

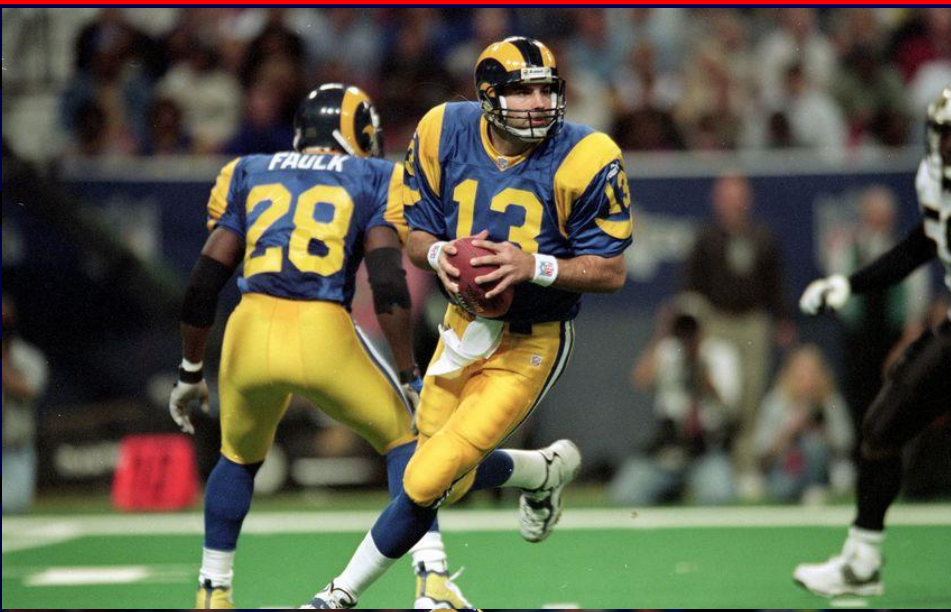
PD-L1 Testing

The What, Why and How in a Growing and Complicated Testing Environment



Richard N. Eisen, M.D.
Phoenix Pathologists
Banner Thunderbird Medical
Center
Chair IHC Committee,
Banner/ LSA

The RAMS ARE BACK?



And for you Dodger Fans!




I set my DVR to record 'The Biggest Loser' and it keeps recording Dodger games.



som^{ee}cards
user card

A white-bordered card with a yellow background. The text is in a black, sans-serif font. Below the text is a black and white illustration of a man in a suit sitting on a chair, looking down at a dog lying on the floor. The logo "som^{ee}cards user card" is in the bottom left corner.

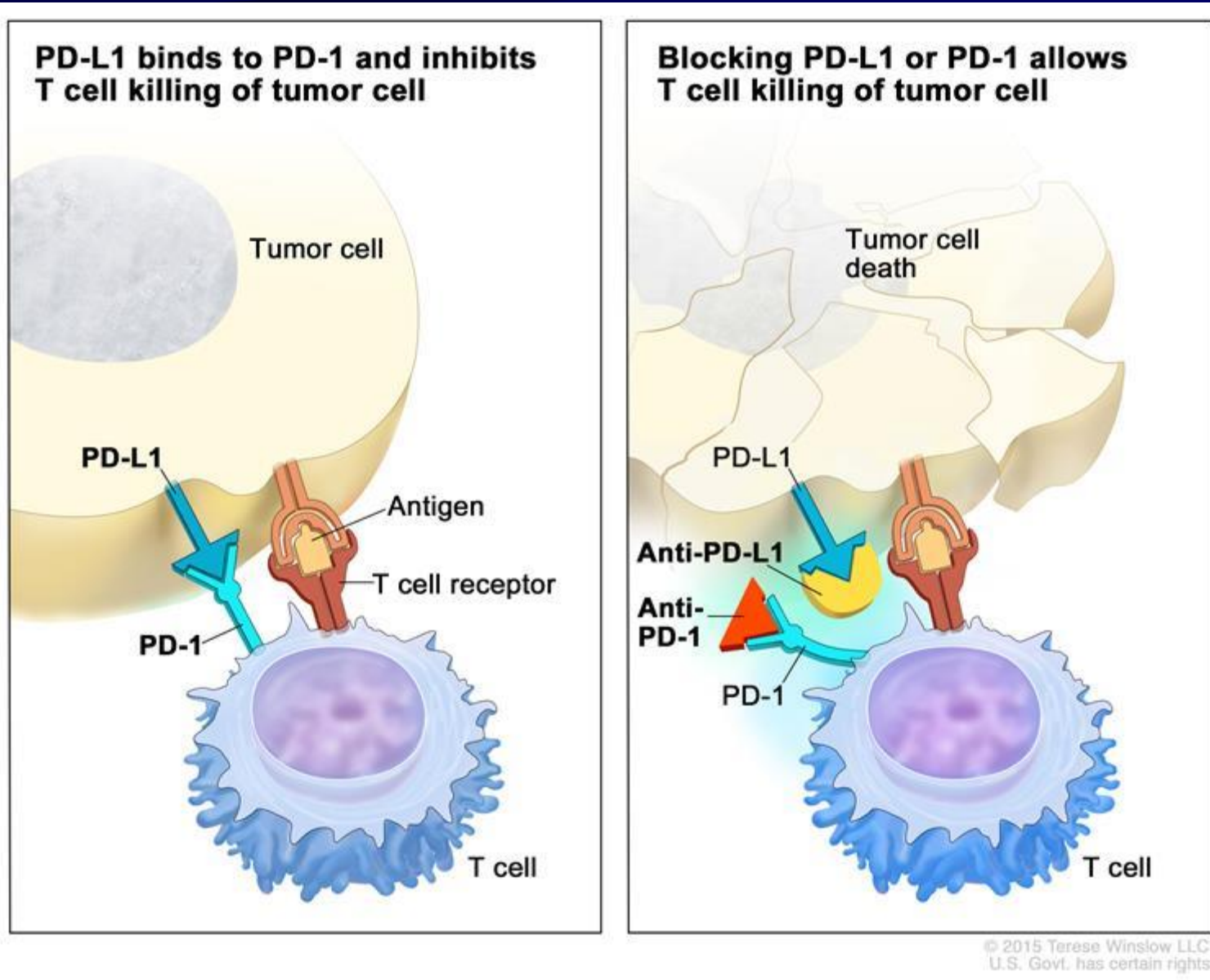
YOU ONLY LIVE ONCE.
DON'T WASTE IT BEING A...
DODGERS FAN

A black poster with orange and white text. The text reads "YOU ONLY LIVE ONCE. DON'T WASTE IT BEING A... DODGERS FAN". Below the text is a white illustration of a man's face and hand, pointing his index finger upwards. The man has a mustache and is wearing a suit and tie.

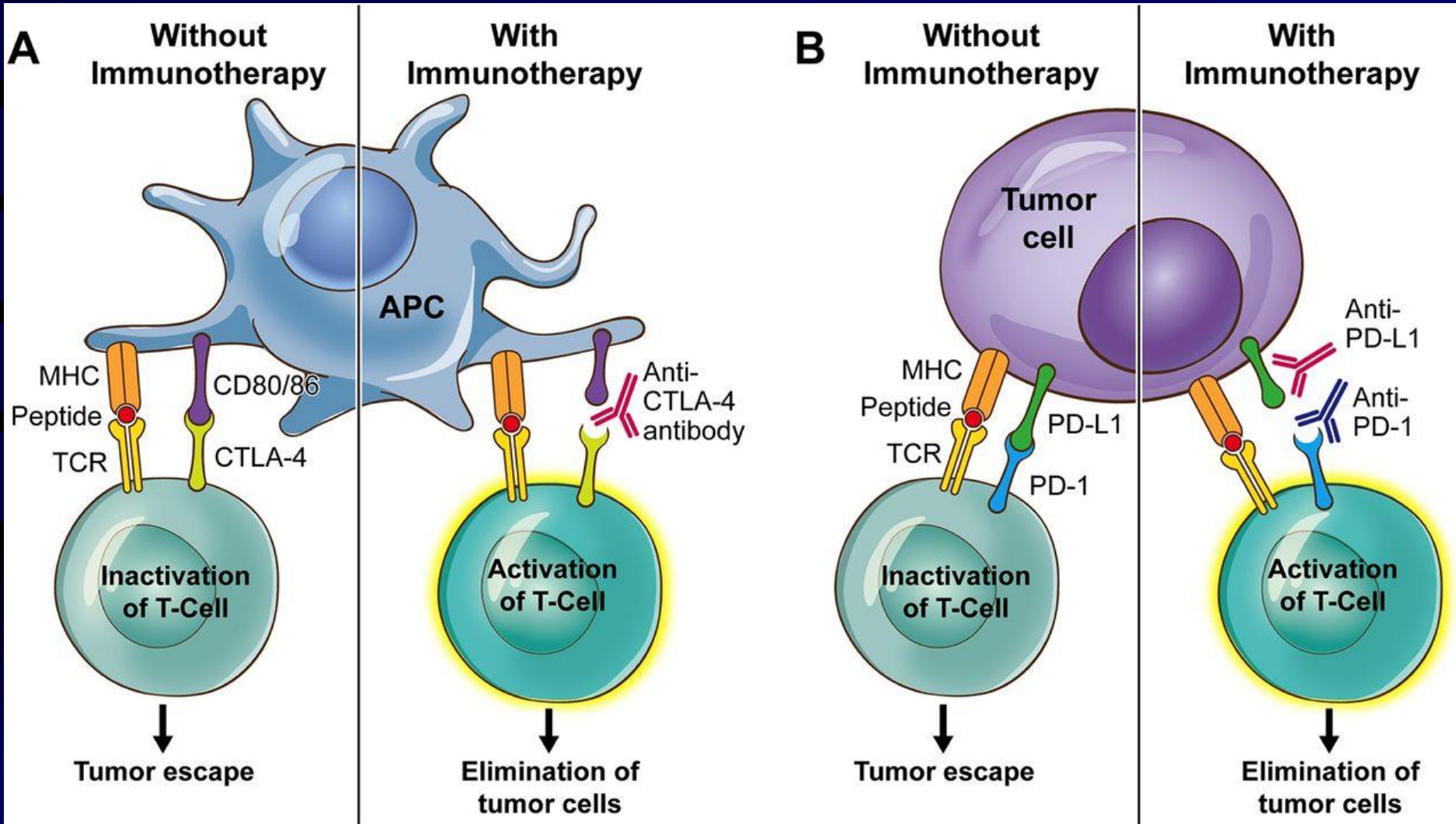
Objectives

1. Review the biology of PD-L1/ PD-1 interaction in tumor biology and principles of immune checkpoint inhibitor therapy.
2. Review the current drug approvals, companion/ complementary PD-L1 test assays and scoring criteria.
3. Discuss scoring with the Dako 22C3 PharmDx assay and associated pitfalls.

