ΠΜΣ ΜΥΟΣΚΕΛΕΤΙΚΗ ΟΓΚΟΛΟΓΙΑ

Οστική μετάσταση: Ανοσοϊστοχημική διερεύνηση

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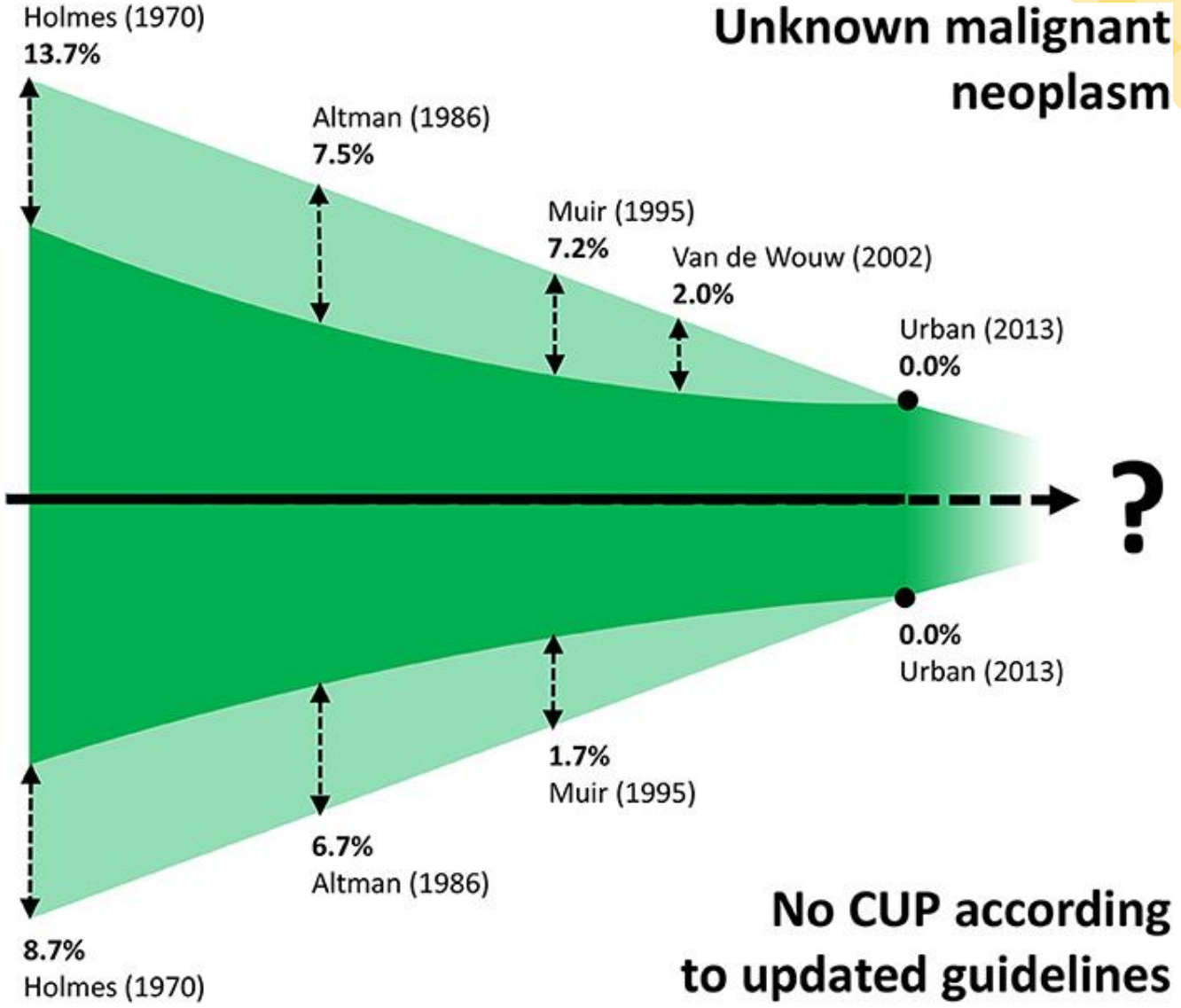
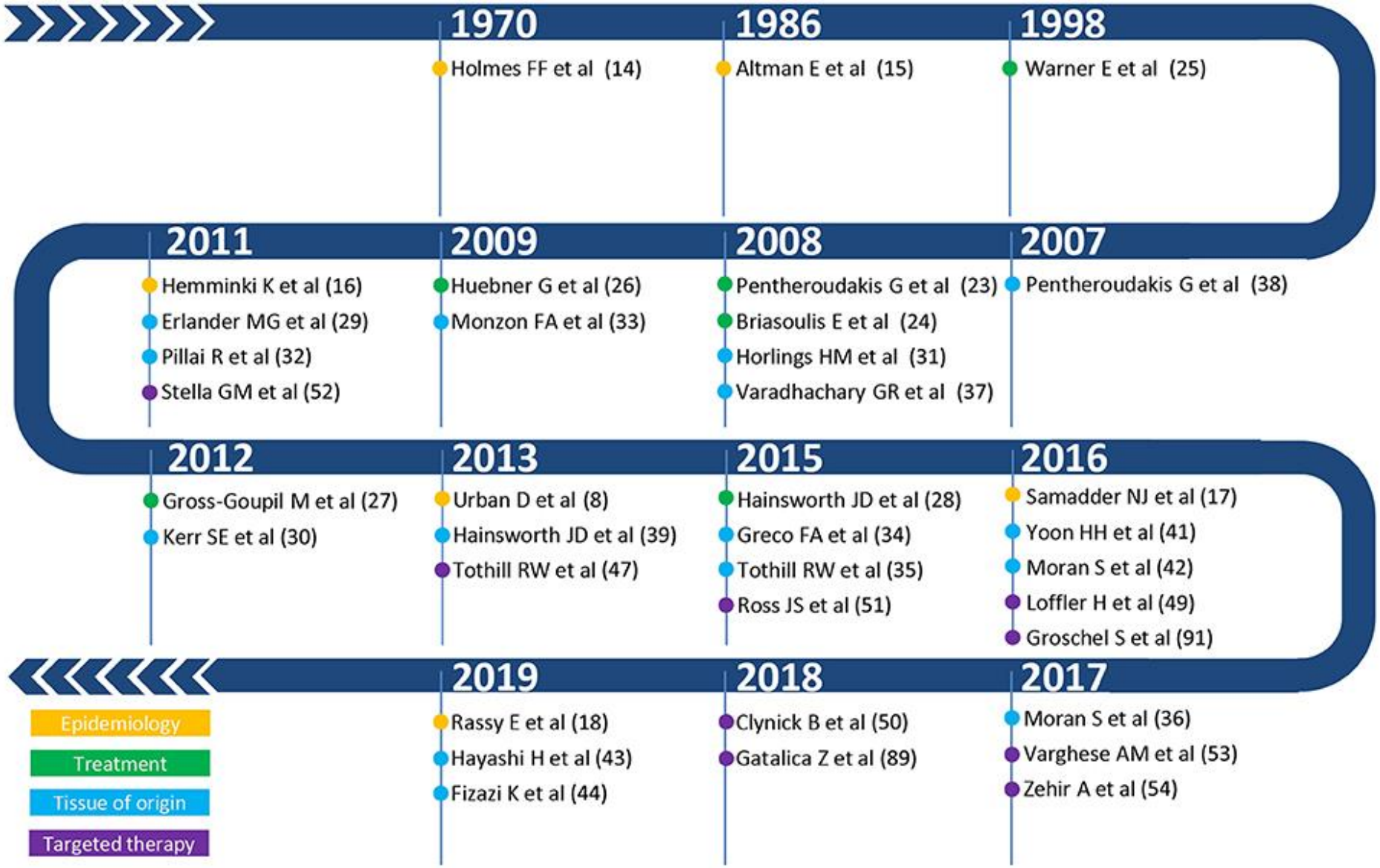
Cancer of unknown primary (CUP)

- A malignant widespread metastatic disease without an identifiable primary site after extensive clinical investigation
  - sixth to eighth most common cancer
  - 2.3% to 5% of new malignancy diagnosed
- Recently, a decline is observed in the diagnosis of CUP, mainly due to improvement in detection of the primary tumors, thus decreasing the unknown primaries.
- a separate disease??
- 80% unfavorable, 20% favorable prognosis
- **Malignant phenotype favors metastases over primary tumor growth**




1970
Holmes FF et al (14)
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Altman E et al (15)
1998
Warner E et al (25)
2011
Hemminki K et al (16)
Erlander MG et al (29)
Pillai R et al (32)
Stella GM et al (52)
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Huebner G et al (26)
Monzon FA et al (33)
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Pentheroudakis G et al (23)
Briasoulis E et al (24)
Horlings HM et al (31)
Varadhachary GR et al (37)
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Gross-Goupil M et al (27)
Kerr SE et al (30)
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Urban D et al (8)
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Tothill RW et al (35)
Ross JS et al (51)
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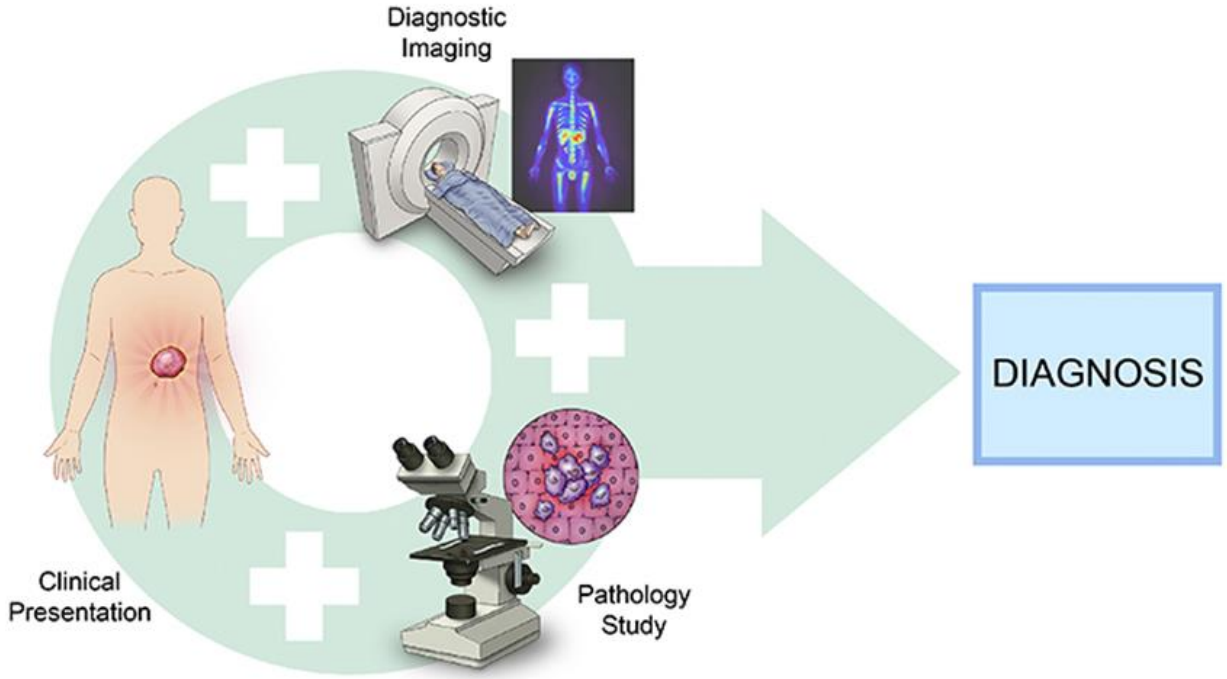
Samadder NJ et al (17)
Yoon HH et al (41)
Moran S et al (42)
Loffler H et al (49)
Groschel S et al (91)
2019
Rassy E et al (18)
Hayashi H et al (43)
Fizazi K et al (44)
2018
Clynick B et al (50)
Gatalica Z et al (89)
2017
Moran S et al (36)
Varghese AM et al (53)
Zehir A et al (54)
Epidemiology
Treatment
Tissue of origin
Targeted therapy

Cancer of unknown primary (CUP)

- The prognosis of CUP is depressing with the **median survival of three to six months** in the previous studies, but according to recent studies, median survival is **less than one year.**
- High risk for developing CUP is seen in **heavy smokers** (26 or more cigarettes/day) and individuals with the lowest quartiles of waist circumference.
- A weak association is observed with the **use of alcohol** consumption and low level of education.
- **Human papillomavirus DNA** plays a role in those with squamous cell carcinoma of unknown primaries in head and neck regions.
- In the diagnosis of CUP, comprehensive medical history, complete physical examination (including genitourinary, rectal exam, and breast examination in women) and necessary laboratory tests are crucial.

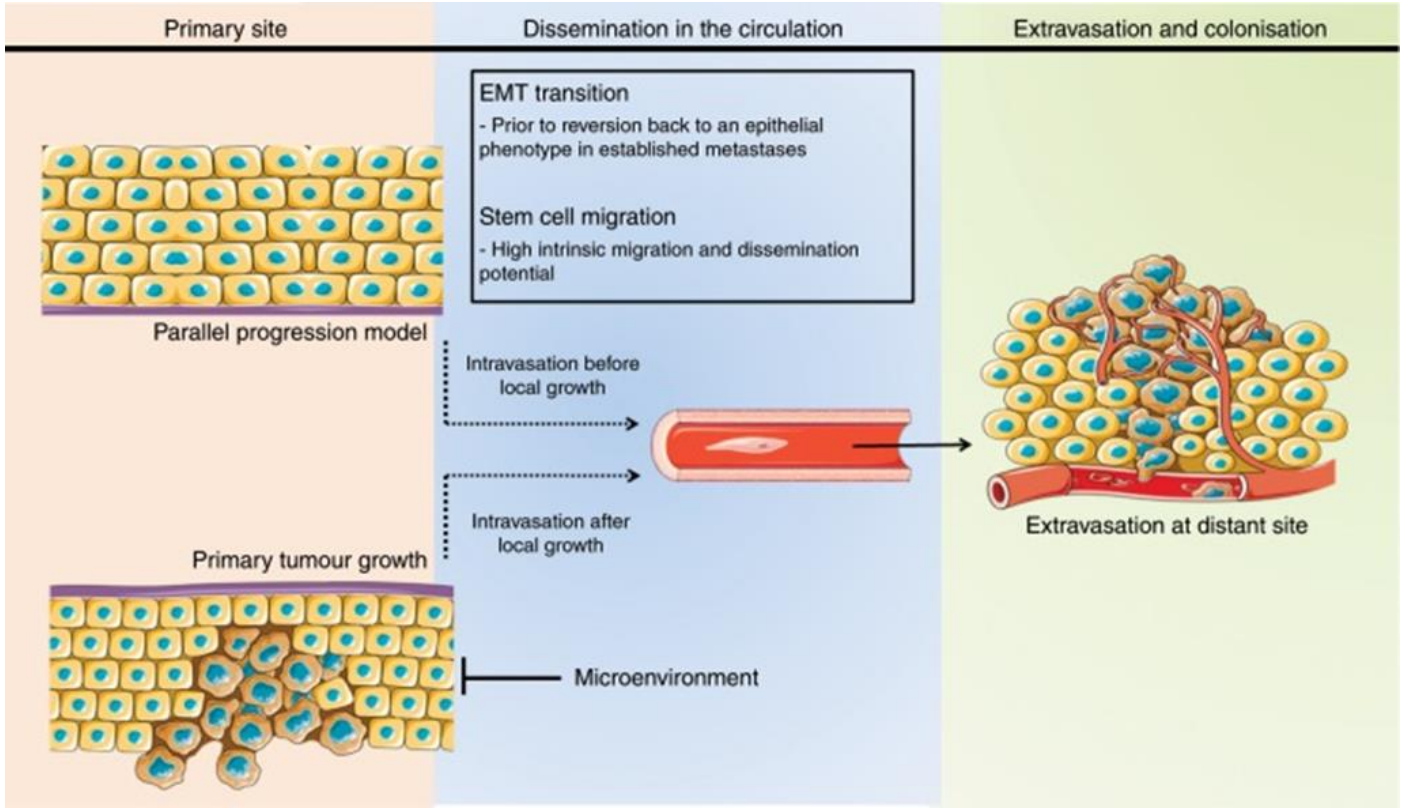


Hypotheses on the pathogenesis of CUP:

1) Stem cell producing cancer without premalignant lesion or primary cancer.

2) A very early primary cancer causes a rapid progression of metastasis.

3) Recently a possible explanation was proposed on the role of chromosomal instability contributing to aggressive disease presentation and chemoresistance



The Guidelines issued by the United Kingdom National Institute of Clinical Excellence (NICE)

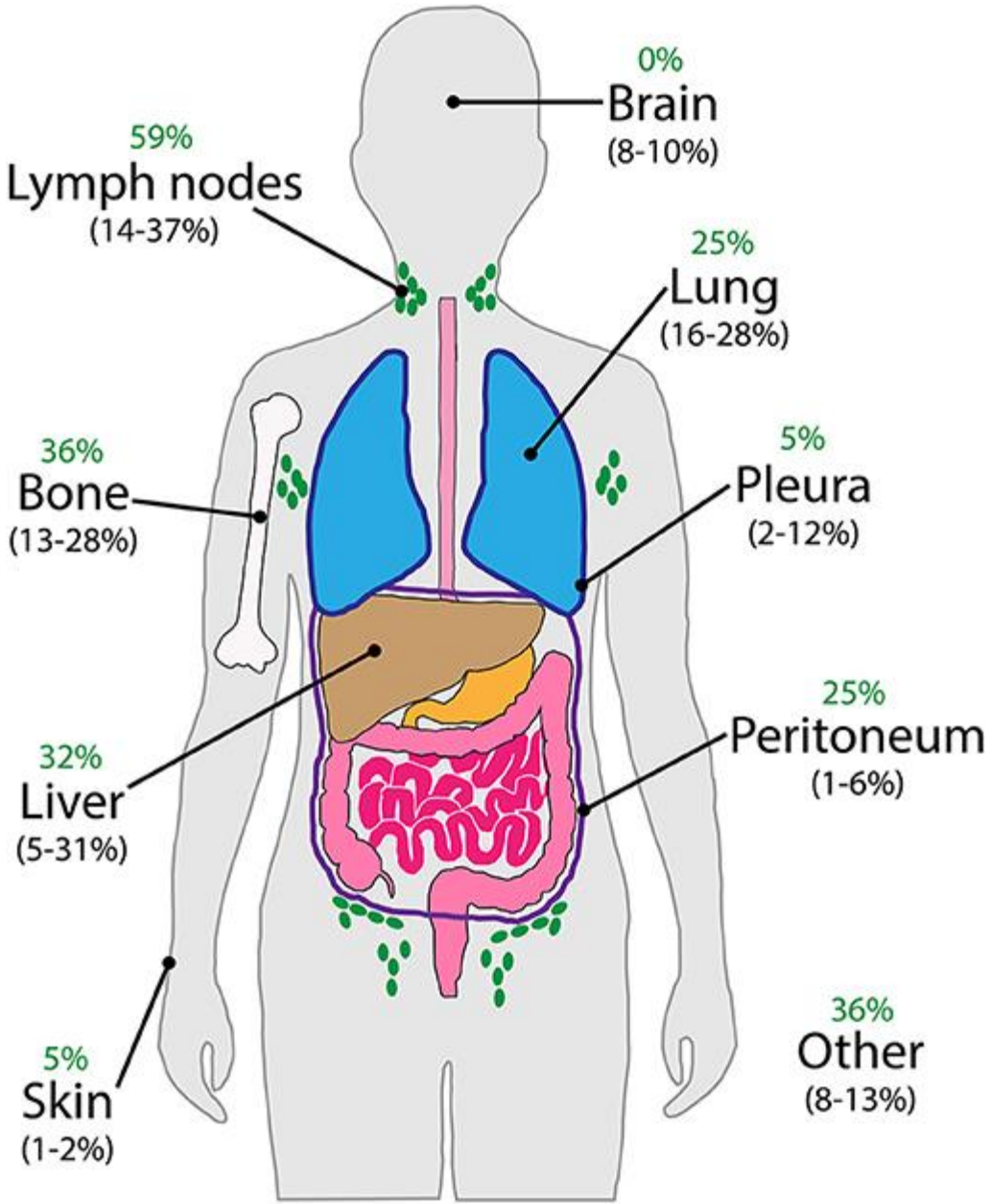
Term	Definition
Malignancy of undefined primary origin (MUO)	Limited tests showed Metastatic malignancy without any clear primary site before the comprehensive investigations are performed.
Provisional CUP (pCUP)	Selected initial tests based on cytology and histology showed metastatic epithelial or neuroendocrine malignancy without any primary site of origin, before specialist evaluation and likely after additional specialized investigations.
Confirmed CUP	Final histology showed metastatic epithelial or neuroendocrine malignancy without any primary site of origin even after the initial tests, specialist evaluation, and likely additional specialized investigations.

