New markers provide new answers in malignant ovarian germ cell tumours

ugr

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WHO 2014

Germ cell tumours	
Dysgerminoma	9060/3
Yolk sac tumour	9071/3
Embryonal carcinoma	9070/3
Non-gestational choriocarcinoma	9100/3
Mature teratoma	9080/0
Immature teratoma	9080/3
Mixed germ cell tumour	9085/3
Monodermal teratoma and somatic-type tumours arising from a dermoid cyst	
Struma ovarii, benign	9090/0
Struma ovarii, malignant	9090/3
Carcinoid	8240/3
Strumal carcinoid	9091/1
Mucinous carcinoid	8243/3
Neuroectodermal-type tumours	
Sebaceous tumours	
Sebaceous adenoma	8410/0
Sebaceous carcinoma	8410/3
Other rare monodermal teratomas	
Carcinomas	
Squamous cell carcinoma	8070/3
Others	
Germ cell - sex cord-stromal tumours	
Gonadoblastoma, including gonadoblastoma with malignant germ cell tumour	9073/1
Mixed germ cell-sex cord-stromal tumour, unclassified	8594/1*

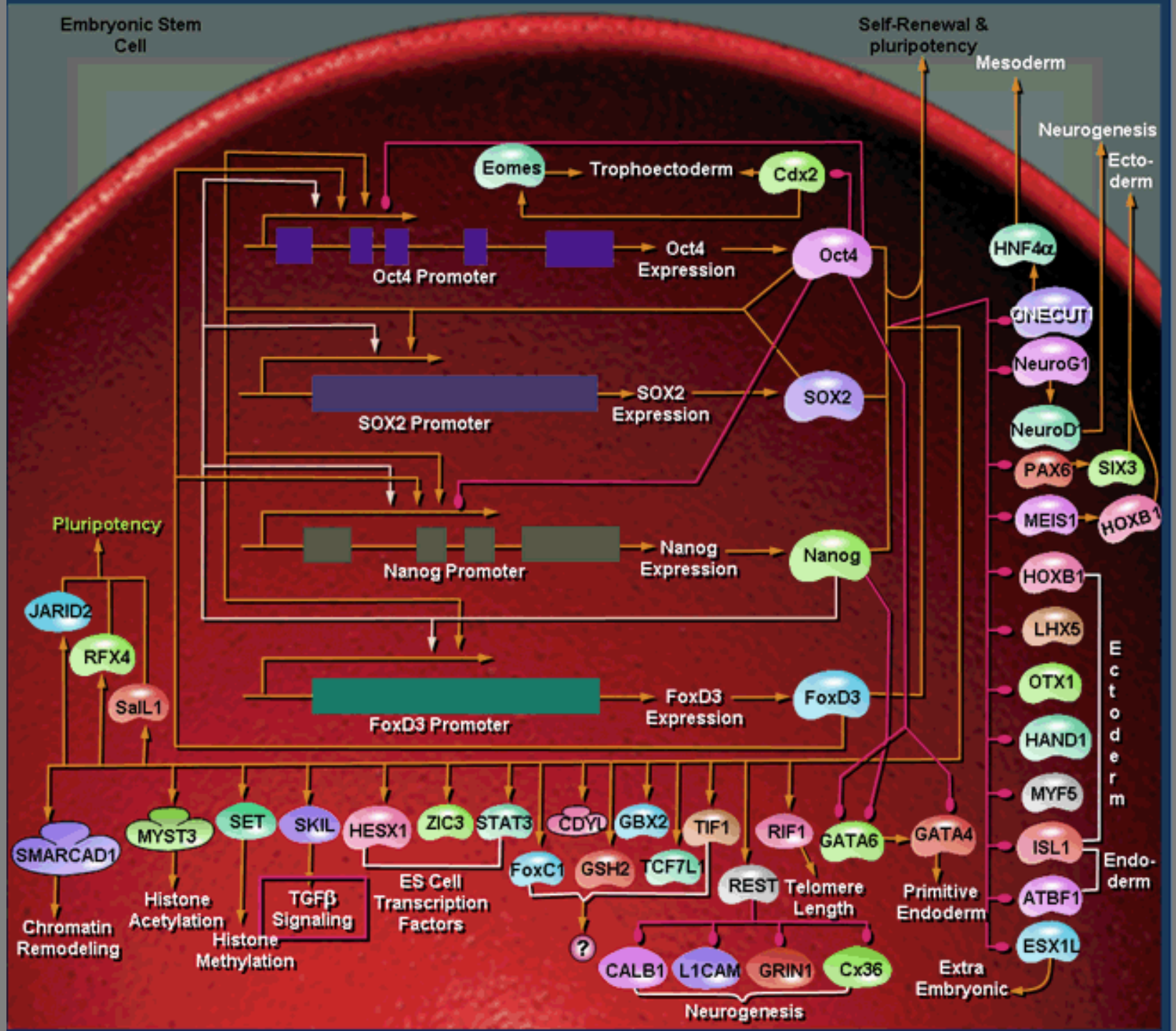
"Germ cell tumours are caricatures of normal embryonal development……" (Pierce 1971)

- **Models of comparative embryology/pathology**

1958,1962

"Germ cell tumours are caricatures of normal embryonal development……" (Pierce 1971)

- **Every normal developmental embryonal stage is caricaturized by a specific GCT type**
- **Each stage has characteristic markers (both stage-specific (SS) and pluripotency -PPM-)**
- **Analysis of expression of these markers (PPM and SS) will lead to a more accurate diagnosis of GCT types**
- **Additional demonstration of tissue-specific markers complement and fine-tune diagnosis based on a PPM expression**

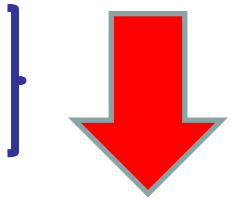

Embryonic Stem Cell
Self-Renewal & pluripotency
Mesoderm
Neurogenesis
Ecto-derm
Eomes
Trophoectoderm
Cdx2
Oct4 Promoter
Oct4 Expression
Oct4
HNF4α
ONECUT
NeuroG1
NeuroD
SOX2 Promoter
SOX2 Expression
SOX2
PAX6
SIX3
MEIS1
HOXB1
Pluripotency
Nanog Promoter
Nanog Expression
Nanog
JARID2
RFX4
SalL1
FoxD3 Promoter
FoxD3 Expression
FoxD3
LHX5
OTX1
HAND1
MYF5
ISL1
ATBF1
ESX1L
Ectoderm
Endo-derm
SMARCAD1
MYST3
SET
SKIL
HESX1
ZIC3
STAT3
CDYL
GBX2
TIF1
RIF1
GATA6
GATA4
FoxC1
GSH2
TCF7L1
REST
Telomere Length
Primitive Endoderm
Chromatin Remodeling
Histone Acetylation
Histone Methylation
TGFβ Signaling
ES Cell Transcription Factors
?
CALB1
L1CAM
GRIN1
Cx36
Neurogenesis
Extra Embryonic
www.SABiosciences.com

Diagnostic self-renewal and pluripotency markers

OCT4 aka POU5F1, OCT3 or OTF3

- Nuclear transcription factor - chromosome 6p21.3
- Blastocyst differentiation
  - Embryonal stem cells of the "inner cell mass"
  - epiblast

gastrulation



Primordial germ cells

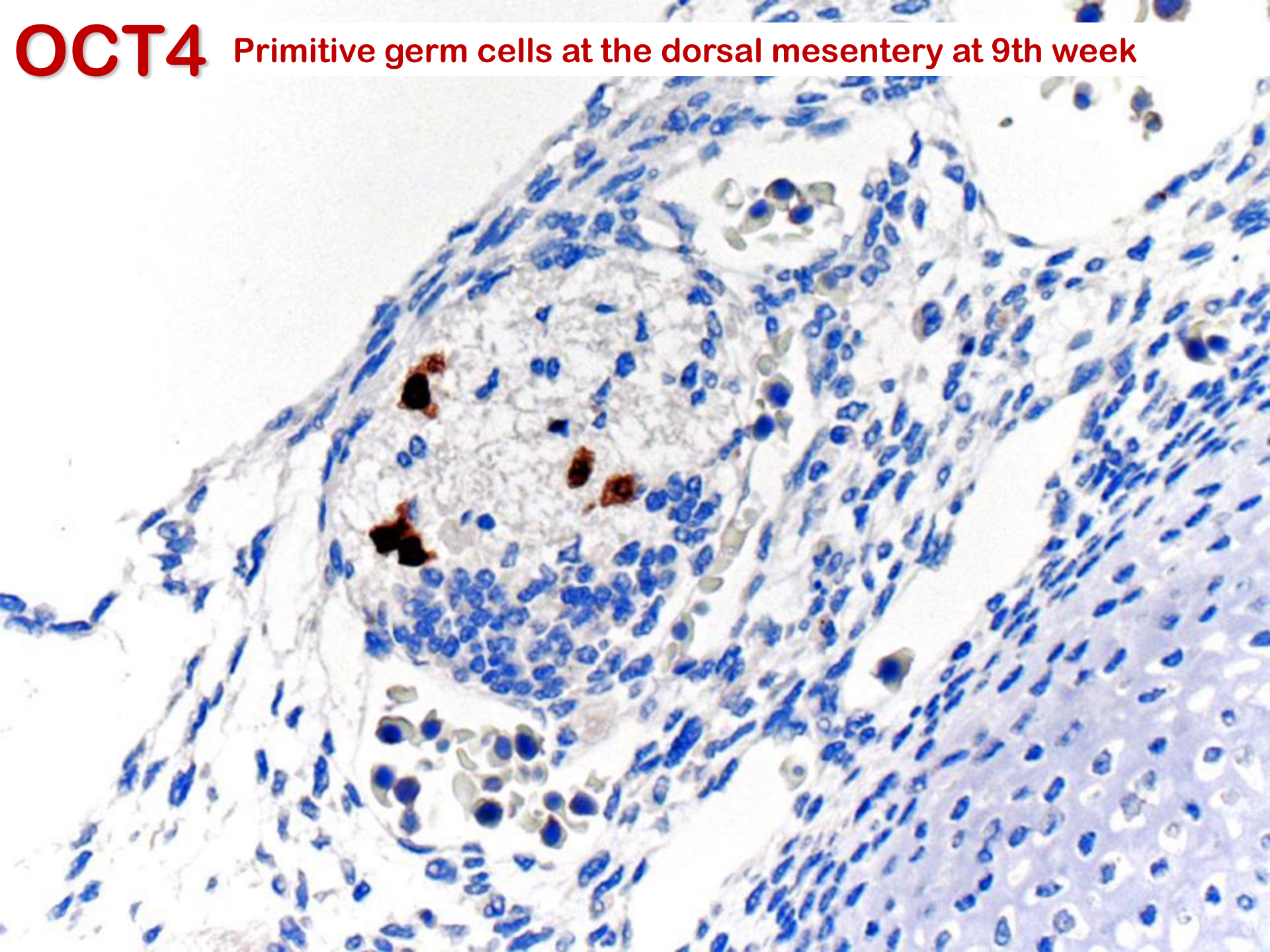
- Inducing pluripotency

Induces pluripotency of mature cells into iPSC

- Earliest marker (Germinoma & Emb Ca.)

OCT4

Primitive germ cells at the dorsal mesentery at 9th week

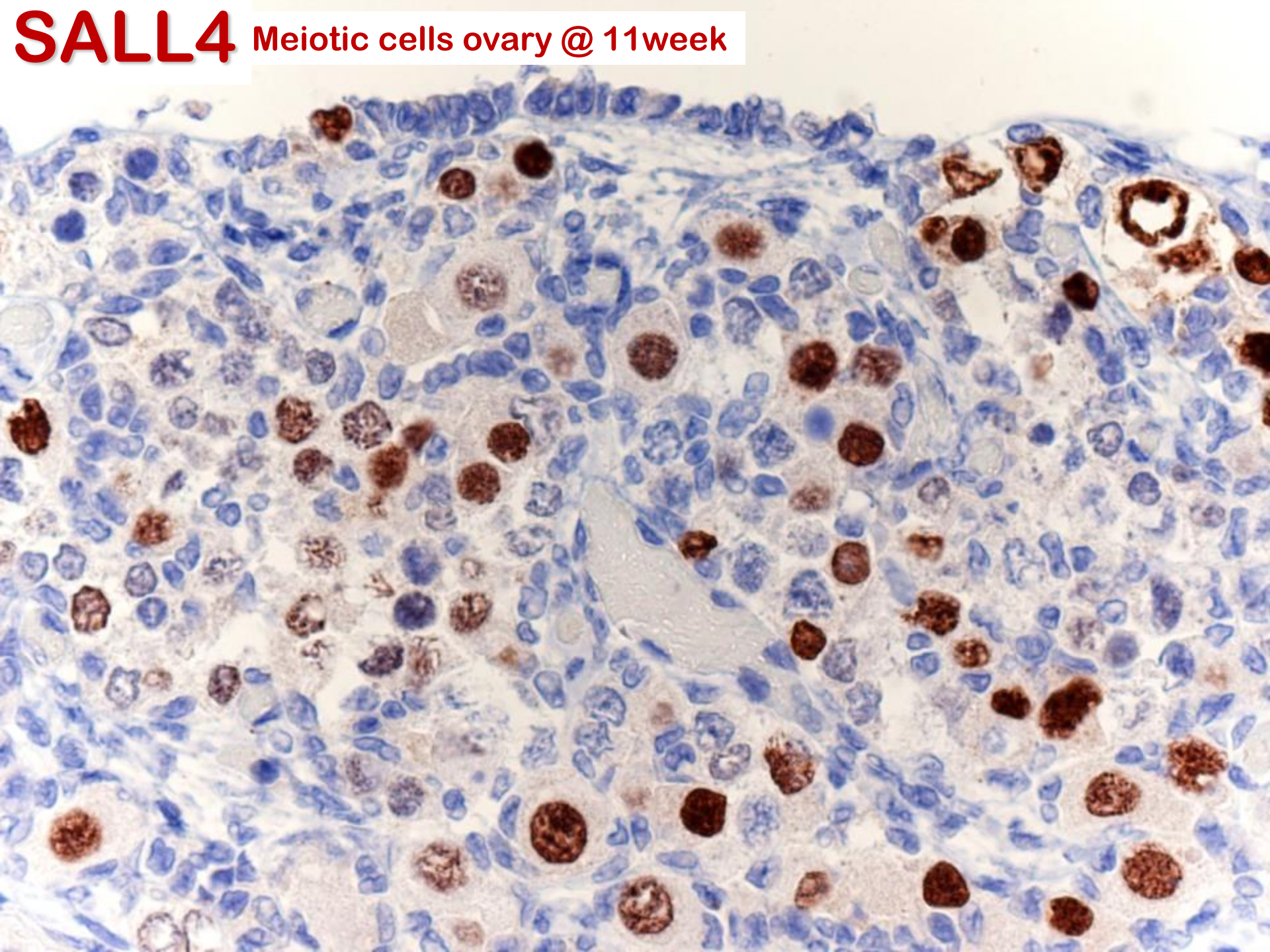


SALL4

- Family of three genes SALL - chromosome 20q13
- Expressed by cells of epiblast and primordial germ cells
  - Mandatory for endodermal differentiation
  - Not implicated in trophectoderm differentiation

- **Expressed by primordial germ cells and embryonal cells retaining pluripotency.**

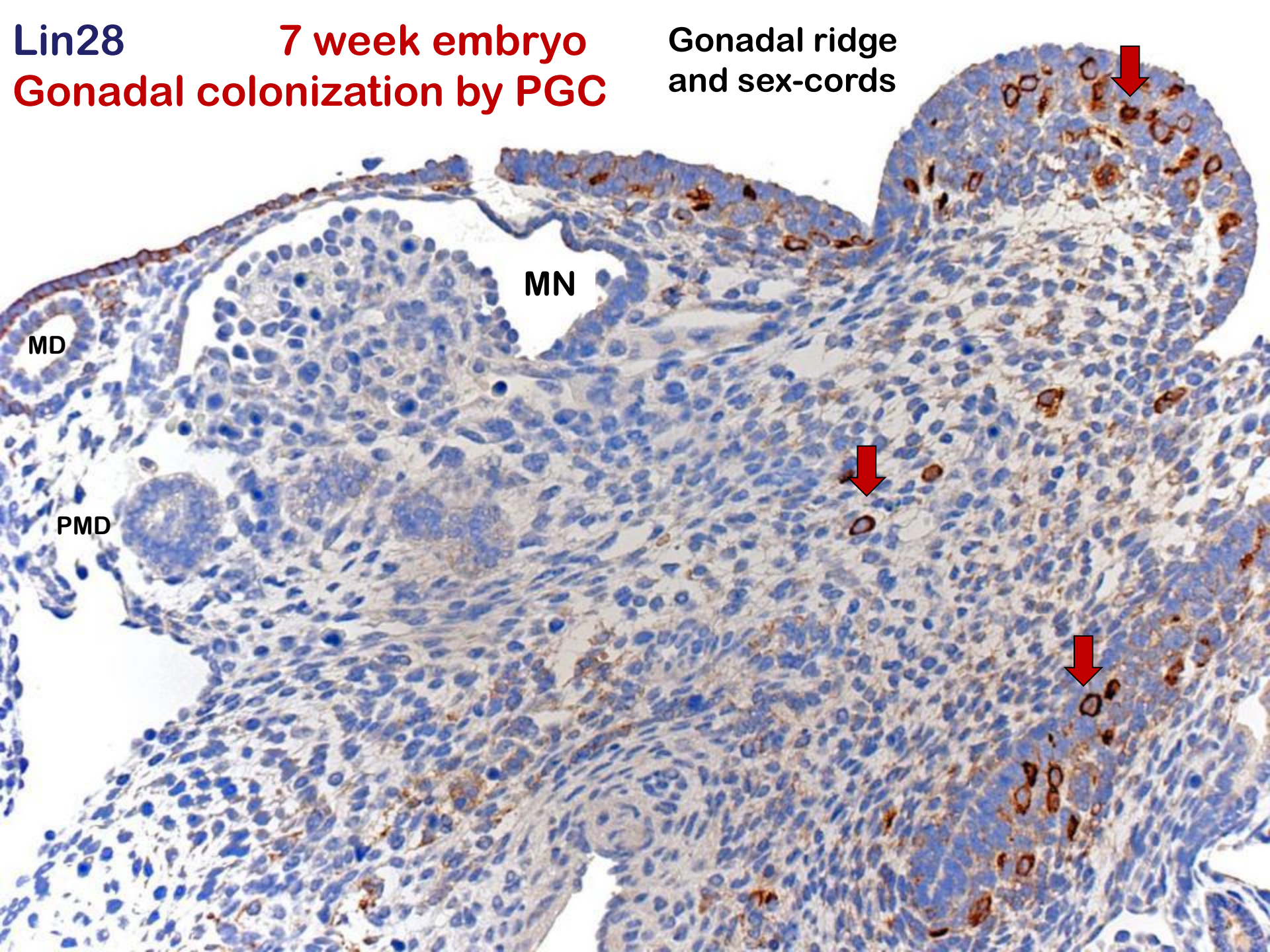
SALL4 Meiotic cells ovary @ 11week



Lin28

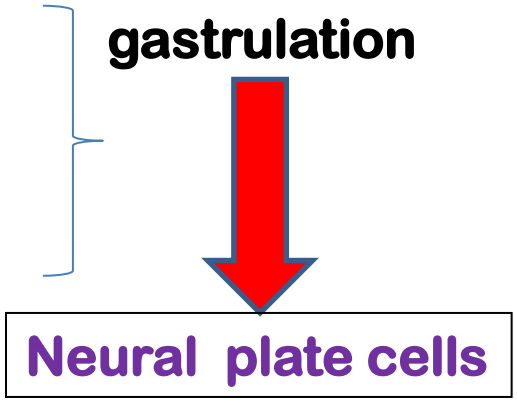
- miRNA binding protein
- Blocks let-7 miRNA activity
- Let-7 diminishes proliferation and induces differentiation
- Lin28 increases proliferation and induces pluripotency

- **Equivalent marker to SALL 4 (exceptions)**


Lin28
7 week embryo
Gonadal colonization by PGC
Gonadal ridge
and sex-cords
MN
MD
PMD

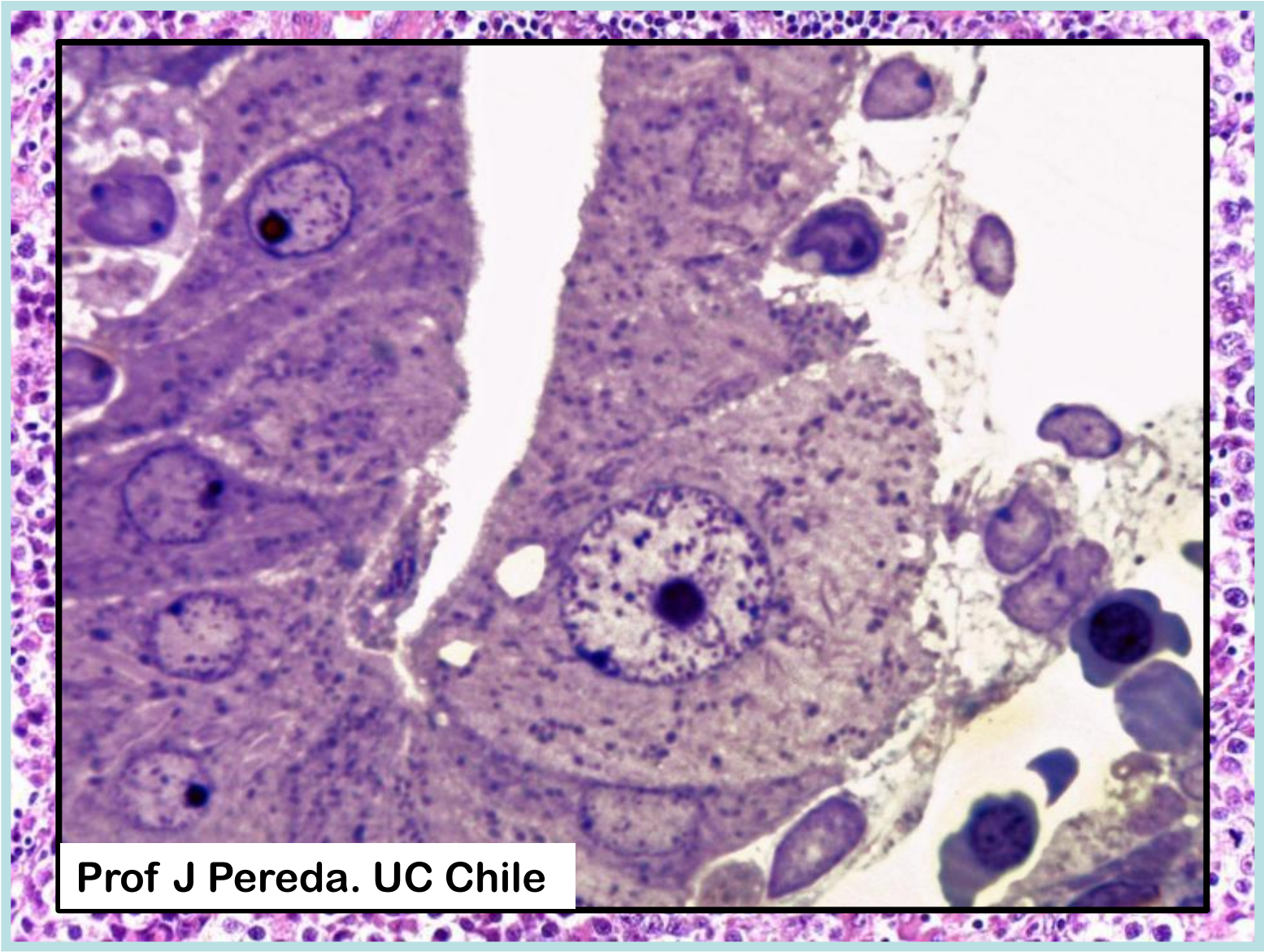
SOX2

- Factor SRY-box2
- Nuclear transcription factor- chromosome 3q26.33
- Responsible for
  - Development of the "inner cell mass"
- Differentiation of the trophectoderm together with CDX2

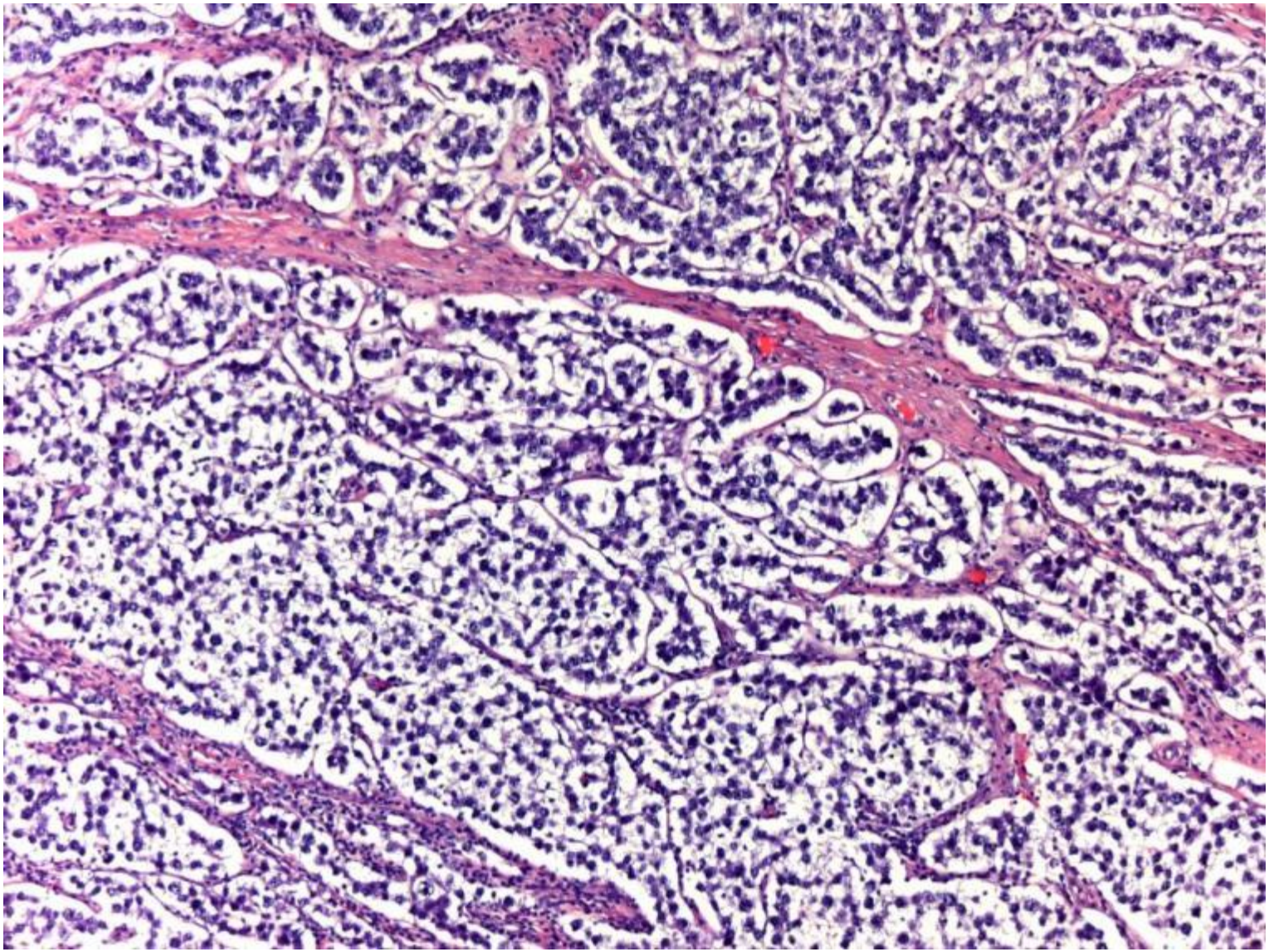


- Expression lost in primordial germ cells
- Ideal marker for embryonal Ca and immature neuroepithelium

