoli, and amphophilic cytoplasm indicating activated state • **Schwannian**: "club-" or "bullet"-shaped nuclei (pointed at one end), typically wavy (look for nuclear palisading) • **Perineurial**: cells are slender and spindled with delicate cytoplasmic processes (storiform pattern) • **GIST (pericyte)**: nucleus is intermediate between smooth muscle (box car) and Schwann cell (pointed). Nuclei can be ridiculously long Other morphologic clues to differentiation are (highly selected examples): • **Vascular**: channels or slit-like spaces with prominent hemorrhage or cytoplasmic vacuoles with RBCs = vascular differentiation (e.g., angiosarcoma or EHE) • **Adipocytic**: presence of lipoblasts. Ironically, some non-adipocytic tumors can have lots of fat (SFT/myofibroblastoma), while some adipocytic tumors (myxoid LPS/dedifferentiated LPS) may not show any fat; lipoblast is only required for diagnosis of pleomorphic LPS
Small round blue cell tumors	Defined as sheets of small round blue cells (duh!). Cells are blue because they have very little cytoplasm. Subtle morphologic hints may be present, but generally diagnosis requires immunostains +/– molecular studies and cytogenetics. DDx is age-dependent (see below).
Tumors with cytoplasmic vacuoles/inclusions	Can be split into several types: • Signet ring cell carcinoma (cytoplasmic mucin vacuole indenting the nucleus): gastric, some lobular breast, urothelial, lung CAs • Rhabdoid tumors (eosinophilic inclusion indenting the nucleus, cells typically discohesive): malignant rhabdoid tumors, carcinomas with rhabdoid component/de-differentiation • Lipoblastic/lipocytic differentiation: lipoblastoma, myxoid liposarcoma, pleomorphic liposarcoma • Intracytoplasmic lumina: vascular tumors such as epithelioid hemangioendothelioma, epithelioid hemangioma, and angiosarcoma may see RBCs in vacuoles, also called "blister" cells • Paranuclear vacuoles: GIST, smooth muscle tumors (bubbly cytoplasm)

Tumor Differentials 101: Tumors by Architectural Pattern (See Glossary in Chapter 16 for definitions)	
Pattern	*Differential diagnosis*
Hemangiopericytoma (HPC)-like pattern (branching staghorn-like vessels)	SFT/hemangiopericytoma (prototype), synovial sarcoma (particularly monophasic), myofibroma/myopericytoma, mesenchymal chondrosarcoma, nasal glomangiopericytoma, nasopharyngeal angiofibroma, MPNST, endometrial stromal sarcoma; may be seen in many other soft tissue lesions (not very specific)
Storiform pattern (cartwheel-like arrangement of cells)	DFSP (prototype), dermatofibroma, perineurioma, some undifferentiated pleomorphic sarcomas
Whorling pattern (concentric growth of tumor cells)	Meningioma (prototype), follicular dendritic cell sarcoma, rarely seen in angiomatoid fibrous histiocytoma, dedifferentiated liposarcoma, and inflammatory myofibroblastic tumor
Nested pattern (packets of cells with intervening stroma)	Neuroendocrine tumors such as pheochromocytoma/paraganglioma (prototype; nested pattern referred to as Zellballen in this setting), ccRCC, urothelial CA, melanoma, PEComa, CCS, and others
Alveolar pattern (nests with central discohesion)	ASPS and alveolar rhabdomyosarcoma (prototypes), nested neoplasms may appear alveolar (e.g., RCC)
Herringbone pattern (fascicles alternating at acute angles)	Fibrosarcoma (infantile or adult type), fibrosarcomatous transformation of DFSP, MPNST, synovial sarcoma, biphenotypic sinonasal sarcoma, spindle cell rhabdomyosarcoma, spindle cell pattern of adamantinoma (rare)
Basaloid tumors (resembling basal cell carcinoma)	Basal cell CA (prototype), basaloid SqCC, HPV-related SqCC, adnexal tumors, adenoid cystic CA, and others
Nuclear palisading in spindle cell tumors	Schwannoma (prototype), smooth muscle tumors, GIST
Biphasic tumors (epithelial and stromal components)	Malignant (both components): sarcomatoid CAs/carcinosarcomas (including the bladder, lung, uterus, etc. – any epithelial organ), biphasic synovial sarcoma, biphasic malignant mesothelioma, pulmonary blastoma, biphasic Wilms tumor, and others Malignant (stroma): malignant phyllodes tumor, Müllerian adenosarcoma Benign: fibroadenoma (breast), adenofibroma/adenomyoma (GYN tract), cystic neoplasms with ovarian-type stroma (mucinous cystic neoplasm of the pancreas, mixed epithelial stromal tumor of the kidney, and others) – stromal cells are ER+, benign mixed tumor (skin/soft tissue), or pleomorphic adenoma (salivary gland)

Abbreviations: *ALCL* anaplastic large-cell lymphoma, *ASPS* alveolar soft part sarcoma, *CCS* clear cell sarcoma of soft tissue, *DFSP* dermatofibrosarcoma protuberans, *DLBCL* diffuse large B-cell lymphoma, *EHE* epithelioid hemangioendothelioma, *GIST* gastrointestinal stromal tumor, *LPS* liposarcoma, *MCC* Merkel cell carcinoma, *MPNST* malignant peripheral nerve sheath tumor, *SFT* solitary fibrous tumor

Potpourri of Differentials	
Tumors with prominent lymphocytes	Carcinoma associated with microsatellite instability (GI, endometrial), seminoma, lymphoepithelioma (LE) and LE-like carcinomas, thymoma, inflammatory myofibroblastic tumor, follicular dendritic cell sarcoma, clear cell CA of ovary, HPV+ oropharyngeal CA
Tumors with prominent neutrophils	Hodgkin lymphoma, neutrophil-rich anaplastic large-cell lymphoma, inflammatory leiomyosarcoma, anaplastic CA of the thyroid, anaplastic CA of the pancreas and lung, sarcomatoid renal cell CA, medullary CA of the kidney, NUT carcinoma
Tumors with prominent eosinophils	Hodgkin lymphoma, Langerhans cell histiocytosis, mast cell tumors, myeloid sarcoma (chloroma), glassy cell carcinoma of the cervix, thyroid sclerosing mucoepidermoid CA with eosinophilia, epithelioid hemangioma (i.e., angiolymphoid hyperplasia with eosinophilia)
Tumors with prominent mast cells	Very non-specific but can be seen in synovial sarcoma, neurofibroma, spindle cell lipoma, myxoid liposarcoma, hemangiopericytoma, hairy cell leukemia (particularly in bone marrow), and others (anything myxoid often has accompanying mast cells)
Tumors with extravasated erythrocytes	Kaposi sarcoma, angiosarcoma, nodular fasciitis, inflammatory myofibroblastic tumor, sinonasal glomangiopericytoma
Tumors associated with granulomas	Classic associations – seminoma, Hodgkin lymphoma, lymphomatoid granulomatosis, Lennert lymphoma, also some carcinomas (e.g., reaction to keratin in SqCC or endometrioid CA with squamous differentiation)
Intranuclear pseudoinclusions	Papillary thyroid CA, hyalinizing trabecular tumor of thyroid, medullary thyroid CA (50%), melanoma, meningioma, pheochromocytoma, lung adenoCA, usual ductal hyperplasia, and others
Hyaline globules	Non-specific but classic associations are yolk sac tumor, Kaposi sarcoma, solid-pseudopapillary tumor of the pancreas, HCC, clear cell CA of GYN tract
Psammoma bodies	Any papillary carcinoma (papillary thyroid CA, serous ovarian CA, papillary CA of the lung, papillary RCC), metanephric adenoma, meningioma (and normal meninges), mesothelioma (and benign mesothelial proliferations in peritoneum), duodenal somatostatinoma
Tumors with melanin pigment	Melanoma (#1, 2, and 3 in the differential), clear cell sarcoma of soft tissue (melanoma of soft parts), Bednar tumor (pigmented dermatofibrosarcoma protuberans; produced by intermixed dendritic cells, not tumor cells). Other neural crest-derived tumors occasionally produce melanin (e.g., melanotic schwannoma, melanotic medulloblastoma)
Mucinous and myxoid tumors	Mucin production is a common feature of carcinomas and soft tissue tumors. Soft tissue mucins are referred to as "myxoid material" to distinguish them from biochemically distinct epithelial mucin. Mucin production is vanishingly rare in melanoma and lymphoma. Colloid CA refers to tumors composed of mainly mucin with only few scattered tumor cells (usually as rows lining colloid at the periphery and floating in mucin as small, inconspicuous clusters). **Common sites of mucinous CA**: the bowel, appendix, ovary, pancreas, lung, breast. Colloid CAs generally considered clinically indolent (except the colon and ovary, mixed data on the lung) **DDx of myxoid soft tissue and bone tumors**: almost ANY soft tissue tumor can be myxoid, at least focally. Major players are intramuscular myxoma, myxofibrosarcoma, myxoid liposarcoma, low-grade fibromyxoid sarcoma, extraskeletal myxoid chondrosarcoma, neurofibroma (myxoid change common), spindle cell lipoma, nodular fasciitis and abdominal fibromatosis (sometimes myxoid), chondromyxoid fibroma, chordoma **Other tumors that may have myxoid change**: malignant mesothelioma, pleomorphic adenoma, myxopapillary ependymoma (filum terminale)
Tumors with squamoid morules (have nuclear β-catenin staining)	Endometrioid CA (low grade), craniopharyngioma (adamantinomatous type), cribriform-morular variant of papillary thyroid CA, pancreatoblastoma, pulmonary blastoma/well-differentiated fetal adenoCA of the lung, basal cell adenoma/adenoCA of salivary glands

Benign Mimics of Malignancy 101 – Watch Out!	
Pleomorphic tumors that are actually NOT high grade (degenerative-type atypia)	Classically, endocrine and NE neoplasms ("NE atypia"), schwannomas ("ancient change"), renal oncocytoma, pleomorphic xantroastrocytoma (PXA), atypical fibroxanthoma (AFX), uterine leiomyomas ("symplastic" change), pleomorphic hyalinizing angiectatic tumor (PHAT). A clue to degenerative nature of atypia is smudgy chromatin and absence of atypical mitoses.
Benign proliferations which may have perineural invasion	Breast sclerosing adenosis, endometriosis, vasitis nodosa, Leydig cell tumors and normal Leydig cells, prostate "benign perineural involvement" (tumor apposed but not surrounding a nerve), pyloric gland metaplasia in the gallbladder, pancreatic islet cells, granular cell tumor
Benign tumors which may have isolated vascular invasion	Pleomorphic adenoma [1, 2], pheochromocytoma/paraganglioma, granular cell tumor [3], giant cell tumor of bone, giant cell tumor of tendon sheath, renal oncocytoma [4]
Benign tumors which may invade the bone (bone invasion ≠ malignancy)	Meningioma, pituitary adenoma, inverted Schneiderian papilloma (extension into the bone occurs as a result of pressure erosion and by itself is not an indication of malignancy)
Benign inclusions in lymph nodes	Müllerian (endometriosis, endosalpingiosis, endocervicosis), nevus (intracapsular location), salivary gland, thyroid (somewhat controversial), mesothelial, breast (heterotopic tissue or benign mechanical transport due to procedure or massage; usually from papillary lesions), renal tubules in patients with large Wilms tumor, lymphangioleiomyomatosis
Benign tumors that can metastasize (!)	Pleomorphic adenoma, uterine benign metastasizing leiomyoma, ameloblastoma, benign fibrous histiocytoma, chondroblastoma, giant cell tumor of the bone, meningioma, pulmonary sclerosing pneumocytoma (formerly sclerosing hemangioma) [5]

Differentials 101: Small Round Blue Cell Tumors (SRBCT)					
Diagnosis	*Age/clinical*	*Location*	*Histologic clues*	*Key immunostains*	*Cytogenetics*
SRBCTs of Adults					
Lymphoma	Any age (type-dependent)	Lymph nodes and any extra-nodal site	No molding (cells are discohesive)	CD45+, CD20 (B cell) or CD3 (T cell), other	Various translocations
Small cell NE carcinoma	Older adults, ectopic hormones, early mets	Any organ	Molding, no nucleoli, "salt and pepper" chromatin, prominent necrosis NE architecture: rosettes, trabeculae (usually subtle)	CK+, NE markers+, TTF-1+ (lung and some non-lung), Neurofilament–, CK20– (opposite to MCC)	
Merkel cell carcinoma (MCC)	60–70 yo	Dermis Head and extremities	Molding, "dusty" vesicular chromatin Rosettes and trabeculae (occasionally)	CK+, NE markers+, always TTF-1–, neurofilament+, CK20+ (punctate), Merkel cell polyomavirus antigen+	
SRBCTs of Young Adults (and Some Children)					
Desmoplastic small round cell tumor (DSRCT)	Mean age 21, M:F = 4:1; rare tumor	Serosal cavities (peritoneum, pleura)	Angulated nests of SRBCs in desmoplastic stroma	WT1+ (C-terminus antibody), CK+, EMA+, NSE+, desmin+/actin–	t(11;22) *EWSR1-WT1*
Synovial sarcoma, poorly differentiated	Any age, typically young adults; mean age 26; 20% <20 yo	Most commonly extremities but can occur almost in any site	High-grade SRBCT, distinction from Ewing sarcoma or other round cell sarcoma usually requires cytogenetics or molecular study	TLE1+, CK/EMA–/+ (patchy at best), poorly-differentiated cases usually CD99+	t(X;18) *SS18- SSX1/2* A small subset can be *SS18* FISH negative but would be positive by other methods such as RT-PCR
Olfactory neuroblastoma (esthesioneuroblastoma)	Bimodal peaks: ages 15 and 55	Roof of nasal fossa (cribriform plate)	Similar to abdominal neuroblastoma: fibrillar rosettes and fibrillar stroma (neuropil), ganglion cells generally absent	NE markers+ (SYN most sensitive), CK can be focal but EMA always –, sustentacular cells S100+, calretinin+	
Small cell osteosarcoma	Bimodal age peaks: 20s and 50s	Around knee (distal femur, proximal tibia)	Tumor osteoid required for diagnosis	SATB2+	
Mesenchymal chondrosarcoma	Typical range 10–40yo (peak 20s and 30s)	Axial skeleton, also in soft tissue	Difficult to recognize if chondroid area is not present	SOX9+ (non-specific), S100+ in chondrocytes	*HEY1-NCOA2*
High-grade myxoid (round cell) liposarcoma	Peak incidence in the 30s	Deep soft tissue of extremities (thigh)	Subtle chicken-wire vessels; variable number of lipoblasts (not required for diagnosis but helpful in recognition)	S100+ in adipocytes (immunostains not helpful for the diagnosis; diagnosis requires cytogenetic or molecular tests)	t(12;16) or t(12;22) *FUS* (or *EWSR1*)-*DDIT3*

Continued on next page…

SRBCTs of Children (and Some Adults) – 2[1]					
Diagnosis	*Age/clinical*	*Location*	*Histologic clues*	*Key immunostains*	*Cytogenetics*
Lymphoblastic lymphoma (LBL) (>80% are T cells)	Peaks in adolescence, rare in adults Boys>>girls #1 pediatric malignancy (together with leukemia)	Thymus (>50%), nodes, spleen, and others	Dense medium-size lymphocytes, blastic ("fine lacey") chromatin, inconspicuous nucleoli No molding (cells are discohesive) Many mitoses, sometimes "starry sky" pattern (similar to Burkitt lymphoma)	CD45 variable, TdT+, CD34+ CD3+ (if T cell) frequently CD99+	Various translocations
Myeloid sarcoma (leukemic infiltrate outside of marrow, aka chloroma)	Wide age range, may be de novo or have history or concurrent leukemia or myeloid disorders	Skin, lymph nodes, and bone but many more organs can be involved	Diffuse, monotonous mononuclear cells that often infiltrate background structures; cells have blastic chromatin with variable nucleoli and scant cytoplasm	CD43+, myeloperoxidase+, lysozyme+, can be CD99 + (pitfall)	Various translocations
Neuroblastoma	Peak age 2 yrs, 90% by age 8, rare in young adults #1 solid extracranial malignancy and #3 overall malignancy (after leukemia/lymphoma and CNS) in kids	Adrenal medulla, sympathetic ganglia	Fibrillar stroma (neuropil) and fibrillar (Homer Wright) rosettes Ganglion cells and Schwannian stroma in better differentiated tumors No molding (cells are evenly spaced apart)	NE markers+, PHOX2B (new marker)	Poor prognosis: N-*myc* amplification, -1p, +17q Good prognosis: age < 1 year, hyperdiploidy
Ewing sarcoma (ES)/PNET (primitive neuroectodermal tumor)[2]	Mean age 11–15 yo, but can occur at any age; rare in ages <5 and > 30 Presents as rapidly growing painful mass. Skeletal form clinically mimics osteomyelitis.	(1) Skeletal: lower extremities and pelvis (2) Soft tissue: paravertebral, extremities, retroperitoneum	Monomorphic uniform cells Vesicular (open) chromatin +/– Homer Wright rosettes Cytoplasmic vacuoles (glycogen/PAS+) No neuropil outside rosettes and no ganglion cells (unlike neuroblastoma)	CD99+, NKX2.2+, NE markers +/–, some may be + for ERG (do not confuse for vascular lesion)	t(11;22) *EWSR1-FLI1* – 90% t(21;22) *EWSR1-ERG* 5% Many more rare fusions
***CIC*-rearranged sarcoma**	Mean age, 24; range 6–62	Trunk and extremities, viscera, rarely the bone	Cytology slightly more atypical than ES. Most have geographic necrosis; some have myxoid changes or spindling of cells	ETV4+ (negative in other SRBCTs), WT1+, variable CD99 (diffuse in only 20% of cases in contrast to ES)	t(4;19) or t(10;19) *CIC-DUX4*
***BCOR*-rearranged sarcoma**	Mean age 15; range 2–44 M>>F Reference: [6]	Bone>>soft tissue>viscera	Round and spindle cells. Resembles poorly different synovial sarcoma	BCOR+, CCNB3+, SATB2+, Cyclin D1+, TLE1+	inv(X) *BCOR-CCNB3* t(X;4) *BCOR-MAML3*
Alveolar rhabdomyosarcoma, solid variant	Peak age 9, can occur up to age 30 (older than embryonal) #1 pediatric sarcoma	Deep muscles of extremities; trunk (distinct from embryonal)	Look for hints of myogenic differentiation: pink cytoplasmic inclusions (cross-striations are rarely evident) and multinucleated wreath-like giant cells Dense chromatin (unlike ES) Cells are discohesive	Desmin+ (can highlight cross-striations), MyoD+, myogenin+ (usually diffuse)	t(2;13) *PAX3- FOXO1* t(1;13) *PAX7- FOXO1*
Wilms tumor (nephroblastoma), blastema predominant	Peak age, 3.5 yo; range 3 mo–6 yrs.; always >3 mo and <16 yrs. of age #1 pediatric renal tumor	Kidney	May see areas with classic triphasic histology Molding present (unlike lymphoma, neuroblastoma) [7]	WT1+	11p13 (*WT1* gene) deletion/mutation, Trisomy 12
Medulloblastoma	Peak age 7 yo; usually <20 yo (70% under age 16)	Cerebellum	High-grade SRBCT Homer Wright rosettes Sometimes nodular architecture	SYN+	Isochromosome 17q
Retinoblastoma	Young children	Retina	Flexner-Wintersteiner rosettes	CRX/OTX3 (new marker)	13q14 (*RB* gene) deletion/mutation
Hepatoblastoma, small-cell variant	90% in kids under age 5	Liver	Diagnosis requires areas of better-differentiated hepatoblastoma		

1. DDx also includes small cell osteosarcoma and mesenchymal chondrosarcoma (see SRBCTs of Young Adults). Note that not all "blastomas" are pediatric small round cell tumors: for example, pulmonary blastoma and hemangioblastoma are tumors of adulthood that are non-SRBCT.

2. Ewing sarcoma (ES) and PNET (peripheral primitive neuroectodermal tumor) are now regarded as morphological manifestations of one tumor type; both are characterized by t(11;22) translocation. In general, there are usually more neuroendocrine features in PNET, whereas ES is thought to be a more undifferentiated tumor. However, there is a considerable overlap in clinical presentation, morphology, and prognosis, and most pathologists no longer separate them. In fact, the term PNET was retired in the most recent soft tissue WHO.

General comment: While not entirely specific (like most stains or morphologic features), many of the soft tissue sarcomas that are associated with specific molecular abnormalities have a distinct cytologic monotony that can serve as a subtle tip. Rare exceptions do occur: some translocation-associated tumors can be pleomorphic (such as myxoinflammatory fibroblastic sarcoma); rarely synovial sarcoma or Ewing sarcoma may show more pleomorphism if they harbor *TP53* mutations.

Abbreviations: *SRBCT* small round blue cell tumor

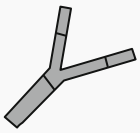
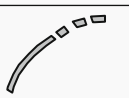
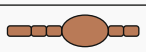
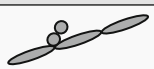
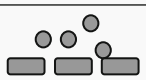
There's a Fungus Among Us! Quick Reference for Histologic Identification of Fungi

Fungi are encountered in two major settings: (1) surface dwellers and colonizers (e.g., mucocutaneous candidiasis and fungus ball, respectively) and (2) infections involving visceral organs/soft tissue. The latter category includes two types of fungi: (1) **opportunistic** (occurring in immunocompromised host) vs. **pathogenic** (able to infect immunocompetent host). Classic opportunistic fungi include *Pneumocystis* and *Zygomycetes*. Classic pathogenic fungi include dimorphic fungi, which exist as molds in nature and yeast in tissue: *Histoplasma* (*Histo*), *Blastomyces* (*Blasto*), *Coccidioides* (*Cocci*), and *Paracoccidioides*. *Cryptococcus* (*Crypto*) is predominantly opportunistic. When you encounter a fungus in visceral organs, your DDx should vary according to the patient's immune status.

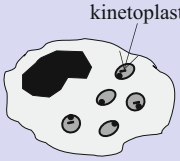
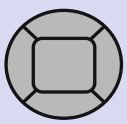
Fungi are generally inconspicuous in H&E sections and are best visualized by "pan-fungal" stains – GMS and PAS – although some larger fungi (*Blasto*, *Cocci*, *Zygomycetes*) are readily visible in H&E. *Crypto* can also be at least suspected on H&E. The sizes of a RBC and a lymphocyte nucleus are ~7 μm; these may be used as a handy-size reference. The most common histologic response to fungi is granulomatous inflammation, but some may manifest with other features, such as granulomas with neutrophils (*Blasto*), granulomas with eosinophils (*Cocci*), or frothy intra-alveolar exudate (*Pneumocystis*).

Organism or disease	Appearance (GMS or PAS)	Organism – key features	Typical tissue reaction	Comment
Budding Yeast in Tissue				
Histoplasma capsulatum	 "tiny critters in a macrophage (MF) and in tissue"	– 2–5 μm – Narrow-based "teardrop" budding (difficult to see in tissue) – Pseudocapsule (faint halo due to retraction artifact) on Giemsa – no true capsule – Mostly oval shapes – Predominantly intracellular but usually spill into surrounding tissue, where organisms tend to remain in clusters – DDx: *Crypto*, *Pneumocystis*, *Candida*, *Penicillium* (rare), and small intracellular protozoa (*Leishmania*)	– Granulomas with fibrocaseous "infarct-like" necrosis – Old lesions typically hyalinize/calcify	– Ohio-Mississippi river valley – Carrier: birds and bats ("cave fever") – Sites: lung, GI, disseminated – Organisms can stain extremely pale with GMS – look closely!
Cryptococcus neoformans	 "soap bubbles"	– 2–15 μm – Narrow-based budding – Highly variable size (unlike *Histo* or *Blasto*, which are uniform) – Variable shape: spherical and elongated (football-shaped) forms – Polysaccharide capsule (mucicarmine+, PAS+, alcian blue+), but some organisms are capsule-deficient. India ink + (historic use only) – Cell wall contains melanin pigment (Fontana-Masson+; pigment not apparent on H&E). Note that positive melanin stain is not entirely specific for *Crypto* since *Cocci*, *Blasto*, and *Sporothrix* can also be positive	– Granulomatous inflammation (+/– necrosis) – Histiocytes with bubbly cytoplasm (where organisms are usually visible by H&E)	– Carrier: pigeons (droppings) – Sites: meningitis, the lung, other deep infections
Blastomyces dermatitidis	 "snowman"	– 8–15 μm – Broad-based budding – Thick double walls ("double contour"), multinucleation – Cell walls can be weakly positive for mucin stains	– Granulomas with neutrophils	– Ohio-Mississippi river valley – Sites: lung, skin, bone, disseminated
Paracoccidioides brasiliensis (aka South American blastomycosis)	 "mariner's wheel"	– 5–30 μm (wide size variation is characteristic) – Large spherule with multiple peripheral narrow-based buds (although diagnostic, the multiple budding cells are usually inconspicuous)		– Africa, Central and South America – Sites: skin, bone, mucous membranes (mimics *Blasto*)
Sporothrix schenckii	 "cigar bodies"	– 2–6 μm – Round or elongated "cigar-shaped" budding yeast, usually rare and difficult to find in tissue – "Asteroid bodies" (Splendore-Hoeppli phenomenon) – crystalline structures representing antigen-antibody complexes. Classic for *Sporo* but not specific		– "Rose-gardener's disease" – Sites: SubQ
Non-budding Spherical Fungi in Tissue				
Coccidioides immitis	 "bag of marbles"	– Thick-walled spherule (50–200 μm) packed with endospores (2–5 μm) – Endospores frequently spill into the surrounding tissue and may resemble *Histoplasma* (but there is no budding) – **DDx:** • *Rhinosporidium*: nasal fungus, much larger than *Cocci*. GMS+ • Myospherulosis: surgical packing material with entrapped RBCs in the nose/sinus. GMS–, PAS–, hemoglobin+	– Granulomas with eosinophils	– Southwest American deserts ("valley fever") – Sites: lung, skin, disseminated
Penicillium marneffei	 "tiny critters in a MF"	– 2–4 μm – Elongated cells with septae (divides by fission, not budding) – predominantly intracellular (like *Histo*) – Mimics *Histo* (but *Penicillium* is non-budding and has no pseudocapsule)		– Southeast Asia – AIDS patients
Pneumocystis jiroveci (formerly *P. carinii)*	 "teacup and saucer" in frothy exudate	– 5–8 μm cyst (seen by GMS), 1–3 μm trophozoites (seen by Giemsa) – Non-budding organisms – GMS: round and crescent (sickle)-shaped cysts (described as a "cup-and-saucer" or "crushed ping-pong balls") with two parenthesis-shaped dots (these are part of the cyst wall) – Giemsa or Diff-Quik: intracystic (up to eight) and free-roaming trophozoites	– Frothy alveolar exudate (but ~10% have a granulomatous response)	– Sites: lung – AIDS patients
Chromoblastomycosis	 "copper pennies"	– 6–12 μm – Brown (melanin-containing) organisms; Fontana-Masson+ – Thick-walled spheres with horizontal and vertical septae ("copper pennies," "medlar bodies," "sclerotic bodies")	– Overlying pseudoepithelio-matous hyperplasia is typical	– Sites: SubQ

There's a Fungus Among Us! Quick Reference for Histologic Identification of Fungi – 2

Organism or disease	Appearance (GMS or PAS)	Key histologic features	Comment
Hyphae in Tissue			
Aspergillus spp. and others **Hyalohyphomycoses** (septate nonpigmented molds, e.g., *Fusarium*)	 "slingshots "	– Thin (2–5-µm-thick) hyphae WITH septae ("septate hyphae") – Frequent dichotomous narrow-angle (45^0) branching (Y-shaped) – When invasive, tends to be angioinvasive – Hyphae tend to grow in a radial "sunburst"-like fashion – Occasional fruiting bodies (in aerated sites) – Definitive diagnosis requires cultures because in tissue, *Aspergillus* is indistinguishable from other hyaline mold, including *Pseudallescheria boydii* and *Fusarium* (both resistant to amphotericin B). Definitive morphologic speciation possible only if diagnostic fruiting bodies are present, which is rare.	Types of *Aspergillus*-related diseases include: 1. Vaso-invasive infections (sinus, lung, disseminated) in immunocompromised host. 2. Allergic bronchopulmonary aspergillosis and allergic fungal sinusitis in atopic host (eos + tigroid mucin+Charcot Leyden crystals) 3. Aspergilloma/mycetoma/fungus ball = colonization of cavities (such as sinuses or cavitary lung disease) 4. Less well-defined form is "subacute" = limited tissue invasive infection in mildly immunocompromised host (like diabetic), occurs in the sinus and lung.
Zygomycetes (*Rhizopus*, *Absidia*, *Mucor)* Disease: Zygomycosis = Mucormycosis = Phycomycosis	 "wide ribbons"	– Wide (6–50-µm-thick) hyphae with INFREQUENT or absent septae – Wide-angle (90^0) branching (branching less frequent than *Aspergillus*) – Undulating, twisting (ribbonlike), often fractured, "empty-looking" hyphae – Angioinvasive – Stain weakly with GMS and PAS; organisms best visualized by H&E – Definitive diagnosis requires culture because treated or degenerating *Aspergillus* may look like *Zygomycetes* *Note regarding the terminology: these organisms are frequently referred to collectively as "Mucor" in pathology, but *Mucor* is only one of several organisms (and not even the most common) in this group.	Aggressive vaso-invasive disease (sinus, disseminated) in immunocompromised host. This is a life-threatening emergency. Why distinguishing *Zygomycetes* vs. *Aspergillus* is important: 1. *Zygomycetes* are more aggressive 2. *Zygomycetes* are treated with amphotericin B. They are resistant to most azoles (except posaconazole).
Dermatophytes (*Microsporum* spp., *Epidermophyton* spp., *Trichophyton* spp.)		– Septate hyphae with rare branching that break into segments (arthroconidia) – 2–3 µm thick – Hyphae confined to the skin, nails, hair	Superficial infections of the and hair ("tinea" or "ringworm")
Phaeohyphomycosis (pigmented molds)		– Septate branching hyphae; may resemble *Aspergillus* in tissue though are often thinner with less branching, have constrictions at their frequent septae and vesicular swellings – Contain melanin (Fontana-Masson+) – Brown pigment sometimes (but not always) evident on H&E	SubQ and deep infections
Yeast and Hyphae in Tissue			
Candida spp.	 "sausage links and yeast"	– 3–5 µm budding yeast – 5–10 µm pseudohyphae: elongated budding yeast joined end-to-end like "sausage links"; occasionally true hyphae (no constrictions) are present – *C. glabrata* is unique in that it does not produce any hyphae; it may mimic *Histo* and other small yeast	Mucocutaneous and deep infections
Pityriasis versicolor *(Malassezia furfur)*	 "spaghetti and meatballs"	– 3–8 µm budding yeast (meatballs) and 5–10 µm fragmented hyphae (spaghetti) often arranged end-to-end – Involves epidermis only, only rarely seen in tissue (skin scraping preferred method of diagnosis)	Site: skin only
Mold-Like Branching Filamentous Bacteria			
Nocardia asteroides		– Delicate narrow (1 µm) beaded filaments; right-angle branching – Gram+, modified AFB (Fite)+, GMS+ – DDx includes *Streptomyces* (AFB–)	Deep infection in immunocompromised host
Actinomyces israelii	 "dust bunnies"	– Delicate narrow (<1 µm) branching filaments intertwined in a dense radiating meshwork – "Sulfur granules" (grossly yellow flecks; do not, in fact, contain sulfur) – Gram+, AFB–, GMS+	Normal commensal inhabitant of the oral cavity May become pathogenic in oropharynx with local tissue damage (such as dental work), may cause draining sinus tracts IUD-related infections

There's a Fungus Among Us! Quick Reference for Histologic Identification of Fungi – 3

Yeastlike Organisms in Tissue			
	Organism	*Key histologic features*	*Comment*
Protozoa	***Leishmania spp*** 	– 2–4 µm round to oval aflagellate amastigotes (extravascular form of organisms) – Amastigotes are intracellular – Transverse paranuclear bar-like kinetoplast – *Leishmania* is a close mimic of *Histoplasma* (look for kinetoplast) – Organisms stain lightly in H&E – GMS–, PAS–, Giemsa+	Visceral leishmaniasis (kala-azar): – Middle East, Africa, India – Sites: reticuloendothelial system (liver, spleen, bone marrow) Cutaneous leishmaniasis: Old World ("oriental sore") and New World ("chicle ulcer") Mucocutaneous leishmaniasis: Central and South America
	Trypanosoma cruzi	– Organisms in tissue look identical to *Leishmania* spp. – *T. gambiense* and *T. rhodesiense* (African trypanosomiasis) are confined to blood and do not invade tissue	*T. cruzi* (Chagas disease): – Central and South America – Usual sites: heart, colon, esophagus
	Toxoplasma gondii	– 5–7 µm crescent-shaped tachyzoites (non-encysted organisms in tissue) – 10–50 µm pseudocysts packed with 2–3 µm round bradyzoites – Basophilic in H&E (unlike yeast) – GMS+, PAS+, Giemsa+	– Worldwide disease, cat vector – Sites: disseminated disease (especially brain) in immunosuppressed patients
	Cryptosporidium	– 2–6 µm round organisms in the brush border of small bowel mucosa – Giemsa+	Chronic diarrhea in immunosuppressed patients
	Cyclospora	– 8–10 µm oocysts in stool – Modified acid fast or safranin stain (stool)+ – Autofluorescence+	
	Cystoisospora belli	– 25–30 µm elliptical organisms interposed between adjacent enterocytes – Giemsa+	
	Microsporidium	– 1–3 µm round organisms in the cytoplasm of enterocytes – Invisible by H&E – Gram+	
Algae	***Prototheca* spp.** 	– 2–12 µm – Sporulating forms are sporangia with up to 20 polygonal or wedge-shaped endospores whose cell walls mold together ("morulas") – GMS+, PAS+	– Two human infections: cutaneous (usually immunosuppresed) and olecranon bursitis (usually otherwise healthy with a history of trauma)
References: [8–10]			

Quick Reference for Histological Identification of Viruses

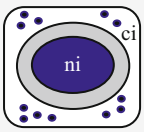
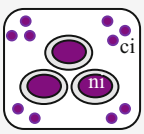
In general, nuclear inclusions are associated with DNA viruses (HSV, CMV, adenovirus, JC, and BK viruses). One major exception is CMV in that in addition to nuclear inclusions, it also forms cytoplasmic inclusions. Some DNA viruses do not have any recognizable cytopathic effects (EBV, HHV8). Note that HPV does not manifest as inclusions but has a unique cytopathic effect (see below).