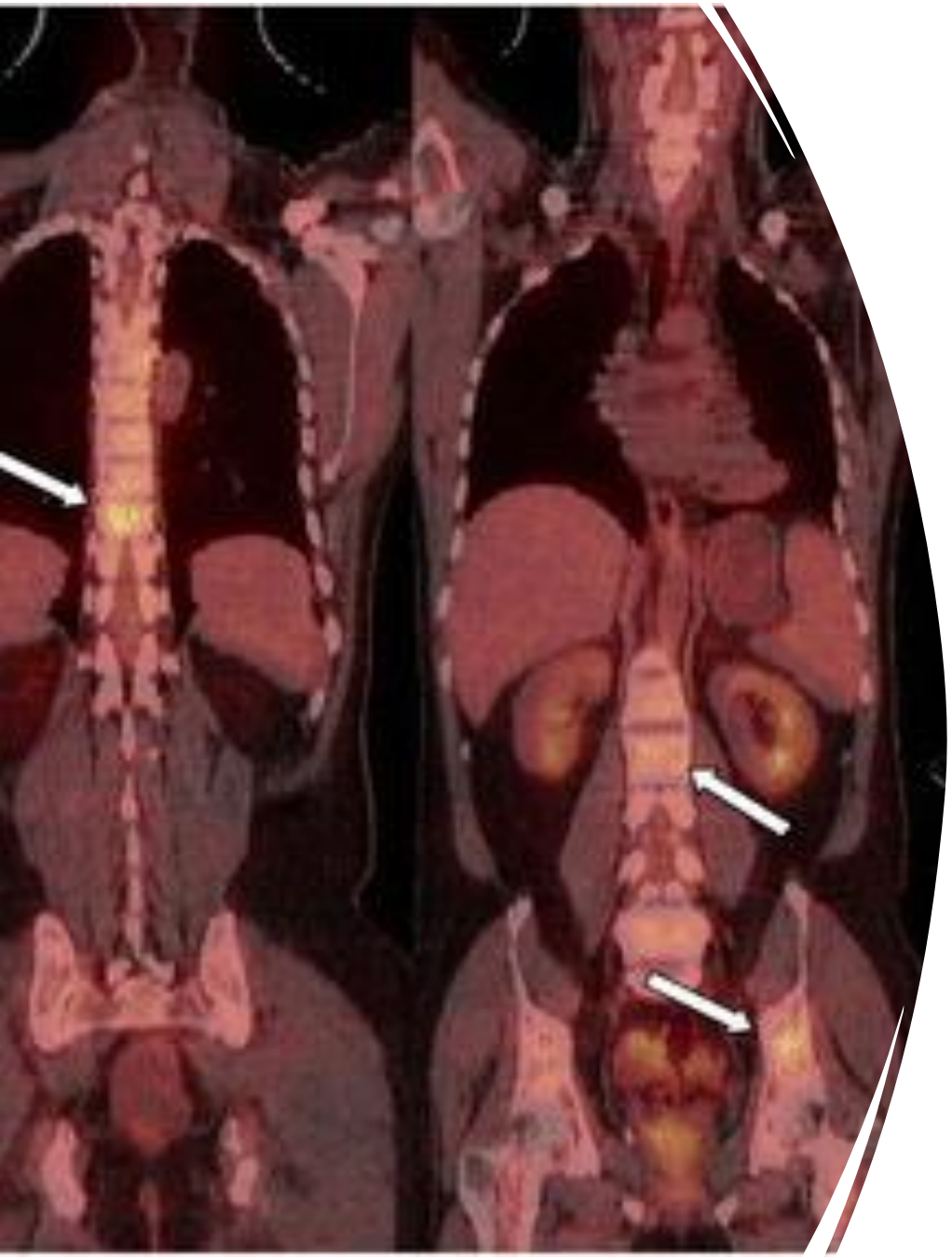
Pathogenesis	
Angiogenesis activation	50–89%
Oncogene over-expression	10–30%
Hypoxia-related proteins and epithelial-mesenchymal transition markers	16–25%
Activation of intracellular signals such as Akt or MAPK	20-35%

TABLE 2: Pathology in the development of the cancer of unknown primary (CUP)

Akt - protein kinase B (PKB), MAPK- mitogen-activated protein kinase.

Cancer of unknown primary (CUP)

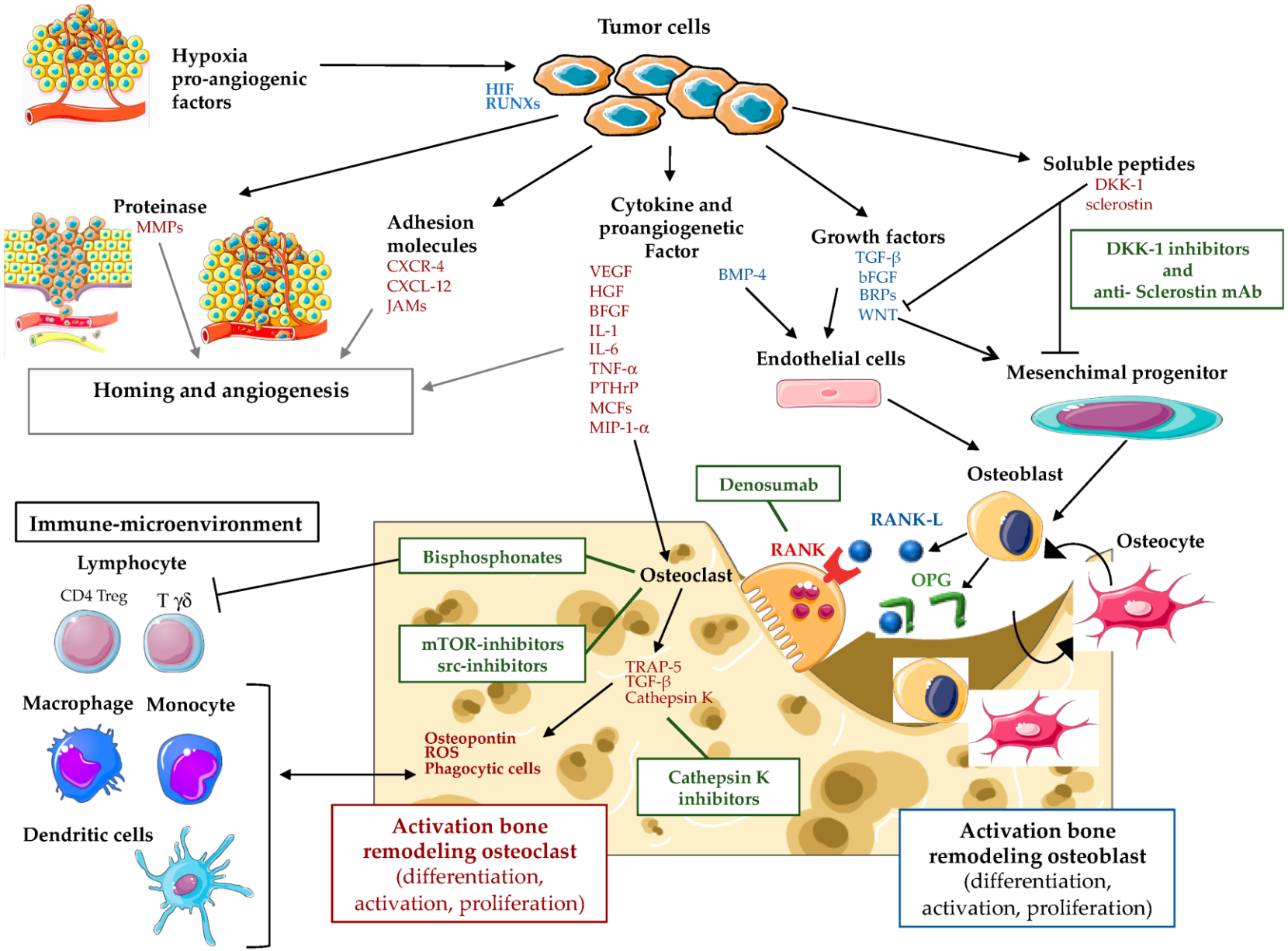


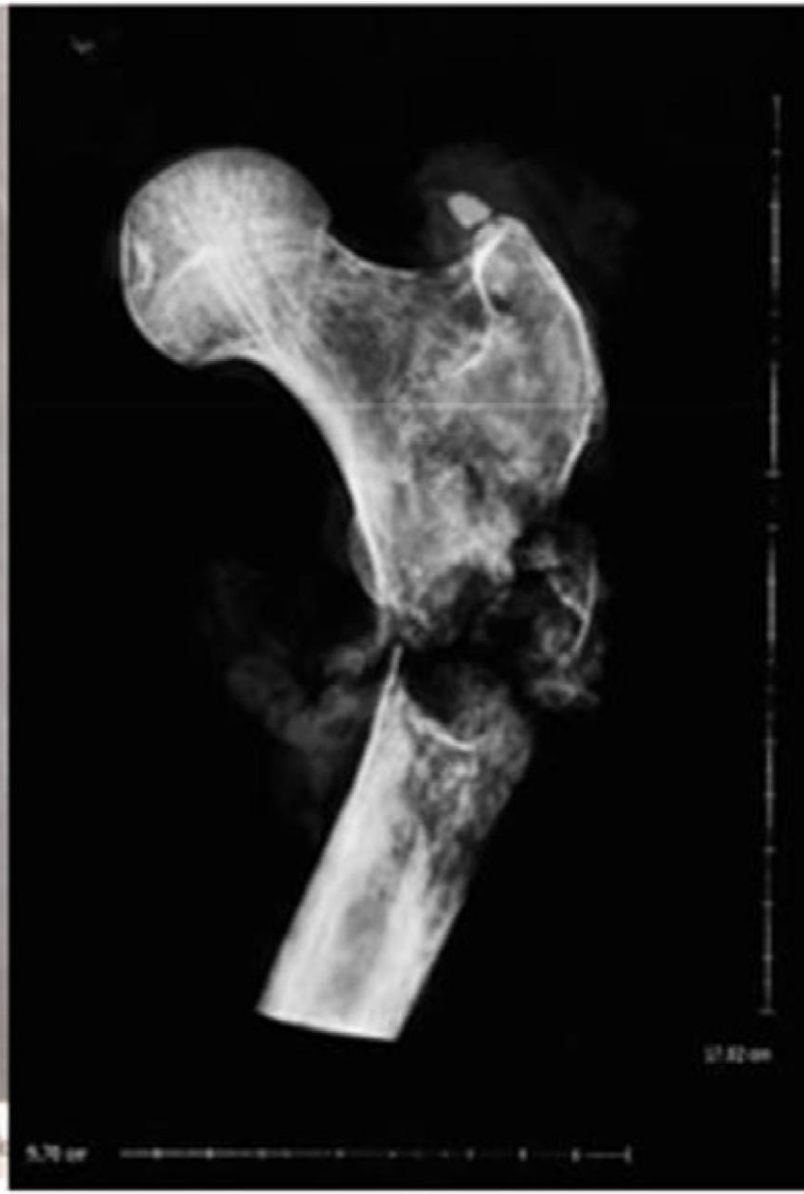


Skeletal metastases of unknown primary (SMUP)

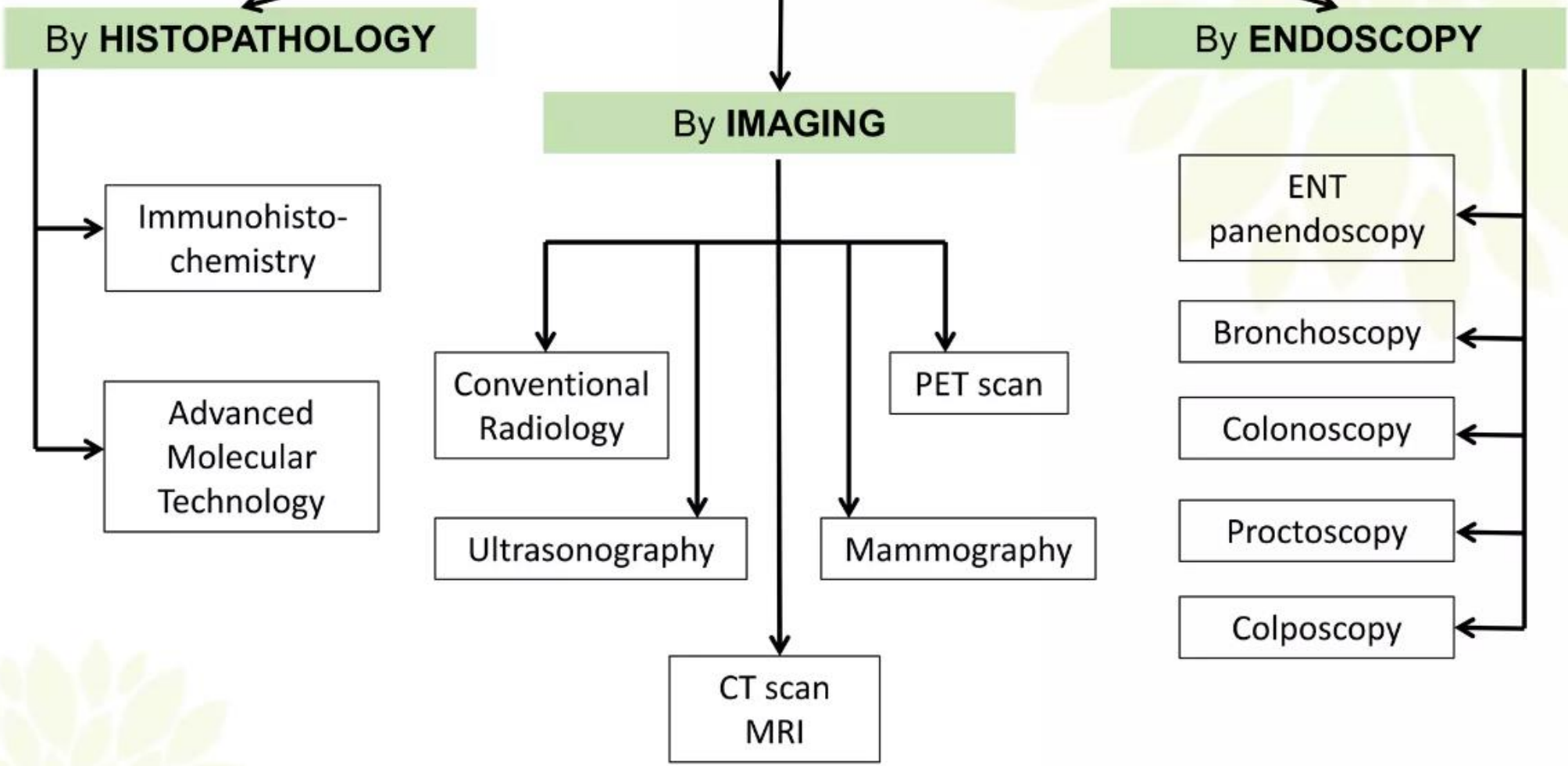
- Epithelial cancers more common
- The skeleton is the third most common site of metastatic cancer after lung and liver (8–23%)
- Spine most common site of SMUP, followed by the pelvis and bones of the extremities
- Lung cancer is the most frequently identified primary tumor (25–67%) across all literature data.
- The other most frequent primary malignancies are multiple myeloma, prostate cancer, breast cancer, kidney, gastrointestinal tumor, and lymphoma.
- Nonetheless, the primary site of bone metastases often remains unidentified despite diagnostic investigations and autoptic examination

Skeletal metastases of unknown primary (SMUP)



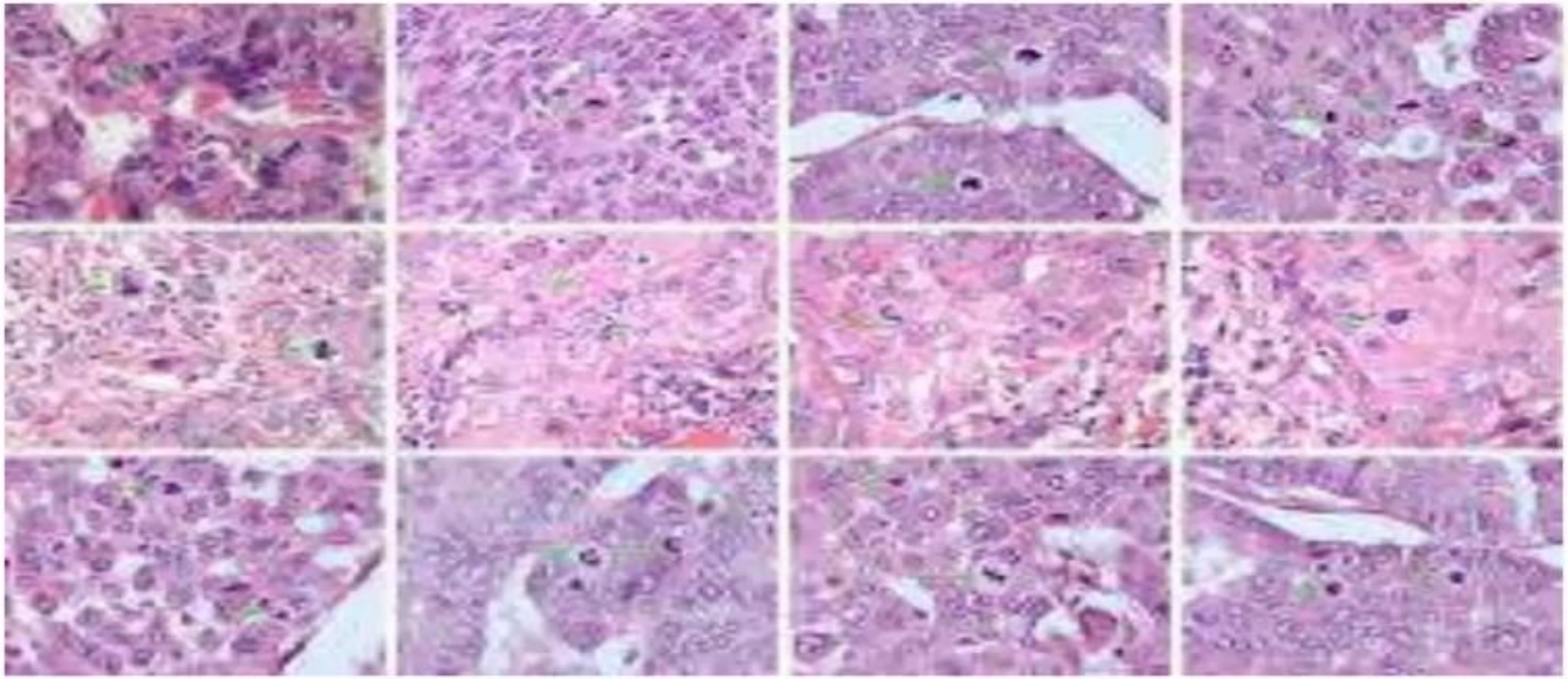


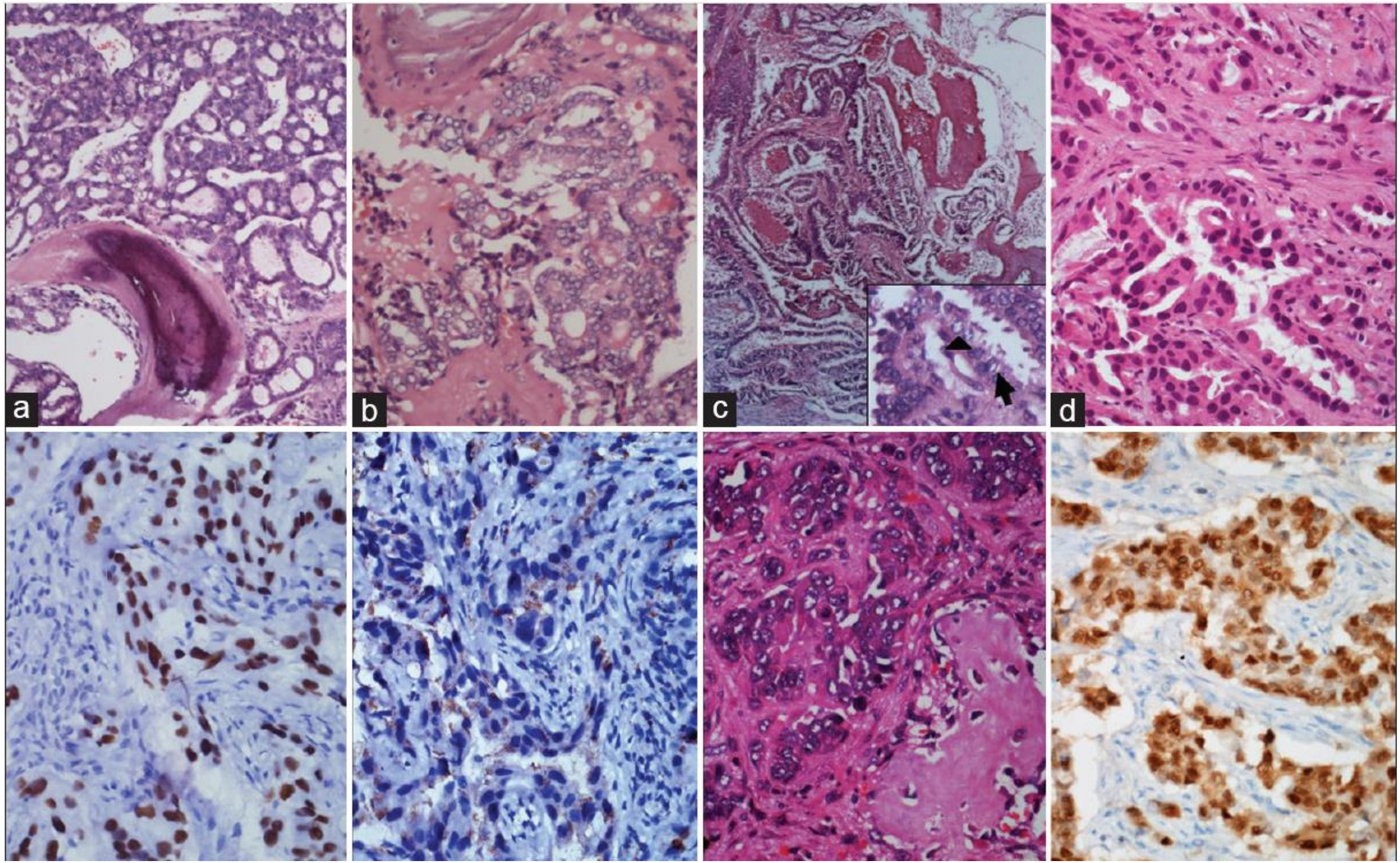
HOW DO WE SEARCH FOR THE PRIMARY ?