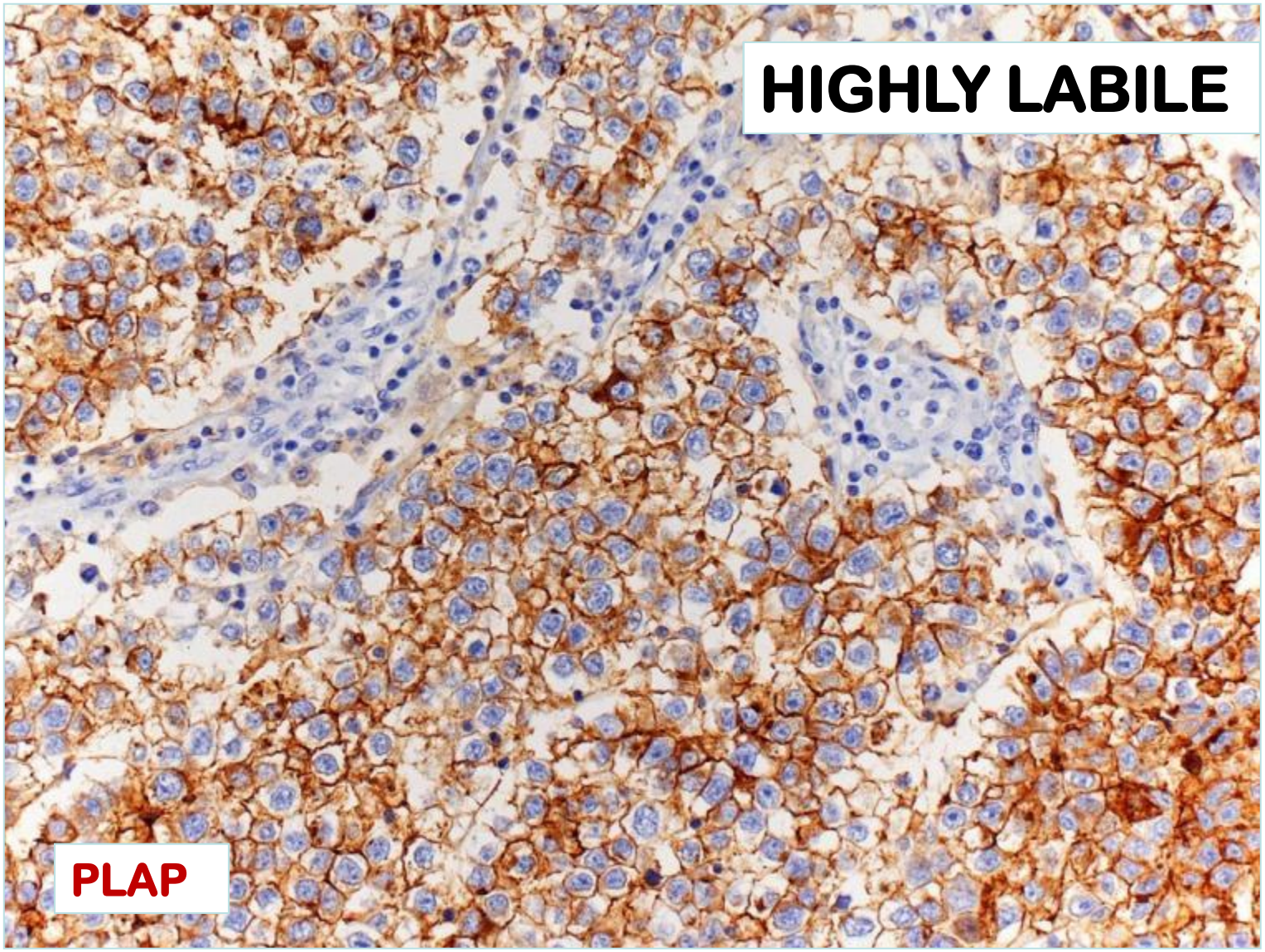
Dysgerminoma



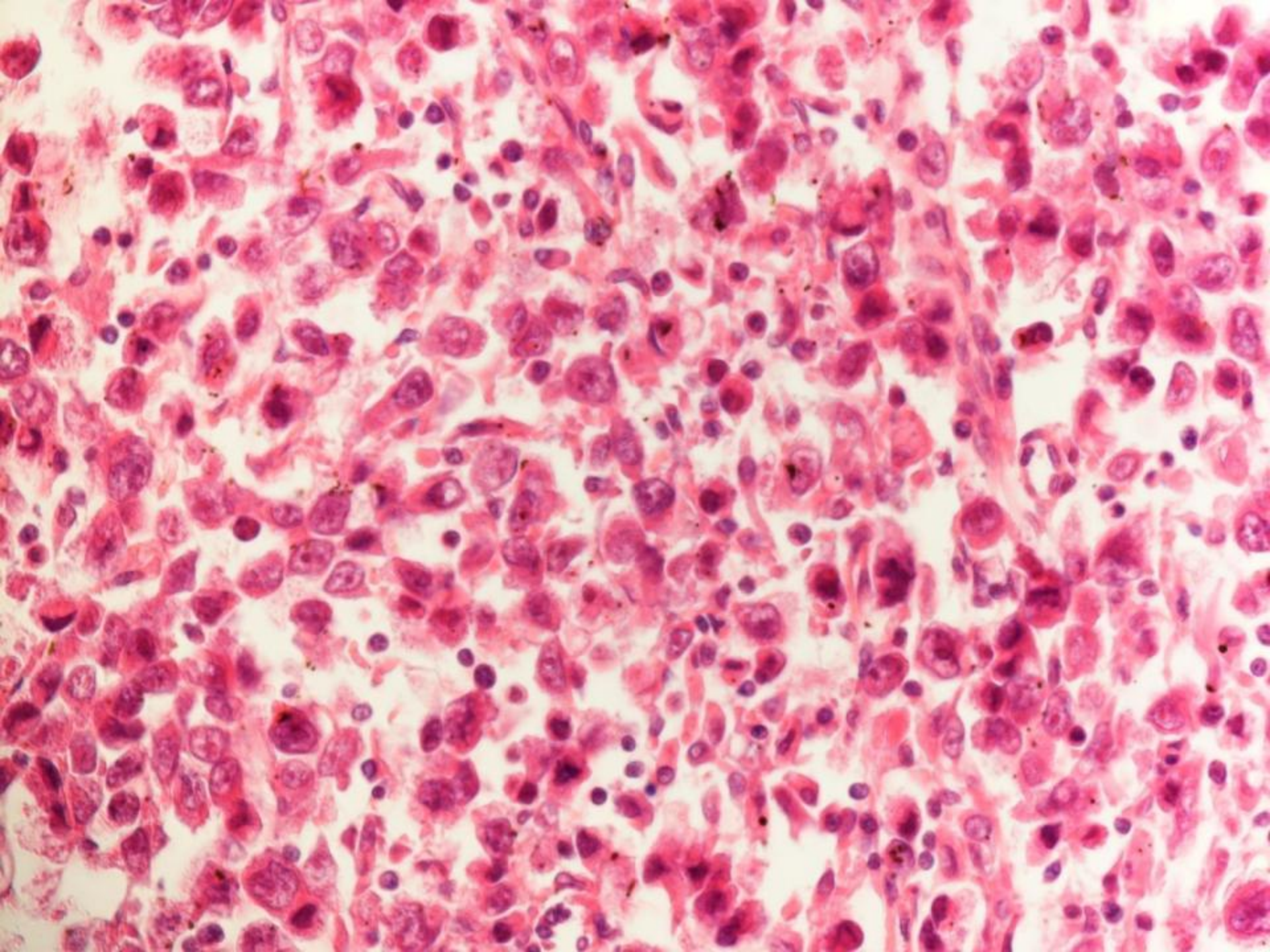
Dysgerminoma variability

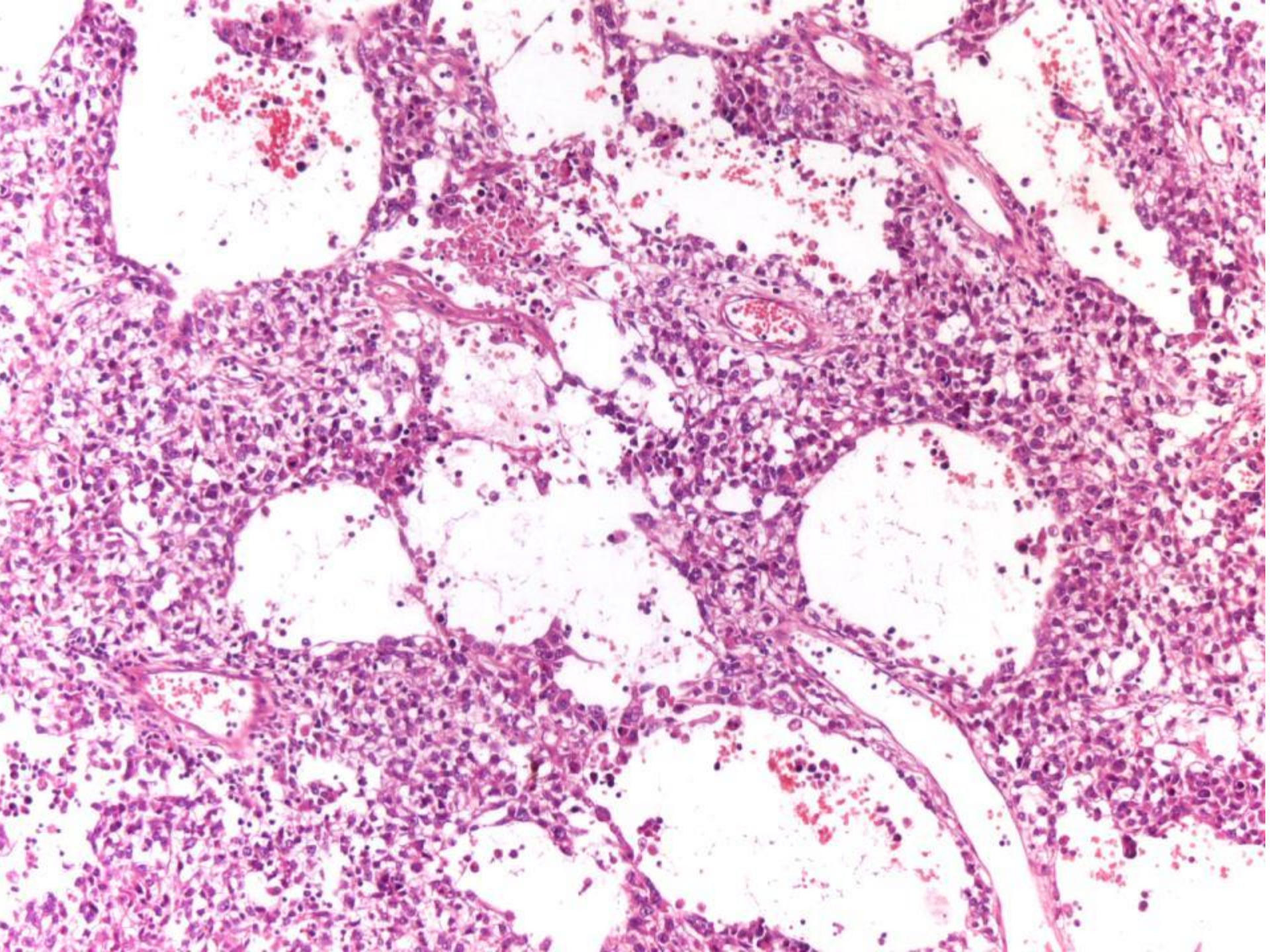


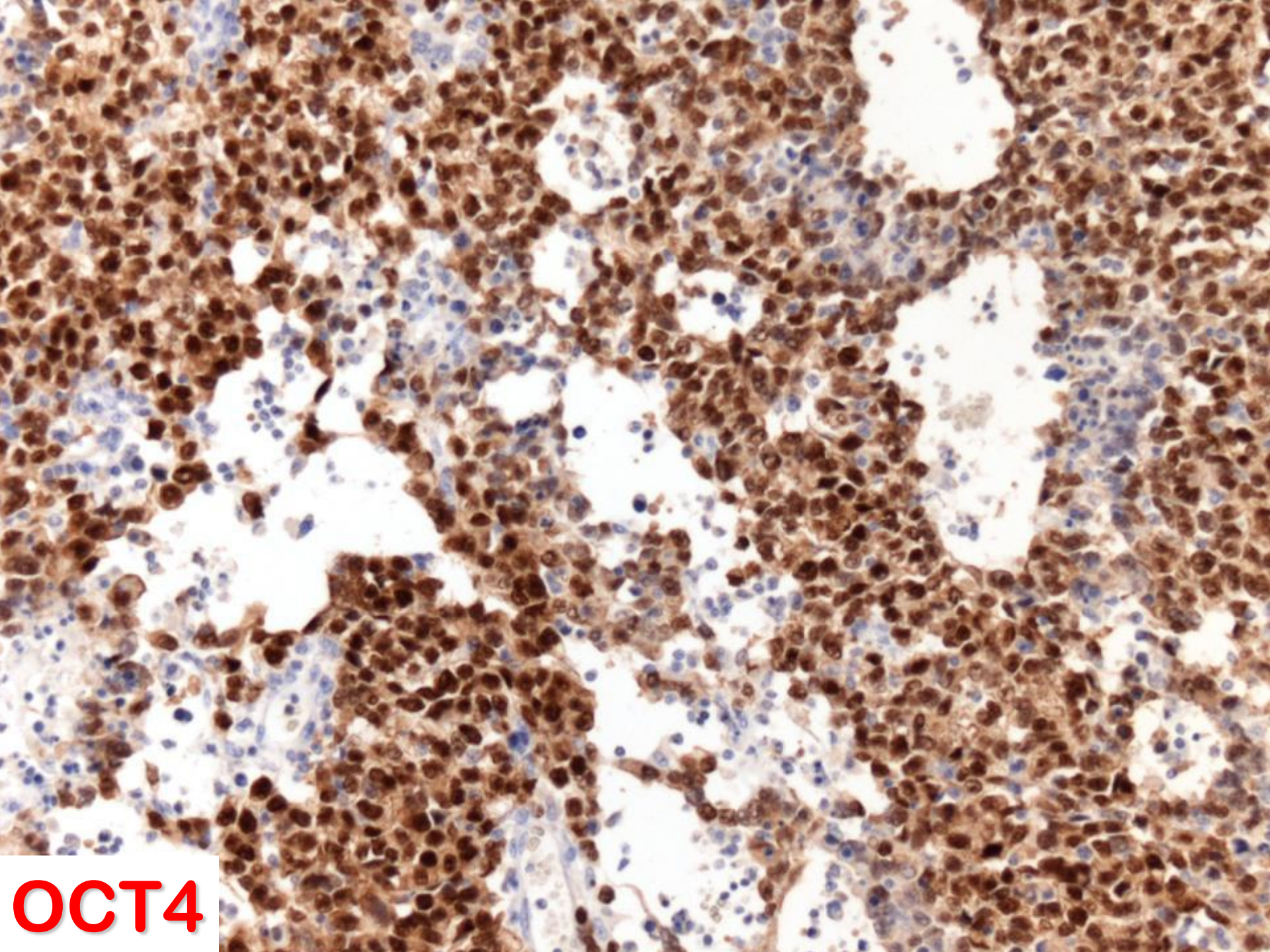
Dysgerminoma - Immuno

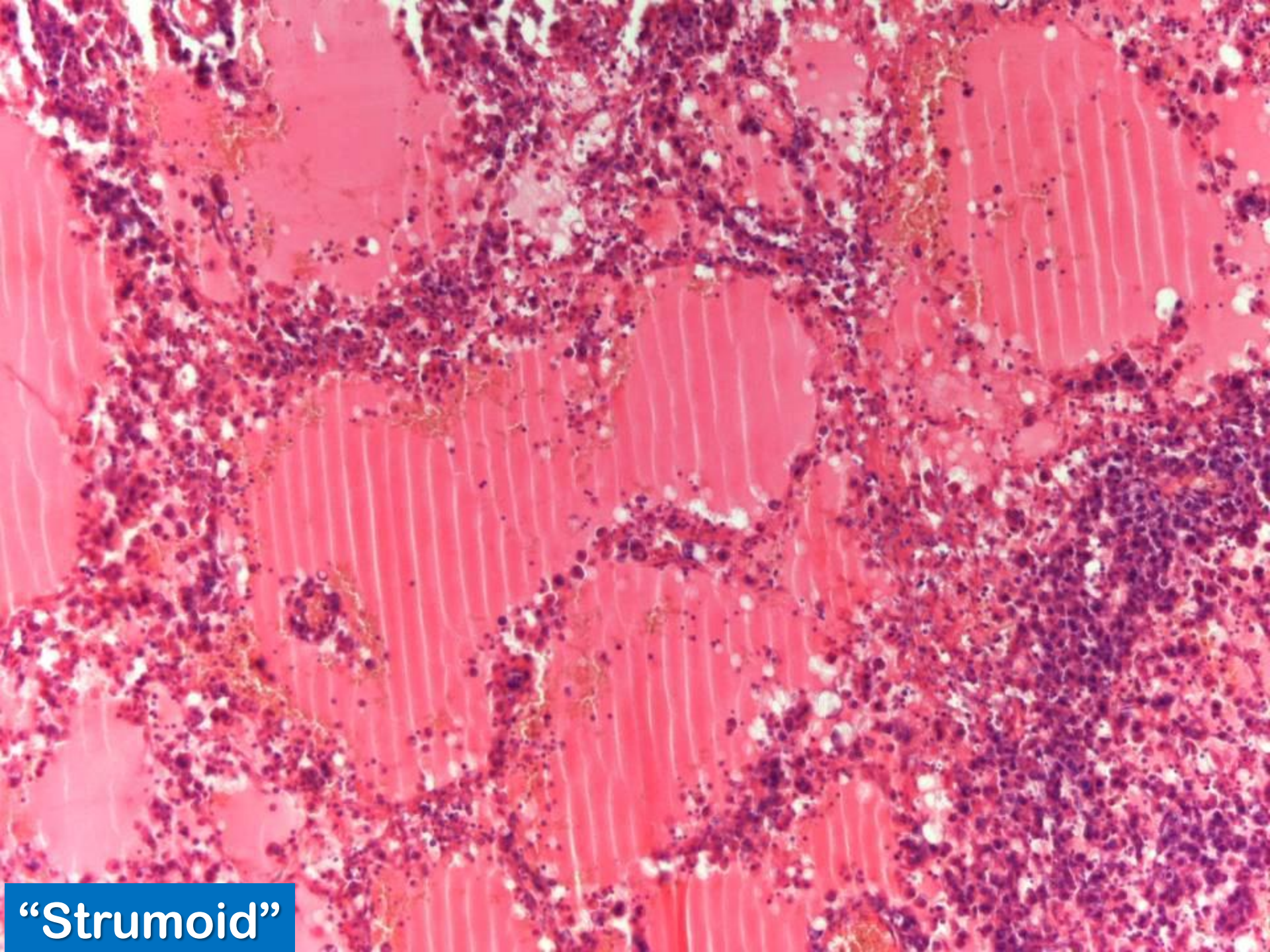


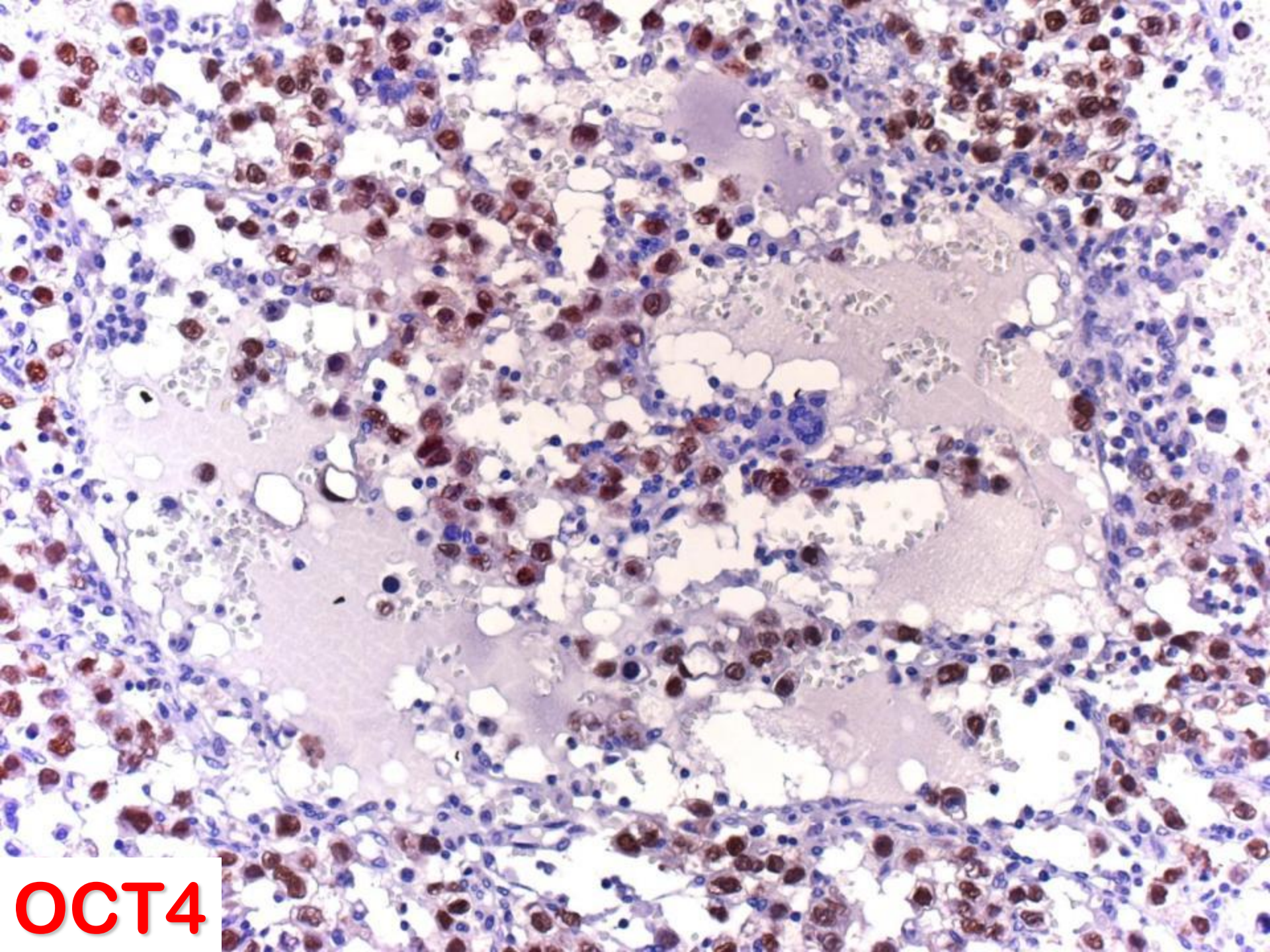
Misinterpretations

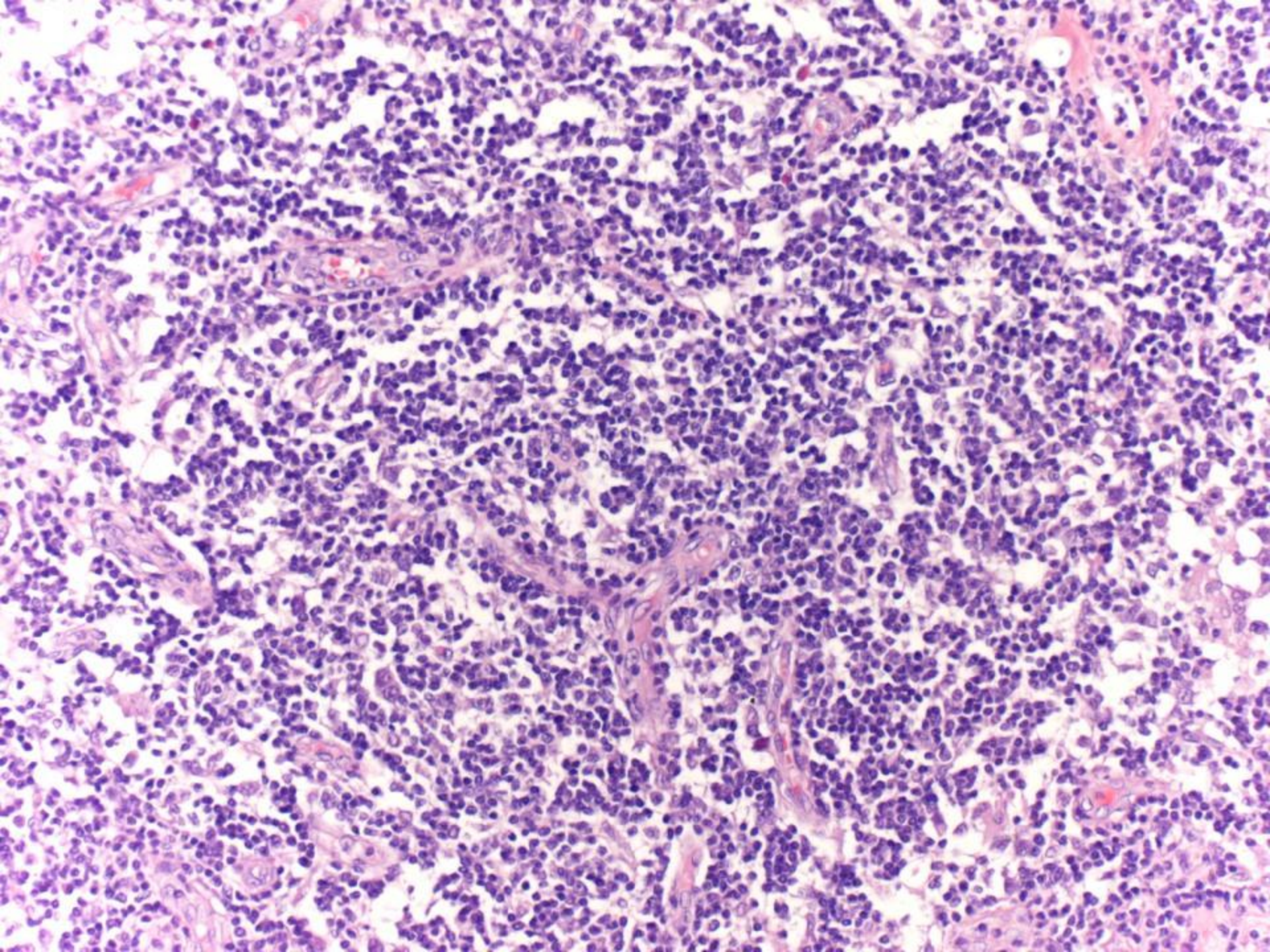


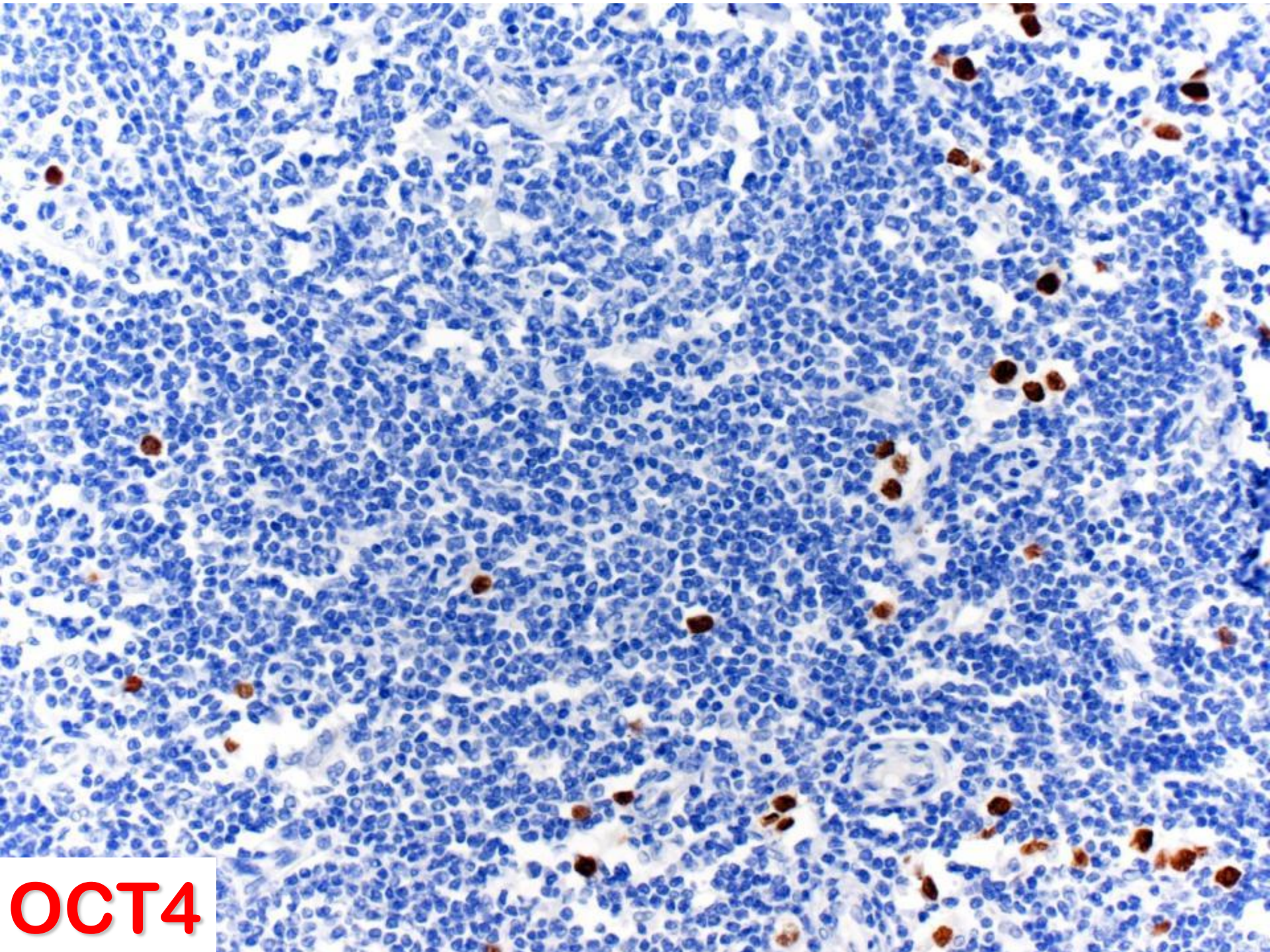



OCT4

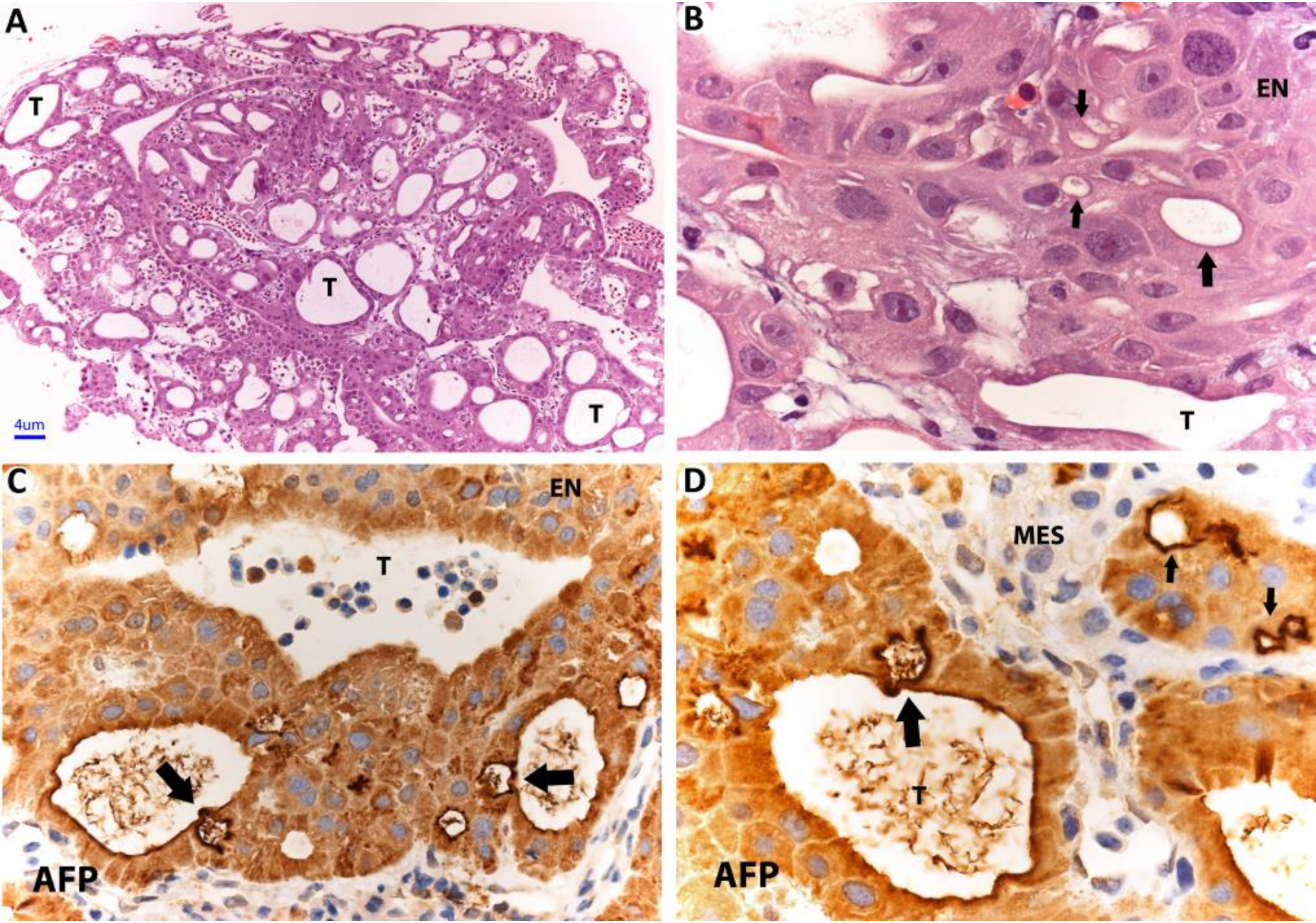

“Strumoid”


OCT4




OCT4

Yolk sac tumours
The primitive endodermal tumours


A
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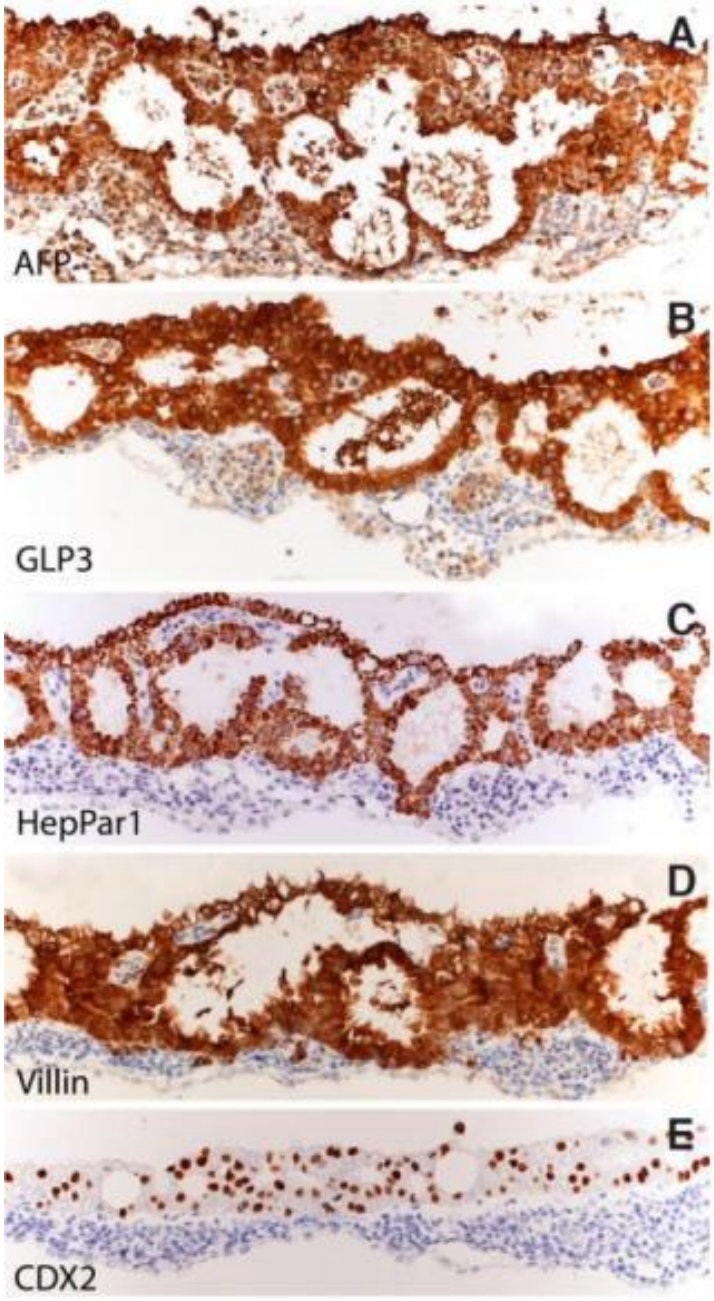
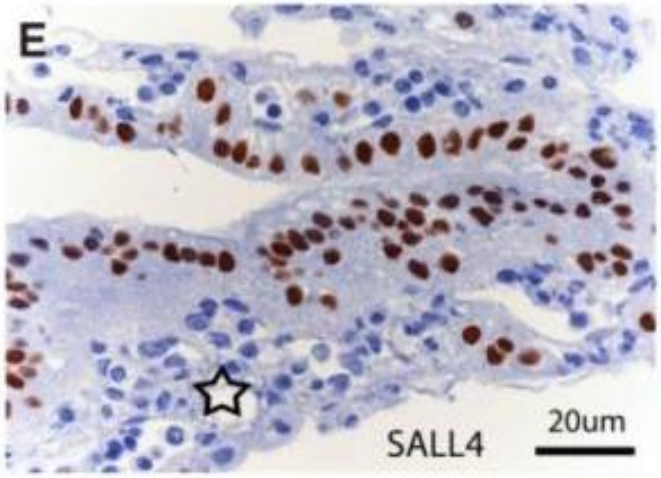
AFP





SHYS IMMUNOPHENOTYPE

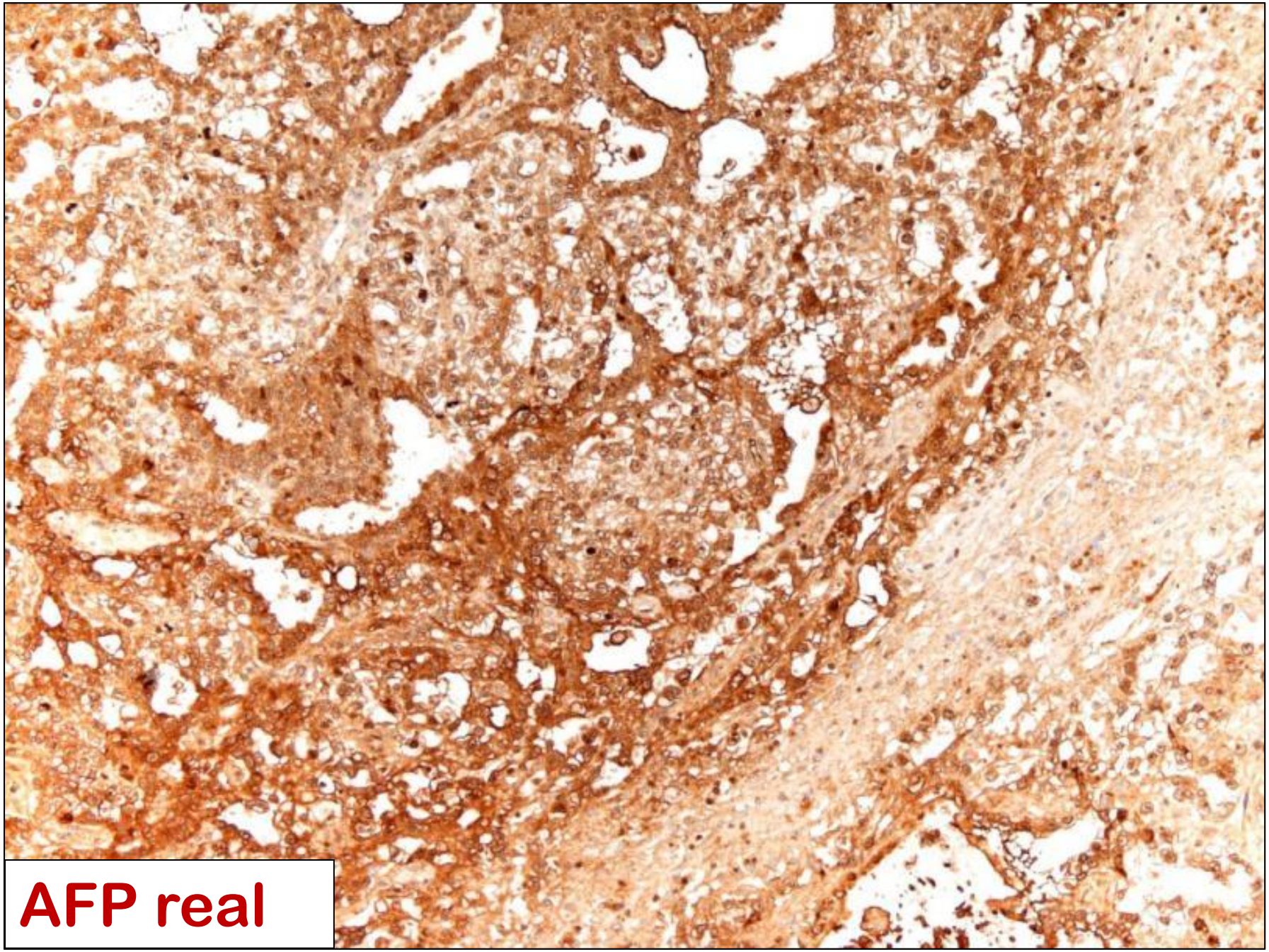
		ANTIBODIES						
Week	**#**	**AFP**	**GLP3**	**HepPar-1**	**Villin**	**CDX2**	**SALL4**	**D2-40**
5-6	1	1/1	-	-	-	-	-	-
7-8	15	15/15	15/15	12/14*	11/12*	10/14*	10/13*	15/15
9-11	10	10/10	10/10	9/10	5/9*	9/10	3/8*	8/9*

All antibodies, except for podoplanin D2-40, were expressed in the endodermal layer. Only podoplanin was positive in the mesothelium.