RNA viruses as a rule do not have recognizable cytopathic changes; few do have cytoplasmic inclusions (RSV, Negri bodies in rabies). Measles is an exception in that it is an RNA virus that forms nuclear inclusions.

Nuclear inclusions of virally infected cells fall into two morphologic categories:

1. **Cowdry type A**: eosinophilic "owl-eye" nuclear inclusion (as in CMV)
2. **Cowdry type B**: (aka "smudge cells") nucleus with a "homogenized, ground glass" chromatin and obliterated nuclear detail

Note that most DNA viruses (HSV, adenovirus) can have nuclear inclusion of either Cowdry A and/or Cowdry B type even within the same lesion. Exception is CMV in that it forms exclusively type A inclusions.

Virus	Appearance	Nuclear inclusions	Cytoplasmic inclusions	Specific features	Infected cell type	Clinical
HSV	 "eggs in a basket" or "pomegranate seeds"	+ (Cowdry A or B), pink, steel gray, or purple	–	The 3 M's: **M**ultinucleation, **M**olding, **M**argination of chromatin (peripheral clearing or "halo effect")	Squamous and some glandular epithelial cells (look at the periphery of an ulcer on mucosal surfaces)	Gingivostomatitis and genital lesions in immunocompetent host. Opportunistic infection of any body site (pneumonia, esophagitis, neurons in encephalitis)
CMV	 "owl-eye nuclear inclusion and cytoplasmic speckles"	+ (Cowdry A), blue	+ (blue speckles)	Nuclear and cytoplasmic enlargement Nuclear inclusion has a prominent halo ("owl eye"), which corresponds to marginated chromatin pushed aside by viral particles.	Stromal and endothelial cells (look at the ulcer base); rarely in epithelial cells	Opportunistic infection of any body site (lung, bowel, retina, neurons in encephalitis)
Adenovirus		+ (Cowdry A or B), blue	–		Epithelial cells (bronchial cells and pneumocytes in the lung)	Opportunistic infections (bladder, kidney, lung, bowel)
HPV	 "koilocyte"	–	–	Clear perinuclear vacuole (Greek *koilos* = hollow), wrinkled (raisin-like) nucleus, binucleation (common), condensed keratohyaline granules typical in skin	Squamous cells	Papillary lesions (warts, condyloma, laryngeal papillomas) and squamous dysplasia and intraepithelial neoplasia (genital organs and rectum)
JC and BK (polyoma)	 "decoy cell"	+ (Cowdry B), blue	-	Nuclear enlargement Non-haloed smudgy (type B) inclusion	JC – brain BK – urothelium (mimics CIS in urine; "decoy" cells)	JC – PML BK – cystitis in immunosuppressed
Measles	 "Warthin-Finkeldey giant cell"	+ (Cowdry A or B), pink	+ (pink speckles)	Giant cells with multinucleation	Depends on the site: -Lung: epithelial cells, most commonly bronchial -Lymph node: lymphoreticular cells (infected cells in lymph node are called Warthin-Finkeldey giant cells) -Brain: oligodendroglia	Pneumonitis, lymphadenitis, SSPE
RSV		–	+ (large pale-pink globs)	Giant cells with multinucleation No nuclear inclusion (this is an RNA virus)	Epithelial cells	Bronchiolitis and pneumonia in children (rarely biopsied)

Abbreviations: *ni* nuclear inclusion, *ci* cytoplasmic inclusion, *PML* progressive multifocal leukoencephalopathy, *SSPE* subacute sclerosing panencephalitis

Tumor Viruses: Quick Reference for Tumor/Viral Associations

Detection of viral molecules is a very helpful adjunct in the diagnosis of the virally induced tumors. In tissue sections, viral proteins can be detected by immunohistochemistry (e.g., EBV-LMP), or viral nucleic acids may be identified by in situ hybridization/ISH (e.g., EBER ISH and HPV by DNA-ISH and more recently RNA- ISH, which is much more sensitive). p16 is NOT a viral protein but an endogenous cell cycle protein that is markedly overexpressed as a result of high-risk HPV infection. In cervical cytology, the specimens are tested for HPV by a DNA-based method (hybrid capture).

Virus	Tumor associations		Detection in tissue
EBV	**Epithelial lesions:** – Nasopharyngeal carcinoma (NPC), aka lymphoepithelial carcinoma (= lymphoepithelioma [LE]) and LE-like carcinomas: ○ EBV (+): NPC and LE-like carcinomas of upper aerodigestive tract (lung, thymus, salivary gland) and stomach ○ EBV (−): LE-like carcinoma of non-aerodigestive tract (bladder, breast, skin, cervix) – Oral hairy leukoplakia – Gastric adenocarcinoma (5%)		1. EBER (EBV encoded early RNA). Most sensitive marker for EBV (in situ hybridization method). IDs all EBV-related tumors. 2. EBV-LMP (late membrane protein). Less sensitive than EBER. IDs PTLD and AIDS-related lymphomas, variable in NPC, Hodgkin, and Burkitt lymphoma, usually negative in plasmablastic lymphoma 3. EBNA (EBV nuclear antigen). Least sensitive marker. IDs PTLD and AIDS-related lymphomas only.
	Lymphoid/heme lesions: – Infectious mononucleosis – Posttransplant lymphoproliferative disease (PTLD) – Classic Hodgkin lymphoma (Mixed cellularity – 70%, AIDS-related) – Non-Hodgkin lymphoma: ○ Burkitt lymphoma (endemic 100%; sporadic 20%) ○ Nasal-type NK/T-cell lymphoma (>95%) ○ Aggressive NK cell leukemia ○ Angioimmunoblastic T-cell lymphoma ○ Lymphomatoid granulomatosis (>95%) ○ CNS lymphoma in AIDS (95%) ○ Plasmablastic lymphoma (HIV) ○ Primary effusion lymphoma (has both EBV and HHV8) ○ EBV+ DLBCL – Germinotropic lymphoproliferative disorder (has both EBV and HHV8) – Inflammatory pseudotumorlike follicular dendritic cell sarcoma – EBV-positive mucocutaneous ulcer		
	Smooth muscle tumors in immunosuppressed (AIDS, transplant)		
HPV	**Female genital tract:** – Squamous dysplasia and carcinoma of the cervix, vagina, vulva (simplex/differentiated VIN and associated SqCC occur in the setting of lichen sclerosus and other dermatoses in older women and are HPV-unrelated)	HSIL and associated SqCC caused by high-risk HPV (16, 18, 31, 33) LSIL is caused by – low-risk HPV (6, 11) in 20% – high-risk HPV in 80% (therefore, high-risk HPV does not distinguish HSIL and LSIL)	1. In situ hybridization for HPV (DNA or RNA) 2. IHC for p16 is a surrogate marker of high-risk HPV Detection of HPV may be used to identify anogenital or oropharyngeal origin of metastatic SqCC of unknown primary. HPV-related SqCC of some (but not all) sites have basaloid morphology: – Sites where HPV-related SqCC are basaloid: oropharynx, penis, vulva – Sites where HPV-related SqCC are either basaloid or conventional: cervix, anus – Sites where basaloid SqCC are unrelated to HPV: breast, lung, non-oropharyngeal head and neck
	– Cervical adenocarcinoma (in situ and invasive)	HPV 18 > 16	
	Penis: – Squamous cell carcinoma, warty and basaloid type (verrucous and papillary SqCC are HPV-unrelated)	HPV 16	
	– Bowenoid papulosis and Erythroplasia de Queyrat	HPV 16	
	Anus: Squamous neoplasia (in situ and invasive) – analogous to cervix	HPV 16, 18	
	Head and neck: – Squamous cell carcinoma of the oropharynx (tonsil and base of tongue)	HPV 16, 18	
	– Laryngeal papillomatosis	HPV 6, 11	
	– Focal epithelial hyperplasia (Heck disease) of oral mucosa	HPV 13, 32	
	– Sinonasal HPV-related multiphenotypic CA	HPV33 and others (not 16 or 18)	
	Mucocutaneous: – Warts (verruca)	HPV 1, 2, 4, 7	
	– Condyloma acuminatum (genital sites)	HPV 6, 11	
HHV8	Kaposi sarcoma, primary effusion lymphoma (also has EBV), germinotropic lymphoproliferative disorder (also has EBV), Castleman disease (multicentric)		HHV8 can be detected by IHC
HTLV1	Adult T-cell leukemia/lymphoma		
Hepatitis B	Hepatocellular carcinoma (Hep C causes HCC indirectly – virus is not present in tumor cells)		HBsAg, HBcAg – rarely used for tumor Dx
Merkel cell polyomavirus	Nearly all Merkel cell carcinomas		Viral antigen can be detected in Merkel cell CA by IHC, (−) in small cell carcinoma

Quick Electron Microscopy Reference for Tumors and Select Non-tumor Diagnoses

By Marina K Baine

General Cell Types	
Epithelial	Desmosomes
Neuroendocrine	Neurosecretory (dense-core) granules
Fibroblast	Abundant rough endoplasmic reticulum
Muscle	Actin filaments
Skeletal muscle	Ribosome-filament complexes Z-band
Smooth muscle	Dense bodies (subplasmallemal) Filaments
Endothelial	Weibel-Palade bodies (elongated, "pear-shaped" storage granules with microtubule-like inclusions appearing striated) Pinocytotic vesicles
General Tumor Types	
Carcinoma	Desmosomes (tight junctions)
Adenocarcinoma	Short luminal ("intestinal type") microvilli Extra- and intracellular lumina Mucin granules Tight junctions
Squamous cell carcinoma	Well-formed intercellular junctions Tonofilaments
Melanoma	Pre-melanosomes and melanosomes
Mesothelioma	Long and thin microvilli Tight junctions Long desmosomes Perinuclear tonofilament bundles
Lymphoma	Abundant polyribosomes Paucity of organelles Devoid of cell junctions
Sarcoma	Absence of true desmosomes and true lumens (most) (Other features vary depending on subtype)
Leukemia	Lineage dependent
Lymphoid	Scant cytoplasm Free ribosomes and polyribosomes Paucity of cell organelles
Myeloid	Abundant cytoplasm Prominent Golgi and rough endoplasmic reticulum +/– Azurophilic granules
Specific Tumor Types and Tumorlike Lesions with Distinctive EM Findings	
Leydig cell tumor	Reinke crystals
Sertoli cell tumor	Charcot-Bottcher crystals
Alveolar soft part sarcoma	Membrane-bound rhomboid crystals with a lattice pattern with a 10-nm periodicity in fibrils (crystal precursor is Golgi) Numerous electron dense vesicles near Golgi
Granular cell tumor	Pleomorphic secondary lysosomes Basal lamina around cell groups Angulate lysosomes ("Gaucher-like") in stromal fibrohistiocytic cells
Schwannoma	Luse bodies (long-spaced collagen) Reduplicated basal lamina
Langerhans cell histiocytosis	Birbeck granules ("tennis racket")
Rosai-Dorfman disease	Emperipolesis of nucleated and nonnucleated blood cells (also seen on light microscopy)
Selected Storage Disorders	
Gaucher disease	Angulated lysosomes
Tay-Sachs disease	Laminated (concentric structure of membranous cytoplasmic bodies)
Niemann-Pick disease	Zebra bodies (aka myelin figures)
Fabry disease	Zebra bodies (aka myelin figures)
Selected Viruses	
VZV/HSV/CMV	Bull's eye appearance in cytoplasm (indistinguishable by EM)
Adenovirus (and other non-enveloped viruses)	Honeycomb (viral particles arranged in paracrystalline arrays)
Papovavirus	Spaghetti and meatballs (virions are both filamentous and spherical)

Chapter 15. Quick Size References

By Marina K Baine, Justin A. Bishop and Natasha Rekhtman

Criteria for "Micro-entities" in Various Organs	
Diagnosis	**Size criteria**
Breast metastases to lymph nodes	Isolated tumor cells: ≤0.2 mm (or ≤200 cells) Micrometastasis: >0.2 mm but <2 mm (and/or >200 cells)
Lung, atypical adenomatous hyperplasia (AAH)	≤0.5 cm (+ low-grade cytology)
Lung, carcinoid tumorlet	<0.5 cm
Gastric microcarcinoid (ECL cell)	<0.5 cm
Pancreatic neuroendocrine microadenoma (formerly islet cell microadenoma)	<0.5 cm
Pituitary microadenoma versus **macroadenoma**	≤1 cm versus >1 cm (this is generally a clinical distinction)
Renal cell papillary adenoma	≤1.5 cm
Thyroid papillary microcarcinoma	≤1 cm (and incidentally found)
Thyroid micromedullary carcinoma	≤1 cm

Criteria for Microinvasion in Various Organs	
Site	**Size criteria for microinvasion**
Breast	≤1 mm
Cervix, squamous cell carcinoma **Stage IA1** **Stage IA2**	Diagnosed by microscopy only, i.e., no grossly visible lesion in a specimen with negative margins ≤3 mm deep and ≤7 mm horizontal extent >3 but ≤5 mm deep and ≤7 mm horizontal extent
Ovary, serous borderline tumor[a]	<5 mm
Ovary, mucinous borderline tumor	<5 mm
Salivary gland, carcinoma ex-mixed tumor	<4–6 mm beyond the tumor capsule (minimally invasive [1])
Upper aerodigestive tract	1–2 mm below the basement membrane
Lung (minimally invasive adenocarcinoma)	≤5 mm focus of invasion in a lepidic-predominant tumor (former bronchioloalveolar carcinoma) that is ≤3 cm in overall size (recently introduced category) [2]
[a]In the 2014 WHO classification, microinvasion is defined as a focus of <5 mm composed of eosinophilic bland glandular cells lining the surface of the lesion. However, when the invasive focus is composed of solid nests or cribriform glands, it should be called "microinvasive carcinoma," rather than serous borderline tumor with focal microinvasion [3]	

Normal Organ Weights/Measurements		
Organ	**Male**	**Female**
Brain	1179–1621 g	1033–1404 g
Thyroid	30–70 g	
Parathyroid (single)	30–40 mg	
Heart	233–383 g	148–296 g
Right lung	155–720 g	101–589 g
Left lung	112–675 g	105–515 g
Liver	968–1860 g	603–1767 g
Spleen	28–226 g	<230 g
Right kidney	81–160 g	38–174 g
Left kidney	83–176 g	35–192 g
Adrenals (combined)	7–10 g	
Testes	5 × 2 × 3 cm, 20–27 g	–
Prostate (by age) 20–30 years 31–60 years 61–80 years	Average 15 g 20 g 40 g	–
Ovaries	–	4 × 3 × 2 cm
Uterus	–	~60 g
For pediatric organ weights, please use Reference [4]		References: [5–11]

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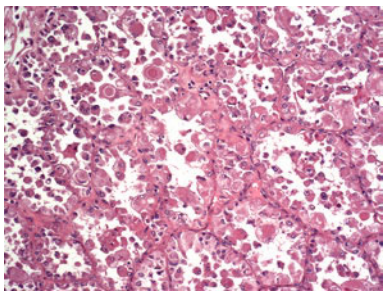
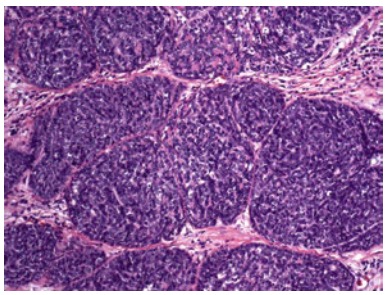
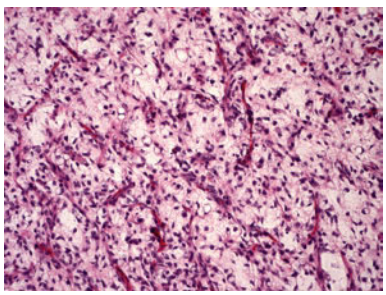
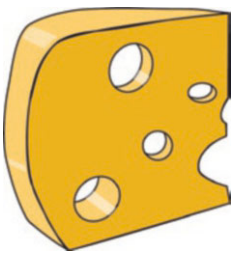
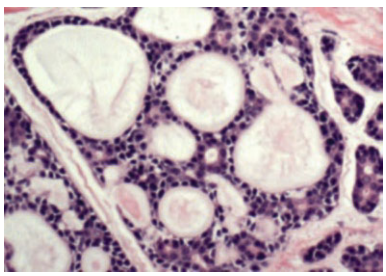
Chapter 16. It Looks Like What? An Illustrated Glossary of Histopathologic Descriptors

By Natasha Rekhtman, Kathryn L Villa, Xiaojun Wu

Color illustrations in the definitions column by Terry Helms and Susan D Weil © Memorial Sloan Kettering Cancer Center 2011 and 2018.
Also see Chapter 2 in *The Practice of Surgical Pathology: A Beginner's Guide to the Diagnostic Process, 2nd Edition* by Diana Molavi.
Thanks to Diana Molavi for many helpful discussions for this section.

Part 1. Solid Tumors

By Natasha Rekhtman and Kathryn L Villa

Descriptors of Architectural Patterns		
Term	*Definition*	*Example(s)*
Alveolar	Resembling lung alveoli. A pattern seen in some tumors in which nests of cells are centrally discohesive (fall apart) and paucicellular, thereby appearing like empty spaces in the lung alveoli.	 Alveolar soft part sarcoma (shown), alveolar rhabdomyosarcoma
Basaloid	Resembling basal cell carcinoma (BCC) in that tumor cells are small, tightly packed, and grow as islands with (or without) peripheral palisading, similar to BCC. Basaloid morphology is typical of some squamous cell carcinomas of the head and neck.	 Basaloid squamous cell carcinoma
Chicken wire	Branching ("crow feet"-like) and anastomosing network of vessels, typically seen in liposarcoma and oligodendroglioma. Calcifications in chondroblastoma are also described as chicken wirelike 	 Vessels in myxoid liposarcoma
Cribriform	Perforated like a sieve; having "Swiss cheese"-like spaces. Origin: Latin *cribrum*, sieve. 	 Adenoid cystic carcinoma (shown), cribriform DCIS

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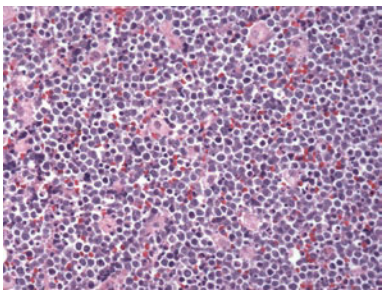
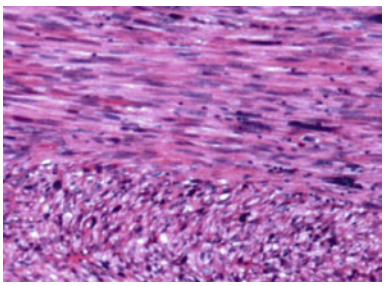
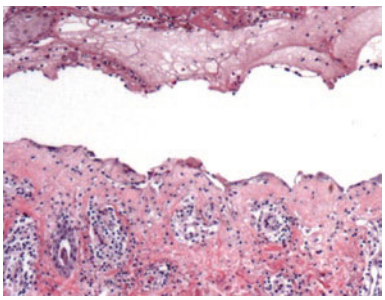
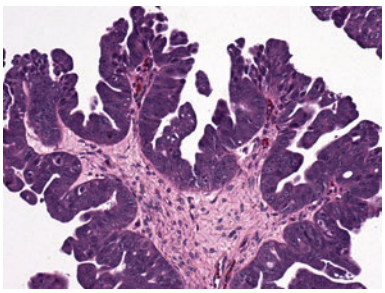
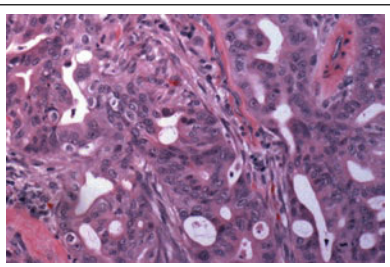
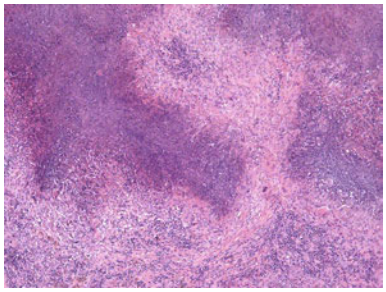
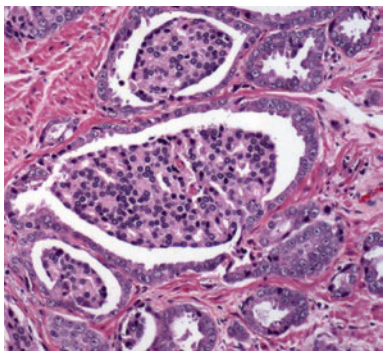
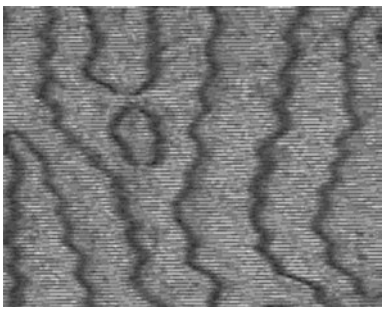
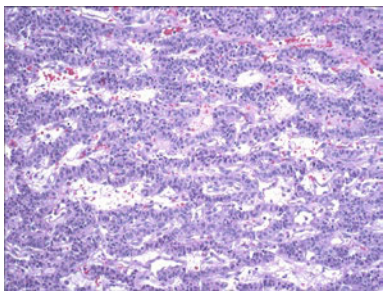
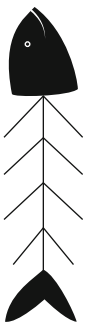
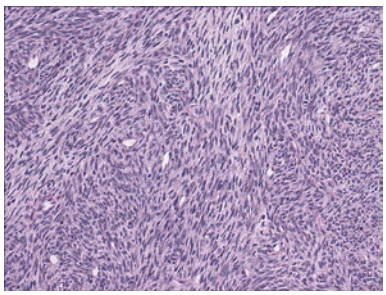
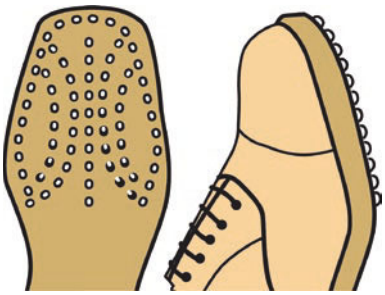
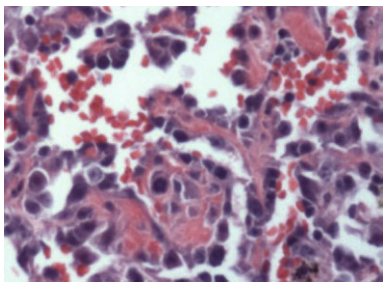
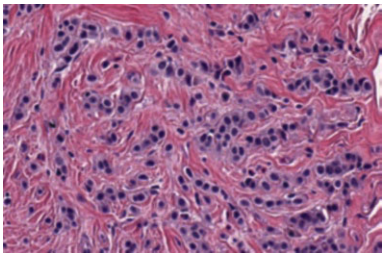
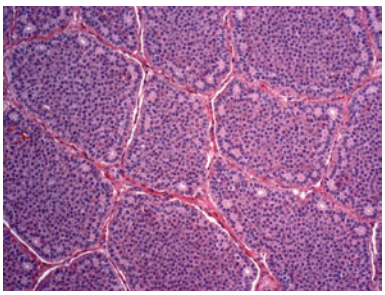
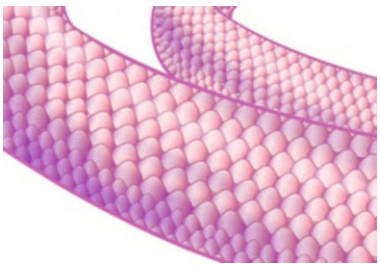
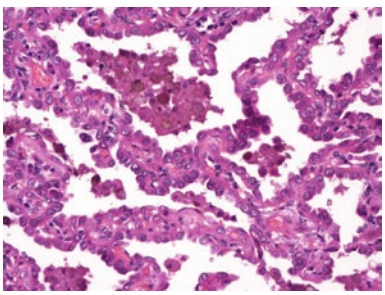
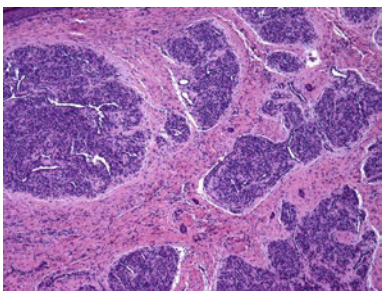
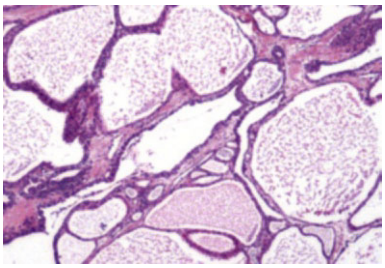
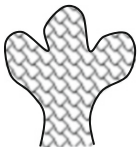
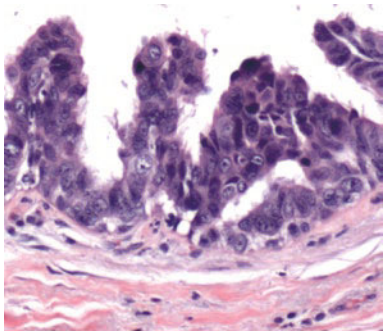
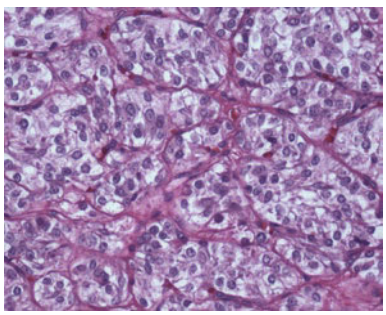
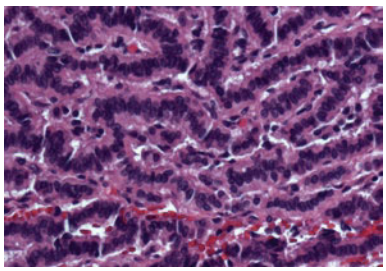
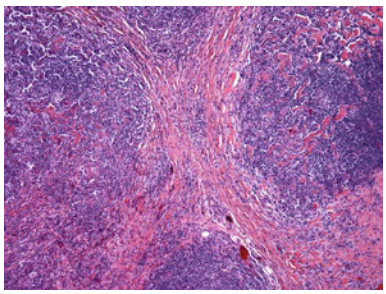
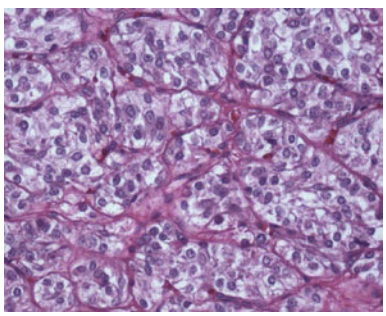
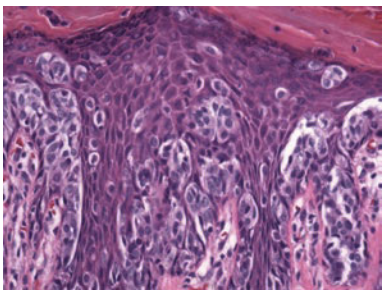
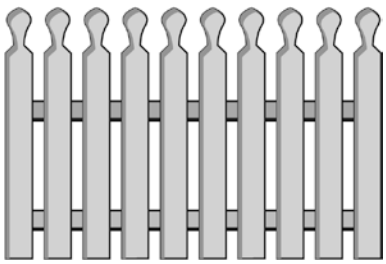
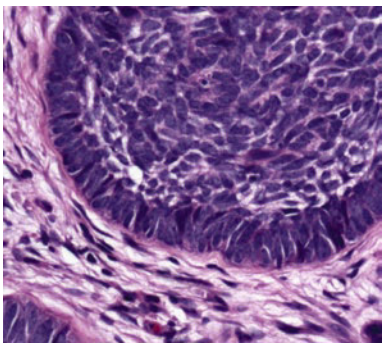
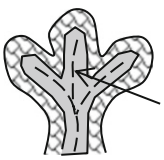
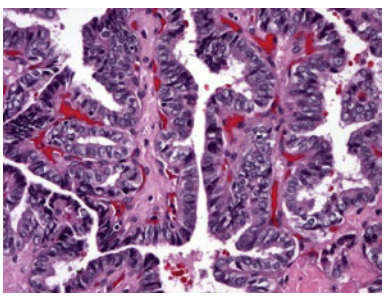
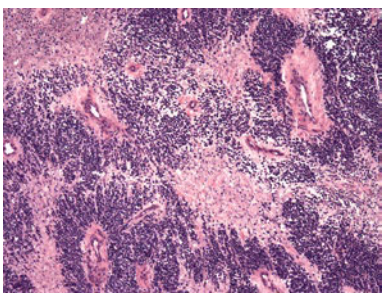
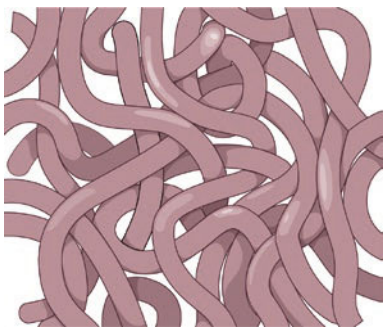
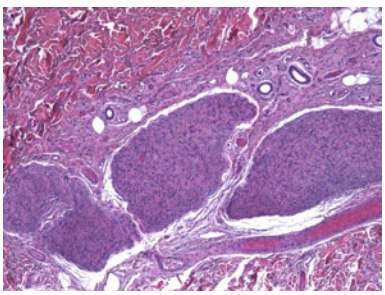
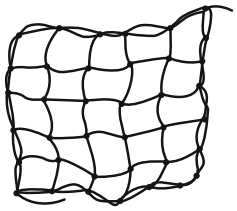
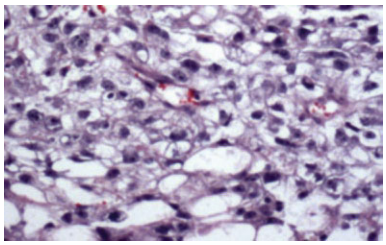
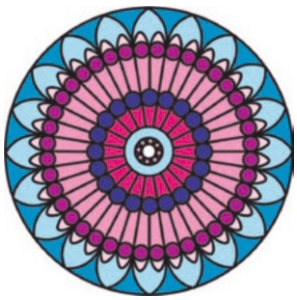
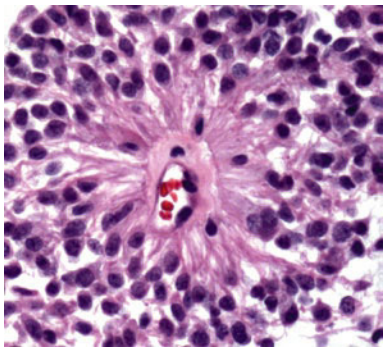
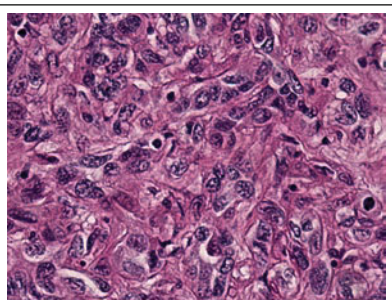
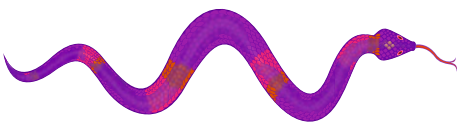
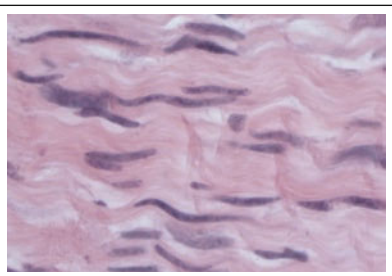
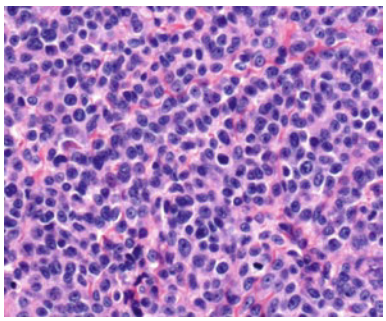
Discohesive (dyshesive)	Falling apart into single cells. In a setting of a poorly differentiated neoplasm, discohesion suggests a lymphoma.	 Lymphoma (shown), LCIS
Fascicular	Fascicle – bundle of cells (as in muscle fascicle). Generally used to describe a bundle of spindle cells streaming in unison (like the school of fish). Fascicles may intersect perpendicularly, as in smooth muscle tumors, or at acute angles, as in fibrosarcoma (see "herringbone" pattern).	 Leiomyosarcoma
Festoon-like **Garland-like**	Undulating appearance, as in a festoon or a garland (an ornament suspended between two points). "Festoon pattern" is used to describe the projection of dermal papillae into blister cavity in some bullous skin diseases as well as undulating cell ribbons in carcinoid tumors and granulosa cell tumors. "Garland necrosis," where undulating collars of tumor project into necrotic center, is typical of colon cancer and epithelioid sarcoma. 	 Pemphigus vulgaris
Filigree-like	Complex intertwining threads (from Latin *filum*, thread), as in filigree style of jewelry. The term is most commonly used to describe complex intertwining papillae in micropapillary serous carcinoma of the ovary (aka the "medusa-head pattern"). In addition, the term has been applied to a pattern of infiltration, wherein the tumor cells are invading as complex cords (such as filigree pattern in Ewing sarcoma). 	 Micropapillary serous carcinoma
Glandular	Forming glands (easy!). This is a defining feature of adenocarcinoma. Sometimes "tubular" and "ductal" are used synonymously with glandular to describe an adenocarcinoma.	 Adenocarcinoma of the colon