By

HISTOPATHOLOGY

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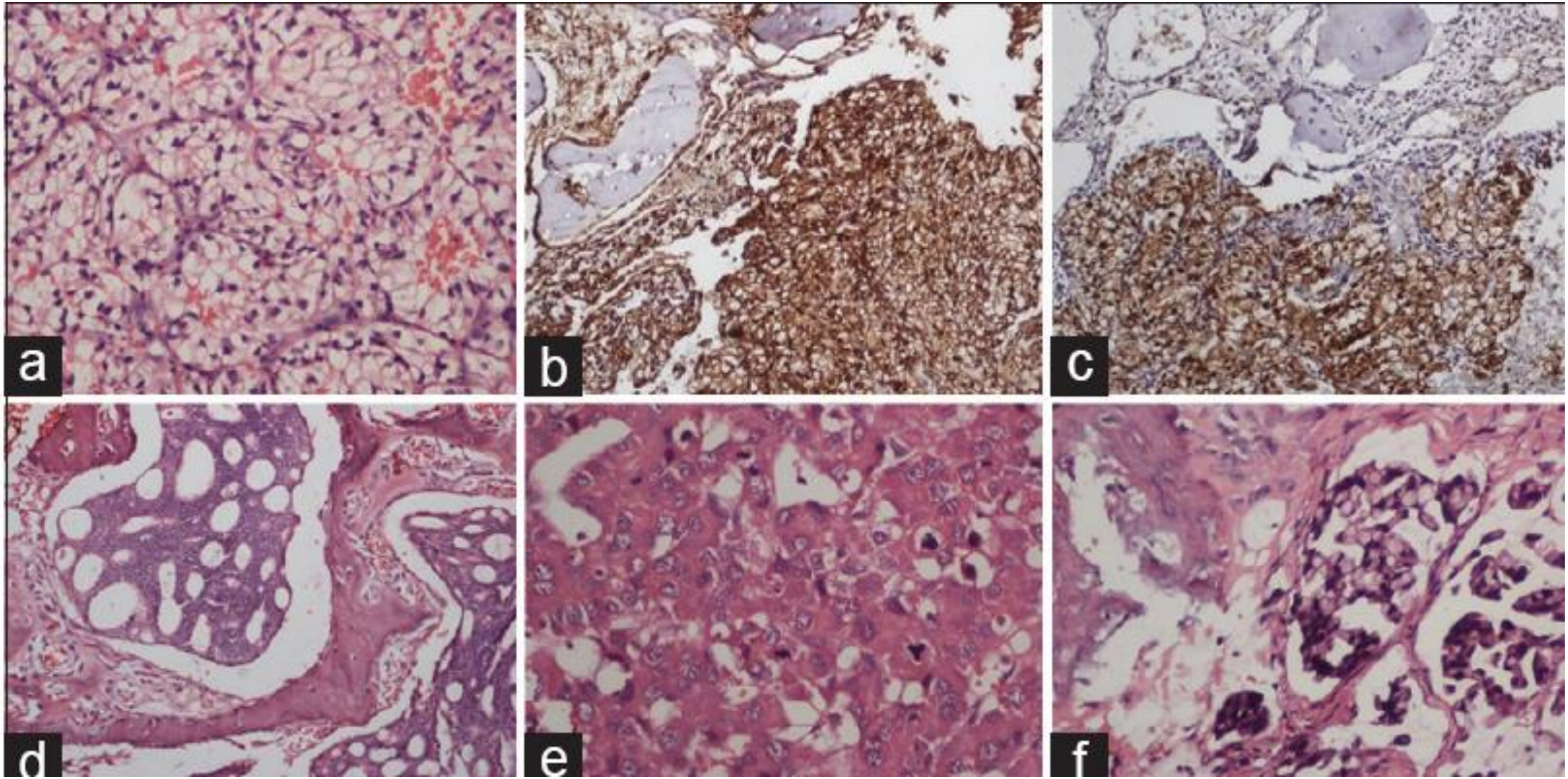
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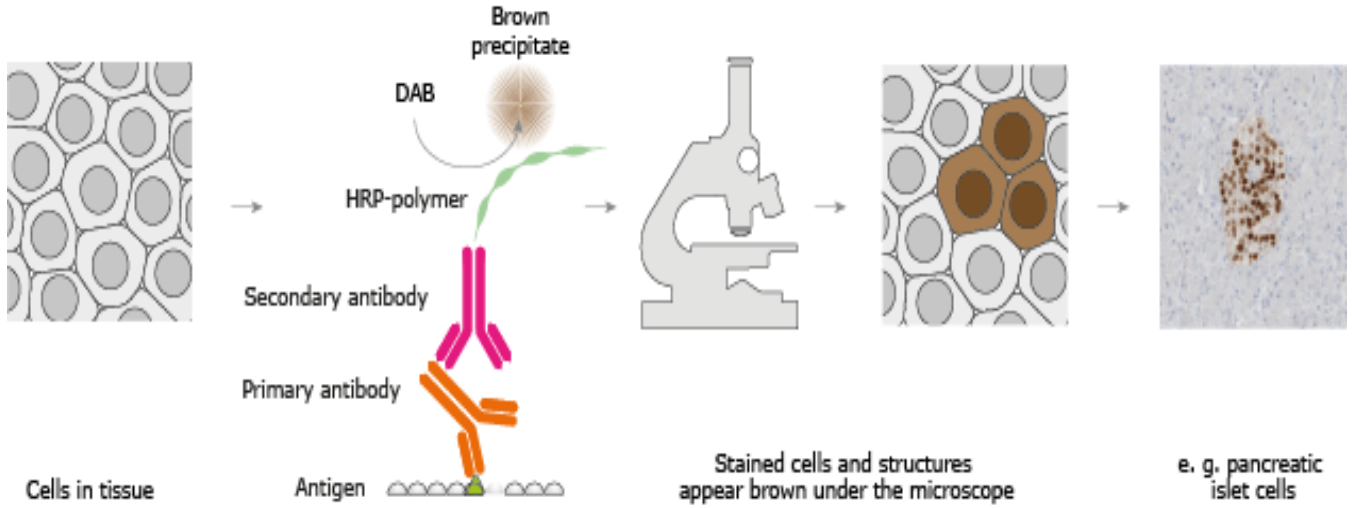
CUP: Immunohistochemistry (IHC)

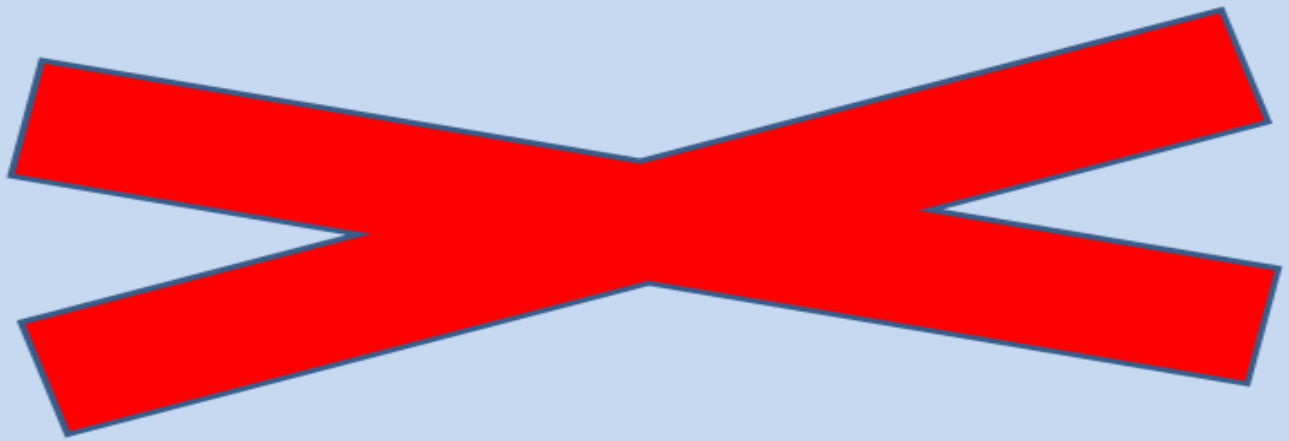
- Low cost/performed easily on paraffin tissue
- New IHC markers/ Abs improved specificity
- Guidance in approximately 90% of undifferentiated malignant tumours
- **Four primary sites (breast, prostate, ovarian, and thyroid**) involving specific effective treatment options and a better prognosis should first be investigated
- In addition, the development of targeted therapies must eliminate a **pulmonary or colorectal origin**.
- **No single antibody is fully sensitive and specific for a particular tumour**



Essentials for right diagnosis

- Quality and quantity of the material
- Quality of standard laboratory procedures
- Quality of immunohistochemistry
- Sufficient number of available antibodies
- Pathologist with sufficient knowledge of morphological spectrum of tumors as well as variation in their immunophenotype and at least basic knowledge of immunohistochemistry!

How to approach the problem?



No “shot gun” immunohistochemistry!

CUP: Initial IHC evaluation

	Lymphoma	Carcinoma	Melanoma	Sarcoma
LCA (CD45)/CD20 /CD3	+	-	-	-
panCK: AE1/AE3, MNF116, OSCAR	-	+	-	-/+
S100, SOX10	-	-/+	+	-/+
Vimentin	-/+	-/+	+	+

CUP: Initial IHC evaluation

Selected IHC Pitfalls

Table 3. Keratin and Vimentin Expression in Carcinomas and Mesenchymal Tumors

Carcinomas That Frequently Express Both	Carcinomas That Rarely Express Both	Mesenchymal Tumors That Frequently Express Both
Renal cell carcinoma	Breast carcinoma	Synovial sarcoma
Anaplastic thyroid carcinoma	Ovarian carcinoma	Desmoplastic small round blue cell tumor
Endometrial carcinoma	Gastrointestinal carcinoma	Epithelioid sarcoma
Thyroid carcinoma	Lung small cell carcinoma	Epithelioid angiosarcoma
Sarcomatoid carcinoma	Lung non–small cell carcinoma	Malignant rhabdoid tumor
Mesothelioma	Prostate carcinoma	Leiomyosarcoma
Myoepithelial carcinoma		Chordoma
Metaplastic breast carcinoma		Adamantinoma

Kandukuri et al, Arch Pathol Lab Med—Vol 141, August 2017

CUP: Initial IHC classification

- **Carcinoma/neuroendocrine tumour**:
  - adenocarcinoma (60%)
  - poorly differentiated carcinoma, including poorly differentiated adenocarcinoma (20%)
  - squamous cell or urothelial carcinoma (5–10%);
  - neuroendocrine tumour (5%)
  - undifferentiated carcinoma
- Lymphoma
- Melanoma
- Sarcoma

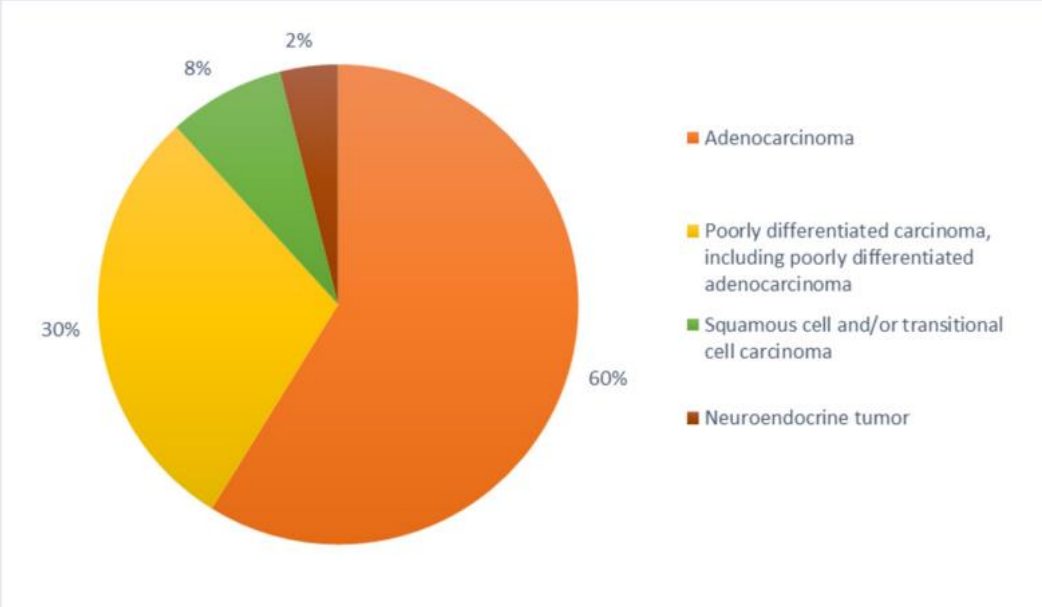


FIGURE 1: Different types of histopathological prevalence in cancer of unknown primary (CUP)

CUP: CK7 and CK20

CK7+/CK20−	CK7+/CK20+	CK7−/CK20+	CK7−/CK20−
Breast carcinoma			
Lung adenocarcinoma			
Endometrial adenocarcinoma			
Endocervical adenocarcinoma	Urothelial carcinoma		Prostate adenocarcinoma
Ovarian (serous) carcinoma	Pancreatic adenocarcinoma		Renal (clear cells)
Cholangiocarcinoma	Ovarian mucinous carcinoma	Colorectal adenocarcinoma	Hepatocellular carcinoma
Small cell lung carcinoma	Bladder adenocarcinoma	Merkel cell carcinoma	Adrenocortical carcinoma
Mesothelioma	Gastric adenocarcinoma	Gastric adenocarcinoma	Non-seminoma germ cell tumours
Thyroid carcinoma	Cholangiocarcinoma		Mesothelioma
Salivary gland tumours			Small cell lung carcinoma
Kidney (papillary)			Gastric adenocarcinoma
Urothelial carcinoma (subset)			
Pancreatic adenocarcinoma			
Gastric adenocarcinoma			

Selves et al, Cancers 2018, 10, 108

CUP: Organ-specific antibodies

Table 2. Summary of Carcinoma (Tumor)-Specific Antibody Reagents

Carcinoma Subtype	Antibodies to:	Localization of Signal	Sensitivity	Specificity	Also Identifies
Breast	Estrogen receptors	Nuclear	Moderate	Moderate	Endometrioid adenocarcinoma, ovarian serous CA
Breast	GCDFP-15	Cytoplasmic	Low	Moderate	Salivary gland, sweat gland tumors
Breast	Mammaglobin	Cytoplasmic	Low	Moderate	Salivary gland, sweat gland tumors
Breast	GATA3	Nuclear	High	Moderate	Salivary gland, transitional cell CAs, skin adnexal tumors
Colorectal and GI	Villin	Membranous brush border	High	Moderate	Subset of lung carcinomas, ovarian and endometrial CAs
Colorectal	CDX2	Nuclear	High	High	Subset of pancreatic, gastric CAs
Hepatocellular	HepPar1	Cytoplasmic	Moderate	High	Hepatoid adenocarcinomas
Hepatocellular	Arginase	Nuclear and cytoplasmic	High	High	Hepatocellular CAs
Lung adenocarcinoma and thyroid, including NE	TTF-1	Nuclear	High	High	Neuroendocrine CAs of other sites
Lung adenocarcinoma	Napsin A	Cytoplasmic	High	High	GYN clear cell CAs, subset of renal cell and thyroid CAs
GYN	PAX8	Nuclear	Very high	Moderate	Thyroid CA, renal cell CA
Ovarian serous	WT1	Nuclear	Very high	High	Mesothelioma
Prostate	Prostate-specific antigen	Cytoplasmic	Very high	Very high	...
Prostate	NKX3.1	Nuclear	Very high	Very high	...
Renal cell	PAX8	Nuclear	Moderate	Moderate	GYN and thyroid CAs
Squamous, transitional cell	p63	Nuclear	Very high	Very high	Thymoma, salivary gland tumors, some neuroendocrine CAs, trophoblastic tumors
Squamous, transitional cell	P40	Nuclear	Very high	Very high	Thymoma, salivary gland tumors, trophoblastic tumors
Thyroid	Thyroglobulin	Cytoplasmic	High	Very high	...
Thyroid	PAX8	Nuclear	Very high	Moderate	GYN and renal CAs
Transitional cell	Uroplakin	Cell membranous	Low	High	...
Transitional cell	GATA3	Nuclear	High	Moderate	Breast cancers, salivary gland CAs, skin adnexal tumors

Abbreviations: CA, carcinoma; GI, gastrointestinal; GYN, gynecologic; NE, neuroendocrine.

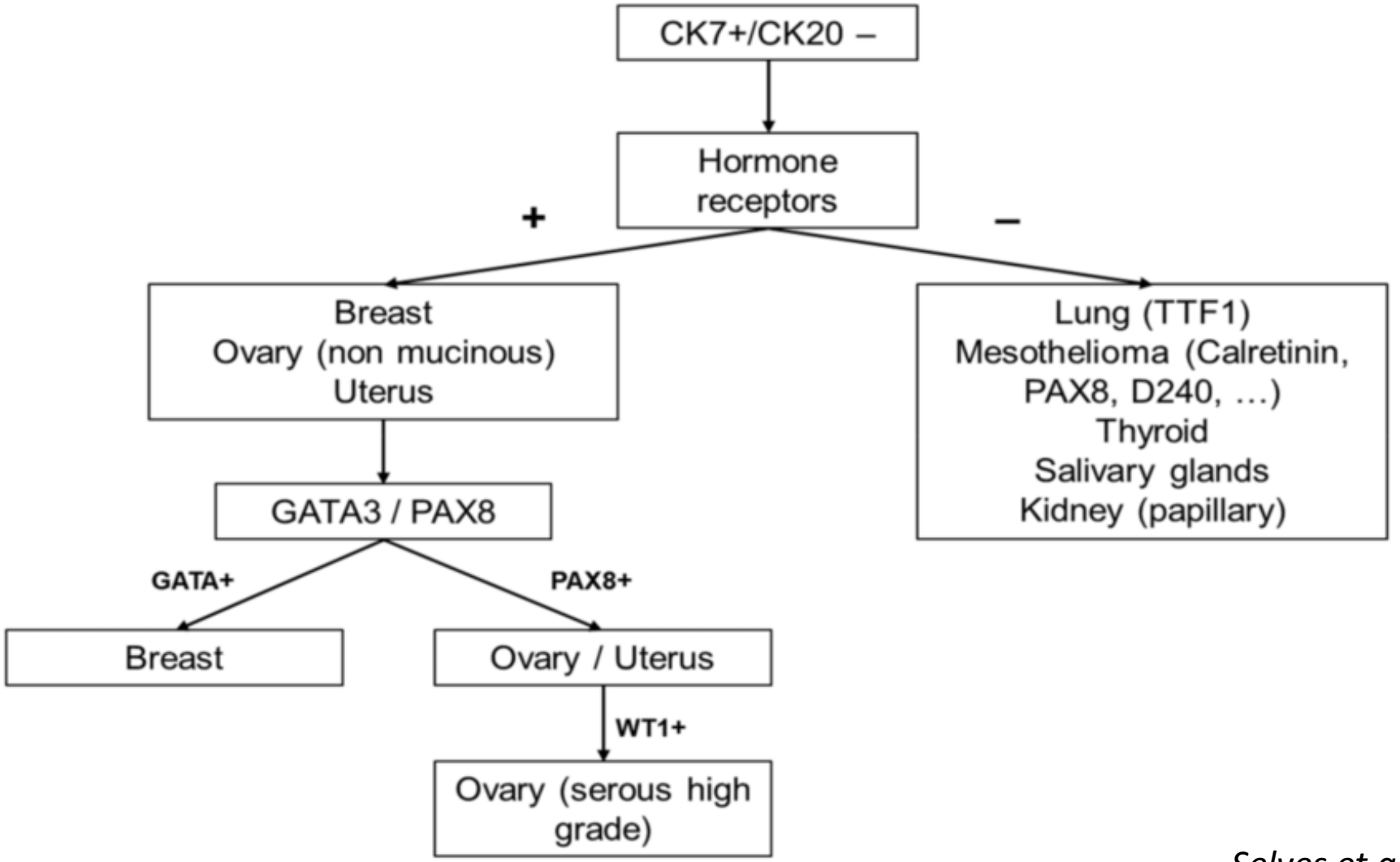
Kandalaft & Gown, *Arch Pathol Lab Med—Vol 140, June 2016*