(*) In some cases, step sections failed to produce a sufficient number of slides to complete the study of some antibodies

Alpha-foetoprotein (AFP)

- Member of the albuminoid gene superfamily secreted by both primitive and SHYS
- Functional binding and transport of ligands
- Immunohistochemical gold standard of YST. However, its negativity does not exclude a diagnosis of YST. Expression is often patchy


AFP real

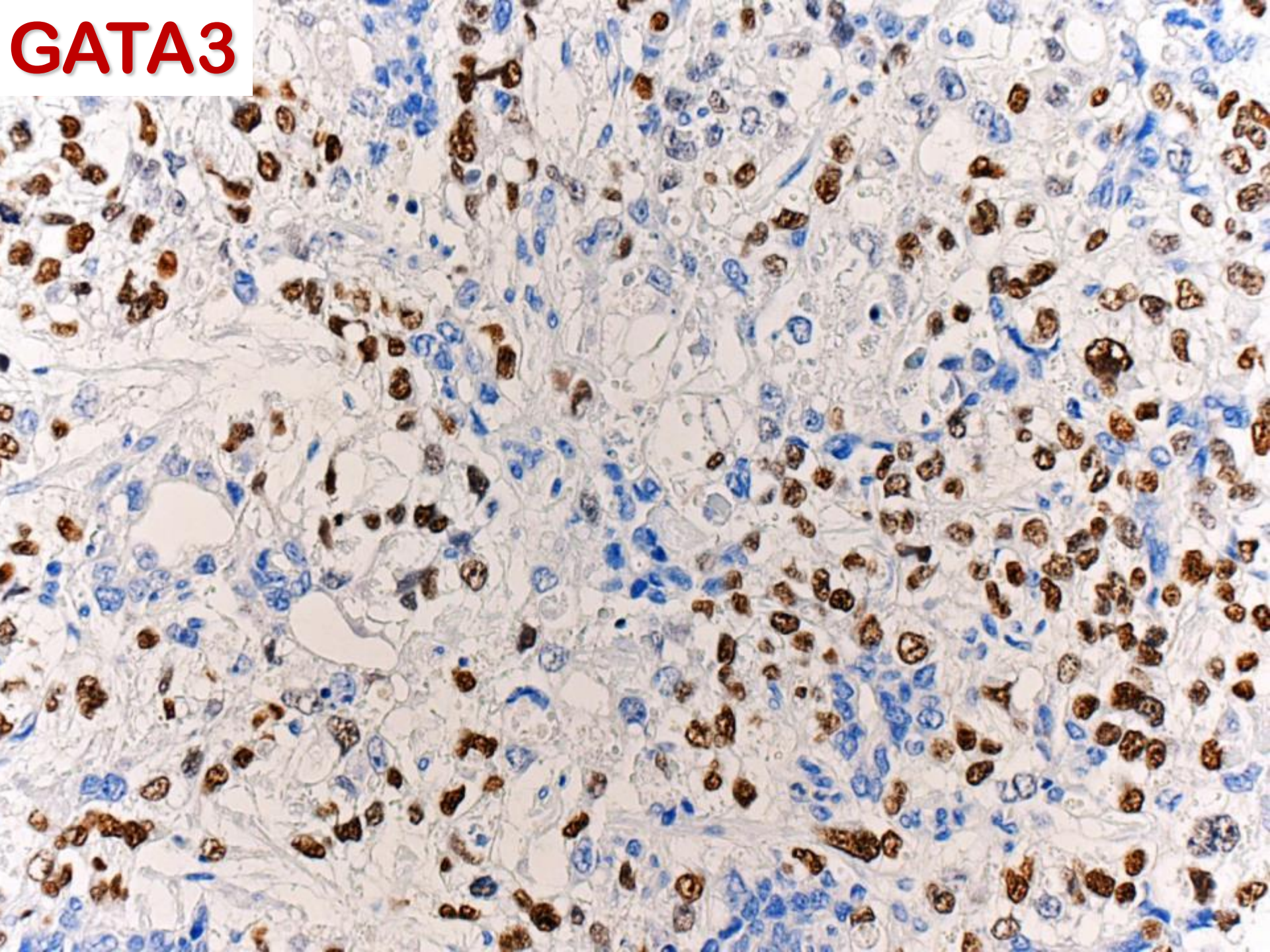
Glypican 3 (GPC3)

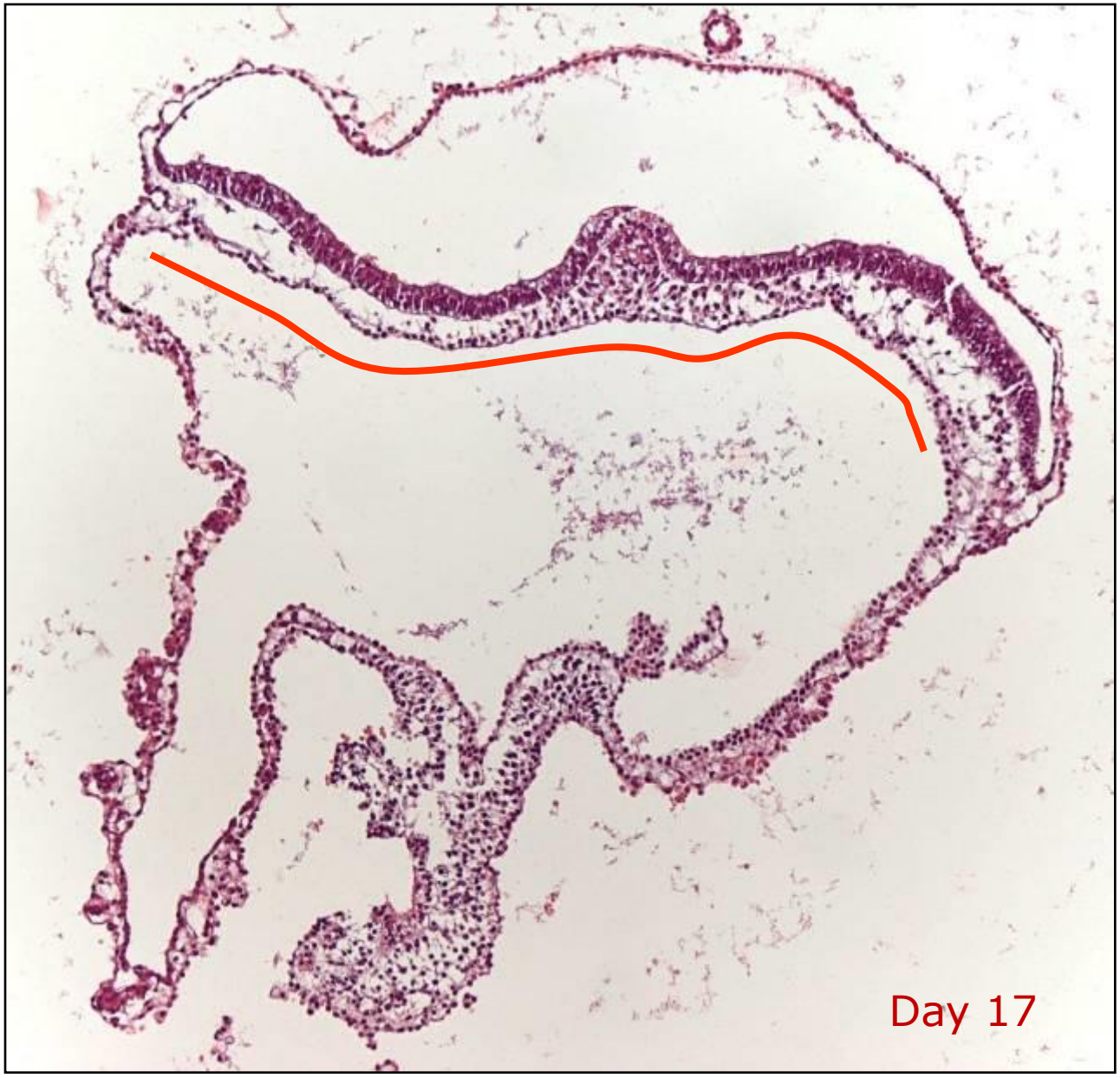
- Expressed in 8-11th week SHYS, but also in developing liver, lung, pancreas, neuroectodermal epithelium and syncytiotrophoblast.
- GPC3 is a sensitive but non-specific marker for YSTs and, to a certain extent, it parallels AFP distribution.
- Consequently, it is positive in other embryonal tumours: neuro, medullo- and nephroblastoma

GATA3

- Transcription factor
- GATA3 participates in differentiation
  - Breast epithelium, urothelium
  - T-cell development

Am J Surg Pathol 2014;38:13


GATA3


Day 17


Day 22

Germ cell tumours

J. Prat
D. Cao
S.G. Carinelli
F.F. Nogales
R. Vang
C.J. Zaloudek

Yolk sac tumour

Definition

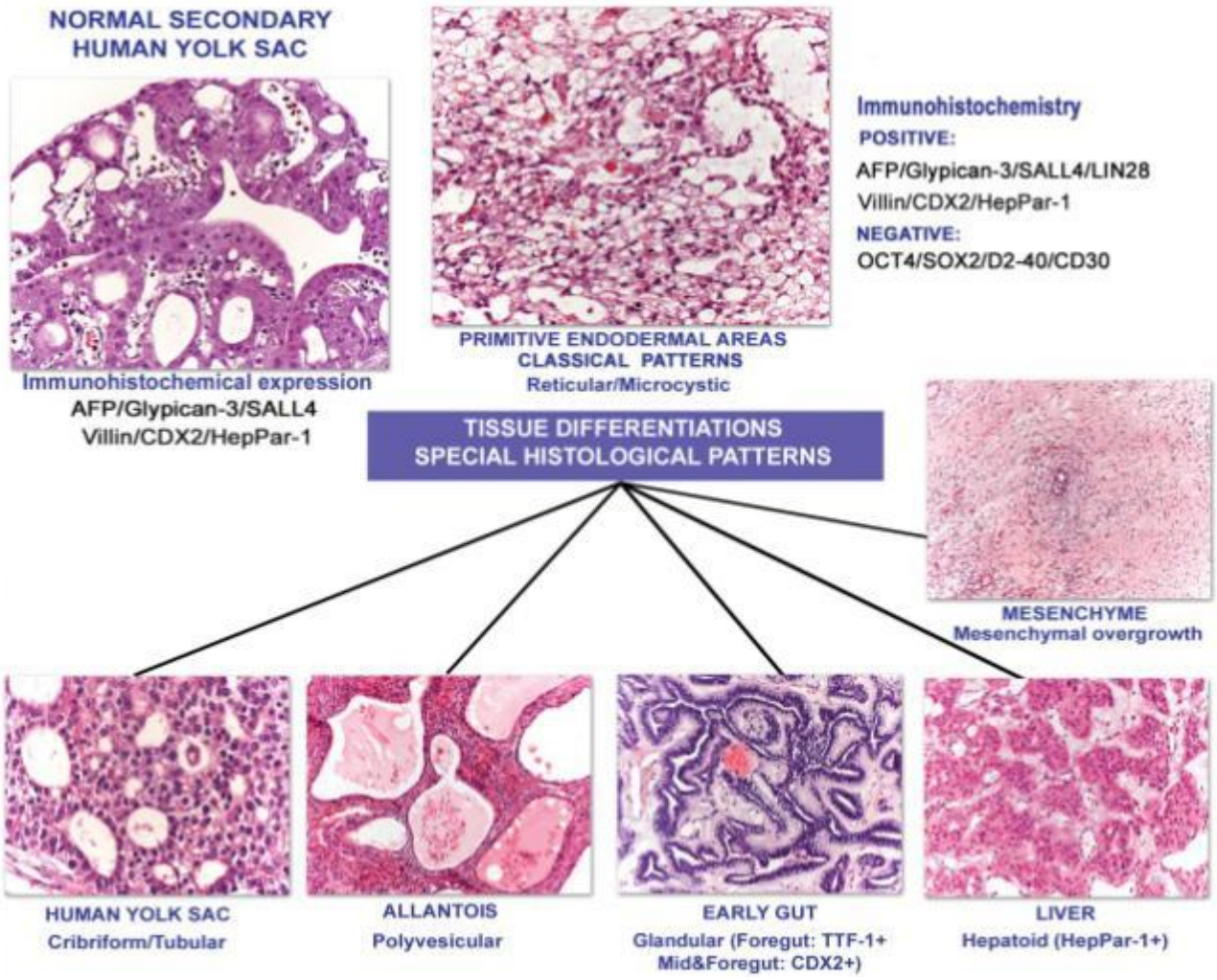
Yolk sac tumour is a primitive germ cell tumour with a variety of distinctive patterns and which may also exhibit differentiation into endodermal structures, ranging from the primitive gut and mesenchyme to the derivatives of extra-embryonal (secondary yolk sac and allantois) and embryonal somatic tissues (intestine, liver and mesenchyme) {1373}.

ICD-O code

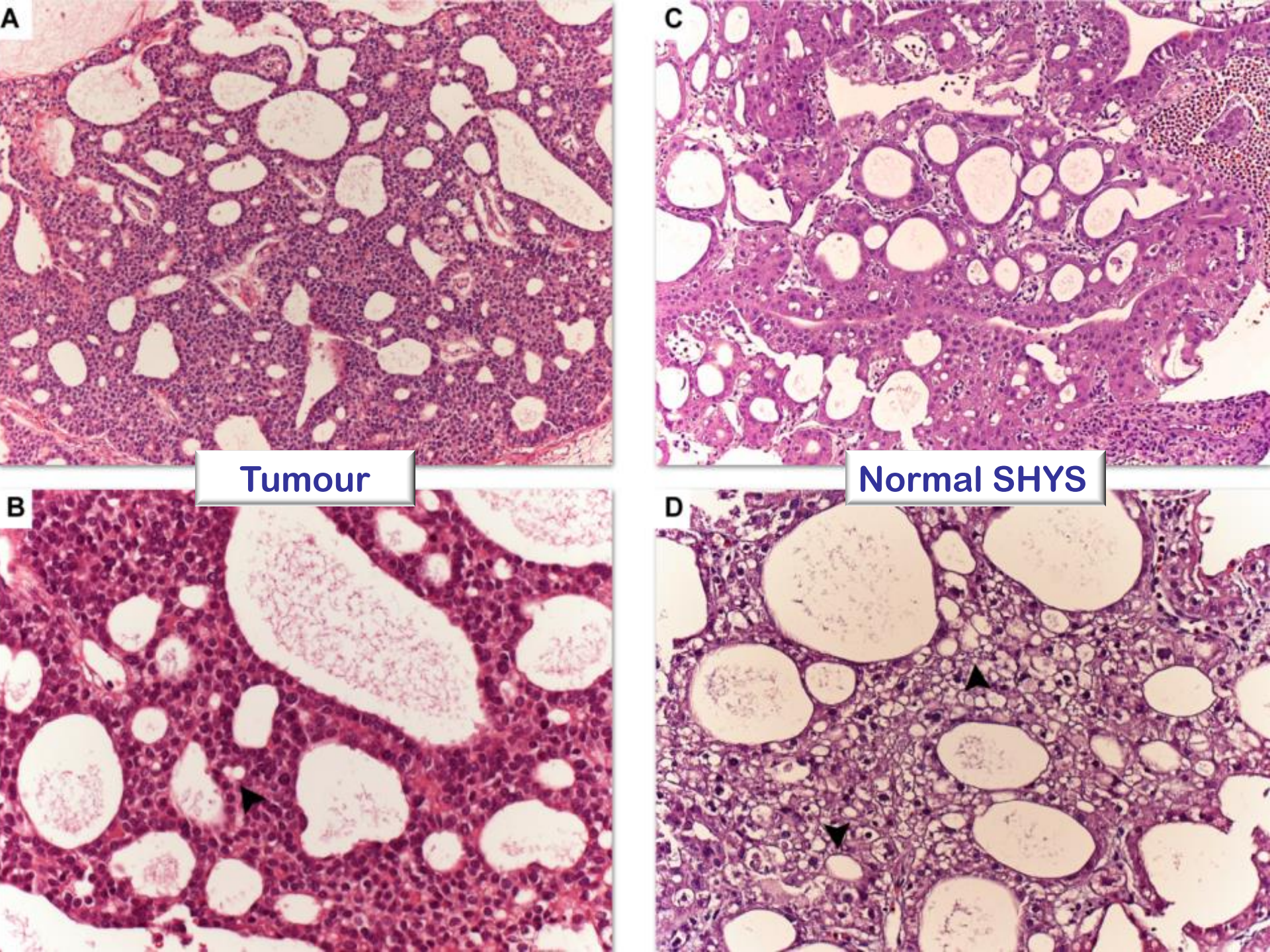
9071/3

Macroscopy

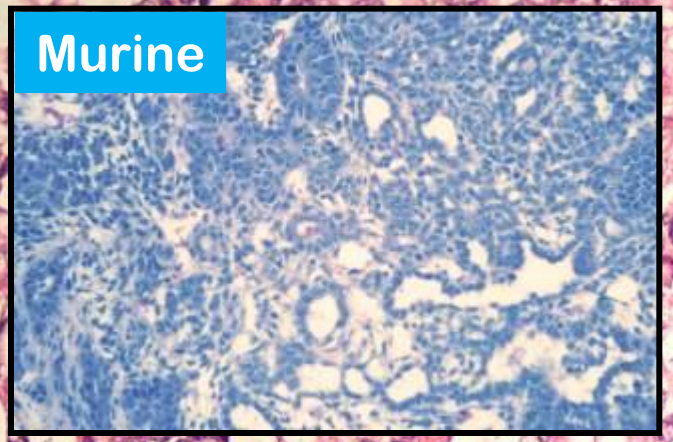
These tumours are large, soft and usually