Geographic	(As in geographic necrosis): large confluent areas of necrosis with an irregular outline resembling outlines of a continent on a map (where water would = viable tumor) 	 Granulomatosis with polyangiitis (Wegener) (shown), small cell carcinoma
Glomeruloid	Resembling a glomerulus: tufts or tangles of vascular or epithelial structures protruding into a clear space. Glomeruloid epithelial structures are diagnostic of prostate cancer, and glomeruloid vascular proliferation is a hallmark feature of glioblastoma.	 Glomeruloid structure in prostate cancer
Gyriform, watered silk, "moiré" pattern	Ribbons or cords of cells that undulate and/or form loops, resembling a topographic map (courtesy of Diana Molavi). From the Greek *gyros*, a circle. 	 Carcinoid tumor (shown), granulosa cell tumor
Herringbone pattern	A pattern resembling the spine of a herring, characterized by alternating cellular fascicles intersecting at acute angles. Prototype is fibrosarcoma, but this pattern may be seen in other neoplasms (e.g., MPNST, synovial sarcoma).  	 Fibrosarcoma
Hobnail	Resembling a large-headed nail (like the ones used to protect the soles of shoes). Describes cells that project into a lumen of a vessel or a gland. 	 Angiosarcoma (shown), clear cell carcinoma of GYN tract

Indian-file (single file)	Cells arranged in linear rows as if one following in the footstep of the other. The term refers to the "notion of Amerindian people's way of walking along a trail" [1] 	 Lobular breast carcinoma
Insular	Literally, resembling an "island" (insula); basically a term for large nests.	 Carcinoid with an insular growth pattern
Lepidic	Scalelike (from Greek *lepis*, scale). The term used to describe the superficial (scalelike) growth pattern of lung adenocarcinoma (formerly called bronchioloalveolar carcinoma). Metastatic mucinous carcinomas to the lung, particularly from the pancreas, can assume this growth pattern! 	 Lepidic adenocarcinoma
Lobular	Referring to an anatomic unit (as in breast lobule). When describing a lesion, the term implies that the lesion has a smooth (non-infiltrative) contour, conforming to or resembling normal anatomic structures. Sometimes used synonymously with nodular. Lobular breast carcinoma relates to the anatomic origin rather than the growth pattern.	 Lobular capillary hemangioma
Microcystic	Tightly packed small cysts, honeycomb-like	 Serous cystadenoma of the pancreas, microcystic

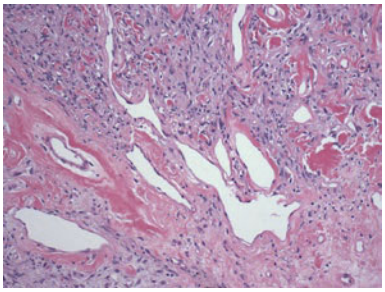
Micropapillary	Papillary-shaped epithelial projections **without** a true fibrovascular core (cells surround avascular cores) 	 Micropapillary DCIS
Nested	Packets or groups of cells separated by stroma	 Pheochromocytoma
Neuroendocrine (NE)	**NE pattern** is defined by two components: architecture and cytology. **NE architecture** refers to formation of nests, ribbons/trabeculae, and rosettes (this is vaguely reminiscent of ribbons and nests formed by normal neuroendocrine structures, such as the pancreatic islets of Langerhans). **NE cytology** refers to cells being monotonous/uniform (not overtly pleomorphic like most carcinomas). Cells have finely stippled "salt and pepper" chromatin with NO prominent nucleoli (see below under "salt and pepper").	 Carcinoid (trabecular/ribbonlike pattern)
Nodular	Refers to a discrete collection of cells with a smooth rounded border. Generally nodules refer to larger cell aggregates than nests	 Nodular sclerosis Hodgkin lymphoma (shown), multinodular thyroid hyperplasia
Organoid	Cells showing some features of organization (into nests, glands, papillae, etc.) rather than growing as sheets – i.e., epithelial organ-like. In an unknown malignancy, the presence of organoid structures argues against a lymphoma. DDx includes carcinoma (most common), few types of sarcoma (most notably alveolar soft part sarcoma), and also melanoma. In practice, the term is used almost exclusively to describe cells aggregating into nests in neuroendocrine neoplasms (pheochromocytoma, carcinoid). The reason for this selective use is completely unclear to us. More intuitively, the term is also used to describe structures resembling various normal organs in a teratoma.	 Pheochromocytoma

Pagetoid	Resembling Paget disease of the nipple in that the large malignant cells ascend singly or in nests in the epidermis in a buckshot-like fashion. True Paget disease (an eponym; named after the person who described it) must be differentiated from other lesions with "pagetoid growth": Bowen disease (squamous cell carcinoma in situ) and melanoma in situ.	 Paget disease of the nipple
Palisading	Cells lining up in parallel arrays; resembling a picket fence. Peripheral palisading is a defining feature of basal cell carcinoma. Palisading in a spindle-cell neoplasm is classic for schwannoma (but a few other tumors, such as smooth muscle neoplasms, can palisade). Also histiocytes may palisade in so-called palisading granulomas (as in a rheumatoid nodule) Origin: French *palissade*, a fence of stakes 	 Basal cell carcinoma
Papillary	Fronds of fingerlike projections containing fibrovascular cores (stroma with blood vessels) that support the overlying epithelium. If papillary structures grow endophytically, the process is called "inverted" (as in inverted papilloma of the bladder or sinuses) Origin: papilla, *nipple* (Latin) or nipple-like protrusion  FV core = Stroma + Vessels	 Papillary thyroid carcinoma
Peritheliomatous	An older term for "perivascular." The term "peritheliomatous growth" relays that the tumor cells are viable around vessel but are necrotic away from vessels. May be a feature of melanoma, small cell carcinoma, or any other very high-grade neoplasm that outgrows its blood supply.	 Melanoma
Plexiform	Resembling or forming a plexus (like the brachial plexus); interwoven network; said to resemble a "bag of worms." Generally a macroscopic term. Origin: from Latin *plexus*, braid. 	 Plexiform neurofibroma

Reticular or **retiform**	Having form of a net, netlike; resembling a complex lattice or spider web. May resemble an irregular honeycomb with spaces of variable size and shape. The term is applied to connective tissue (reticular framework in liver, spleen, lymph nodes) as well as to describe a pattern in neoplasms (classically a feature of yolk sac tumors) Origin: from Latin *rete*, net 	 Yolk sac tumor, reticular pattern
Rosette (also see table below)	Origin: rosette – structure with a circular arrangement of parts radiating out from the center resembling the petals of a rose. In pathology, "rosette" is a term used to describe structures with cells arranged radially around a central point. Different from a gland in that basally cell are not supported by a basement membrane (but merge with the rest of tumor cells) See table below for details on rosettes and pseudorosettes  Window rosette in a cathedral	 Ependymoma (perivascular pseudorosette)
Sarcomatoid	Term generally applied to spindle-cell growth in a carcinoma (or mesothelioma)	 Sarcomatoid renal cell carcinoma
Serpiginous or **serpentine**	Snake (serpent)-like: undulating, wavy 	 Wavy nuclei of neurofibroma (shown) wavy spaces in usual duct hyperplasia of the breast
Sheetlike or solid pattern	A sea of back-to-back cells with no particular architecture	 Any poorly differentiated malignancy (lymphoma [shown], carcinoma, melanoma, sarcoma)

Staghorn (hemangiopericytoma-like) **vessels**

Branching thin-walled vessels that have staghorn or antler-like shapes. Prototype is hemangiopericytoma (HPC).

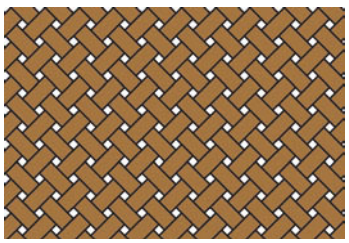


Solitary fibrous tumor

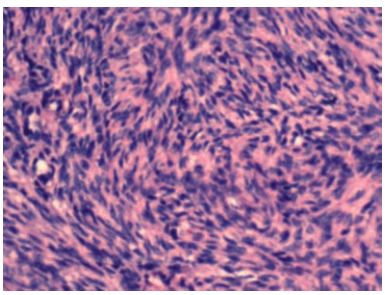
Storiform

Short fascicles of spindle cells that intersect or intertwine at various angles, thereby resembling the weaving of a **doormat** OR **cartwheel**, **pinwheel**, or **starburst.**

Storea is Latin for woven straw mat [2]



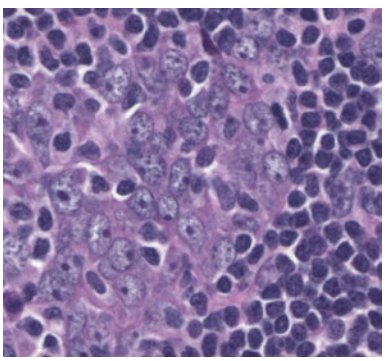
The storiform pattern is also said to resemble a **cartwheel**, **pinwheel**, or **starburst** in that intersecting spindle cells have radial orientation emanating from a common point



Prototype is DFSP (shown): both the doormat-like and cartwheel-like arrangement of cells can be discerned (with some imagination)

Syncytial

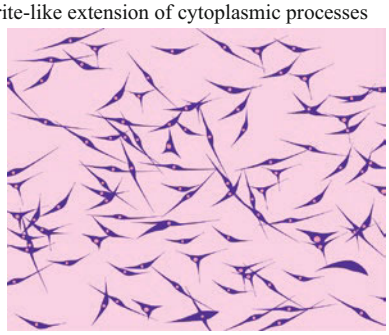
From Greek: *syn-* – "together." Tissue characterized by cytoplasmic continuity or having indistinct cell borders. Prototypical examples are skeletal muscle and granulomas. Also a characteristic feature of several tumors



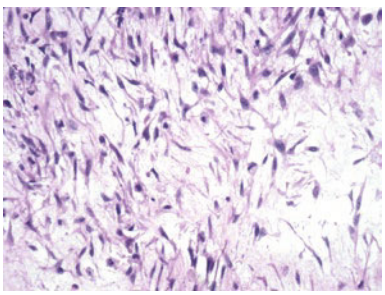
Lymphoepithelioma-like carcinoma (shown), medullary carcinoma of the breast

Tissue culture-like growth pattern

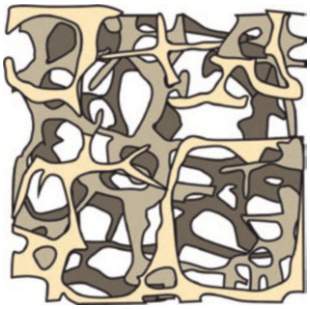
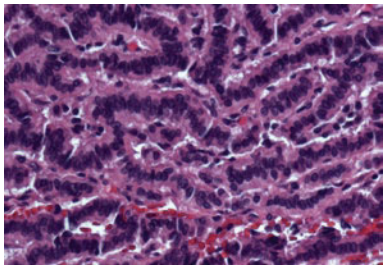
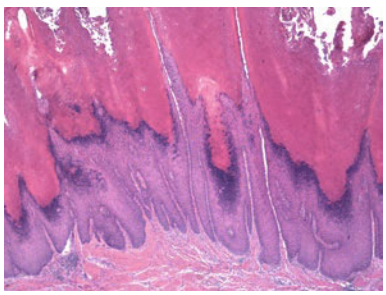
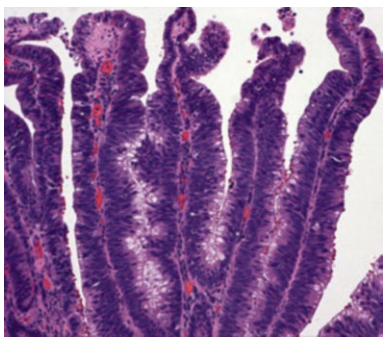
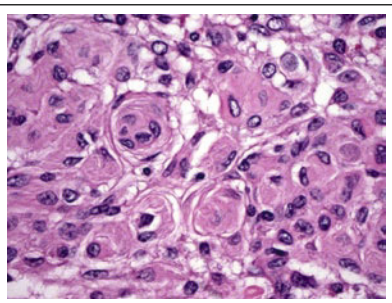
Pattern resembling the growth of cultured fibroblasts in a tissue culture plate: spindle cells are loosely arranged and are randomly oriented; cells have plump nuclei and dendrite-like extension of cytoplasmic processes



Cultured fibroblasts growing in a Petri dish



Inflammatory myofibroblastic tumor (shown), nodular fasciitis

Trabecular	Origin: Latin *trabes*, beam-like.[3] The term is used to describe the "beam-like" arrangement of cells in rows, cords, ribbons, or strands; cells may form parallel arrays or tram-tracks (as in trabecular pattern in carcinoid tumors). In addition, the term describes the beam-like strands of connective tissue (as in splenic trabeculae) or macroscopic beam-like structures (as in trabecular bone).  Trabecular bone	 Carcinoid, trabecular pattern
Verrucous Verrucoid Verruciform Warty	Resembling a verruca (clinical wart) – a lesion with pointed church spire-like epithelium covered by hyperkeratosis (abundant keratin). "Verrucous carcinoma" and "warty carcinoma" are specific entities of mucosal surfaces, which have additional defining features (e.g., broad pushing border in the former). Note that warts are HPV-related lesions, containing koilocytes (cells with crinkly nuclei, perinuclear halos) +/– chunky keratohyaline granules. Some lesions with "verrucous" architecture are also HPV-related, even if koilocytes are not prominent (e.g., some verrucous carcinomas), whereas other verrucous lesions are HPV-unrelated (e.g., verrucoid keratosis of the skin, verruciform xanthoma, verrucous dysplasia of the mucosa).	 Verrucous carcinoma
Villous	Having fingerlike projections. In essence same as papillary, except that, by convention, when referring to intestinal epithelium, the process is called villous.	 Villous adenoma of the colon
Whorled	Cells in a spiral or circular arrangement. Prototypical tumor with true 360-degree whorls is meningioma. Other tumors with whorls include follicular dendritic cell sarcoma, also rarely seen in angiomatoid fibrous histiocytoma, dedifferentiated liposarcoma, and inflammatory myofibroblastic tumors 	 Meningioma
Zellballen	German for "cellular ball." Basically another term for nested. The term is usually applied to pheochromocytoma and paraganglioma, where nests are surrounded by supportive (sustentacular) cells and vascular stroma	See pheochromocytoma under "nested"

Rosettes at a Glance

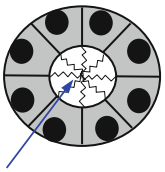
Rosettes are structures with radial arrangement of cells around a central point (like rose petals). In general, the presence of rosettes in an unknown neoplasm is a clue to its **neuroglial differentiation** (neuroblastic, neuroendocrine, or ependymal). In addition, rosette-like structure may be present in several non-neuroglial neoplasms: thymoma (type A), ovarian granulosa cell tumor (Call-Exner bodies), and others.

The terminology of "pseudo" versus "true" rosettes is inconsistently applied. In general, the term "pseudo" is applied to perivascular rosettes to distinguish them from "true" rosettes, which do not surround a central vessel.

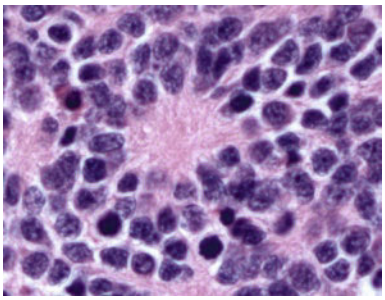
Homer Wright rosettes (aka **fibrillary** or **neuroblastic** rosettes) have a central tangle of fibrillar processes (neuropil). There is no central vessel or lumen.

DDx: fibrillar rosettes are **pathognomonic for neuroblastic tumors** (tumors with primitive neuron differentiation) – neuroblastoma, medulloblastoma, PNET, pineoblastoma, retinoblastoma.

(Named after one person, James Homer Wright, which is why there is no hyphen between the names.)



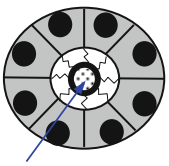
Fibrillar processes (neurites)



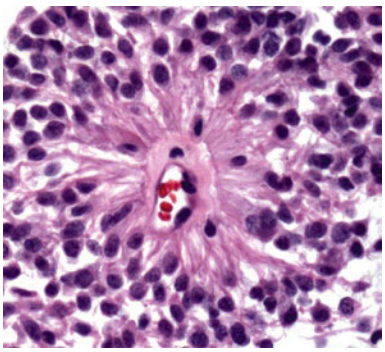
Neuroblastoma

Perivascular pseudorosettes: as above, but fibrillar processes are projecting toward a central blood vessel, resembling "spokes around the hub of wheel." Prototype is ependymoma.

DDx: **ependymoma** (prominent in nearly all cases), central neurocytoma, rarely neuroblastic tumors (neuroblastoma, medulloblastoma, PNET).



Fibrillar processes around a blood vessel

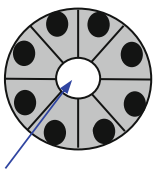


Ependymoma

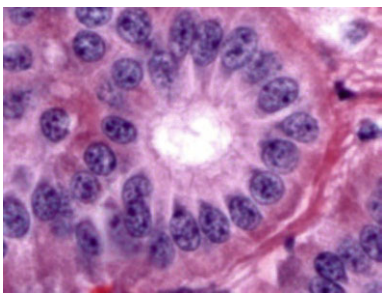
Luminal rosettes: cells polarized around a central lumen. Lumen is formed by apical cell borders. These are different from a gland in that the cells are not surrounded by a basement membrane. While in a gland of adenocarcinoma nuclei generally are aligned (as if in a circle), in a rosette nuclei are stratified relative to each other. In some cases (such as in carcinoid), this distinction can be tricky.

DDx:

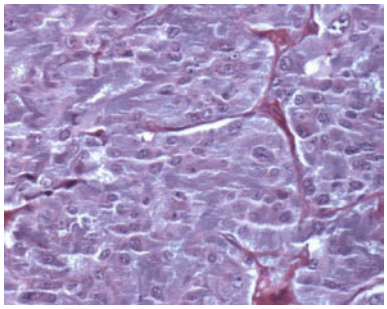
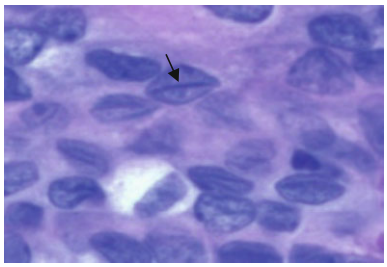
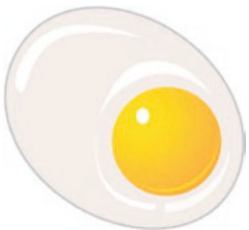
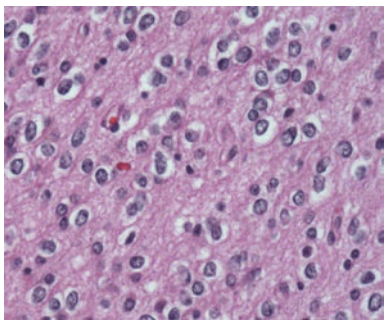
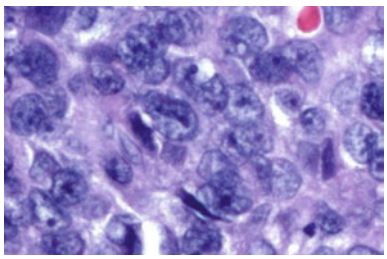
- **Ependymoma**: "true ependymal rosettes" – recapitulate embryonic ependymal canal. These are less numerous than perivascular pseudorosettes.
- **Retinoblastoma:** "Flexner-Wintersteiner rosettes" – photoreceptor-like differentiation**.** Lumen has hypereosinophilic border.
- **Neuroendocrine neoplasms** (e.g., carcinoid, islet cell tumor). Rosettes are commonly present.