Diagnostic Workflow of CK7+/CK20– CUPs

Primary Site of Origin	Immunostaining Profile
Breast [8,14–17]	ER+/PgR+, GATA3+, GCDFP15−/+, MGB+/−, TFF1−
Ovary (serous) [17–21]	PAX8+, ER+, WT1+, TTF1−, TFF3−, GATA3−
Ovary (clear cell) [17–21]	pVHL+, HNF-1β+, Napsin A+, AFP−, WT1−, ER−, GPC3−
Endometrium [17–21]	ER+, PAX8+, Vimentin+
Uterine cervix [17–21]	p16+, HPV+, CEA+, PR−, PAX2−, PAX8+/−
Lung [22–24]	TTF1+, Napsin A+, GATA3−
Thyroid (papillary/follicular) [23–25]	TTF1+, Thyroglobulin+, PAX8+
Thyroid (medullary) [23–25]	TTF1+, Calcitonin+, CEA+
Stomach [26–29]	CEA+, CDX2−/+, MUC1−/+, MUC5AC−/+, CDH17+/−, TTF1−
Oesophagus [26–29]	CDX2+/−, CEA+, CDH17+, MUC1−/+, MUC5AC−/+, SATB2−
Pancreas [26–29]	DPC4−/+, CK17+/−, pVHL−, Maspin+, S100P+, MUC5AC+
Urinary bladder [17–21]	GATA3+, p63+, CK5/6+, p40+, S100P+, CK903+, UPII+/−
Thymus [19–21]	CD5+/−, p63+/−, PAX8+/−, CD117+/−, Glut1+/−
Salivary (ductal) [16,17,30]	GATA3+, AR+, GCDFP-15+
Mesothelioma [30–34]	Calretinin+, WT1+, CK5/6+, TTF1−, CEA−, BerP4−

Selves et al, Cancers 2018, 10, 108

Diagnostic Workflow of CK7+/CK20– CUPs



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Diagnostic Workflow of CK7+/CK20– CUPs

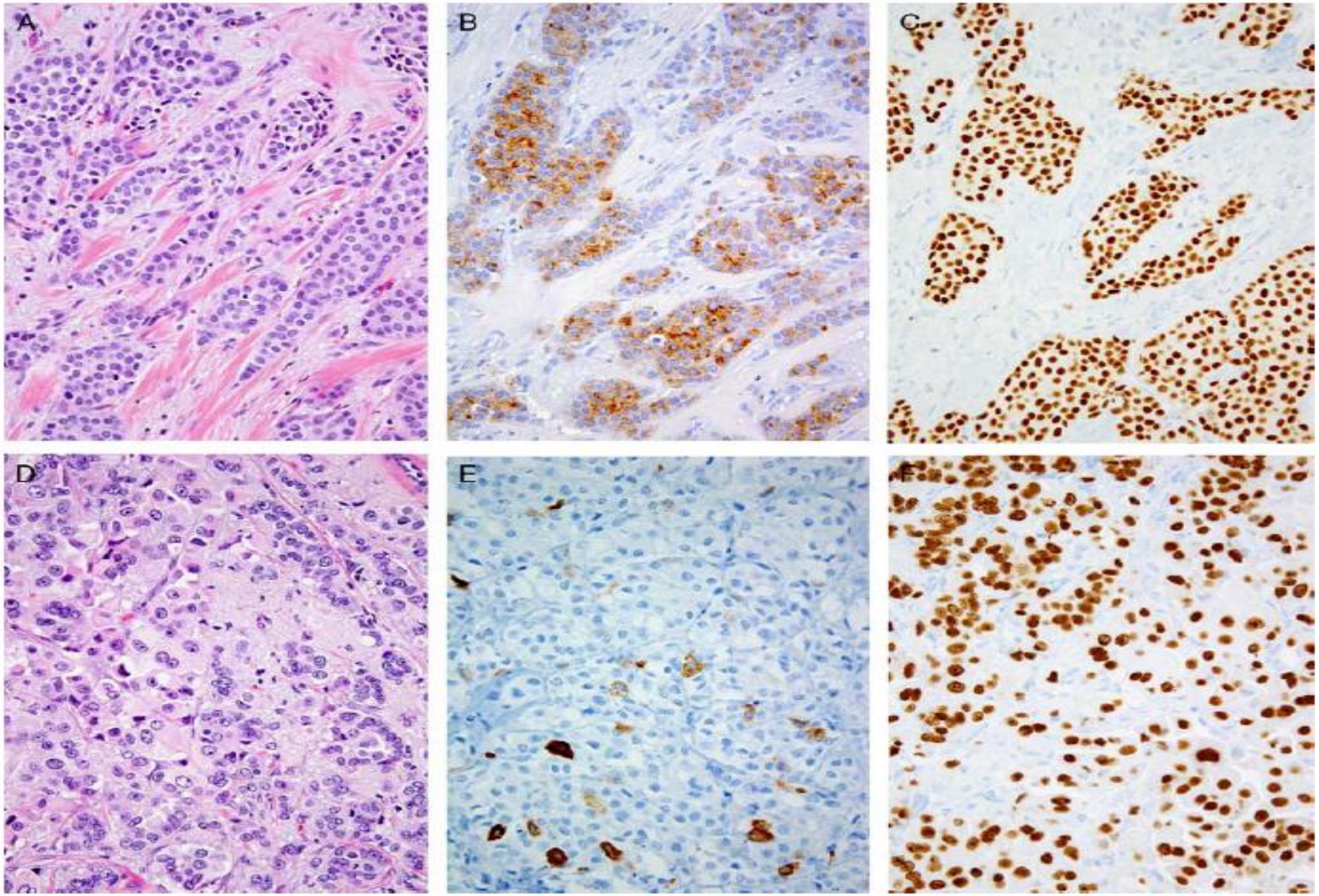
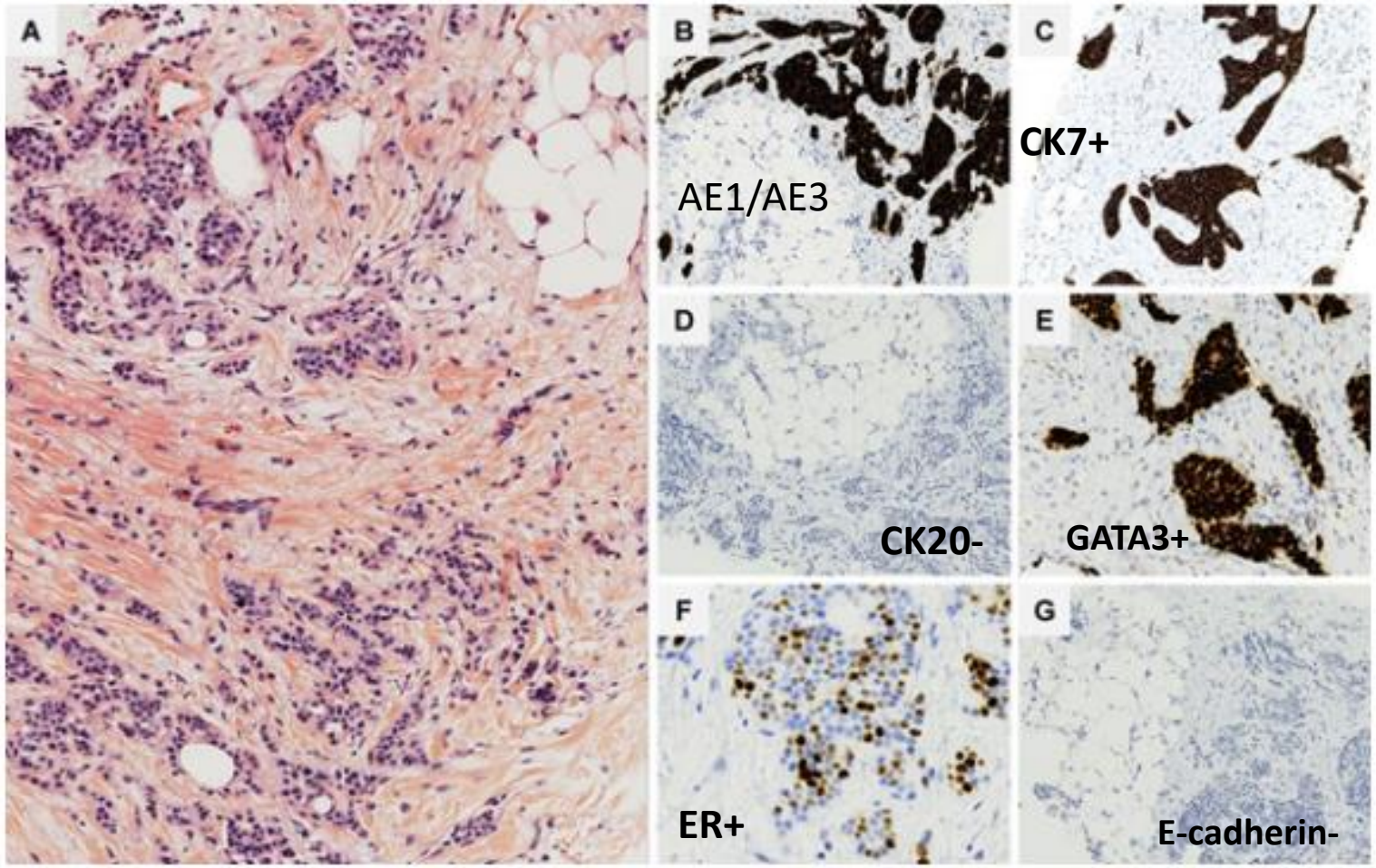


FIGURE 1. Mammaglobin, GCDFP-15, and GATA3 in breast carcinomas. A moderately differentiated invasive ductal carcinoma (A) shows diffuse cytoplasmic staining for GCDFP-15 with variable intensity (B) and diffuse strong GATA3 nuclear expression (C). In contrast, a more poorly differentiated invasive ductal carcinoma (D) shows only limited cytoplasmic staining for mammaglobin (E), but GATA3 nuclear expression remains diffuse and strong (F) (A and D: hematoxylin and eosin, ×400; B, C, E, and F: immunoperoxidase,

Diagnostic Workflow of CK7+/CK20– CUPs



GATA3

Very sensitive marker for breast carcinomas and urothelial carcinomas

43% of TNBC

54% of metaplastic BC

90% of metastatic BC

skin adnexal tumors

↓↓ endometrial, pancreatic, and salivary gland carcinomas

most mesotheliomas, chromophobe renal cell, trophoblastic germ cell neoplasms, and paragangliomas

Diagnostic Workflow of CK7+/CK20– CUPs

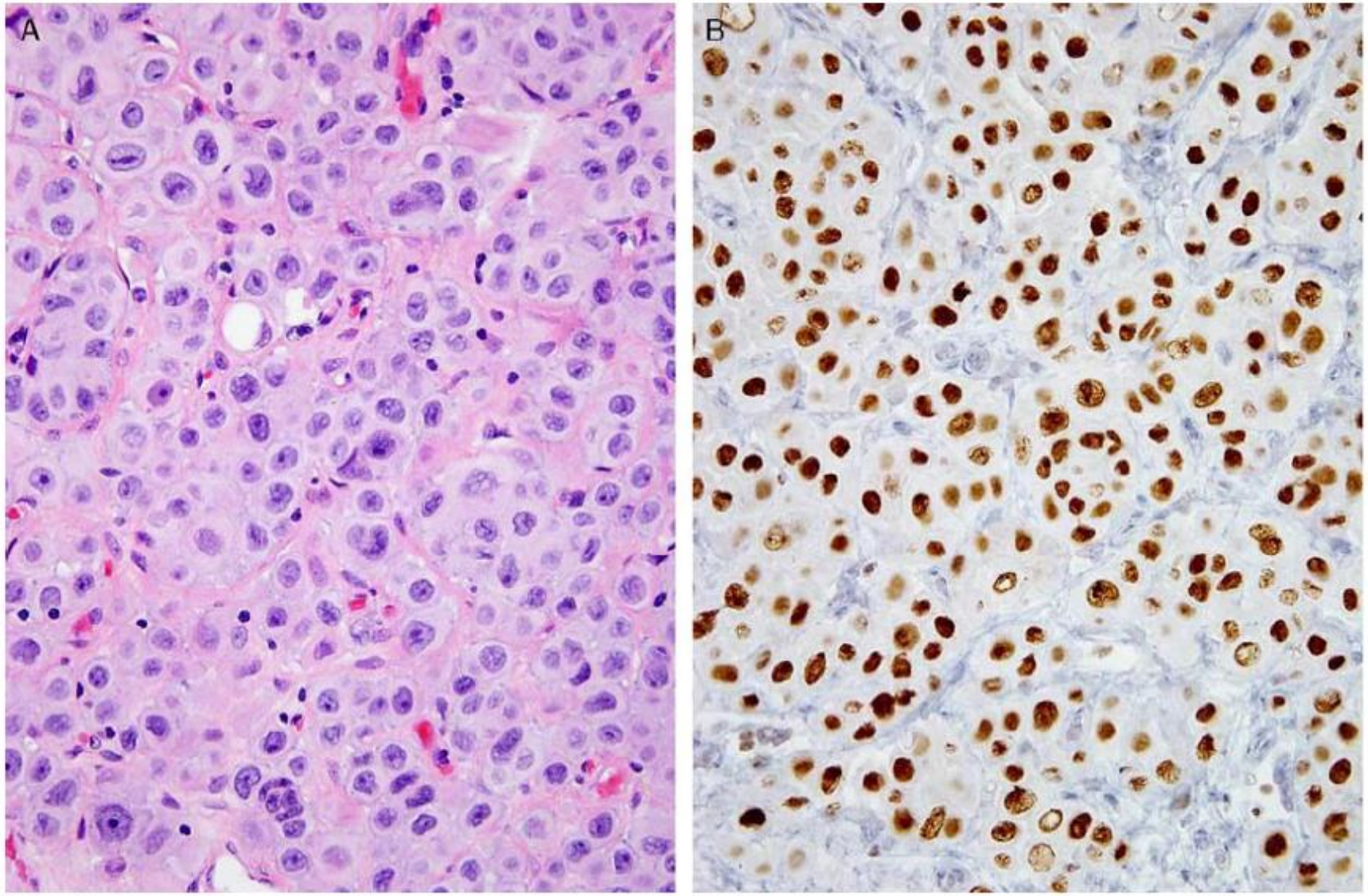
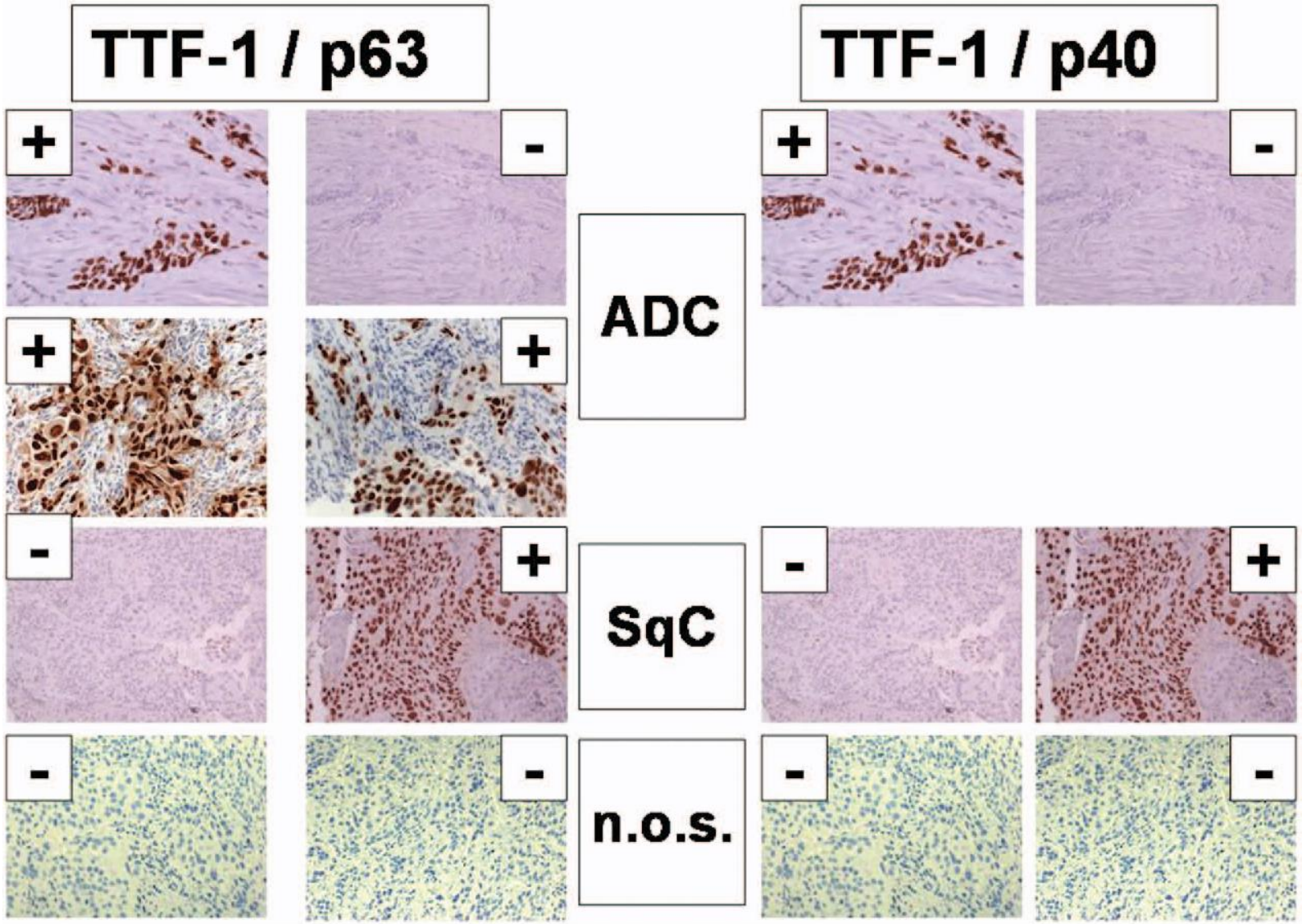


FIGURE 7. GATA3 in urothelial carcinoma. A high-grade urothelial carcinoma (A: hematoxylin and eosin, ×400) shows diffuse, strong nuclear GATA3 expression (B: immunoperoxidase, ×400).

