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Undifferentiated panel: Ca vs Mel vs La

Keratin AE1/3 mix, 90%+ of Ca

Not CK7 or 20 or 5/6 to start with
EMA, CEA as backups

S100

95%+ of Mel, but 10% of Ca are +
SOX10 may be better, more sensitive and specific
HMB45, MelanA less sensitive, very specific

LCA or CD20 90% of lymphomas

Things that might be negative

Anaplastic large cell lymphoma - use CD30, ALK, CD3

Plasmacytoma – use CD138, kappa, lambda

Sarcomas – use various markers, esp vascular CD31, CD34

Spindled/sarcomatoid carcinoma – use CK5/6 and p63

Liver – use HepPar1

Adrenal – use Inhibin, MelanA, SF1

Seminoma/germinoma – use OCT3/4 or SALL4

Carcinoma – primary site

What is the DDx? Choose complementary antibodies (check the history first)

CK7/20: USE THE TABLES

Most use is for ADENOCARCINOMAS, Little data for undifferentiated Ca

UNKNOWN PRIMARY: immunohistochemistry			
CK7+20+		CK7-20+	
Ovary mucinous	90%	Colorectal adeno	80%
Transitional cell	65%	Merkel cell	70%
Pancreas adeno	65%	Gastric adeno	35%
Cholangio	65%	<u>Excluded tumors</u>	≤ 5%
Gastric adeno	40%	<i>Breast; Carcinoid lung;</i>	
<u>Excluded tumors</u>	≤ 5%	<i>Cholangio; Esoph squam;</i>	
<i>Carcinoid; Germ cell; Esoph squam; Head/neck squam; Hepatocellular; Lung small cell & squam; Ovary non-mucinous; Renal adeno</i>		<i>Germ cell; Lung all types; Hepatocellular; Ovary; Pancreas adeno; Renal adeno; Transitional cell; Uterus endometrioid</i>	
CK7+20-		CK7-20-	
Ovary non-mucinous	100%	Adrenal	100%
Thyroid (all 3 types)	100%	Seminoma & YST	95%
Breast	90%	Prostate	85%
Lung adeno	90%	Hepatocellular	80%
Uterus endometrioid	85%	Renal adeno	80%
Embryonal	80%	Carcinoid GI & lung	80%
Mesothelioma	65%	Lung small cell & squam	75%
Transitional cell	35%	Esoph squam	70%
Pancreas adeno	30%	Head/neck squam	70%
Cholangio	30%	Mesothelioma	35%
<u>Excluded tumors</u>	≤ 5%	<u>Excluded tumors</u>	≤ 5%
<i>Colorectal adeno; Ovary mucinous, Seminoma, Yolk sac tumor</i>		<i>Breast; Cholangio; Lung adeno; Ovary; Pancreas adeno; Transitional cell</i>	

Breast Carcinoma

GATA3 90%, BRST2 (GCDFP15) 60%+, quite specific (salivary and skin adnexal tumors pos)
 ER 75%+, PR 60%+ (lung most neg to focal/weak but up to 5% strong pos in one report)
 S100 15%+, (lung neg)
 CK7+20- 90% (lung is also 7+20-)
 Mammaglobulin breast 85%, cholangio, GI, lung adenoca 10-20%

ER, PR +

- Breast
- Ovary
- Endometrium
- Papillary thyroid
- Skin adnexal tumors
- Sarcomas
- Meningioma (PR only)
- Solid-pseudopapillary neoplasm of pancreas (PR only)

Breast carcinoma type

E cadherin

Ductal positive, Lobular negative
Works for invasive and in situ

Breast vs Lung Panel

BRST2 (60%) or GATA3 (90%) Breast pos Lung neg
ER, PR favor breast but not sensitive
TTF1 (70%) or Napsin (80%) - Lung pos Breast neg

Metaplastic/Sarcomatoid Carcinoma

CK5/6 about 50%
P63 60%
AE1/3 40%
Smooth muscle actin 70%

Breast invasive vs in situ

Myoepithelial cells absent in invasion
Smooth muscle actin stains myoepithelial cells; myofibroblasts frequently confusing
Calponin cytoplasmic, a bit more specific than actin, easier to interpret at low power than p63
P63 nuclear, most specific but may be harder to see at low power if not strong
Calponin vs p63: personal preference or use both
Occasionally get divergent results
Usually go with the positive

Breast papilloma vs in situ

Calponin and/or p63
Are myoepithelial cells present throughout the lesion?