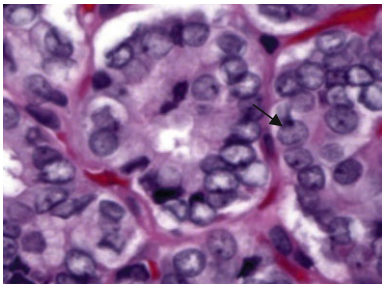
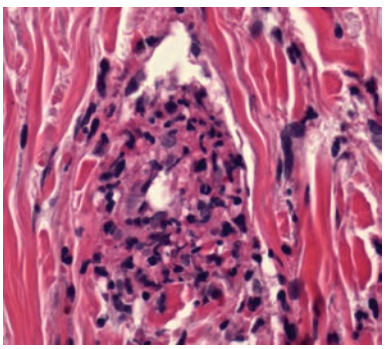
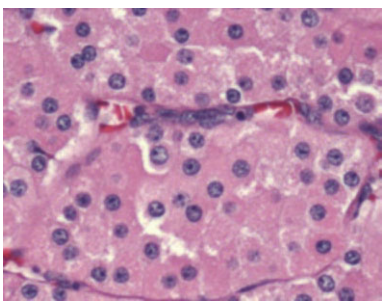
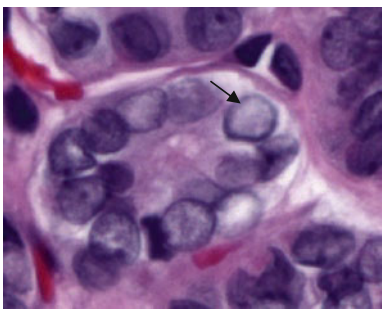


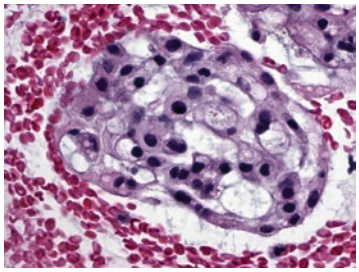
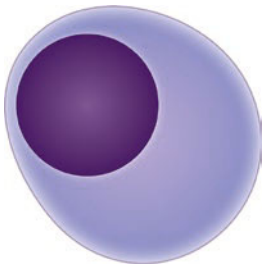
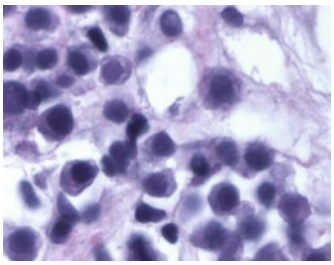
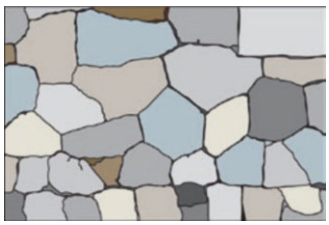
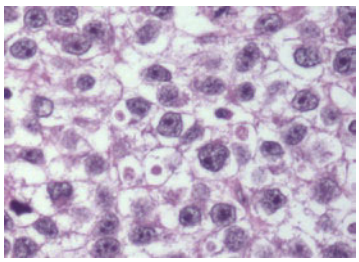
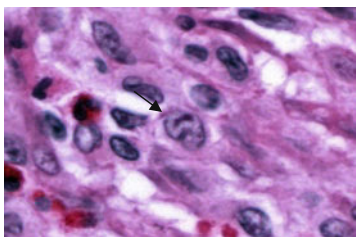
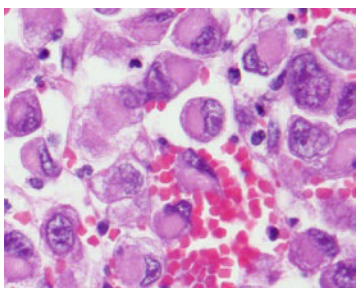
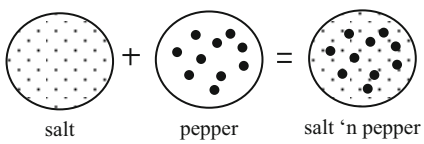
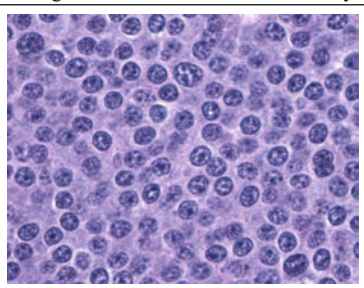
Lumen

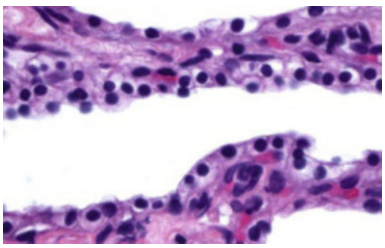
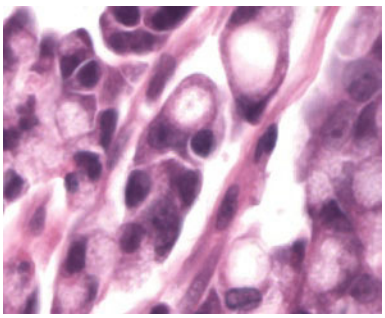
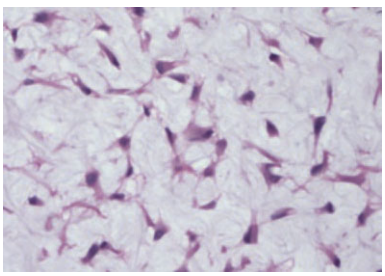
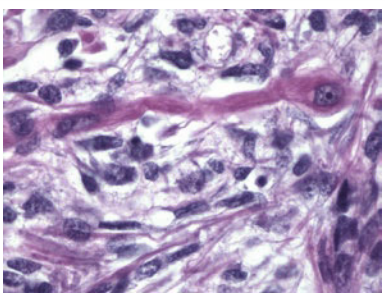
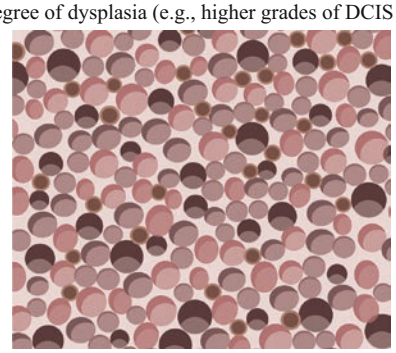
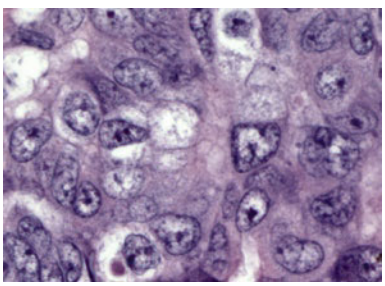


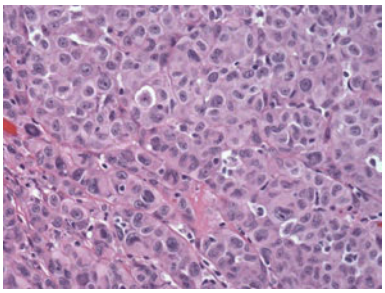
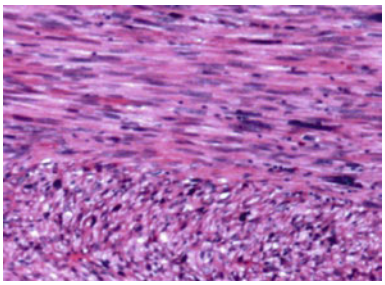
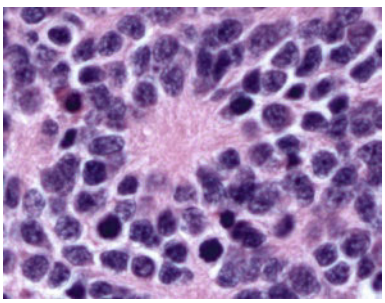
Carcinoid

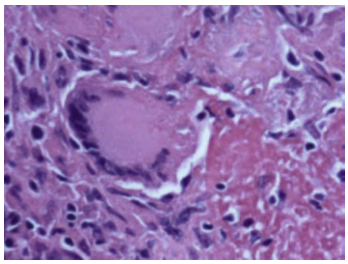
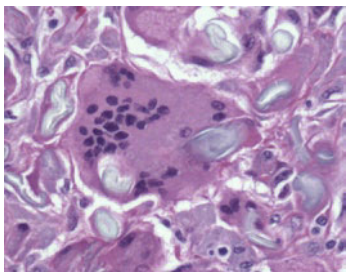
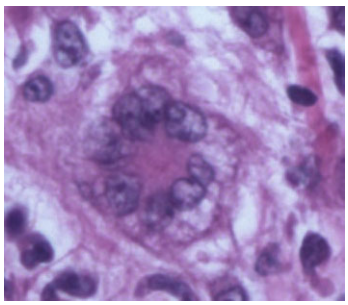
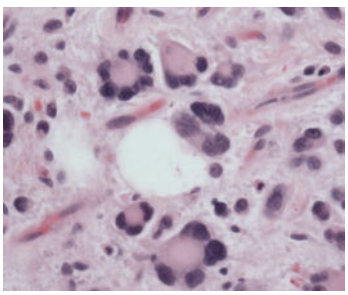
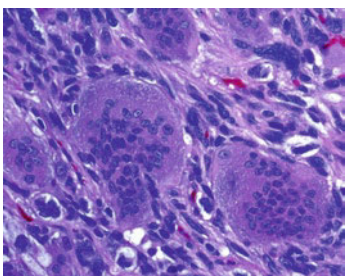
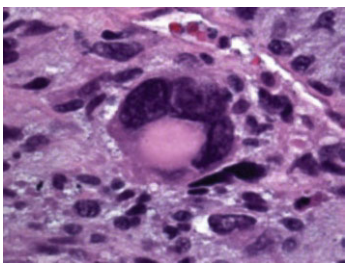
Cytologic Descriptors		
Term	*Definition/appearance*	*Example*
Amphophilic (cytoplasm)	Color that is a mixture of blue and pink due to affinity for both hematoxylin (blue dye) and eosin (pink dye), resulting in an intermediate purplish color	 Pheochromocytoma
Cerebriform (nucleus)	Convoluted outline like in cerebral cortex; flowerlike; classic descriptor nuclei in peripheral T-cell lymphomas	Sezary cells in mycosis fungoides (see Heme Glossary)
Coffee bean-like (nucleus)	Containing a longitudinal groove or fold 	 Papillary thyroid cancer (shown), granulosa cell tumor
Fried egg-like cell	Cells with a perfectly round nucleus, surrounded by either a clear halo (oligodendroglioma) or by pale cytoplasm (chondroblastoma). Remember the poultry theme in both of these tumors: chicken-wire vasculature in the former and chicken-wire calcifications in the latter. 	 Oligodendroglioma
Grooved (nucleus)	Containing an indentation or a cleft; synonymous with reniform, grooved, or cleaved (see coffee bean-like)	 Urothelial cells, papillary thyroid cancer (longitudinal groove), granulosa cell tumor (shown), "buttock lymphocytes" in follicular lymphoma

Ground glass	Glass that has been ground or etched to create a roughened nontransparent surface; "frosted glass," as seen in glass bottle stoppers In pathology, the term is classically used to describe optical clearing of nuclei (washed-out look with loss of chromatin detail) in papillary thyroid cancer and homogenized cytoplasmic inclusions of hepatitis B virus-infected hepatocytes. In radiology, the term is used to describe hazy opacities characteristic of interstitial pneumonias, lepidic adenocarcinomas (buzzword is "GGO," ground-glass opacity), or fibrous dysplasia of the bone. 	 Papillary thyroid cancer: note the optically cleared ("ground-glass") nuclei
Leukocytoclastic	Fragmentation of neutrophils; looks like blue cell dust	 Leukocytoclastic vasculitis
Oncocytic cell, Hürthle cell, oxyphil cell	Having abundant pink granular cytoplasm (due to lots of mitochondria) and bland small round nuclei. Oncocytic neoplasms are grossly mahogany brown (e.g., oncocytic neoplasms of the kidney or salivary gland). Oncocytic cells are called Hürthle or Askanazy cells in the thyroid and oxyphil cells in the pituitary and parathyroid (all terms are basically synonymous, but different names are inconveniently applied in different organs). Origin: oncocyte is Greek for "swollen cells"	 Oncocytoma (salivary)
Orphan Annie eyes-like (nucleus)	Little Orphan Annie is a character in a comic strip, who has "vacant circles for eyes." The term is used interchangeably with "ground glass" to describe optical clearing of nuclei in papillary thyroid cancer.  2018 Little Orphan Annie ® Tribune Content Agency, LLC. Redrawn with permission	 Papillary thyroid cancer (see under "ground glass" above)

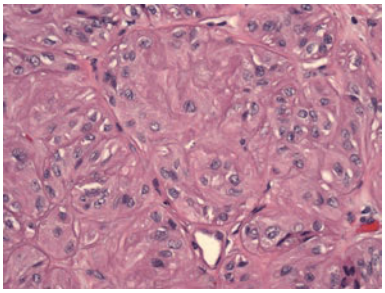
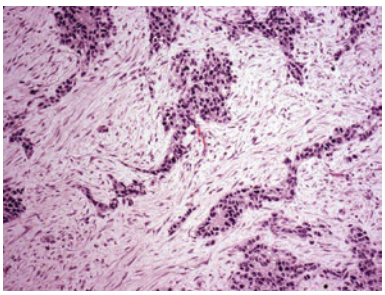
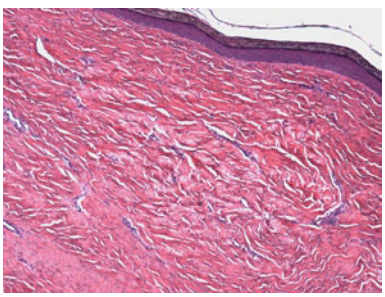
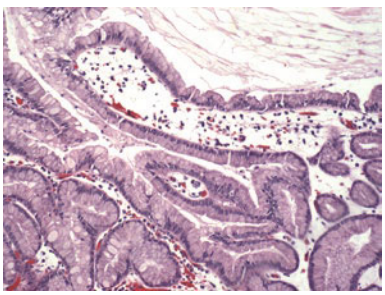
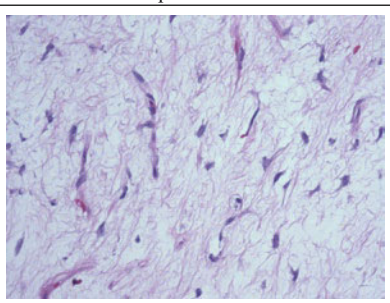
Physaliphorous	Foamy (cytoplasm). Origin: Greek *physalis*, bubbles or vacuoles; *phoros*, bearing	 Chordoma
Plasmacytoid	Resembling plasma cell in that the nucleus is eccentrically placed and is round (unlike signet-ring cell, in which the nucleus is eccentric but is indented due to cytoplasmic mucin) 	 Plasmacytoid variant of urothelial carcinoma (shown), neuroendocrine neoplasms
Polygonal	Relatively vague descriptor typically denoting tumor cells with sharp "squared" cell membranes (fitting together like boulders). Classic examples are squamous cell carcinoma and seminoma. 	 Seminoma
Reniform (nucleus)	Kidney-shaped, indented, containing a groove Origin: Latin *renes*, kidneys 	 Histiocytes in Langerhans cell histiocytosis
Rhabdoid	Cells with bright pink globular cytoplasmic inclusions resembling primitive skeletal muscle cells (rhabdomyoblasts). Usually accompanied by vesicular nuclei with prominent nucleoli. Prototypes are pediatric malignant rhabdoid tumors (MRT) of the kidney, extrarenal/soft tissue, and brain (known as atypical teratoid/rhabdoid tumors). Inclusions are aggregates of intermediate filaments. There are many tumors that have rhabdoid appearance, where it is usually a sign of poor differentiation. Melanoma can look rhabdoid and must be ruled out!	 Malignant rhabdoid tumor of the kidney
Salt and pepper chromatin (neuroendocrine chromatin)	Granular finely speckled chromatin, which is evenly distributed throughout the nucleus; consists of intermixed finer and larger particles (like salt and pepper). Is a defining feature of neuroendocrine neoplasms.  salt + pepper = salt 'n pepper Diagram based on Atkinson, Atlas of Diagnostic Cytopathology	 Carcinoid

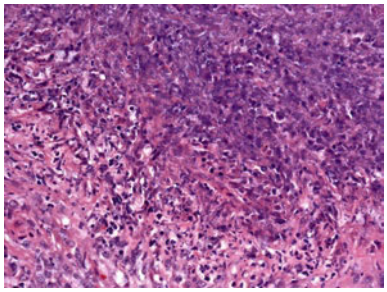
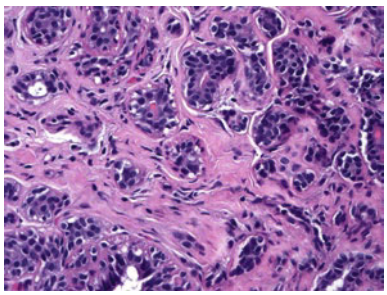
Serous	"Serous fluid" refers to clear watery fluid, which resembles *serum*. "Serous cells" are those cells that secrete serous fluid (as opposed to mucinous secretions and cells). There are four types of serous cells/lesions: 1. Serous cells of the salivary gland (have zymogen granules). 2. Serous tumors of the ovary. 3. Serous cysts of various organs (e.g., serous cystadenoma of the pancreas). 4. Serous cells lining body cavities/serosal surfaces (pleura, peritoneum pericardium); these are referred to as mesothelial cells. Note that the above cells and lesions are histologically dissimilar, and they bear the same designation ("serous") only by virtue of their ability to elaborate clear secretions, NOT because of histologic relatedness.	 Serous cystadenoma of the pancreas
Signet ring	Cytoplasmic vacuole (usually containing mucin) that compresses the nucleus. Signet, bookmark or seal; signet ring, portable version of a signet. [4] 	 Gastric carcinoma, signet ring-type
Stellate cell	Star-shaped, with several pointed cytoplasmic tails; typical morphology of fibroblasts and myofibroblasts 	 Chondromyxoid fibroma
Strap cell	Strap-shaped (elongated like a strap of a shoulder bag; tadpole-like). Describes the shape of rhabdomyoblasts in rhabdomyosarcoma. With rare luck, cross-striations may be visible. 	 Rhabdomyosarcoma
Vesicular (chromatin)	Chromatin that appears porous or bubbly. This occurs due to clumping of the chromatin, which leaves behind cleared-out spaces resembling vesicles. Nuclei appear light, almost transparent. Frequently vesicular appearance correlates with a higher degree of dysplasia (e.g., higher grades of DCIS are vesicular). 	 High-grade DCIS (shown), embryonal carcinoma

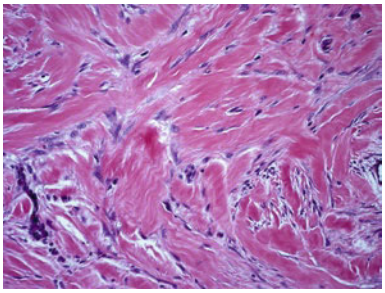
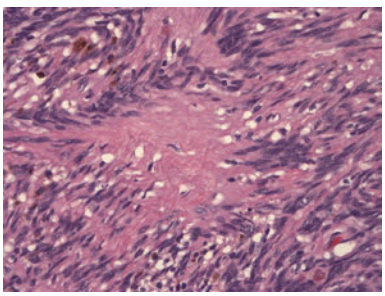
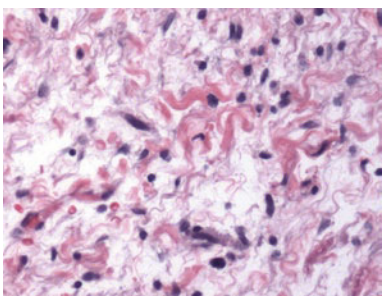
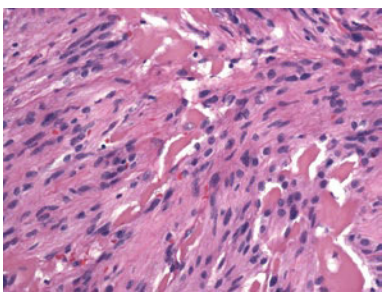
General Descriptors of Cell Shape (used to define 3 broad groups of tumor differentials)		
Epithelioid	Resembling epithelial cells in that the cells have plump round to oval nuclei (as opposed to spindle or small cells), abundant cytoplasm, and well-defined cell borders. The term also usually implies that the cells are cohesive (as epithelioid histiocytes in a granuloma). In a setting of a poorly differentiated neoplasm, the term "epithelioid" implies that a neoplasm looks like carcinoma, but this could still be epithelioid melanoma, sarcoma, or even lymphoma.	 DDx: carcinoma, epithelioid melanoma (shown), epithelioid sarcoma, large cell lymphoma
Spindle (fusiform)	Cells that are elongated and have tapered (pointed) ends. Usually both the nucleus and cytoplasm are elongated, but the term still applies for cells with spindle-shaped cytoplasmic outline but rounded (or only slightly oval) nucleus. In a setting of malignant neoplasm, the differential diagnosis includes sarcoma (e.g., muscle, neural, vascular) AND spindle-cell variants of carcinoma (sarcomatoid carcinoma) and melanoma Origin: "fusiform" is derived from the Latin "fusus" meaning "spindle" 	 DDx: sarcoma (shown leiomyosarcoma), sarcomatoid carcinoma, melanoma
Small round blue cell	Sheets of small round blue cells. Cells are blue because they have very little cytoplasm. Subtle morphologic clues may be present (as in rosettes in neuroblastoma and PNET) but generally diagnosis requires immunostains +/– molecular studies +/– cytogenetics. DDx is age-dependent (see "Potpourri of Differentials" section in Chapter 14).	 DDx (for pediatric SRBCT): neuroblastoma (shown), Ewing/PNET, alveolar rhabdomyosarcoma, Wilms, lymphoblastic lymphoma, and others

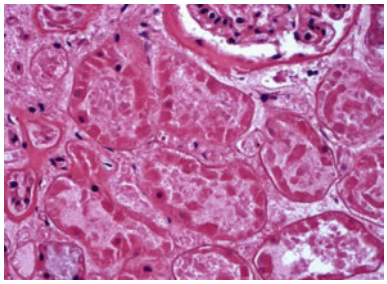
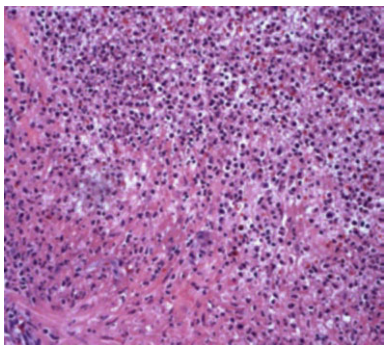
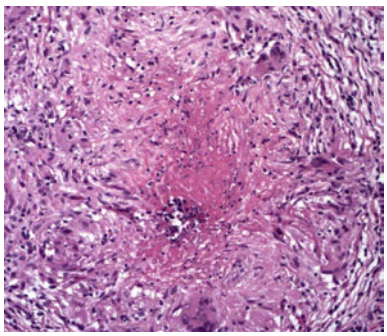
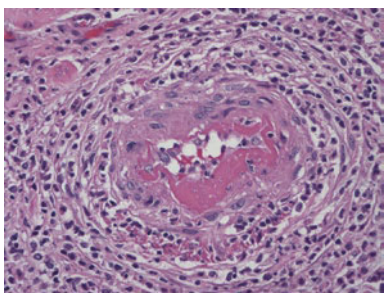
Descriptors of Giant cells (For more images and differentials, see http://www.granuloma.homestead.com/)		
Langhans-type giant cells	Peripheral semicircular nuclei (horseshoe-like); characteristic of TB Named after Theodor Langhans, who was not the same person as Paul Langerhans of the Langerhans cell and islets of Langerhans eponyms	
Foreign body-type giant cells	Haphazardly arranged nuclei often aggregating toward the center of the cell; characteristic of reaction to a foreign body	
Touton giant cells	A full ring of nuclei with eosinophilic cytoplasm centrally and foamy cytoplasm at the periphery. Seen in lesions with high lipid content such as xanthoma, juvenile xanthogranuloma, and fat necrosis; also common in dermatofibroma. Named after Karl Touton, a German dermatologist	
Floret cell	Wreath of hyperchromatic peripheral nuclei. Hallmark of pleomorphic lipoma, but these cells may rarely be present in liposarcoma as well.	
Osteoclast-like giant cell	Multiple **bland** central nuclei, ruffled cell membrane (feature well seen in smears but not in H&E sections). Seen in giant cell-rich tumors of bone and soft tissue (e.g., giant cell tumor) and admixed in various high-grade carcinomas (e.g., pancreatic undifferentiated carcinoma with osteoclast-like giant cells). In the latter case, these cells are nonneoplastic (as may be confirmed by negative CKs and positive CD68 by IHC)	
Tumor giant cells	Pleomorphic (scary-looking) cytology; featured in anaplastic carcinomas and high-grade sarcomas	
Viral infections	HSV, respiratory syncytial virus (RSV), and measles	

Descriptors of Granulomas and Differential Diagnoses		
Term	*Definition/appearance*	*Differential diagnosis*
Granuloma NOS	Granuloma – rounded aggregate of histiocytes. Histiocytes in a granuloma have abundant cytoplasm (are "epithelioid") and indistinct cell borders such that they seem to merge into a syncytium. Giant cells are usually but not always present. If syncytial aggregates of histiocytes/giant cells are in sheets (rather than in a discreet nodule), the preferred term is "granulomatous inflammation" rather than granuloma.	– Foreign body or particles (suture, beryllium) – Sarcoidosis – Infection (frequently but not always granulomas are necrotizing) – Autoimmune (Crohn, hypersensitivity pneumonitis, primary biliary cirrhosis) – Drug reactions – Chronic granulomatous disease (rare)
Necrotizing (caseating) granuloma	Caseating: cheese-like appearance (strictly speaking a macroscopic term) Necrotizing: microscopic counterpart to "caseating"	– Infection (TB, fungus) – Sarcoidosis (minimal necrosis is allowed) – Also see necrobiotic granulomas
Suppurative granuloma	Granulomas with central collections of neutrophils. Coalescent abscesses in granulomas have been termed "stellate microabscesses".	Classic differential: – Cat scratch disease (*Bartonella henselae*) – Lymphogranuloma venereum (*Chlamydia trachomatis*) – Tularemia – *Yersinia* Also r/o mycobacteria, fungi
Necrobiotic granuloma	See below for definition of "necrobiosis"	– Granuloma annulare (central mucin) – Rheumatoid nodule – Necrobiosis lipoidica diabeticorum (shins; cake-like horizontal layers) – Granulomatosis with polyangiitis (Wegener) ("blue" necrobiosis) – Post-transurethral resection (TUR) granuloma (prostate, bladder)
"Special" granulomas	Either frank misnomers (pyogenic granuloma) or lesions where histiocytes are present but are not prominent (plasma cell granuloma). This is because the term is loosely applied (usually by non-pathologists) to refer to a nodule (as in a clinical term "suture granuloma").	– Plasma cell granuloma (lung) = inflammatory myofibroblastic tumor – Eosinophilic granuloma (lung) = Langerhans cell histiocytosis – Pulmonary hyalinizing granuloma = sclerosing lesion analogous to sclerosing mediastinitis – Pyogenic granuloma = lobular capillary hemangioma – Lethal midline granuloma = NK/T lymphoma nasal type

Descriptors of Stroma		
Term	*Definition/appearance*	*Example*
Hyaline	Origin: from Latin *hyalinus*, transparent or nearly so and homogeneous. [5] The term is etiologically nonspecific. There is no one molecule that corresponds to "hyaline" material. Instead, "hyaline" is a descriptive term, which refers to any material that looks homogeneous (non-fibrillar, nongranular), glassy, almost refractile (like hyaline cartilage) and usually bright pink on H&E. The term is used to describe a variety of processes, including: – *Hyalinized collagen* (as in hyalinized fibroadenoma): the term refers to collagen which has lost its fibrillar quality and appears homogenized and glassy; the molecular basis for this change is unknown; usually a feature of a long-standing benign process – *Hyalinized vessels* (as in schwannoma or nephrosclerosis): the term refers to deposition of glassy pink material in vessel walls; here hyaline material consists of extravasated plasma proteins and basement membrane matrix – Others: *hyaline membranes* in the lung (fibrin), *Russell bodies* in plasma cells (immunoglobulins), *Mallory hyaline* in the liver (cytokeratins), *hyaline globules* in various tumors, etc.	 Hyalinizing trabecular adenoma of the thyroid
Desmoplastic	Host response to a neoplasm manifesting as fibroblast proliferation and deposition of collagen	 Desmoplastic small round cell tumor
Fibrotic, fibrosis	Having an abundant collagen deposition. Sometimes sclerotic is used synonymously with fibrotic (see below).	 Scar
Mucinous (colloid)	Slimy viscous material (a component of mucus) that looks purplish and stringy on H&E. Carcinomas may produce abundant mucin (e.g., mucinous or colloid carcinomas, mucoepidermoid carcinoma). Strictly speaking NOT stromal as mucin is secreted by epithelial cells into luminal spaces, but mucin pools may dissect into and through the stroma.	 Intraductal papillary mucinous neoplasm of the pancreas
Myxoid	Gelatinous material produced by soft tissue cells. Resembles epithelial mucin, but is not as thick (looks like watered-down mucin – it is not as blue or stringy). By convention, mucoid material produced by soft tissues is called "myxoid" rather than mucinous material. These substances are biochemically distinct: stromal mucin contains hyaluronic acid, whereas epithelial mucin does not (see section on Special Stains for details). **Chondromyxoid** material is bluish and refractile, like cartilage.	 Myxoid liposarcoma

Necrobiotic	In surgical pathology, the term "necrobiosis" or "bionecrosis" is generally used to describe the blue-red granular necrosis ("granular soup") characteristic of granulomatosis with polyangiitis (Wegener). The granularity represents karyorrhectic debris (nuclear dust) from degenerated neutrophils superimposed on degenerated collagen. In the dermpath literature, necrobiosis is used to indicate "collagenolysis" or degeneration of collagen, wherein collagen takes on an amorphous bluish appearance (nuclear dust may not be prominent). When surrounded by epithelioid histiocytes, the process is called "necrobiotic granuloma" (as seen in necrobiosis lipoidica diabeticorum and granuloma annulare). [6, 7]	 Granulomatosis with polyangiitis (shown), necrobiosis lipoidica diabeticorum, granuloma annulare
Sclerotic (sclerosis)	Etiologically nonspecific term describing "thickening or hardening." May be used to describe microscopic fibrosis/collagen deposition (as in sclerosing adenosis of the breast, sclerotic glomeruli, systemic sclerosis), formation of macroscopically firm plaques (as in multiple sclerosis), or vessel hardening (arteriosclerosis).	 Sclerosing adenosis

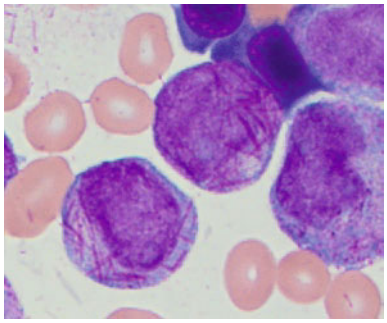
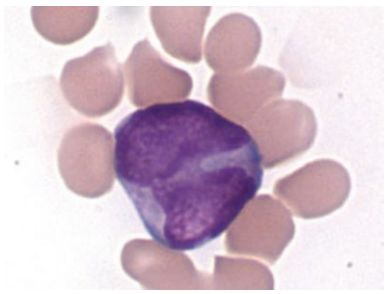
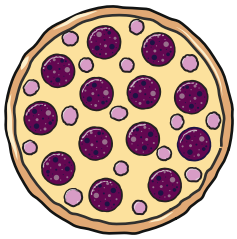
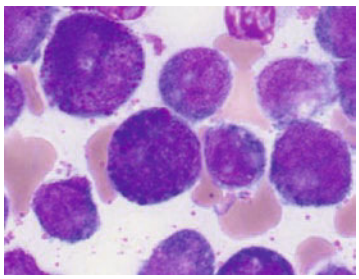
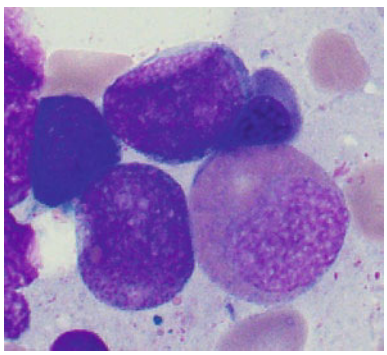
Descriptors of Collagen		
Term	*Definition/appearance*	*Example*
Keloidal collagen	Thick ropey bundles of eosinophilic hyalinized (bright pink and glassy) collagen. Prototype is a keloid. This type of collagen is typical of fibromatosis, solitary fibrous tumor, and spindle-cell lipoma.	 Keloid (shown)
Amianthoid collagen	Resembling amianth: earth flax or mountain flax; composed of delicate filaments resembling threads of silk. [8] The term is used to describe collagen nodules with fibrillar "frayed" border (as in palisaded myofibroblastoma).	 Palisaded myofibroblastoma
Carrot shavings-like collagen	Long wavy, curly, wiry strands of collagen. A classic feature of neurofibroma and malignant peripheral nerve sheath tumor (MPNST).	 Neurofibroma (shown), MPNST
Skeinoid fibers	Collagen fibers "so-named after their peculiar appearance by electron microscopy simulating skeins of yarn" [9]. By light microscopy, these are rounded collagen globules or short fibers. Present in ~15% of gastrointestinal stromal tumors (GIST).	 GIST