Diagnostic Workflow of CK7+/CK20– CUPs

- **TTF-1**
- **Lung**
- Adeno well-differentiated (~100%)
- Adeno poorly differentiated (~50%)
- 90% SCLC 50% LCCLC
- 50% carcinoid tumors
- **Thyroid**
- 90% except anaplastic variant
- **↓↓ ovarian, endometrial and colorectal**



Diagnostic Workflow of CK7+/CK20– CUPs

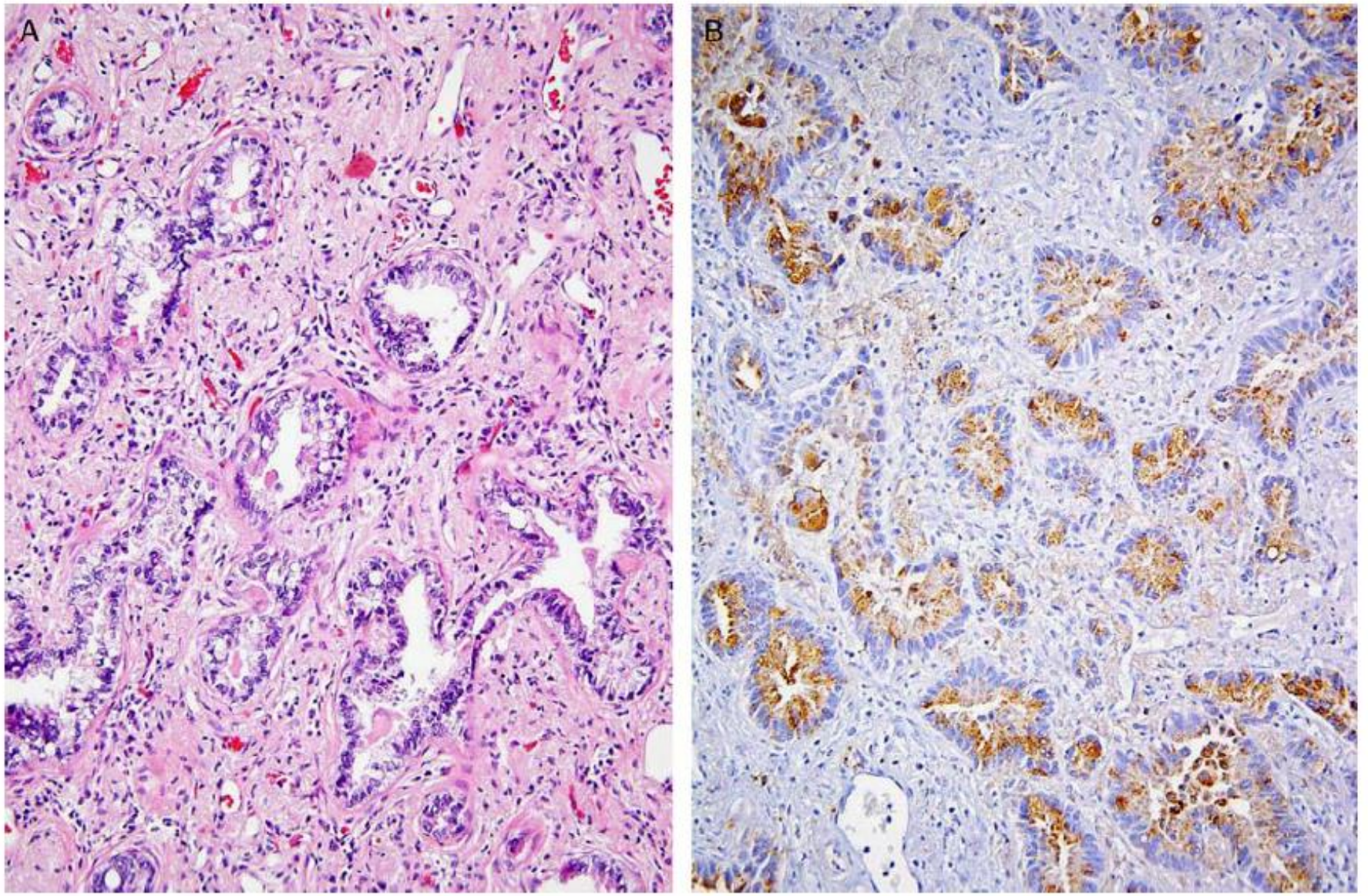
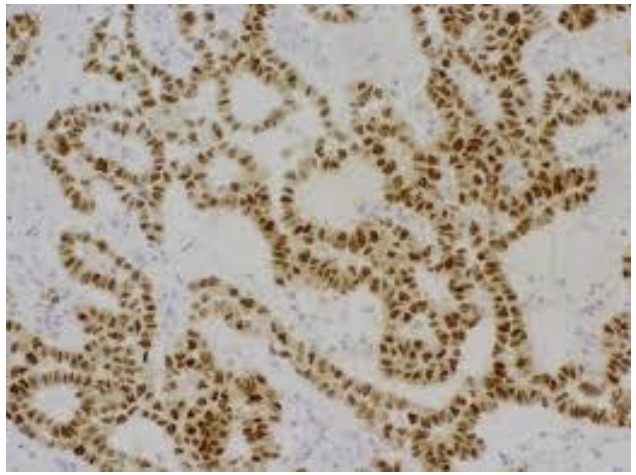


FIGURE 2. Napsin A in pulmonary adenocarcinoma. A moderately differentiated pulmonary adenocarcinoma (A: hematoxylin and

Diagnostic Workflow of CK7+/CK20– CUPs

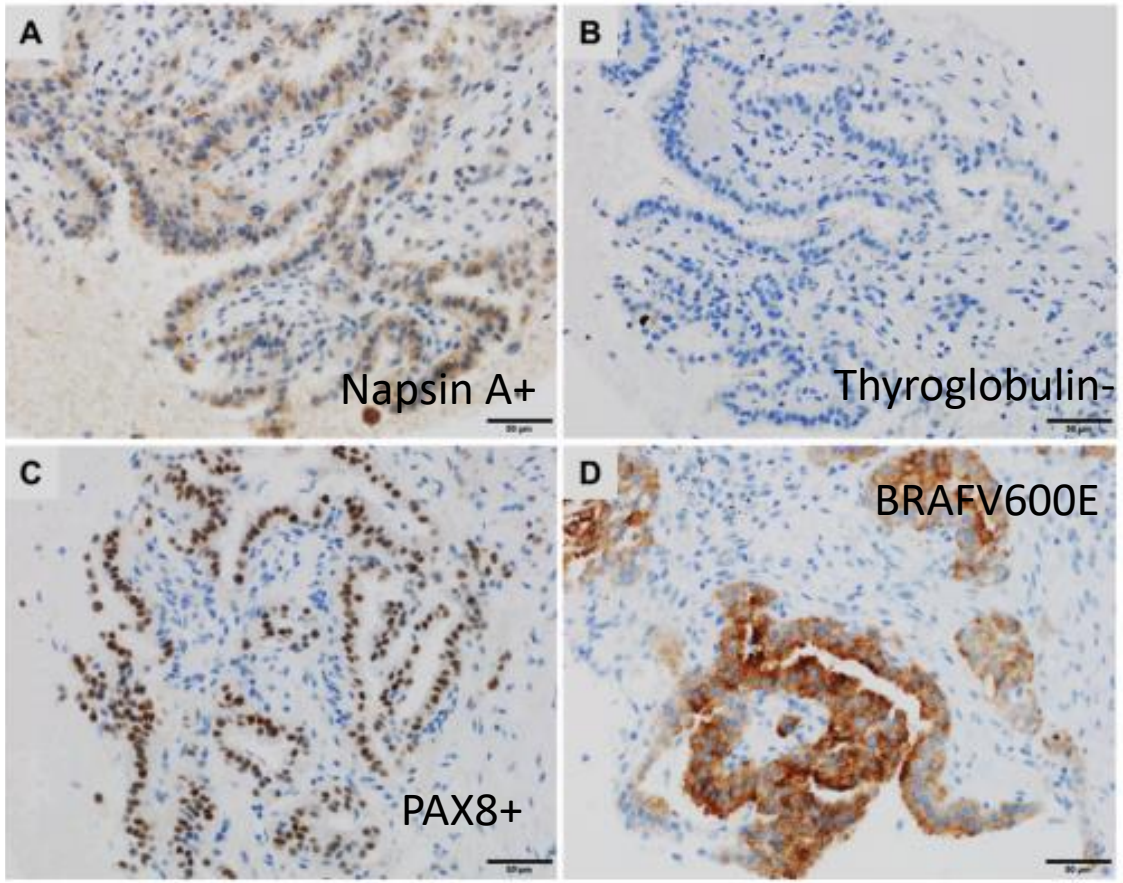
Napsin A

Lund Adeno
Renal cell Ca
Papilary thyroid Ca
Endometrial Ca
Clear cell ovarian Ca



Clear cell ovarian Ca

Lung metastasis of a papillary thyroid carcinoma



PAX8

Müllerian tumours (ovarian and endometrial),
Renal cell carcinomas
Thyroid carcinomas FC origin

PAX8 : DD. gynaecologic carcinomas from non-gynaecologic malignancies including malignant mesotheliomas, GI cancers and breast Ca
CK7+/CK20−/ER+

Diagnostic Workflow of CK7+/CK20– CUPs

WT1

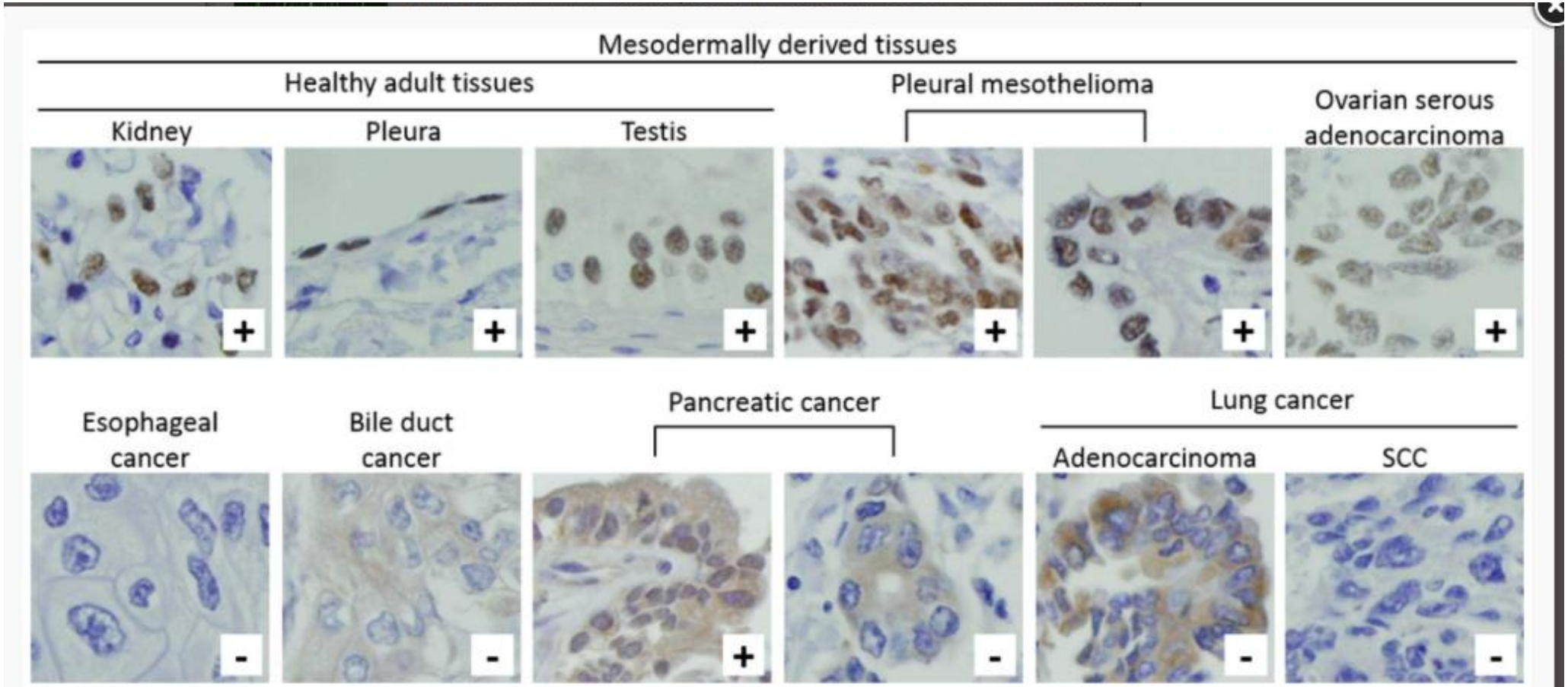
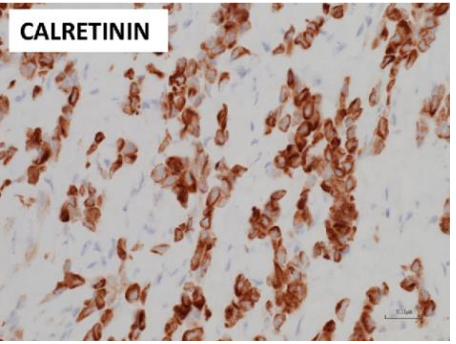
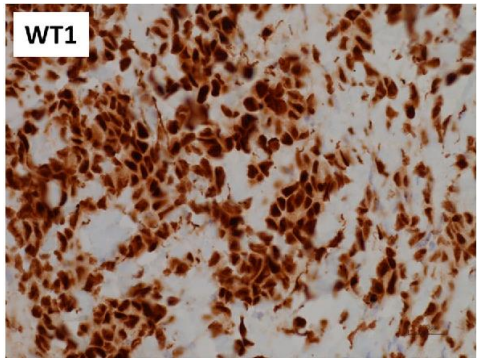


Figure 7 - Nuclear immunostaining by 6F-H2 was barely detectable in four types of solid cancers. Representative immunohistochemical staining of healthy and malignant tissue samples using the 6F-H2 monoclonal antibody. '+' represents positive staining of the entire nucleus; '−' represents negative staining of the entire nucleus. SCC, squamous cell carcinoma.

Peritoneal carcinomatosis CK7+/CK20–

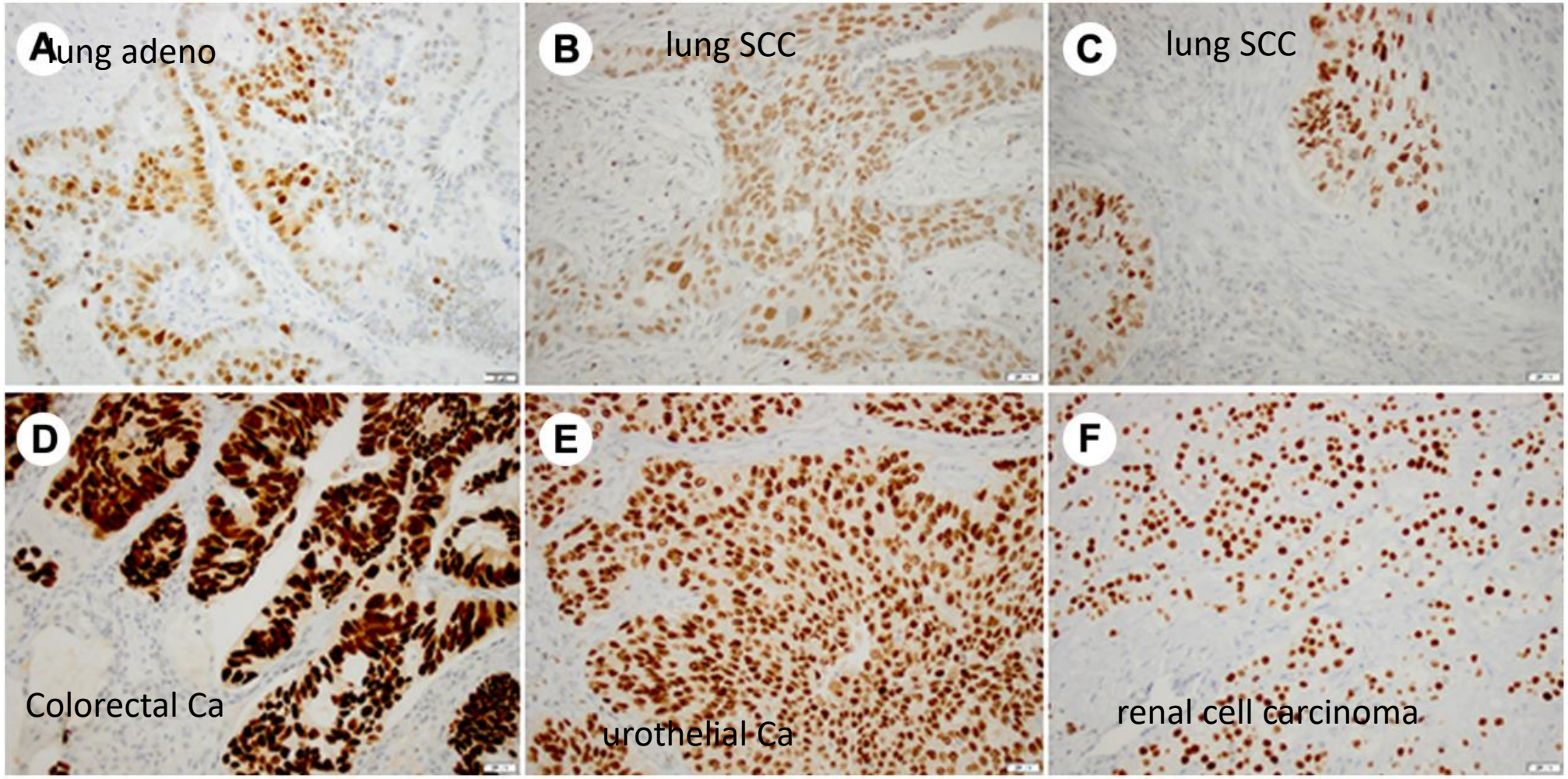


Mesothelial Markers	
Calretinin	Useful. Positive in 85–100% of MPMs. Positivity in 0–38% of PSPCs prevents its use as a single differential marker.
D2-40	Potentially useful. Positive in 93–96% of MPMs but also focal positivity in 13–65% of PSPCs; additional data are needed.
CK5/6	Limited use. Positive in 53–100% of MPMs but also focal positivity in 22–35% of PSPCs.
WT1	Not useful. Positive in 43–93% of MPMs and positive in 89–93% of PSPCs.
PSPCs Markers	
MOC-31	Very useful. Positive in 98% of PSPCs and 5% of MPMs.
PAX8	Very useful. Positive in most Mullerian carcinomas and negative in MPMs.
BG8	Very useful. Positive in 73% of PSPCs and 3–9% of MPMs.
BerEP4	Useful. Positive in 83–100% of PSPCs and in 9–13% of MPMs.
B72.3	Limited use. Positive in 65–100% of PSPCs and focal expression in 0–3% of MPMs.
CEA	Not useful. Positive in only 0–45% of PSPCs and negative in MPMs but sensitivity too low compared to other markers.
Oestrogen receptor	Useful. Positive in 60–93% of PSPCs and 0–8% of MPMs.
Progesterone receptor	Limited use. Lower sensitivity than oestrogen receptors in PSPCs, negative in MPMs. Can be useful when positive.
Mesothelioma vs. Non-Gynaecological Adenocarcinoma (Biliary, Pancreas, Stomach, Colon)	
Calretinin	Very useful. Positive in 85–100% of MPMs but also positive in 10% of pancreatic ADCs, limited value as single marker.
WT1	Very useful. Positive in 43–93% of MPMs, 3% of gastric ADCs and 0% of pancreatic ADCs.
D2-40	Potentially useful. Positive in 93–96% of MPMs, negative in gastric and pancreatic ADCs (limited data).
CK5/6	Not useful. Positive in 53–100% of MPMs and 38% of pancreatic ADCs.
MOC-31	Very useful. Positive in 5% of MPMs and 87% of ADCs.
BG8	Very useful. Positive in 3–9% of MPMs and 89% of ADCs.
CEA	Very useful. Negative in MPMs and positive in 81% of ADCs.
B72.3	Very useful. Positive in 0–3% of MPMs, 84% of pancreatic ADCs, 89% of biliary ADCs, 98% colon ADCs.
BerEP4	Useful. Positive in 9–13% of MPMs et >98% pancreatic and gastric ADCs.
CDX2	Useful. Positive in 90–100% of colon ADCs, 80% small intestine ADCs, 70% of gastric ADCs and negative in MPMs.



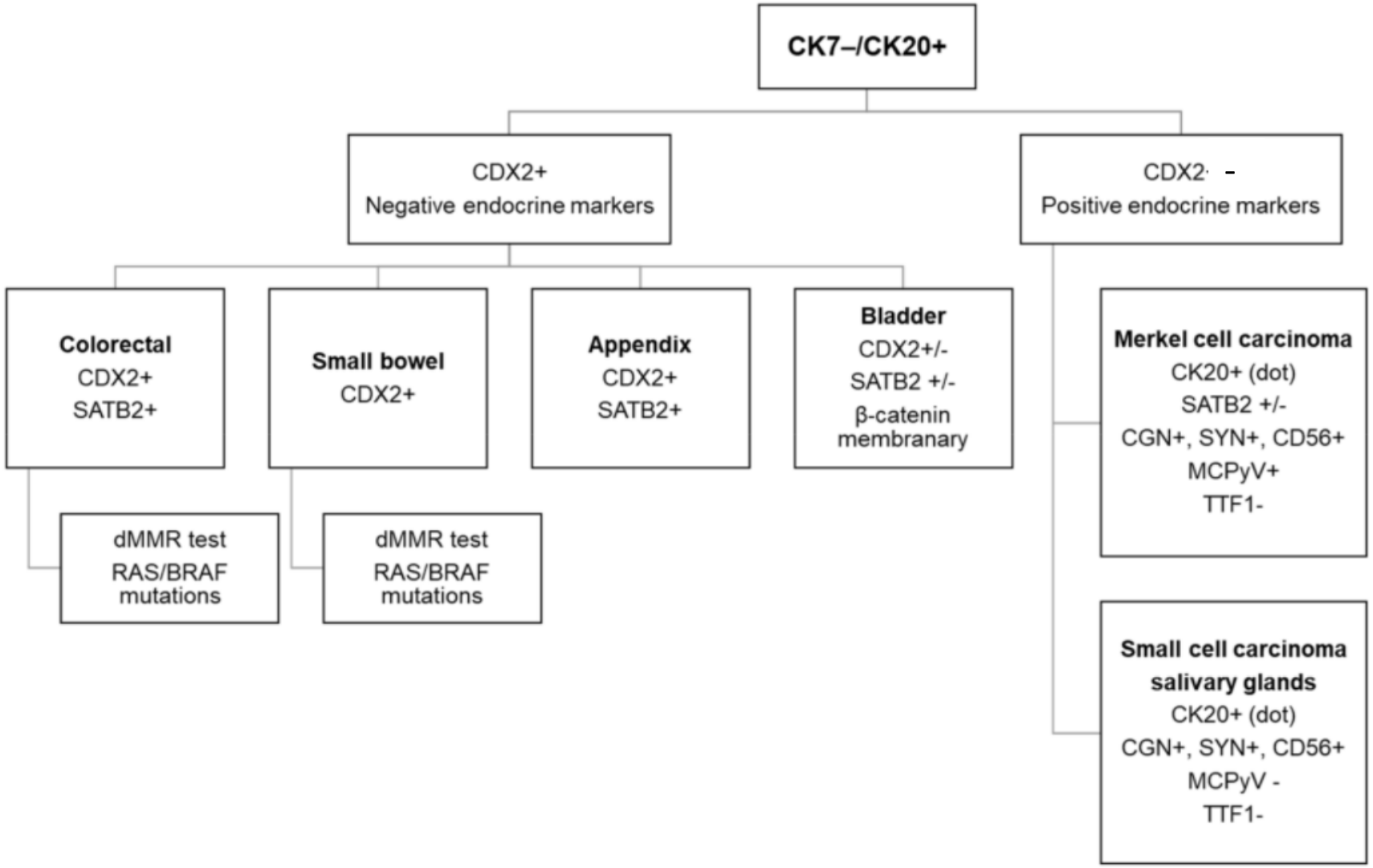
Selves et al, Cancers 2018, 10, 108

Diagnostic Workflow of CK7+/CK20– CUPs



Vidarsdottir et al, Human Pathology, 84, 2019, 221-230

Diagnostic Workflow of CK7-/CK20+ CUPs



Selves et al, Cancers 2018, 10, 108

Diagnostic Workflow of CK7-/CK20+ CUPs

TABLE 4. Expression of SATB2 in Carcinomas From Various Primary Sites

Primary Site	Positive Cases (%)
Colorectal and appendiceal	80-100
Renal	25-35
Gastroesophageal	10-20*
Pancreatic/biliary	10-20*
Mullerian	10-20*
Pulmonary	10-20*
Bladder: urothelial carcinoma	10-20*
Prostatic	5-15*
Breast	5-15*
Thyroid	< 5