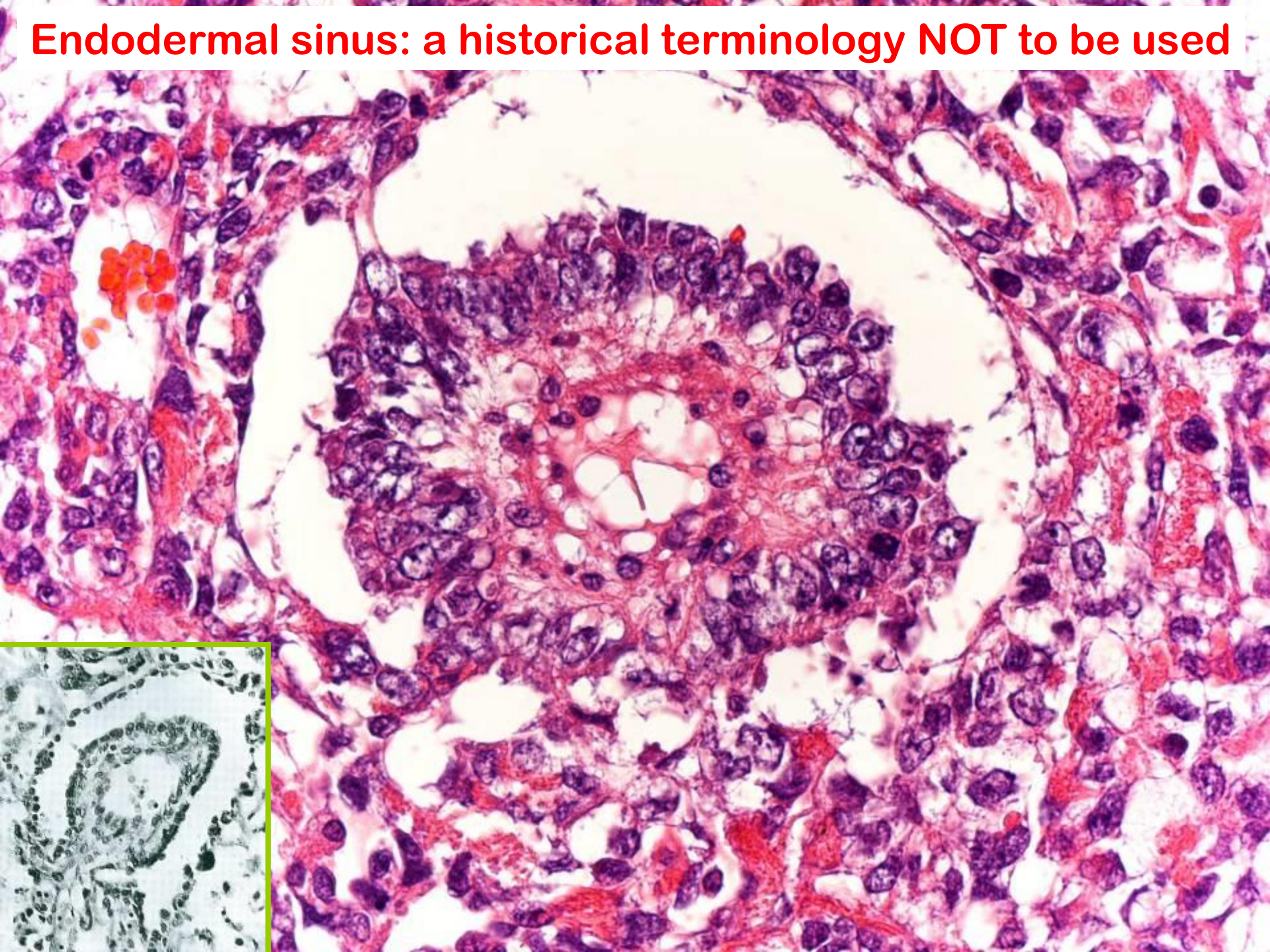
WHO 2014

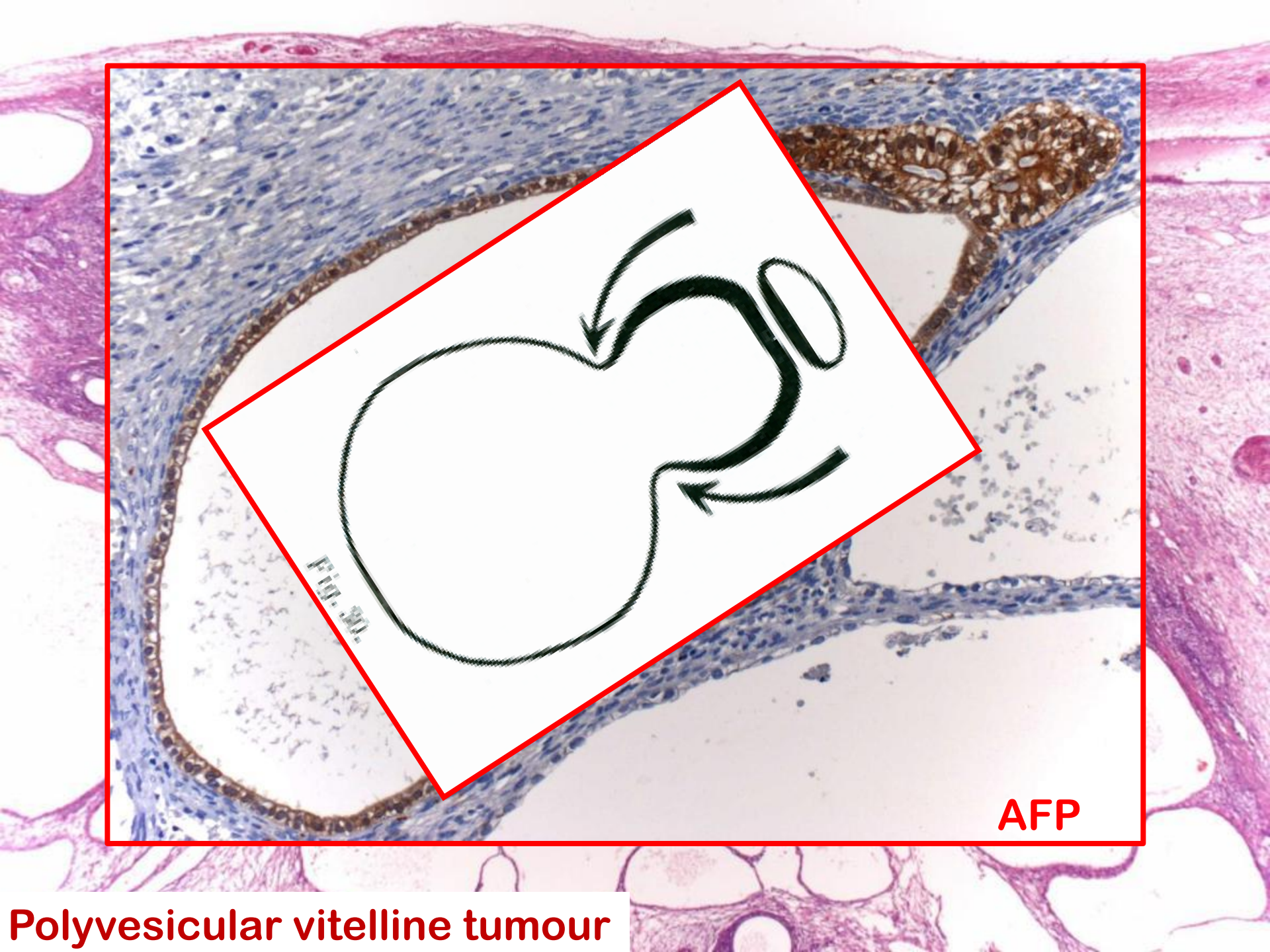

A
C
Tumour
Normal SHYS
B
D

Classic microcystic

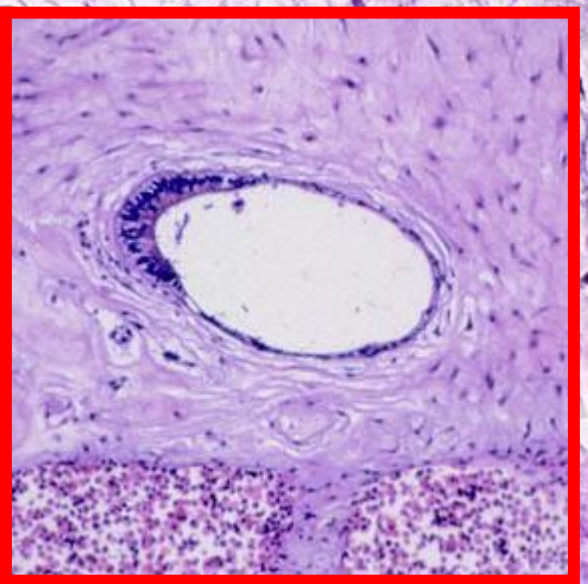


Endodermal sinus: a historical terminology NOT to be used



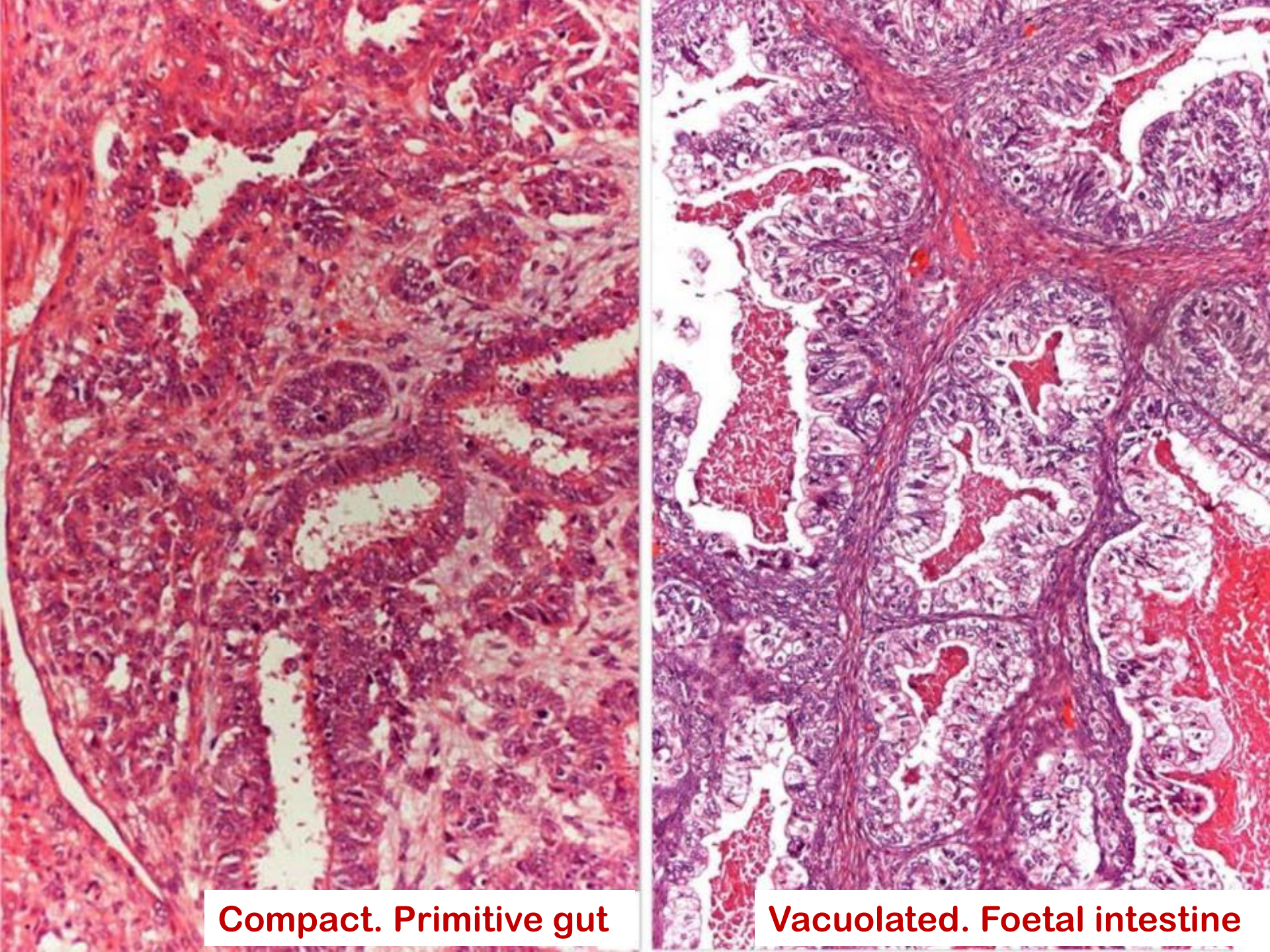


Polyvesicular vitelline tumour

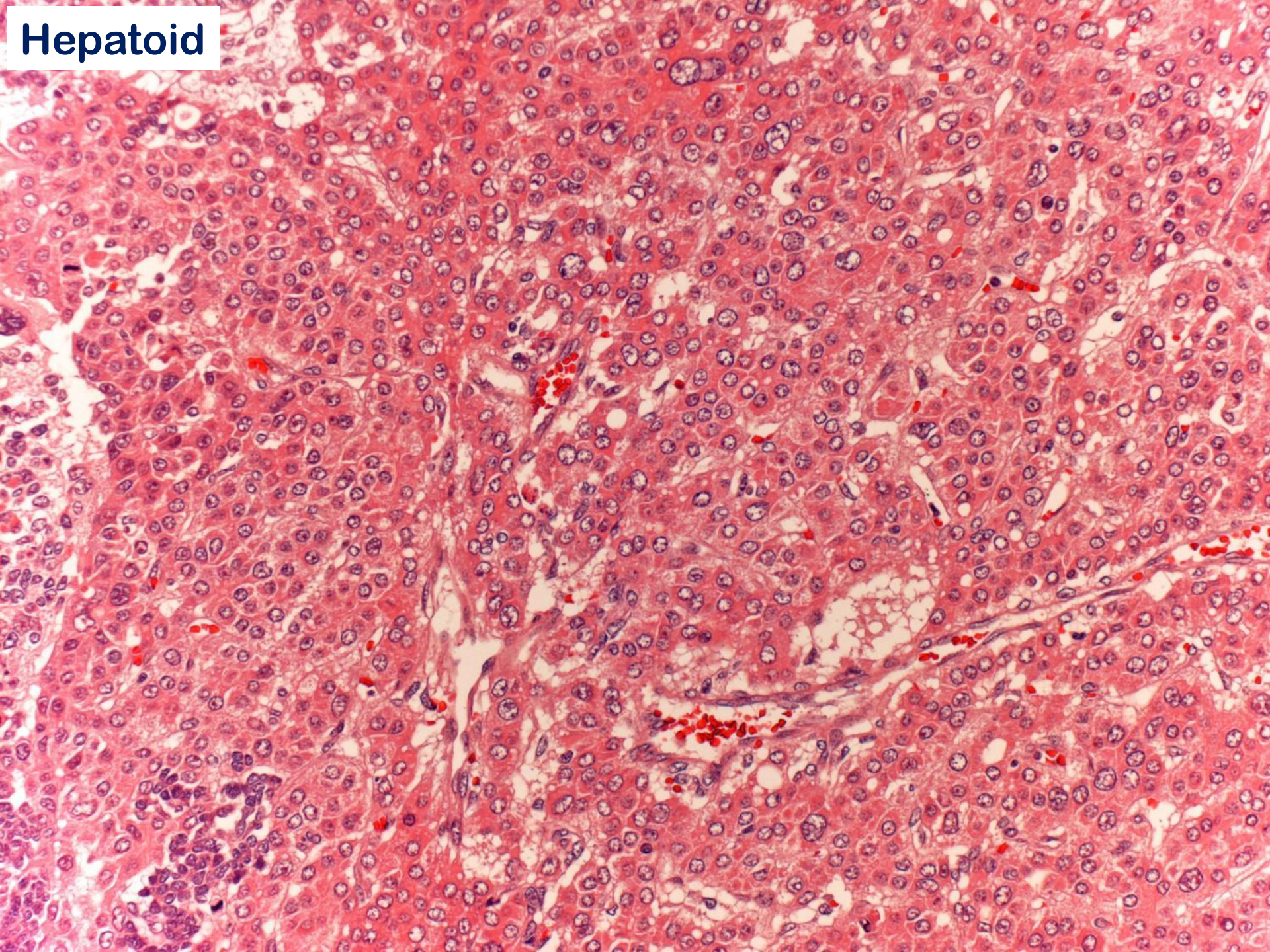


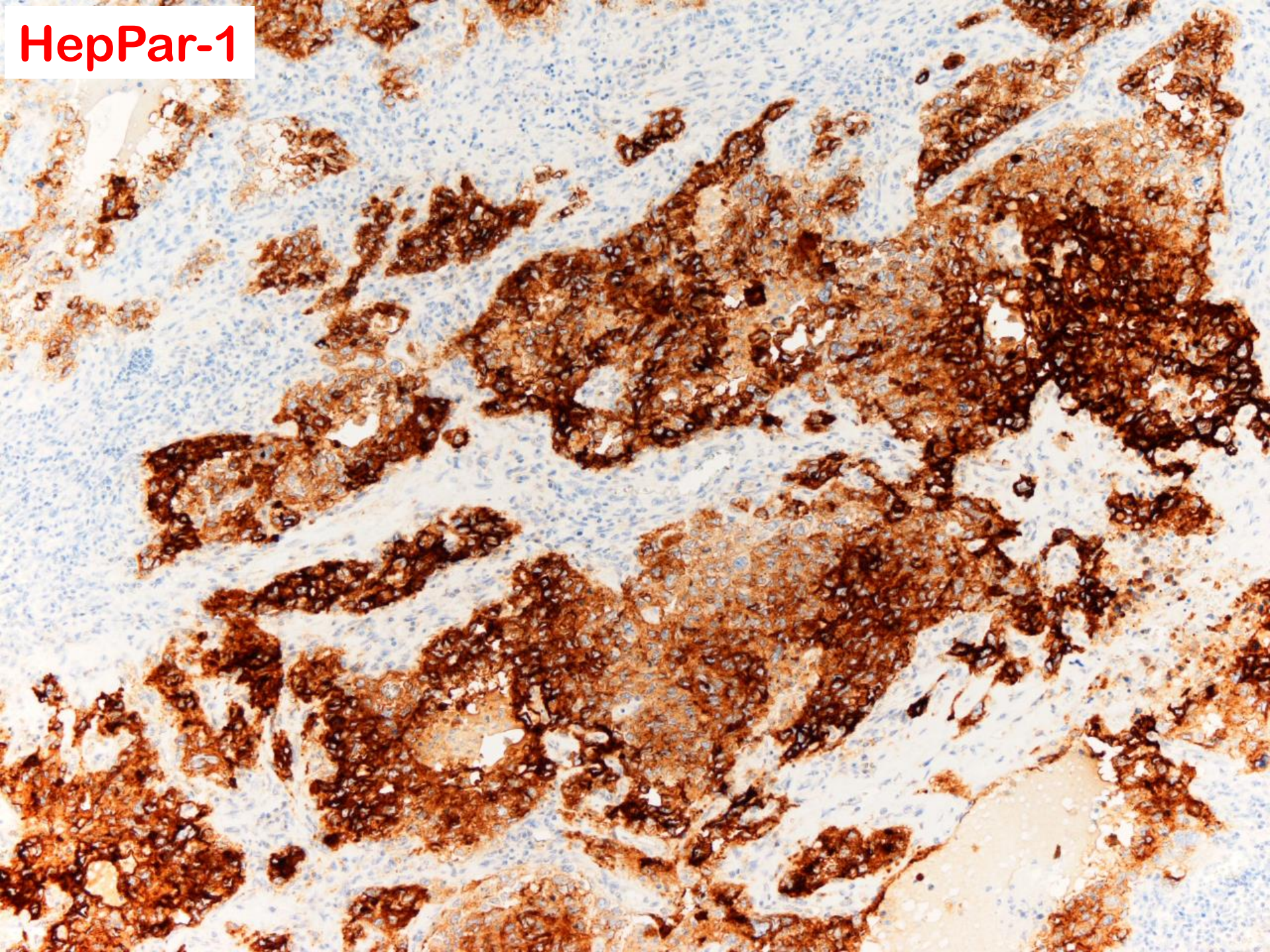
- 1: Heifetz et al. Immature teratomas in children: pathologic considerations: a report from the combined Pediatric Oncology Group/Children's Cancer Group. Am J Surg Pathol 1998;22:1115.

….morphologic diagnoses that were most frequently misinterpreted by contributing pathologists included the failure to recognize two well-differentiated patterns of YST (the **hepatoid** pattern resembling fetal liver and the **well-differentiated glandular** pattern resembling fetal lung or intestine).


Compact. Primitive gut
Vacuolated. Foetal intestine

Hepatoid




HepPar-1

Histopathology

Histopathology 2014 DOI: 10.1111/his.12373

A diagnostic immunohistochemical panel for yolk sac (primitive endodermal) tumours based on an immunohistochemical comparison with the human yolk sac

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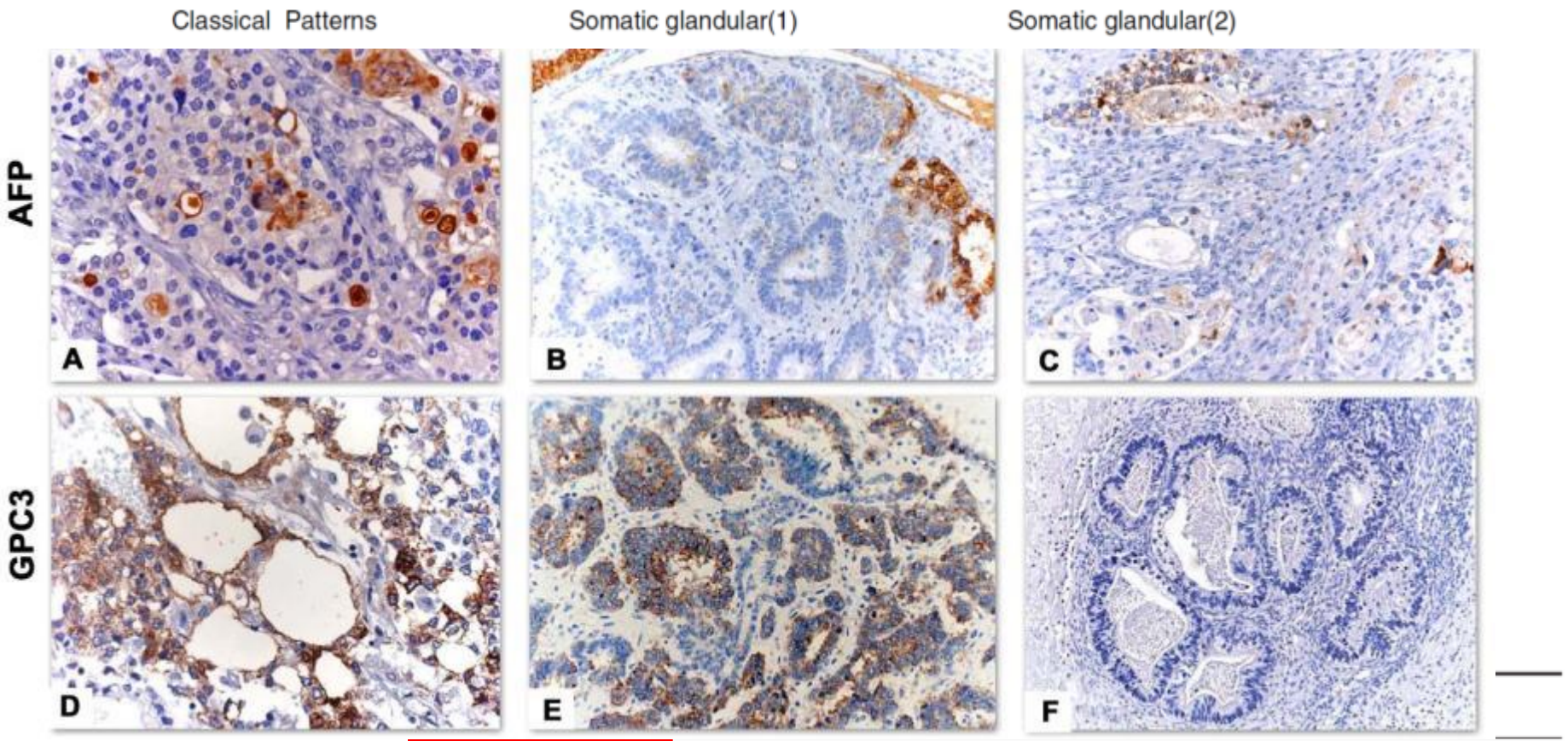
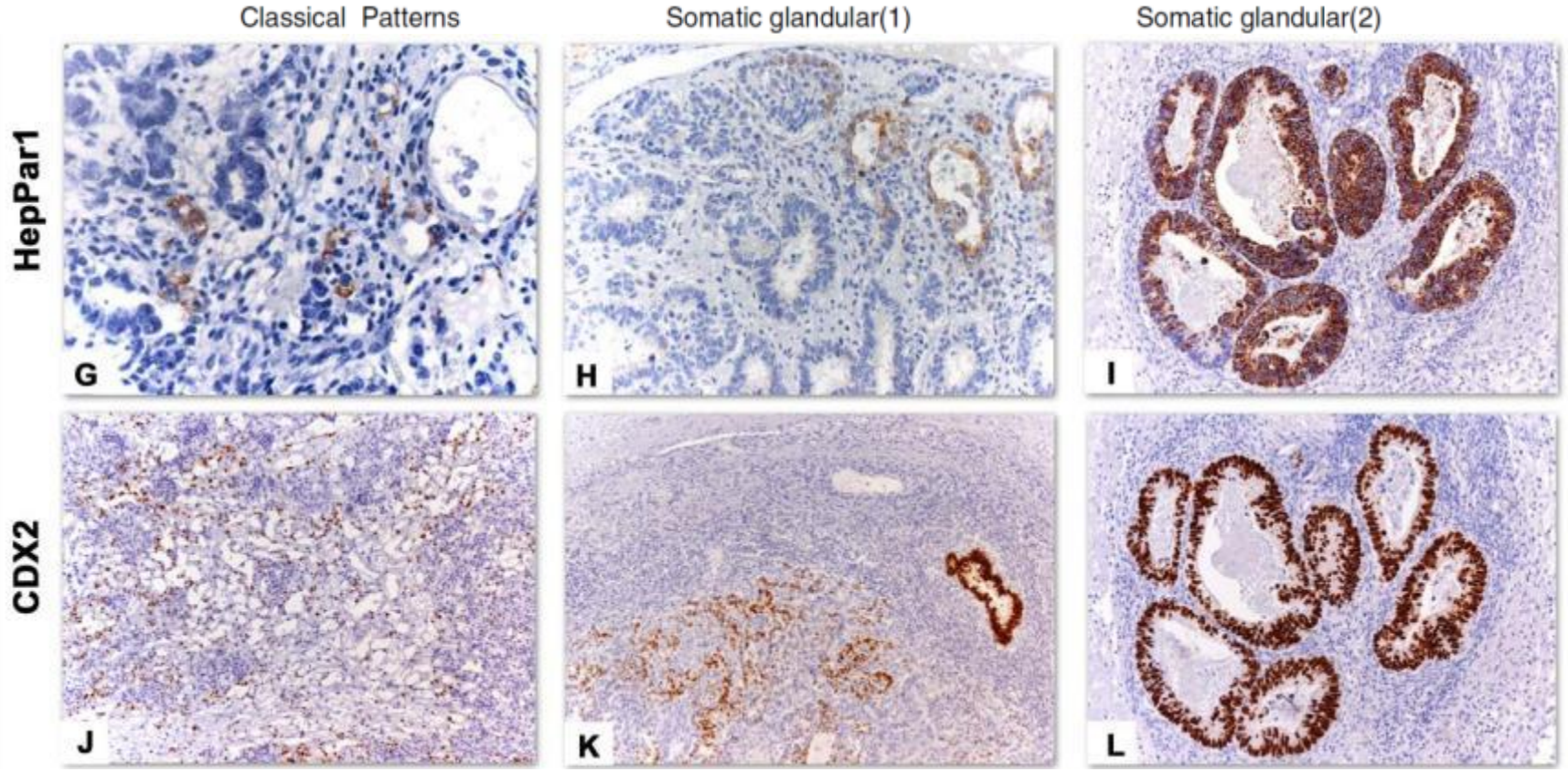
(2014) *Histopathology*



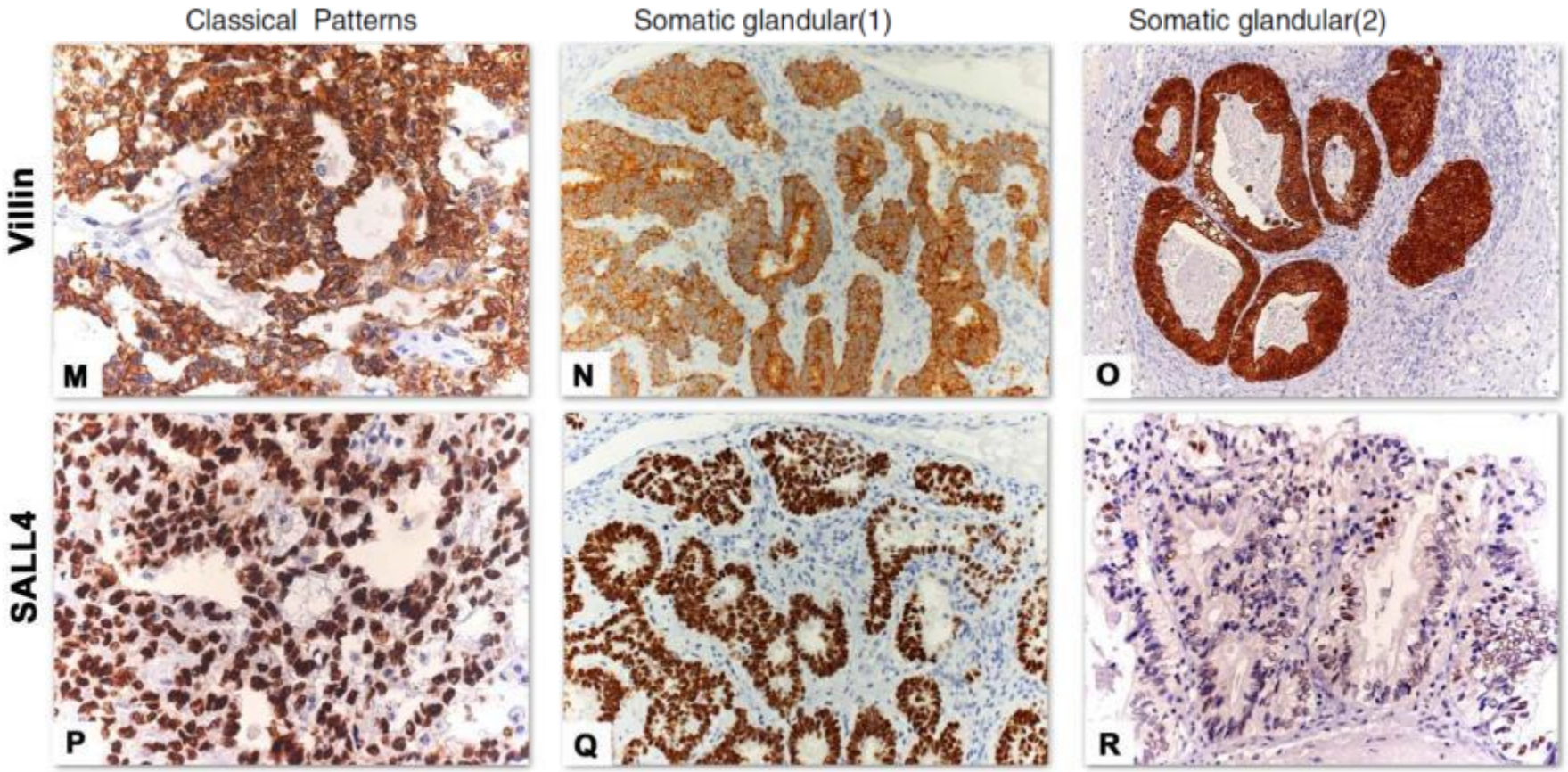
Histology	AFP	GPC3	HepPar–1	CDX2	Villin	TTF–1	SALL4	LIN28
Classical patterns								
Microcystic/reticular	14/14 H	14/14 D	14/14 F	14/14 F	14/14 D	–	13/13D	10/10 D
Polyvesicular	1/1 H	1/1 D	1/1 F	1/1 F	1/1 D	1/1	1/1 D	1/1 D
Hepatoid	1/1 H	1/1 D	1/1 D	1/1 H	1/1 D	–	1/1 D	1/1 H
Somatic glandular patterns	7/9 F	7/9 H	7/9 F	7/9 H	9/9D	3/5F	8/9 D	4/5 D
Normal human yolk sac[6]	D	D	D	D	D	ND	D	1/1 (5th week) 0/6 (7–8th weeks)

H, heterogeneous; D, diffuse; F, focal; ND, not done.



	Immunohistochemical staining results							
Histology	AFP	GPC3	HepPar–1	CDX2	Villin	TTF–1	SALL4	LIN28
Classical patterns								
Microcystic/reticular	14/14 H	14/14 D	14/14 F	14/14 F	14/14 D	–	13/13D	10/10 D
Polyvesicular	1/1 H	1/1 D	1/1 F	1/1 F	1/1 D	1/1	1/1 D	1/1 D
Hepatoid	1/1 H	1/1 D	1/1 D	1/1 H	1/1 D	–	1/1 D	1/1 H
Somatic glandular patterns	7/9 F	7/9 H	7/9 F	7/9 H	9/9D	3/5F	8/9 D	4/5 D
Normal human yolk sac[6]	D	D	D	D	D	ND	D	1/1 (5th week) 0/6 (7–8th weeks)

H, heterogeneous; D, diffuse; F, focal; ND, not done.



Immunohistochemical staining results

Histology	AFP	GPC3	HepPar–1	CDX2	Villin	TTF–1	SALL4	LIN28
Classical patterns								
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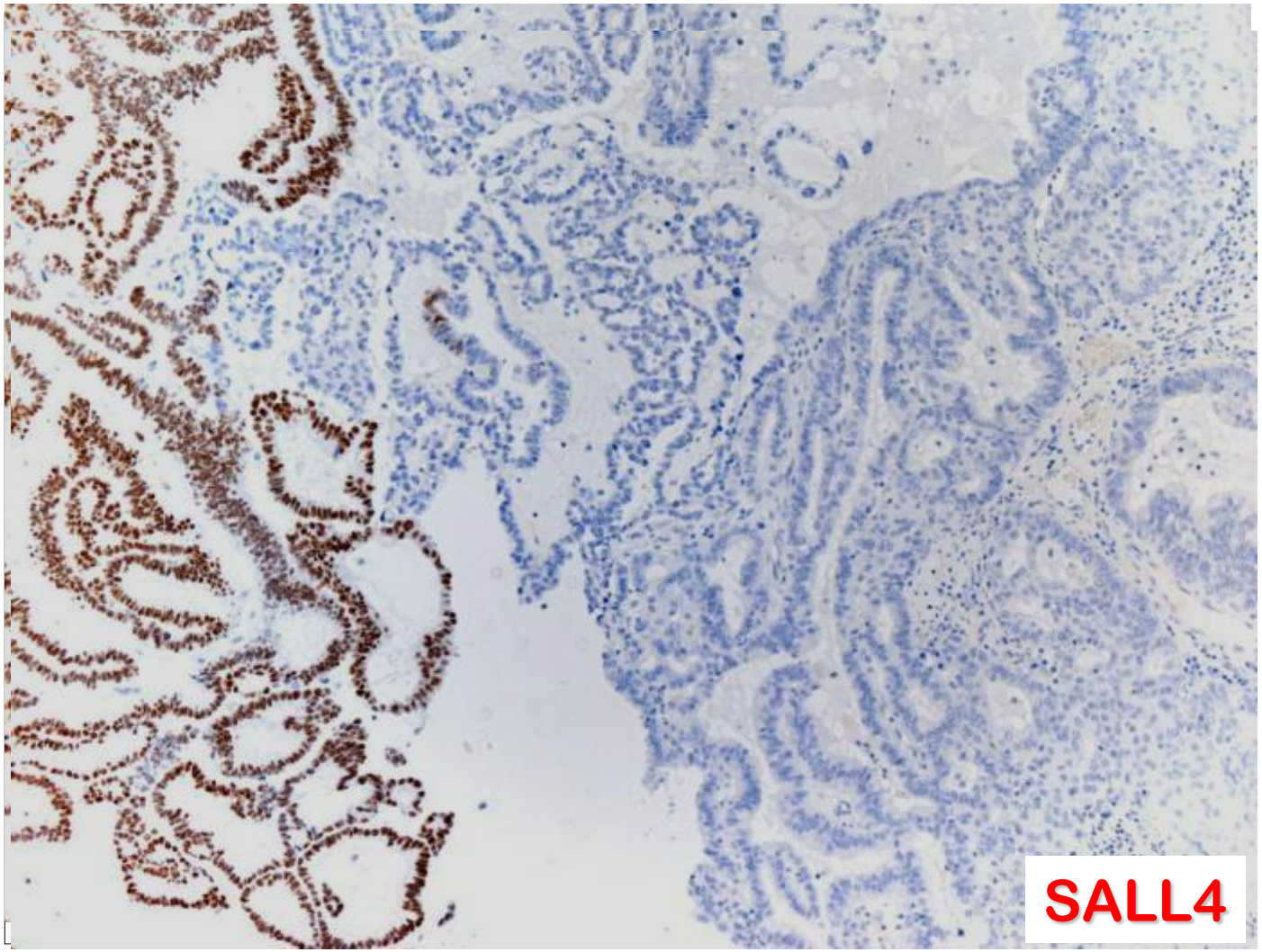
Polyvesicular	1/1 H	1/1 D	1/1 F	1/1 F	1/1 D	1/1	1/1 D	1/1 D
Hepatoid	1/1 H	1/1 D	1/1 D	1/1 H	1/1 D	–	1/1 D	1/1 H
Somatic glandular patterns	7/9 F	7/9 H	7/9 F	7/9 H	9/9D	3/5F	8/9 D	4/5 D
Normal human yolk sac[6]	D	D	D	D	D	ND	D	1/1 (5th week) 0/6 (7–8th weeks)

H, heterogeneous; D, diffuse; F, focal; ND, not done.

Interpretation problems in special patterns.

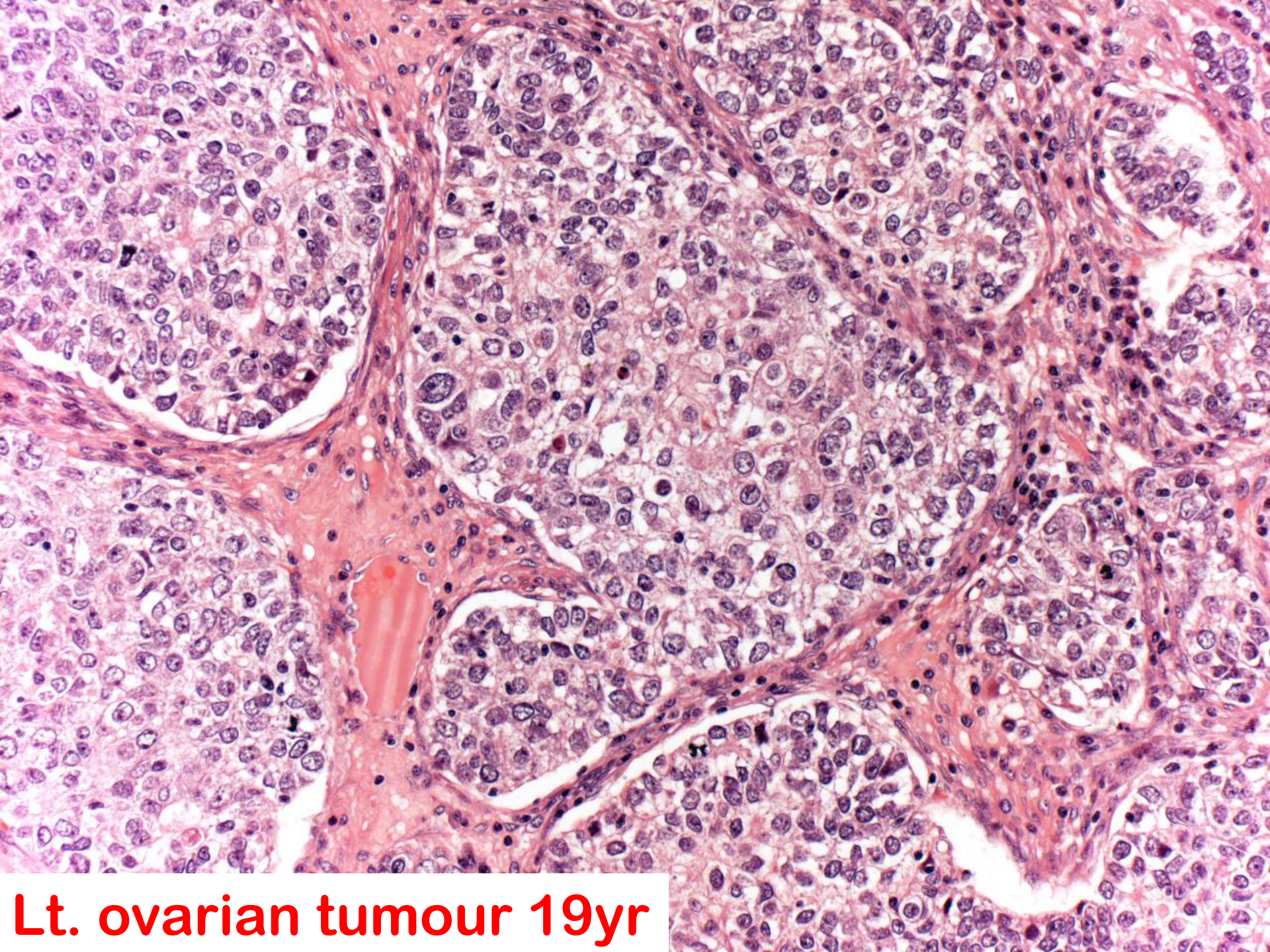
- In absence of classical patterns
- In older age groups
  - Associated to somatic tumours