Descriptors of Necrosis		
Term	*Definition/appearance*	*Example*
Coagulative necrosis	Dead cells are seen as cell shadows or ghosts but the overall architecture is preserved (as if mummified)	 Ischemia (except brain, where ischemia causes liquefaction)
Liquefactive necrosis	Dead cells are completely digested away and normal architecture is obliterated. Usually caused by infection and is associated with inflammatory cells.	 Abscess
Caseating (caseous) necrosis	Central necrosis within a granuloma. Named after the "cheesy" macroscopic appearance.	 TB
Fibrinoid necrosis	Fibrin-like. The term "fibrinoid necrosis" is mainly applied to necrotic vessels with deposition of pink hyaline material (as in leukocytoclastic vasculitis). It consists of plasma proteins, including fibrin.	 Fibrinoid necrosis in vasculitis
Necrobiotic	See above in the Descriptors of Stroma	

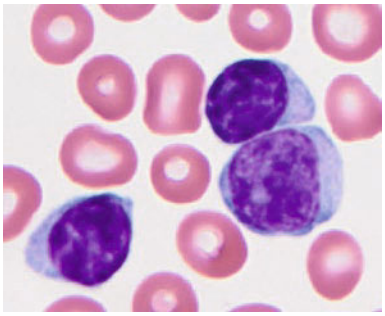
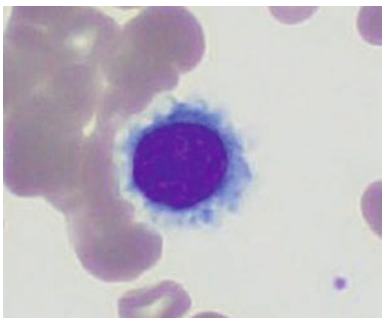
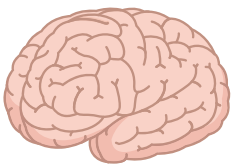
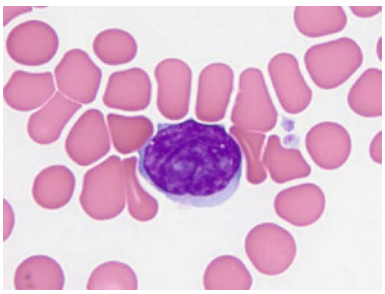
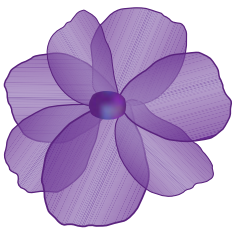
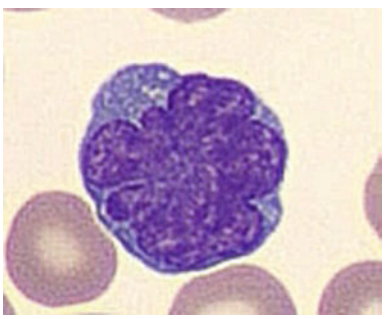
Common Dermatopathologic Descriptors		
Term	*Definition/appearance*	*Example*
Acanthosis	Increased thickness of the epidermis	Psoriasis
Acantholysis	The loss of cohesion between keratinocytes	Acantholytic squamous cell carcinoma, pemphigus vulgaris
Dyskeratosis	Premature keratinization of individual keratinocytes before they have reached the surface layer. Dyskeratotic cells have a dense pink cytoplasm and they usually become rounded.	Squamous dysplasia, erythema multiforme, graft-versus-host disease
Grenz zone	A narrow area of uninvolved dermis between the upper edge of a dermal lesion (neoplastic or inflammatory) and the epidermis. "Grenz" is German for "border"	Dermatofibroma (Grenz zone present) versus dermatofibrosarcoma protuberans (Grenz zone absent)
Hyperkeratosis	Increased thickness of the stratum corneum (clinically a scale)	Actinic keratosis
Lentiginous	Junctional (dermo-epidermal) growth; used in reference to melanocytes	Lentigo maligna
Lichenoid dermatitis (synonymous with "interface dermatitis")	Junctional (dermal-epidermal or DE) inflammatory process, manifesting as band-like lymphocytic infiltrate ("lichenoid infiltrate") and basal cell damage. Basal cell damage leads to formation of vesicles at DE junction ("basilar vasculopathy" or "liquefaction degeneration").	Lichen planus (prototype)
Orthokeratosis	Normal "basket-weave" pattern of the stratum corneum (no nuclear retention as in parakeratosis)	Normal skin
Parakeratosis	Retention of nuclei in the stratum corneum. Reflects a hyperproliferative state. Normal on mucous membranes.	Psoriasis
Papillomatosis	Upward displacement of the dermal papillae, giving skin surface a fingerlike or "church-spire"-like appearance	Verruca vulgaris (clinical wart)
Spongiosis	Intercellular edema in the epidermis seen as an increase in intercellular spaces	Allergic contact dermatitis

Part 2 Hematopoietic System

By Xiaojun Wu

Morphologic Identification of Blasts (Selected Examples)*		
Diagnosis	*Definition/appearance*	*Example*
Hypergranular APL with t(15;17)	Abundant azurophilic (burgundy-colored) granules with abundant **Auer rods**, elongated clumps/needles of azurophilic granules (Auer is an eponym; named after the person who described them)	
Hypogranular/ microgranular APL with t(15;17)	The majority of blasts have irregular nuclear contours (monocytic appearing) with bilobed "**butterfly**" nuclei and sparsely granular cytoplasm. The typical hypergranular promyelocytic blasts are often identified but much fewer in numbers. 	
AML with inv (16)	Numerous immature eosinophilic precursors with both abnormal large eosinophilic and large basophilic dual granules (**pizza cells**). Background blasts with myelomonocytic morphology (irregular nuclear contours, nuclear grooves/folds, cytoplasmic vacuoles and eosinophilia). 	
AML with t(8;21)	Large blasts with abundant basophilic cytoplasm, often containing numerous azurophilic granules. The granules vary from fine dusty to large orange-pink and coarse (pseudo-Chédiak-Higashi granules). Auer rods can be found. There is also a perinuclear clearing (hof).	

*Final diagnosis of AML always requires flow cytometry +/− cytogenetics, but distinctive morphologic features of some blasts can give a good preliminary clue to the diagnosis.

Morphologic Identification of Lymphoma Cells (Selected Examples)		
Diagnosis	*Definition/appearance*	*Example*
CLL/SLL	**CLL cells:** Small and round Chunky chromatin (**soccer ball pattern**), inconspicuous nucleoli Scant cytoplasm  **Prolymphocyte:** Intermediate to large, oval Finer chromatin with central prominent nucleoli Abundant cytoplasm	
Hairy cell leukemia	Small to medium size Oval- or indented-shaped nuclei with moderately condensed chromatin and inconspicuous nucleoli Abundant pale blue cytoplasm with circumferential delicate, **hair-like cytoplasmic projections** 	 Image courtesy of Dr. Wei Wang from MDACC
Sezary syndrome	Sezary cells: Hyperconvoluted **cerebriform** (resembling gyri and sulci of brain) nuclei 	
Adult T-cell leukemia/ lymphoma (ATLL)	**Flower cells** with multilobated nuclei and deeply basophilic cytoplasm 	

References

Chapter 1. Immunostains: Introduction

1. Moll R, Franke WW, Schiller DL, et al. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell. 1982;31:11–24.
2. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. Histopathology. 2002;40:403–39.
3. Cooper D, Schermer A, Sun TT. Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: strategies, applications, and limitations. Lab Invest. 1985;52:243–56.
4. Dabbs DJ. Diagnostic immunohistochemistry: theranostic and genomic applications. 3rd ed. Philadelphia: Saunders/Elsevier; 2010.: United States 2010. xviii, 941 p. : ill. pp.
5. Moll R, Divo M, Langbein L. The human keratins: biology and pathology. Histochem Cell Biol. 2008;129:705–33.
6. Taylor CR, Cote RJ. Immunomicroscopy: a diagnostic tool for the surgical pathologist. 3rd ed. Philadelphia: Saunders/Elsevier; 2006.: United States 2006. xiv, 531 p. : ill., port. pp.
7. Schaefer IM, Agaimy A, Fletcher CD, et al. Claudin-4 expression distinguishes SWI/SNF complex-deficient undifferentiated carcinomas from sarcomas. Mod Pathol. 2017;30:539–48.
8. Zhang PJ, Shah M, Spiegel GW, et al. Cytokeratin 7 immunoreactivity in rectal adenocarcinomas. Appl Immunohistochem Mol Morphol. 2003;11:306–10.
9. Chen ZM, Wang HL. Alteration of cytokeratin 7 and cytokeratin 20 expression profile is uniquely associated with tumorigenesis of primary adenocarcinoma of the small intestine. Am J Surg Pathol. 2004;28:1352–9.
10. Leduc C, Zhang L, Oz B, et al. Thoracic myoepithelial tumors: a pathologic and molecular study of 8 cases with review of the literature. Am J Surg Pathol. 2016;40:212–23.
11. Rooper LM, Bishop JA, Westra WH. INSM1 is a sensitive and specific marker of neuroendocrine differentiation in head and neck tumors. Am J Surg Pathol. 2018;42:665–71.
12. Miettinen M, McCue PA, Sarlomo-Rikala M, et al. Sox10–a marker for not only schwannian and melanocytic neoplasms but also myoepithelial cell tumors of soft tissue: a systematic analysis of 5134 tumors. Am J Surg Pathol. 2015;39:826–35.
13. Ordonez NG. Value of SOX10 immunostaining in tumor diagnosis. Adv Anat Pathol. 2013;20:275–83.
14. Shahid M, Mubeen A, Tse J, et al. Branched chain in situ hybridization for albumin as a marker of hepatocellular differentiation: evaluation of manual and automated in situ hybridization platforms. Am J Surg Pathol. 2015;39:25–34.
15. Bahrami A, Gown AM, Baird GS, et al. Aberrant expression of epithelial and neuroendocrine markers in alveolar rhabdomyosarcoma: a potentially serious diagnostic pitfall. Mod Pathol. 2008;21:795–806.
16. Antonescu CR, Owosho AA, Zhang L, et al. Sarcomas with CIC-rearrangements are a distinct pathologic entity with aggressive outcome: a clinicopathologic and molecular study of 115 cases. Am J Surg Pathol. 2017;41:941–9.
17. Hung YP, Lee JP, Bellizzi AM, et al. PHOX2B reliably distinguishes neuroblastoma among small round blue cell tumours. Histopathology. 2017;71:786–94.
18. Terry J, Calicchio ML, Rodriguez-Galindo C, et al. Immunohistochemical expression of CRX in extracranial malignant small round cell tumors. Am J Surg Pathol. 2012;36:1165–9.
19. Carpentieri DF, Nichols K, Chou PM, et al. The expression of WT1 in the differentiation of rhabdomyosarcoma from other pediatric small round blue cell tumors. Mod Pathol. 2002;15:1080–6.
20. Schaefer IM, Fletcher CD, Hornick JL. Loss of H3K27 trimethylation distinguishes malignant peripheral nerve sheath tumors from histologic mimics. Mod Pathol. 2016;29:4–13.
21. Montgomery E, Lee JH, Abraham SC, et al. Superficial fibromatoses are genetically distinct from deep fibromatoses. Mod Pathol. 2001;14:695–701.
22. Charville GW, Wang WL, Ingram DR, et al. EWSR1 fusion proteins mediate PAX7 expression in Ewing sarcoma. Mod Pathol. 2017;30:1312–20.
23. Nonaka D, Papaxoinis G, Mansoor W. Diagnostic utility of orthopedia homeobox (OTP) in pulmonary carcinoid tumors. Am J Surg Pathol. 2016;40:738–44.
24. Sangoi AR, Ohgami RS, Pai RK, et al. PAX8 expression reliably distinguishes pancreatic well-differentiated neuroendocrine tumors from ileal and pulmonary well-differentiated neuroendocrine tumors and pancreatic acinar cell carcinoma. Mod Pathol. 2011;24:412–24.
25. Yang Z, Klimstra DS, Hruban RH, et al. Immunohistochemical characterization of the origins of metastatic well-differentiated neuroendocrine tumors to the liver. Am J Surg Pathol. 2017;41:915–22.

Chapter 2. Immunostains: Solid Tumors

1. Werling RW, Hwang H, Yaziji H, et al. Immunohistochemical distinction of invasive from noninvasive breast lesions: a comparative study of p63 versus calponin and smooth muscle myosin heavy chain. Am J Surg Pathol. 2003;27:82–90.
2. Bratthauer GL, Moinfar F, Stamatakos MD, et al. Combined E-cadherin and high molecular weight cytokeratin immunoprofile differentiates lobular, ductal, and hybrid mammary intraepithelial neoplasias. Hum Pathol. 2002;33:620–7.
3. Grin A, O'Malley FP, Mulligan AM. Cytokeratin 5 and estrogen receptor immunohistochemistry as a useful adjunct in identifying atypical papillary lesions on breast needle core biopsy. Am J Surg Pathol. 2009;33:1615–23.
4. Collins LC, Schnitt SJ. Papillary lesions of the breast: selected diagnostic and management issues. Histopathology. 2008;52:20–9.
5. Tse GM, Tan PH, Moriya T. The role of immunohistochemistry in the differential diagnosis of papillary lesions of the breast. J Clin Pathol. 2009;62:407–13.
6. Cimino-Mathews A, Sharma R, Illei PB, et al. A subset of malignant phyllodes tumors express p63 and p40: a diagnostic pitfall in breast core needle biopsies. Am J Surg Pathol. 2014;38:1689–96.
7. Lacroix-Triki M, Geyer FC, Lambros MB, et al. beta-catenin/Wnt signalling pathway in fibromatosis, metaplastic carcinomas and phyllodes tumours of the breast. Mod Pathol. 2010;23:1438–48.
8. Tse GM, Tan PH, Lui PC, et al. Spindle cell lesions of the breast–the pathologic differential diagnosis. Breast Cancer Res Treat. 2008;109:199–207.
9. Goldblum JR, Lamps LW, McKenney JK, et al., editors. Rosai and Ackerman's surgical pathology. Vol. 11th ed. Philadelphia: Elsevier; 2017. p. 2306.

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10. Amin MB, Epstein JI, Ulbright TM, et al. Best practices recommendations in the application of immunohistochemistry in urologic pathology: report from the International Society of Urological Pathology consensus conference. Am J Surg Pathol. 2014;38:1017–22.
11. Gurel B, Ali TZ, Montgomery EA, et al. NKX3.1 as a marker of prostatic origin in metastatic tumors. Am J Surg Pathol. 2010;34:1097–105.
12. Sheridan T, Herawi M, Epstein JI, et al. The role of P501S and PSA in the diagnosis of metastatic adenocarcinoma of the prostate. Am J Surg Pathol. 2007;31:1351–5.
13. Uchida K, Ross H, Lotan T, et al. DeltaNp63 (p40) expression in prostatic adenocarcinoma with diffuse p63 positivity. Hum Pathol. 2015;46:384–9.
14. Epstein JI, Netto JG. Biopsy interpretation of the prostate, Biopsy Interpretation Series. 4th ed. Philadelphia; Fourth edition (September 18, 2007):: LWW; 2008. p. 368.
15. Amin MB, Trpkov K, Lopez-Beltran A, et al. Best practices recommendations in the application of immunohistochemistry in the bladder lesions: report from the International Society of Urologic Pathology consensus conference. Am J Surg Pathol. 2014;38:e20–34.
16. Herawi M, Drew PA, Pan CC, et al. Clear cell adenocarcinoma of the bladder and urethra: cases diffusely mimicking nephrogenic adenoma. Hum Pathol. 2010;41:594–601.
17. Sun K, Huan Y, Unger PD. Clear cell adenocarcinoma of urinary bladder and urethra: another urinary tract lesion immunoreactive for P504S. Arch Pathol Lab Med. 2008;132:1417–22.
18. Tong GX, Weeden EM, Hamele-Bena D, et al. Expression of PAX8 in nephrogenic adenoma and clear cell adenocarcinoma of the lower urinary tract: evidence of related histogenesis? Am J Surg Pathol. 2008;32:1380–7.
19. Chuang AY, DeMarzo AM, Veltri RW, et al. Immunohistochemical differentiation of high-grade prostate carcinoma from urothelial carcinoma. Am J Surg Pathol. 2007;31:1246–55.
20. Dabbs DJ. Diagnostic immunohistochemistry: theranostic and genomic applications. 3rd ed. Philadelphia: Saunders/Elsevier; 2010.: United States 2010. xviii, 941 p. : ill. pp.
21. Oh WJ, Chung AM, Kim JS, et al. Differential immunohistochemical profiles for distinguishing prostate carcinoma and urothelial carcinoma. J Pathol Transl Med. 2016;50:345–54.
22. Wang HY, Mills SE. KIT and RCC are useful in distinguishing chromophobe renal cell carcinoma from the granular variant of clear cell renal cell carcinoma. Am J Surg Pathol. 2005;29:640–6.
23. Gobbo S, Eble JN, Grignon DJ, et al. Clear cell papillary renal cell carcinoma: a distinct histopathologic and molecular genetic entity. Am J Surg Pathol. 2008;32:1239–45.
24. Moch H, Humphrey PA, Ulbright TM, et al., editors. WHO classification of tumours of the urinary system and male genital organs. 4th ed. Lyon, France: IARC Press; 2016.
25. Chen YB, Brannon AR, Toubaji A, et al. Hereditary leiomyomatosis and renal cell carcinoma syndrome-associated renal cancer: recognition of the syndrome by pathologic features and the utility of detecting aberrant succination by immunohistochemistry. Am J Surg Pathol. 2014;38:627–37.
26. Kryvenko ON, Jorda M, Argani P, et al. Diagnostic approach to eosinophilic renal neoplasms. Arch Pathol Lab Med. 2014;138:1531–41.
27. Williamson SR, Eble JN, Amin MB, et al. Succinate dehydrogenase-deficient renal cell carcinoma: detailed characterization of 11 tumors defining a unique subtype of renal cell carcinoma. Mod Pathol. 2015;28:80–94.
28. Zhong M, De Angelo P, Osborne L, et al. Translocation renal cell carcinomas in adults: a single-institution experience. Am J Surg Pathol. 2012;36:654–62.
29. Reuter VE, Argani P, Zhou M, et al. Best practices recommendations in the application of immunohistochemistry in the kidney tumors: report from the International Society of Urologic Pathology consensus conference. Am J Surg Pathol. 2014;38:e35–49.
30. Nonaka D, Wang BY, Edmondson D, et al. A study of gata3 and phox2b expression in tumors of the autonomic nervous system. Am J Surg Pathol. 2013;37:1236–41.
31. Lee JP, Hung YP, O'Dorisio TM, et al. Examination of PHOX2B in adult neuroendocrine neoplasms reveals relatively frequent expression in phaeochromocytomas and paragangliomas. Histopathology. 2017;71:503–10.
32. Hung YP, Lee JP, Bellizzi AM, et al. PHOX2B reliably distinguishes neuroblastoma among small round blue cell tumours. Histopathology. 2017;71:786–94.
33. Browning L, Bailey D, Parker A. D2-40 is a sensitive and specific marker in differentiating primary adrenal cortical tumours from both metastatic clear cell renal cell carcinoma and phaeochromocytoma. J Clin Pathol. 2008;61:293–6.
34. Cao D, Li J, Guo CC, et al. SALL4 is a novel diagnostic marker for testicular germ cell tumors. Am J Surg Pathol. 2009;33:1065–77.
35. Jones TD, Ulbright TM, Eble JN, et al. OCT4 staining in testicular tumors: a sensitive and specific marker for seminoma and embryonal carcinoma. Am J Surg Pathol. 2004;28:935–40.
36. Lau SK, Weiss LM, Chu PG. D2-40 immunohistochemistry in the differential diagnosis of seminoma and embryonal carcinoma: a comparative immunohistochemical study with KIT (CD117) and CD30. Mod Pathol. 2007;20:320–5.
37. Alvarado-Cabrero I, Hernandez-Toriz N, Paner GP. Clinicopathologic analysis of choriocarcinoma as a pure or predominant component of germ cell tumor of the testis. Am J Surg Pathol. 2014;38:111–8.
38. Nonaka D. Differential expression of SOX2 and SOX17 in testicular germ cell tumors. Am J Clin Pathol. 2009;131:731–6.
39. Osman H, Cheng L, Ulbright TM, et al. The utility of CDX2, GATA3, and DOG1 in the diagnosis of testicular neoplasms: an immunohistochemical study of 109 cases. Hum Pathol. 2016;48:18–24.
40. Ulbright TM, Tickoo SK, Berney DM, et al. Best practices recommendations in the application of immunohistochemistry in testicular tumors: report from the International Society of Urological Pathology consensus conference. Am J Surg Pathol. 2014;38:e50–9.
41. Magers MJ, Kao CS, Cole CD, et al. “Somatic-type” malignancies arising from testicular germ cell tumors: a clinicopathologic study of 124 cases with emphasis on glandular tumors supporting frequent yolk sac tumor origin. Am J Surg Pathol. 2014;38:1396–409.
42. Rosai J, Carcangiu ML, DeLellis RA. Undifferentiated (Anaplastic) carcinoma. In: Tumors of the thyroid gland. Washington, DC: ARP Press; 1992. p. 135–59.
43. Mendelsohn G, Wells SA Jr, Baylin SB. Relationship of tissue carcinoembryonic antigen and calcitonin to tumor virulence in medullary thyroid carcinoma. An immunohistochemical study in early, localized, and virulent disseminated stages of disease. Cancer. 1984;54:657–62.
44. Bishop JA, Sharma R, Westra WH. PAX8 immunostaining of anaplastic thyroid carcinoma: a reliable means of discerning thyroid origin for undifferentiated tumors of the head and neck. Hum Pathol. 2011;42:1873–7.
45. Nonaka D, Tang Y, Chiriboga L, et al. Diagnostic utility of thyroid transcription factors Pax8 and TTF-2 (FoxE1) in thyroid epithelial neoplasms. Mod Pathol. 2008;21:192–200.
46. Gill AJ, Clarkson A, Gimm O, et al. Loss of nuclear expression of parafibromin distinguishes parathyroid carcinomas and hyperparathyroidism-jaw tumor (HPT-JT) syndrome-related adenomas from sporadic parathyroid adenomas and hyperplasias. Am J Surg Pathol. 2006;30:1140–9.
47. El-Naggar AK, Chan JKC, Grandis JR, et al., editors. WHO classification of head and neck tumors. 4th ed. Lyon, France: Lyon International Agency for Research on Cancer; 2017.

48. Lo EJ, Bell D, Woo JS, et al. Human papillomavirus and WHO type I nasopharyngeal carcinoma. Laryngoscope. 2010;120:1990–7.
49. Maxwell JH, Kumar B, Feng FY, et al. HPV-positive/p16-positive/EBV-negative nasopharyngeal carcinoma in white North Americans. Head Neck. 2010;32:562–7.
50. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst. 2000;92:709–20.
51. Bishop JA, Westra WH. NUT midline carcinomas of the sinonasal tract. Am J Surg Pathol. 2012;36:1216–21.
52. Haack H, Johnson LA, Fry CJ, et al. Diagnosis of NUT midline carcinoma using a NUT-specific monoclonal antibody. Am J Surg Pathol. 2009;33:984–91.
53. Rooper LM, Bishop JA. Sinonasal small round blue cell tumors: an immunohistochemical approach. Surg Pathol Clin. 2017;10:103–23.
54. Wenig BM. Undifferentiated malignant neoplasms of the sinonasal tract. Arch Pathol Lab Med. 2009;133:699–712.
55. Begum S, Westra WH. Basaloid squamous cell carcinoma of the head and neck is a mixed variant that can be further resolved by HPV status. Am J Surg Pathol. 2008;32:1044–50.
56. Bilodeau EA, Acquafondata M, Barnes EL, et al. A comparative analysis of LEF-1 in odontogenic and salivary tumors. Hum Pathol. 2015;46:255–9.
57. Bishop JA, Alaggio R, Zhang L, et al. Adamantinoma-like Ewing family tumors of the head and neck: a pitfall in the differential diagnosis of basaloid and myoepithelial carcinomas. Am J Surg Pathol. 2015;39:1267–74.
58. Bishop JA, Sciubba JJ, Westra WH. Squamous cell carcinoma of the oral cavity and oropharynx. Surg Pathol Clin. 2011;4:1127–51.
59. Emanuel P, Wang B, Wu M, et al. p63 Immunohistochemistry in the distinction of adenoid cystic carcinoma from basaloid squamous cell carcinoma. Mod Pathol. 2005;18:645–50.
60. Jo VY, Sholl LM, Krane JF. Distinctive patterns of CTNNB1 (beta-Catenin) alterations in salivary gland basal cell adenoma and basal cell adenocarcinoma. Am J Surg Pathol. 2016;40:1143–50.
61. Serrano MF, El-Mofty SK, Gnepp DR, et al. Utility of high molecular weight cytokeratins, but not p63, in the differential diagnosis of neuroendocrine and basaloid carcinomas of the head and neck. Hum Pathol. 2008;39:591–8.
62. Kawahara A, Harada H, Abe H, et al. Nuclear beta-catenin expression in basal cell adenomas of salivary gland. J Oral Pathol Med. 2011;40:460–6.
63. Chenevert J, Duvvuri U, Chiosea S, et al. DOG1: a novel marker of salivary acinar and intercalated duct differentiation. Mod Pathol. 2012;25:919–29.
64. Schwartz LE, Begum S, Westra WH, et al. GATA3 immunohistochemical expression in salivary gland neoplasms. Head Neck Pathol. 2013;7:311–5.
65. Schmitt AC, Griffith CC, Cohen C, et al. LEF-1: diagnostic utility in distinguishing basaloid neoplasms of the salivary gland. Diagn Cytopathol. 2017;45:1078–83.
66. Bishop JA, Yonescu R, Batista D, et al. Utility of mammaglobin immunohistochemistry as a proxy marker for the ETV6-NTRK3 translocation in the diagnosis of salivary mammary analogue secretory carcinoma. Hum Pathol. 2013;44:1982–8.
67. Brill LB 2nd, Kanner WA, Fehr A, et al. Analysis of MYB expression and MYB-NFIB gene fusions in adenoid cystic carcinoma and other salivary neoplasms. Mod Pathol. 2011;24:1169–76.
68. Rooney SL, Robinson RA. Immunohistochemical expression of MYB in salivary gland basal cell adenocarcinoma and basal cell adenoma. J Oral Pathol Med. 2017;46:798–802.
69. West RB, Kong C, Clarke N, et al. MYB expression and translocation in adenoid cystic carcinomas and other salivary gland tumors with clinicopathologic correlation. Am J Surg Pathol. 2011;35:92–9.
70. Katabi N, Xu B, Jungbluth AA, et al. PLAG1 immunohistochemistry is a sensitive marker for pleomorphic adenoma: a comparative study with PLAG1 genetic abnormalities. Histopathology. 2018;72:285–93.
71. Hsieh MS, Lee YH, Chang YL. SOX10-positive salivary gland tumors: a growing list, including mammary analogue secretory carcinoma of the salivary gland, sialoblastoma, low-grade salivary duct carcinoma, basal cell adenoma/adenocarcinoma, and a subgroup of mucoepidermoid carcinoma. Hum Pathol. 2016;56:134–42.
72. Ohtomo R, Mori T, Shibata S, et al. SOX10 is a novel marker of acinus and intercalated duct differentiation in salivary gland tumors: a clue to the histogenesis for tumor diagnosis. Mod Pathol. 2013;26:1041–50.
73. Ellis GL, Auclair PL. Tumors of the salivary glands, AFIP Atlas of Tumor Pathology: Series 4. Washington, DC: ARP Press; 2008.
74. Ji H, Isacson C, Seidman JD, et al. Cytokeratins 7 and 20, Dpc4, and MUC5AC in the distinction of metastatic mucinous carcinomas in the ovary from primary ovarian mucinous tumors: Dpc4 assists in identifying metastatic pancreatic carcinomas. Int J Gynecol Pathol. 2002;21:391–400.
75. Rooper L, Sharma R, Bishop JA. Polymorphous low grade adenocarcinoma has a consistent p63+/p40- immunophenotype that helps distinguish it from adenoid cystic carcinoma and cellular pleomorphic adenoma. Head Neck Pathol. 2015;9:79–84.
76. Parwani AV, Geradts J, Caspers E, et al. Immunohistochemical and genetic analysis of non-small cell and small cell gallbladder carcinoma and their precursor lesions. Mod Pathol. 2003;16:299–308.
77. La Rosa S, Adsay V, Albarello L, et al. Clinicopathologic study of 62 acinar cell carcinomas of the pancreas: insights into the morphology and immunophenotype and search for prognostic markers. Am J Surg Pathol. 2012;36:1782–95.
78. Singhi AD, Lilo M, Hruban RH, et al. Overexpression of lymphoid enhancer-binding factor 1 (LEF1) in solid-pseudopapillary neoplasms of the pancreas. Mod Pathol. 2014;27:1355–63.
79. Kim EK, Jang M, Park M, et al. LEF1, TFE3, and AR are putative diagnostic markers of solid pseudopapillary neoplasms. Oncotarget. 2017;8:93404–13.
80. Guo Y, Yuan F, Deng H, et al. Paranuclear dot-like immunostaining for CD99: a unique staining pattern for diagnosing solid-pseudopapillary neoplasm of the pancreas. Am J Surg Pathol. 2011;35:799–806.
81. Adsay NV, Merati K, Andea A, et al. The dichotomy in the preinvasive neoplasia to invasive carcinoma sequence in the pancreas: differential expression of MUC1 and MUC2 supports the existence of two separate pathways of carcinogenesis. Mod Pathol. 2002;15:1087–95.
82. Moniaux N, Andrianifahanana M, Brand RE, et al. Multiple roles of mucins in pancreatic cancer, a lethal and challenging malignancy. Br J Cancer. 2004;91:1633–8.
83. Kloppel G, Detlefsen S, Chari ST, et al. Autoimmune pancreatitis: the clinicopathological characteristics of the subtype with granulocytic epithelial lesions. J Gastroenterol. 2010;45:787–93.
84. Deshpande V, Zen Y, Chan JK, et al. Consensus statement on the pathology of IgG4-related disease. Mod Pathol. 2012;25:1181–92.
85. Dhall D, Suriawinata AA, Tang LH, et al. Use of immunohistochemistry for IgG4 in the distinction of autoimmune pancreatitis from peritumoral pancreatitis. Hum Pathol. 2010;41:643–52.