Lung Adenocarcinoma

CK7+20- 90%, TTF1 70% (also thyroid +), Napsin 80% (also some RCCa +)
CD56 5%, CK5/6 10%, p63 0-25% focal, PAX8 neg

Lung squamous Ca, both basaloid and usual types

CK7-20- 70% (CK7+20- 25%)
TTF1/Napsin neg, CD56 0-10%, PAX8 neg
P63, 34BE12 and CK5/6+ 100%

Lung small cell (oat cell) Ca

CK7-20- 90%, CK20 rare
TTF1 90%, CD56 95%
P63 neg, CK5/6 neg, 34BE12 scattered pos cells 12%, no confluent positive cases
Synaptophysin 50%, Chromogranin 40%
Keratin usually absent to patchy or dot like

Oat vs Merkel cell

TTF1 Oat 90%, Merkel neg
CK20 Oat neg, Merkel 90%
Keratin may be dot like for both
Chromogranin and synaptophysin variable in both
Merkel 35-75% pos for TdT, do not confuse with ALL

Mesothelioma

Reactive vs neoplastic mesothelium:

EMA, IMP3, Glut1, p53: favor mesothelioma if positive

Desmin favors reactive if positive

	Pleural Mesothelioma	Lung Adenoca
Calretinin	90% (nuclear)	15%
CK5/6	90% (Benign mesos freq neg)	10%
D2-40 (podoplanin)	90%	0-7%
WT1	90%	25%
TTF1 (nucl) / Napsin (cytopl)	neg	70-80%
CD15	very rare	95%
CEA	very rare	90%
BerEp4	15%	95%
MOC31	10%	100%
B72.3 (TAG72)	5-15%	85%

Lung vs Mesothelioma Panel

Two or three of each of meso pos and lung pos markers should be enough

WT1 not useful vs lung

D2-40

Kaposi sarcoma 100%

Angiosarcoma 72%

Seminoma 100%

Nonseminomatous germ cell negative

Mesothelioma 90%

Lung adenocarcinoma 0-7%

Various carcinomas 20-40%

Dermatofibroma 100%

	Ovary serous	Peritoneal mesothelioma
Calretinin	7-34%, rare in our studies	100% (nuclear)
CK5/6	17%	100%
Thrombomodulin	30%	74%
PAX8	75-90%	9% (weak)
BerEp4	100%	9%
CD15	60%	neg
S100	27%	neg
B72.3 (TAG72)	80%	neg
MOC31	97%	3%

WT1 stains both (but good for ovary vs breast)

Ovary serous vs mesothelioma panel

BerEp4 Ovary+

MOC31 Ovary+

PAX8 Ovary+

Calretinin Mesothelioma+

GYN

Ovary vs Breast PAX8

Ovary surface epithelial carcinomas 70-100%

Only mucinous 10-59%

Breast negative

Ovary Serous Ca

CK7+20- 100%

WT1 90%

90% mesotheliomas, 25% lung adenoca, 5% breast

Neg: colorectal, endometrial (incl pap serous), panc, bile duct, ovary mucinous

WT1 other reactivity

90% DSRCT

75% Wilms

55% Rhabdo

12% Neuroblastoma

0% PNET

30% Burkitt and lymphoblastic

Pos AML

Angiosarcoma (cytoplasmic)

Ovary Mucinous

CK7+20+ 95%

MUC2 and MUC5AC various mucinous tumors +

Ovary, appendix, colon, breast, stomach, endocervix

CDX2 mucinous ovary and GI tract all sites, pancreas +

Ovary CK7/20

Serous 7+20- 100%

Mucinous 7+20+ 95% (rule out stomach, pancreas, bile duct)

Colorectum 7-20+ 90%

Endometrial vs Endocervical (applies to glandular areas only)

	Endometrial	Endocervical
ER and vimentin	Usually diffuse pos	Usually neg or focal
P16 and CEA	Usually neg or focal	usually positive

Endometrial stromal vs Smooth muscle

	Muscle	Stromal
Caldesmon	70%	5%
CD10	35%	95%
Desmin	90%	only in areas of muscle differentiation
B-catenin	neg	50-100%

Complete mole p57 neg vs partial mole and hydropic change p57 pos

MUC4 may be useful for identifying implantation site trophoblast

GI Tract

Colorectum

CK7-20+ 90%

MUC2 and MUC5AC various mucinous tumors +

Ovary, appendix, colon, breast, stomach, endocervix

CDX2

Colorectal >90%

Mucinous carcinomas of all organs including ovary, pancreas positive from 40-100%

<5% lung, prostate, breast, ovarian serous

Carcinoids: ileum and appendix >90%, other sites variable but frequently negative, lung negative

TTF1 stains 50% of pulmonary and 0% of GI.