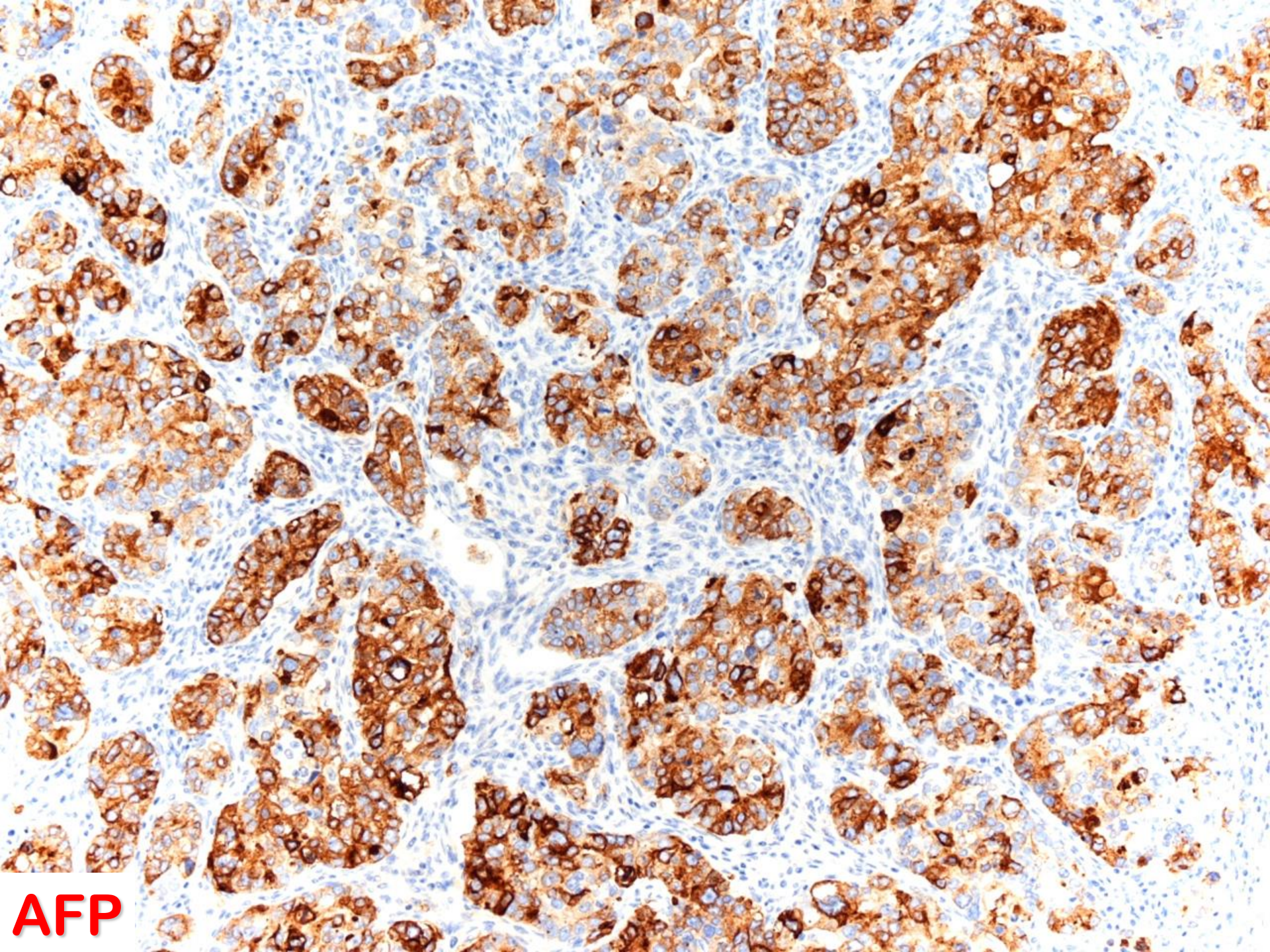
Markers differentiate YST from somatic tumours

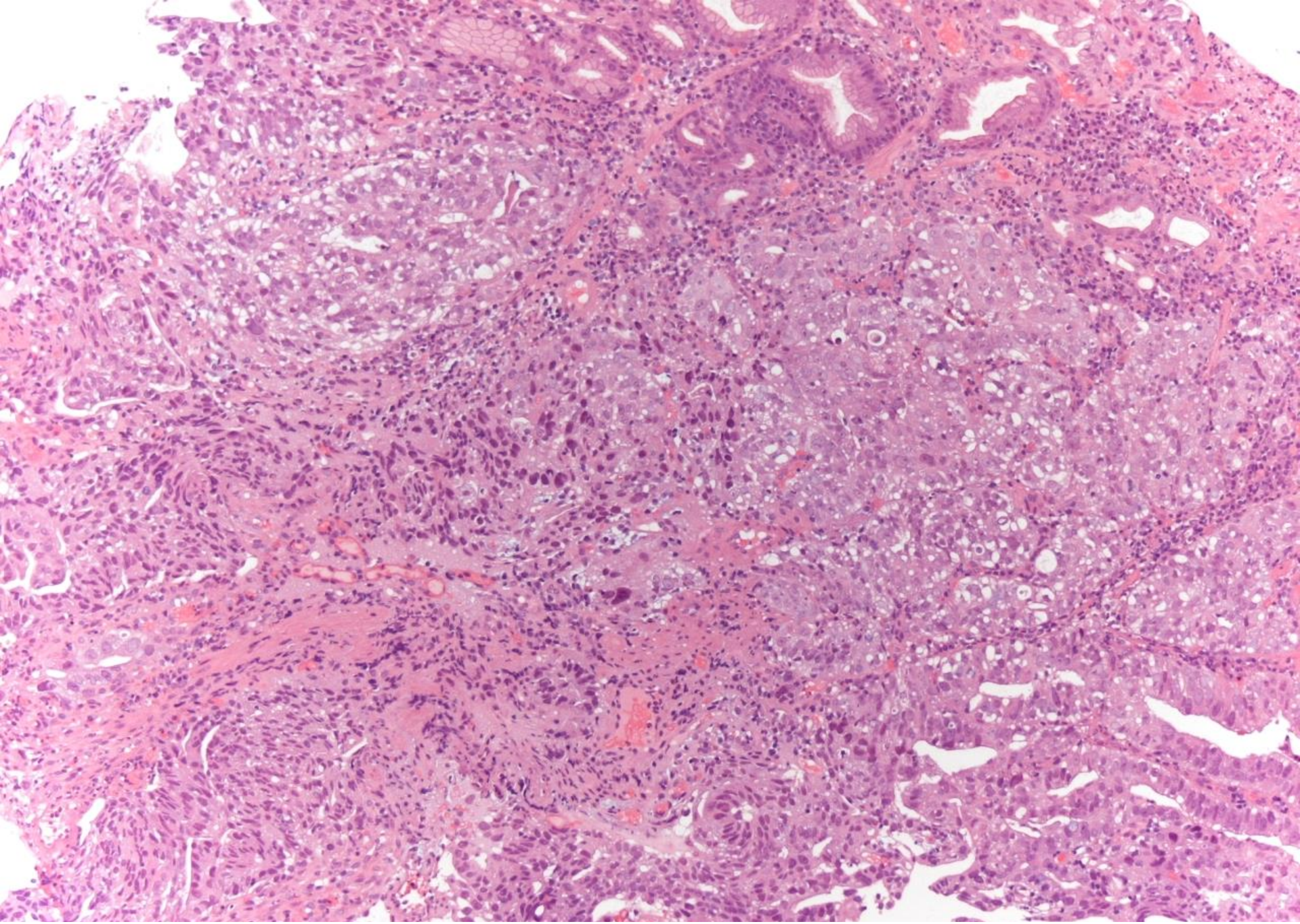


Interpretation problems

- In absence of classical patterns
- In older age groups
  - Associated to somatic tumours
  - Gastric carcinoma metastases


Lt. ovarian tumour 19yr

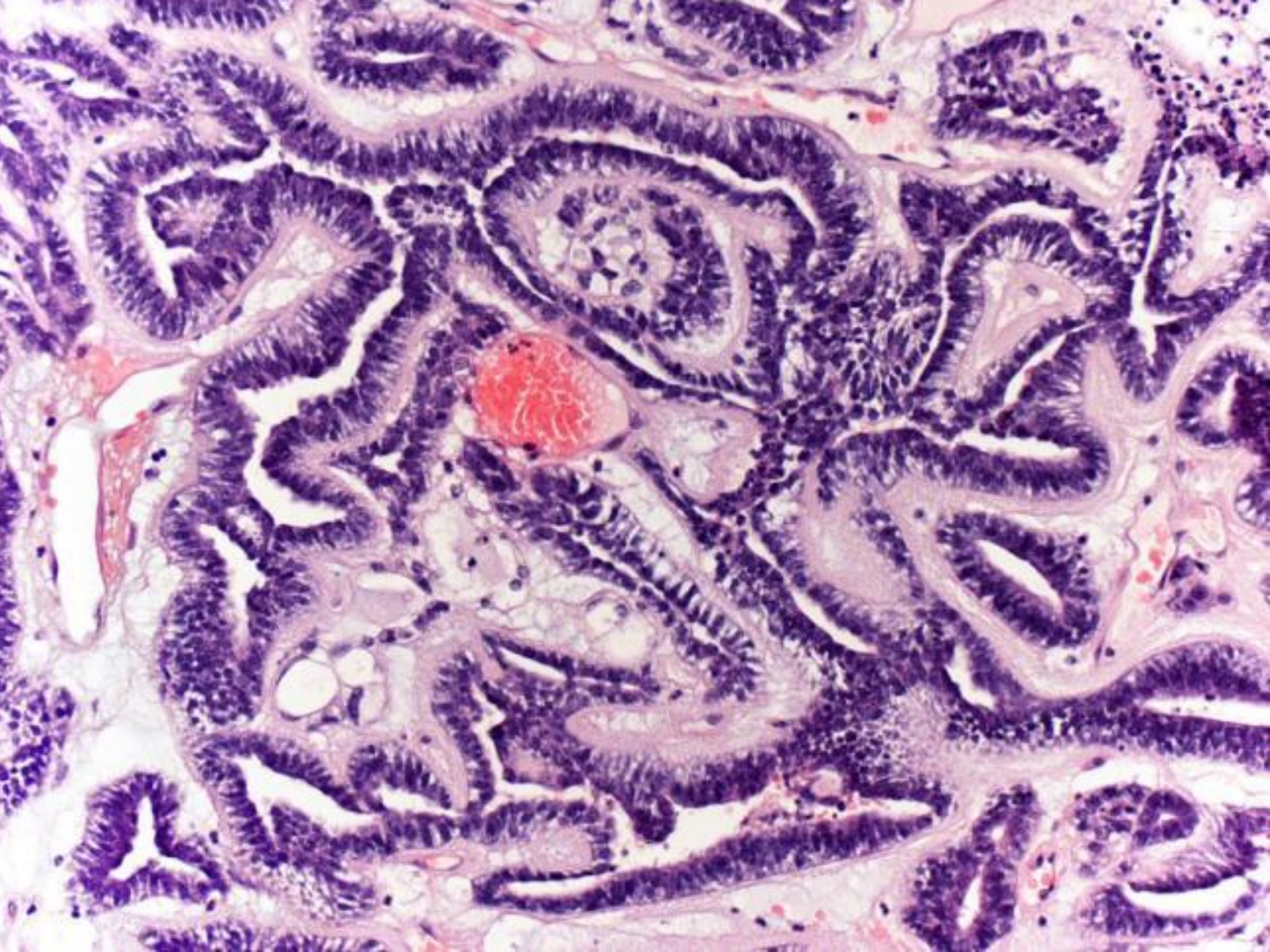

AFP

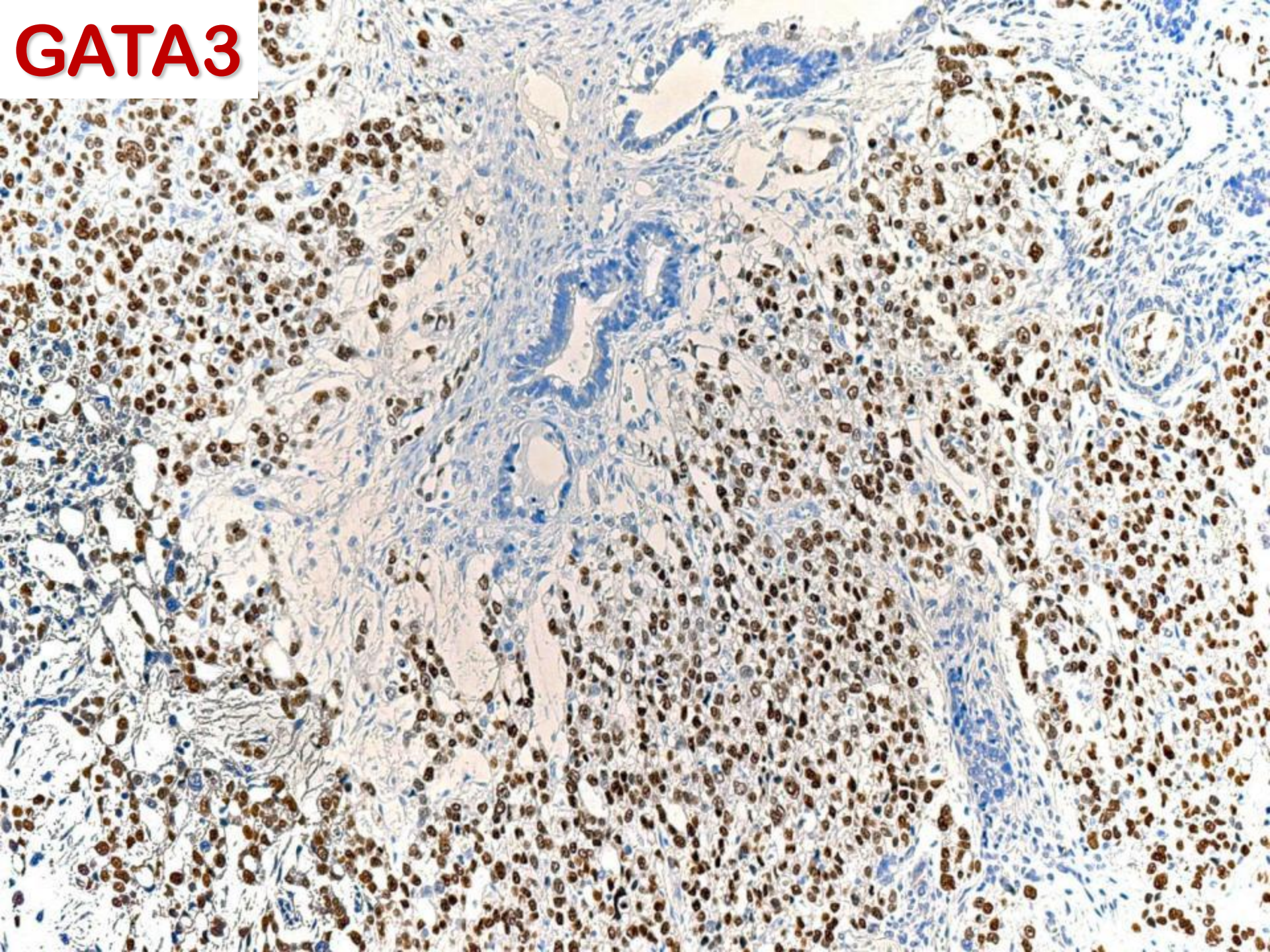


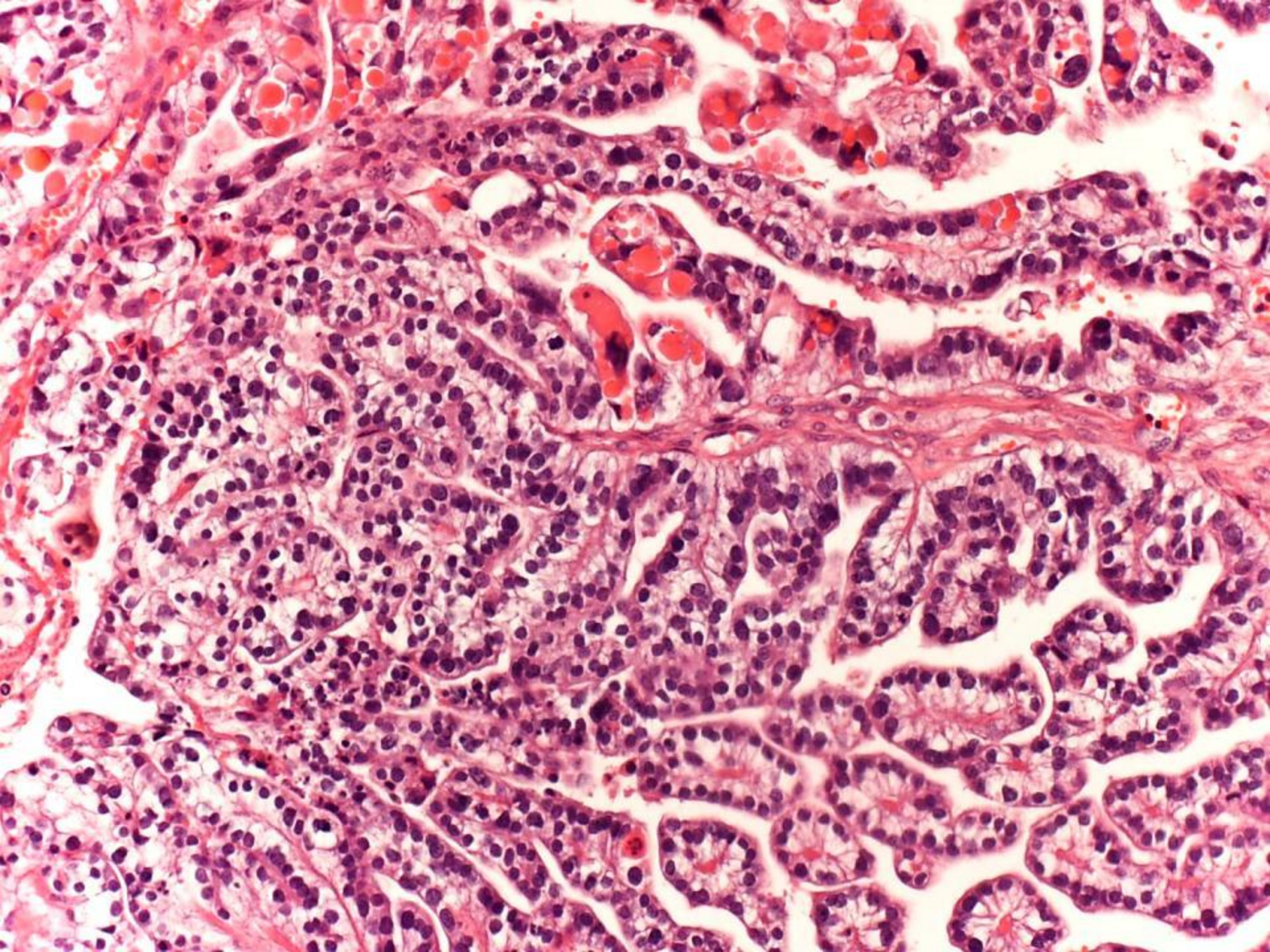
Clear-cell, alpha-foetoprotein-producing gastric carcinoma with hepatoid differentiation.

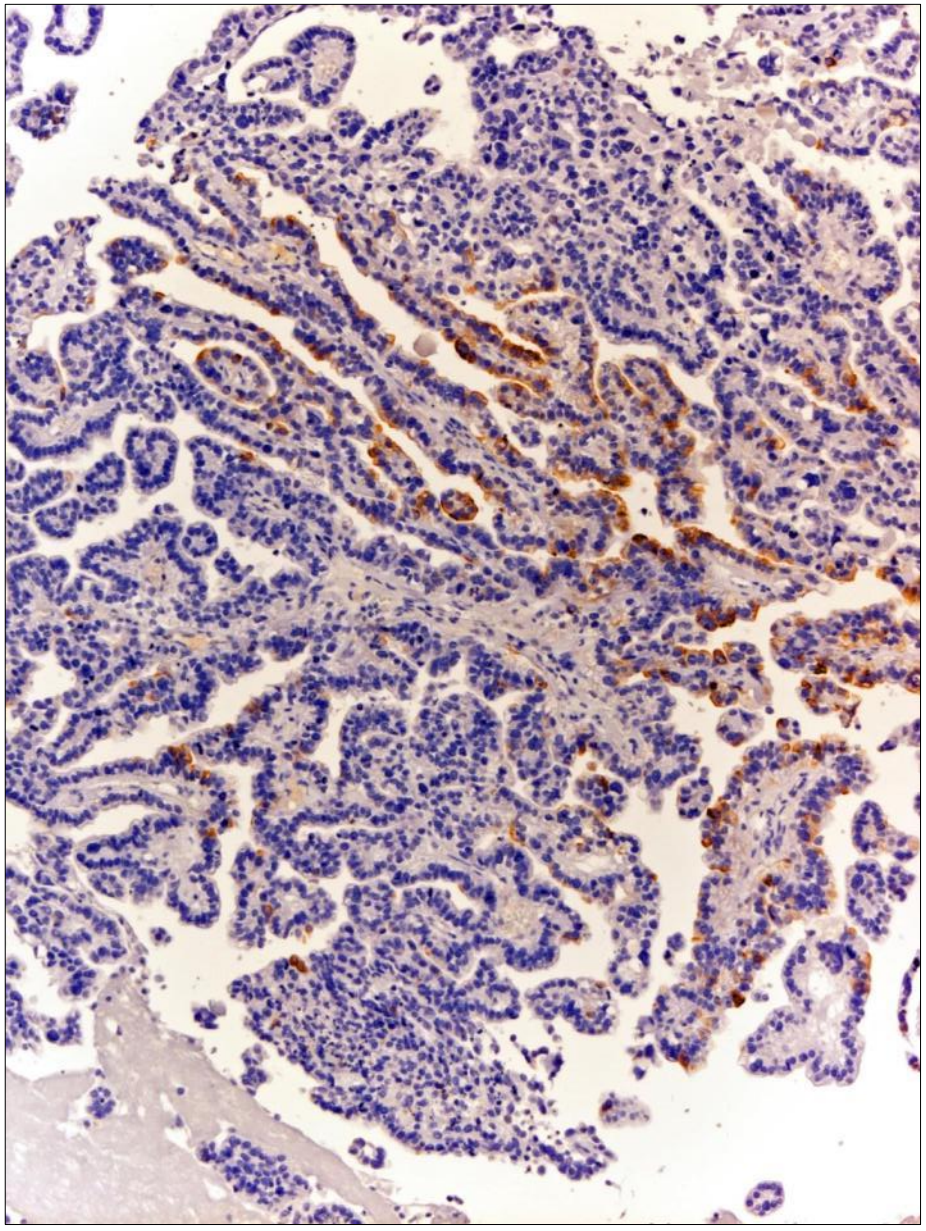
Interpretation problems

- Glandular patterns
  - Compact glands
  - Vacuolated (intestinal)
  - Papillary