86. Chen ZE, Lin F. Application of immunohistochemistry in gastrointestinal and liver neoplasms: new markers and evolving practice. Arch Pathol Lab Med. 2015;139:14–23.
87. Klimstra DS, Pitman MB, Hruban RH. An algorithmic approach to the diagnosis of pancreatic neoplasms. Arch Pathol Lab Med. 2009;133:454–64.
88. Lin F, Shi J, Zhu S, et al. Cadherin-17 and SATB2 are sensitive and specific immunomarkers for medullary carcinoma of the large intestine. Arch Pathol Lab Med. 2014;138:1015–26.
89. Wang HL, Kim CJ, Koo J, et al. Practical immunohistochemistry in neoplastic pathology of the gastrointestinal tract, liver, biliary tract, and pancreas. Arch Pathol Lab Med. 2017;141:1155–80.
90. Patil DT, Rubin BP. Gastrointestinal stromal tumor: advances in diagnosis and management. Arch Pathol Lab Med. 2011;135:1298–310.
91. Virani N, Pang J, Lew M. Cytologic and immunohistochemical evaluation of low-grade spindle cell lesions of the gastrointestinal tract. Arch Pathol Lab Med. 2016;140:1038–44.
92. Kane MF, Loda M, Gaida GM, et al. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res. 1997;57:808–11.
93. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol. 2008;26:5783–8.
94. Metcalf AM, Spurdle AB. Endometrial tumour BRAF mutations and MLH1 promoter methylation as predictors of germline mismatch repair gene mutation status: a literature review. Fam Cancer. 2014;13:1–12.
95. Buchanan DD, Rosty C, Clendenning M, et al. Clinical problems of colorectal cancer and endometrial cancer cases with unknown cause of tumor mismatch repair deficiency (suspected Lynch syndrome). Appl Clin Genet. 2014;7:183–93.
96. Hatch SB, Lightfoot HM Jr, Garwacki CP, et al. Microsatellite instability testing in colorectal carcinoma: choice of markers affects sensitivity of detection of mismatch repair-deficient tumors. Clin Cancer Res. 2005;11:2180–7.
97. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med. 2003;349:247–57.
98. Watanabe T, Wu TT, Catalano PJ, et al. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. N Engl J Med. 2001;344:1196–206.
99. De Jong AE, Morreau H, Van Puijenbroek M, et al. The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. Gastroenterology. 2004;126:42–8.
100. Iino H, Simms L, Young J, et al. DNA microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. Gut. 2000;47:37–42.
101. Muller CI, Schulmann K, Reinacher-Schick A, et al. Predictive and prognostic value of microsatellite instability in patients with advanced colorectal cancer treated with a fluoropyrimidine and oxaliplatin containing first-line chemotherapy. A report of the AIO Colorectal Study Group. Int J Colorectal Dis. 2008;23:1033–9.
102. Pino MS, Mino-Kenudson M, Wildemore BM, et al. Deficient DNA mismatch repair is common in Lynch syndrome-associated colorectal adenomas. J Mol Diagn. 2009;11:238–47.
103. Shahid M, Mubeen A, Tse J, et al. Branched chain in situ hybridization for albumin as a marker of hepatocellular differentiation: evaluation of manual and automated in situ hybridization platforms. Am J Surg Pathol. 2015;39:25–34.
104. Chandan VS, Shah SS, Torbenson MS, et al. Arginase-1 is frequently positive in hepatoid adenocarcinomas. Hum Pathol. 2016;55:11–6.
105. Lau SK, Prakash S, Geller SA, et al. Comparative immunohistochemical profile of hepatocellular carcinoma, cholangiocarcinoma, and metastatic adenocarcinoma. Hum Pathol. 2002;33:1175–81.
106. Wang FH, Yip YC, Zhang M, et al. Diagnostic utility of glypican-3 for hepatocellular carcinoma on liver needle biopsy. J Clin Pathol. 2010;63:599–603.
107. Yan BC, Gong C, Song J, et al. Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. Am J Surg Pathol. 2010;34:1147–54.
108. Coston WM, Loera S, Lau SK, et al. Distinction of hepatocellular carcinoma from benign hepatic mimickers using Glypican-3 and CD34 immunohistochemistry. Am J Surg Pathol. 2008;32:433–44.
109. Libbrecht L, Severi T, Cassiman D, et al. Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. Am J Surg Pathol. 2006;30:1405–11.
110. Wang HL, Anatelli FA, Zhai Q, et al. Glypican-3 is a useful diagnostic marker that distinguishes hepatocellular carcinoma from benign hepatocellular mass lesions. Arch Pathol Lab Med. 2008;132(11):1723–8.
111. Bioulac-Sage P, Balabaud C, Zucman-Rossi J. Subtype classification of hepatocellular adenoma. Dig Surg. 2010;27:39–45.
112. Dhingra S, Fiel MI. Update on the new classification of hepatic adenomas: clinical, molecular, and pathologic characteristics. Arch Pathol Lab Med. 2014;138:1090–7.
113. Travis WD, Brambilla E, Burke AP, et al. WHO classification of tumours of the lung, pleura, thymus and heart. Lyon, France: IARC Press; 2015. 334 pp.
114. Siami K, McCluggage WG, Ordonez NG, et al. Thyroid transcription factor-1 expression in endometrial and endocervical adenocarcinomas. Am J Surg Pathol. 2007;31:1759–63.
115. Kubba LA, McCluggage WG, Liu J, et al. Thyroid transcription factor-1 expression in ovarian epithelial neoplasms. Mod Pathol. 2008;21:485–90.
116. Rekhtman N, Kazi S. Nonspecific reactivity of polyclonal napsin a antibody in mucinous adenocarcinomas of various sites: a word of caution. Arch Pathol Lab Med. 2015;139:434–6.
117. Bishop JA, Teruya-Feldstein J, Westra WH, et al. p40 (DeltaNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol. 2012;25:405–15.
118. Rekhtman N. Neuroendocrine tumors of the lung: an update. Arch Pathol Lab Med. 2010;134:1628–38.
119. Cimino-Mathews A, Subhawong AP, Illei PB, et al. GATA3 expression in breast carcinoma: utility in triple-negative, sarcomatoid, and metastatic carcinomas. Hum Pathol. 2013;44:1341–9.
120. Dabbs DJ, Landreneau RJ, Liu Y, et al. Detection of estrogen receptor by immunohistochemistry in pulmonary adenocarcinoma. Ann Thorac Surg. 2002;73:403–5; discussion 6.
121. Miettinen M, McCue PA, Sarlomo-Rikala M, et al. GATA3: a multispecific but potentially useful marker in surgical pathology: a systematic analysis of 2500 epithelial and nonepithelial tumors. Am J Surg Pathol. 2014;38:13–22.
122. Takeda Y, Tsuta K, Shibuki Y, et al. Analysis of expression patterns of breast cancer-specific markers (mammaglobin and gross cystic disease fluid protein 15) in lung and pleural tumors. Arch Pathol Lab Med. 2008;132:239–43.
123. Wang LJ, Greaves WO, Sabo E, et al. GCDFP-15 positive and TTF-1 negative primary lung neoplasms: a tissue microarray study of 381 primary lung tumors. Appl Immunohistochem Mol Morphol. 2009;17:505–11.

124. Tian W, Guner G, Miyamoto H, et al. Utility of uroplakin II expression as a marker of urothelial carcinoma. Hum Pathol. 2015;46:58–64.
125. Gaffey MJ, Mills SE, Askin FB. Minute pulmonary meningothelial-like nodules. A clinicopathologic study of so-called minute pulmonary chemodectoma. Am J Surg Pathol. 1988;12:167–75.
126. Ordonez NG. Value of claudin-4 immunostaining in the diagnosis of mesothelioma. Am J Clin Pathol. 2013;139:611–9.
127. Chapel DB, Husain AN, Krausz T, et al. PAX8 expression in a subset of malignant peritoneal mesotheliomas and benign mesothelium has diagnostic implications in the differential diagnosis of ovarian serous carcinoma. Am J Surg Pathol. 2017;41:1675–82.
128. Carella R, Deleonardi G, D'Errico A, et al. Immunohistochemical panels for differentiating epithelial malignant mesothelioma from lung adenocarcinoma: a study with logistic regression analysis. Am J Surg Pathol. 2001;25:43–50.
129. Husain AN, Colby TV, Ordonez NG, et al. Guidelines for pathologic diagnosis of malignant mesothelioma 2017 update of the consensus statement from the International Mesothelioma Interest Group. Arch Pathol Lab Med. 2018;142:89–108.
130. Ordonez NG. The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. Am J Surg Pathol. 2003;27:1031–51.
131. Sheffield BS, Hwang HC, Lee AF, et al. BAP1 immunohistochemistry and p16 FISH to separate benign from malignant mesothelial proliferations. Am J Surg Pathol. 2015;39:977–82.
132. Tandon RT, Jimenez-Cortez Y, Taub R, et al. Immunohistochemistry in peritoneal mesothelioma: a single-center experience of 244 cases. Arch Pathol Lab Med. 2018;142:236–42.
133. Hida T, Hamasaki M, Matsumoto S, et al. Immunohistochemical detection of MTAP and BAP1 protein loss for mesothelioma diagnosis: comparison with 9p21 FISH and BAP1 immunohistochemistry. Lung Cancer. 2017;104:98–105.
134. Dorfman DM, Shahsafaei A, Chan JK. Thymic carcinomas, but not thymomas and carcinomas of other sites, show CD5 immunoreactivity. Am J Surg Pathol. 1997;21:936–40.
135. Kojika M, Ishii G, Yoshida J, et al. Immunohistochemical differential diagnosis between thymic carcinoma and type B3 thymoma: diagnostic utility of hypoxic marker, GLUT-1, in thymic epithelial neoplasms. Mod Pathol. 2009;22:1341–50.
136. Nakagawa K, Matsuno Y, Kunitoh H, et al. Immunohistochemical KIT (CD117) expression in thymic epithelial tumors. Chest. 2005;128:140–4.
137. Kao YC, Owosho AA, Sung YS, et al. BCOR-CCNB3 fusion positive sarcomas: a clinicopathologic and molecular analysis of 36 cases with comparison to morphologic spectrum and clinical behavior of other round cell sarcomas. Am J Surg Pathol. 2018;42:604–15.
138. Miettinen M, Wang Z, Lasota J, et al. Nuclear Brachyury expression is consistent in chordoma, common in germ cell tumors and small cell carcinomas, and rare in other carcinomas and sarcomas: an immunohistochemical study of 5229 cases. Am J Surg Pathol. 2015;39:1305–12.
139. Doyle LA, Fletcher CD, Hornick JL. Nuclear expression of CAMTA1 distinguishes epithelioid hemangioendothelioma from histologic mimics. Am J Surg Pathol. 2016;40:94–102.
140. Hornick JL, Dal Cin P, Fletcher CD. Loss of INI1 expression is characteristic of both conventional and proximal-type epithelioid sarcoma. Am J Surg Pathol. 2009;33:542–50.
141. Stockman DL, Hornick JL, Deavers MT, et al. ERG and FLI1 protein expression in epithelioid sarcoma. Mod Pathol. 2014;27:496–501.
142. Doyle LA, Moller E, Dal Cin P, et al. MUC4 is a highly sensitive and specific marker for low-grade fibromyxoid sarcoma. Am J Surg Pathol. 2011;35:733–41.
143. Schaefer IM, Agaimy A, Fletcher CD, et al. Claudin-4 expression distinguishes SWI/SNF complex-deficient undifferentiated carcinomas from sarcomas. Mod Pathol. 2017;30:539–48.
144. Agaram NP, Sung YS, Zhang L, et al. Dichotomy of genetic abnormalities in PEComas with therapeutic implications. Am J Surg Pathol. 2015;39:813–25.
145. Argani P, Aulmann S, Illei PB, et al. A distinctive subset of PEComas harbors TFE3 gene fusions. Am J Surg Pathol. 2010;34:1395–406.
146. Jagdis A, Rubin BP, Tubbs RR, et al. Prospective evaluation of TLE1 as a diagnostic immunohistochemical marker in synovial sarcoma. Am J Surg Pathol. 2009;33:1743–51.
147. Kosemehmetoglu K, Vrana JA, Folpe AL. TLE1 expression is not specific for synovial sarcoma: a whole section study of 163 soft tissue and bone neoplasms. Mod Pathol. 2009;22:872–8.
148. Hornick JL. Practical soft tissue pathology: a diagnostic approach. Philadelphia: Elsevier/Saunders; 2013.
149. Hornick JL. Novel uses of immunohistochemistry in the diagnosis and classification of soft tissue tumors. Mod Pathol. 2014;27(Suppl 1):S47–63.
150. Louis DN, Ohgaki H, Wiestler OD, et al. WHO classification of tumours of the central nervous system, vol. 1. 4th ed. Lyon: IARC Press; 2016.
151. Ogura R, Tsukamoto Y, Natsumeda M, et al. Immunohistochemical profiles of IDH1, MGMT and P53: practical significance for prognostication of patients with diffuse gliomas. Neuropathology. 2015;35:324–35.
152. Brat DJ, Verhaak RG, Aldape KD, et al. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med. 2015;372:2481–98.
153. Burger PC, Scheithauer BW. Tumors of the central nervous system, AFIP Atlas of Tumor Pathology: Series 4. 1st ed. Washington, DC: American Registry of Pathology; 2007.. 596 pp
154. Barresi V, Vitarelli E, Branca G, et al. Expression of brachyury in hemangioblastoma: potential use in differential diagnosis. Am J Surg Pathol. 2012;36:1052–7.
155. Boulagnon-Rombi C, Fleury C, Fichel C, et al. Immunohistochemical approach to the differential diagnosis of meningiomas and their mimics. J Neuropathol Exp Neurol. 2017;76:289–98.
156. Carney EM, Banerjee P, Ellis CL, et al. PAX2(-)/PAX8(-)/inhibin A(+) immunoprofile in hemangioblastoma: a helpful combination in the differential diagnosis with metastatic clear cell renal cell carcinoma to the central nervous system. Am J Surg Pathol. 2011;35:262–7.
157. Hasselblatt M, Nolte KW, Paulus W. Angiomatous meningioma: a clinicopathologic study of 38 cases. Am J Surg Pathol. 2004;28:390–3.
158. Hoang MP, Amirkhan RH. Inhibin alpha distinguishes hemangioblastoma from clear cell renal cell carcinoma. Am J Surg Pathol. 2003;27:1152–6.
159. Rivera AL, Takei H, Zhai J, et al. Useful immunohistochemical markers in differentiating hemangioblastoma versus metastatic renal cell carcinoma. Neuropathology. 2010;30:580–5.
160. Takei H, Powell SZ. Novel immunohistochemical markers in the diagnosis of nonglial tumors of nervous system. Adv Anat Pathol. 2010;17:150–3.
161. Cho HY, Lee M, Takei H, et al. Immunohistochemical comparison of chordoma with chondrosarcoma, myxopapillary ependymoma, and chordoid meningioma. Appl Immunohistochem Mol Morphol. 2009;17:131–8.
162. Huse JT, Pasha TL, Zhang PJ. D2-40 functions as an effective chondroid marker distinguishing true chondroid tumors from chordoma. Acta Neuropathol. 2007;113:87–94.
163. Sangoi AR, Dulai MS, Beck AH, et al. Distinguishing chordoid meningiomas from their histologic mimics: an immunohistochemical evaluation. Am J Surg Pathol. 2009;33:669–81.
164. Kobel M, Ronnett BM, Singh N, et al. Interpretation of P53 immunohistochemistry in endometrial carcinomas: toward increased reproducibility. Int J Gynecol Pathol. 2019;38 Suppl 1:S123–31.

165. Comin CE, Saieva C, Messerini L. h-caldesmon, calretinin, estrogen receptor, and Ber-EP4: a useful combination of immunohistochemical markers for differentiating epithelioid peritoneal mesothelioma from serous papillary carcinoma of the ovary. Am J Surg Pathol. 2007;31:1139–48.
166. Nofech-Mozes S, Khalifa MA, Ismiil N, et al. Immunophenotyping of serous carcinoma of the female genital tract. Mod Pathol. 2008;21:1147–55.
167. Ordonez NG. Value of immunohistochemistry in distinguishing peritoneal mesothelioma from serous carcinoma of the ovary and peritoneum: a review and update. Adv Anat Pathol. 2006;13:16–25.
168. Conlon N, Silva A, Guerra E, et al. Loss of SMARCA4 expression is both sensitive and specific for the diagnosis of small cell carcinoma of ovary, hypercalcemic type. Am J Surg Pathol. 2016;40:395–403.
169. Al-Agha OM, Huwait HF, Chow C, et al. FOXL2 is a sensitive and specific marker for sex cord-stromal tumors of the ovary. Am J Surg Pathol. 2011;35:484–94.
170. Baker PM, Oliva E. Immunohistochemistry as a tool in the differential diagnosis of ovarian tumors: an update. Int J Gynecol Pathol. 2005;24:39–55.
171. McCluggage WG, Young RH. Immunohistochemistry as a diagnostic aid in the evaluation of ovarian tumors. Semin Diagn Pathol. 2005;22:3–32.
172. Zhao C, Vinh TN, McManus K, et al. Identification of the most sensitive and robust immunohistochemical markers in different categories of ovarian sex cord-stromal tumors. Am J Surg Pathol. 2009;33:354–66.
173. Kobel M, Kalloger SE, Carrick J, et al. A limited panel of immunomarkers can reliably distinguish between clear cell and high-grade serous carcinoma of the ovary. Am J Surg Pathol. 2009;33:14–21.
174. Lax SF, Pizer ES, Ronnett BM, et al. Clear cell carcinoma of the endometrium is characterized by a distinctive profile of p53, Ki-67, estrogen, and progesterone receptor expression. Hum Pathol. 1998;29:551–8.
175. Reid-Nicholson M, Iyengar P, Hummer AJ, et al. Immunophenotypic diversity of endometrial adenocarcinomas: implications for differential diagnosis. Mod Pathol. 2006;19:1091–100.
176. Yemelyanova A, Ji H, Shih Ie M, et al. Utility of p16 expression for distinction of uterine serous carcinomas from endometrial endometrioid and endocervical adenocarcinomas: immunohistochemical analysis of 201 cases. Am J Surg Pathol. 2009;33:1504–14.
177. Carlson JW, Jarboe EA, Kindelberger D, et al. Serous tubal intraepithelial carcinoma: diagnostic reproducibility and its implications. Int J Gynecol Pathol. 2010;29:310–4.
178. Park KJ, Kiyokawa T, Soslow RA, et al. Unusual endocervical adenocarcinomas: an immunohistochemical analysis with molecular detection of human papillomavirus. Am J Surg Pathol. 2011;35:633–46.
179. Ronnett BM. Endocervical adenocarcinoma: selected diagnostic challenges. Mod Pathol. 2016;29(Suppl 1):S12–28.
180. Busca A, Gulavita P, Parra-Herran C, et al. IFITM1 outperforms CD10 in differentiating low-grade endometrial stromal sarcomas from smooth muscle neoplasms of the uterus. Int J Gynecol Pathol. 2018;37:372–8.
181. Chiang S, Lee CH, Stewart CJR, et al. BCOR is a robust diagnostic immunohistochemical marker of genetically diverse high-grade endometrial stromal sarcoma, including tumors exhibiting variant morphology. Mod Pathol. 2017;30:1251–61.
182. Lee CH, Ali RH, Rouzbahman M, et al. Cyclin D1 as a diagnostic immunomarker for endometrial stromal sarcoma with YWHAE-FAM22 rearrangement. Am J Surg Pathol. 2012;36:1562–70.
183. Hoang L, Chiang S, Lee CH. Endometrial stromal sarcomas and related neoplasms: new developments and diagnostic considerations. Pathology. 2018;50:162–77.
184. Oliva E, Young RH, Amin MB, et al. An immunohistochemical analysis of endometrial stromal and smooth muscle tumors of the uterus: a study of 54 cases emphasizing the importance of using a panel because of overlap in immunoreactivity for individual antibodies. Am J Surg Pathol. 2002;26:403–12.
185. Irving JA, Carinelli S, Prat J. Uterine tumors resembling ovarian sex cord tumors are polyphenotypic neoplasms with true sex cord differentiation. Mod Pathol. 2006;19:17–24.
186. Czernobilsky B. Uterine tumors resembling ovarian sex cord tumors: an update. Int J Gynecol Pathol. 2008;27:229–35.
187. Shih IM. Trophogram, an immunohistochemistry-based algorithmic approach, in the differential diagnosis of trophoblastic tumors and tumorlike lesions. Ann Diagn Pathol. 2007;11:228–34.
188. Castrillon DH, Sun D, Weremowicz S, et al. Discrimination of complete hydatidiform mole from its mimics by immunohistochemistry of the paternally imprinted gene product p57KIP2. Am J Surg Pathol. 2001;25:1225–30.
189. Kipp BR, Ketterling RP, Oberg TN, et al. Comparison of fluorescence in situ hybridization, p57 immunostaining, flow cytometry, and digital image analysis for diagnosing molar and nonmolar products of conception. Am J Clin Pathol. 2010;133:196–204.
190. Prieto VG, Shea CR. Use of immunohistochemistry in melanocytic lesions. J Cutan Pathol. 2008;35(Suppl 2):1–10.
191. Bandarchi B, Ma L, Marginean C, et al. D2-40, a novel immunohistochemical marker in differentiating dermatofibroma from dermatofibrosarcoma protuberans. Mod Pathol. 2010;23:434–8.
192. Stanoszek LM, Wang GY, Harms PW. Histologic mimics of basal cell carcinoma. Arch Pathol Lab Med. 2017;141:1490–502.
193. Busam KJ, Jungbluth AA, Rekthman N, et al. Merkel cell polyomavirus expression in merkel cell carcinomas and its absence in combined tumors and pulmonary neuroendocrine carcinomas. Am J Surg Pathol. 2009;33:1378–85.
194. Pulitzer MP, Brannon AR, Berger MF, et al. Cutaneous squamous and neuroendocrine carcinoma: genetically and immunohistochemically different from Merkel cell carcinoma. Mod Pathol. 2015;28:1023–32.
195. Gleason BC, Calder KB, Cibull TL, et al. Utility of p63 in the differential diagnosis of atypical fibroxanthoma and spindle cell squamous cell carcinoma. J Cutan Pathol. 2009;36:543–7.
196. Miller K, Goodlad JR, Brenn T. Pleomorphic dermal sarcoma: adverse histologic features predict aggressive behavior and allow distinction from atypical fibroxanthoma. Am J Surg Pathol. 2012;36:1317–26.
197. Tardio JC, Pinedo F, Aramburu JA, et al. Pleomorphic dermal sarcoma: a more aggressive neoplasm than previously estimated. J Cutan Pathol. 2016;43:101–12.
198. Goldblum JR, Hart WR. Perianal Paget's disease: a histologic and immunohistochemical study of 11 cases with and without associated rectal adenocarcinoma. Am J Surg Pathol. 1998;22:170–9.
199. Morbeck D, Tregnago AC, Baiocchi G, et al. GATA3 expression in primary vulvar Paget disease: a potential pitfall leading to misdiagnosis of pagetoid urothelial intraepithelial neoplasia. Histopathology. 2017;70:435–41.

Chapter 3. Immunostains: Hematopoietic System

1. Swerdlow S, Campo E, Harris N, et al. WHO classification of tumors of haematopoietic and lymphoid tissues. (Revised 4th ed.). Lyon, France: IARC Press; 2017.
2. Ioachim H, Medeiros L. The normal lymph node. In: Ioachim H, Medeiros L, editors. Ioachim's lymph node pathology. 4th ed. Lippincott Williams & Wilkins: Philadelphia; 2009. p. 2–15.

3. Bell A, Sallah S, Diggs L. Hematopoiesis. In: Bell A, Sallah S, Diggs L, editors. Morphology of human blood cells. 7th ed. Abbott Park: Abbott Laboratory; 2005. p. 1–12.
4. Sagaert X, Sprangers B, De Wolf-Peeters C. The dynamics of the B follicle: understanding the normal counterpart of B-cell-derived malignancies. Leukemia. 2007;21:1378–86.
5. Dictor M, Ek S, Sundberg M, et al. Strong lymphoid nuclear expression of SOX11 transcription factor defines lymphoblastic neoplasms, mantle cell lymphoma and Burkitt's lymphoma. Haematologica. 2009;94:1563–8.
6. Navarro A, Clot G, Royo C, et al. Molecular subsets of mantle cell lymphoma defined by the IGHV mutational status and SOX11 expression have distinct biologic and clinical features. Cancer research. 2012;72:5307–16.
7. Soldini D, Valera A, Sole C, et al. Assessment of SOX11 expression in routine lymphoma tissue sections: characterization of new monoclonal antibodies for diagnosis of mantle cell lymphoma. Am J Surg Pathol. 2014;38:86–93.
8. Younes SF, Beck AH, Lossos IS, et al. Immunoarchitectural patterns in follicular lymphoma: efficacy of HGAL and LMO2 in the detection of the interfollicular and diffuse components. Am J Surg Pathol. 2010;34:1266–76.
9. Menter T, Gasser A, Juskevicius D, Dirnhofer S, Tzankov A. Diagnostic utility of the germinal center-associated markers GCET1, HGAL, and LMO2 in hematolymphoid neoplasms. Appl Immunohistochem Mol Morphol. 2015;23:491–8.
10. Lorsbach RB, Hsi ED, Dogan A, Fend F. Plasma cell myeloma and related neoplasms. Am J Clin Pathol. 2011;136:168–82.
11. Hsi ED, Lorsbach RB, Fend F, Dogan A. Plasmablastic lymphoma and related disorders. Am J Clin Pathol. 2011;136:183–94.
12. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004;103:275–82.
13. Berglund M, Thunberg U, Amini RM, et al. Evaluation of immunophenotype in diffuse large B-cell lymphoma and its impact on prognosis. Modern Pathol: Off J US Can Acad Pathol Inc. 2005;18:1113–20.
14. Choi WW, Weisenburger DD, Greiner TC, et al. A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. Clin Cancer Res. 2009;15:5494–502.
15. Friedberg JW. How I treat double-hit lymphoma. Blood. 2017;130:590–6.
16. Pileri S, Jones DM, Jaffe R, et al. Histiocytic and dendritic cell neoplasms. In: Swerdlow SH, Campo E, Harris NL, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Revised 4th ed. Lyon, France: IARC; 2017.

Chapter 4. Predictive Markers

1. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med. 2010;134:e48–72.
2. Wolff AC, Hammond ME, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. Arch Pathol Lab Med. 2018;142(11):1364–82.
3. DAKO HercepTest. A manual for interpretation. https://www.agilent.com/cs/library/usermanuals/public/28630_herceptest_interpretation_manual-breast_ihc_row.pdf.
4. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol. 2013;31:3997–4013.
5. Ruschoff J, Dietel M, Baretton G, et al. HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. Virchows Arch. 2010;457:299–307.
6. Abrahao-Machado LF, Scapulatempo-Neto C. HER2 testing in gastric cancer: an update. World J Gastroenterol. 2016;22:4619–25.
7. DAKO PD-L1 IHC 22C3 pharmDx. Interpretation manual. https://www.agilent.com/cs/library/usermanuals/public/29158_pd-l1-ihc-22C3-pharmdx-nsclc-interpretation-manual.pdf.
8. Brown NF, Carter T, Mulholland P. Dabrafenib in BRAFV600-mutated anaplastic pleomorphic xanthoastrocytoma. CNS Oncol. 2017;6:5–9.
9. Chamberlain MC. Salvage therapy with BRAF inhibitors for recurrent pleomorphic xanthoastrocytoma: a retrospective case series. J Neurooncol. 2013;114:237–40.
10. Lindel K, Beer KT, Laissue J, et al. Human papillomavirus positive squamous cell carcinoma of the oropharynx: a radiosensitive subgroup of head and neck carcinoma. Cancer. 2001;92:805–13.
11. Lewis JS Jr, Beadle B, Bishop JA, et al. Human papillomavirus testing in head and neck carcinomas: guideline from the College of American Pathologists. Arch Pathol Lab Med. 2018;142:559–97.
12. Ziemann F, Arenz A, Preising S, et al. Increased sensitivity of HPV-positive head and neck cancer cell lines to x-irradiation +/− cisplatin due to decreased expression of E6 and E7 oncoproteins and enhanced apoptosis. Am J Cancer Res. 2015;5:1017–31.
13. Limaye SA, Posner MR, Krane JF, et al. Trastuzumab for the treatment of salivary duct carcinoma. Oncologist. 2013;18:294–300.
14. Brose MS, Cabanillas ME, Cohen EE, et al. Vemurafenib in patients with BRAF(V600E)-positive metastatic or unresectable papillary thyroid cancer refractory to radioactive iodine: a non-randomised, multicentre, open-label, phase 2 trial. Lancet Oncol. 2016;17:1272–82.
15. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010;376:687–97.
16. Dalerba P, Sahoo D, Paik S, et al. CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N Engl J Med. 2016;374:211–22.

Chapter 5. Immunostains: Antibody Index – Solid Tumors

1. Matoso A, Singh K, Jacob R, et al. Comparison of thyroid transcription factor-1 expression by 2 monoclonal antibodies in pulmonary and nonpulmonary primary tumors. Appl Immunohistochem Mol Morphol. 2010;18:142–9.
2. Zhang PJ, Gao HG, Pasha TL, Litzky L, Livolsi VA. TTF-1 expression in ovarian and uterine epithelial neoplasia and its potential significance, an immunohistochemical assessment with multiple monoclonal antibodies and different secondary detection systems. Int J Gynecol Pathol. 2009;28:10–8.
3. Tran L, Mattsson JS, Nodin B, et al. Various antibody clones of napsin A, thyroid transcription factor 1, and p40 and comparisons with cytokeratin 5 and p63 in histopathologic diagnostics of non-small cell lung carcinoma. Appl Immunohistochem Mol Morphol. 2016;24:648–59.
4. Klebe S, Swalling A, Jonavicius L, Henderson DW. An immunohistochemical comparison of two TTF-1 monoclonal antibodies in atypical squamous lesions and sarcomatoid carcinoma of the lung, and pleural malignant mesothelioma. J Clin Pathol. 2016;69:136–41.