Stomach, Pancreas, Bile Duct

No great markers

Wide range of CK7&20

Stomach 50%+ HepPar1

CA19-9 not specific for pancreas

Pos: Colon, ovary, lung, cholangio

0-5%: Liver, breast, mesothelioma

Esophageal adenocarcinoma frequently TTF1 and napsin +

GU & Germ Cell

Prostate – well/mod diff

PSA or NKX3.1 and PSAP (prost acid phos) 95% pos

All very specific, one is usually enough if untreated, mod diff carcinoma

ERG very specific but 50% sensitive

Prostate vs TCCa (high grade or treated Ca)

For poorly diff TCCa: GATA3 may be most sensitive and specific

Backups p63 (85%) and HMWCK (CK5/6 or 34BE12 60%)

Uroplakin and thrombomodulin do not work for poorly diff TCCa

For poorly diff prostate ca, NKX3.1 more sensitive (>90%) than PSA (may be neg in 10%)

Both very specific

Prostate Ca vs benign

In general, for small foci, IPOX can prove benignancy but not prove malignancy

Small foci that stain as cancer are usually best left at ASAP (see surpathcriteria)

Basal epithelial cells (not myoepithelial cells) absent in carcinoma

High molecular weight keratin - 34BE12 or CK5/6 cytoplasmic, p63 nuclear

Basal cells may be patchy

Presence of a basal layer virtually rules out invasive Ca (Gold standard?)

Intraductal carcinoma is an exception (see surpathcriteria)

Rare cases of prostate carcinoma reported as p63 positive

Not a basal layer, but single layer glands

HMWCK negative, Racemase positive

P504S (Racemase, AMACR)

Positive in Ca and PIN, negative in benign

At least moderate, circumluminal stain

Faint or patchy stain may be seen in benign

Lots of other carcinomas stain – this is not specific for prostate origin

PIN2-PIN4 cocktails

P504S (cytoplasmic), p63 (nuclear) +/- high molecular weight keratin

Especially useful if only one slide

P504S helps highlight the area of interest – go down and look for basal cells

For small foci, destain H&E instead of recutting block

Germ cell tumors

General

EMA neg

SALL4, OCT3/4 and CD30 are quite specific for germ cell

PLAP and ckit stain many other carcinomas also

Seminoma - Keratin dots, focal or negative, rarely strong and diffuse

Embryonal Ca - HCG, AFP 20-30%, serum test may be better

Yolk sac Ca - AFP may be scant, serum test may be better

Germ cell tumors differential, % positive

	Sem.	EmbCa	YST	ChorioCa
CD117, ckit	90	0	0	0 few cases
D2-40, podoplanin	100 diffuse	30 focal	0	0 few cases
OCT3/4, POU5F1	100	100	0	0
SALL4	100	100	100	100 few cases
NANOG	100	100	9	
SOX2	0	100	0	
CD30	5	90	20	0
CK7	5	80	0	+ few cases
Glypican 3	0	0-8	100	30-100
EMA	2	5	3	50

Miscellaneous

Renal cell carcinoma

CK7-20- 80%neg for both

Keratin, EMA, CD10 90% but not specific

PAX8 >80% (best for mets)

SF1, CDX2, P63 negative

RCCma 85%? (not in our hands)

Major types of RCCa (see surpathcriteria for details and other types)

	Vimentin	CD117	CK7	CAIX
Clear cell	>85%	<5%	neg/focal	100%
Papillary	>90	<20	20-80	50%
Chromophobe	0	>80%	>70	neg
Oncocytoma	0	>90%	neg/scat	neg

Hepatocellular Ca

70-90% HepPar1

50% gastric

Occasional adrenal, yolk sac, colon, lung, ovary, endocervix

CholangioCa and pancreatic Ca can be + in up to 15%

85-100% Arginase1

CholangioCa and pancreatic Ca 0-8%

<20% EMA, CD15, mCEA, MOC31

80% mets and cholangiocarcinoma are positive for these markers

<50% Canalicular staining: CD10 & pCEA, quite specific if branching

88% Glypican 3

Hepatic adenoma, FNH, cirrhosis negative

Other carcinomas 3%

CD34 stains sinusoidal lining in HCC but not normal sinusoid lining cells

Keratin and EMA

Keratin+ EMA-

Hepatocellular Ca
Germ cell tumors
Granulosa cell tumor (keratin 30-50%)

Keratin- EMA+

Meningioma
Pulmonary sclerosing hemangioma (keratin+/-)
Small cell ca and renal cell ca frequently EMA strong, keratin weak

Adrenal cortical carcinoma

SF1 85%, PAX8 negative
MelanA, Inhibin 90% - less in carcinomas than adenomas?
Calretinin, Synaptophysin 60-90%
Keratin, EMA 0-15%

CD10

RCCa 90%
Endometrioid ovary, prostate, HCCa, TCCa, SqCC 50-60%
Melanoma, oat cell, pancreas 30-50%%
Lung adeno, colorectal, breast, serous ovary, stomach 10-20%
Several sarcomas may stain (MFH, fibrosarcoma, MPNST...)