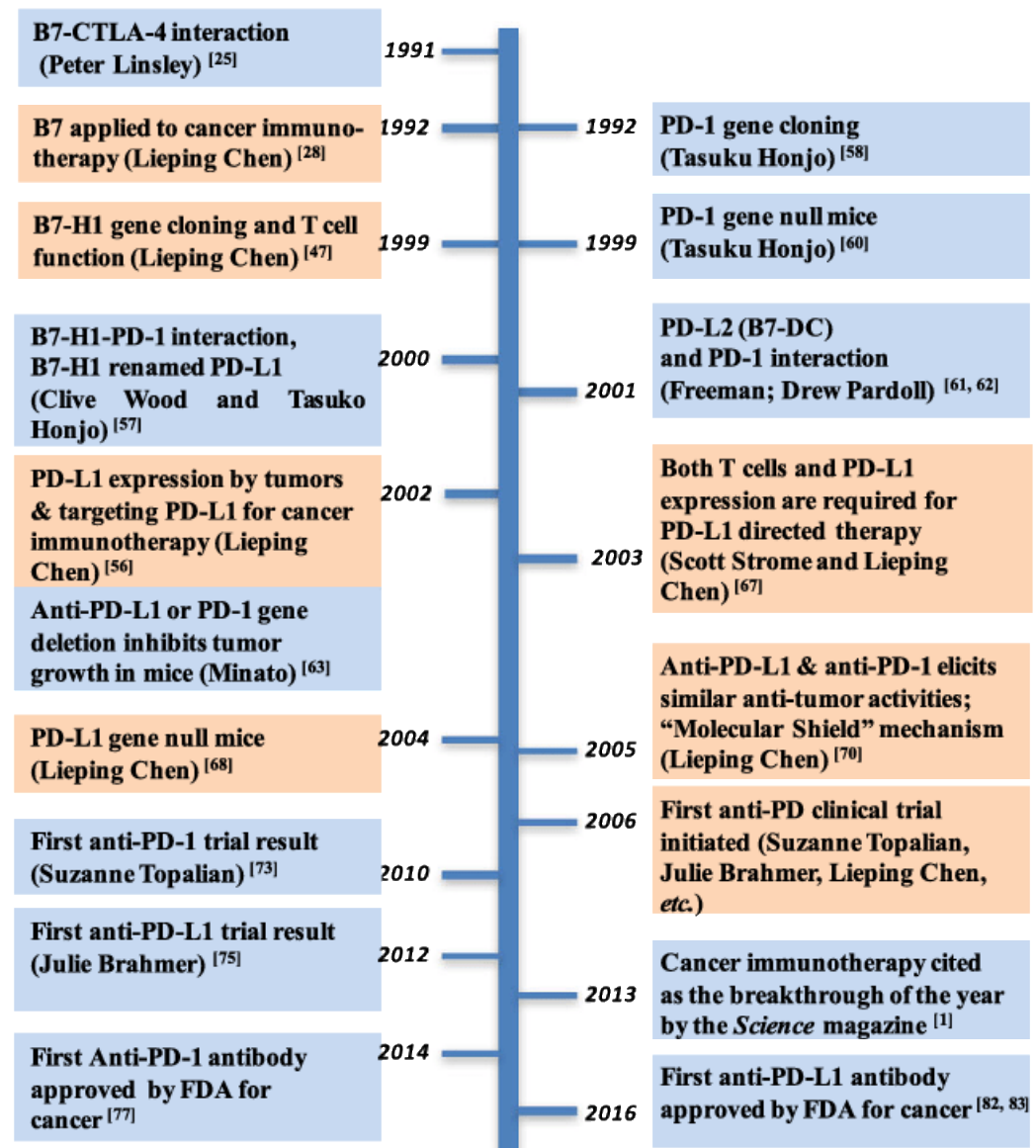
Principles of Immune Checkpoint Therapy



Principles of Immune Checkpoint Therapy

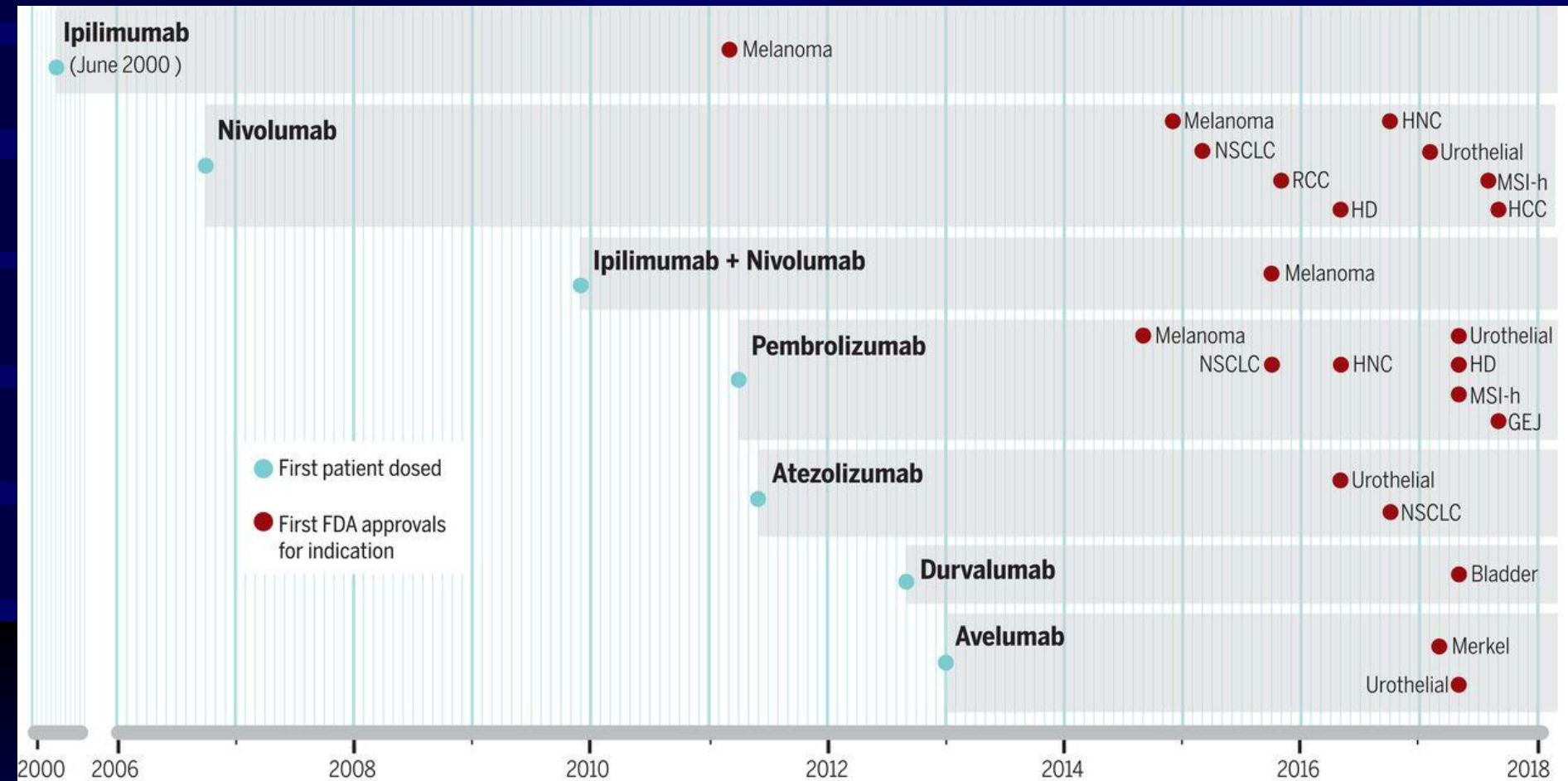


Immune Checkpoint Therapy Timeline



Journal of Hematology & Oncology 10(1)
December 2017

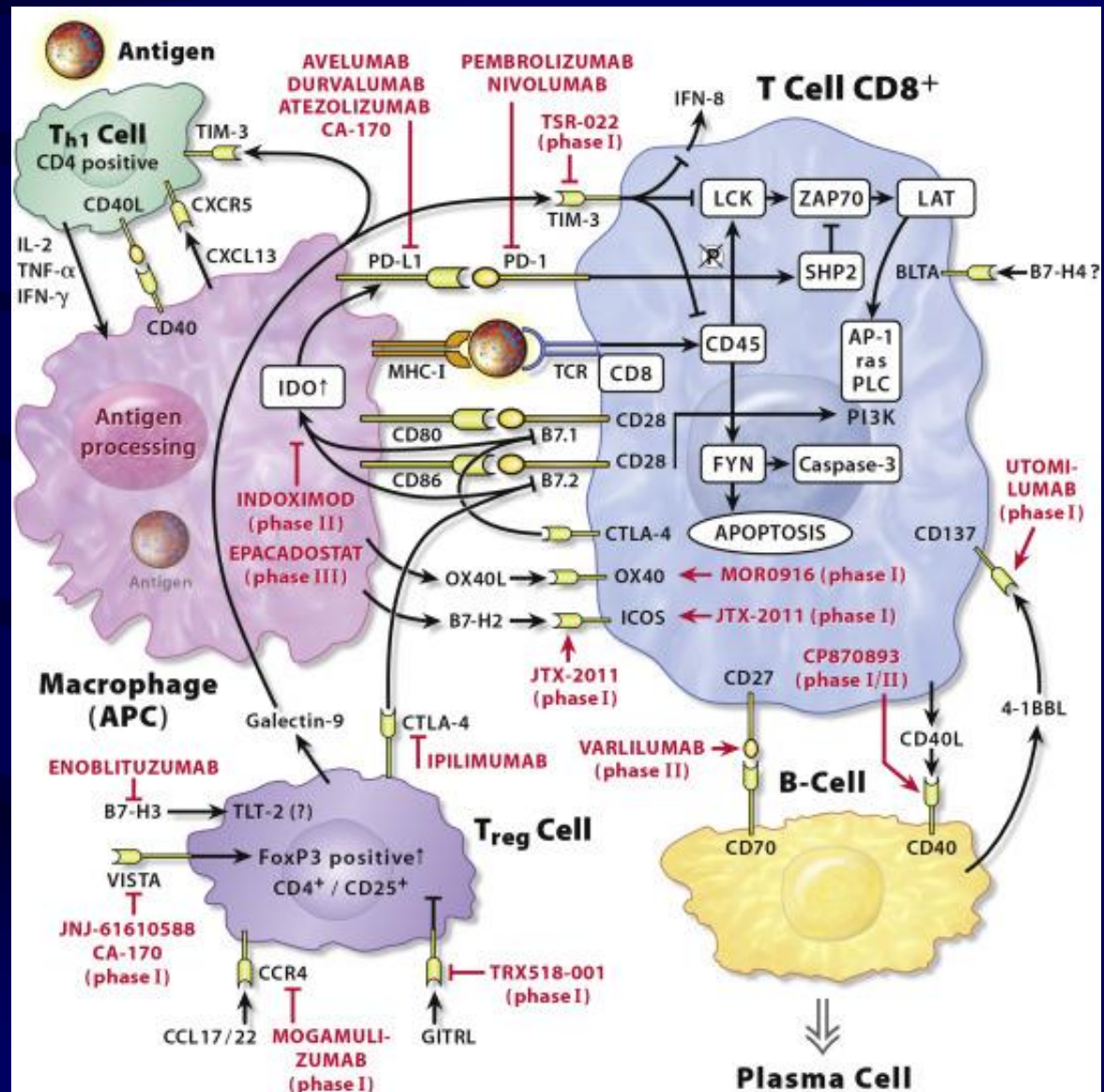
Immune Checkpoint Therapy Timeline



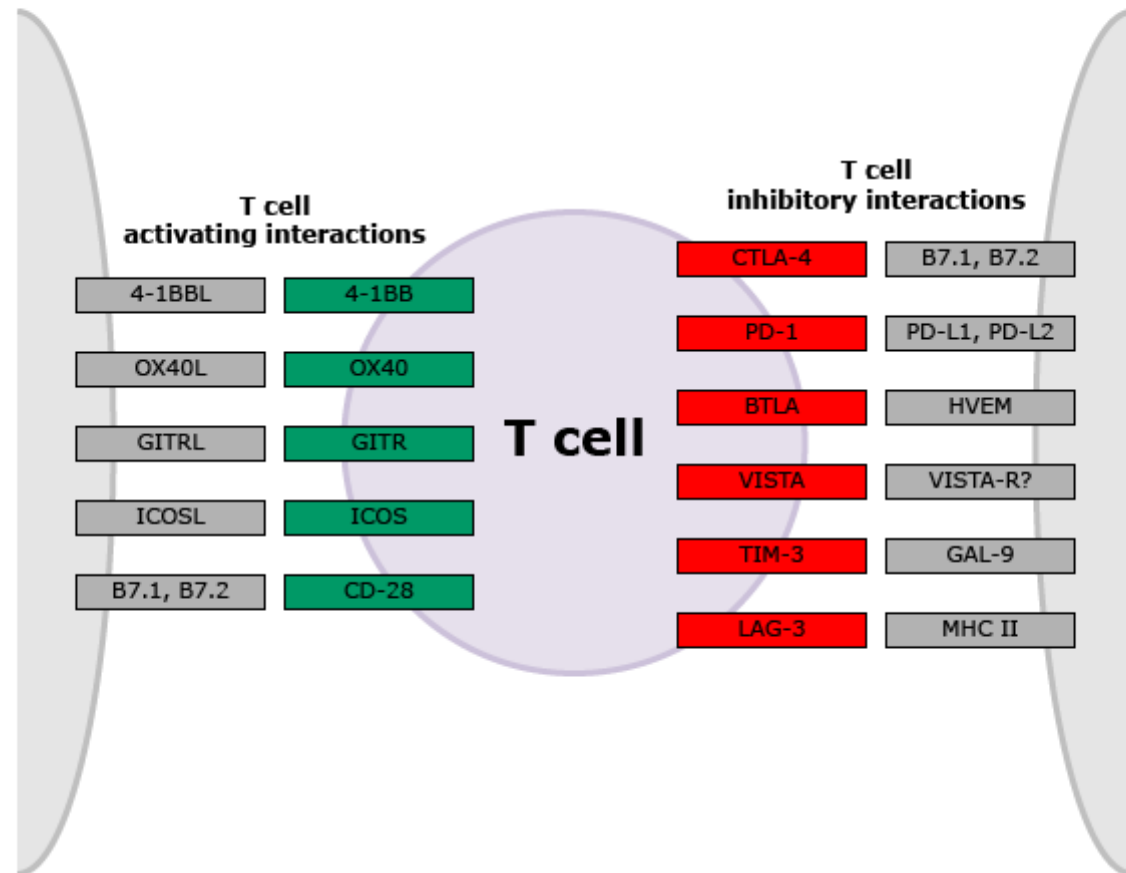
Ribas and Wolchok; *Science* 23 Mar 2018:
Vol. 359, Issue 6382.

Principles of Immune Checkpoint Therapy

European Journal of
Cancer
Volume 74, March
2017



A simplified view of co-stimulatory and co-inhibitory ligand-receptor pairs that regulate T cell activity



Note that some binding partners involving some molecules, such as VISTA, are still being explored. Many additional co-stimulatory and co-inhibitory molecules (not shown) are involved in T cell activity and in the tumor microenvironment.

Original figure modified for this publication. Callahan MK, Postow MA, Wolchok JD.

Immunomodulatory therapy for melanoma: ipilimumab and beyond. Clin Dermatol 2013; 31:191.

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Other
Pathways

Response rates with immunotherapy

1. Meta-analysis of a number of trials report better response rates and progression free survival than chemotherapy in most solid tumors to date.
2. Responses are best in advanced melanoma, especially in first line therapy with combined nivolumab and ipilumimab (39% and 58% 3 year PFS and OS; better in BRAF mutated tumors).
3. Rates of pseudoprogression (response after initial period of progression) range from 1.3 to 8%.
4. Rates of hyperprogression (rapid progression after initiation of therapy) range from 4-29%.

Issues with PD-L1 expression testing

1. Indications for immunotherapy continue to evolve with new cancers acquiring FDA approval for treatment with pembrolizumab and other drugs on a regular basis.
2. Certain immunotherapy drug approvals are tied specific FDA approved PD-L1 IHC assays, either as companion diagnostics (required for treatment) or complementary diagnostics (optional) or not at all
3. Determining which test assay to implement and how to validate has been a source of confusion and difficulty for laboratories.

Immunotherapy
drug approvals
anti-PD-1
anti-PD-L1
anti-CTLA-4

+Med. DLBCL
Cervical ca
Small cell lung ca
Triple negative
breast ca

Table 1. Anti-PD-1/PD-L1 immunotherapies, indications and diagnostic assay requirement

Cancer type	Drug(s)	Drug target(s)	Indications in US			Indications in EU	
Melanoma	Pembrolizumab	PD-1	Unresectable or metastatic			Unresectable or metastatic	
	Nivolumab	PD-1					
	Nivolumab + ipilimumab	PD-1, CTLA-4					
Non-small-cell lung cancer	Nivolumab	PD-1	Metastatic disease with progression on/after platinum-based chemotherapy or after FDA-approved treatment if EGFR+ or ALK+			Locally advanced or metastatic disease after prior chemotherapy in adults	
	Atezolizumab	PD-L1				If progression after chemotherapy or after targeted treatment if EGFR+ or ALK+	
	Pembrolizumab	PD-1	1 st line with pemetrexed & carboplatin	1 st line monotherapy if EGFR-/ALK-	2 nd line monotherapy if progression on/after platinum-based chemotherapy or after FDA-approved treatment if EGFR+ or ALK+	1st line monotherapy if EGFR-/ALK-	If progression on/after platinum-based chemotherapy or after targeted treatment if EGFR+ or ALK+
Renal cell carcinoma	Nivolumab	PD-1	Advanced disease after prior anti-angiogenic therapy			Advanced disease after prior therapy	
Classical Hodgkin lymphoma	Nivolumab	PD-1	Relapsed or progressed disease after auto-HSCT and BV, or 3 or more lines of therapy including auto-HSCT			Relapsed or refractory disease after auto-HSCT and treatment with BV	
	Pembrolizumab	PD-1	With refractory disease or who have relapsed after 3 or more prior lines of therapy			Relapsed or refractory disease after auto-HSCT and BV, or are transplant-ineligible and have failed BV	
Bladder cancer	Atezolizumab	PD-L1	Locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-based chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy			Locally advanced unresectable or metastatic urothelial carcinoma after prior platinum-based chemotherapy or considered cisplatin ineligible	
	Nivolumab	PD-1				Locally advanced unresectable or metastatic urothelial carcinoma after failure of prior platinum-based chemotherapy	
	Durvalumab	PD-L1	Locally advanced or metastatic urothelial carcinoma not eligible for cisplatin-containing chemotherapy or who have disease progression during or following platinum-based chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy			NO	
	Avelumab	PD-L1				NO	
	Pembrolizumab	PD-1				Locally advanced or metastatic urothelial carcinoma not eligible for cisplatin-containing chemotherapy or who have disease progression during or following platinum-based chemotherapy	
Head and neck cancer	Pembrolizumab	PD-1	Recurrent or metastatic squamous cell carcinoma with disease progression on or after platinum-based therapy			NO	
	Nivolumab	PD-1				Squamous cell cancer progression on or after platinum-based therapy	
Merkel cell carcinoma	Avelumab	PD-L1	Metastatic disease			Metastatic disease	
Gastric cancer	Pembrolizumab	PD-1	Recurrent locally advanced or metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma with disease progression on or after two or more prior lines of therapy including fluoropyrimidine- and platinum-containing chemotherapy and, if appropriate, HER2/neu-targeted therapy			NO	
Liver cancer	Nivolumab	PD-1	Hepatocellular carcinoma previously treated with sorafenib			NO	
MSI-H or dMMR-deficient solid tumours	Pembrolizumab	PD-1	Unresectable or metastatic solid tumours that have progressed following prior treatment and who have no satisfactory alternative treatment options, or colorectal cancer that has progressed following treatment with fluoropyrimidine, oxaliplatin, and irinotecan			NO	
MSI-H or dMMR-deficient colorectal tumours	Nivolumab	PD-1	Metastatic colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan			NO	

Immunotherapy Drugs and Diagnostic Tests

Drug	Indications	Diagnostic Test
Nivolumab (Opdivo)	NSCLC- 2nd line Melanoma Bladder Colorectal-2 nd line	Dako 28-8-complement. Dako 28-8-complement. Dako 28-8-complement. MMR IHC/ MSI PCR
Atezolizumab (Tecentriq)	Bladder- 1 st or 2nd NSCLC- 2 nd line Met/ unresect. triple negative breast ca	Vent. SP142- companion. No specific test required. FDA approved test. (Vent)
Durvalumab (Imfinzi)	Bladder- 2 nd line	Vent. SP263-complement.
Avelumab (Bavencio)	Merkel cell- 1 st line Bladder- 2 nd line	None required Dako 73-10 FDA approved
Ipilumimab (Yervoy)	Melanoma	None

Immunotherapy Drugs and Diagnostic Tests

Drug	Indications	Diagnostic Test
Pembrolizumab (Keytruda)	NSCLC- 1st line	Dako 22C3-companion*
	NSCLC- 2 nd line	Dako 22C3-companion#
	Bladder ca-2 nd line	Dako 22C3-companion@
	GE junc.-gastric	Dako 22C3-companion@
	Cervical- 2 nd line	Dako 22C3-companion@
	Head & neck SCC	Optional
	Hodgkin- 2 nd line	None required
	Prim. Med. NHL	None required
	Melanoma	None required
	Liver-HCC-2 nd line	None required
	Merkel cell ca	None required
	Lung-small cell ca	None required
	MMR def-MSI solid tumors 2 nd line	MMR IHC/ MSI PCR

*TPS \geq 50% #TPS \geq 1% @CPS \geq 1

Commercially available anti-PD-L1 clones

E1L3N, E1J2J, SP142, 28-8, 22C3, SP263, 73-10,
EPR1161-2, 7G11

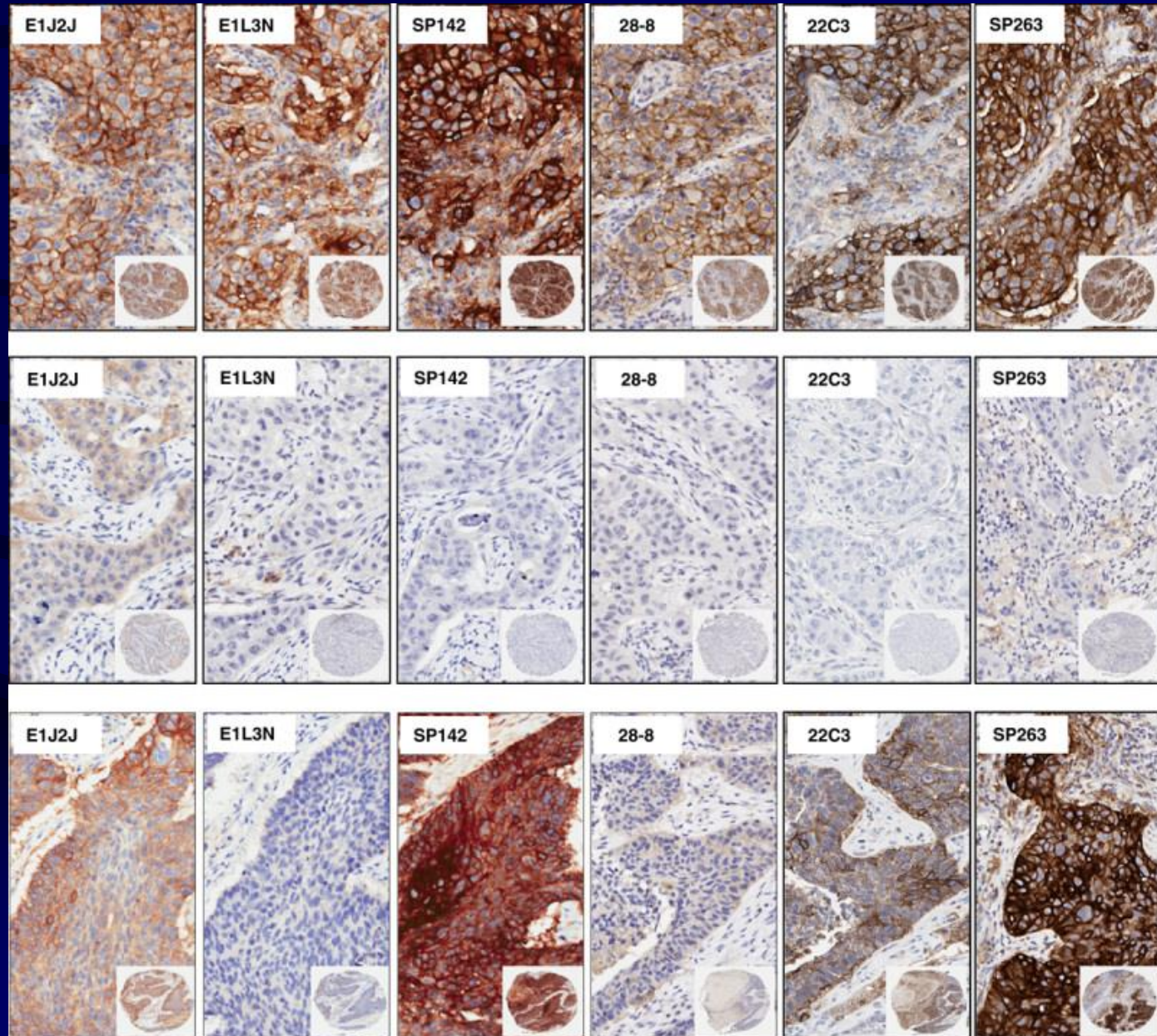
Blueprint study 1: 28-8, 22C3 and SP263 were found to be equivalent across the case set (n=39).

Blueprint 2: added clone 73-10, slightly superior (n=81).
SP142 detected fewer positive cases than the others.

8 commercial clones tested on **259 NSCLC's**:

E1L3N, E1J2J, SP142, 28-8, 22C3, and SP263 found to be equivalent with **SP263** showing the best performance.
(Para ER et al, Appl Immunohistochem Mol Morphol. 2018 Feb;26(2):83-93).

Cell line- positive control-Dako- PharmDx-22C3



Para ER et al, Appl Immunohistochem Mol Morphol. 2018 Feb;26(2):83-93.

Options for PD-L1 expression testing

1. Bring in the most commonly used assay (Dako 22C3) for the most prescribed drug (pembrolizumab) and validate for only those indications. Send out for the others.
2. Bring in one assay and validate for all approved drug-tumor combinations (i.e. E1L3N; not attractive for most labs given the additional validation requirements as a non-FDA approved LDT).
3. Bring in all 4 FDA approved assays (Dako 22C3, Dako 28-8, Ventana SP142 and Ventana SP263).