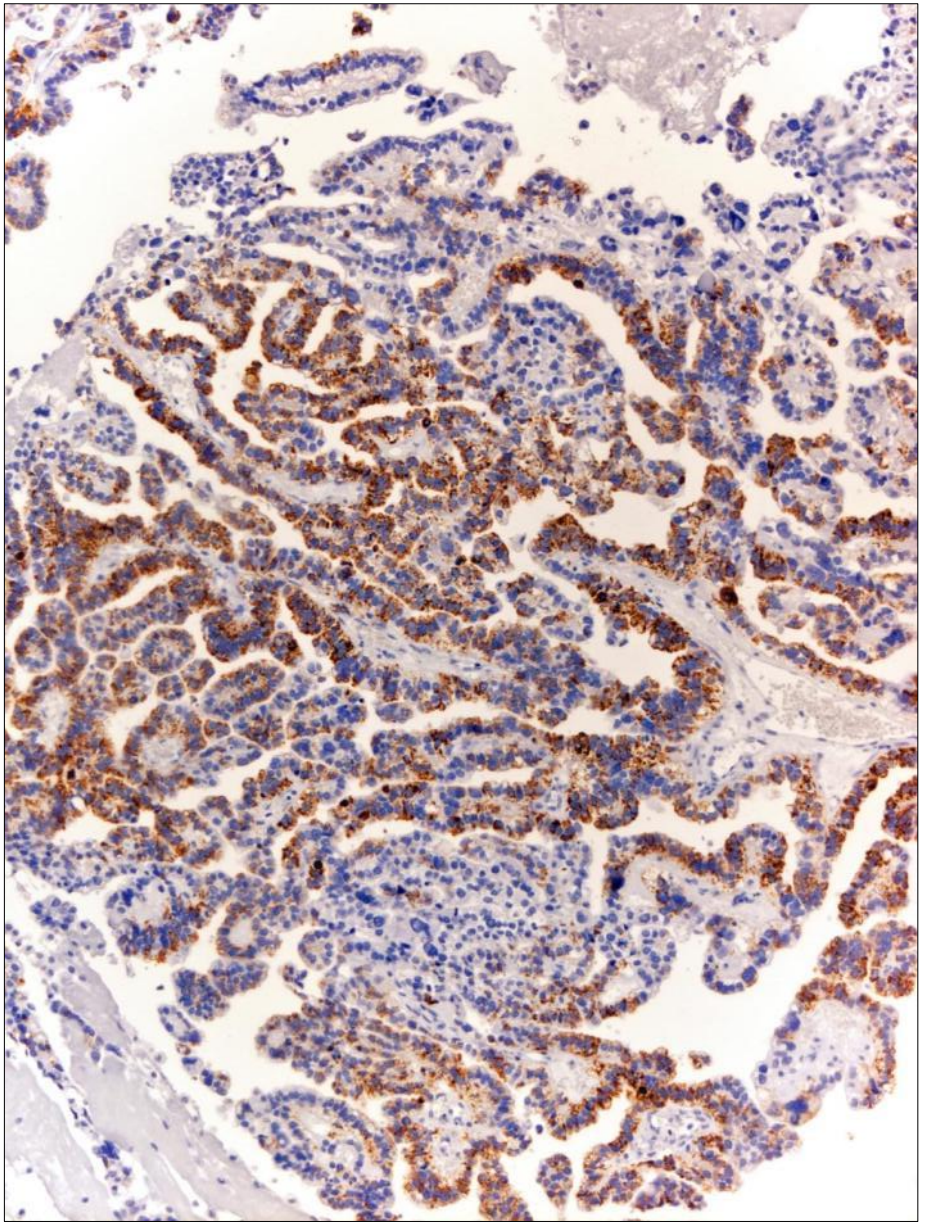



GATA3





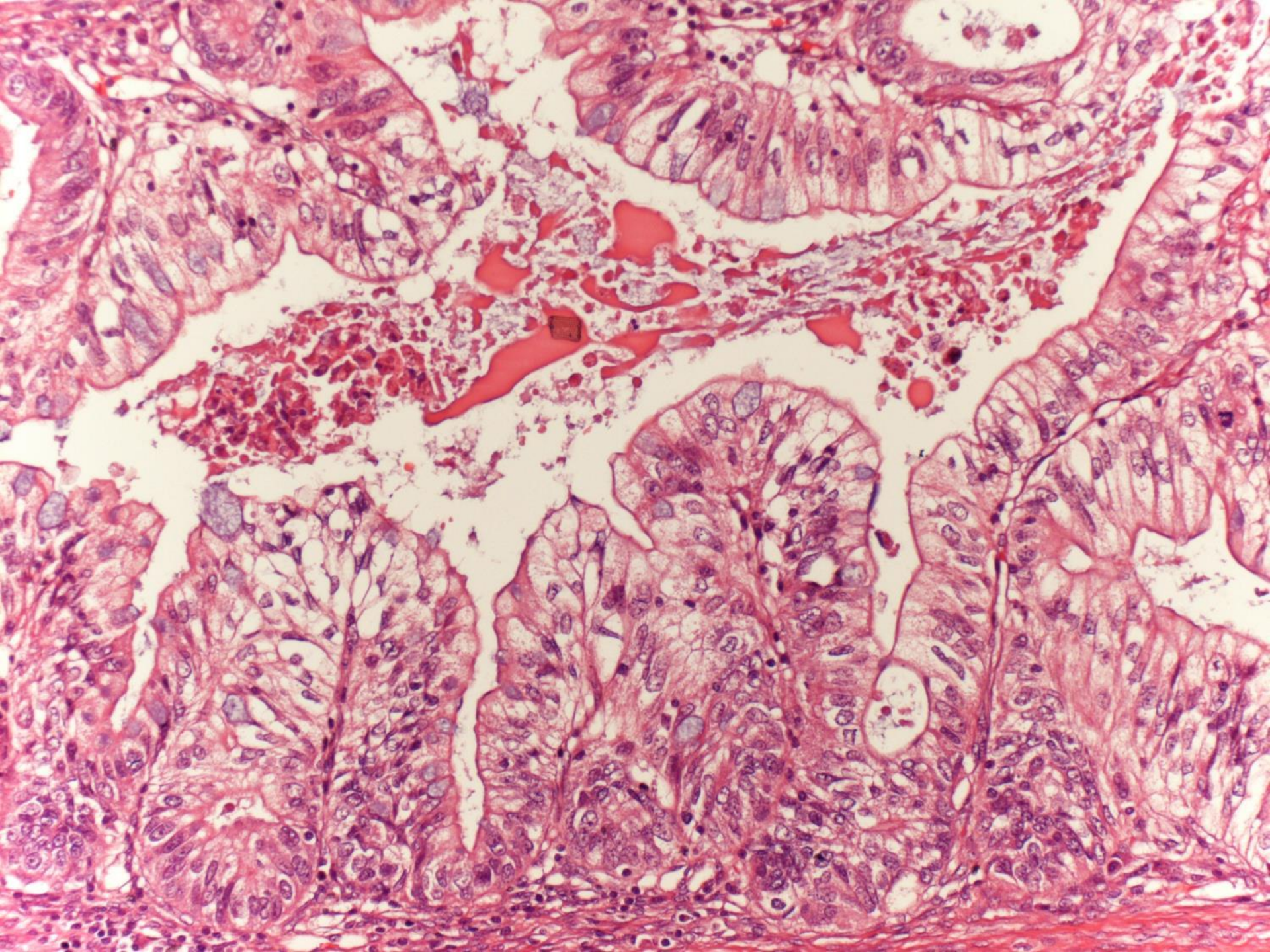
AFP

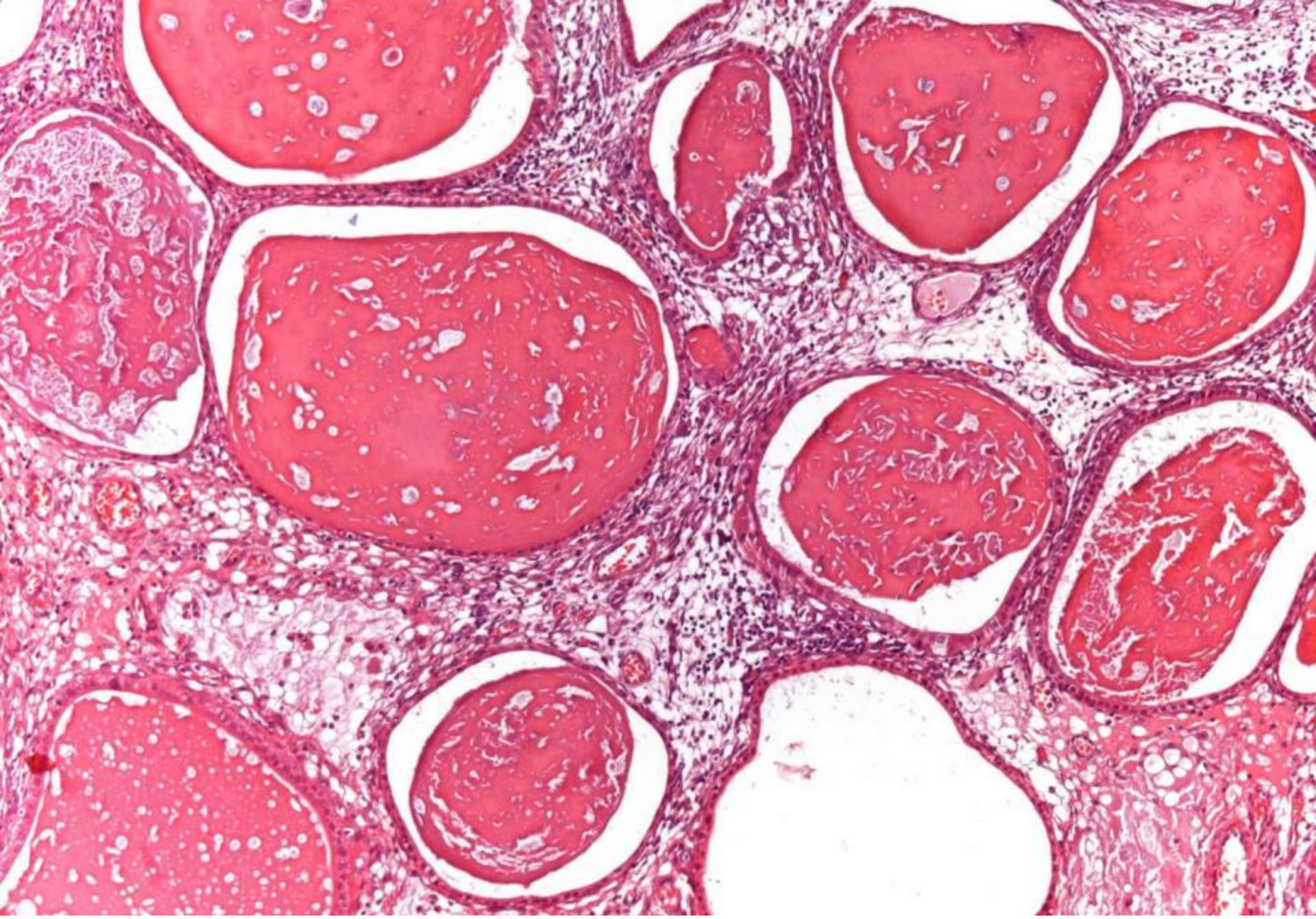


HepPar1

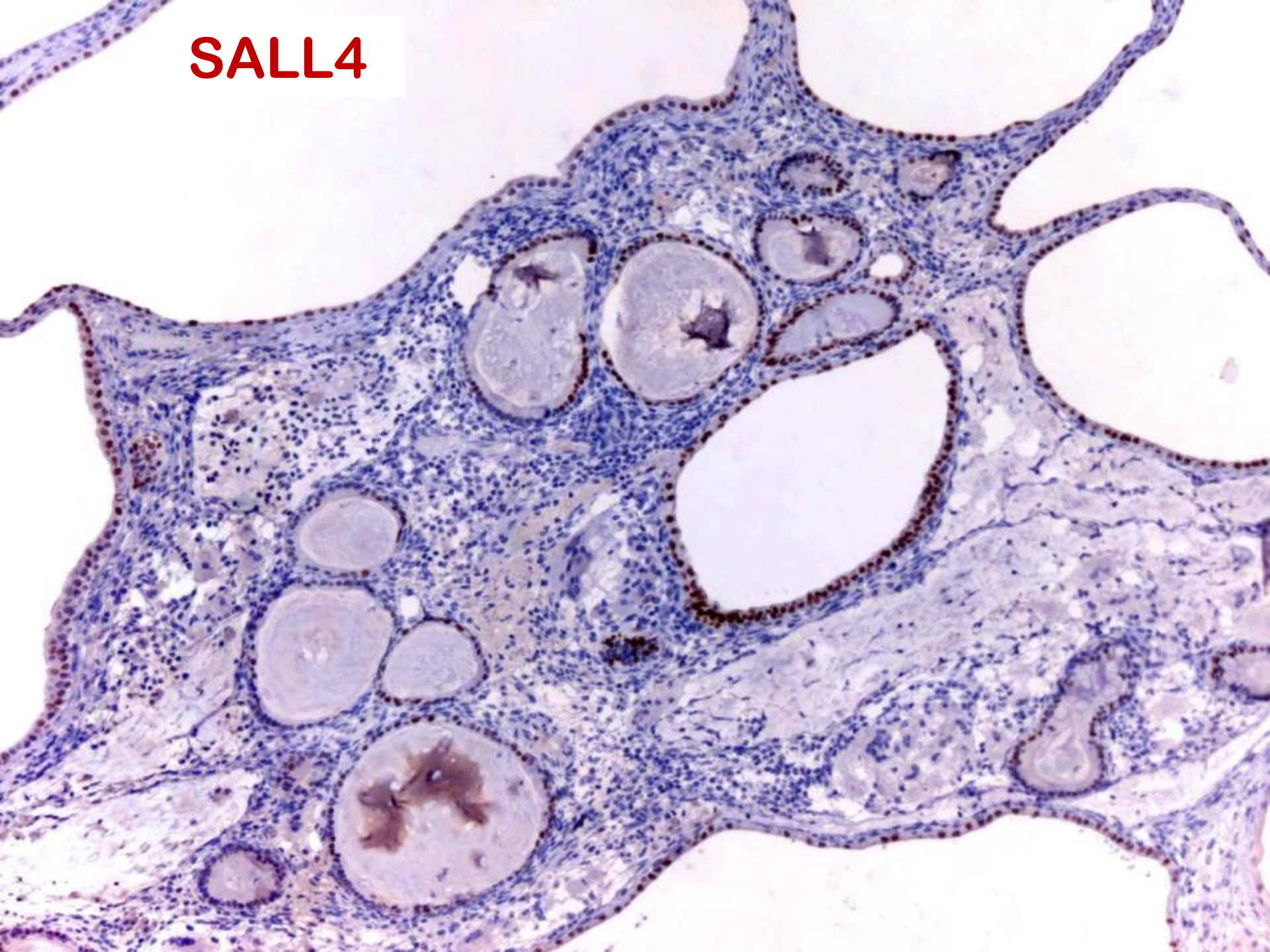
Interpretation problems

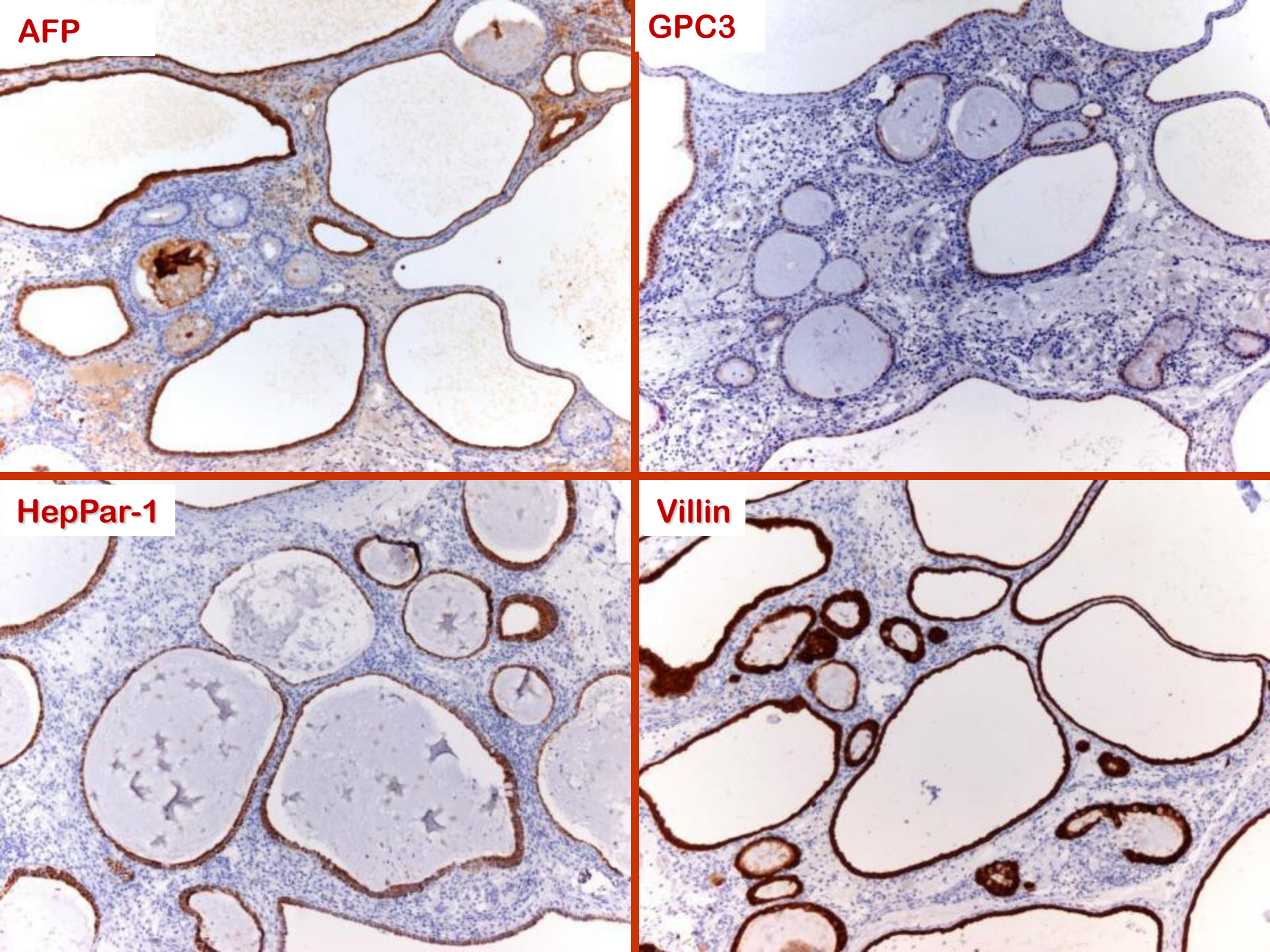
- Glandular patterns
  - Compact glands
  - Vacuolated (intestinal)
  - Papillary
- Mimicking intestinal-type mucinous tumours





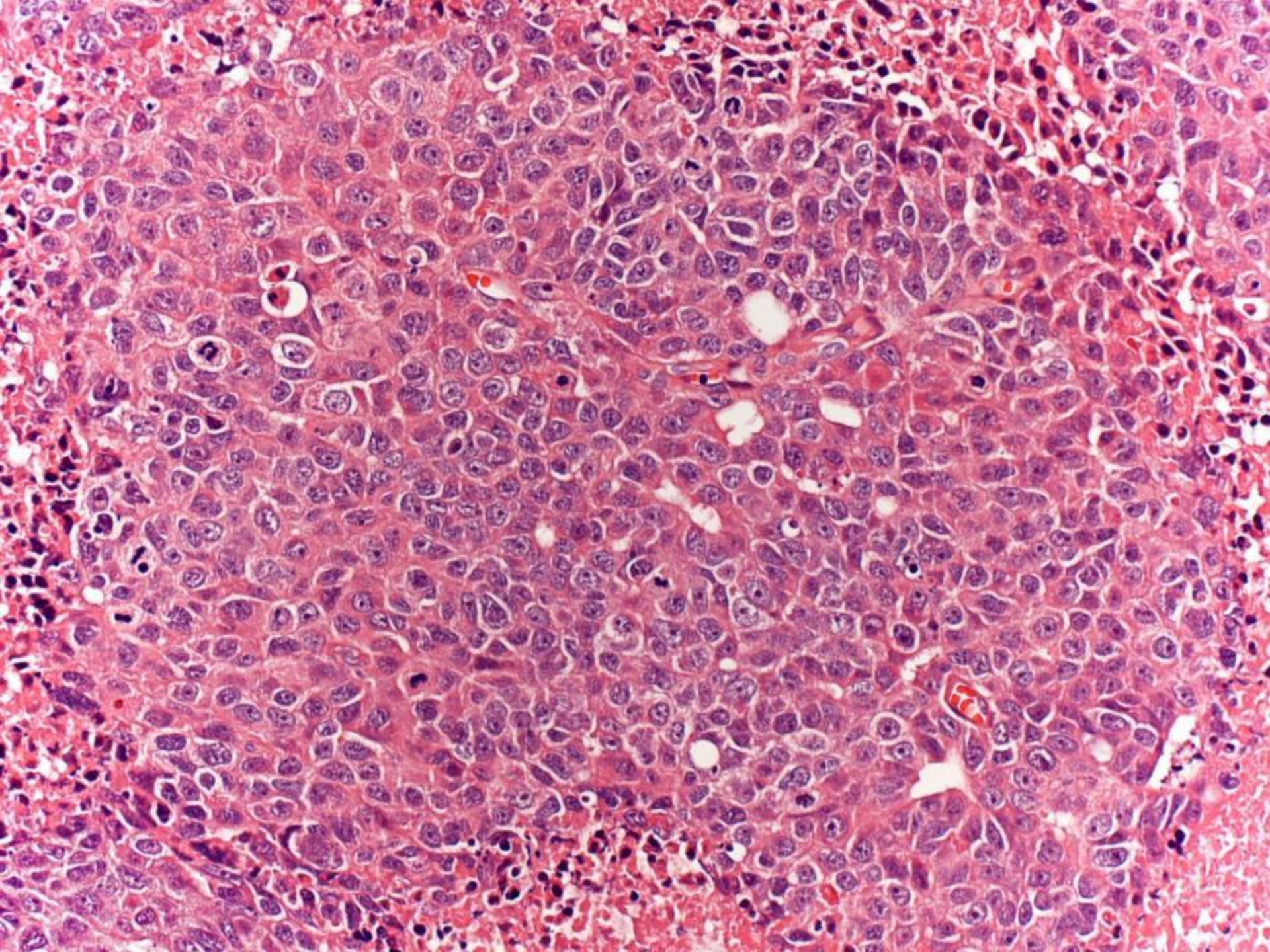
20cm ovarian tumour in a 24 yr old


SALL4


AFP
GPC3
HepPar-1
Villin

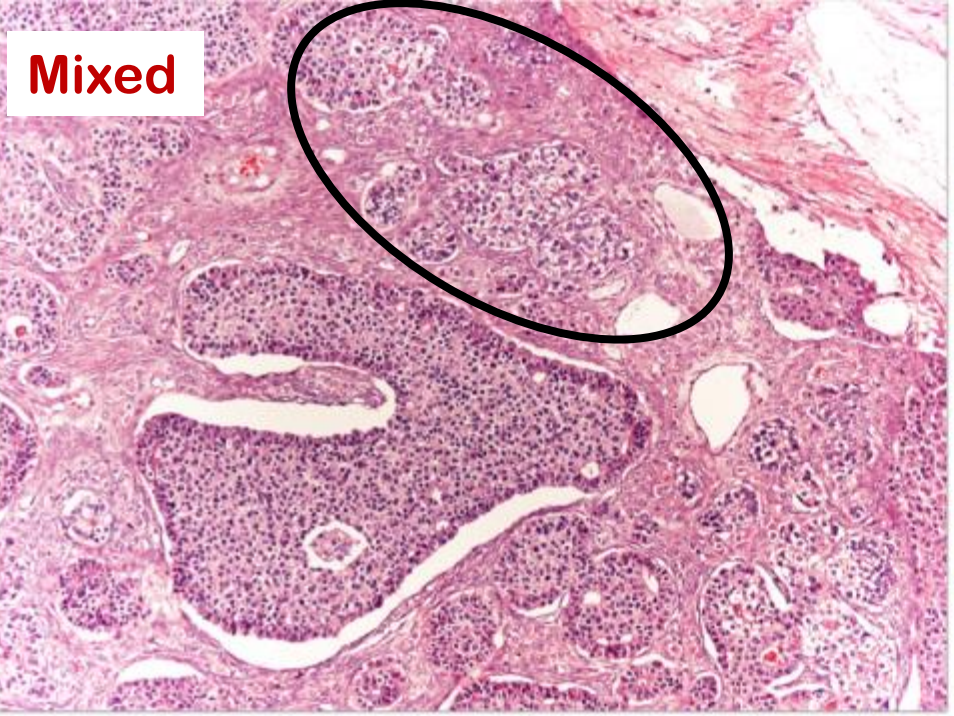
Interpretation problems

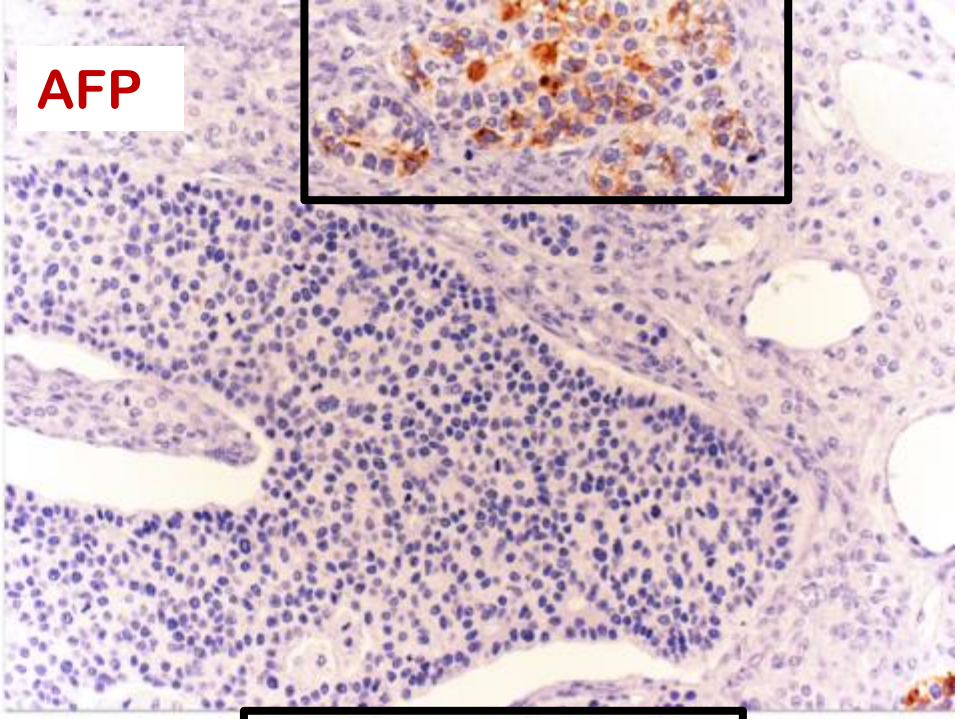
- Glandular patterns
  - Compact glands
  - Vacuolated (intestinal)
  - Papillary
- Mimicking intestinal-type mucinous tumours
- Solid



Interpretation problems

- Glandular patterns
  - Compact glands
  - Vacuolated (intestinal)
- Mimicking intestinal-type mucinous tumours
- Solid
- Carcinoid-associated


Mixed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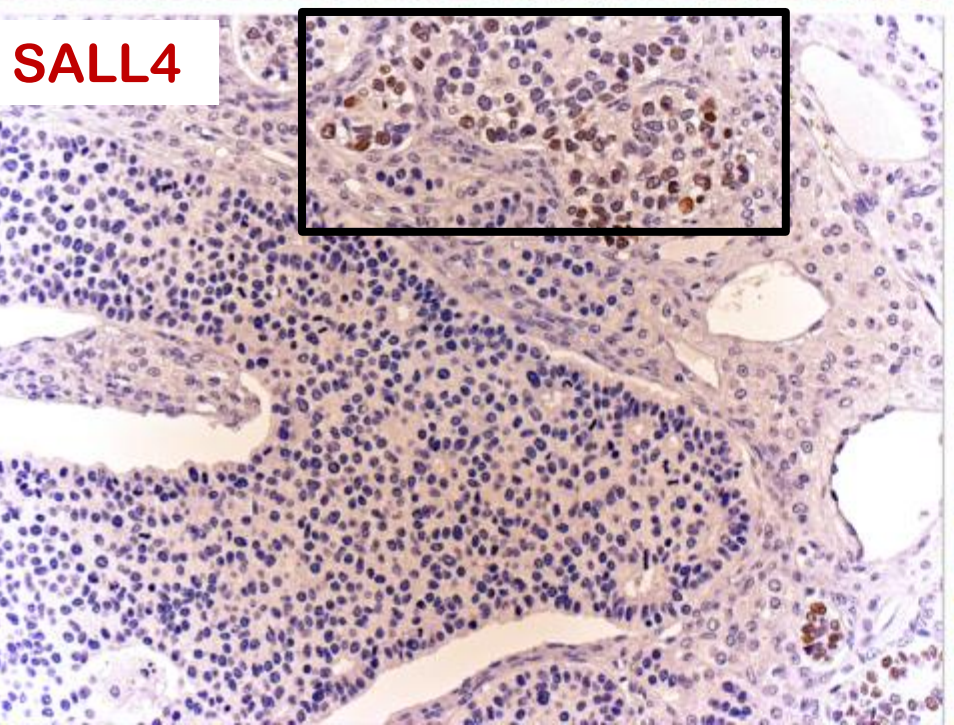
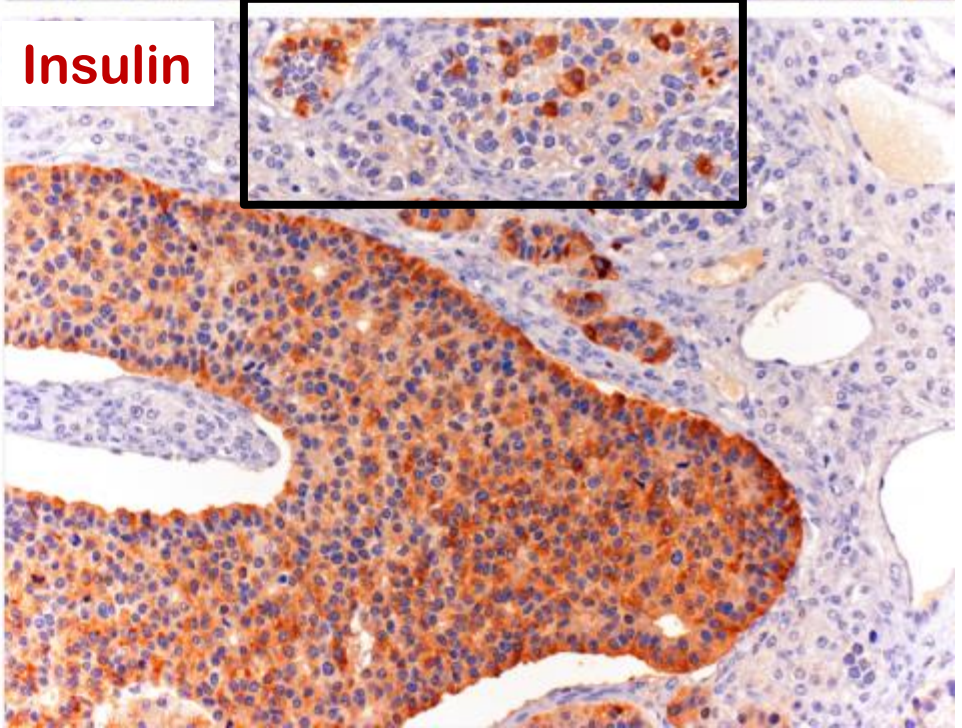
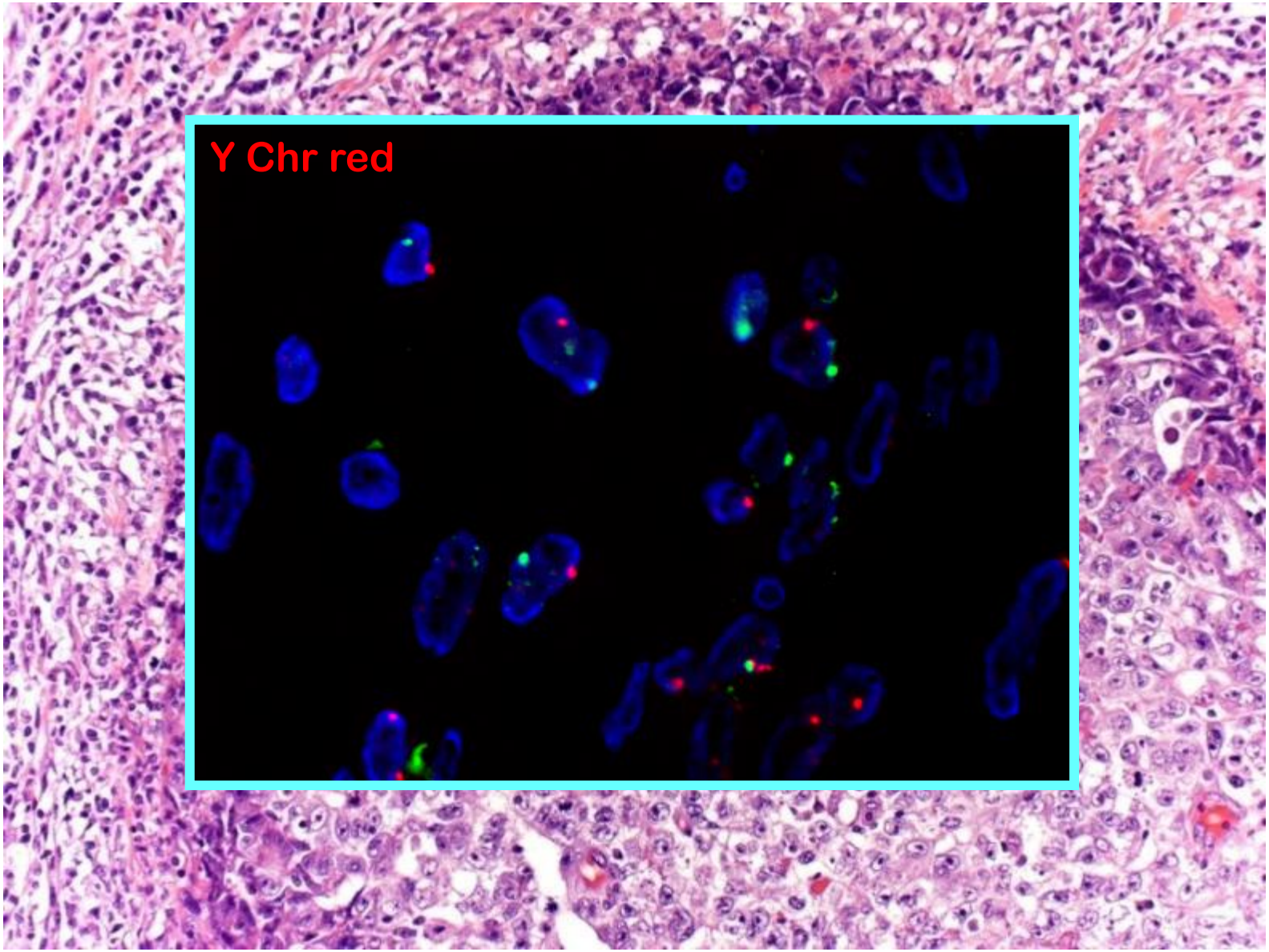
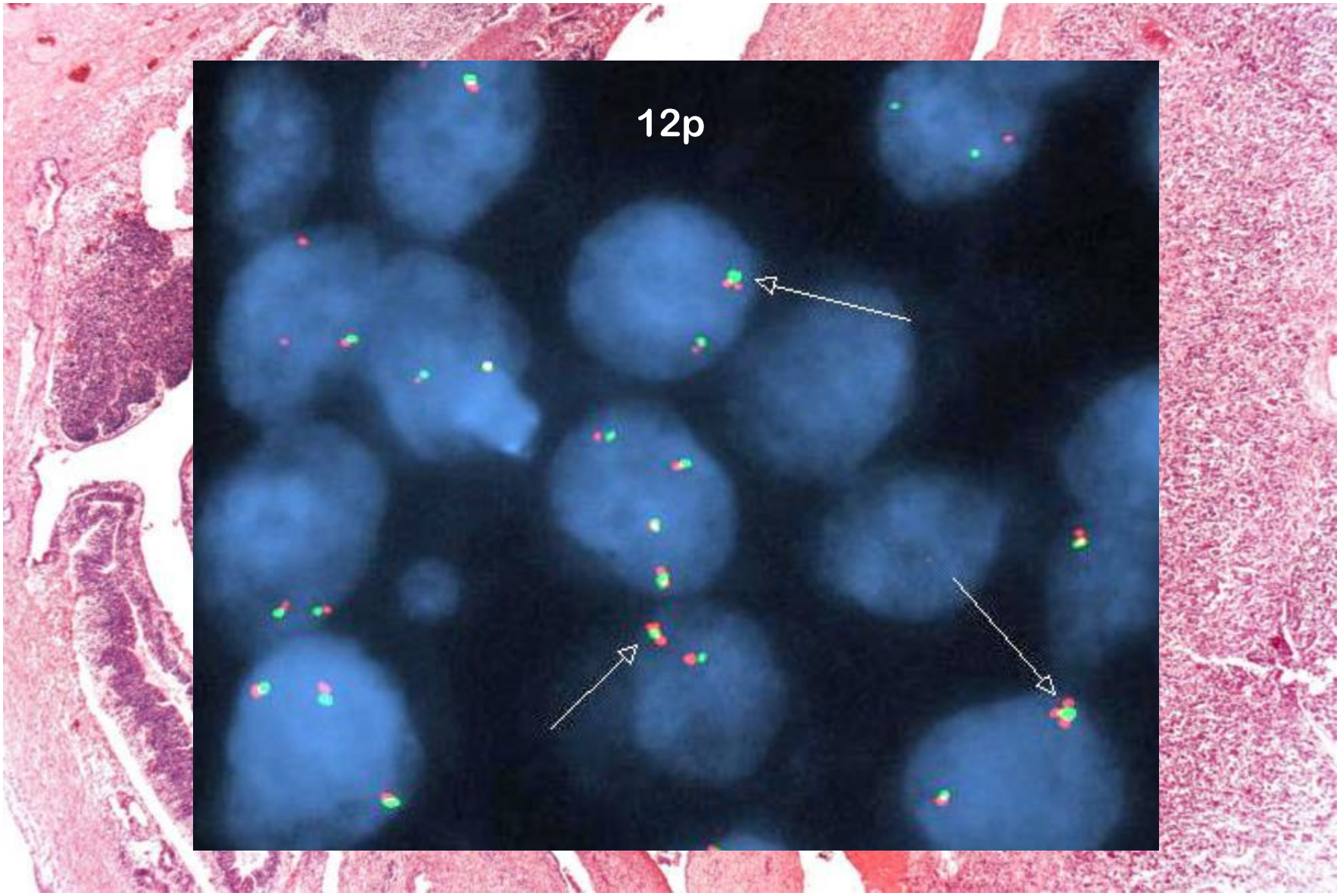

SALL4


Insulin

Embryonal Carcinoma

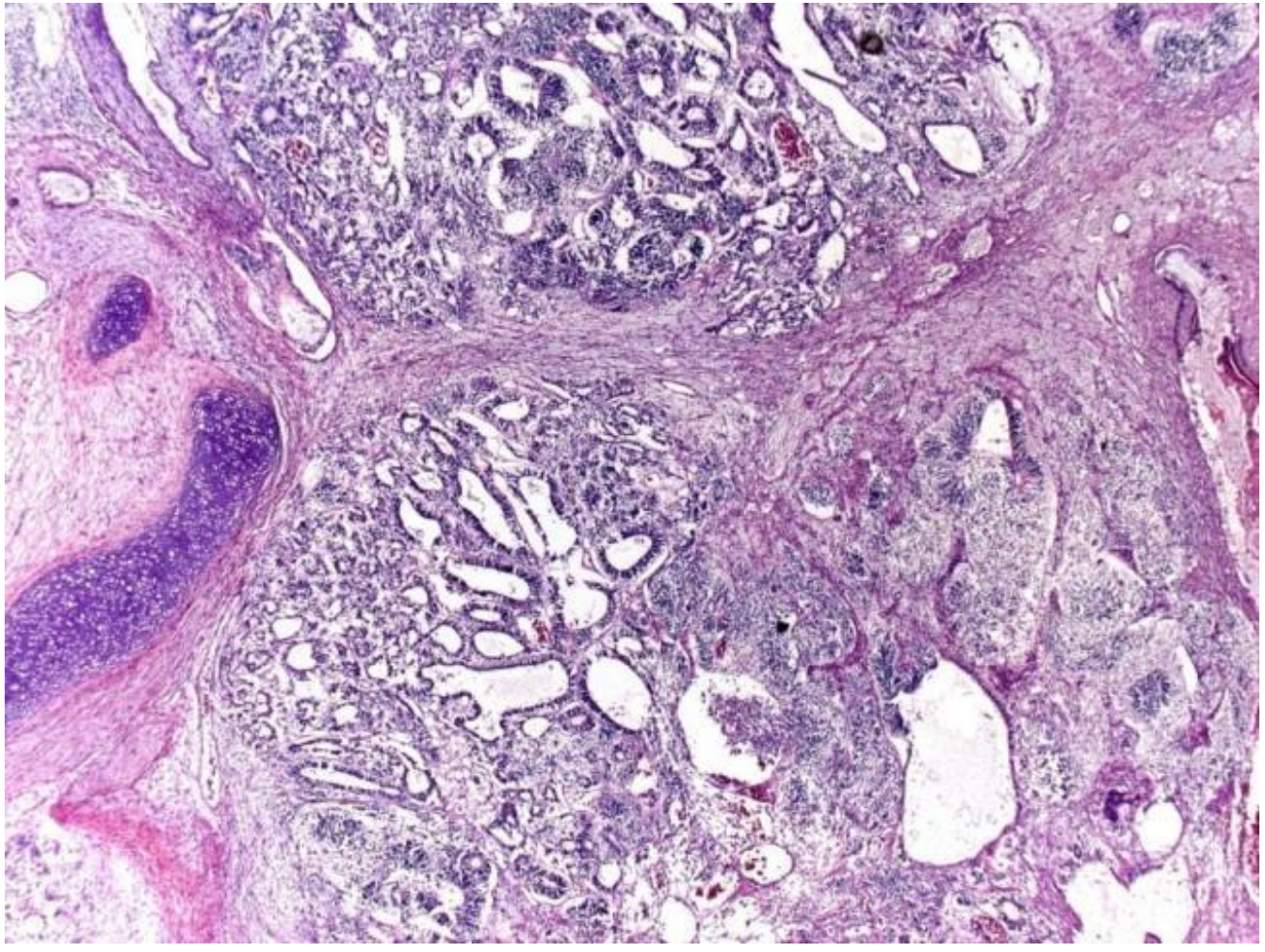


Mixed germ cell tumour



Immature teratomas

Grade 1	Immature neuroectoderm ≤ 1lpf (4x)
Grade 2	Immature neuroectoderm > 1lpf ≤ 3cba (4x)
Grade 3	Immature neuroectoderm > 3lpf (4x)