Thyroid Ca

Follicular or papillary - TTF1, thyroglobulin+
Medullary - TTF1, calcitonin, chromogranin +

Thymus

Thymoma PAX8+, p63+, CD5 neg except B3 may be positive
Thymic carcinoma PAX8+, p63+, CD5+
Lymphocytes of normal thymus and thymoma have immature T phenotype

Squamous Ca – all sites

Can't separate sites by IPOX
P63, CK5/6 and 34BE12 more sensitive than AE1 or CAM5.2
Majority are CK7-20-, PAX8-
Cutaneous CEA-

Spindled/sarcomatoid Ca

CK5/6 and p63 frequently better than AE1
About half are keratin negative
About half may be smooth muscle actin positive

Sustentacular cells (S100+. quite specific if definite)

Paraganglioma
Esthesioneuroblastoma
Neuroblastoma
Carcinoid (infrequent)
R/O interdigitating cells

Derm IPOX - selected topics

DF vs DFSP

Factor 13a, increased in DF, not in DFSP

Not easy to interpret

Lesional cells or reactive cells? Entrapped around edges in some cases?

CD34 - 75% DFSP

Occasional cases seem to have a bit of both F13a and CD34

D2-40 DF positive, DFSP negative/weak (one report)

Nestin DFSP positive, DF neg/focal

Desmoplastic melanoma

S100, SOX10 95-100%

Both stain nerve sheath tumors

Interdigitating cells S100+, SOX10 neg

HMB45, MelanA neg or almost always neg

Literature mixes desmoplastic with spindled

Spindled melanomas usually pos for HMB45 and MelanA

May have SMA+ myofibroblasts

Intraepidermal melanocytic processes

MelanA, MITF and SOX10 are better than S100

CK5/6 is a good complementary stain

Use a red chromogen to avoid mistaking melanin pigment for positive stain

Some reports of MelanA nonspecificity in actinic keratoses

Dermal nevus vs invasive melanoma

Dermal nevus HMB45 neg, Ki67 <5%

Melanoma frequently HMB45 pos, Ki67 >10%

Ki67 may stain reactive lymphocytes etc

Atypical fibroxanthoma

Defined as keratin, S100 negative

Should also be p63, SOX10 neg

Spindled, not desmoplastic, melanoma usually the differential

Spindled carcinomas 50% keratin negative

CD68 50% (Histiocyte marker, nonspecific – also in carcinoma, melanoma)

CD163 better

Smooth muscle actin 40%

Leiomyosarcoma, some spindled SCC +

CD10 and Procollagen 1 >90%, strong reactions appear relatively specific

BerEp4 BCC and sebaceous carcinoma +, SCC neg

Soft tissue tumors Keratin+

Synovial sarcoma

Keratin, EMA: Biphasic 100%, Monophasic 50%

S100 30%

TLE1 95%, occasionally in schwannoma, sft/hpc

Temperamental IPOX stain, FISH is better

CD99 50% (not specific, see CD99 and bcl2 notes below)

bcl2 >90% (not specific, see CD99 and bcl2 notes below)

Epithelioid sarcoma (both proximal and distal)

Keratin, EMA essentially 100%, INI1 negative
CD34 50%, Carcinomas are negative

(Intra-abdominal) desmoplastic small round cell tumor (DSRCT)

Co-expresses desmin and keratin >90%
WT1 90%, CD15, BerEp4 70%
Calretinin, CK5/6 0-15%
CD99 35% (not specific, see CD99 and bcl2 notes below)

Other keratin+ soft tissue tumors

Inflammatory myofibroblastic tumor 30-77%
Extra-renal rhabdoid tumor 100% (INI1 loss in 85%)
Epithelioid angiosarcoma 0-50%
May also be seen in nerve sheath sarcomas (35%)
Occasionally seen in MFH (up to 17%) and smooth muscle tumors (dot-like)
Rhabdomyosarcoma reported 5-50%