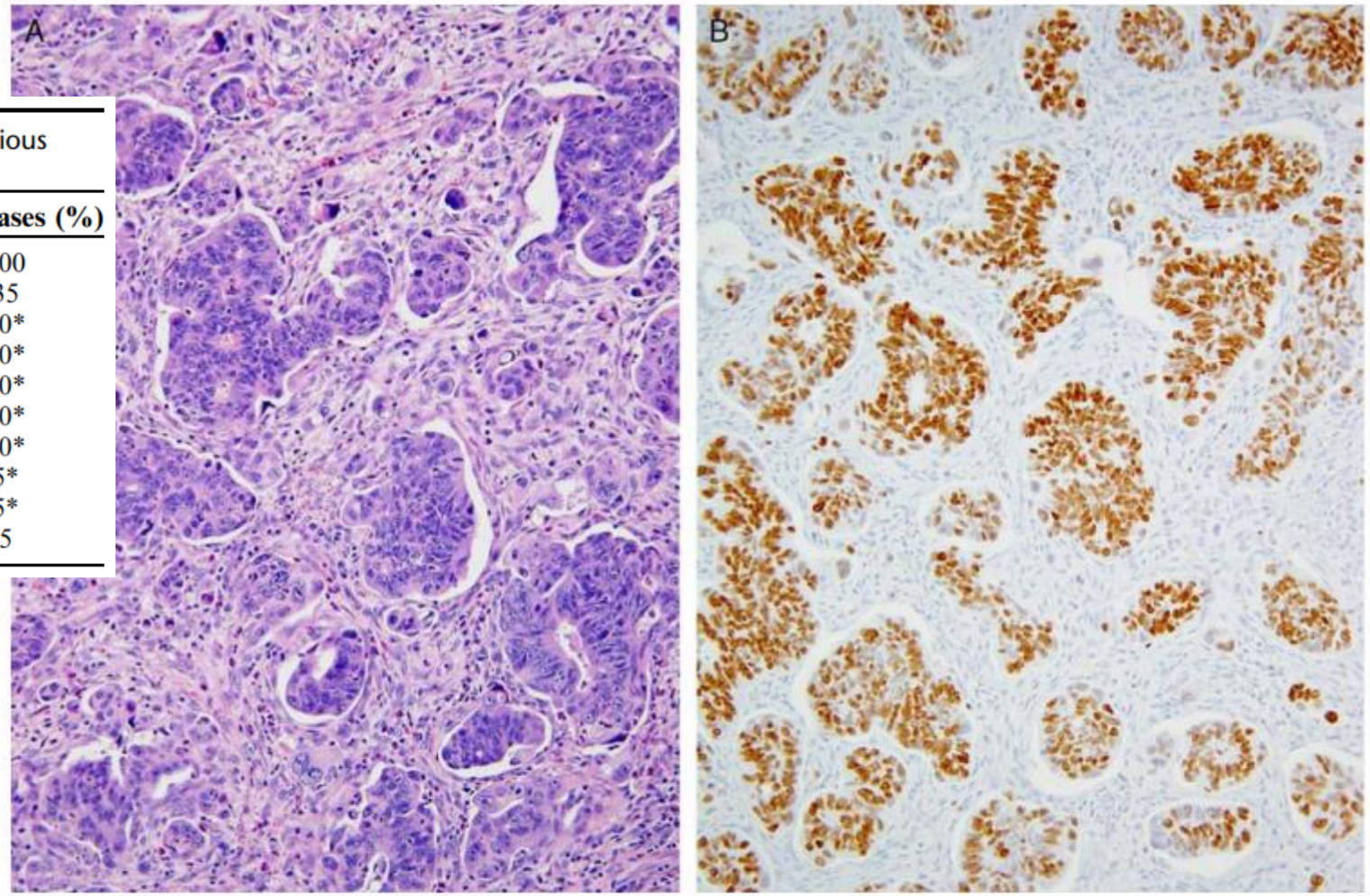


FIGURE 6. SATB2 in colorectal adenocarcinoma. A poorly differentiated colonic adenocarcinoma (A: hematoxylin and eosin, ×400) shows diffuse, strong nuclear staining for SATB2 (B: immunoperoxidase, ×400).

Conner and Hornick, Adv Anat Pathol 2015;22:149–167

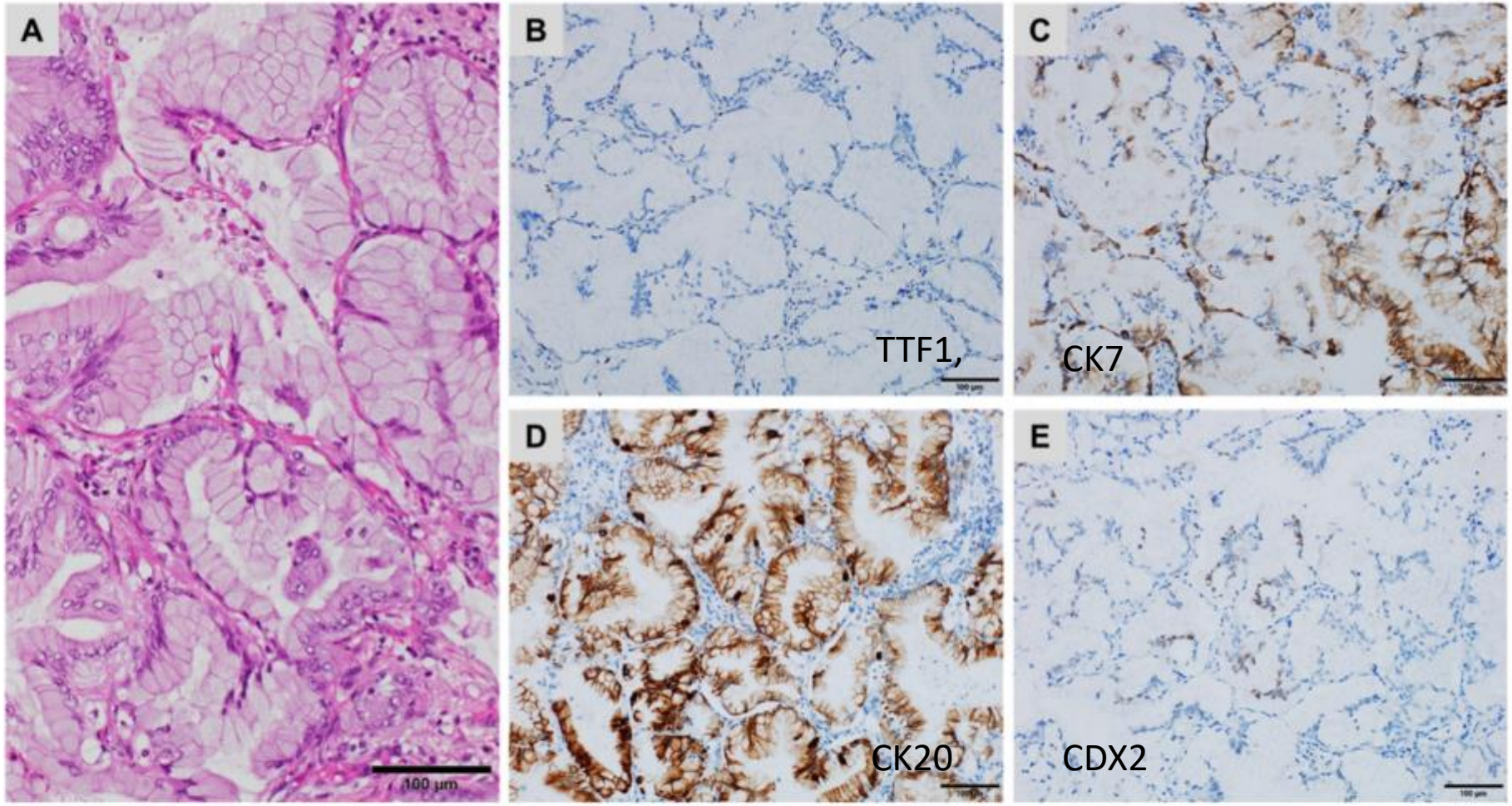
Diagnostic Workflow of CK7+/CK20+ CUPs

Table 4. IHC tumour staining patterns in the differential diagnosis of CUPs expressing CK7+/CK20+ [1,7].

Primary Origin Site	Immunostaining Profile
Lung (mucinous) [49]	TTF1−/+, CK7−/+, CDX2−/+
Pancreas [26,27,50,51]	Maspin A+, S100P+, IMP-3+, pVHL−, SMAD4−/+, MUC5AC+, CDX2−/+
Stomach [26,27,50,51]	CEA+, CDX2−/+, MUC1−/+, MUC5AC−/+, CDH17+/−, TTF1−
Oesophagus [26,27,50,51]	CEA+, MUC5AC+/−, CDH17+, MUC1−/+, CDX2−/+
Ovary (mucinous) [7,52]	DPC4+, CA-12.5+, CDX2+/−
Urinary bladder [17–21]	GATA3+, p63+, p40+, CK5/6+, CK20+/−, S100P+, CK903+, UPIII+/−
Small intestine [39]	CDX2+, CDH17+, Villin+/−, MUC5AC+/−
NUT midline carcinoma [53–55]	CK7+/−, CK20+/−, p40+/−, NUT

Selves et al, Cancers 2018, 10, 108

Diagnostic Workflow of CK7+/CK20+ CUPs

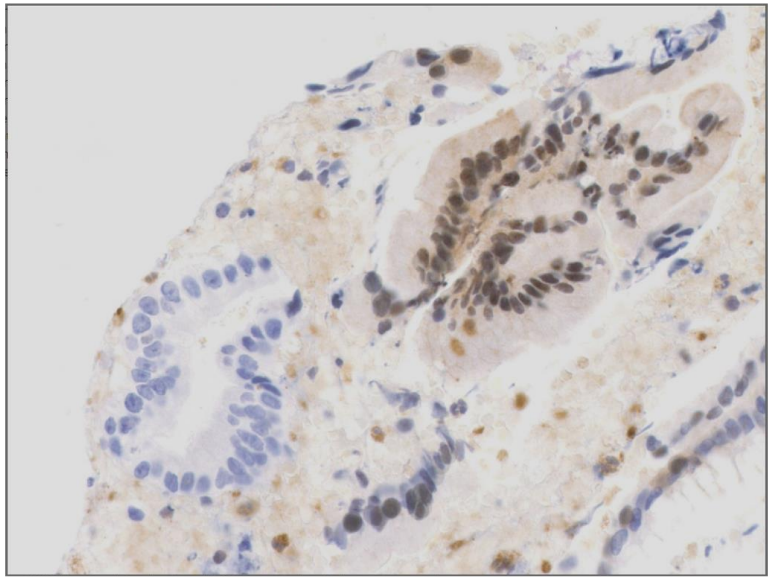


Primary mucinous pulmonary adenocarcinoma

Diagnostic Workflow of CK7+/CK20+ CUPs

Table 4. Ancillary Tests for Distinguishing Nonneoplastic Duct Epithelium and PDAC

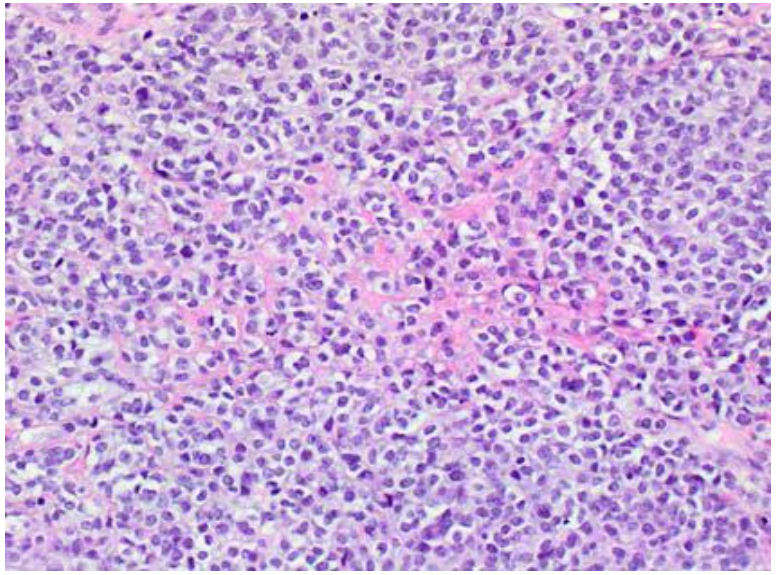
Antibody or Genetic Mutation	Pattern in PDAC	Comment
KRAS mutation analysis	Mutations identified	Also occur in PanIN
P16	Loss of nuclear expression	Occurs in PanIN
P53	Strong nuclear expression in 60%	Strong nuclear expression or total loss (null pattern)
SMAD4/DPC4	Loss of expression in 50%-60%	
CK17	Cytoplasmic expression	
S100P	Nuclear or nuclear and cytoplasmic	Also expressed in gastric epithelium
IMP3	Cytoplasmic expression	Weaker and less diffuse than maspin and S100P; may be focal or patchy
Maspin	Nuclear or nuclear and cytoplasmic	Also identified in PanIN; expressed in gastric mucosa and duodenal epithelium
Mesothelin	Cytoplasmic	May be expressed in nonneoplastic ducts and may be focal in PDAC
pVHL	Weak to absent membranous expression	Strong membranous expression in normal epithelium

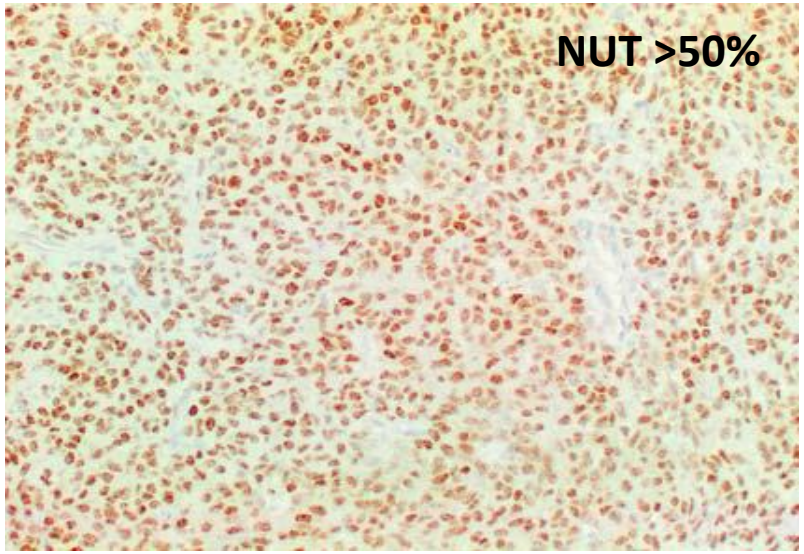


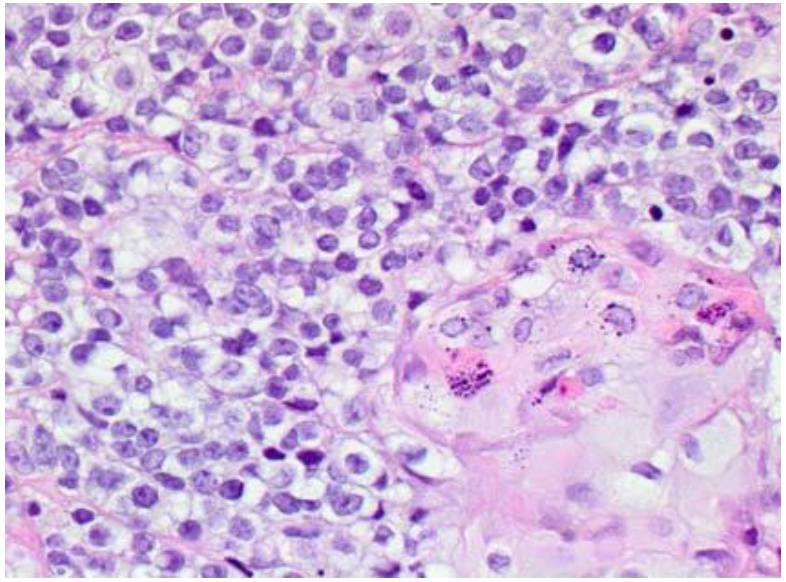
Pancreatic ductal adenocarcinoma shows absent SMAD4/DPC4 expression, whereas (*Right*) the reactive duct epithelium in the same specimen shows preserved nuclear expression.

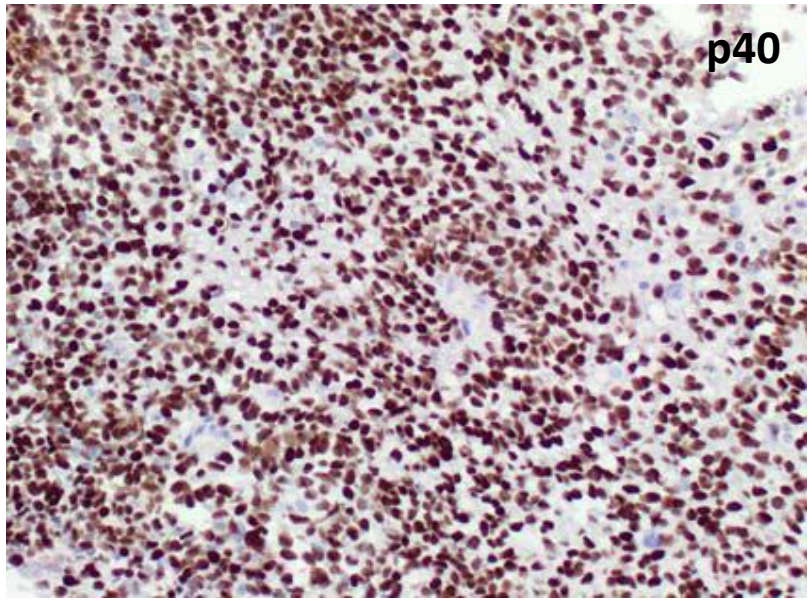
Diagnostic Workflow of CK7+/CK20+ CUPs

NUT midline carcinomas









Diagnostic Workflow of CK7-/CK20- CUPs

Table 5. IHC tumour staining patterns in the differential diagnosis of CUPs expressing CK7−/CK20− [1,7].