SQL/ LSA chose to option # 1 for the immediate future

Considerations before testing

It is imperative to know which immunotherapy drug is being considered for use and the indications for its use.

For example, advanced NSCLC with pembrolizumab.

For PD-L1 testing using the Dako 22C3 PharmDx assay, currently the only test offered by SQL, the indications are treatment with pembrolizumab for:

Advanced NSCLC:	testing required (CD)
Urothelial cancer:	testing required
Gastric or GE junction:	testing required
Cervical cancer:	testing required
Head & Neck SCC, Melanoma, Hodgkin, Med. DLBCL, Merkel, HCC, small cell lung ca:	optional

Tumors for which the DAKO 22C3 PharmDx is not required to be used to guide immunotherapy

Bladder, lung cancer, triple neg. breast ca treated with atezoluzimab (Tecentriq, Vent. SP142 for bladder /breast required- companion diagnostic)

Bladder cancer treated with durvalumab (Imfinzi, Vent. SP263 complementary (optional) or avelumab (no test).

Melanoma, NSCLC, bladder or colorectal treated with nivolumab (first 3: Dako 28.8 complementary, colorectal: MMR/MSI) or ipilumimab (none required).

All other solid tumors or lymphomas (other than Hodgkin or Med. DLBCL) considered for 2nd/3rd line pembrolizumab (Keytruda) therapy require MMR deficient or MSI +

No PD-L1 test approved to guide therapy (4/1/19)

Breast ca other than triple negative and Prostate carcinomas

Pancreatic and biliary carcinomas

Small intestinal carcinomas

Endocrine malignancies

Non-squamous, non-melanoma head and neck cancers

Neuroendocrine carcinomas including pulmonary small cell

Non-Hodgkin lymphoma other than primary Med. DLBCL

Renal cell and adrenal cortical carcinomas

Gynecologic malignancies other than cervical

Primary CNS tumors

Soft tissue and bone sarcomas

Cutaneous malignancies other than melanoma and Merkel cell

Most pediatric malignancies unless noted in slides 3 and 4

Any solid tumor failing standard treatment may be treated with pembrolizumab if proven MMR deficient/ MS unstable



Scoring criteria- Dako- PharmDx

For Non-small cell lung cancer: Tumor Proportion Score:

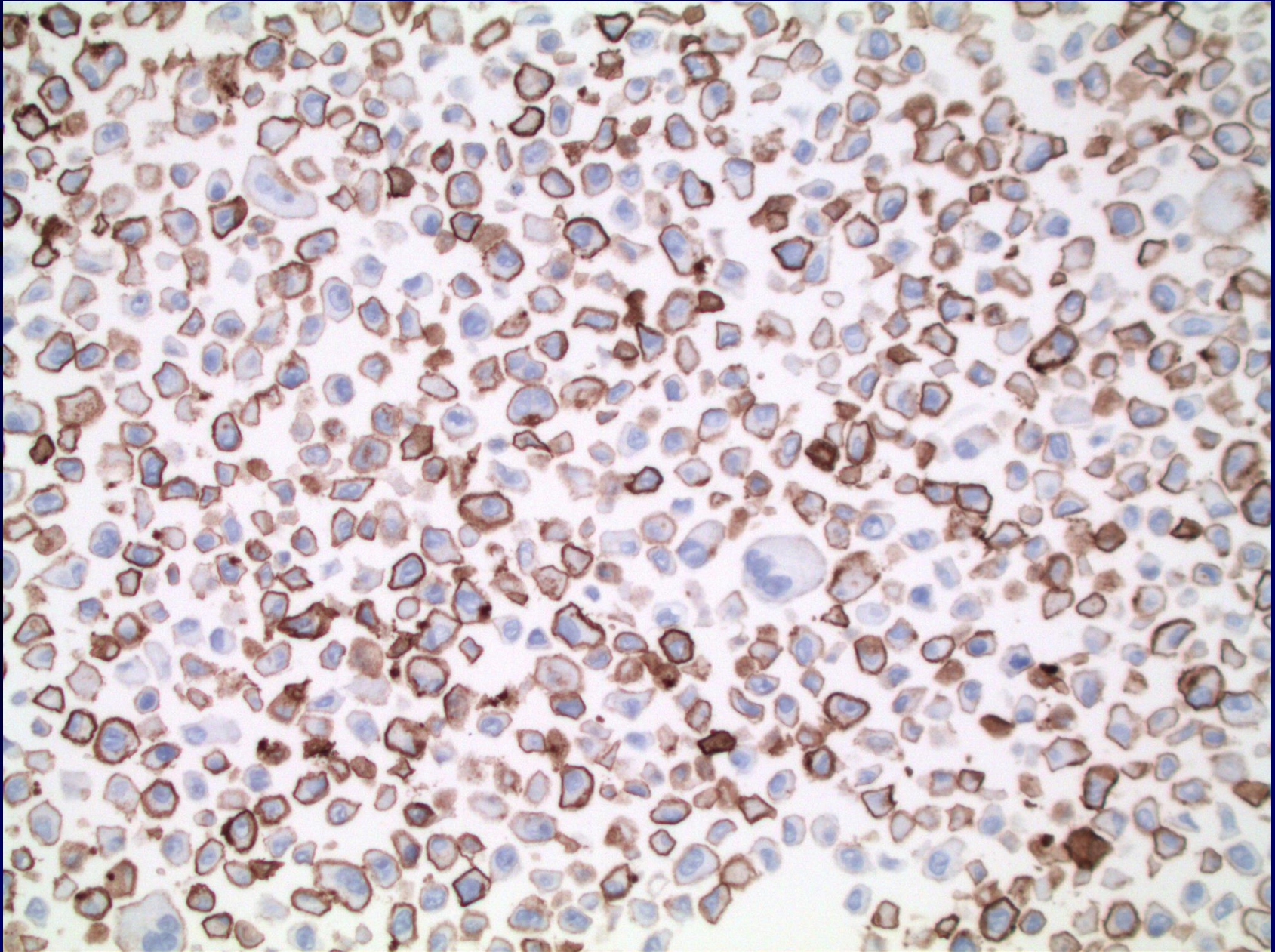
viable tumor cells labeling/ total # viable tumor cells =
0-100%

Labeling must be membranous, partial or complete.

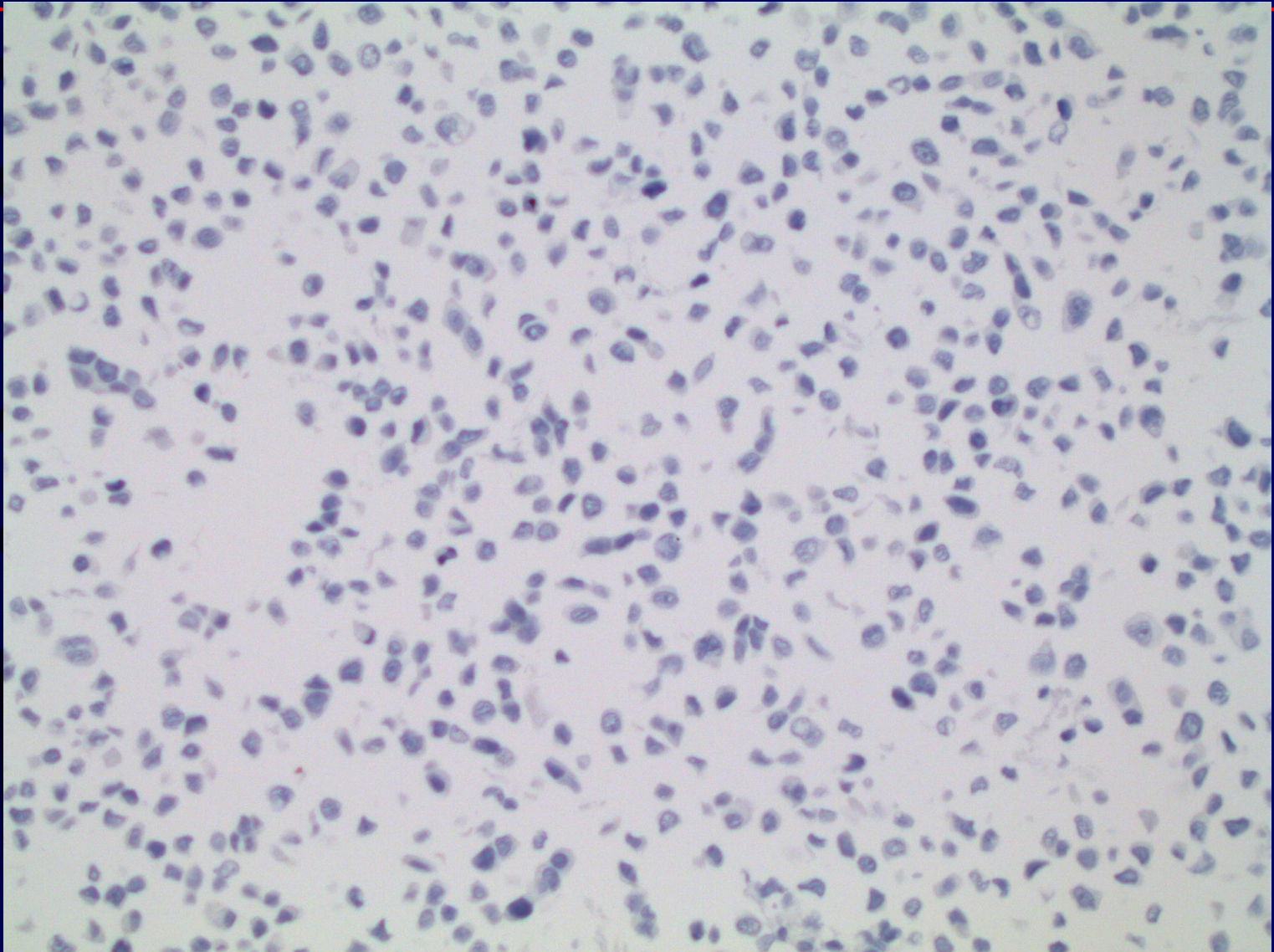
Do not score cytoplasmic only, crushed or necrotic cells
or immune cells.

<1% is negative; 1-49% is positive low expression;
≥50% is positive, high expression.

Cell line- positive control-Dako- PharmDx-22C3

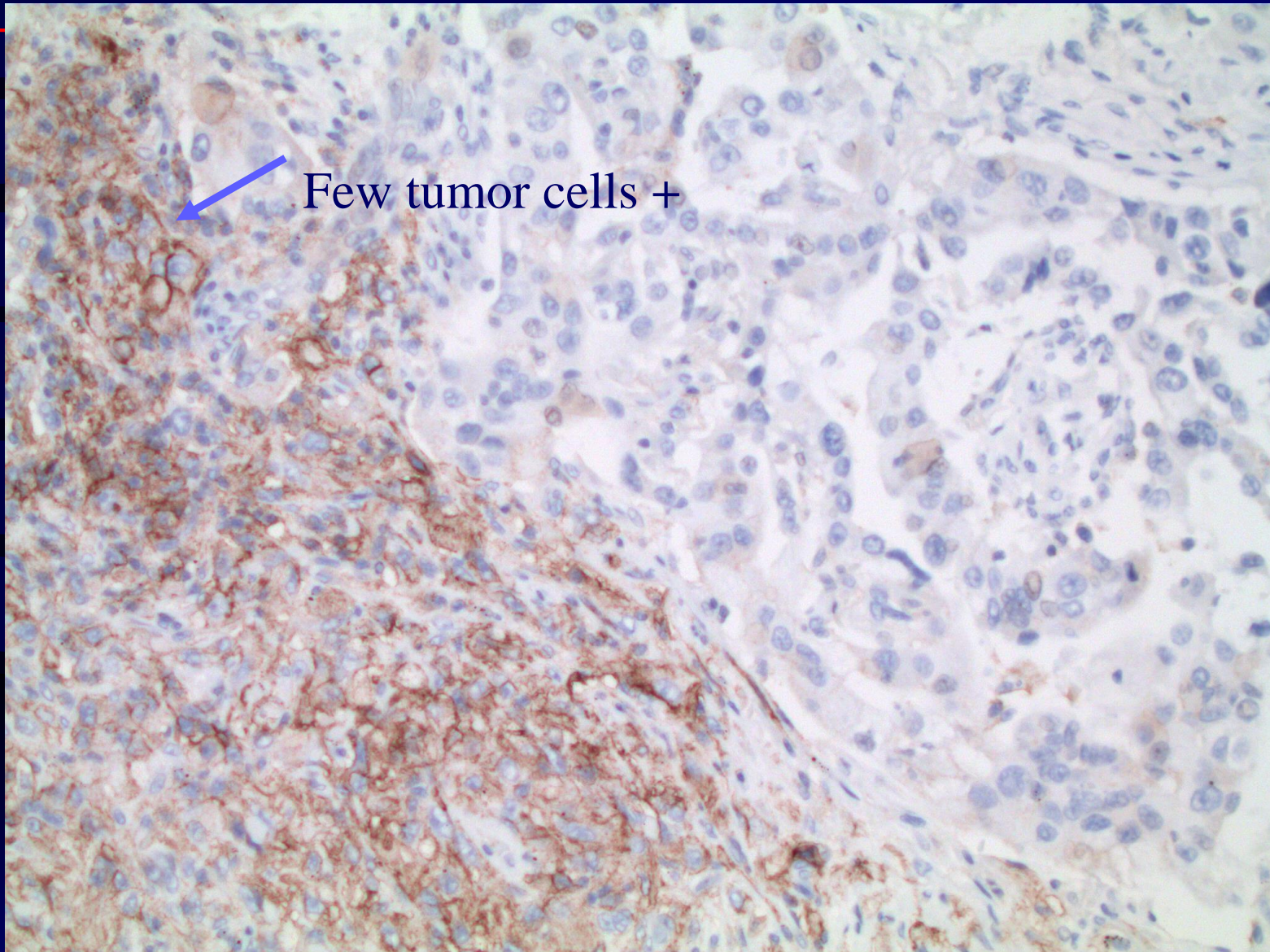


Cell line-negative control-Dako- PharmDx-22C3



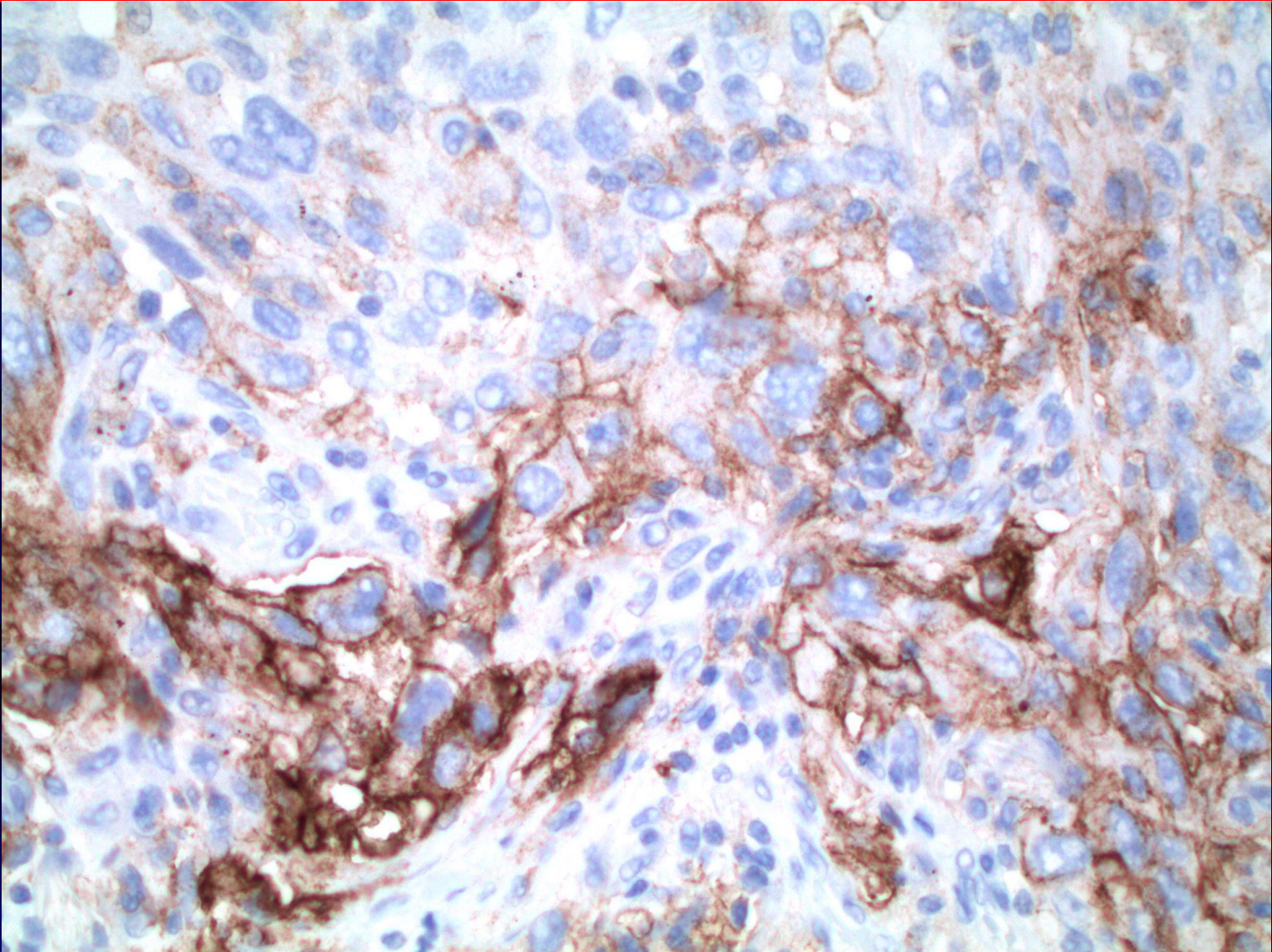
One set of positive and neg. cell line controls are performed for each run