5. Toriyama A, Mori T, Sekine S, et al. Utility of PAX8 mouse monoclonal antibody in the diagnosis of thyroid, thymic, pleural and lung tumours: a comparison with polyclonal PAX8 antibody. Histopathology. 2014;65:465–72.
6. Ordonez NG. Value of PAX 8 immunostaining in tumor diagnosis: a review and update. Adv Anat Pathol. 2012;19:140–51.
7. Rekhtman N, Kazi S. Nonspecific reactivity of polyclonal napsin a antibody in mucinous adenocarcinomas of various sites: a word of caution. Arch Pathol Lab Med. 2015;139:434–6.
8. Acs B, Kulka J, Kovacs KA, et al. Comparison of 5 Ki-67 antibodies regarding reproducibility and capacity to predict prognosis in breast cancer: does the antibody matter? Hum Pathol. 2017;65:31–40.
9. Focke CM, Burger H, van Diest PJ, et al. Interlaboratory variability of Ki67 staining in breast cancer. Eur J Cancer. 2017;84:219–27.
10. Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med. 2010;134:e48–72.
11. Dabbs DJ, Landreneau RJ, Liu Y, et al. Detection of estrogen receptor by immunohistochemistry in pulmonary adenocarcinoma. Ann Thorac Surg. 2002;73:403–5; discussion 6.
12. Hakima L, Schlesinger K, Sunkara J, Karabakhtsian RG. Differential expression of various clones of estrogen receptor in cell block preparation of lung adenocarcinoma. Diagn Cytopathol. 2018;46:390–4.
13. Gomez-Fernandez C, Mejias A, Walker G, Nadji M. Immunohistochemical expression of estrogen receptor in adenocarcinomas of the lung: the antibody factor. Appl Immunohistochem Mol Morphol. 2010;18:137–41.
14. Arnold MA, Schoenfield L, Limketkai BN, Arnold CA. Diagnostic pitfalls of differentiating desmoplastic small round cell tumor (DSRCT) from Wilms tumor (WT): overlapping morphologic and immunohistochemical features. Am J Surg Pathol. 2014;38:1220–6.
15. Murphy AJ, Bishop K, Pereira C, et al. A new molecular variant of desmoplastic small round cell tumor: significance of WT1 immunostaining in this entity. Hum Pathol. 2008;39:1763–70.
16. Stockman DL, Hornick JL, Deavers MT, et al. ERG and FLI1 protein expression in epithelioid sarcoma. Mod Pathol. 2014;27:496–501.
17. Miettinen M, Wang Z, Sarlomo-Rikala M, et al. ERG expression in epithelioid sarcoma: a diagnostic pitfall. Am J Surg Pathol. 2013;37:1580–5.
18. Taheri D, Zahavi DJ, Del Carmen Rodriguez M, et al. For staining of ALK protein, the novel D5F3 antibody demonstrates superior overall performance in terms of intensity and extent of staining in comparison to the currently used ALK1 antibody. Virchows Arch. 2016;469:345–50.
19. Ostler DA, Prieto VG, Reed JA, et al. Adipophilin expression in sebaceous tumors and other cutaneous lesions with clear cell histology: an immunohistochemical study of 117 cases. Mod Pathol. 2010;23:567–73.
20. Muthusamy K, Halbert G, Roberts F. Immunohistochemical staining for adipophilin, perilipin and TIP47. J Clin Pathol. 2006;59:1166–70.
21. Yan BC, Gong C, Song J, et al. Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. Am J Surg Pathol. 2010;34:1147–54.
22. Sheffield BS, Hwang HC, Lee AF, et al. BAP1 immunohistochemistry and p16 FISH to separate benign from malignant mesothelial proliferations. Am J Surg Pathol. 2015;39:977–82.
23. Andrici J, Gill AJ, Hornick JL. Next generation immunohistochemistry: emerging substitutes to genetic testing? Semin Diagn Pathol. 2018;35:161–9.
24. Conlon N, Silva A, Guerra E, et al. Loss of SMARCA4 expression is both sensitive and specific for the diagnosis of small cell carcinoma of ovary, hypercalcemic type. Am J Surg Pathol. 2016;40:395–403.
25. Shain AH, Giacomini CP, Matsukuma K, et al. Convergent structural alterations define SWItch/Sucrose NonFermentable (SWI/SNF) chromatin remodeler as a central tumor suppressive complex in pancreatic cancer. Proc Natl Acad Sci U S A. 2012;109:E252–9.
26. Strehl JD, Wachter DL, Fiedler J, et al. Pattern of SMARCB1 (INI1) and SMARCA4 (BRG1) in poorly differentiated endometrioid adenocarcinoma of the uterus: analysis of a series with emphasis on a novel SMARCA4-deficient dedifferentiated rhabdoid variant. Ann Diagn Pathol. 2015;19:198–202.
27. Herpel E, Rieker RJ, Dienemann H, et al. SMARCA4 and SMARCA2 deficiency in non-small cell lung cancer: immunohistochemical survey of 316 consecutive specimens. Ann Diagn Pathol. 2017;26:47–51.
28. Agaimy A, Fuchs F, Moskalev EA, et al. SMARCA4-deficient pulmonary adenocarcinoma: clinicopathological, immunohistochemical, and molecular characteristics of a novel aggressive neoplasm with a consistent TTF1(neg)/CK7(pos)/HepPar-1(pos) immunophenotype. Virchows Arch. 2017;471:599–609.
29. Agaimy A, Daum O, Markl B, et al. SWI/SNF complex-deficient undifferentiated/rhabdoid carcinomas of the gastrointestinal tract: a series of 13 cases highlighting mutually exclusive loss of SMARCA4 and SMARCA2 and frequent co-inactivation of SMARCB1 and SMARCA2. Am J Surg Pathol. 2016;40:544–53.
30. Doyle LA, Fletcher CD, Hornick JL. Nuclear expression of CAMTA1 distinguishes epithelioid hemangioendothelioma from histologic mimics. Am J Surg Pathol. 2016;40:94–102.
31. Miettinen M. Immunohistochemistry of soft tissue tumours – review with emphasis on 10 markers. Histopathology. 2014;64:101–18.
32. Guo Y, Yuan F, Deng H, et al. Paranuclear dot-like immunostaining for CD99: a unique staining pattern for diagnosing solid-pseudopapillary neoplasm of the pancreas. Am J Surg Pathol. 2011;35:799–806.
33. Chen ZE, Lin F. Application of immunohistochemistry in gastrointestinal and liver neoplasms: new markers and evolving practice. Arch Pathol Lab Med. 2015;139:14–23.
34. Lin F, Shi J, Zhu S, et al. Cadherin-17 and SATB2 are sensitive and specific immunomarkers for medullary carcinoma of the large intestine. Arch Pathol Lab Med. 2014;138:1015–26.
35. Thway K, Flora R, Shah C, Olmos D, Fisher C. Diagnostic utility of p16, CDK4, and MDM2 as an immunohistochemical panel in distinguishing well-differentiated and dedifferentiated liposarcomas from other adipocytic tumors. Am J Surg Pathol. 2012;36:462–9.
36. Bing Z, Pasha T, Tomaszewski JE, Zhang P. CDX2 expression in yolk sac component of testicular germ cell tumors. Int J Surg Pathol. 2009;17:373–7.
37. Terry J, Calicchio ML, Rodriguez-Galindo C, Perez-Atayde AR. Immunohistochemical expression of CRX in extracranial malignant small round cell tumors. Am J Surg Pathol. 2012;36:1165–9.
38. Lau SK, Weiss LM, Chu PG. D2-40 immunohistochemistry in the differential diagnosis of seminoma and embryonal carcinoma: a comparative immunohistochemical study with KIT (CD117) and CD30. Mod Pathol. 2007;20:320–5.
39. Kalof AN, Cooper K. D2-40 immunohistochemistry–so far! Adv Anat Pathol. 2009;16:62–4.
40. Ordonez NG. Podoplanin: a novel diagnostic immunohistochemical marker. Adv Anat Pathol. 2006;13:83–8.
41. Miettinen M, Wang ZF, Lasota J. DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases. Am J Surg Pathol. 2009;33:1401–8.

42. Carter CS, Skala SL, Chinnaiyan AM, et al. Immunohistochemical characterization of Fumarate Hydratase (FH) and Succinate Dehydrogenase (SDH) in cutaneous leiomyomas for detection of familial cancer syndromes. Am J Surg Pathol. 2017;41:801–9.
43. Buelow B, Cohen J, Nagymanyoki Z, et al. Immunohistochemistry for 2-Succinocysteine (2SC) and Fumarate Hydratase (FH) in cutaneous leiomyomas may aid in identification of patients with HLRCC (Hereditary Leiomyomatosis and Renal Cell Carcinoma Syndrome). Am J Surg Pathol. 2016;40:982–8.
44. Ellison DW, Dalton J, Kocak M, et al. Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. Acta Neuropathol. 2011;121:381–96.
45. Chlapek P, Zitterbart K, Kren L, et al. Uniformity under in vitro conditions: changes in the phenotype of cancer cell lines derived from different medulloblastoma subgroups. PloS one. 2017;12:e0172552.
46. Egashira N, Takekoshi S, Takei M, Teramoto A, Osamura RY. Expression of FOXL2 in human normal pituitaries and pituitary adenomas. Mod Pathol. 2011;24:765–73.
47. Kandil DH, Cooper K. Glypican-3: a novel diagnostic marker for hepatocellular carcinoma and more. Adv Anat Pathol. 2009;16:125–9.
48. Vivero M, Doyle LA, Fletcher CD, Mertens F, Hornick JL. GRIA2 is a novel diagnostic marker for solitary fibrous tumour identified through gene expression profiling. Histopathology. 2014;65:71–80.
49. Hornick JL, Dal Cin P, Fletcher CD. Loss of INI1 expression is characteristic of both conventional and proximal-type epithelioid sarcoma. Am J Surg Pathol. 2009;33:542–50.
50. Yang Z, Klimstra DS, Hruban RH, Tang LH. Immunohistochemical characterization of the origins of metastatic well-differentiated neuroendocrine tumors to the liver. Am J Surg Pathol. 2017;41:915–22.
51. Lau SK, Chu PG, Weiss LM. Immunohistochemical expression of Langerin in Langerhans cell histiocytosis and non-Langerhans cell histiocytic disorders. Am J Surg Pathol. 2008;32:615–9.
52. Korshunov A, Ryzhova M, Jones DT, et al. LIN28A immunoreactivity is a potent diagnostic marker of embryonal tumor with multilayered rosettes (ETMR). Acta Neuropathol. 2012;124:875–81.
53. Rao S, Rajeswarie RT, Chickabasaviah Yasha T, et al. LIN28A, a sensitive immunohistochemical marker for Embryonal Tumor with Multilayered Rosettes (ETMR), is also positive in a subset of Atypical Teratoid/Rhabdoid Tumor (AT/RT). Childs Nerv Syst. 2017;33:1953–9.
54. Zafrakas M, Petschke B, Donner A, et al. Expression analysis of mammaglobin A (SCGB2A2) and lipophilin B (SCGB1D2) in more than 300 human tumors and matching normal tissues reveals their co-expression in gynecologic malignancies. BMC Cancer. 2006;6:88.
55. Bhargava R, Beriwal S, Dabbs DJ. Mammaglobin vs GCDFP-15: an immunohistologic validation survey for sensitivity and specificity. Am J Clin Pathol. 2007;127:103–13.
56. Weaver J, Rao P, Goldblum JR, et al. Can MDM2 analytical tests performed on core needle biopsy be relied upon to diagnose well-differentiated liposarcoma? Mod Pathol. 2010;23:1301–6.
57. Kashima T, Halai D, Ye H, et al. Sensitivity of MDM2 amplification and unexpected multiple faint alphoid 12 (alpha 12 satellite sequences) signals in atypical lipomatous tumor. Mod Pathol. 2012;25:1384–96.
58. Dujardin F, Binh MB, Bouvier C, et al. MDM2 and CDK4 immunohistochemistry is a valuable tool in the differential diagnosis of low-grade osteosarcomas and other primary fibro-osseous lesions of the bone. Mod Pathol. 2011;24:624–37.
59. Doyle LA, Moller E, Dal Cin P, et al. MUC4 is a highly sensitive and specific marker for low-grade fibromyxoid sarcoma. Am J Surg Pathol. 2011;35:733–41.
60. Doyle LA, Wang WL, Dal Cin P, et al. MUC4 is a sensitive and extremely useful marker for sclerosing epithelioid fibrosarcoma: association with FUS gene rearrangement. Am J Surg Pathol. 2012;36:1444–51.
61. Mawas AS, Amatya VJ, Kushitani K, et al. MUC4 immunohistochemistry is useful in distinguishing epithelioid mesothelioma from adenocarcinoma and squamous cell carcinoma of the lung. Sci Rep. 2018;8:134.
62. Shibuya R, Matsuyama A, Nakamoto M, et al. The combination of CD99 and NKX2.2, a transcriptional target of EWSR1-FLI1, is highly specific for the diagnosis of Ewing sarcoma. Virchows Arch. 2014;465:599–605.
63. McCuiston A, Bishop JA. Usefulness of NKX2.2 Immunohistochemistry for distinguishing Ewing sarcoma from other sinonasal small round blue cell tumors. Head Neck Pathol. 2018;12:89–94.
64. Ligon KL, Alberta JA, Kho AT, et al. The oligodendroglial lineage marker OLIG2 is universally expressed in diffuse gliomas. J Neuropathol Exp Neurol. 2004;63:499–509.
65. Bishop JA, Teruya-Feldstein J, Westra WH, et al. p40 (DeltaNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol. 2012;25:405–15.
66. Gill AJ, Clarkson A, Gimm O, et al. Loss of nuclear expression of parafibromin distinguishes parathyroid carcinomas and hyperparathyroidism-jaw tumor (HPT-JT) syndrome-related adenomas from sporadic parathyroid adenomas and hyperplasias. Am J Surg Pathol. 2006;30:1140–9.
67. Nonaka D, Tang Y, Chiriboga L, Rivera M, Ghossein R. Diagnostic utility of thyroid transcription factors Pax8 and TTF-2 (FoxE1) in thyroid epithelial neoplasms. Mod Pathol. 2008;21:192–200.
68. Bishop JA, Sharma R, Westra WH. PAX8 immunostaining of anaplastic thyroid carcinoma: a reliable means of discerning thyroid origin for undifferentiated tumors of the head and neck. Hum Pathol. 2011;42:1873–7.
69. Weissferdt A, Moran CA. Pax8 expression in thymic epithelial neoplasms: an immunohistochemical analysis. Am J Surg Pathol. 2011;35:1305–10.
70. Lee JP, Hung YP, O'Dorisio TM, et al. Examination of PHOX2B in adult neuroendocrine neoplasms reveals relatively frequent expression in phaeochromocytomas and paragangliomas. Histopathology. 2017;71:503–10.
71. Hung YP, Lee JP, Bellizzi AM, Hornick JL. PHOX2B reliably distinguishes neuroblastoma among small round blue cell tumours. Histopathology. 2017;71:786–94.
72. McDonald WC, Banerji N, McDonald KN, et al. Steroidogenic factor 1, Pit-1, and adrenocorticotropic hormone: a rational starting place for the immunohistochemical characterization of pituitary adenoma. Arch Pathol Lab Med. 2017;141:104–12.
73. Katabi N, Xu B, Jungbluth AA, et al. PLAG1 immunohistochemistry is a sensitive marker for pleomorphic adenoma: a comparative study with PLAG1 genetic abnormalities. Histopathology. 2018;72:285–93.
74. Sheridan T, Herawi M, Epstein JI, Illei PB. The role of P501S and PSA in the diagnosis of metastatic adenocarcinoma of the prostate. Am J Surg Pathol. 2007;31:1351–5.
75. Epstein JI, Netto GJ. Biopsy interpretation of the prostate, Biopsy Interpretation Series. 4th ed. Philadelphia. Fourth edition (September 18, 2007): LWW; 2008. p. 368.
76. Conner JR, Hornick JL. SATB2 is a novel marker of osteoblastic differentiation in bone and soft tissue tumours. Histopathology. 2013;63:36–49.
77. Gopalan A, Dhall D, Olgac S, et al. Testicular mixed germ cell tumors: a morphological and immunohistochemical study using stem cell markers, OCT3/4, SOX2 and GDF3, with emphasis on morphologically difficult-to-classify areas. Mod Pathol. 2009;22:1066–74.
78. Nonaka D, Chiriboga L, Rubin BP. Sox10: a pan-schwannian and melanocytic marker. Am J Surg Pathol. 2008;32:1291–8.

79. Elston MS, Meyer-Rochow GY, Conaglen HM, et al. Increased SSTR2A and SSTR3 expression in succinate dehydrogenase-deficient pheochromocytomas and paragangliomas. Hum Pathol. 2015;46:390–6.
80. Menke JR, Raleigh DR, Gown AM, et al. Somatostatin receptor 2a is a more sensitive diagnostic marker of meningioma than epithelial membrane antigen. Acta Neuropathol. 2015;130:441–3.
81. Doyle LA, Tao D, Marino-Enriquez A. STAT6 is amplified in a subset of dedifferentiated liposarcoma. Mod Pathol. 2014;27:1231–7.
82. Argani P, Aulmann S, Illei PB, et al. A distinctive subset of PEComas harbors TFE3 gene fusions. Am J Surg Pathol. 2010;34:1395–406.
83. Hung YP, Fletcher CD, Hornick JL. Evaluation of ETV4 and WT1 expression in CIC-rearranged sarcomas and histologic mimics. Mod Pathol. 2016;29:1324–34.

Chapter 6. Immunostains: Antibody Index – Hematopoietic System

1. Chan JKC, Lamant L, Algar E, et al. ALK(+) histiocytosis: a novel type of systemic histiocytic proliferative disorder of early infancy. Blood. 2008;112:2965–8.
2. McCune RC, Syrbu SI, Vasef MA. Expression profiling of transcription factors Pax-5, Oct-1, Oct-2, BOB.1, and PU.1 in Hodgkin's and non-Hodgkin's lymphomas: a comparative study using high throughput tissue microarrays. Mod Pathol: Off J US Can Acad Pathol Inc. 2006;19:1010–8.
3. Erber WN, Asbahr H, Rule SA, Scott CS. Unique immunophenotype of acute promyelocytic leukaemia as defined by CD9 and CD68 antibodies. Br J Haematol. 1994;88:101–4.
4. Di Noto R, Mirabelli P, Del Vecchio L. Flow cytometry analysis of acute promyelocytic leukemia: the power of 'surface hematology'. Leukemia. 2007;21:4–8.
5. Veltroni M, De Zen L, Sanzari MC, et al. Expression of CD58 in normal, regenerating and leukemic bone marrow B cells: implications for the detection of minimal residual disease in acute lymphocytic leukemia. Haematologica. 2003;88:1245–52.
6. Paiva B, Gutierrez NC, Chen X, et al. Clinical significance of CD81 expression by clonal plasma cells in high-risk smoldering and symptomatic multiple myeloma patients. Leukemia. 2012;26:1862–9.
7. Sung CO, Ko YH, Park S, Kim K, Kim W. Immunoreactivity of CD99 in non-Hodgkin's lymphoma: unexpected frequent expression in ALK-positive anaplastic large cell lymphoma. J Kor Med Sci. 2005;20:952–6.
8. Dorfman DM, Shahsafaei A, Alonso MA. Utility of CD200 immunostaining in the diagnosis of primary mediastinal large B cell lymphoma: comparison with MAL, CD23, and other markers. Mod Pathol: Off J US Can Acad Pathol Inc. 2012;25:1637–43.
9. Vermi W, Lonardi S, Bosisio D, et al. Identification of CXCL13 as a new marker for follicular dendritic cell sarcoma. J Pathol. 2008;216:356–64.
10. Dupuis J, Boye K, Martin N, et al. Expression of CXCL13 by neoplastic cells in angioimmunoblastic T-cell lymphoma (AITL): a new diagnostic marker providing evidence that AITL derives from follicular helper T cells. Am J Surg Pathol. 2006;30:490–4.
11. Lau SK, Chu PG, Weiss LM. Immunohistochemical expression of langerin in langerhans cell histiocytosis and non-langerhans cell histiocytic disorders. Am J Surg Pathol. 2008;32:615–9.
12. Copie-Bergman C, Gaulard P, Maouche-Chretien L, et al. The MAL gene is expressed in primary mediastinal large B-cell lymphoma. Blood. 1999;94:3567–75.
13. Bishop JA, Teruya-Feldstein J, Westra WH, et al. p40 (DeltaNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol. 2012;25:405–15.
14. Dictor M, Ek S, Sundberg M, et al. Strong lymphoid nuclear expression of SOX11 transcription factor defines lymphoblastic neoplasms, mantle cell lymphoma and Burkitt's lymphoma. Haematologica. 2009;94:1563–8.

Chapter 7. Special Stains

1. Lodish HBA, Kaiser CA, Krieger M, Scott MP, Bretscher A, et al. Molecular cell biology. 6th ed. New York: W.H. Freeman & Co Ltd; 2007. p. 973.
2. Klatt E. Special stains in histology. http://library.med.utah.edu/WebPath/HISTHTML/STAINS/STAINS.html.
3. Bancroft JD, Gamble M. Theory and practice of histological techniques. London: Churchill Livingstone; 2008.
4. Kitagawa Y, Ikebe D, Hara T, et al. Enhanced detection of lymphovascular invasion in small rectal neuroendocrine tumors using D2-40 and Elastica van Gieson immunohistochemical analysis. Cancer Med. 2016;5:3121–7.

Chapter 8. Grading (and Classification) Quick Reference: Solid Tumors

1. Fan F. Tumors of the breast. In: Damjanov I, Fan F, editors. Cancer grading manual. 2nd ed. New York: Springer; 2013. p. 131–44.
2. Neumann WL, Genta RM. Tumors of the digestive system. In: Damjanov I, Fan F, editors. Cancer grading manual. 2nd ed. New York: Springer; 2013. p. 51–64.
3. Westra WH. The changing face of head and neck cancer in the 21st century: the impact of HPV on the epidemiology and pathology of oral cancer. Head Neck Pathol. 2009;3:78–81.
4. Bishop JA, Sciubba JJ, Westra WH. Squamous cell carcinoma of the oral cavity and oropharynx. Surg Pathol Clin. 2011;4:1127–51.
5. El-Naggar AK, JKC C, Grandis JR, et al., editors. WHO classification of head and neck tumors. 4th ed. Lyon, France: IARC Press; 2017.
6. Lester SC, Bose S, Chen YY, et al. Protocol for the examination of specimens from patients with ductal carcinoma in situ of the breast. Arch Pathol Lab Med. 2009;133:15–25.
7. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 1991;19:403–10.
8. Lkhani SR, Ellis IO, Schnitt SJ, editors. WHO classification of tumours of the breast. Lyon, France: IARC Press; 2012.
9. Epstein JI, Egevad L, Amin MB, et al. The 2014 International Society of Urological Pathology (ISUP) consensus conference on gleason grading of prostatic carcinoma: definition of grading patterns and proposal for a new grading system. Am J Surg Pathol. 2016;40:244–52.
10. Moch H, Humphrey PA, Ulbright TM, et al., editors. WHO classification of tumours of the urinary system and male genital organs. 4th ed. Lyon, France: IARC Press; 2016.
11. Dome JS, Cotton CA, Perlman EJ, et al. Treatment of anaplastic histology Wilms' tumor: results from the fifth National Wilms' Tumor Study. J Clin Oncol. 2006;24:2352–8.
12. Miyamoto H, Miller JS, Fajardo DA, et al. Non-invasive papillary urothelial neoplasms: the 2004 WHO/ISUP classification system. Pathol Int. 2010;60:1–8.
13. Ghossein R. Update to the College of American Pathologists reporting on thyroid carcinomas. Head Neck Pathol. 2009;3:86–93.

14. Kakudo K, Bai Y, Katayama S, et al. Classification of follicular cell tumors of the thyroid gland: analysis involving Japanese patients from one institute. Pathol Int. 2009;59:359–67.
15. Volante M, Collini P, Nikiforov YE, et al. Poorly differentiated thyroid carcinoma: the Turin proposal for the use of uniform diagnostic criteria and an algorithmic diagnostic approach. Am J Surg Pathol. 2007;31:1256–64.
16. Lloyd RV, Osamura RY, Klöppel G, et al., editors. WHO classification of tumours of endocrine organs, vol. 10. 4th ed. Lyon, France: IARC Press; 2017.
17. Hiltzik D, Carlson DL, Tuttle RM, et al. Poorly differentiated thyroid carcinomas defined on the basis of mitosis and necrosis: a clinicopathologic study of 58 patients. Cancer. 2006;106:1286–95.
18. Ellis GL, Auclair PL. Tumors of the salivary glands, AFIP Atlas of Tumor Pathology: Series 4. Washington, DC: ARP Press; 2008.
19. Shiboski SC, Shiboski CH, Criswell L, et al. American College of Rheumatology classification criteria for Sjogren's syndrome: a data-driven, expert consensus approach in the Sjogren's International Collaborative Clinical Alliance cohort. Arthritis Care Res (Hoboken). 2012;64:475–87.
20. Cleary KR, Batsakis JG. Biopsy of the lip and Sjogren's syndrome. Ann Otol Rhinol Laryngol. 1990;99:323–5.
21. Greenspan JS, Daniels TE, Talal N, et al. The histopathology of Sjogren's syndrome in labial salivary gland biopsies. Oral Surg Oral Med Oral Pathol. 1974;37:217–29.
22. Ainechi S, Lee H. Updates on precancerous lesions of the biliary tract: biliary precancerous lesion. Arch Pathol Lab Med. 2016;140:1285–9.
23. Basturk O, Hong SM, Wood LD, et al. A revised classification system and recommendations from the Baltimore consensus meeting for neoplastic precursor lesions in the pancreas. Am J Surg Pathol. 2015;39:1730–41.
24. Bosman FT, Carneiro F, Hruban RH, et al., editors. WHO classification of tumours of the digestive system. 4th ed. Lyon, France: IARC Press; 2010.
25. Hruban RH, Takaori K, Klimstra DS, et al. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. Am J Surg Pathol. 2004;28:977–87.
26. Hruban RH, Bishop JA, Pitman M, et al. Tumors of the pancreas, Afip Atlas of Tumor Pathology; 4th Series Fascicle 6. 6th ed. Washington, DC: American Registry of Pathology; 2007.. 422 pp
27. Zen Y, Adsay NV, Bardadin K, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. Mod Pathol. 2007;20:701–9.
28. Maitra A, Fukushima N, Takaori K, et al. Precursors to invasive pancreatic cancer. Adv Anat Pathol. 2005;12:81–91.
29. Montgomery E, Bronner MP, Goldblum JR, et al. Reproducibility of the diagnosis of dysplasia in Barrett esophagus: a reaffirmation. Hum Pathol. 2001;32:368–78.
30. Odze RD. Diagnosis and grading of dysplasia in Barrett's oesophagus. J Clin Pathol. 2006;59:1029–38.
31. Theise ND. Liver biopsy assessment in chronic viral hepatitis: a personal, practical approach. Mod Pathol. 2007;20(Suppl 1):S3–14.
32. Rekhtman N. Neuroendocrine tumors of the lung: an update. Arch Pathol Lab Med. 2010;134:1628–38.
33. Travis WD, Brambilla E, Burke AP, et al. WHO classification of tumours of the lung, pleura, thymus and heart. Lyon, France: IARC Press; 2015. p. 334.
34. Kloppel G, Klimstra DS, Hruban RH, et al. Pancreatic neuroendocrine tumors: updated on the new World Health Organization classification. AJSP: Reviews & Reports. 2017;22:233–9.
35. Shimada H, Ambros IM, Dehner LP, et al. The international neuroblastoma pathology classification (the Shimada system). Cancer. 1999;86:364–72.
36. Guillou L, Coindre JM, Bonichon F, et al. Comparative study of the National Cancer Institute and French Federation of Cancer Centers Sarcoma Group grading systems in a population of 410 adult patients with soft tissue sarcoma. J Clin Oncol. 1997;15:350–62.
37. Fletcher CDM, Bridge JA. In: Hogendoorn PCW, Mertens F, editors. WHO classification of tumours of soft tissue and bone. 4th ed. Lyon: IARC WHO Classification of Tumours; 2013.
38. Oliveira AM, Nascimento AG. Grading in soft tissue tumors: principles and problems. Skeletal Radiol. 2001;30:543–59.
39. Coindre JM. Grading of soft tissue sarcomas: review and update. Arch Pathol Lab Med. 2006;130:1448–53.
40. Deyrup AT, Weiss SW. Grading of soft tissue sarcomas: the challenge of providing precise information in an imprecise world. Histopathology. 2006;48:42–50.
41. Recommendations for reporting soft tissue sarcomas. Association of Directors of Anatomic and Surgical Pathology. Am J Clin Pathol. 1999;111:594–8.
42. Fisher C. Standards and datasets for reporting cancers: dataset for cancer histopathology reports on soft tissue sarcomas. Royal College of Pathologist. In: November 2009.
43. Louis DN, Ohgaki H, Wiestler OD, et al. WHO classification of tumours of the central nervous system, vol. 1. 4th ed. Lyon: IARC Press; 2016.
44. Prat J, Damjanov I. Tumors of the female genital organs. In: Damjanov I, Fan F, editors. Cancer grading manual. 2nd ed. New York., LLC: Springer; 2013. p. 223.
45. Bell SW, Kempson RL, Hendrickson MR. Problematic uterine smooth muscle neoplasms. A clinicopathologic study of 213 cases. Am J Surg Pathol. 1994;18:535–58.
46. Kurman RJ, Carcangiu ML, Herrington CS, et al. WHO classification of tumours of female reproductive organs, vol. 6. 4th ed. Lyon, France: IARC Press; 2014.
47. Mills AM, Longacre TA. Smooth muscle tumors of the female genital tract. Surg Pathol Clin. 2009;2:625–77.
48. Redline RW, Faye-Petersen O, Heller D, et al. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2003;6:435–48.
49. Redline RW, Heller D, Keating S, et al. Placental diagnostic criteria and clinical correlation–a workshop report. Placenta. 2005;26(Suppl A):S114–7.
50. Stewart S, Fishbein MC, Snell GI, et al. Revision of the 1996 working formulation for the standardization of nomenclature in the diagnosis of lung rejection. J Heart Lung Transplant. 2007;26:1229–42.
51. Berry G, Burke M, Andersen C, et al. Pathology of pulmonary antibody-mediated rejection: 2012 update from the Pathology Council of the ISHLT. J Heart Lung Transplant. 2013;32:14–21.
52. Hammond MEH, Revelo MP, Miller DV, et al. ISHLT pathology antibody mediated rejection score correlates with increased risk of cardiovascular mortality: a retrospective validation analysis. J Heart Lung Transplant. 2016;35:320–5.
53. Sale GE, Shulman HM, McDonald GB, et al. Gastrointestinal graft-versus-host disease in man. A clinicopathologic study of the rectal biopsy. Am J Surg Pathol. 1979;3:291–9.
54. Snover DC. Graft-versus-host disease of the gastrointestinal tract. Am J Surg Pathol. 1990;14(Suppl 1):101–8.
55. Hurt MA. Review of practical dermatopathology by Ronald P. Rapini. Dermatol Pract Concept. 2013;3:51–2.
56. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology. 1997;25:658–63.