Primary Site	IHC Profile
Prostate [58–62]	PSA+, NKX3.1+/−, PSAP+, P504S+, ERG+/−
Colon (medullary) [29,35,36]	SATB2+, CDH17+, TFF3+/−, Calretinin+/−, CDX2−/+
Renal [21,63]	CD10+, PAX8+, Vimentin+, pVHL+, RCCMa+, Inhibin−, TTF1−, CEA−
Liver [64]	HepPar1+, CD10+, pCEA+, mCEA−, AFP+, Glypican-3+, Arginase-1+, CK19−
Adrenal (cortical) [4,7,10]	Melan A+, Calretinin+, Inhibin A+, Synaptophysin+, Chromograni−, CEA−
Germ cell tumours [4,7,10]	CD117+, OCT4+, CD30+, Glypican-3+, PLAP+, SALL4+, NANOG+

Diagnostic Workflow of CK7-/CK20- CUPs

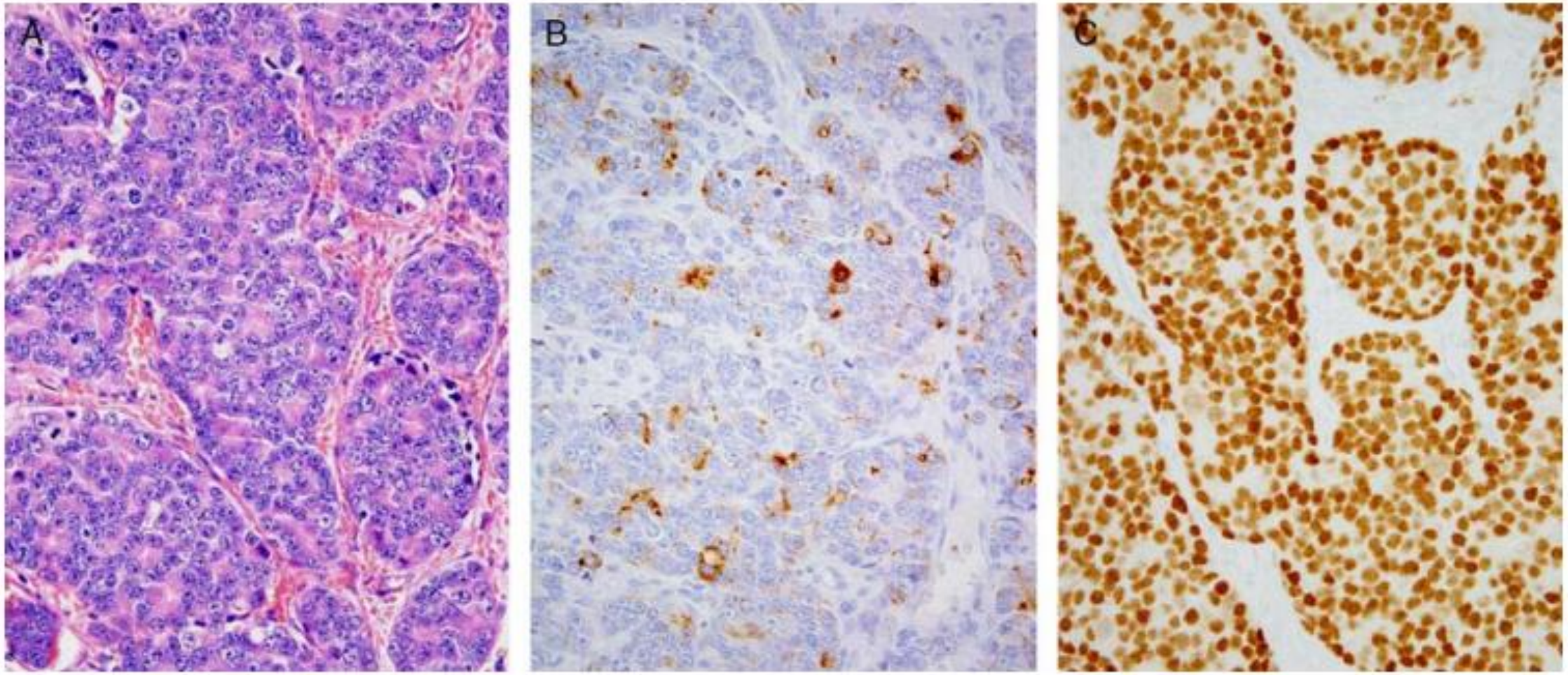
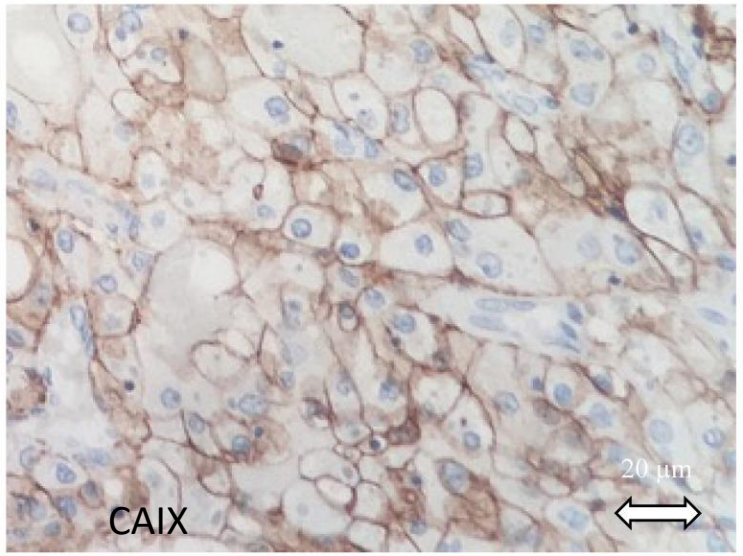


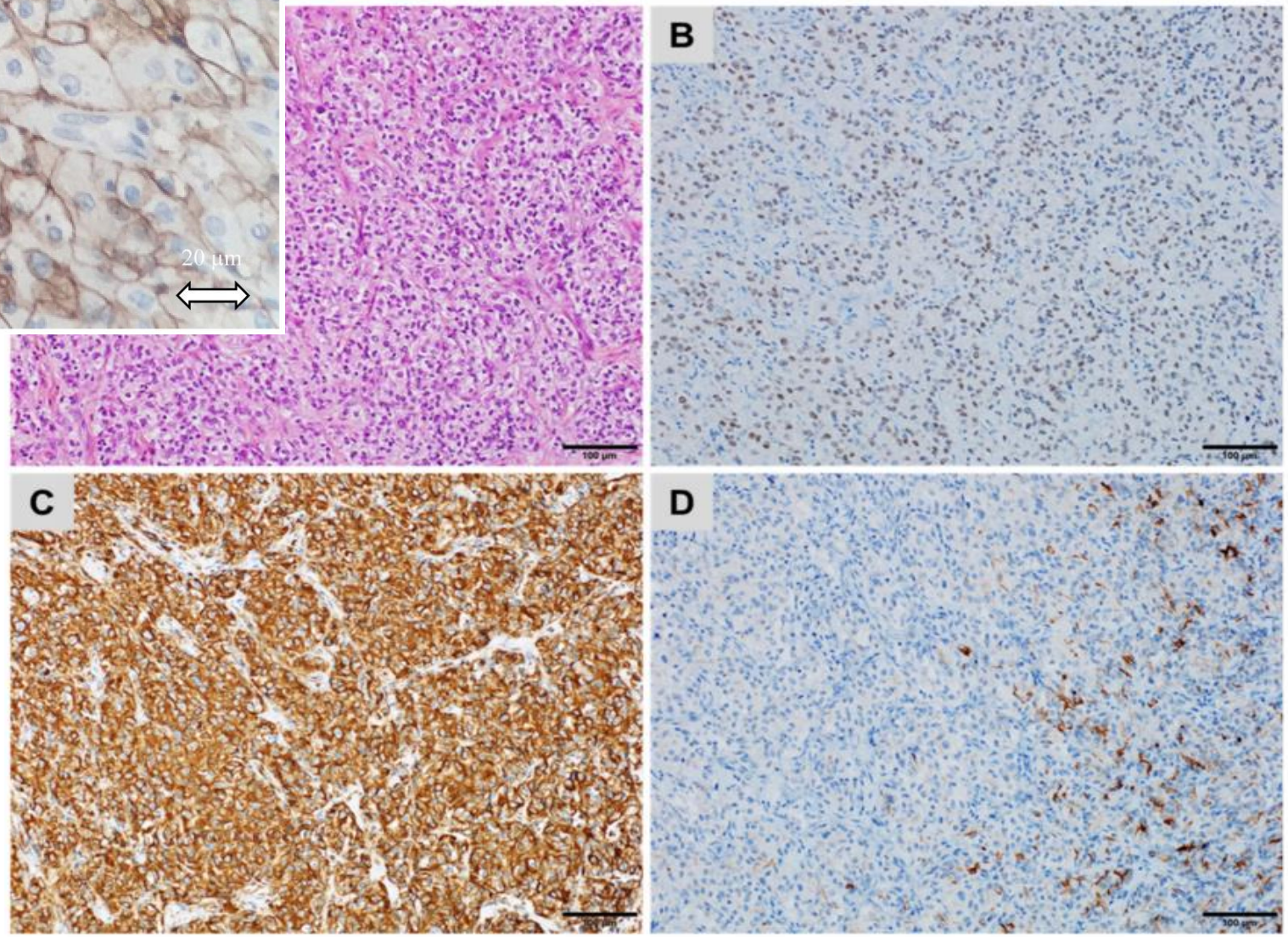
FIGURE 4. PSA and NKX3-1 in prostatic adenocarcinoma. A metastatic high-grade prostatic adenocarcinoma (A: hematoxylin and eosin, ×400) shows cytoplasmic staining for PSA in a small subset of cells (B) and diffuse, strong nuclear staining for NKX3-1 (C) (B and C: immunoperoxidase, ×400).

Diagnostic Workflow of CK7-/CK20- CUPs



CAIX

Metastatic renal cell carcinoma



PAX8

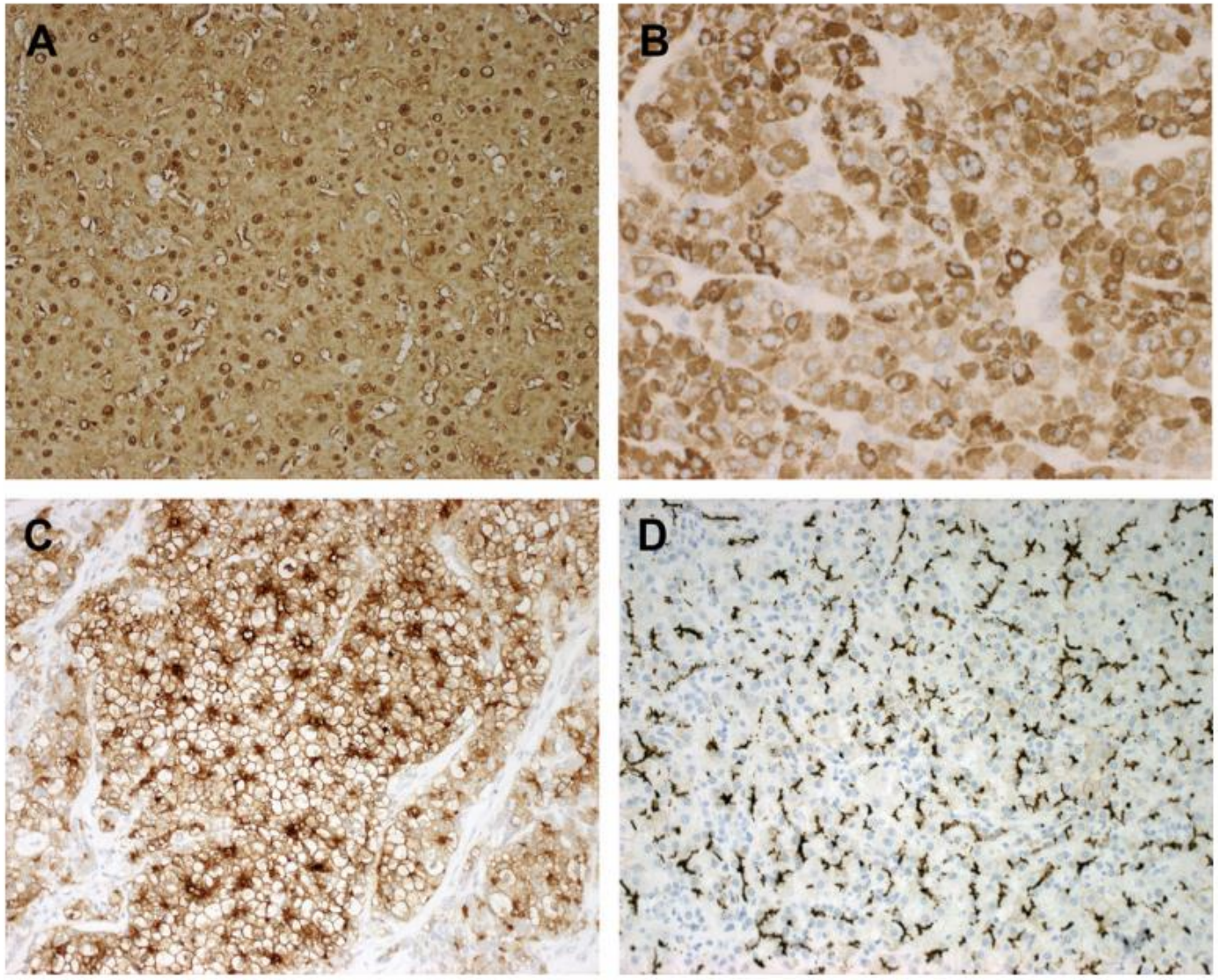
Vimentin

CD10

Diagnostic Workflow of CK7-/CK20- CUPs

Hepatocellular carcinoma (HCC)

Arg-1



Hep Par-1

Also: 1-19% of lung, pancreas, stomach, ovaries, and adrenal cortex

GPC-3

pCEA

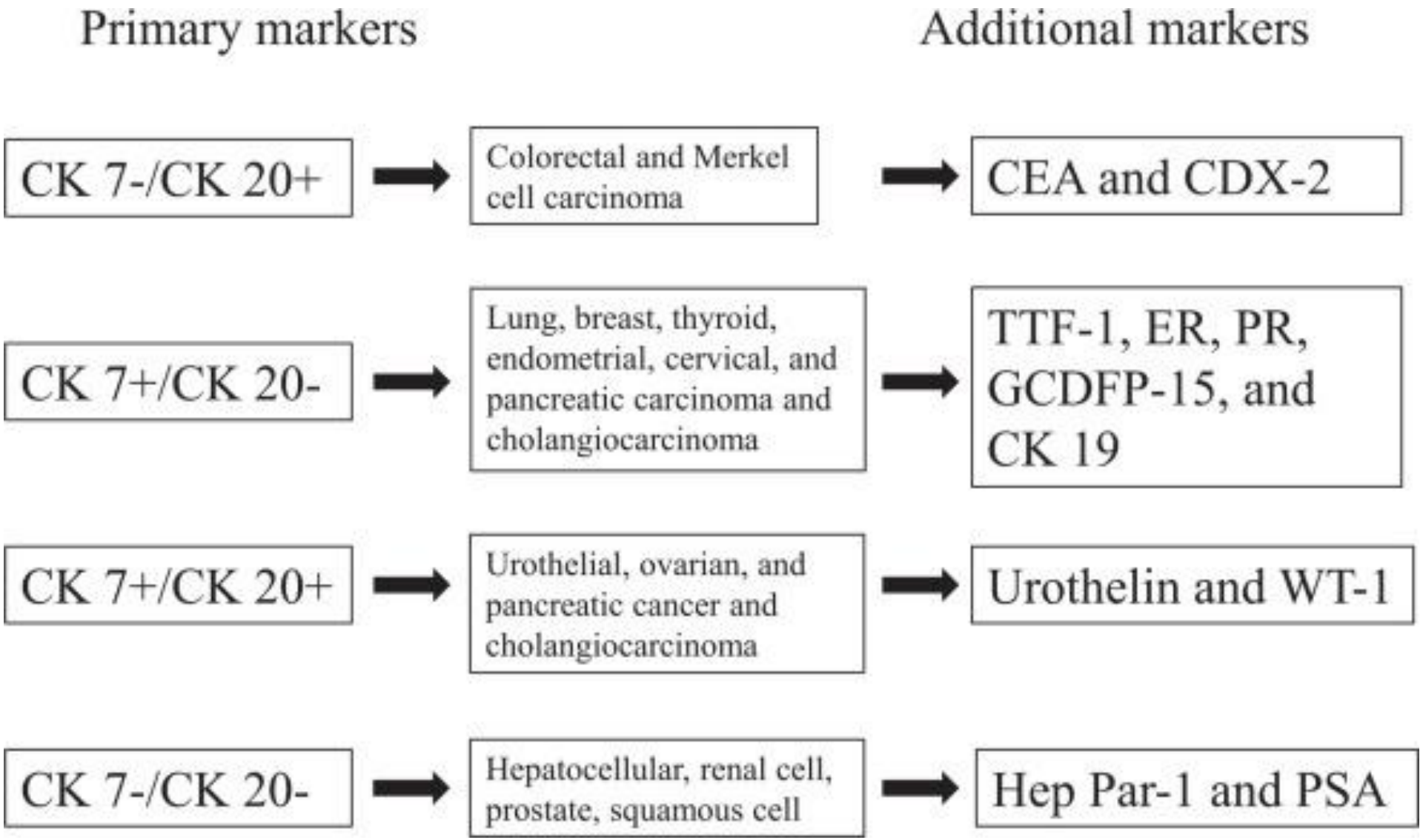
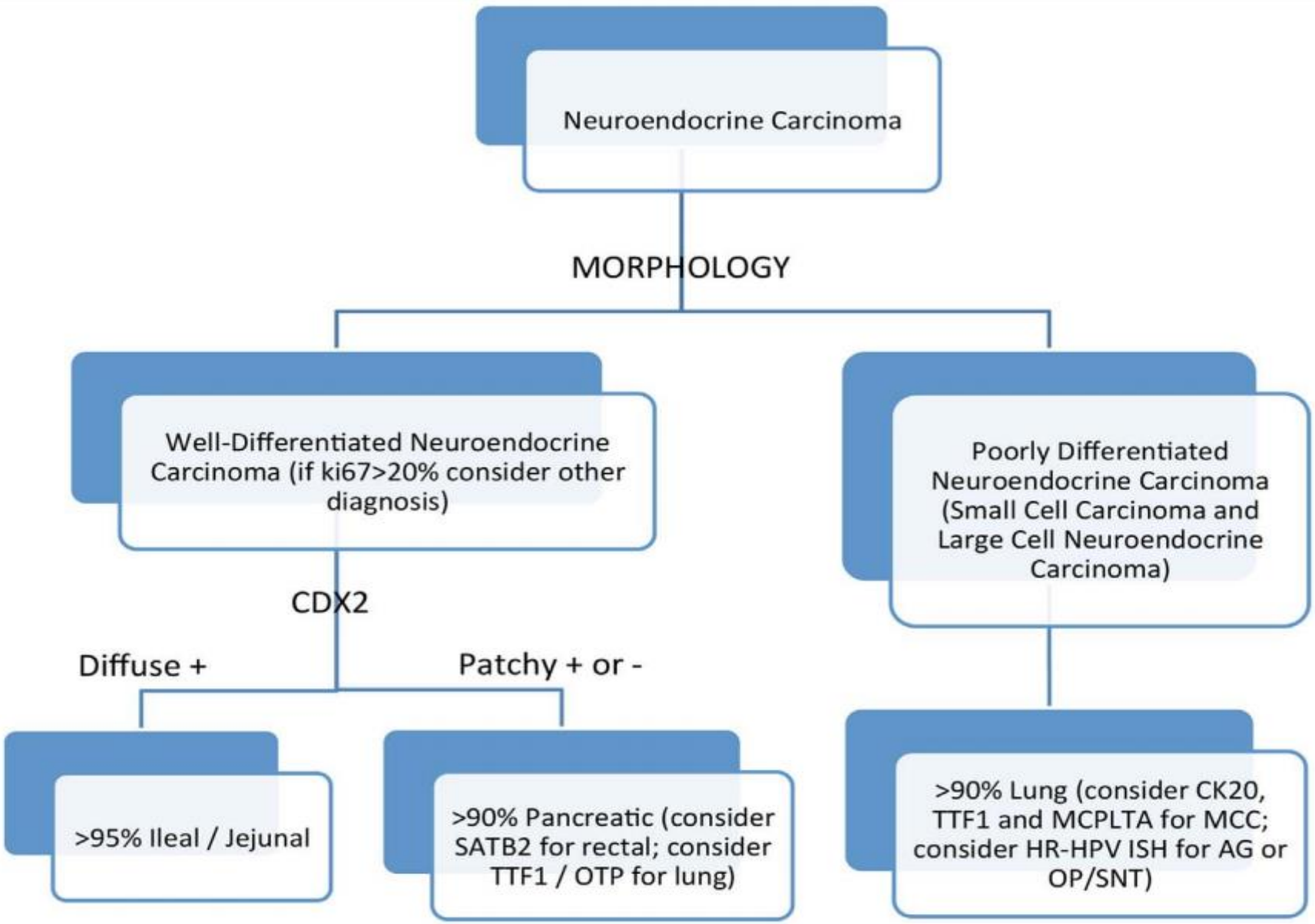

Primary markers
Additional markers
CK 7-/CK 20+
Colorectal and Merkel cell carcinoma
CEA and CDX-2
CK 7+/CK 20-
Lung, breast, thyroid, endometrial, cervical, and pancreatic carcinoma and cholangiocarcinoma
TTF-1, ER, PR, GCDFP-15, and CK 19
CK 7+/CK 20+
Urothelial, ovarian, and pancreatic cancer and cholangiocarcinoma
Urothelin and WT-1
CK 7-/CK 20-
Hepatocellular, renal cell, prostate, squamous cell
Hep Par-1 and PSA

Table 1 **Modal distribution of cytokeratins CK7 and CK20 in some of the types of carcinomas most frequently associated with bone metastases.**

Types of carcinoma	CK7	CK20
Lung adenocarcinoma	+	–
Ductal carcinoma of the breast	+	–
Endometrial adenocarcinoma	+	–
Prostate adenocarcinoma	–	–
Renal cell carcinoma	–	–
Colorectal adenocarcinoma	–	+

Endocrine CUPs



E.B. Stelow, H. Yaziji, Seminars in Diagnostic Pathology, 2018

Squamous Cell CUPs

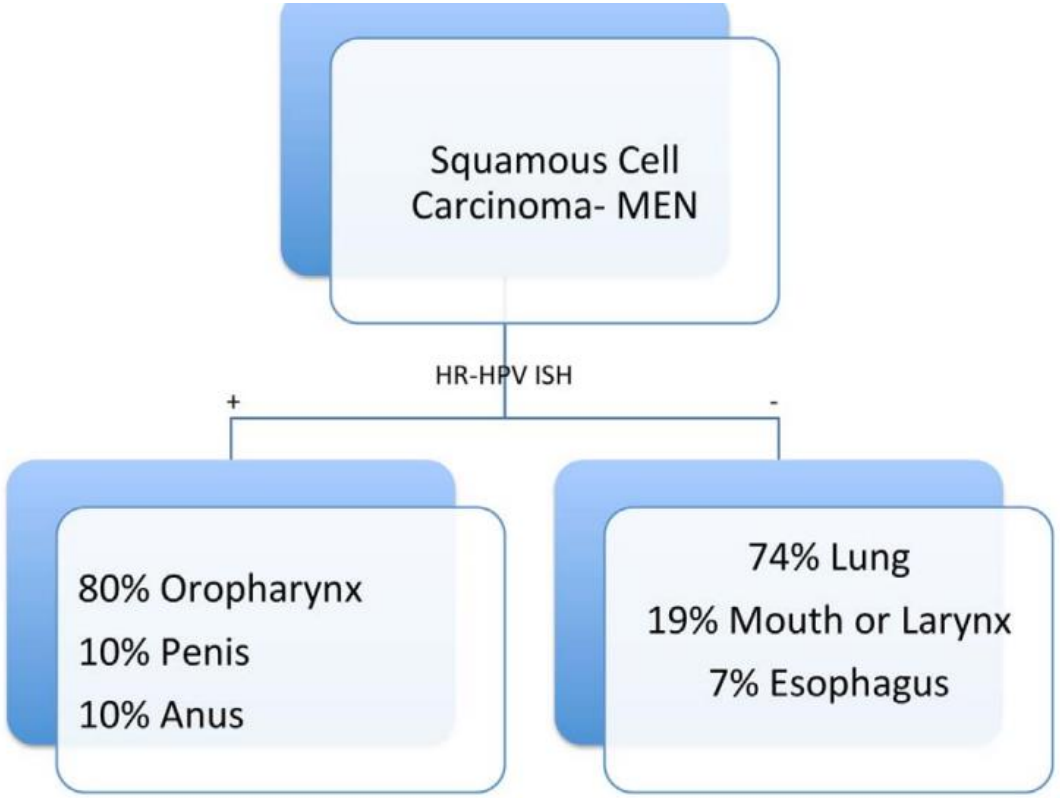


Fig. 2. Use of HR-HPV ISH with squamous cell carcinoma in men based on incident mortality rates.