Tissue control-Dako- PharmDx- 22C3



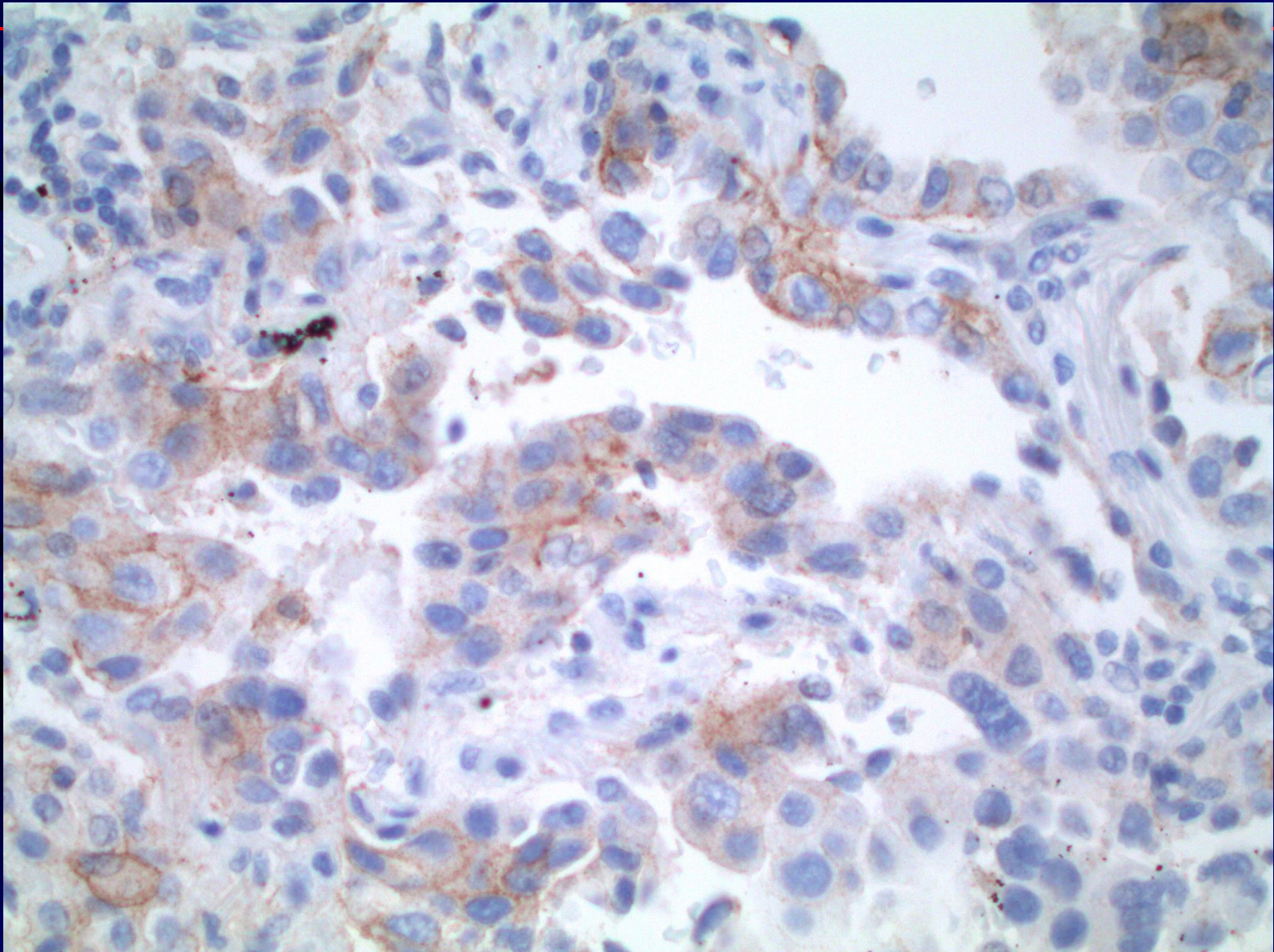
Immune cells and rare tumor cells are immunoreactive

Tissue control-Dako- PharmDx- 22C3



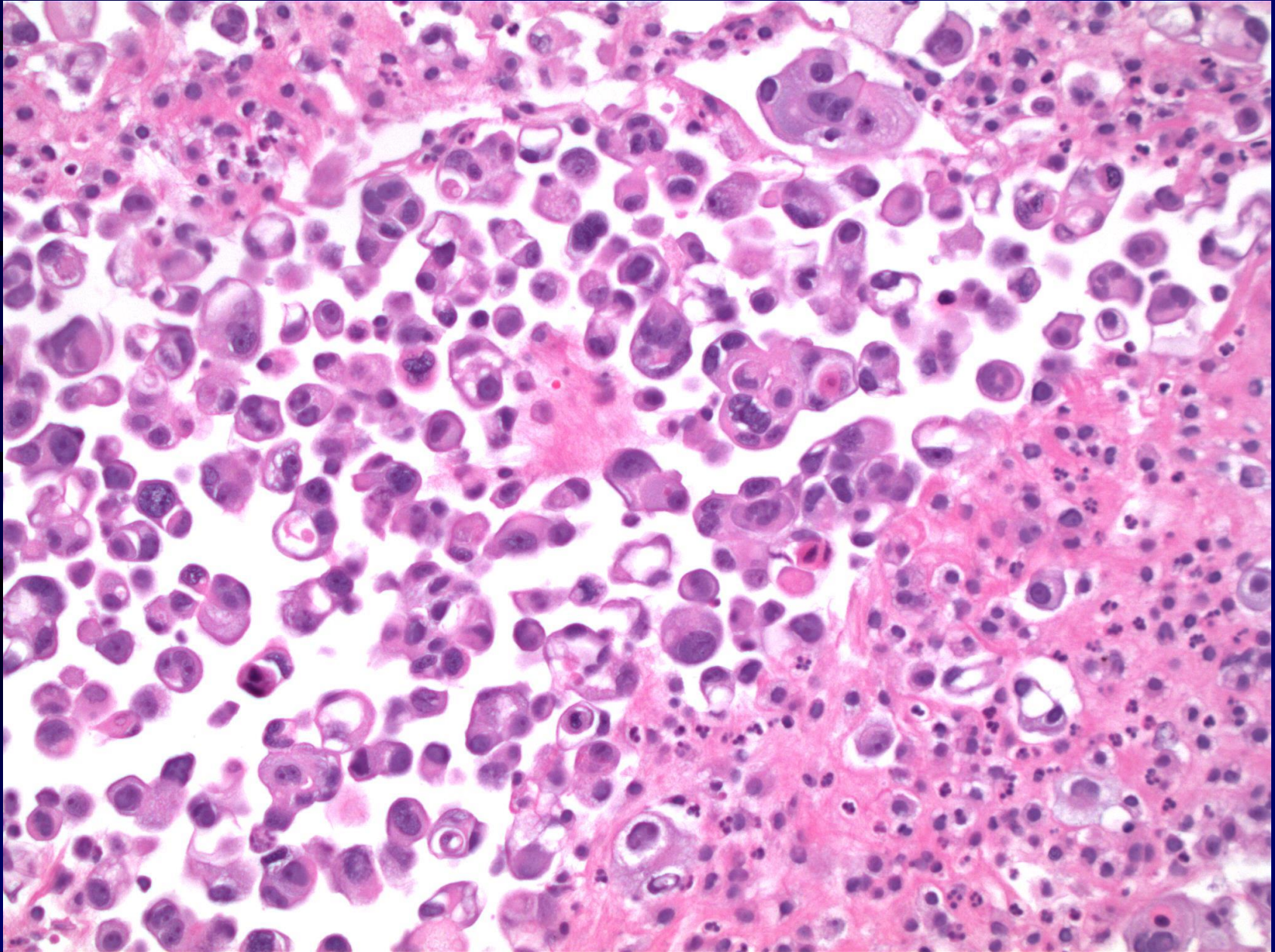
Immune cells and tumor cells are immunoreactive

Tissue control-Dako- PharmDx- 22C3

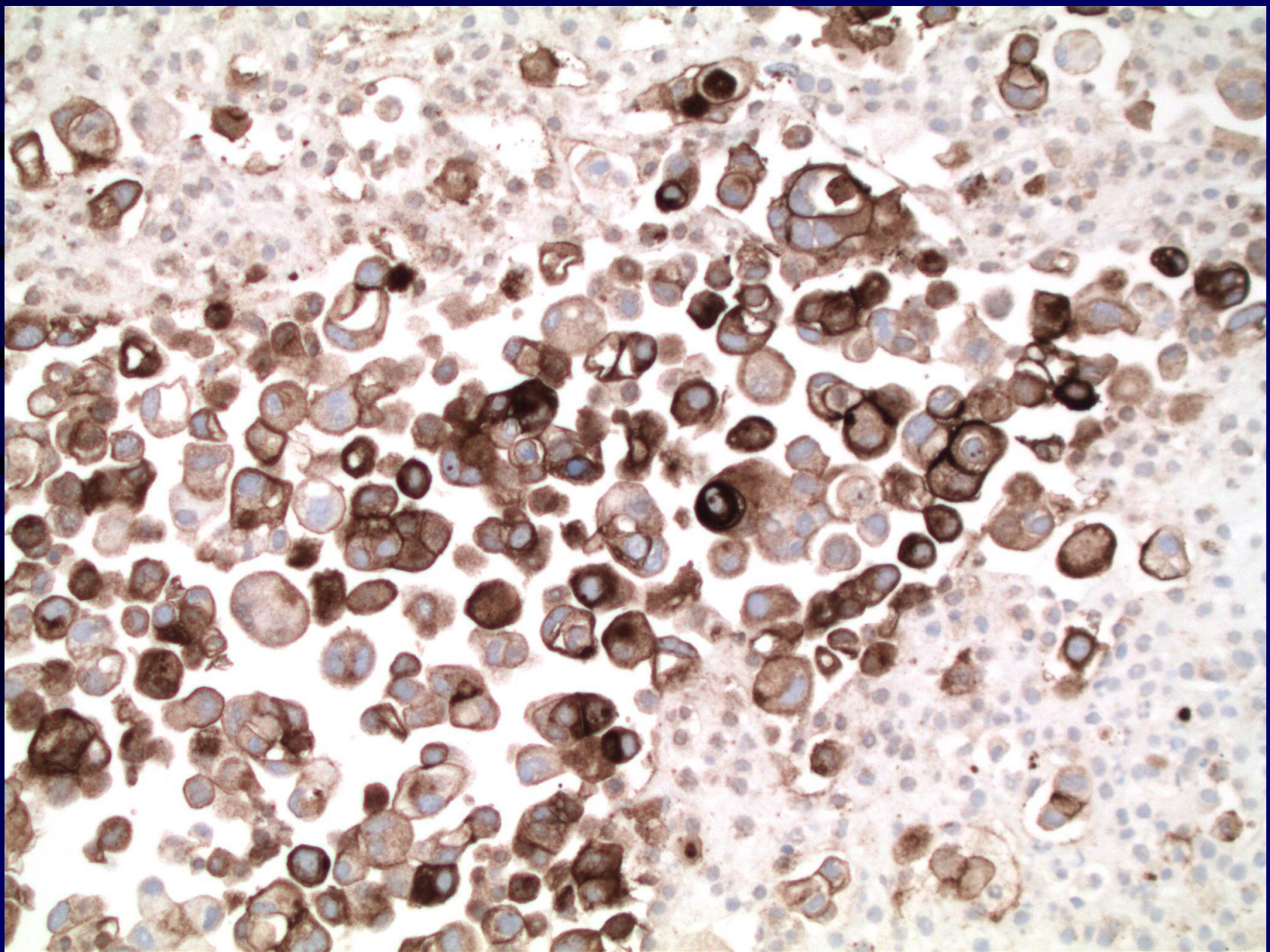


Immune cells and tumor cells are immunoreactive

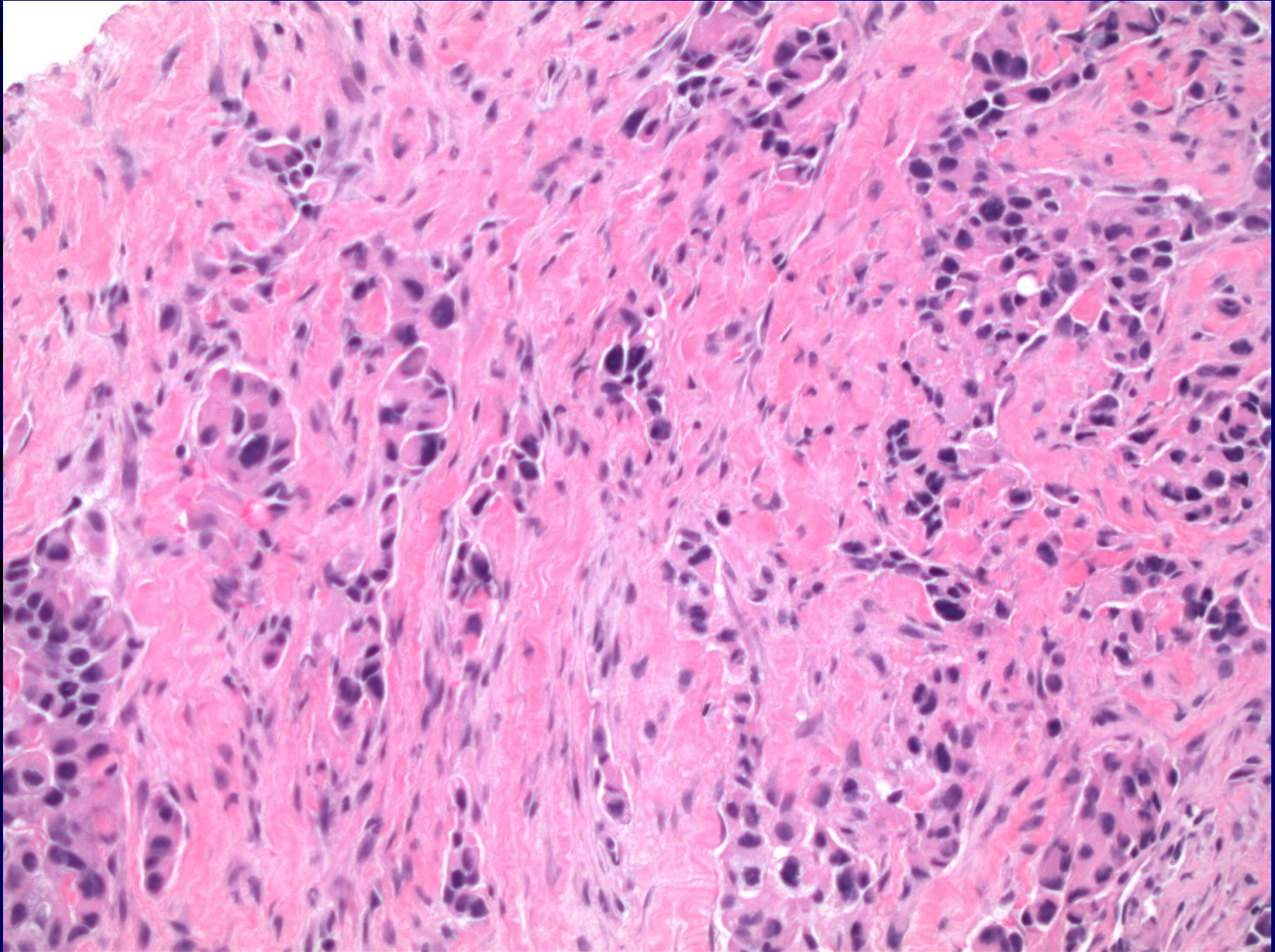
Met lung ca-pl. fluid-Dako- PharmDx- 22C3



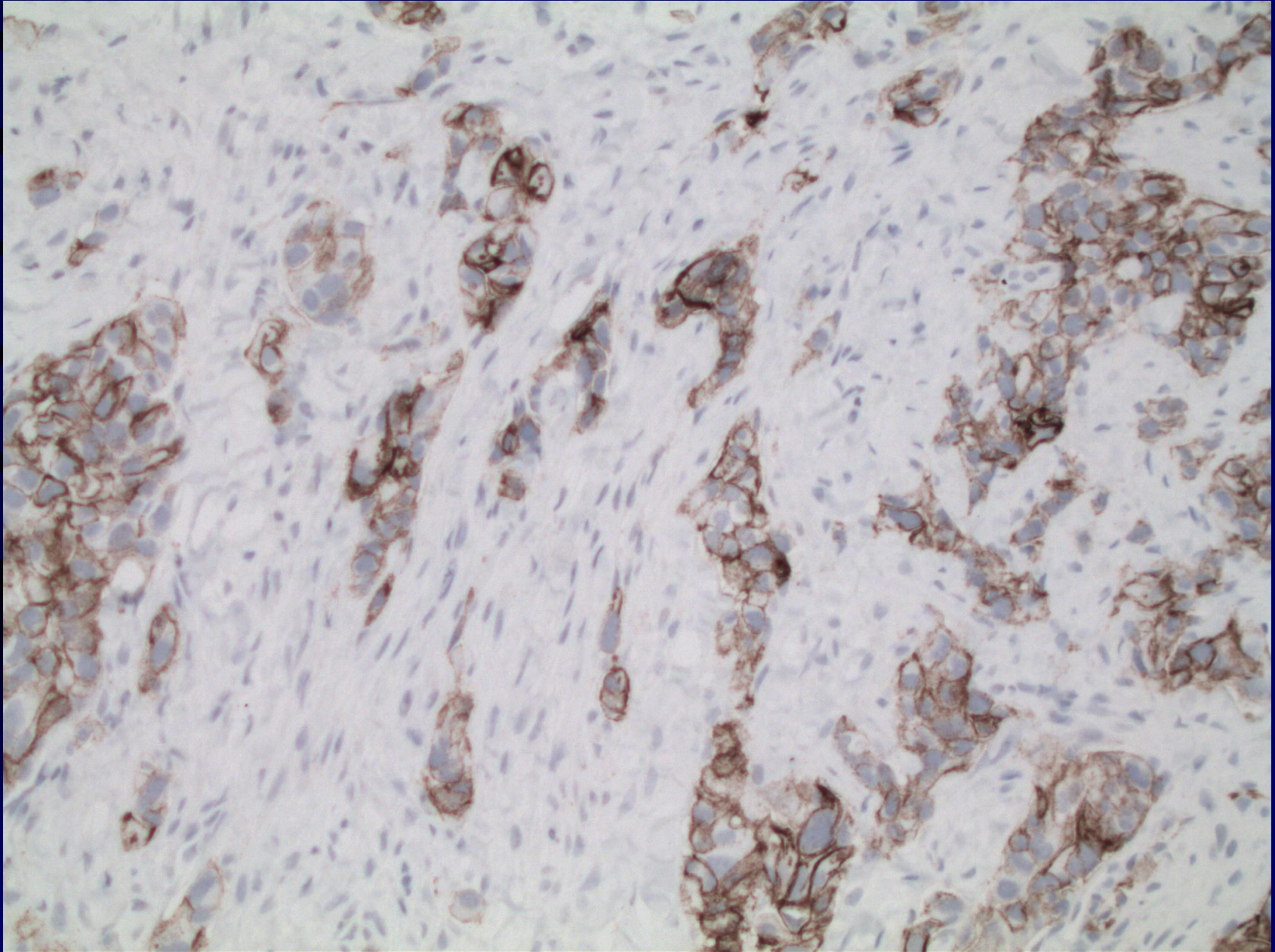
Met lung adenoca-pleural fluid- TPS 100%