57. Demetris AJ, Bellamy C, Hubscher SG, et al. 2016 comprehensive update of the Banff Working Group on liver allograft pathology: introduction of antibody-mediated rejection. Am J Transplant. 2016;16:2816–35.
58. Aubert S, Wacrenier A, Leroy X, et al. Weiss system revisited: a clinicopathologic and immunohistochemical study of 49 adrenocortical tumors. Am J Surg Pathol. 2002;26:1612–9.
59. Weiss LM. Comparative histologic study of 43 metastasizing and nonmetastasizing adrenocortical tumors. Am J Surg Pathol. 1984;8:163–9.
60. Bisceglia M, Ludovico O, Di Mattia A, et al. Adrenocortical oncocytic tumors: report of 10 cases and review of the literature. Int J Surg Pathol. 2004;12:231–43.
61. Wong DD, Spagnolo DV, Bisceglia M, et al. Oncocytic adrenocortical neoplasms–a clinicopathologic study of 13 new cases emphasizing the importance of their recognition. Hum Pathol. 2011;42:489–99.
62. Papotti M, Libe R, Duregon E, et al. The Weiss score and beyond–histopathology for adrenocortical carcinoma. Horm Cancer. 2011;2:333–40.
63. Beuschlein F, Weigel J, Saeger W, et al. Major prognostic role of Ki67 in localized adrenocortical carcinoma after complete resection. J Clin Endocrinol Metab. 2015;100:841–9.
64. Duregon E, Molinaro L, Volante M, et al. Comparative diagnostic and prognostic performances of the hematoxylin-eosin and phospho-histone H3 mitotic count and Ki-67 index in adrenocortical carcinoma. Mod Pathol. 2014;27:1246–54.
65. Pennanen M, Heiskanen I, Sane T, et al. Helsinki score-a novel model for prediction of metastases in adrenocortical carcinomas. Hum Pathol. 2015;46(3):404–10.
66. Duregon E, Cappallesso R, Maffeis V, et al. Validation of the prognostic role of the "Helsinki Score" in 225 cases of adrenocortical carcinoma. Hum Pathol. 2017;62:1–7.
67. Sbragia L, Oliveira-Filho AG, Vassallo J, et al. Adrenocortical tumors in Brazilian children: immunohistochemical markers and prognostic factors. Arch Pathol Lab Med. 2005;129:1127–31.
68. Wieneke JA, Thompson LD, Heffess CS. Adrenal cortical neoplasms in the pediatric population: a clinicopathologic and immunophenotypic analysis of 83 patients. Am J Surg Pathol. 2003;27:867–81.
69. Thompson LD. Pheochromocytoma of the Adrenal gland Scaled Score (PASS) to separate benign from malignant neoplasms: a clinicopathologic and immunophenotypic study of 100 cases. Am J Surg Pathol. 2002;26:551–66.
70. Kimura N, Takayanagi R, Takizawa N, et al. Pathological grading for predicting metastasis in phaeochromocytoma and paraganglioma. Endocr Relat Cancer. 2014;21:405–14.
71. Gill AJ, Clarkson A, Gimm O, et al. Loss of nuclear expression of parafibromin distinguishes parathyroid carcinomas and hyperparathyroidism-jaw tumor (HPT-JT) syndrome-related adenomas from sporadic parathyroid adenomas and hyperplasias. Am J Surg Pathol. 2006;30:1140–9.
72. Delellis RA. Challenging lesions in the differential diagnosis of endocrine tumors: parathyroid carcinoma. Endocr Pathol. 2008;19:221–5.
73. Joensuu H, Vehtari A, Riihimaki J, et al. Risk of recurrence of gastrointestinal stromal tumour after surgery: an analysis of pooled population-based cohorts. Lancet Oncol. 2012;13:265–74.
74. Demetri GD, von Mehren M, Antonescu CR, et al. NCCN Task Force report: update on the management of patients with gastrointestinal stromal tumors. J Natl Compr Canc Netw. 2010;8(Suppl 2):S1–41; quiz S2–4.
75. Miettinen M, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. Semin Diagn Pathol. 2006;23:70–83.
76. England DM, Hochholzer L, McCarthy MJ. Localized benign and malignant fibrous tumors of the pleura. A clinicopathologic review of 223 cases. Am J Surg Pathol. 1989;13:640–58.
77. Fine SW, McCarthy DM, Chan TY, et al. Malignant solitary fibrous tumor of the kidney: report of a case and comprehensive review of the literature. Arch Pathol Lab Med. 2006;130:857–61.
78. Gold JS, Antonescu CR, Hajdu C, et al. Clinicopathologic correlates of solitary fibrous tumors. Cancer. 2002;94:1057–68.
79. Folpe AL, Mentzel T, Lehr HA, et al. Perivascular epithelioid cell neoplasms of soft tissue and gynecologic origin: a clinicopathologic study of 26 cases and review of the literature. Am J Surg Pathol. 2005;29:1558–75.
80. Conlon N, Soslow RA, Murali R. Perivascular epithelioid tumours (PEComas) of the gynaecological tract. J Clin Pathol. 2015;68:418–26.
81. Schoolmeester JK, Howitt BE, Hirsch MS, et al. Perivascular epithelioid cell neoplasm (PEComa) of the gynecologic tract: clinicopathologic and immunohistochemical characterization of 16 cases. Am J Surg Pathol. 2014;38:176–88.
82. Folpe AL, Kwiatkowski DJ. Perivascular epithelioid cell neoplasms: pathology and pathogenesis. Hum Pathol. 2010;41:1–15.

Chapter 9. Grading (and Classification) Quick Reference: Hematopoietic System

1. Swerdlow S, Campo E, Harris N, et al. WHO classification of tumors of haematopoietic and lymphoid tissues. (Revised 4th ed.). Lyon, France: IARC Press; 2017.
2. Malcovati L, Papaemmanuil E, Bowen DT, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood. 2011;118:6239–46.
3. Papaemmanuil E, Cazzola M, Boultwood J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med. 2011;365:1384–95.
4. Patnaik MM, Hanson CA, Sulai NH, et al. Prognostic irrelevance of ring sideroblast percentage in World Health Organization-defined myelodysplastic syndromes without excess blasts. Blood. 2012;119:5674–7.
5. Mufti GJ, Bennett JM, Goasguen J, et al. Diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts. Haematologica. 2008;93:1712–7.

Chapter 10. Tumor Genetics and Cytogenetics: Solid Tumors

1. Wang W, Lazar AJ. Applications of molecular testing to differential diagnosis (Chapter 18). In: Hornick JL, editor. Practical soft tissue pathology: a diagnostic approach: A volume in the pattern recognition series. 2nd ed; 2019.
2. Kao YC, Owosho AA, Sung YS, et al. BCOR-CCNB3 fusion positive sarcomas: a clinicopathologic and molecular analysis of 36 cases with comparison to morphologic spectrum and clinical behavior of other round cell sarcomas. Am J Surg Pathol. 2018;42:604–15.
3. Kao YC, Sung YS, Zhang L, et al. BCOR overexpression is a highly sensitive marker in round cell sarcomas with BCOR genetic abnormalities. Am J Surg Pathol. 2016;40:1670–8.
4. Le Guellec S, Velasco V, Perot G, et al. ETV4 is a useful marker for the diagnosis of CIC-rearranged undifferentiated round-cell sarcomas: a study of 127 cases including mimicking lesions. Mod Pathol. 2016;29:1523–31.

5. Shibuya R, Matsuyama A, Shiba E, et al. CAMTA1 is a useful immunohistochemical marker for diagnosing epithelioid haemangioendothelioma. Histopathology. 2015;67:827–35.
6. Hung YP, Fletcher CD, Hornick JL. Evaluation of NKX2-2 expression in round cell sarcomas and other tumors with EWSR1 rearrangement: imperfect specificity for Ewing sarcoma. Mod Pathol. 2016;29:370–80.
7. Yoshida A, Sekine S, Tsuta K, et al. NKX2.2 is a useful immunohistochemical marker for Ewing sarcoma. Am J Surg Pathol. 2012;36:993–9.
8. Antonescu CR, Suurmeijer AJ, Zhang L, et al. Molecular characterization of inflammatory myofibroblastic tumors with frequent ALK and ROS1 gene fusions and rare novel RET rearrangement. Am J Surg Pathol. 2015;39:957–67.
9. Mertens F, Fletcher CD, Antonescu CR, et al. Clinicopathologic and molecular genetic characterization of low-grade fibromyxoid sarcoma, and cloning of a novel FUS/CREB3L1 fusion gene. Lab Invest. 2005;85:408–15.
10. Gleason BC, Fletcher CD. Myoepithelial carcinoma of soft tissue in children: an aggressive neoplasm analyzed in a series of 29 cases. Am J Surg Pathol. 2007;31:1813–24.
11. Brandal P, Panagopoulos I, Bjerkehagen B, et al. t(19;22)(q13;q12) Translocation leading to the novel fusion gene EWSR1-ZNF444 in soft tissue myoepithelial carcinoma. Genes Chromosomes Cancer. 2009;48:1051–6.
12. Erickson-Johnson MR, Chou MM, Evers BR, et al. Nodular fasciitis: a novel model of transient neoplasia induced by MYH9-USP6 gene fusion. Lab Invest. 2011;91:1427–33.
13. Argani P, Aulmann S, Illei PB, et al. A distinctive subset of PEComas harbors TFE3 gene fusions. Am J Surg Pathol. 2010;34:1395–406.
14. Williamson D, Missiaglia E, de Reynies A, et al. Fusion gene-negative alveolar rhabdomyosarcoma is clinically and molecularly indistinguishable from embryonal rhabdomyosarcoma. J Clin Oncol. 2010;28:2151–8.
15. Doyle LA, Wang WL, Dal Cin P, et al. MUC4 is a sensitive and extremely useful marker for sclerosing epithelioid fibrosarcoma: association with FUS gene rearrangement. Am J Surg Pathol. 2012;36:1444–51.
16. Brill LB 2nd, Kanner WA, Fehr A, et al. Analysis of MYB expression and MYB-NFIB gene fusions in adenoid cystic carcinoma and other salivary neoplasms. Mod Pathol. 2011;24:1169–76.
17. Kim J, Geyer FC, Martelotto LG, et al. MYBL1 rearrangements and MYB amplification in breast adenoid cystic carcinomas lacking the MYB-NFIB fusion gene. J Pathol. 2018;244:143–50.
18. Fehr A, Kovacs A, Loning T, et al. The MYB-NFIB gene fusion-a novel genetic link between adenoid cystic carcinoma and dermal cylindroma. J Pathol. 2011;224:322–7.
19. Bishop JA, Yonescu R, Epstein JI, et al. A subset of prostatic basal cell carcinomas harbor the MYB rearrangement of adenoid cystic carcinoma. Hum Pathol. 2015;46:1204–8.
20. Jo VY, Sholl LM, Krane JF. Distinctive patterns of CTNNB1 (beta-Catenin) alterations in salivary gland basal cell adenoma and basal cell adenocarcinoma. Am J Surg Pathol. 2016;40:1143–50.
21. Wilson TC, Ma D, Tilak A, et al. Next-generation sequencing in salivary gland basal cell adenocarcinoma and basal cell adenoma. Head Neck Pathol. 2016;10:494–500.
22. Shah AA, LeGallo RD, van Zante A, et al. EWSR1 genetic rearrangements in salivary gland tumors: a specific and very common feature of hyalinizing clear cell carcinoma. Am J Surg Pathol. 2013;37:571–8.
23. Antonescu CR, Katabi N, Zhang L, et al. EWSR1-ATF1 fusion is a novel and consistent finding in hyalinizing clear-cell carcinoma of salivary gland. Genes Chromosomes Cancer. 2011;50:559–70.
24. Dalin MG, Desrichard A, Katabi N, et al. Comprehensive molecular characterization of salivary duct carcinoma reveals actionable targets and similarity to apocrine breast cancer. Clin Cancer Res. 2016;22:4623–33.
25. El Hallani S, Udager AM, Bell D, et al. Epithelial-myoepithelial carcinoma: frequent morphologic and molecular evidence of preexisting pleomorphic adenoma, common HRAS mutations in PLAG1-intact and HMGA2-intact cases, and occasional TP53, FBXW7, and SMARCB1 alterations in high-grade cases. Am J Surg Pathol. 2018;42:18–27.
26. Weinreb I, Bishop JA, Chiosea SI, et al. Recurrent RET gene rearrangements in intraductal carcinomas of salivary gland. Am J Surg Pathol. 2018;42:442–52.
27. Okabe M, Miyabe S, Nagatsuka H, et al. MECT1-MAML2 fusion transcript defines a favorable subset of mucoepidermoid carcinoma. Clin Cancer Res. 2006;12:3902–7.
28. Seethala RR, Chiosea SI. MAML2 status in mucoepidermoid carcinoma can no longer be considered a prognostic marker. Am J Surg Pathol. 2016;40:1151–3.
29. Bishop JA, Cowan ML, Shum CH, et al. MAML2 rearrangements in variant forms of mucoepidermoid carcinoma: ancillary diagnostic testing for the ciliated and warthin-like variants. Am J Surg Pathol. 2018;42:130–6.
30. Winnes M, Molne L, Suurkula M, et al. Frequent fusion of the CRTC1 and MAML2 genes in clear cell variants of cutaneous hidradenomas. Genes Chromosomes Cancer. 2007;46:559–63.
31. Katabi N, Ghossein R, Ho A, et al. Consistent PLAG1 and HMGA2 abnormalities distinguish carcinoma ex-pleomorphic adenoma from its de novo counterparts. Hum Pathol. 2015;46:26–33.
32. Persson F, Andren Y, Winnes M, et al. High-resolution genomic profiling of adenomas and carcinomas of the salivary glands reveals amplification, rearrangement, and fusion of HMGA2. Genes Chromosomes Cancer. 2009;48:69–82.
33. Mito JK, Jo VY, Chiosea SI, et al. HMGA2 is a specific immunohistochemical marker for pleomorphic adenoma and carcinoma ex-pleomorphic adenoma. Histopathology. 2017;71:511–21.
34. Martins C, Fonseca I, Roque L, et al. PLAG1 gene alterations in salivary gland pleomorphic adenoma and carcinoma ex-pleomorphic adenoma: a combined study using chromosome banding, in situ hybridization and immunocytochemistry. Mod Pathol. 2005;18:1048–55.
35. Weinreb I, Piscuoglio S, Martelotto LG, et al. Hotspot activating PRKD1 somatic mutations in polymorphous low-grade adenocarcinomas of the salivary glands. Nat Genet. 2014;46:1166–9.
36. Weinreb I, Zhang L, Tirunagari LM, et al. Novel PRKD gene rearrangements and variant fusions in cribriform adenocarcinoma of salivary gland origin. Genes Chromosomes Cancer. 2014;53:845–56.
37. Clauditz TS, Reiff M, Gravert L, et al. Human epidermal growth factor receptor 2 (HER2) in salivary gland carcinomas. Pathology. 2011;43:459–64.
38. Chiosea SI, Williams L, Griffith CC, et al. Molecular characterization of apocrine salivary duct carcinoma. Am J Surg Pathol. 2015;39:744–52.
39. Griffith CC, Seethala RR, Luvison A, et al. PIK3CA mutations and PTEN loss in salivary duct carcinomas. Am J Surg Pathol. 2013;37:1201–7.
40. Skalova A, Vanecek T, Sima R, et al. Mammary analogue secretory carcinoma of salivary glands, containing the ETV6-NTRK3 fusion gene: a hitherto undescribed salivary gland tumor entity. Am J Surg Pathol. 2010;34:599–608.
41. Skalova A, Vanecek T, Martinek P, et al. Molecular profiling of mammary analog secretory carcinoma revealed a subset of tumors harboring a novel ETV6-RET translocation: report of 10 cases. Am J Surg Pathol. 2018;42:234–46.

42. Brat DJ, Verhaak RG, Aldape KD, et al. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med. 2015;372:2481–98.
43. Bandopadhayay P, Ramkissoon LA, Jain P, et al. MYB-QKI rearrangements in angiocentric glioma drive tumorigenicity through a tripartite mechanism. Nat Genet. 2016;48:273–82.
44. Taylor MD, Northcott PA, Korshunov A, et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol. 2012;123:465–72.
45. Argani P, Lae M, Hutchinson B, et al. Renal carcinomas with the t(6;11)(p21;q12): clinicopathologic features and demonstration of the specific alpha-TFEB gene fusion by immunohistochemistry, RT-PCR, and DNA PCR. Am J Surg Pathol. 2005;29:230–40.
46. Karlsson J, Valind A, Gisselsson D. BCOR internal tandem duplication and YWHAE-NUTM2B/E fusion are mutually exclusive events in clear cell sarcoma of the kidney. Genes Chromosomes Cancer. 2016;55:120–3.
47. Pugh TJ, Morozova O, Attiyeh EF, et al. The genetic landscape of high-risk neuroblastoma. Nat Genet. 2013;45:279–84.
48. Nicolai S, Pieraccioli M, Peschiaroli A, et al. Neuroblastoma: oncogenic mechanisms and therapeutic exploitation of necroptosis. Cell Death Dis. 2015;6:e2010.
49. Woodman SE, Davies MA. Targeting KIT in melanoma: a paradigm of molecular medicine and targeted therapeutics. Biochem Pharmacol. 2010;80:568–74.
50. Haack H, Johnson LA, Fry CJ, et al. Diagnosis of NUT midline carcinoma using a NUT-specific monoclonal antibody. Am J Surg Pathol. 2009;33:984–91.
51. Fine SW, Gopalan A, Leversha MA, et al. TMPRSS2-ERG gene fusion is associated with low Gleason scores and not with high-grade morphological features. Mod Pathol. 2010;23:1325–33.
52. Park K, Tomlins SA, Mudaliar KM, et al. Antibody-based detection of ERG rearrangement-positive prostate cancer. Neoplasia. 2010;12:590–8.
53. Marino-Enriquez A, Lauria A, Przybyl J, et al. BCOR internal tandem duplication in high-grade uterine sarcomas. Am J Surg Pathol. 2018;42:335–41.
54. Chiang S, Lee CH, Stewart CJR, et al. BCOR is a robust diagnostic immunohistochemical marker of genetically diverse high-grade endometrial stromal sarcoma, including tumors exhibiting variant morphology. Mod Pathol. 2017;30:1251–61.
55. Lewis N, Soslow RA, Delair DF, et al. ZC3H7B-BCOR high-grade endometrial stromal sarcomas: a report of 17 cases of a newly defined entity. Mod Pathol. 2018;31:674–84.

Chapter 11. Tumor Genetics and Cytogenetics: Hematopoietic System

1. Swerdlow S, et al. WHO classification of tumors of haematopoietic and lymphoid tissues. (Revised 4th ed.). Lyon, France: IARC Press; 2017.

Chapter 12. Tumor Syndromes

1. Agaimy A, Hartmann A. Uncovering hereditary tumor syndromes: emerging role of surgical pathology. Semin Diagn Pathol. 2018;35:154–60.
2. Hansel DE. Genetic alterations and histopathologic findings in familial renal cell carcinoma. Histol Histopathol. 2006;21:437–44.
3. Genetics Home Reference https://ghr.nlm.nih.gov/.
4. Orphanet: an Online Database of Rare Diseases and Orphan Drugs http://www.orpha.net.
5. Kohlmann W, Gruber SB. Lynch syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews((R)). Seattle, WA: University of Washington, Seattle University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved; 1993.
6. Jass JR. Role of the pathologist in the diagnosis of hereditary non-polyposis colorectal cancer. Dis Markers. 2004;20:215–24.
7. Giardiello FM, Welsh SB, Hamilton SR, et al. Increased risk of cancer in the Peutz-Jeghers syndrome. N Engl J Med. 1987;316:1511–4.
8. Rustgi AK. The genetics of hereditary colon cancer. Genes Dev. 2007;21:2525–38.
9. Jasperson KW, Tuohy TM, Neklason DW, et al. Hereditary and familial colon cancer. Gastroenterology. 2010;138:2044–58.
10. Kimonis VE, Goldstein AM, Pastakia B, et al. Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome. Am J Med Genet. 1997;69:299–308.
11. Lloyd RV, Osamura RY, Klöppel G, et al., editors. WHO classification of tumours of endocrine organs, vol. 10. 4th ed. Lyon, France: IARC Press; 2017.
12. Andrici J, Gill AJ, Hornick JL. Next generation immunohistochemistry: emerging substitutes to genetic testing? Semin Diagn Pathol. 2018;35:161–9.
13. Gill AJ. Succinate dehydrogenase (SDH)-deficient neoplasia. Histopathology. 2018;72:106–16.
14. Barletta JA, Hornick JL. Succinate dehydrogenase-deficient tumors: diagnostic advances and clinical implications. Adv Anat Pathol. 2012;19:193–203.
15. Merino MJ, Torres-Cabala C, Pinto P, et al. The morphologic spectrum of kidney tumors in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome. Am J Surg Pathol. 2007;31:1578–85.
16. Cox R, Vang R, Epstein JI. Papillary cystadenoma of the epididymis and broad ligament: morphologic and immunohistochemical overlap with clear cell papillary renal cell carcinoma. Am J Surg Pathol. 2014;38:713–8.
17. Ferner RE. Neurofibromatosis 1 and neurofibromatosis 2: a twenty first century perspective. Lancet Neurol. 2007;6:340–51.
18. Dehner LP, Messinger YH, Schultz KA, et al. Pleuropulmonary blastoma: evolution of an entity as an entry into a familial tumor predisposition syndrome. Pediatr Dev Pathol. 2015;18:504–11.
19. Burkart AL, Sheridan T, Lewin M, et al. Do sporadic Peutz-Jeghers polyps exist? Experience of a large teaching hospital. Am J Surg Pathol. 2007;31:1209–14.
20. Anlauf M, Schlenger R, Perren A, et al. Microadenomatosis of the endocrine pancreas in patients with and without the multiple endocrine neoplasia type 1 syndrome. Am J Surg Pathol. 2006;30:560–74.
21. Perigny M, Hammel P, Corcos O, et al. Pancreatic endocrine microadenomatosis in patients with von Hippel-Lindau disease: characterization by VHL/HIF pathway proteins expression. Am J Surg Pathol. 2009;33:739–48.
22. Greenson JK, Huang SC, Herron C, et al. Pathologic predictors of microsatellite instability in colorectal cancer. Am J Surg Pathol. 2009;33:126–33.
23. Jenkins MA, Hayashi S, O'Shea AM, et al. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterology. 2007;133:48–56.

24. Shia J, Ellis NA, Paty PB, et al. Value of histopathology in predicting microsatellite instability in hereditary nonpolyposis colorectal cancer and sporadic colorectal cancer. Am J Surg Pathol. 2003;27:1407–17.
25. Yurgelun MB, Kulke MH, Fuchs CS, et al. Cancer susceptibility gene mutations in individuals with colorectal cancer. J Clin Oncol. 2017;35:1086–95.
26. Boland CR, Shike M. Report from the Jerusalem workshop on Lynch syndrome-hereditary nonpolyposis colorectal cancer. Gastroenterology. 2010;138:2197 e1–7.
27. Buza N, Ziai J, Hui P. Mismatch repair deficiency testing in clinical practice. Expert Rev Mol Diagn. 2016;16:591–604.
28. National Comprehensive Cancer Network. Genetic/familial high-risk assessment: Colorectal. (Version 3.2017). https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf. Accessed 25 April 2018.
29. Vasen HF, Mecklin JP, Khan PM, et al. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum. 1991;34:424–5.
30. Vasen HF, Watson P, Mecklin JP, et al. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology. 1999;116:1453–6.
31. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004;96:261–8.
32. Garg K, Soslow RA. Lynch syndrome (hereditary non-polyposis colorectal cancer) and endometrial carcinoma. J Clin Pathol. 2009;62:679–84.

Chapter 13. Quick Clinical References for Pathologists

1. Schueller G, Herold CJ. Lung metastases. Cancer Imaging. 2003;3:126–8.
2. Umeki S. Association of miliary lung metastases and bone metastases in bronchogenic carcinoma. Chest. 1993;104:948–50.
3. Luna MA, Pfaltz M. Cysts of the neck, unknown primary tumor, and neck dissection. In: Diagnostic surgical pathology of the head and neck. Philadelphia: W.B. Saunders; 2009.
4. Marchevsky AM, Gupta R, Balzer B. Diagnosis of metastatic neoplasms: a clinicopathologic and morphologic approach. Arch Pathol Lab Med. 2010;134:194–206.
5. Amin MB, Edge S, Green F, et al. AJCC cancer staging manual. 8th ed. New York: Springer International Publishing; 2017.. 1032 pp
6. Burtis CA, Ashwood ER, Bruns DE, editors. Teitz textbook of clinical chemistry and molecular diagnostics. 4th ed. Philadelphia: Saunders; 2005.

Chapter 14. Potpourri of Quick Morphologic References

1. Altini M, Coleman H, Kienle F. Intra-vascular tumour in pleomorphic adenomas–a report of four cases. Histopathology. 1997;31:55–9.
2. Coleman H, Altini M. Intravascular tumour in intra-oral pleomorphic adenomas: a diagnostic and therapeutic dilemma. Histopathology. 1999;35:439–44.
3. Cserni G, Bori R, Sejben I. Vascular invasion demonstrated by elastic stain-a common phenomenon in benign granular cell tumors. Virchows Arch. 2009;454:211–5.
4. Wobker SE, Przybycin CG, Sircar K, et al. Renal oncocytoma with vascular invasion: a series of 22 cases. Hum Pathol. 2016;58:1–6.
5. Miyagawa-Hayashino A, Tazelaar HD, Langel DJ, et al. Pulmonary sclerosing hemangioma with lymph node metastases: report of 4 cases. Arch Pathol Lab Med. 2003;127:321–5.
6. Kao YC, Owosho AA, Sung YS, et al. BCOR-CCNB3 fusion positive sarcomas: a clinicopathologic and molecular analysis of 36 cases with comparison to morphologic spectrum and clinical behavior of other round cell sarcomas. Am J Surg Pathol. 2018;42:604–15.
7. Argani P, Beckwith JB. Renal Neoplasms of Childhood. In: Sternberg's diagnostic surgical pathology, vol. 2. 5th ed: LWW; 2009. p. 1799–829.
8. Larone DH. Medically important fungi. Washington, DC: ASM Press; 2002.
9. Chandler FW, Watts JC. Pathologic diagnosis of fungal infections. Chicago: ASCP Press; 1987.
10. Merz WG, Roberts GD. Algorithms for detection and identification of fungi. In: Manual of clinical microbiology. Washington, DC: American Society for Microbiology Press; 2003. p. 1668–85.

Chapter 15. Quick Size References

1. El-Naggar AK, JKC C, Grandis JR, et al., editors. WHO classification of head and neck tumors. 4th ed. Lyon, France: IARC Press; 2017.
2. Borczuk AC, Qian F, Kazeros A, et al. Invasive size is an independent predictor of survival in pulmonary adenocarcinoma. Am J Surg Pathol. 2009;33:462–9.
3. Kurman RJ, Carcangiu ML, Herrington CS, et al. WHO classification of tumours of female reproductive organs, vol. 6. 4th ed. Lyon, France: IARC Press; 2014.
4. Pryce JW, Bamber AR, Ashworth MT, et al. Reference ranges for organ weights of infants at autopsy: results of >1,000 consecutive cases from a single centre. BMC Clin Pathol. 2014;14:18.
5. Daffary S, Chakravarti S. Manual of obstetrics. 3rd ed. New Delhi: Elsevier; 2011. p. 1–16.
6. Molina DK, DiMaio VJ. Normal organ weights in men: part II-the brain, lungs, liver, spleen, and kidneys. Am J Forensic Med Pathol. 2012;33:368–72.
7. Molina DK, DiMaio VJ. Normal organ weights in men: part I-the heart. Am J Forensic Med Pathol. 2012;33:362–7.
8. Molina DK, DiMaio VJ. Normal organ weights in women: part II-the brain, lungs, liver, spleen, and kidneys. Am J Forensic Med Pathol. 2015;36:182–7.
9. Molina DK, DiMaio VJ. Normal organ weights in women: part I-the heart. Am J Forensic Med Pathol. 2015;36:176–81.
10. Neville AM, O'Hare MJ. Chapter 4: structure of the adult cortex. In: The human adrenal cortex: pathology and biology, an integrated approach. Berlin; New York: Springer; 1982.. pp xiv, 354 p., 1 p. of plates.
11. Nieschlag E, Behre HM, Ahlen HV. Chapter 6. Diagnosis of male infertility and hypogonadism. In: Andrology: male reproductive health and dysfunction. 2nd ed. New York: Springer; 2001.. pp xx, 454 p.

Chapter 16. It Looks Like WHAT? An Illustrated Glossary of Histopathologic Descriptors

1. Indian file definition, http://www.yourdictionary.com/indian-file.
2. Storiform, http://www.biology-online.org/dictionary/Storiform.
3. Trabecular definition, http://www.yourdictionary.com/trabecular.
4. Signet ring definition, http://www.yourdictionary.com/signet-ring.
5. Hyaline definition, http://www.yourdictionary.com/hyaline.
6. Lynch JM, Barrett TL. Collagenolytic (necrobiotic) granulomas: part II – the 'red' granulomas. J Cutan Pathol. 2004;31:409–18.
7. Lynch JM, Barrett TL. Collagenolytic (necrobiotic) granulomas: part 1 – the "blue" granulomas. J Cutan Pathol. 2004;31:353–61.
8. Amianthoid http://www.biology-online.org/dictionary/Amianthoid.
9. Min KW. Stromal elements for tumor diagnosis: a brief review of diagnostic electron microscopic features. Ultrastruct Pathol. 2005;29:305–18.

Subject Index

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Common conversions:

1in = 2.54cm

1cm = 0.39in

1kg = 2.2lb

1lb = 0.45kg

1g = 0.04oz

1oz = 28g

1gallon = 3.8L

1L = 0.26gallons