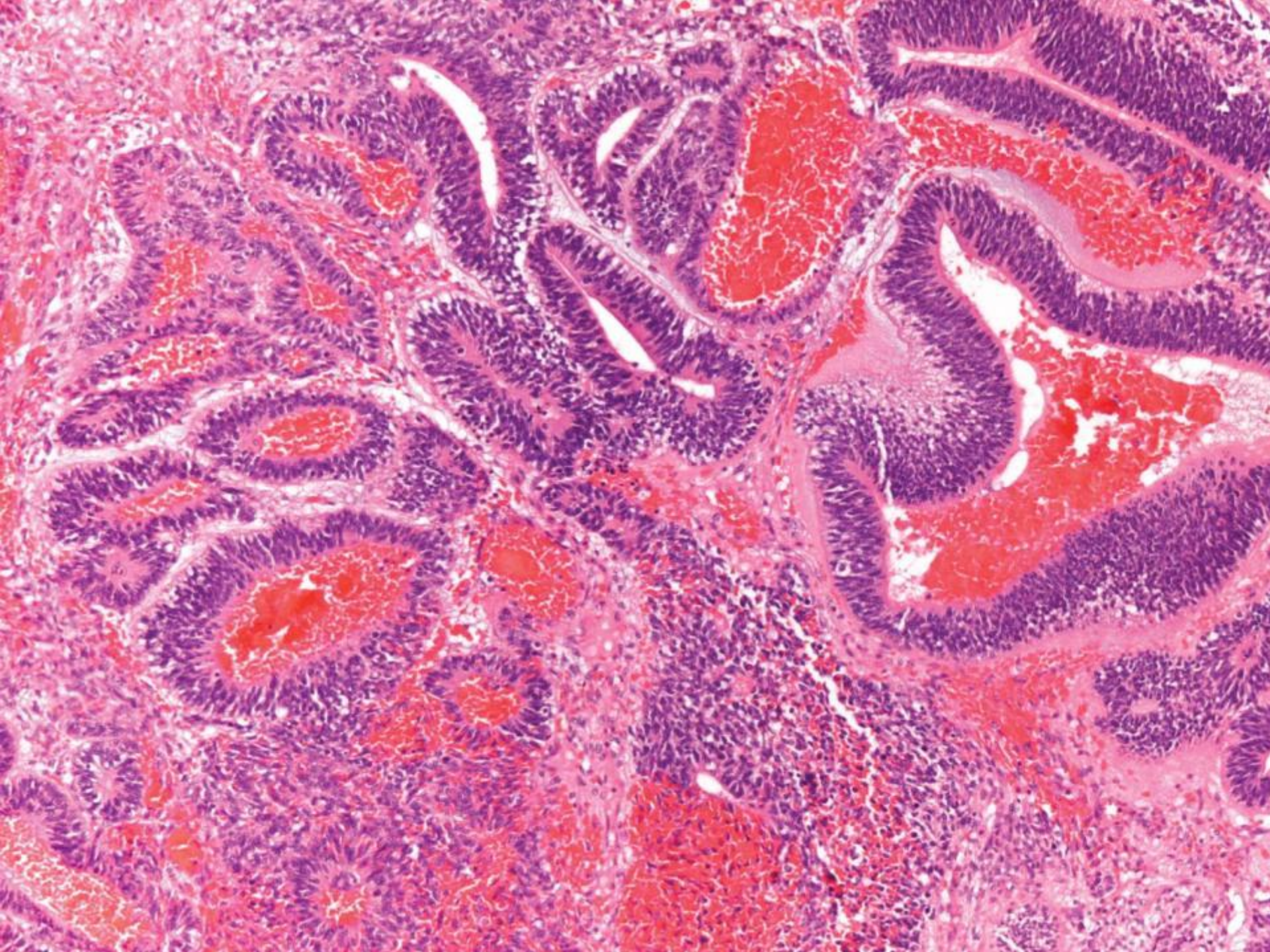
Immature teratoma

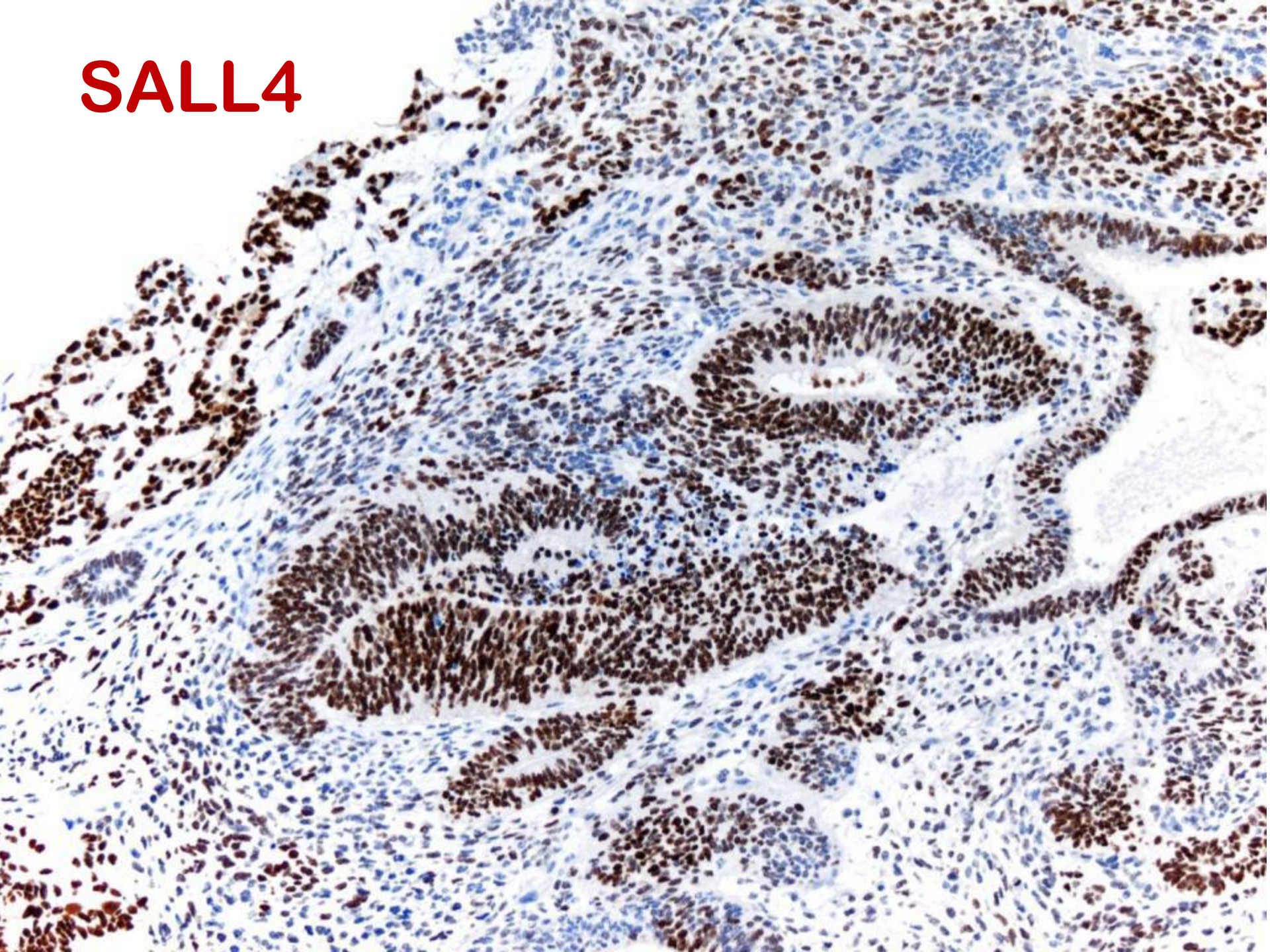
Immature teratomas usually present with non-specific mass related symptoms but occasionally the history is noteworthy because an ipsilateral dermoid cyst has been resected previously.[46] The risk of an immature teratoma in such patients may be increased if the dermoid cysts are bilateral, multiple, or associated with rupture.[47] The median diameter is over 15 cm and the predominantly solid cut surface is fleshy, gray to pink, often with associated variably sized cysts, focal haemorrhage and necrosis (Figure 14). Although an associated dermoid cyst is grossly evident in 25% of tumours, the overall features are in most cases in marked contrast to those of dermoid cysts.

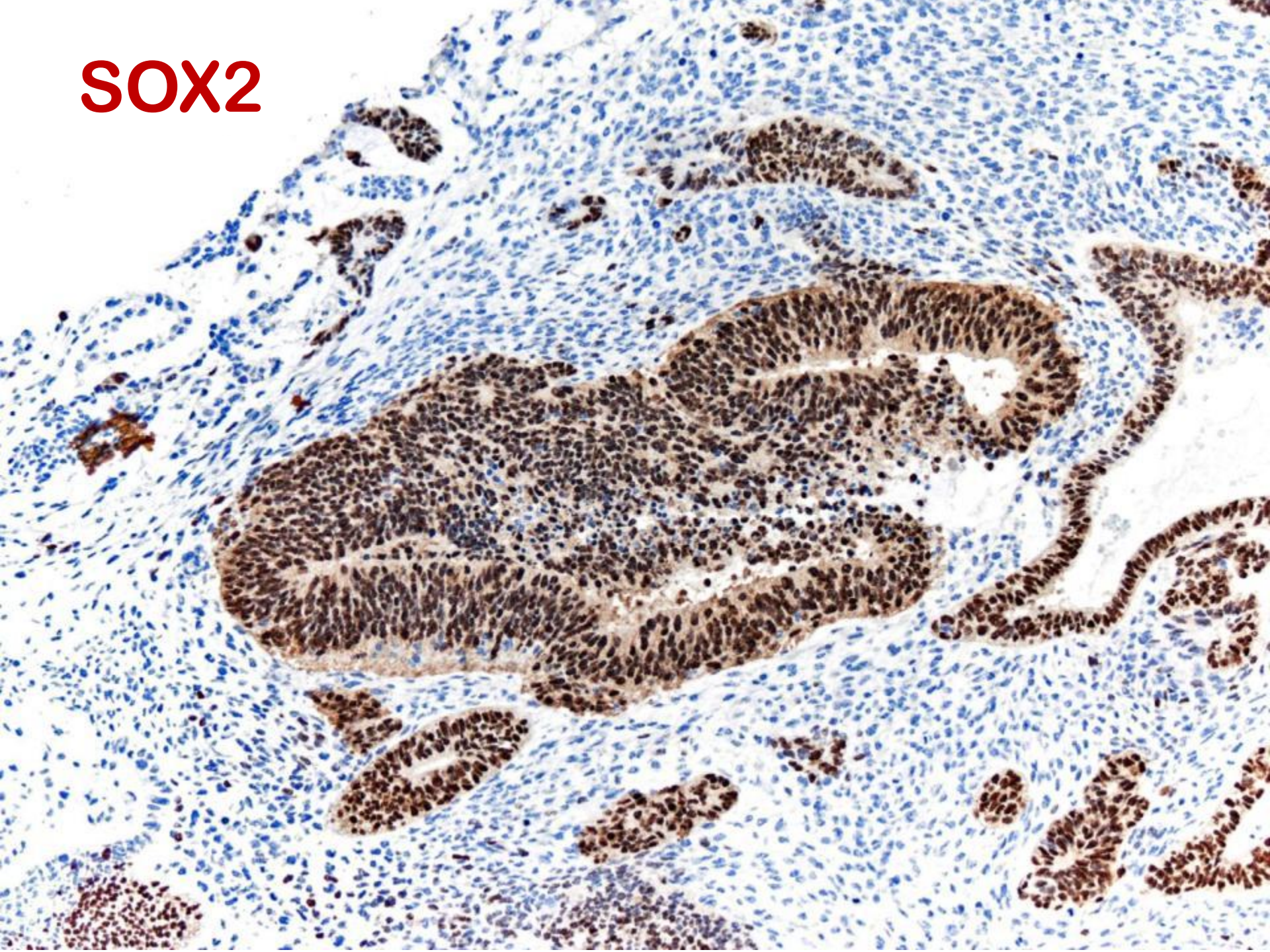
The tumours are graded based on the degree of immaturity of the neural tissue (Figure 15). Grade 1 tumours contain rare foci of immature neural tissue (<1 low-power-field [LPF] in any one slide), while grade 2 and grade 3 tumours contain 2–3 LPFs or 4 or more LPFs of immature neural tissue in any one slide respectively. A 2-grade system has been proposed: low-grade (grade 1) and high-grade (grades 2 and 3) based on outcome, as patients with grade 1 immature teratomas do not need adjuvant treatment and have a good outcome.[48] Embryoid bodies may be present in immature teratoma, and in fact they are not an uncommon finding (Figure 16). They reflect high-grade imma-

Immature tubular structures in IT

- Neural tubules

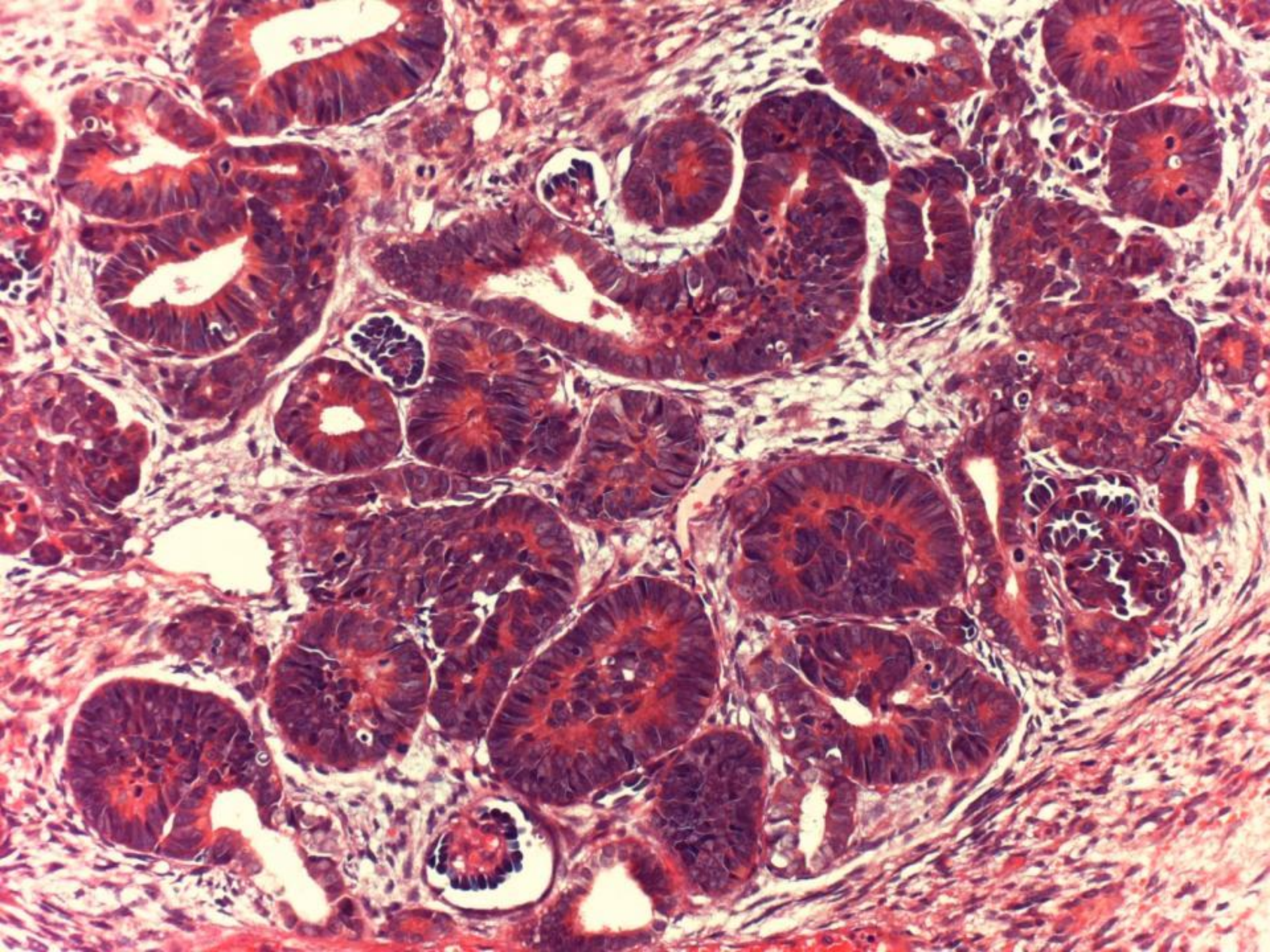



SALL4


SOX2

Immature tubular structures in IT

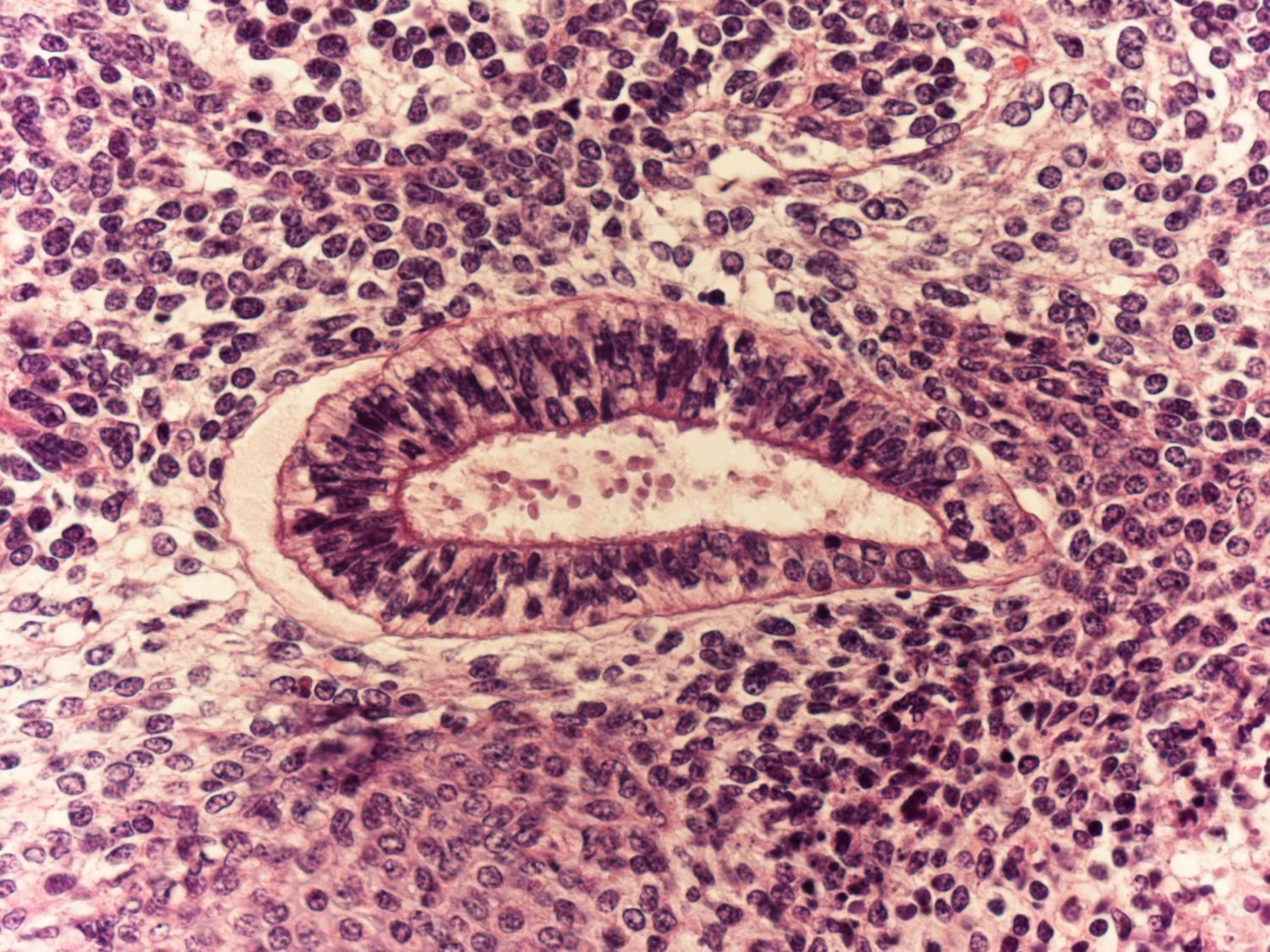
- Neural tubules
- Nephrogenic tubules



Immature tubular structures in IT

- Neural tubules
- Nephrogenic tubules
- Endodermal tubules

Immature endodermal areas are also present in high grade immature teratoma and its presence may imply aggressive behaviour

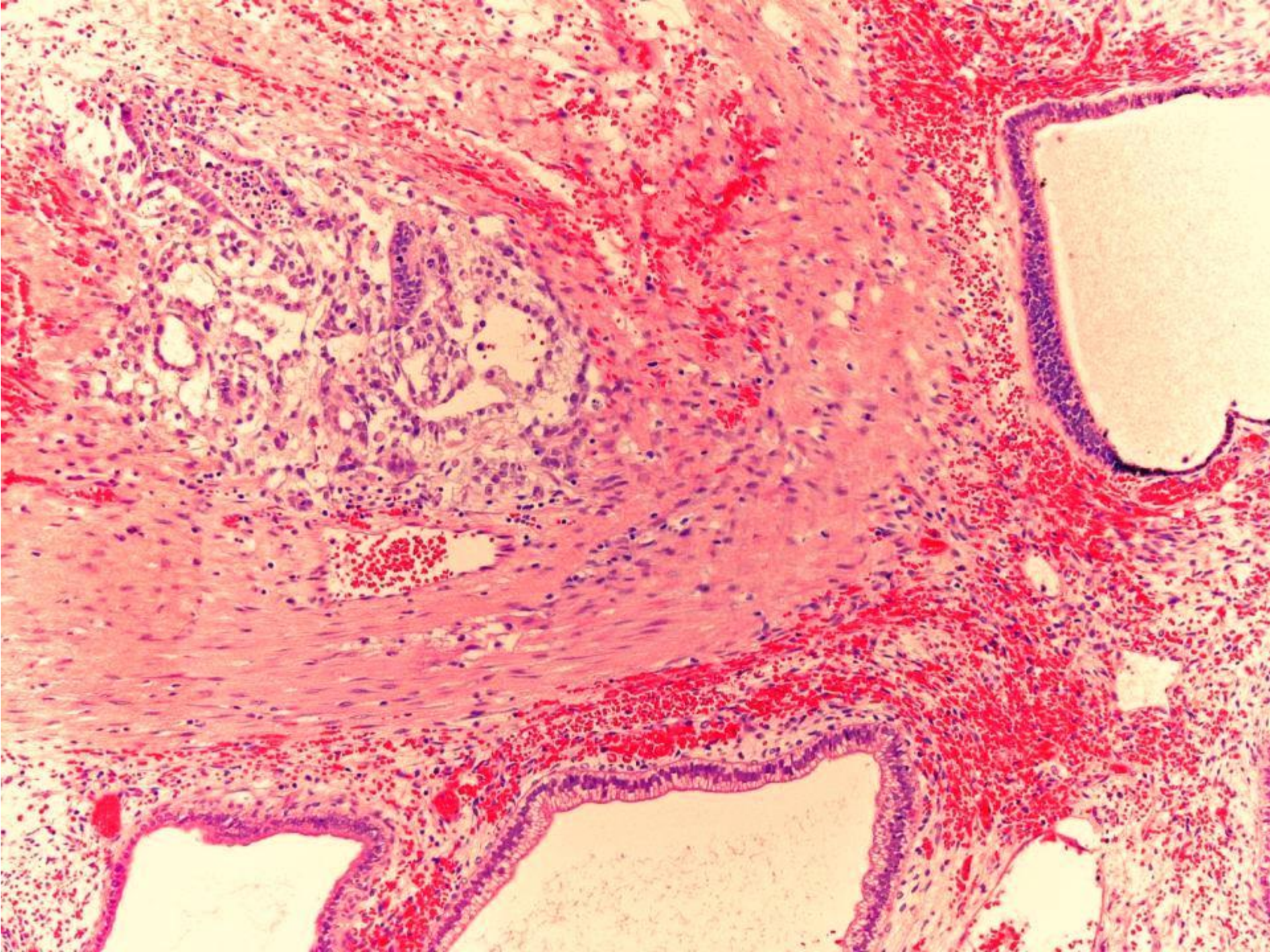


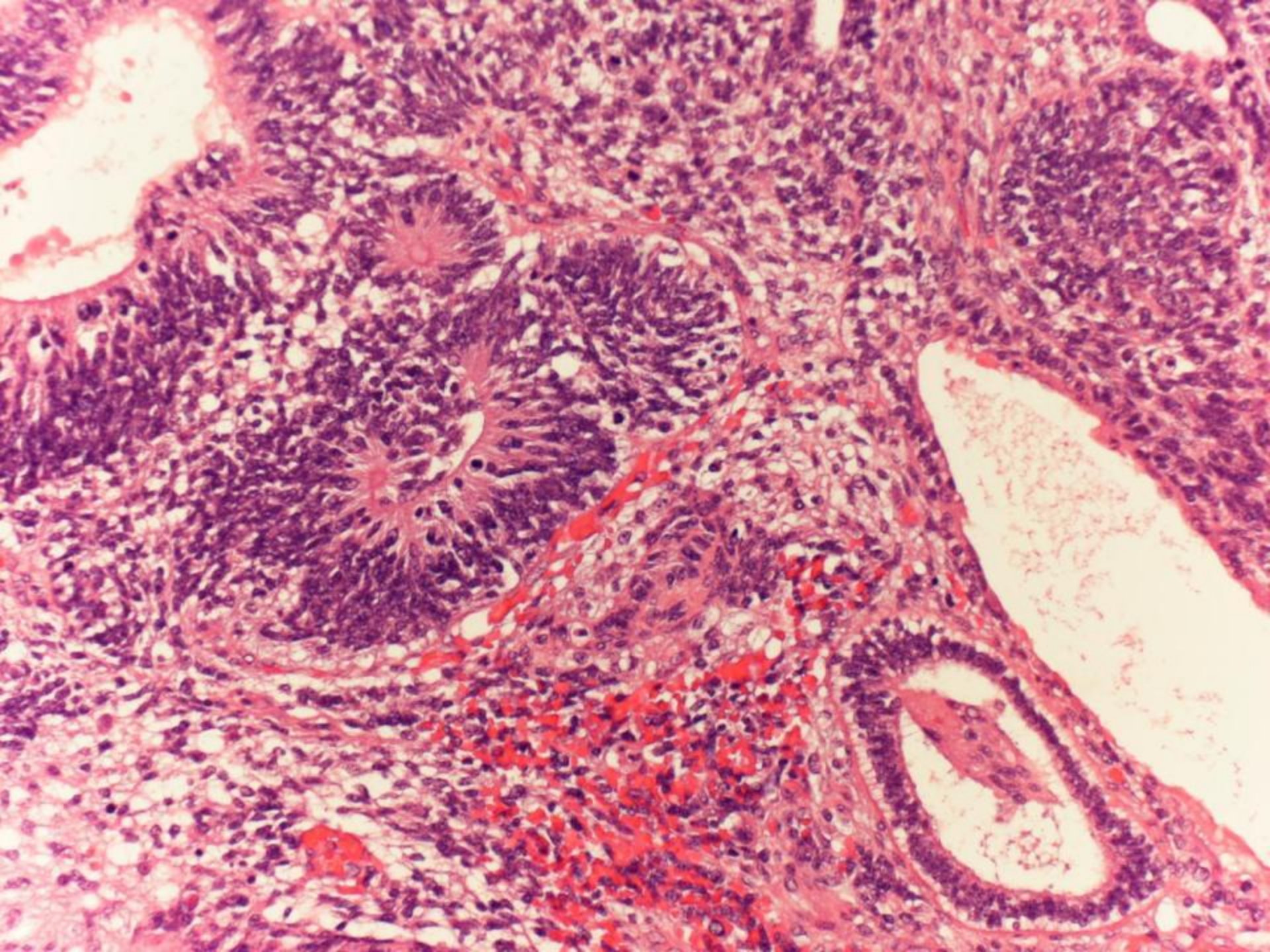
Immature teratoma and endodermal areas

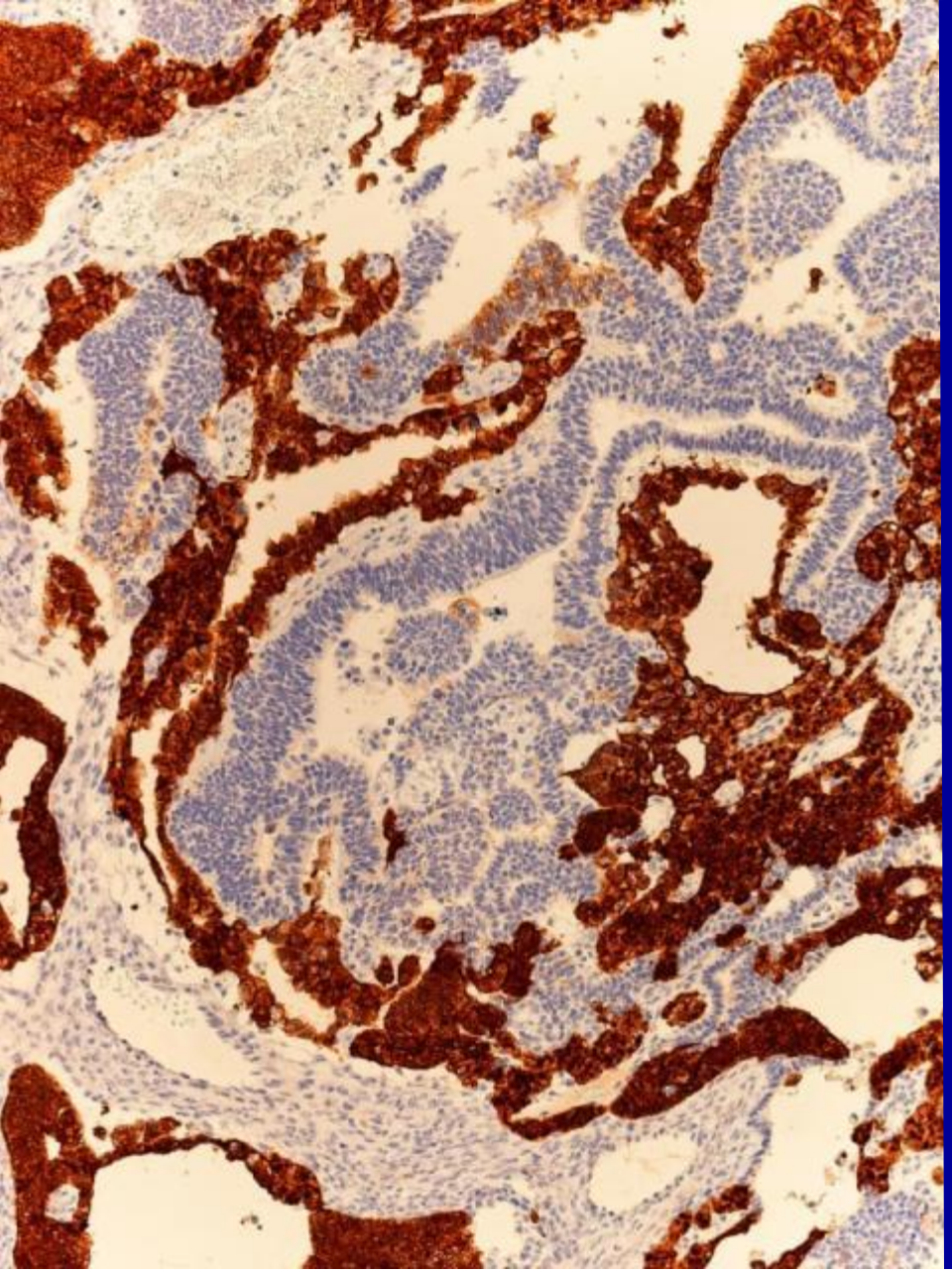
- 1: Heifetz et al. Immature teratomas in children: pathologic considerations: a report from the combined Pediatric Oncology Group/Children's Cancer Group. Am J Surg Pathol 1998;22:1115.

Overall 2- to 6-year survival rate was 96% and was related to the presence of YST….