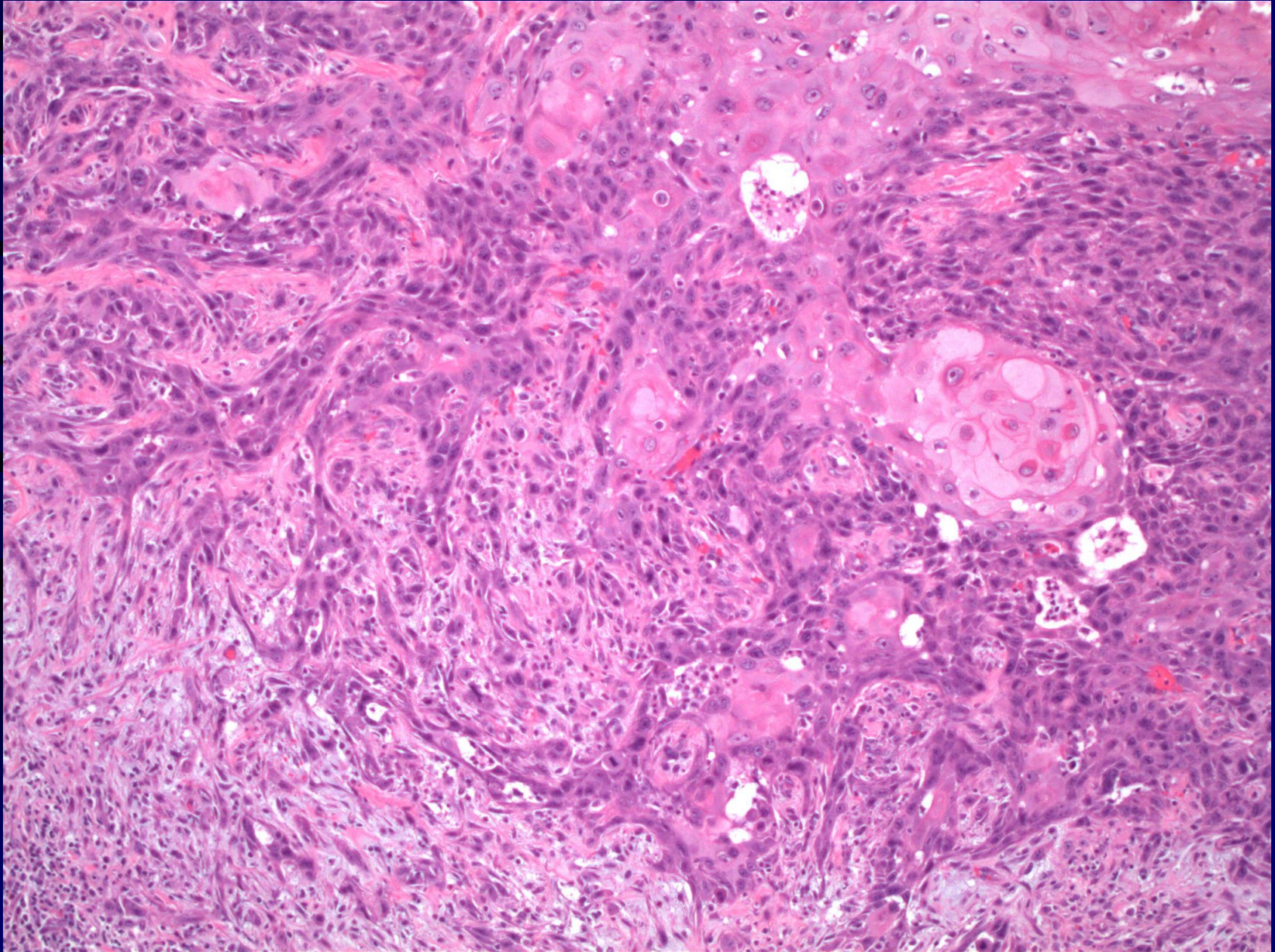
Met lung adenoca-soft tissue-TPS 80%



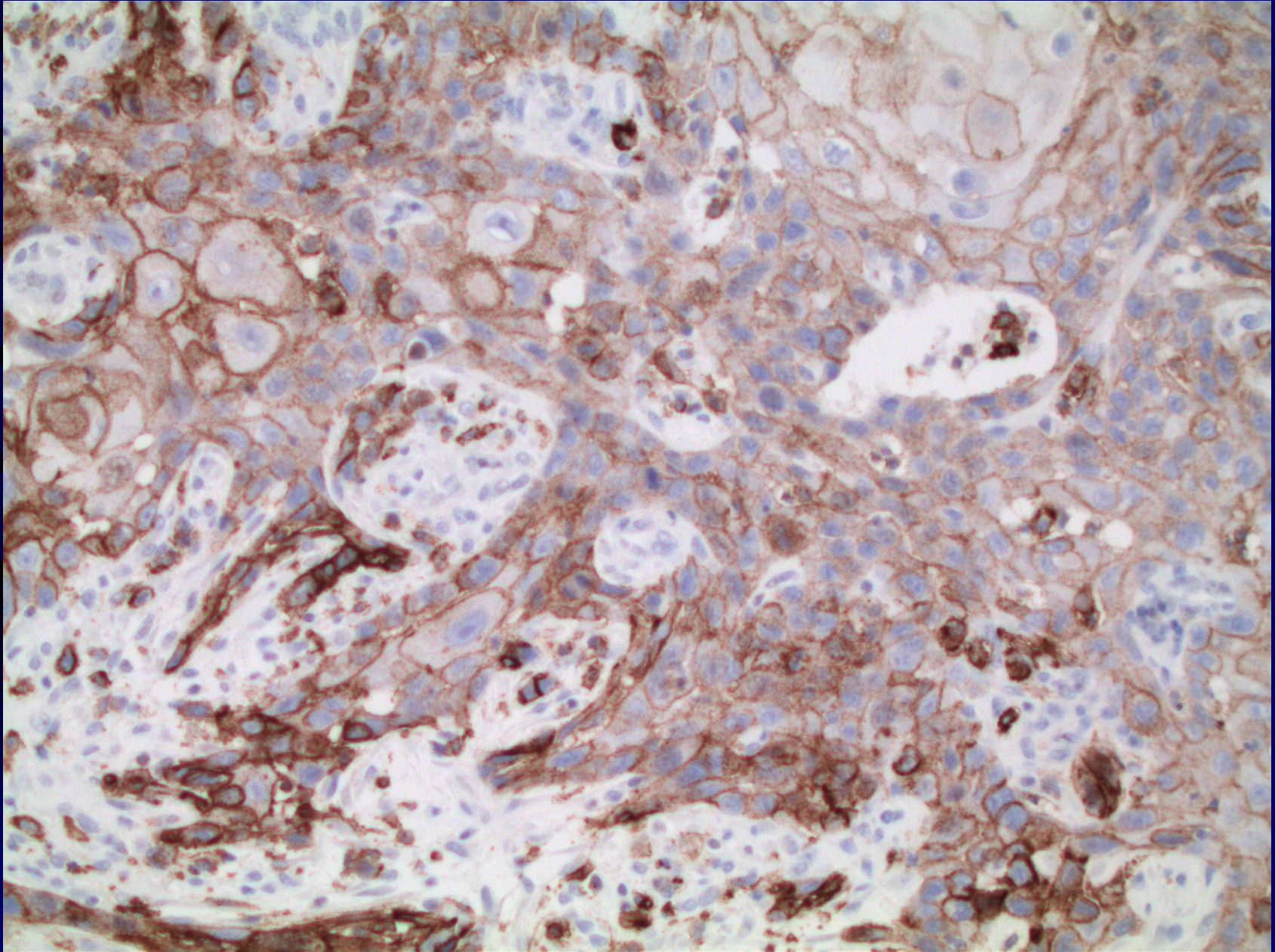
Met lung adenoca-soft tissue-TPS 80%



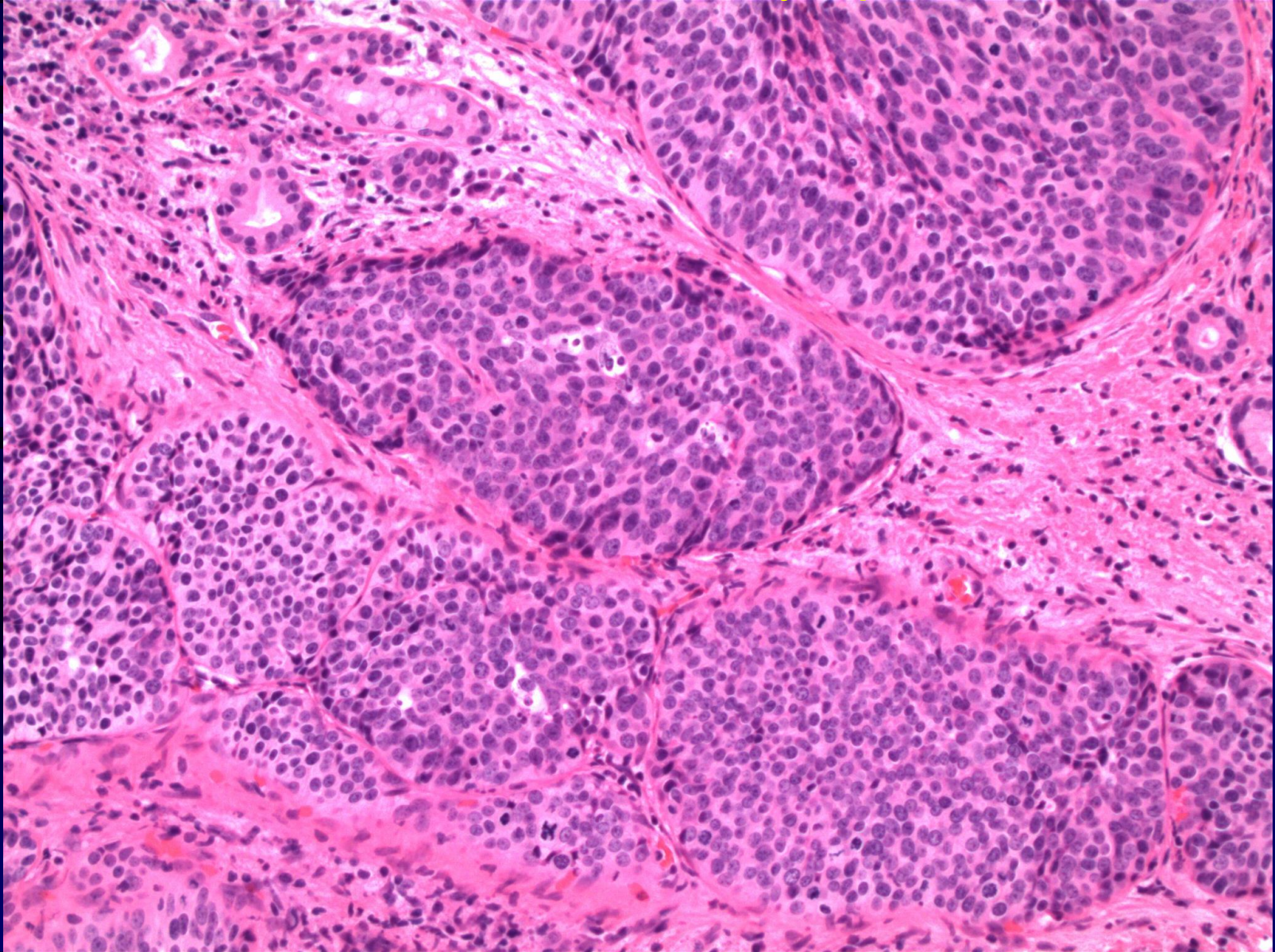
Oral cavity SCCA- TPS 70-80%



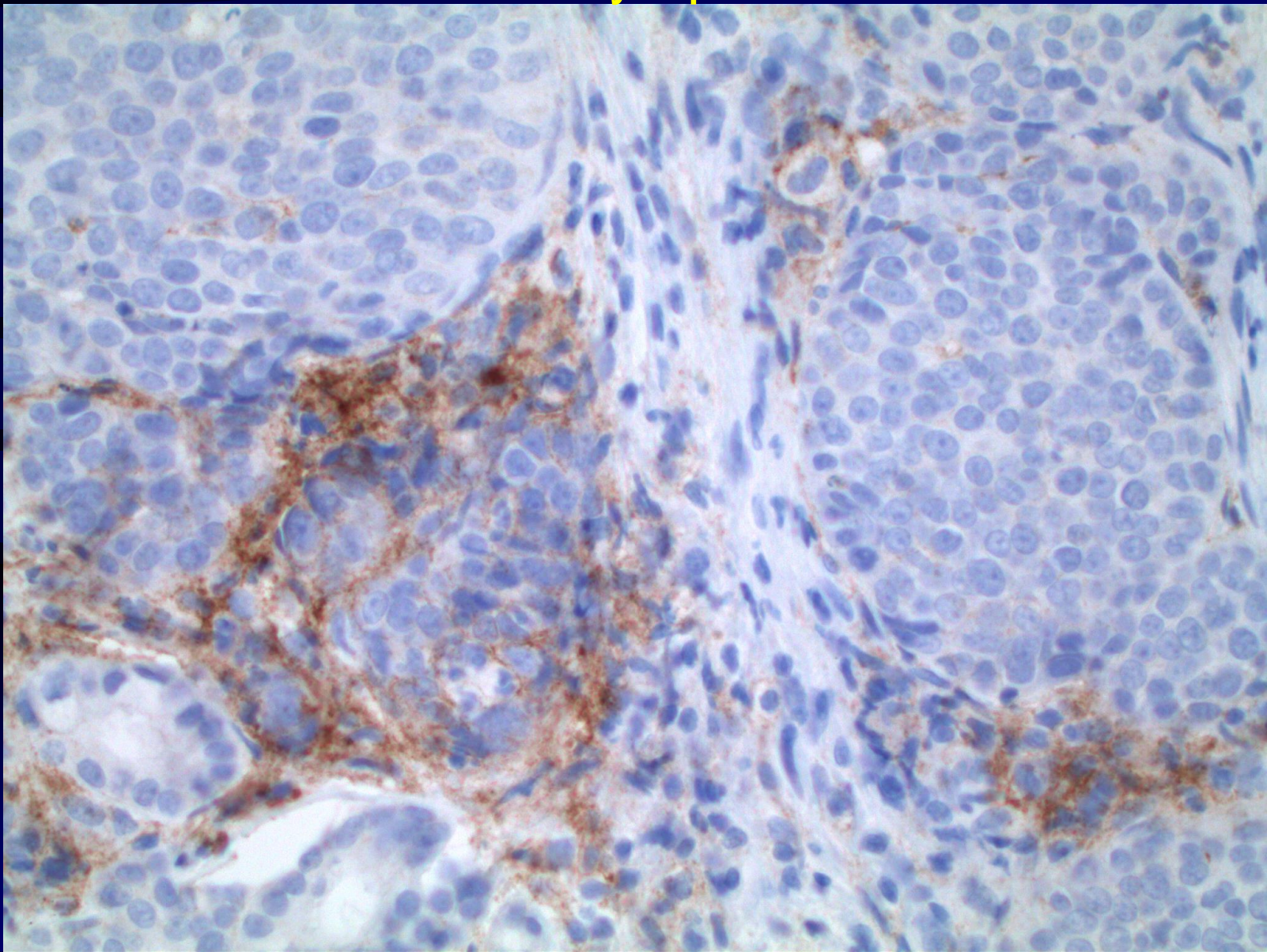
Oral cavity SCCA- TPS 70-80%



Nasal SCCA- TPS 0%: Intratumoral lymphs reactive

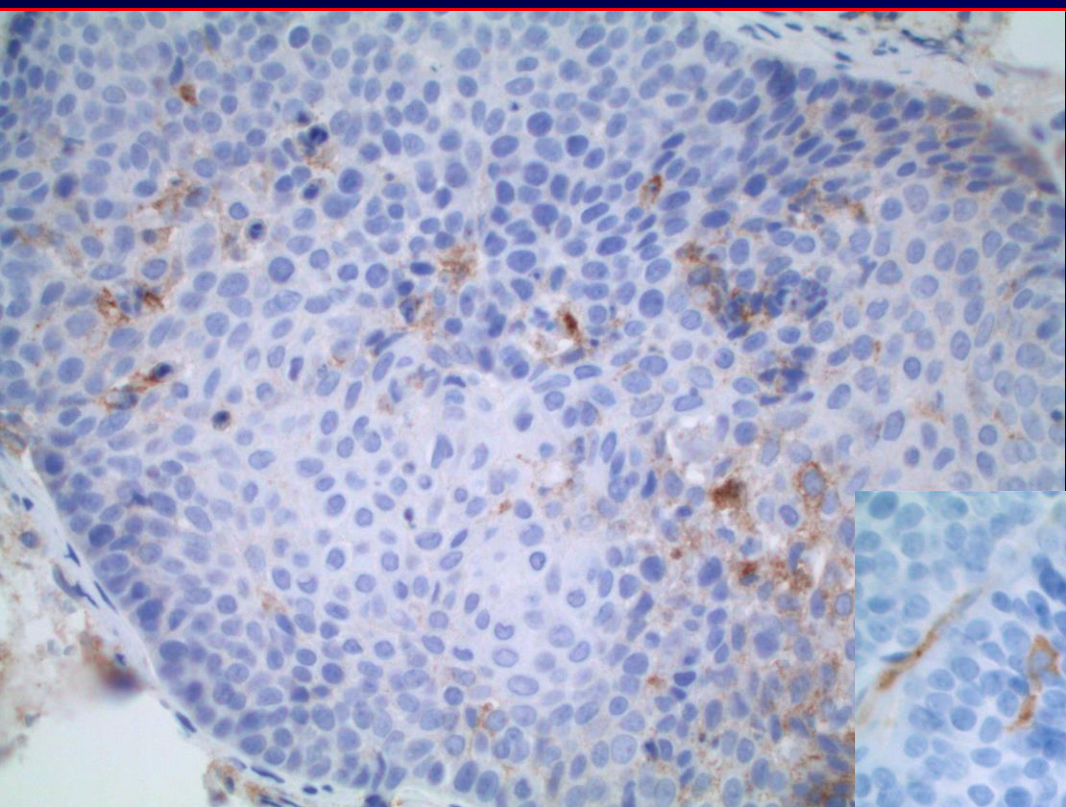


Nasal cavity SCCA- TPS <1% Intratumoral lymphs reactive



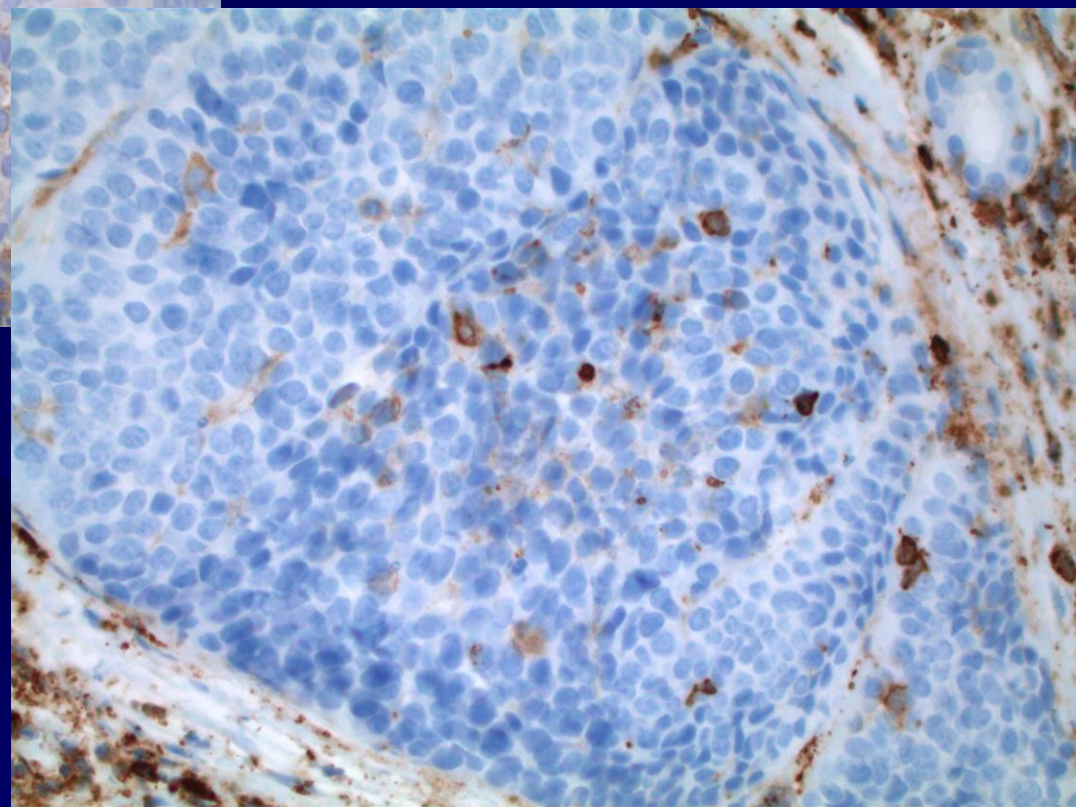
Nasal cavity SCCA-
TPS 0%
Intratumoral lymphs
reactive