Soft tissue – CD34+

Vascular

ERG new marker, may be most sensitive and specific
CD31 does well on high grade tumors
 Also stains histiocytes
CD34 does better on lower grade tumors
 Stains various other tumors
D2-40 lymphatic marker, stains 100% of Kaposi and 70% of angiosarcomas
Frequently use multiple markers
Epithelioid hemangioendothelioma – frequently only the lumens stain
Keratin 30-50% esp epithelioid tumors

GIST

DOG1 appears to be more sensitive and specific than CD117 or CD34
CD117 (c-kit) 90%
 Quite specific among spindle cell tumors
 Stains lots of carcinomas, melanomas
CD34 75%
 Quite specific vs carcinoma, melanoma (better for epithelioid tumors)
 Stains lots of spindle cell tumors

Epithelioid sarcoma CD34 50% (see above under Soft Tissue Keratin+)

DFSP CD34 75% (see above, Derm lesions)

Solitary fibrous tumor / HPC

CD34 SFT 90%, HPC 75%
STAT6 appears to be very sensitive and specific
CD99, bcl2 90% (not specific, see below)
Neg: keratin, actin, desmin, S100

Spindle cell lipoma / Pleomorphic Lipoma

CD34 100%
 Other fatty tumors only scattered dendritic cells
 Some dediff liposarcs positive
CD99, bcl2 >90% (not specific, see below)

Pleomorphic hyalinizing angiectatic tumor (PHAT)

CD34 80% large cells+
Factor 13a many pos small spindle cells
S100, keratin, actin neg

Mammary type myofibroblastoma

CD34 90%+
Desmin pos
Smooth muscle actin variable
Keratin, S100 negative

Soft tissue tumors - other markers +

Rhabdomyosarcoma

Desmin, muscle specific actin 50-100%
Myogenin more specific, less sensitive
SMA occasional + cells
CD56 50-100%
Keratin 5-50%
CD99 0-50%

Nerve sheath

S100, SOX10
Benign virtually 100%, Malignant 30-50%
HMB45, MelanA pos indicates spindled melanoma
(Pigmented nerve sheath tumors also positive)
Negative result is indeterminate
CD34 stains some dendritic cells but not typically the neoplastic cells
True S100+ CD34+ is unusual phenotype
Perineurioma: CD34+, EMA+(may be focal, faint), GLUT1+, S100 neg

PNET/Ewing

CD99 >90% (Not specific)
Keratin 15%
S100, synaptophysin 0-50%, chromogranin neg
Actin, desmin very rare
INI1: PNET/Ewing, Wilms, DSRCT 100%; Rhabdoid tumor 0%
FLI1 specific but not sensitive

CD99: soft tissue tumors

>90%: SFT, Sp cell lipoma, PNET/Ewing
20-60%: Synovial sarc, HPC, MFH, Osteosarc, Fibrosarc, Leiomyosarc, DSRCT
Before you use CD99, be sure it distinguishes between your candidates

bcl2: soft tissue tumors

>90%: Kaposi, Leiomyosarc, Spindle cell lipoma, GIST, SFT, Synovial sarc, Melanoma,
Nerve sheath tumors, PNET
50%: DFSP, MFH, Fibrosarc
Before you use bcl2, be sure it distinguishes between your candidates

Angiomyolipoma/PEComa (Perivascular Epithelioid Cell – oma)

HMB45, SMA definitional
MelanA, MITF pos
S100 variable

Atypical lipomatous tumor and dedifferentiated liposarcoma

MDM2 & CDK4 95-100% but difficult to interpret

FISH is better for MDM2

Myxofibrosarcoma 40%, MFH 10%, Spindle cell lipoma 10%, Myxoid liposarcoma 4%

Pleomorphic liposarcoma neg

p16 reported as sensitive and more specific than MDM2 staining

Fibromatosis beta catenin 80-90% (nuclear), smooth muscle actin variable

Most others in ddx negative except SFT and synovial sarcoma

S100, CD34, CD117 neg

Desmoplastic small round cell tumor

Desmin and keratin pos, see above under Soft Tissue Keratin+

Alveolar soft part sarcoma

TFE3 strong nuclear +, muscle markers variably positive

Low grade fibromyxoid sarcoma & sclerosing epithelioid fibrosarcoma

MUC4 relatively sensitive and specific, except for 30% of monophasic synovial sarcoma

Soft tissue – No good markers

MFH - CD68 stains cells with lysosomes, not specific

CD163 stains only 4%

Fibrosarcoma

IPOX mostly to rule out things in the differential dx of this group

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