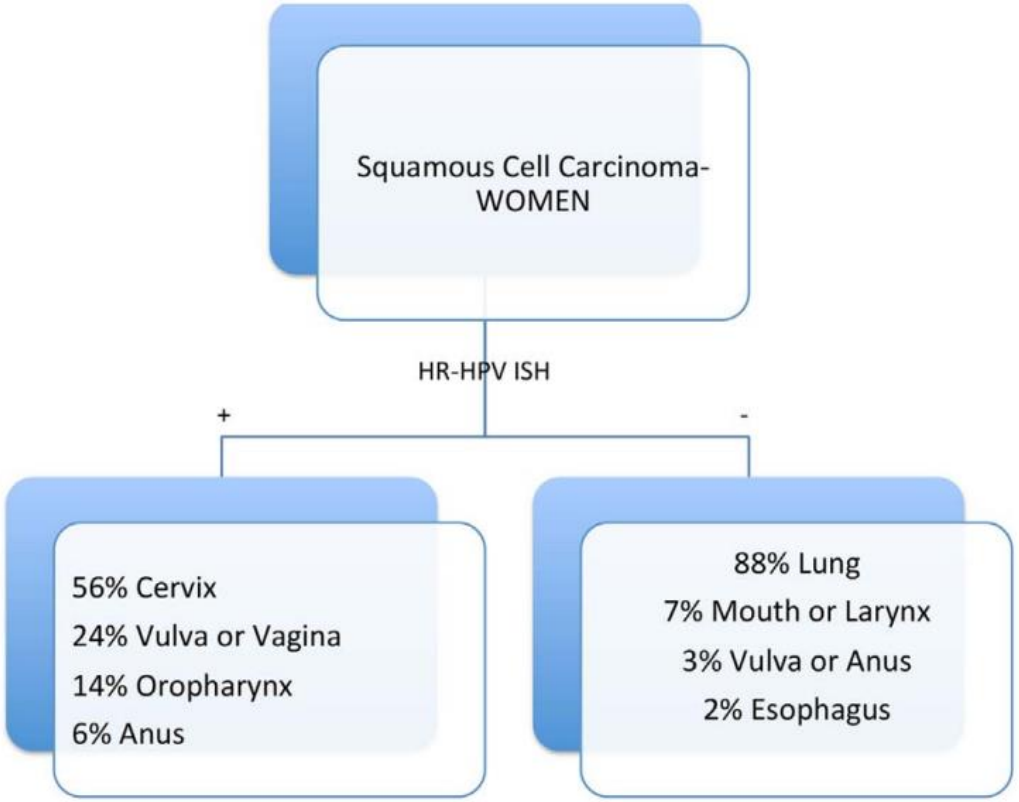


Fig. 3. Use of HR-HPV ISH with squamous cell carcinoma of women based on incident mortality rates.

E.B. Stelow, H. Yaziji, Seminars in Diagnostic Pathology, 2018

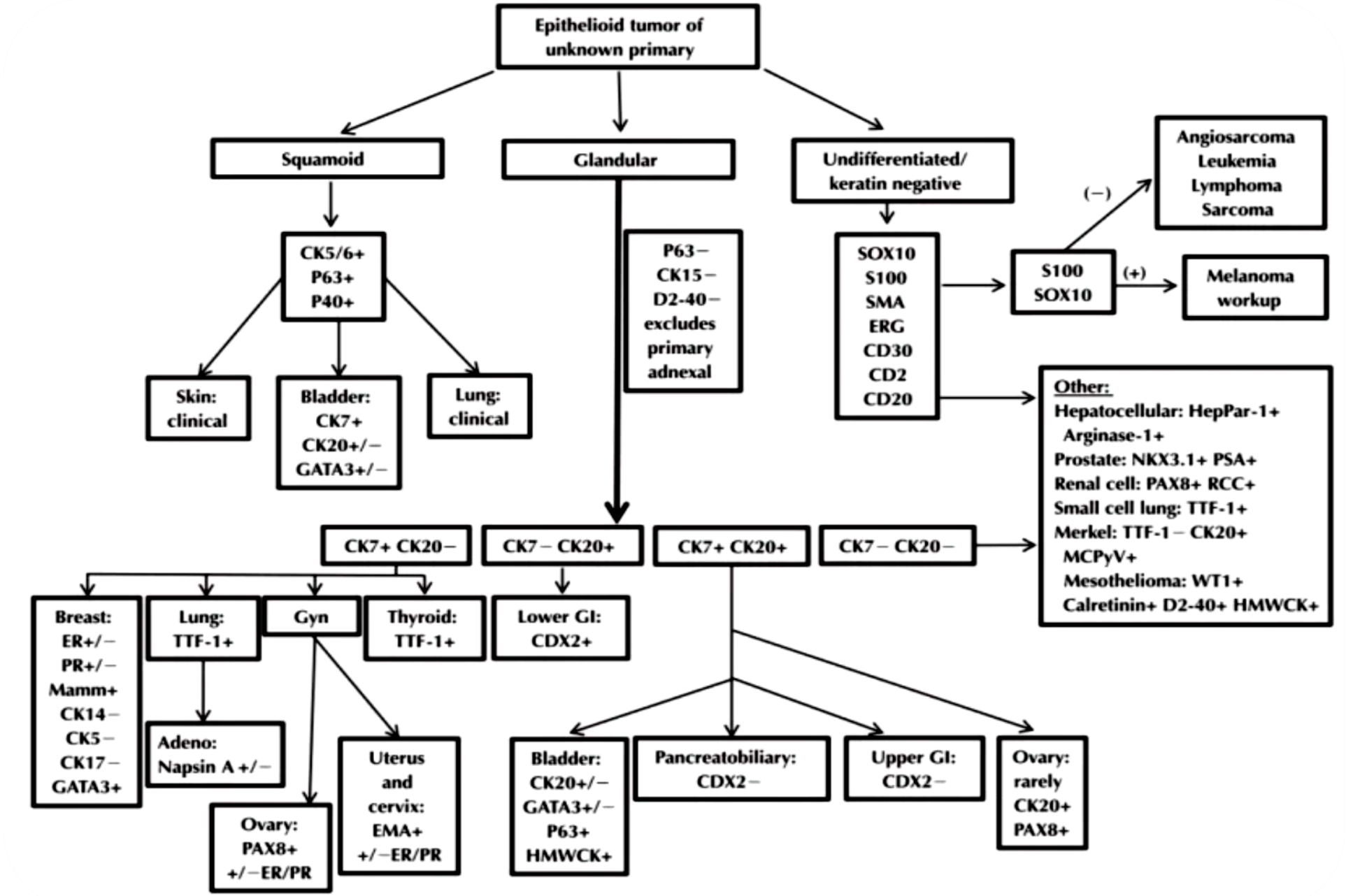
Squamous/*urothelial* **Cell CUPs**

- Not site-specific markers
- High specificity and sensitivity for SCC when used in conjunction
- P63, p40, CK5
  - Also *breast, ovarian, pancreatic, and endometrioid carcinomas (50%)*
  - *Myoepithelial differentiation* (eg, adenoid cystic and other salivary gland carcinomas)
  - *Trophoblastic differentiation*

Table 6. Tumour-specific markers useful for the diagnosis of various biphasic connective tumours.

Type of Tumour	IHC Markers
Synovial sarcoma [83,84]	AE1/AE3+/−, EMA+/− (epithelial cells), S100+ (spindle cells), CD34+
Dedifferentiated liposarcoma [85–87]	MDM2+, CDK4+, HMGA2+
Mixed tumour/myoepithelioma of soft tissue [88,89]	AE1/AE3+, EMA+, S100+, SMA+/−, p63+/−, GFAP+/−, INI-1−
MPNST [90,91]	S100+, AE1/AE3−, EMA− (spindle cells)
Hamartomatous ectopic thymoma [92]	AE1/AE3+, CK5/6+, CK14+, SMA+, CD34+ (spindle cells), Desmin−, S100−
Biphasic mesothelioma(epithelial component) [31,32]	AE1/AE3+, CK5/6+, Calretinin+, WT1+
Germ cell tumours [92]	AE1/AE3+, SALL-4+, CD117+, OCT-4+
Malignant mixed Mullerian tumours (“carcinosarcoma”) [93]	AE1/AE3+, PAX8+, WT1+Sarcomatoid components: Desmin+, Myogenin, Caldesmon+, S100+

CUP: Systemic diagnostic approach

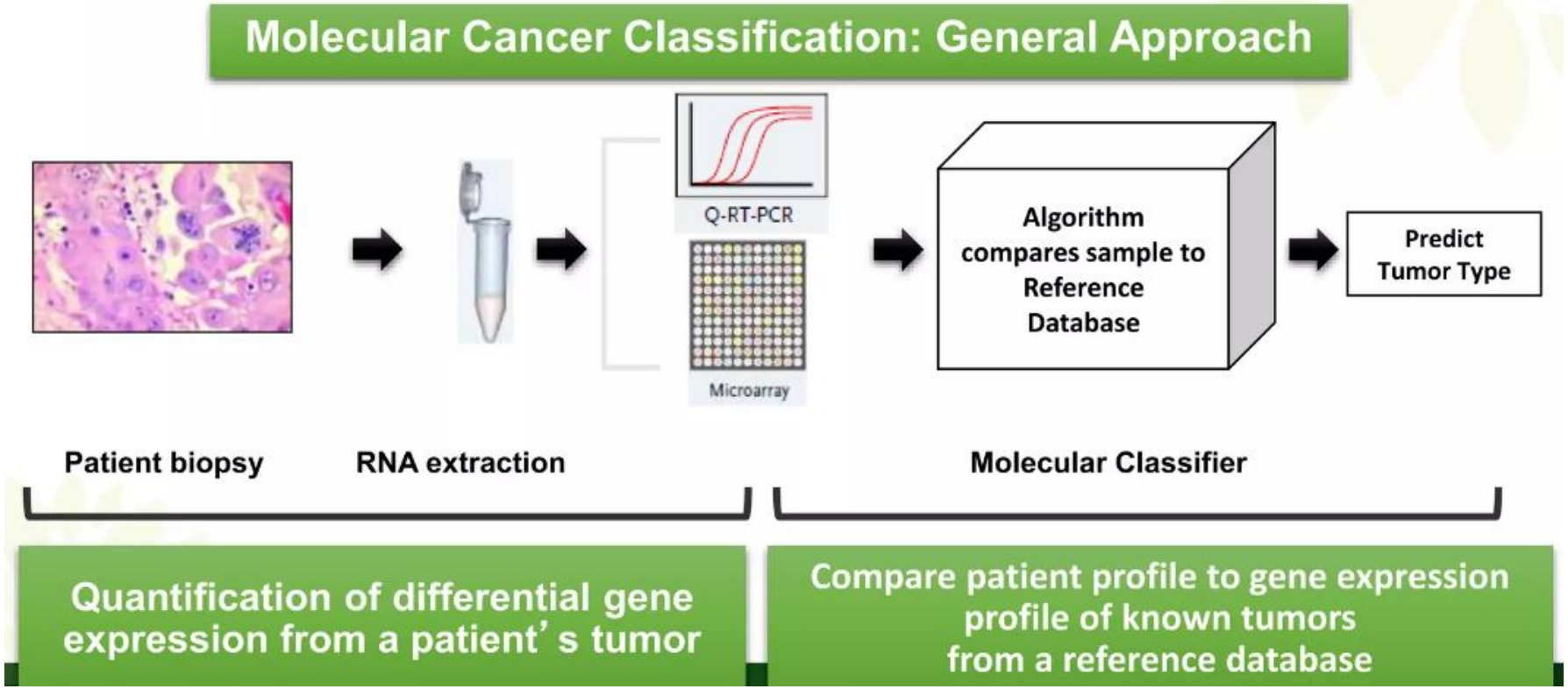


Complementary Tools for Diagnosis in CUPs

- Several gene signatures using **reverse transcriptase polymerase chain reaction (RT-PCR)** or a **microarray-based gene expression analysis** have been proposed
- These assays were validated on tumour series of known primary origin and now allow determination of sites of origin for CUPs by analysing the rate of concordance of the genomic profile with that of a metastasis of known origin.
- A 92-gene real-time RT-PCR assay (CancerTYPE ID®; bioTheranostics, Inc., San Diego, CA, USA) can identify the primary cancer for metastases of known origin in 85% of cases and for CUPs for which the primary origin was found in 15/20 cases (75%) In a prospective series of 247 CUPs, 98% of cases had a tissue of origin predicted by the assay
- European randomized, phase III, multi-centric trial GEFCAPI04 (Clinicaltrials.gov/NCT01540058)
    - The purpose of this trial is to determine whether or not a strategy based on molecular analysis is effective in improving the progression free survival rates of patients with CUPs. The CancerTYPE ID is performed on formalin-fixed paraffin-embedded (FFPE) tumour specimens with at least 300 tumour cells (excluding decalcified bone samples).
- A classifier of cancer types based on the microarray DNA methylation signature (EPICUP®, Bellvitge Biomedical Research Institute, Barcelona, Spain)

Molecular Analysis: General Approach

- In recent years, molecular cancer classification has emerged as a standardized, objective technique to help identify tumor type in patients with CUP
- Concept: neoplasms retain gene expression profile based on cellular origin; this profile can be exploited to identify tumor type



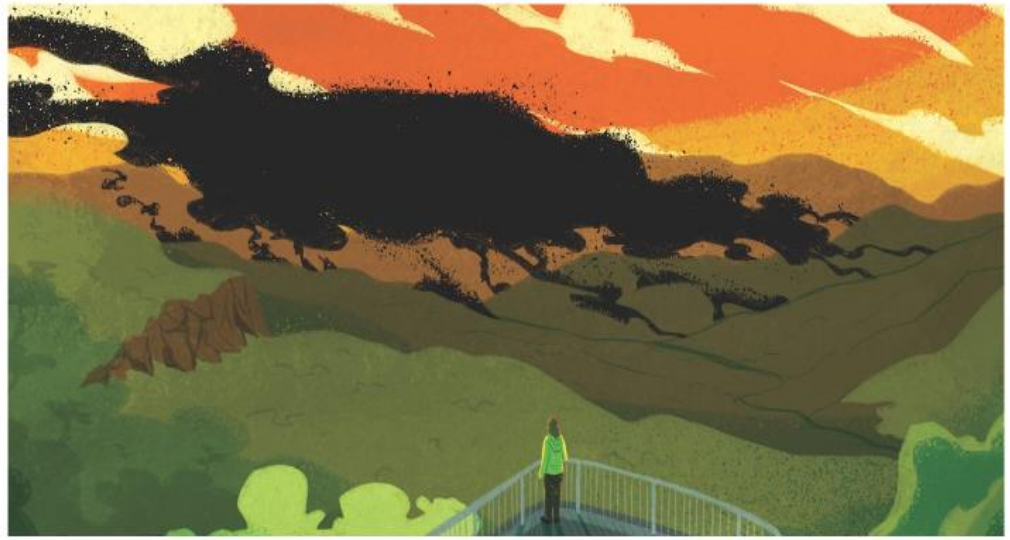
GENE EXPRESSION PROFILING

ASSAY	PLATFORM	TISSUE	NO. OF TUMOR TYPES	NO. OF GENES	ACCURACY IN KNOWN TUMORS (%)
1.) Veridex	RT-PCR	FFPE	6 and "other"	10	76
2.) Pathwork Diagnostics Tissue of Origin Test	cDNA microarray	Frozen / FFPE	15	1500	89
3.) Rosetta Genomics MiReview met	RT-PCR miRNA	FFPE	22	48 miRNAs	86
4.) bioTheranostics CancerType ID	RT-PCR mRNA	FFPE	39 (including subypes)	92	86

Complementary Tools for Prediction of Treatment Response in CUPs

- A next-generation sequencing analysis (FoundationOne®, Foundation Medicine, Cambridge, MA, USA) of 237 genes showed that 96% of CUPs harboured at least one genetic alteration. Of these, about 85% had an alteration that could be used to guide the treatment.
- The extended diagnostic requirement of increasingly limited material provided by minimally invasive biopsy techniques and the cost-effectiveness of the DNA-based assays are major challenges for pathology departments.
- There is a need for an efficient diagnostic screening algorithm method of molecular alterations using IHC.
- IHC may provide an efficient screening tool for “druggable” genomic alterations by taking advantage of a growing list of available mutation-specific antibodies (i.e., ALK rearrangements, D5F3 and 5A4 clones; ROS1 rearrangements, clone D4D6; BRAF V600E mutation, clone VE1; EGFR exon 19 E746_A750del, 6B6 clone; EGFR exon 21 L858R mutation, 43B2 clone) [78,79].
- Recently, PD-1 and PD-L1, detected by IHC, have been identified as immune therapy biomarkers in various solid malignancies including CUPs, which may open up an unexplored avenue for treatment with anti-PD-1/PD-L1 antibodies in these patients
- One of the main causes of technical failure (15% to 25%) of molecular analyses is the low number of tumour cells in biopsies

Do we still need to know or have an idea of where this has come from???



Confirmed CUP - treat as per clinical subtype

Favourable subtype	
Subtype	**Treat as analogous tumour**
Poorly differentiated NE carcinomas of unknown primary	Poorly differentiated NE carcinomas with known primary
Well-differentiated NE tumour of unknown primary	Well-differentiated NE tumour of known primary site
Serous papillary peritoneal adenocarcinomas (female)	Ovarian cancer
Female patient with isolated axillary nodal metastasis	Breast cancer
SCC of non-supraclavicular cervical lymph nodes	Head/neck SCC
CUP with a colorectal IHC (CK20+, CK7–, CDX2+)	Metastatic colorectal cancer
Solitary metastatic deposit of unknown primary	Single metastasis
Blastic bone metastasis; IHC/serum PSA expression (male)	Prostate cancer

Unfavourable subtype	
Example	**Treatment**
Adenocarcinoma metastatic to liver or other organs	Doublet platinum-based chemotherapy (PS 0-1 and normal LDH) vs best supportive palliative care
Non-papillary malignant ascites (adenocarcinoma)	
Multiple cerebral metastases (adenocarcinoma or SCC)	
Multiple lung/pleural metastases (adenocarcinoma)	
Multiple metastatic bone metastases (adenocarcinoma)	

In conclusion

- Immunohistochemistry is an efficient and cost-effective approach to identifying site of origin in CUP.
- It is accessible to most anatomic pathologists and can be performed on relatively limited amounts of formalin-fixed paraffine mbedded tumor tissue.
- The lineage-specific transcription factors described above have proven to be particularly sensitive and specific markers, even in many cases of histologically poorly differentiated carcinomas. Many other emerging markers hold exciting promise as new immunohistochemical targets.
- Only by exercising a systematic approach to working up CUPs can an accurate specific diagnosis be reached. A systematic approach is also required to preserve tissue for potential molecular or other ancillary testing for most cases in the routine practice.
- The proportion of CUP cases in which pathologists can confidently assign primary site will continue to increase.


Thank you for your attention!