CD45

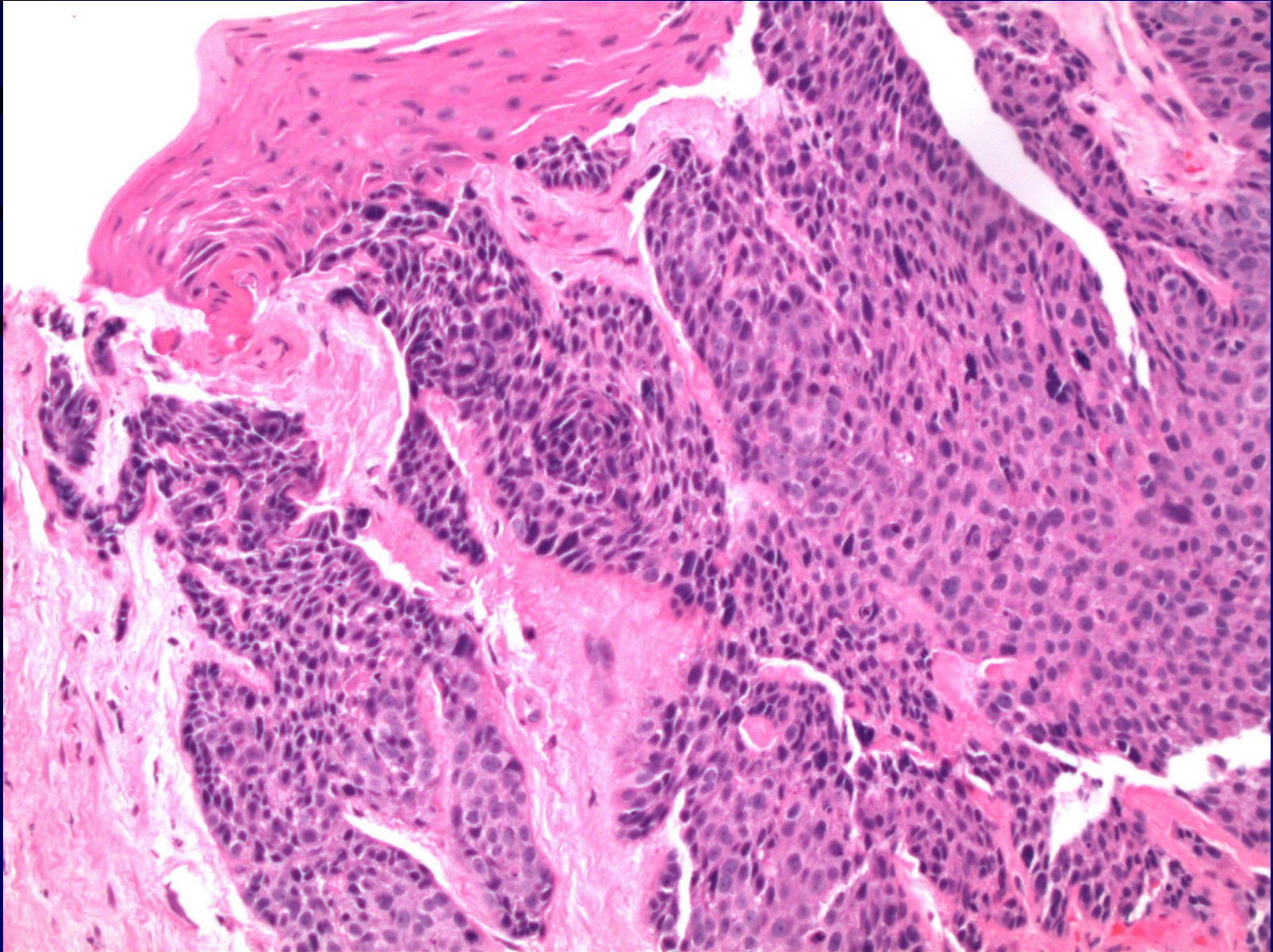


PD-L1

Parallel CD45
can be useful

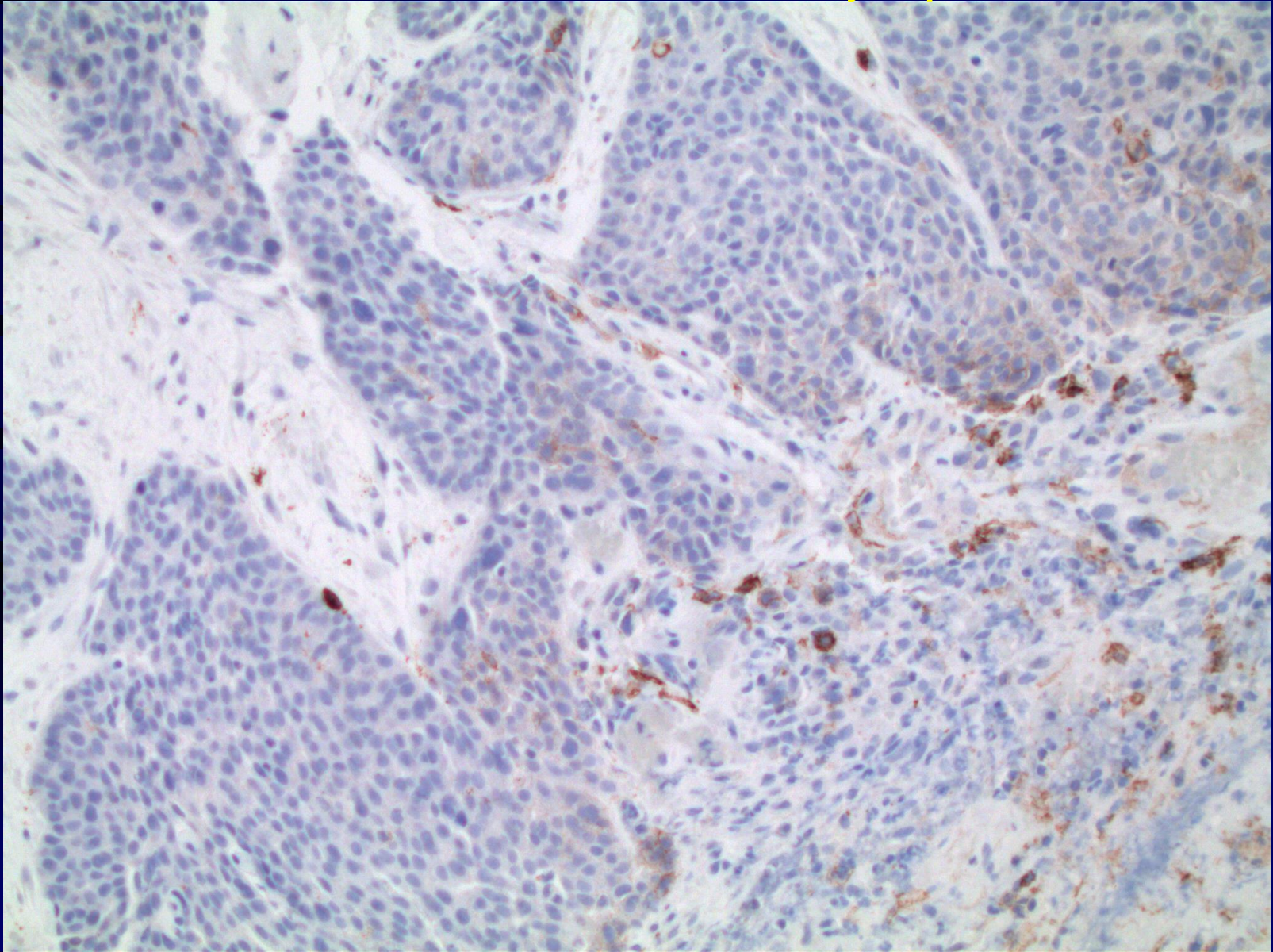


Oropharyngeal SCCA- TPS 1% Intratumoral immune cells also reactive

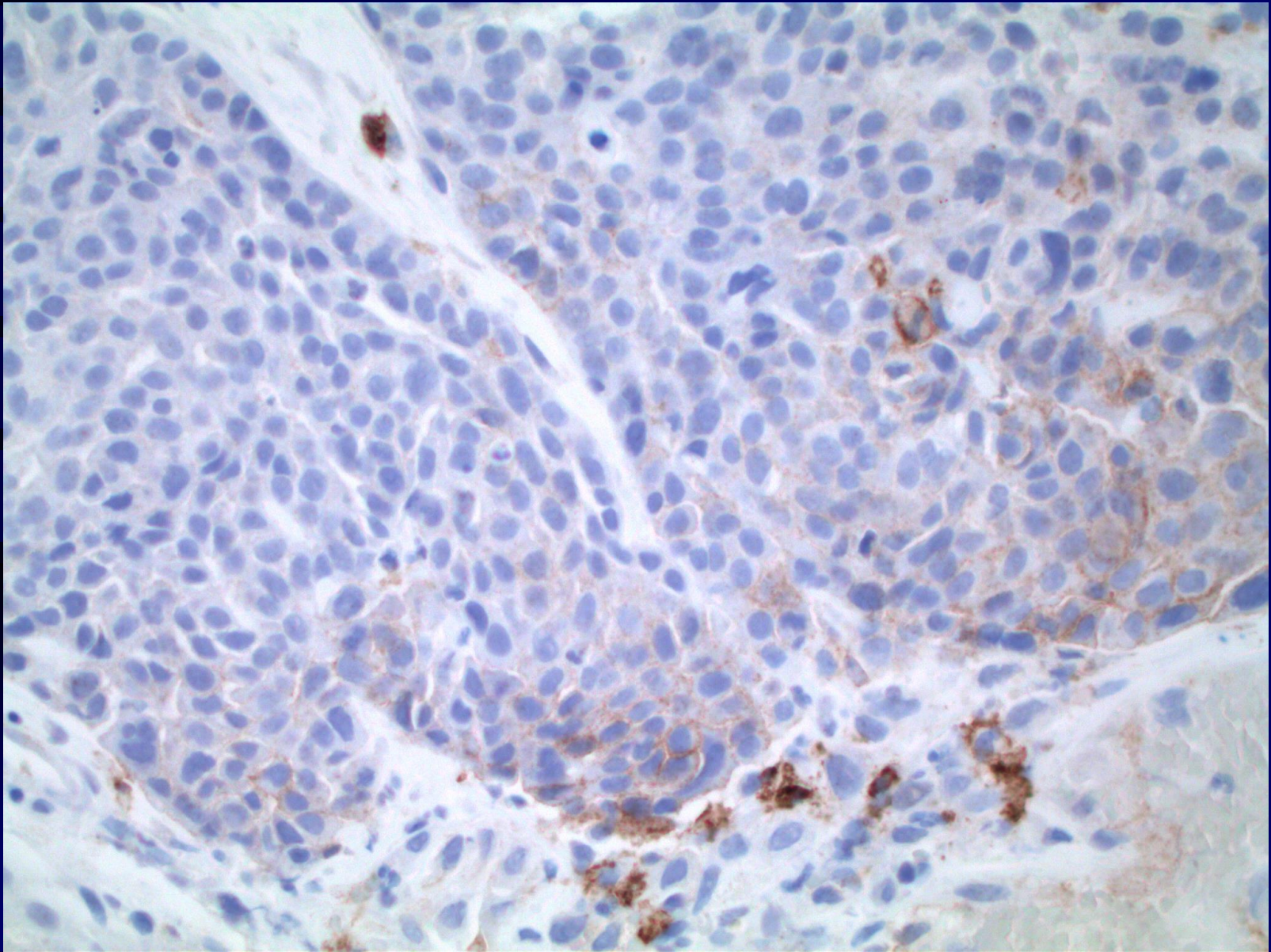


Oropharyngeal SCCA- TPS 1%

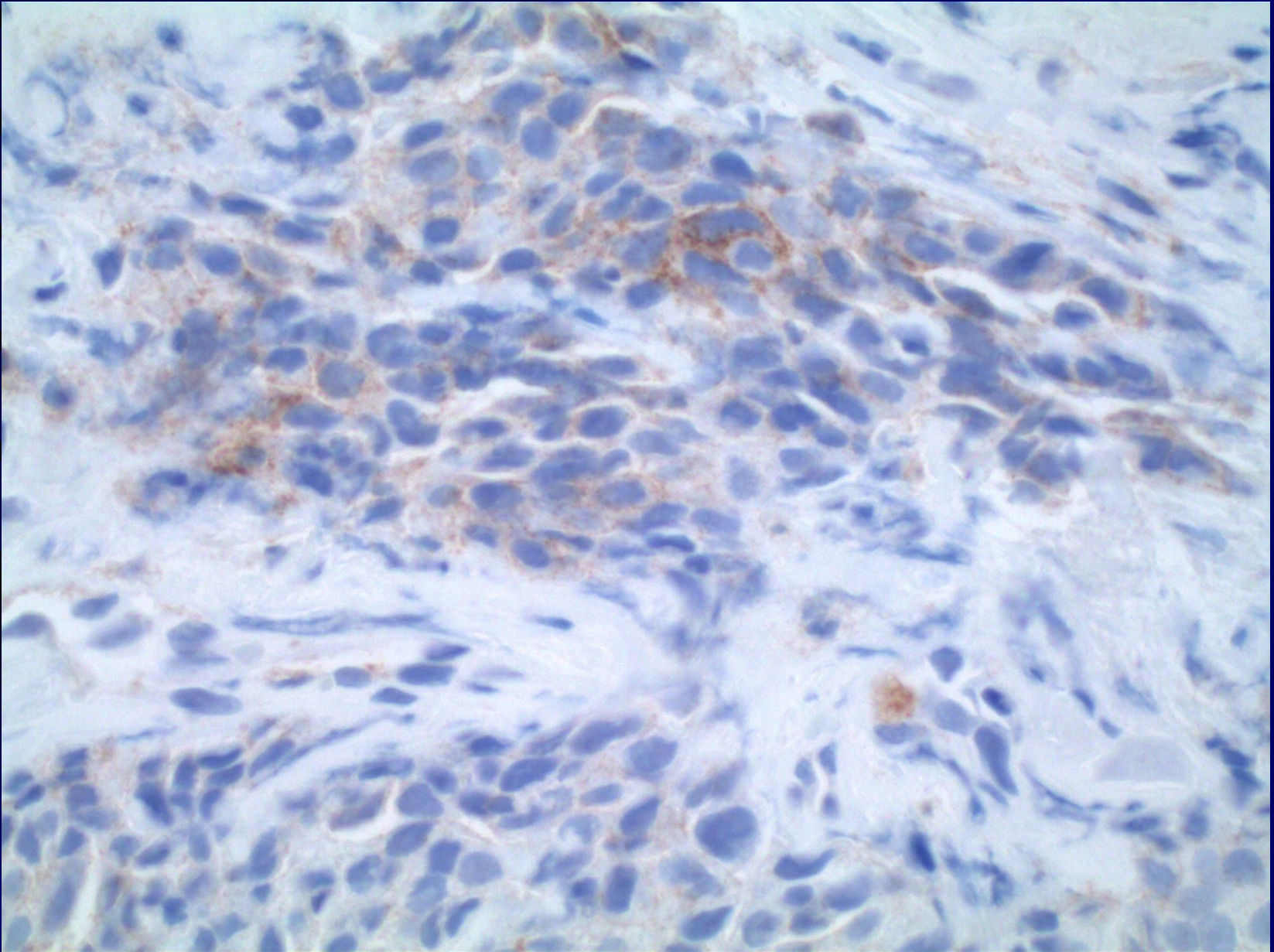
1% tumor cells- intratumoral lymphs reactive