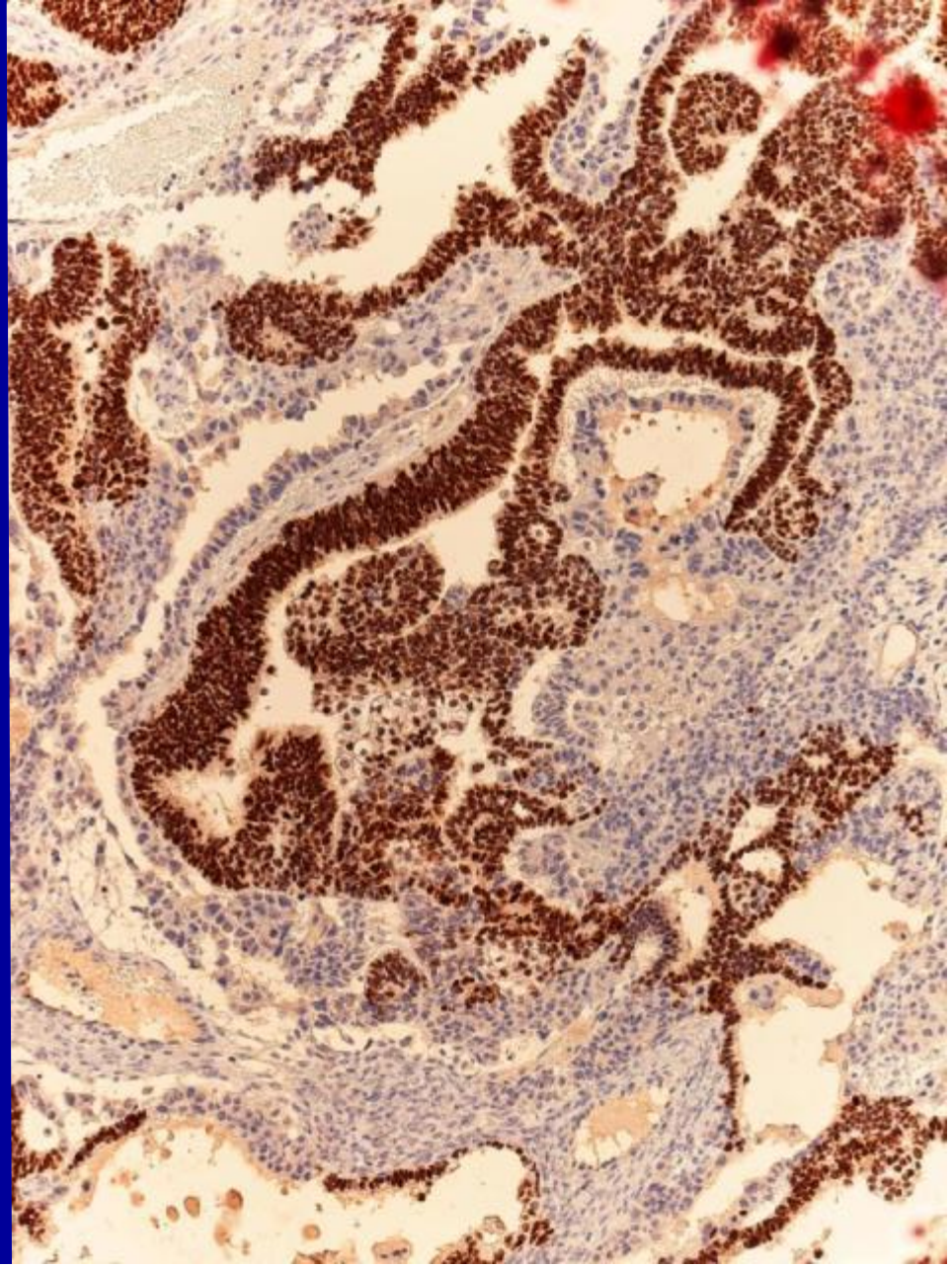
….the presence of microscopic foci of YST, rather than the grade of IT, *per se*, is the only valid predictor of recurrence in pediatric IT at any site.





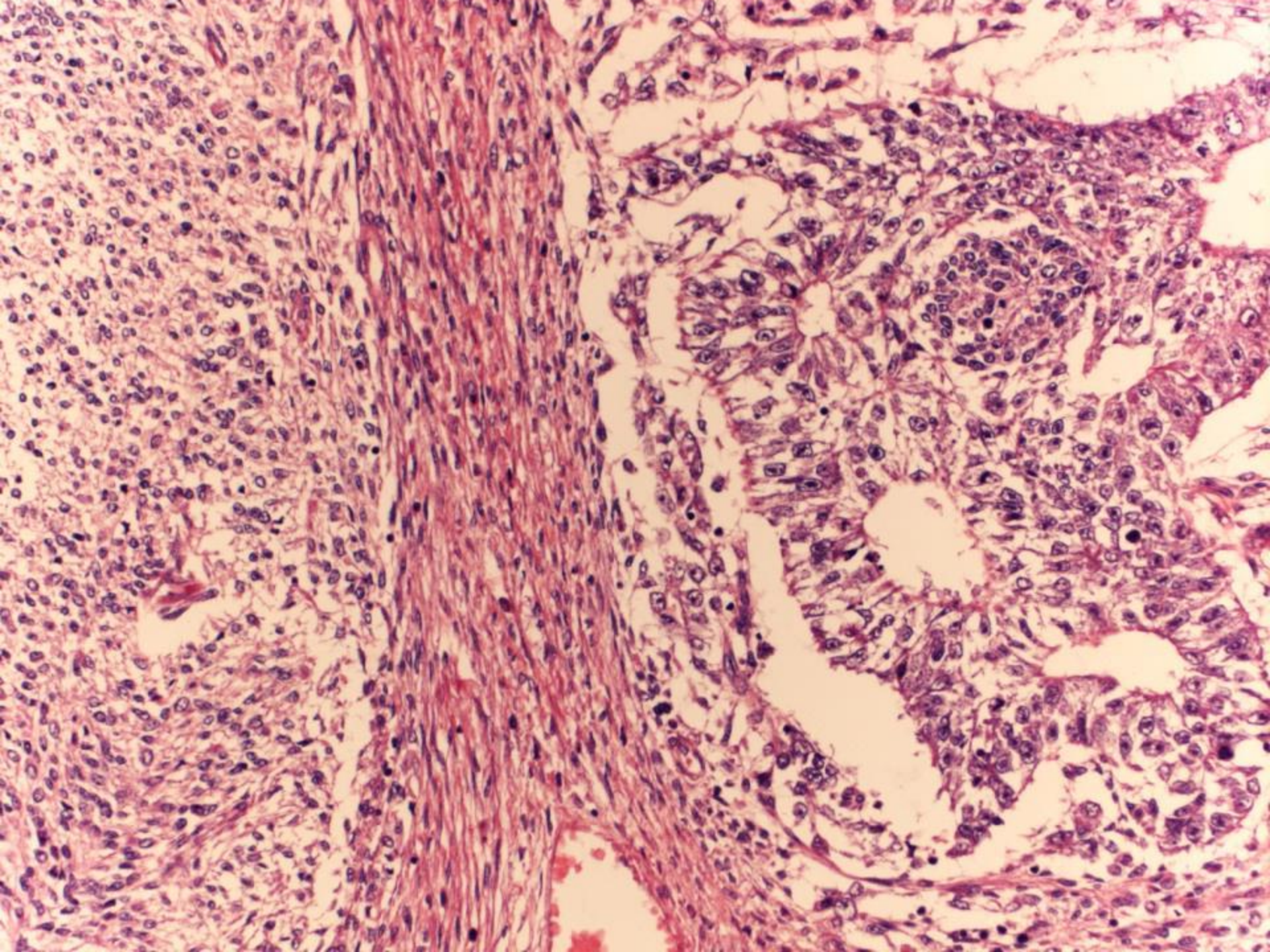


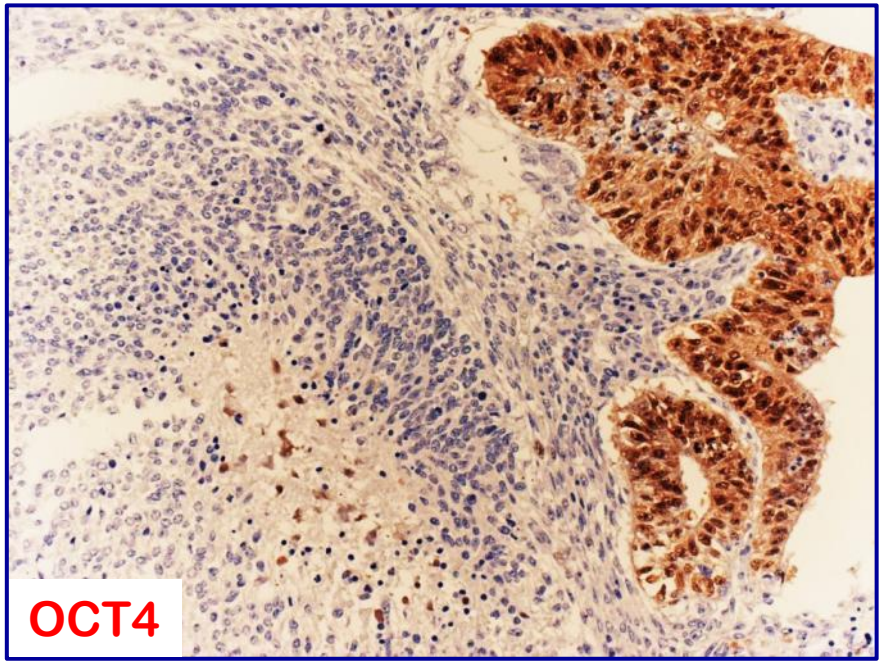
Villin

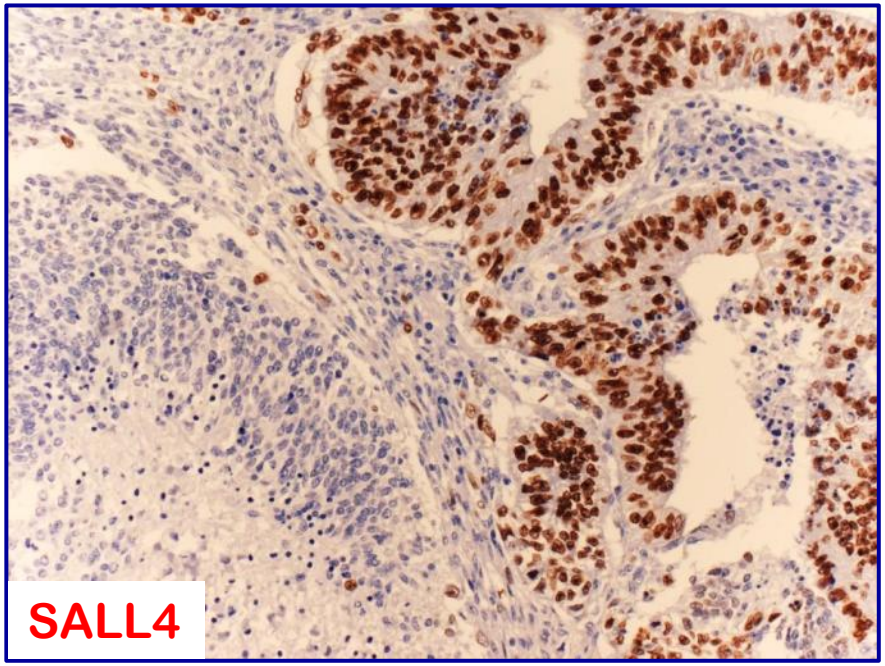


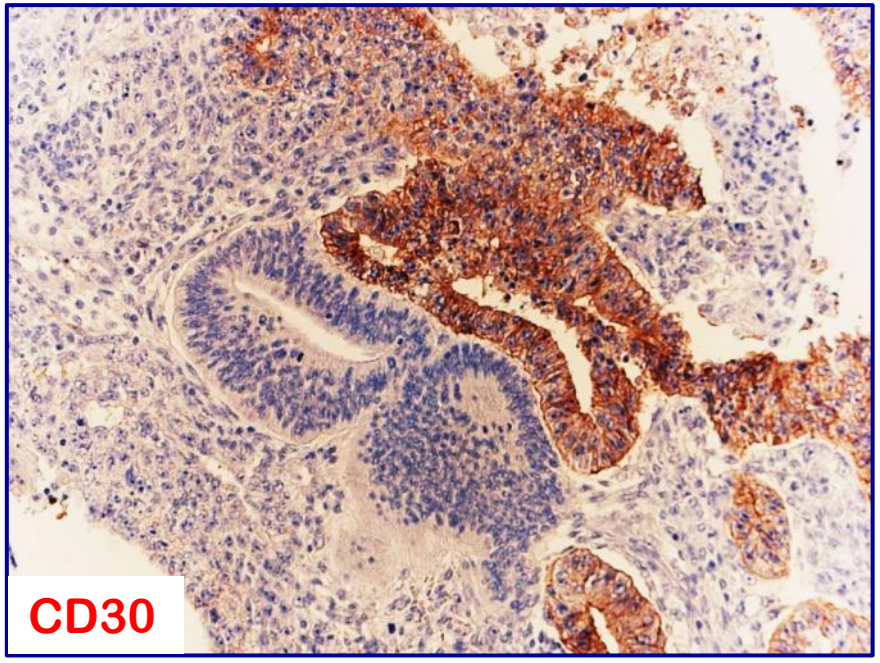
SOX2

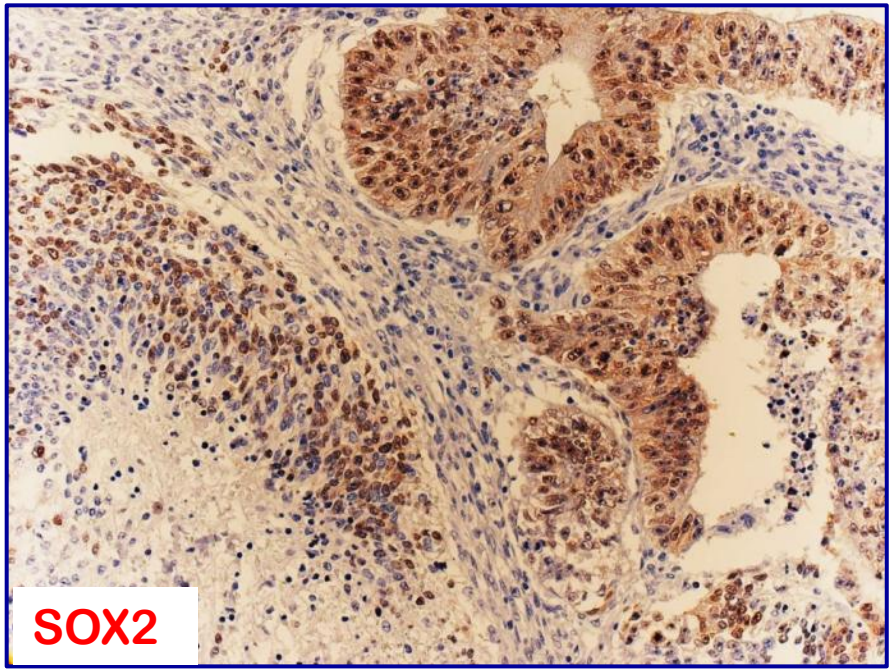
Embryonal Carcinoma-like areas can be present in high grade immature teratomas



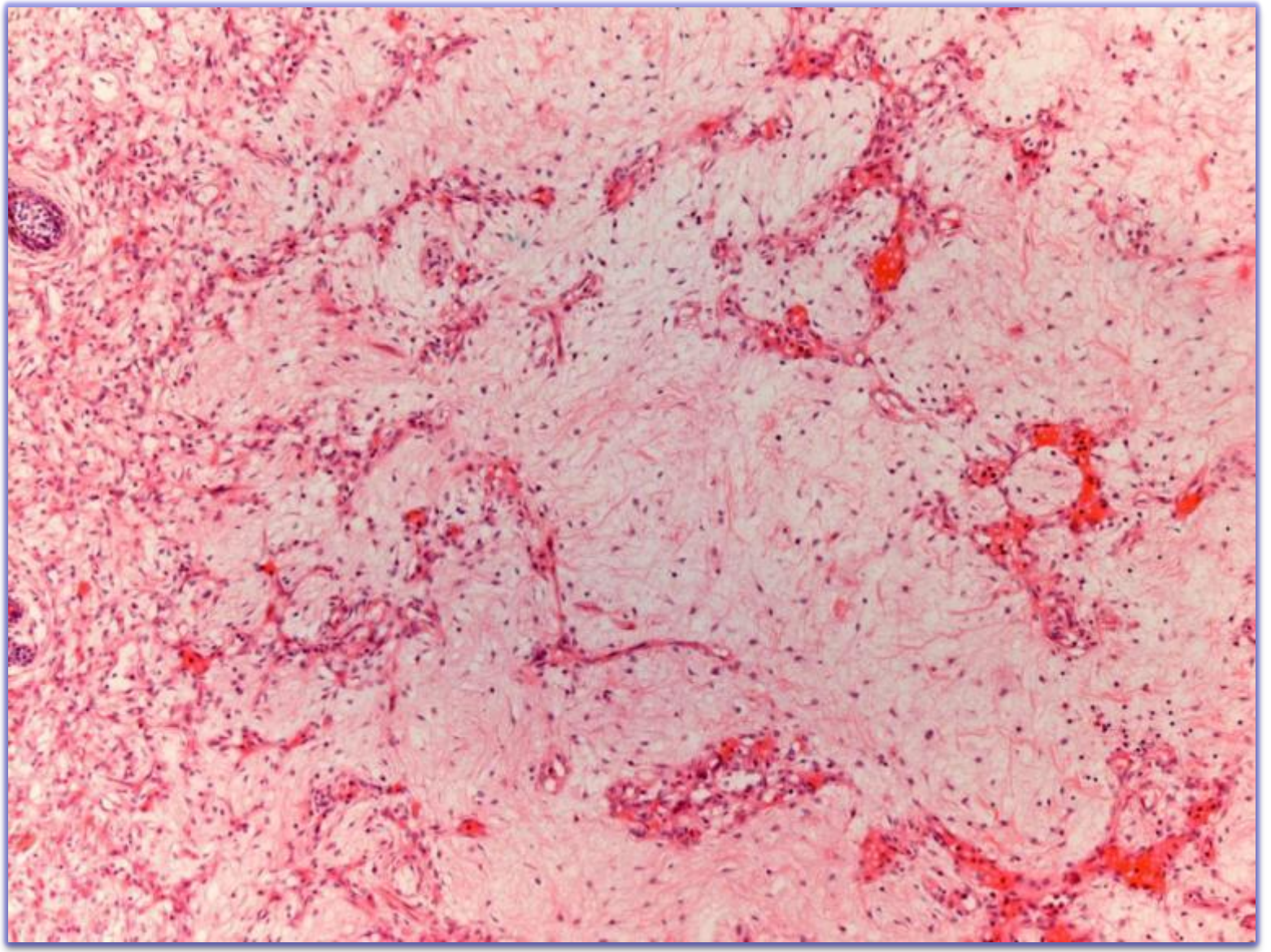

OCT4


SALL4


CD30


SOX2

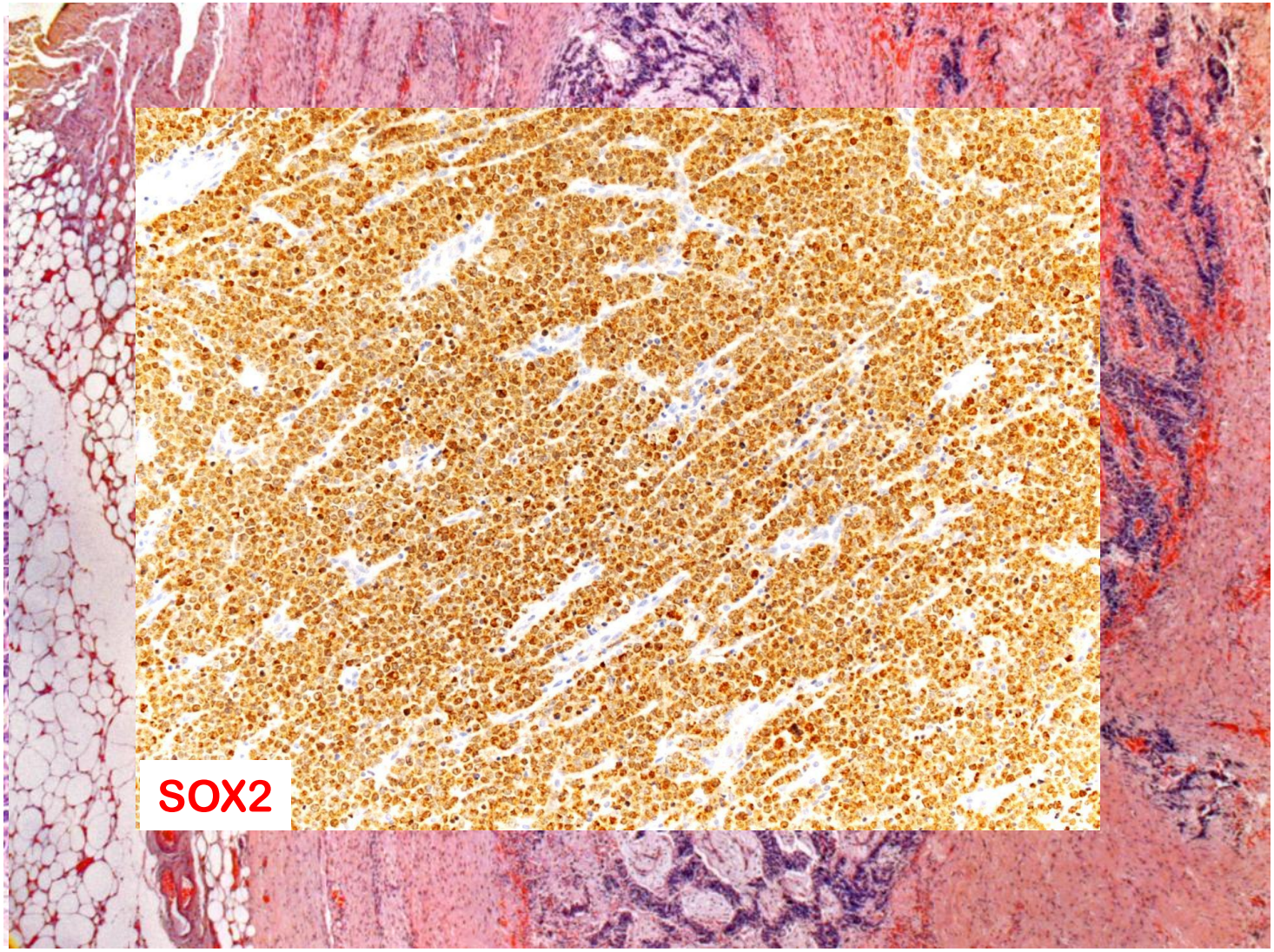
Immature mesenchyme in IT



Immature teratoma grading

- Should be comprehensive of neural/endodermal/mesenchymal immature areas
- Grading facilitated by PPM expression analysis (SALL4/SOX2/OCT4)
- General assessment of tissue immaturity rather than mixed GCT

PNET and IT (MT)



Classification conundrums

PRIMORDIAL GERM CELLS

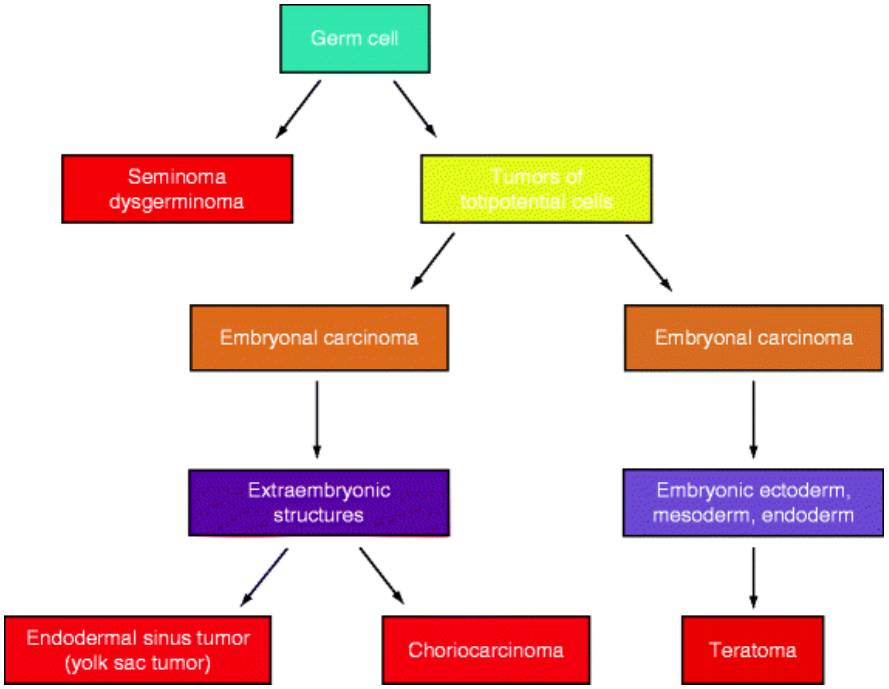
SEMINOMA

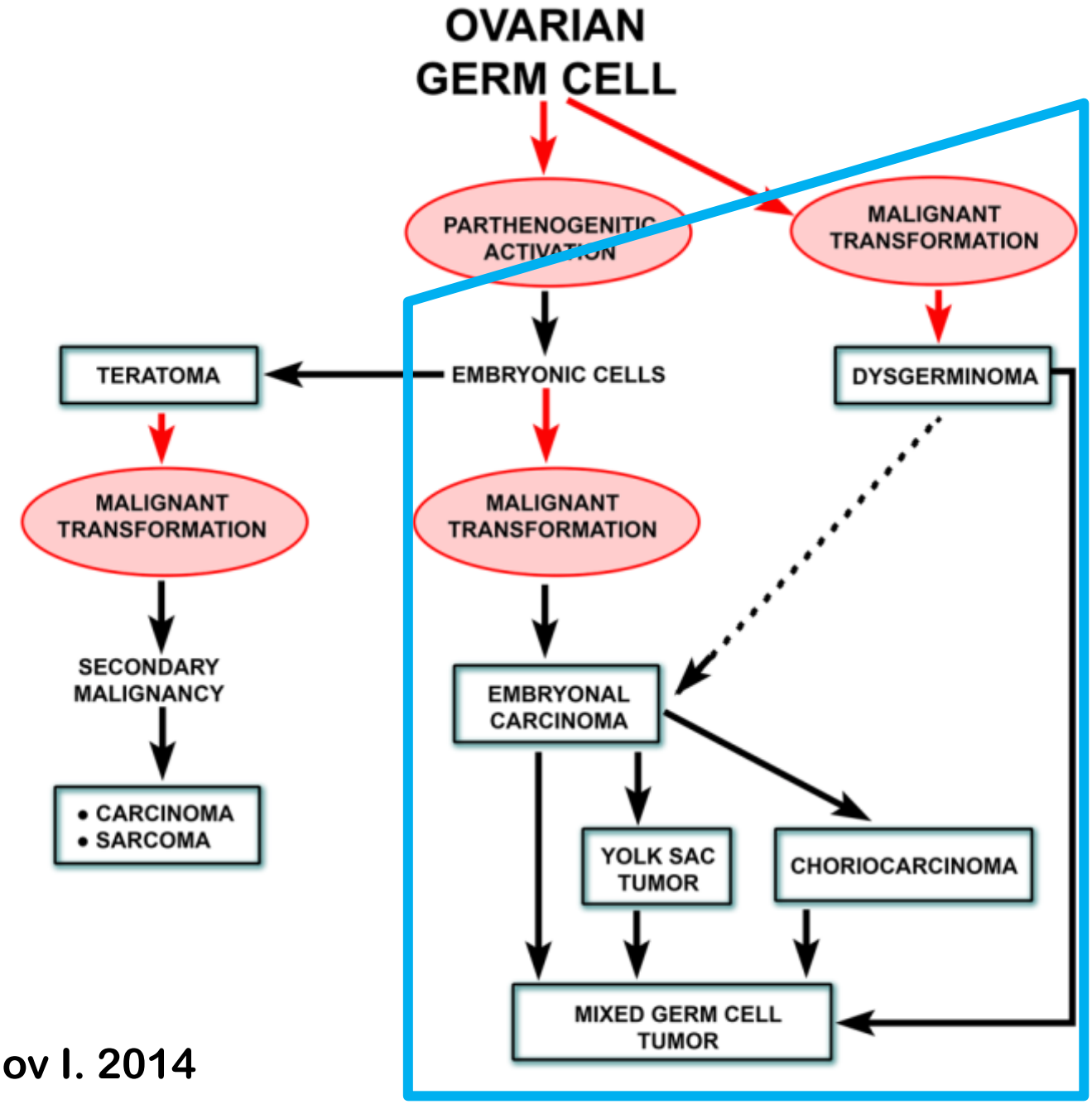
EMBRYONAL CARCINOMA

YOLK SAC TUMOUR

CHORIOCARCINOMA

TERATOMA



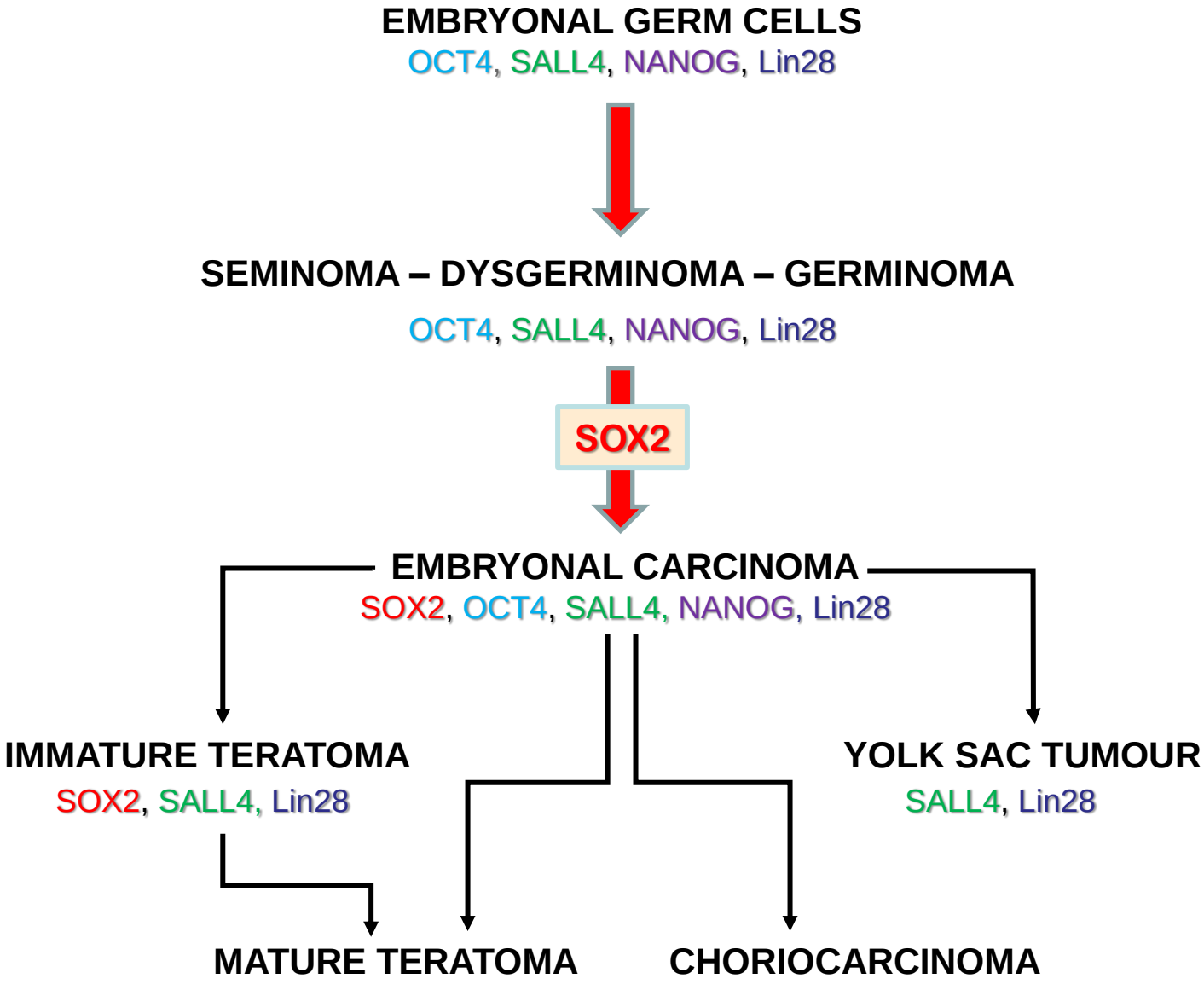


Damjanov I. 2014

Comparative Immunohistochemical Expression in Malignant Ovarian Germ Cell Tumors of Classic, Pluripotency, and Somatic Differentiation Markers

	Immunohistochemical Markers											
	Classic					Pluripotency			Somatic Differentiation			
Tumor	PLAP	CD30	AFP	GLP3	D2-40	OCT3/4	SOX2	SALL4	Villin	CDX2	HepPar-1	TTF1
Dysgerminoma	+	−	−	−	+	+	−	+	−	−	−	−
Yolk sac tumor	+/−	−	+	+	+/−	−	−	+	+ INT	+ INT	+ HEP	+ FRG
Immature teratoma	−	−	−	−	−	−	−	+	−	−	−	−
	−		+ END	+ NEP	+ STR		+ NEP		+ INT	+ END		
Embryonal carcinoma	+	+	−	+ Focal	+/− Apical	+	+	+	NA	−	−	−
Choriocarcinoma	−	−	−	−	−	−	−	−	−	−	−	−
	+SYNC			+SYNC								

Abbreviations: AFP, α-fetoprotein; END, endodermal; FRG, foregut; GLP3, glypican3; HEP, hepatic; INT, intestinal; NA, not available; NEP, neuroepithelium; PLAP, placental alkaline phosphatase; STR, stroma; SYNC, syncytiotrophoblast; TTF1, thyroid transcription factor 1.


EMBRYONAL GERM CELLS
OCT4, SALL4, NANOG, Lin28
SEMINOMA – DYSGERMINOMA – GERMINOMA
OCT4, SALL4, NANOG, Lin28
SOX2
EMBRYONAL CARCINOMA
SOX2, OCT4, SALL4, NANOG, Lin28
IMMATURE TERATOMA
SOX2, SALL4, Lin28
YOLK SAC TUMOUR
SALL4, Lin28
MATURE TERATOMA
CHORIOCARCINOMA