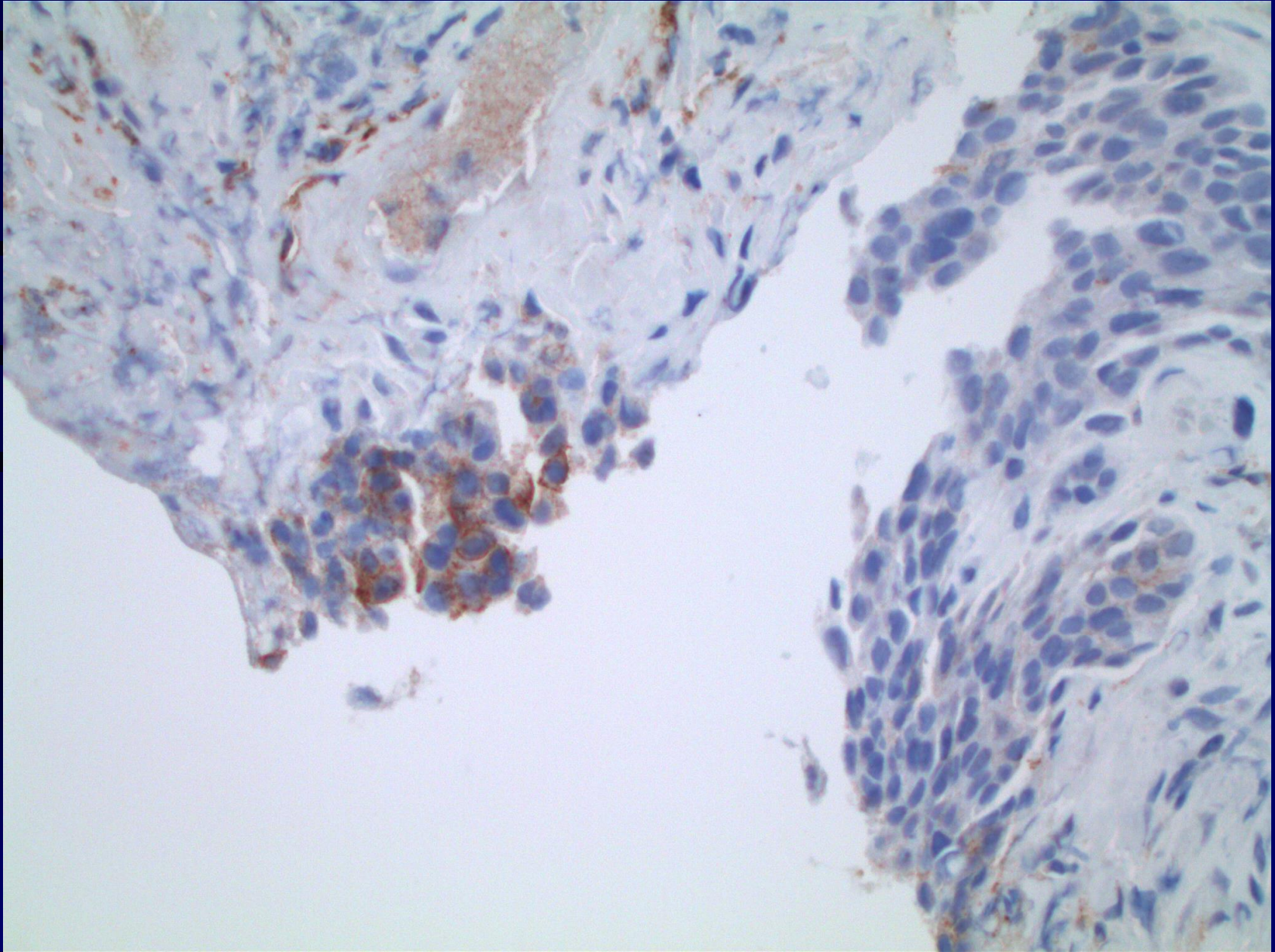
Oropharyngeal SCCA- TPS 1% Peritumoral immune cells also reactive



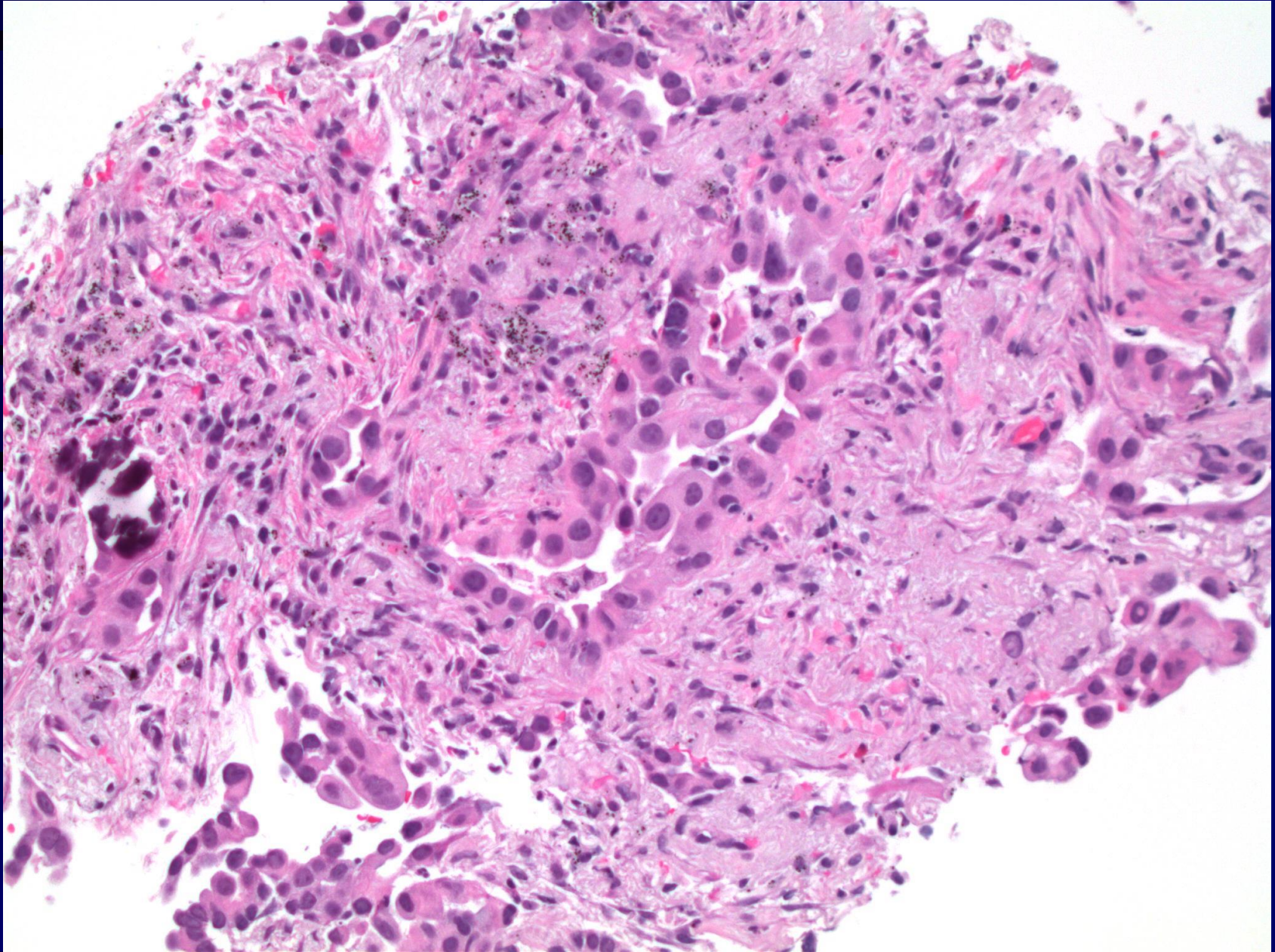
Oropharyngeal SCCA- TPS 1%



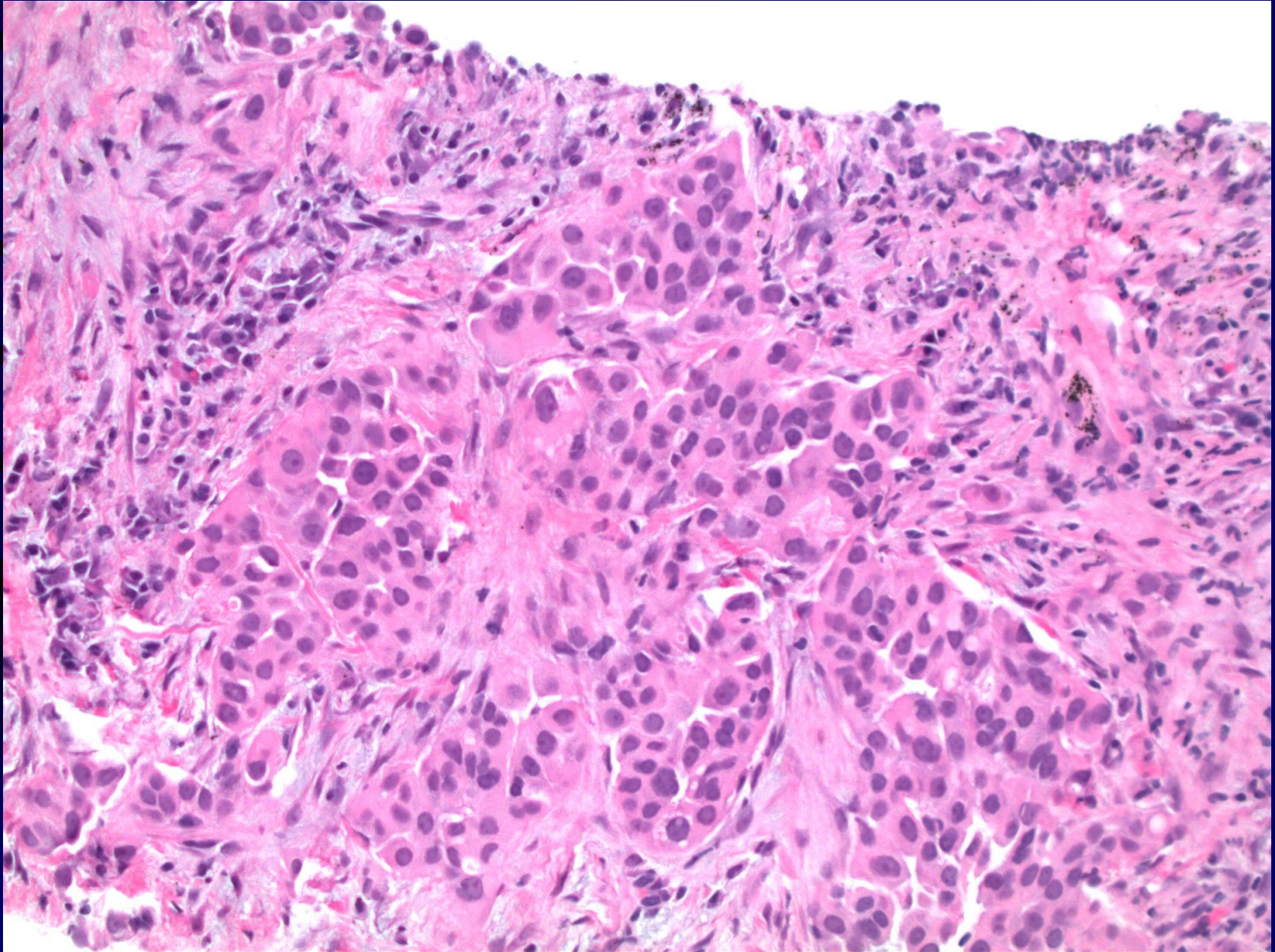
Oropharyngeal SCCA- TPS 1%



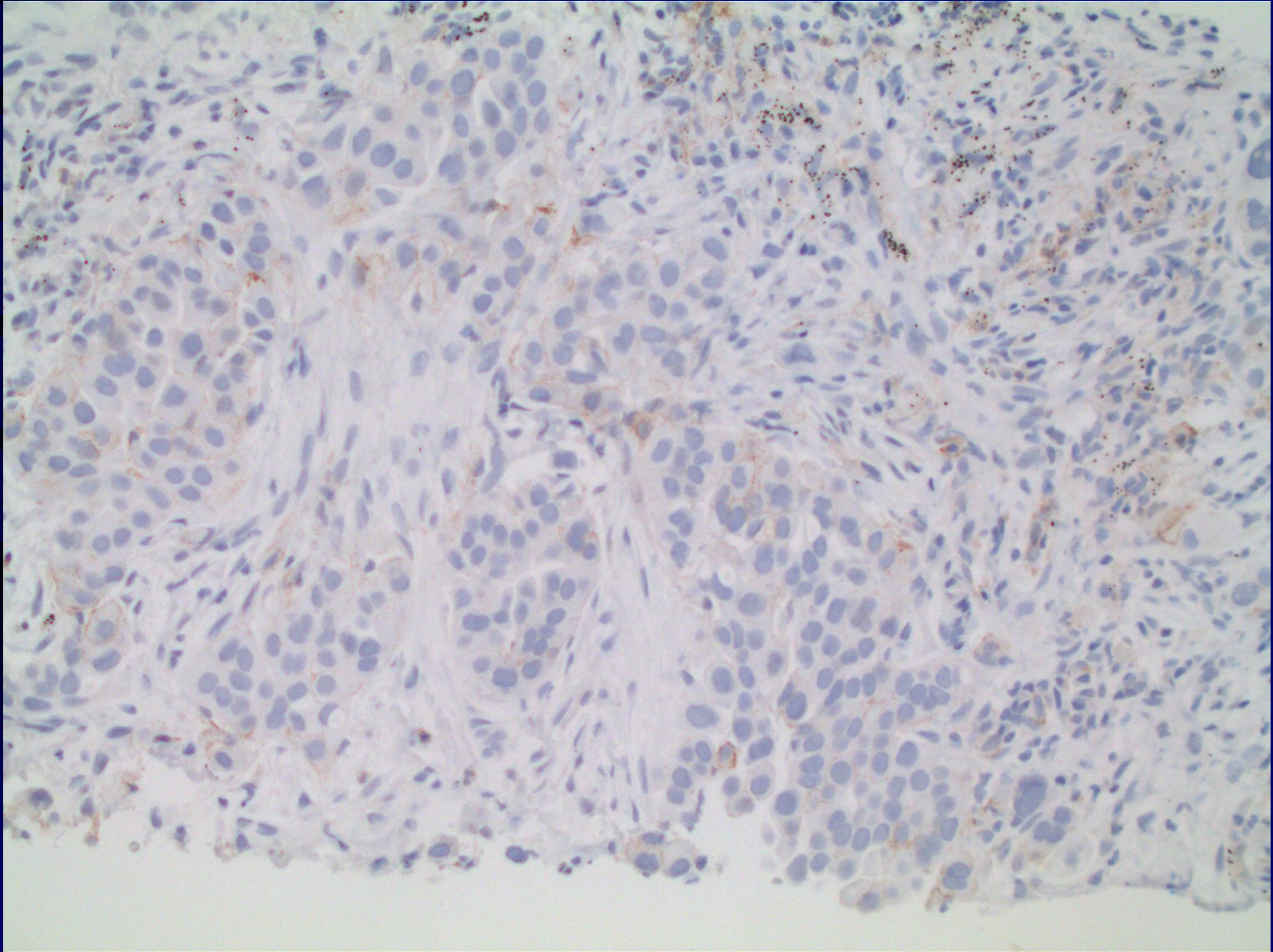
Lung-adenoca-core-TPS 15%



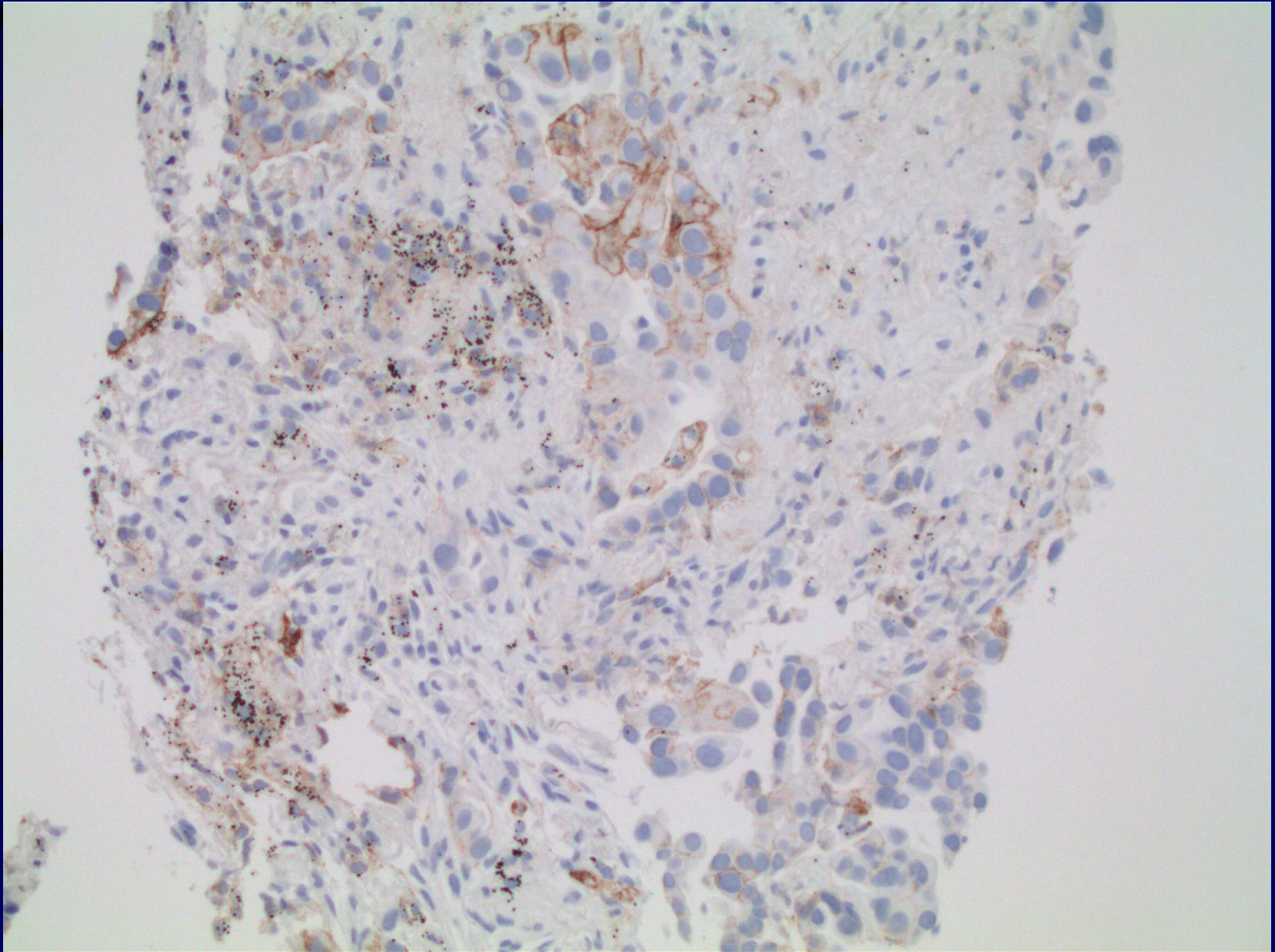
Lung-adenoca-core-TPS 15%



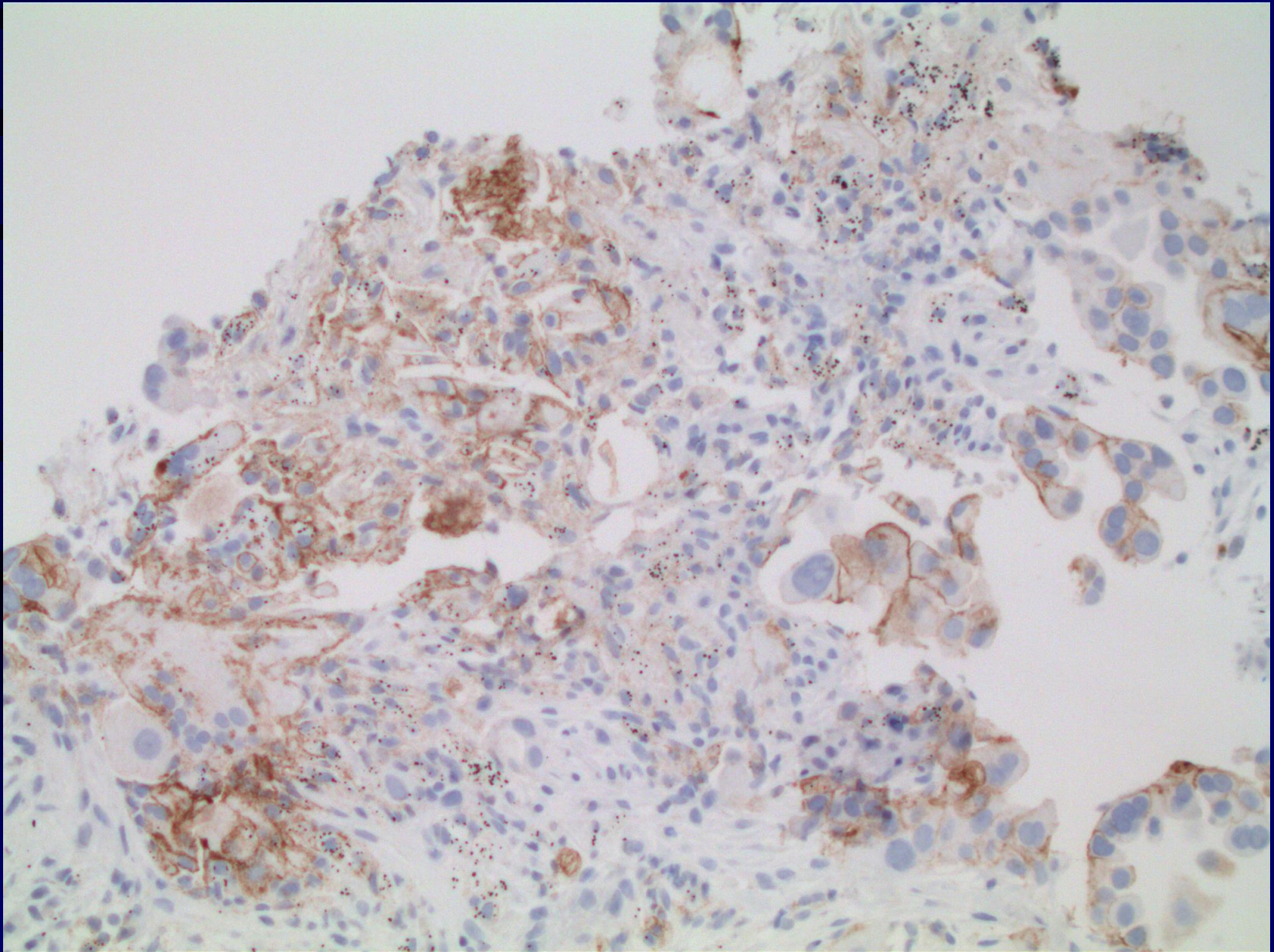
Lung-adenoca-core-TPS 15%- area 1 Intratumoral immune cells also reactive



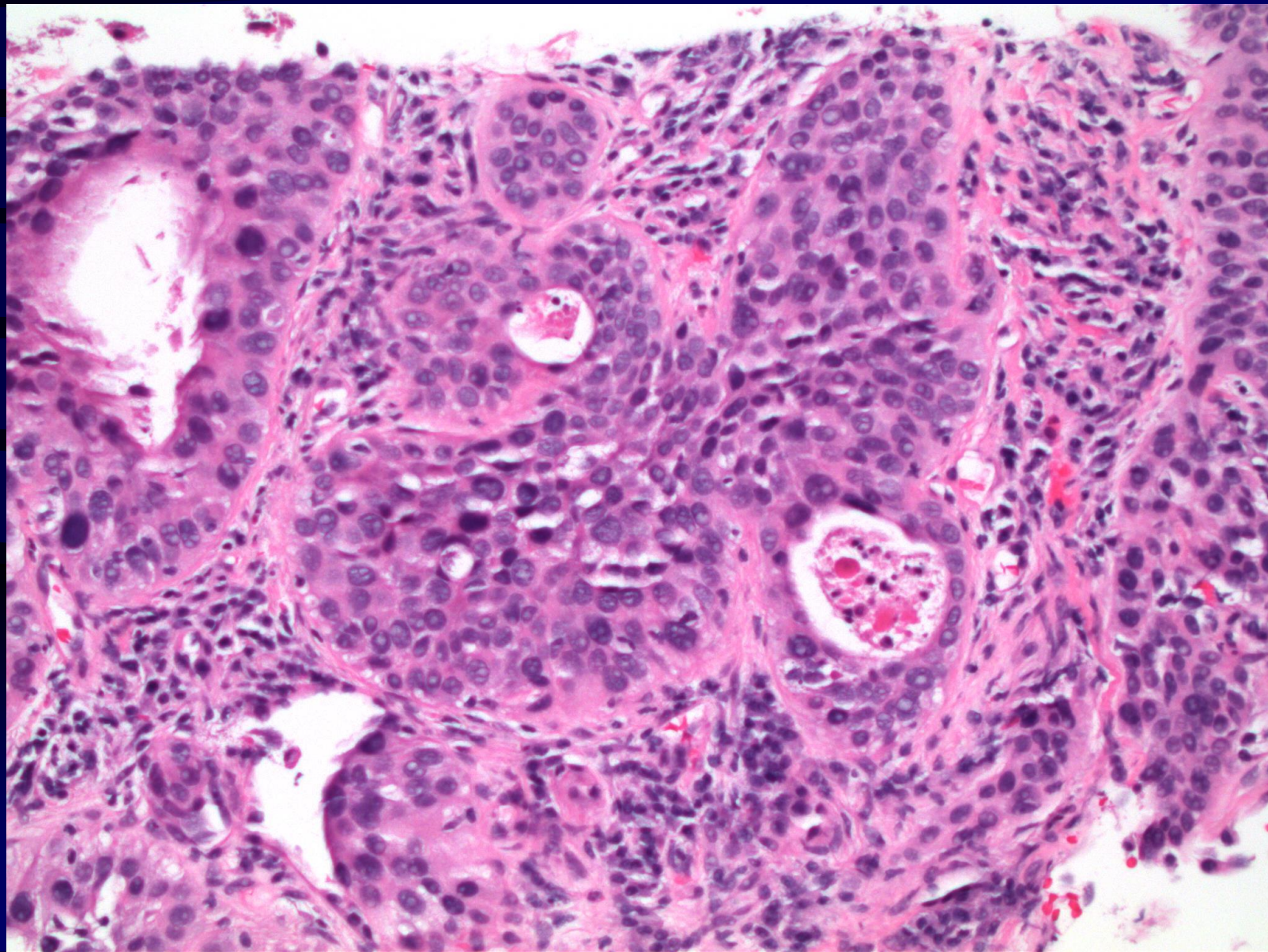
Lung-adenoca-core-TPS 15%- area 2



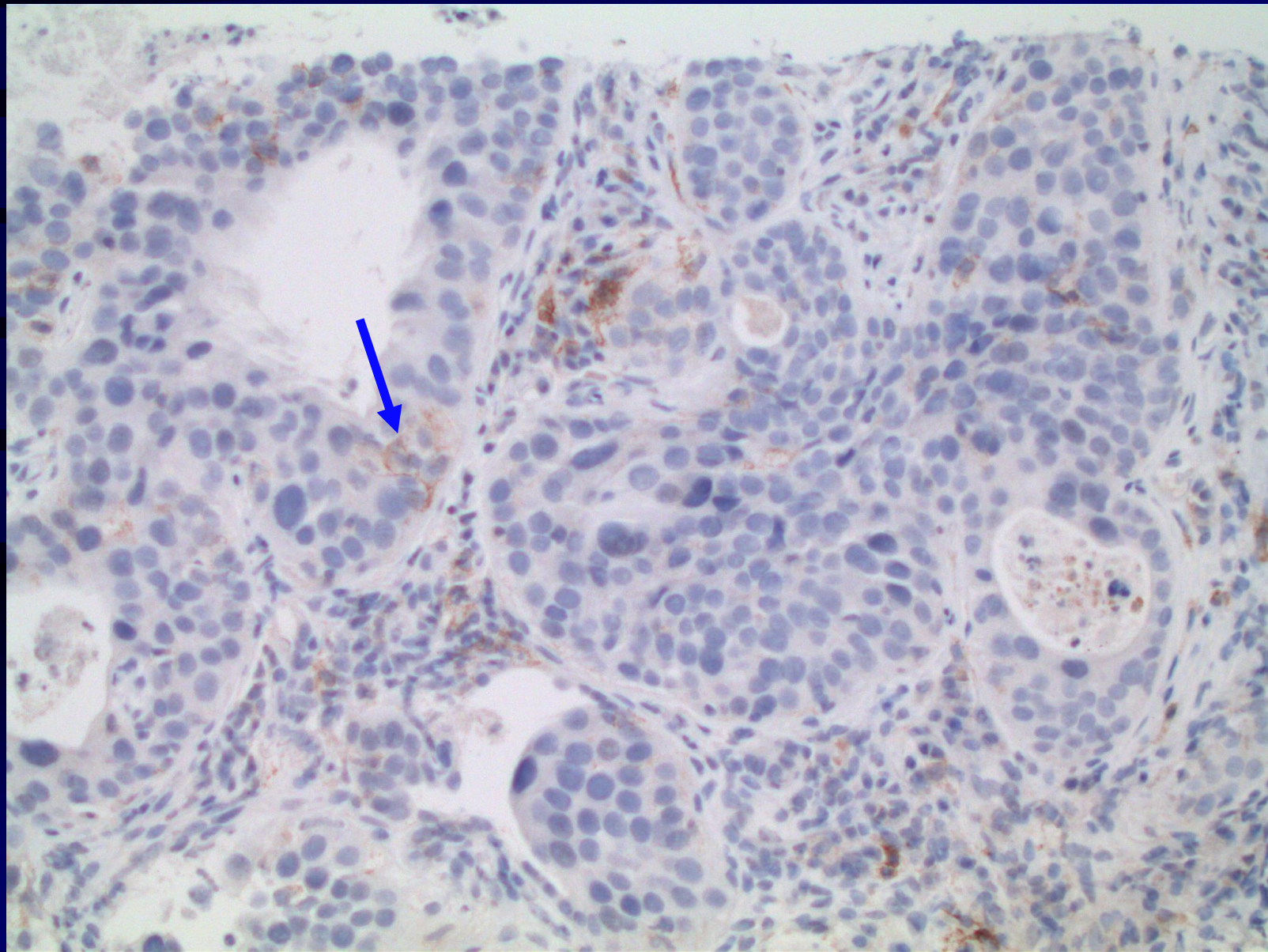
Lung-adenoca-core-TPS 15%- area 3



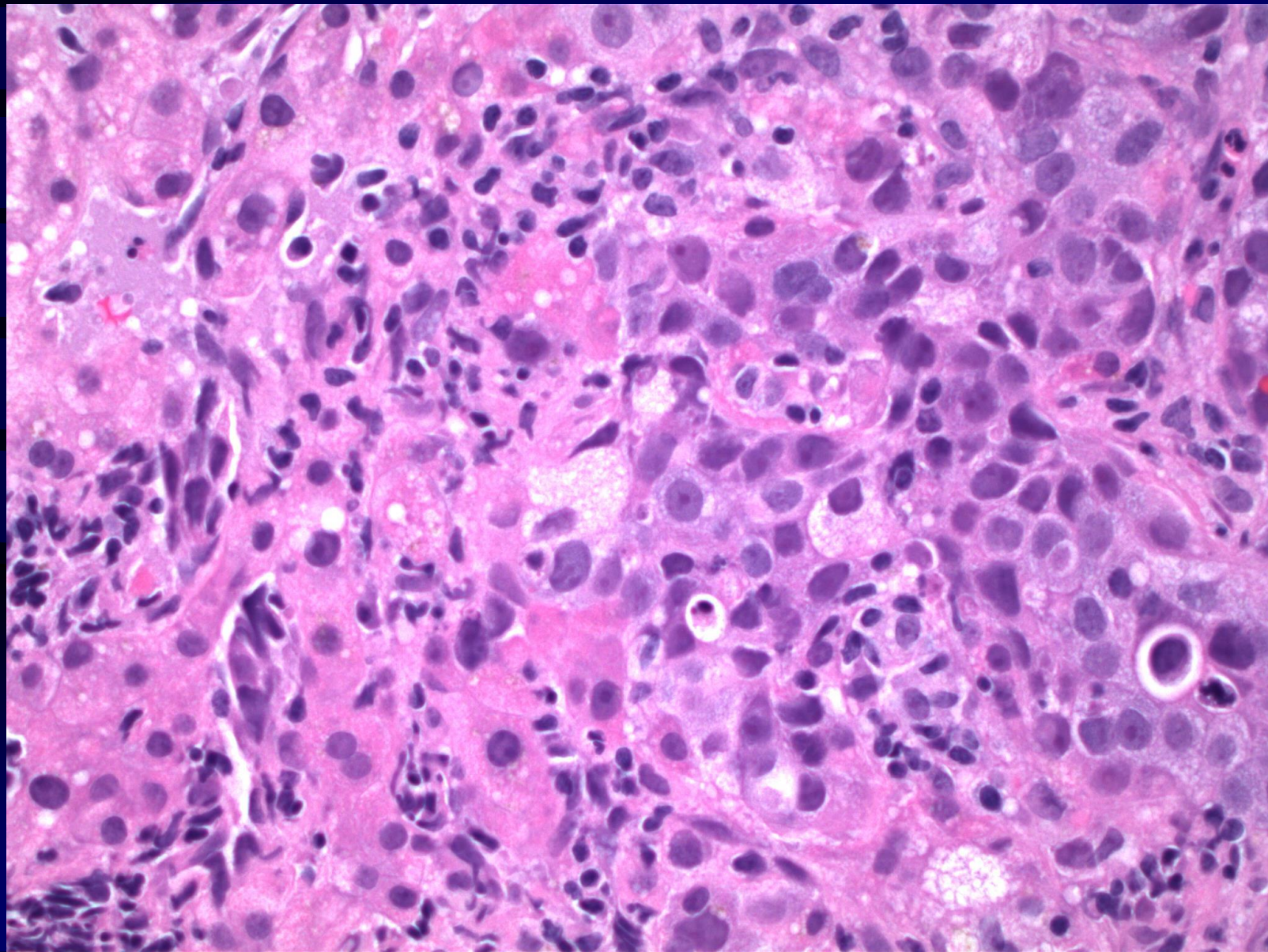
Lung-SCCA-core-TPS <1% Intratumoral immune cells also reactive



Lung-SCCA-core-TPS <1% immune cells also reactive

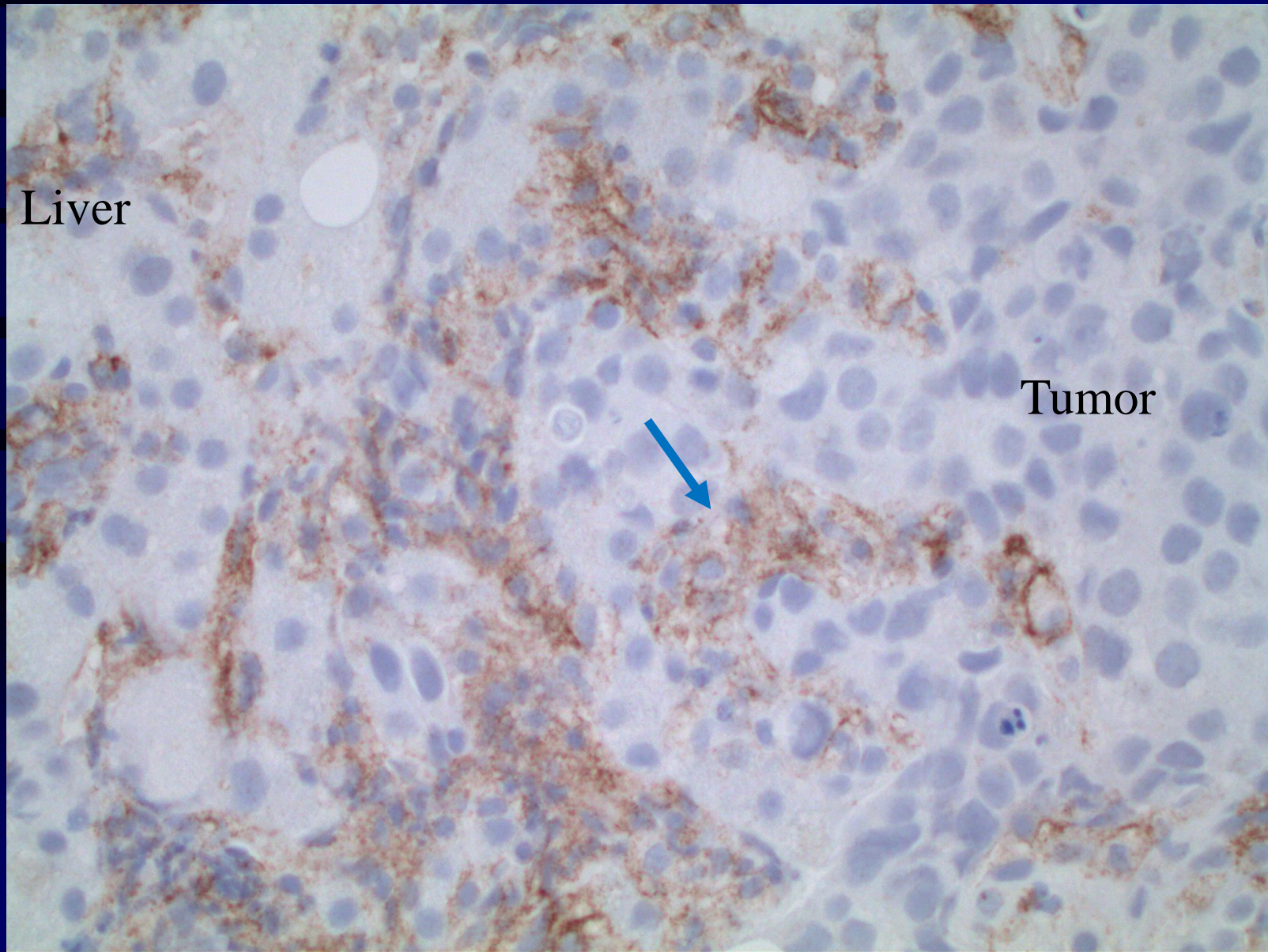


Liver-met lung adenoca-TPS 0% Intratumoral immune cells reactive

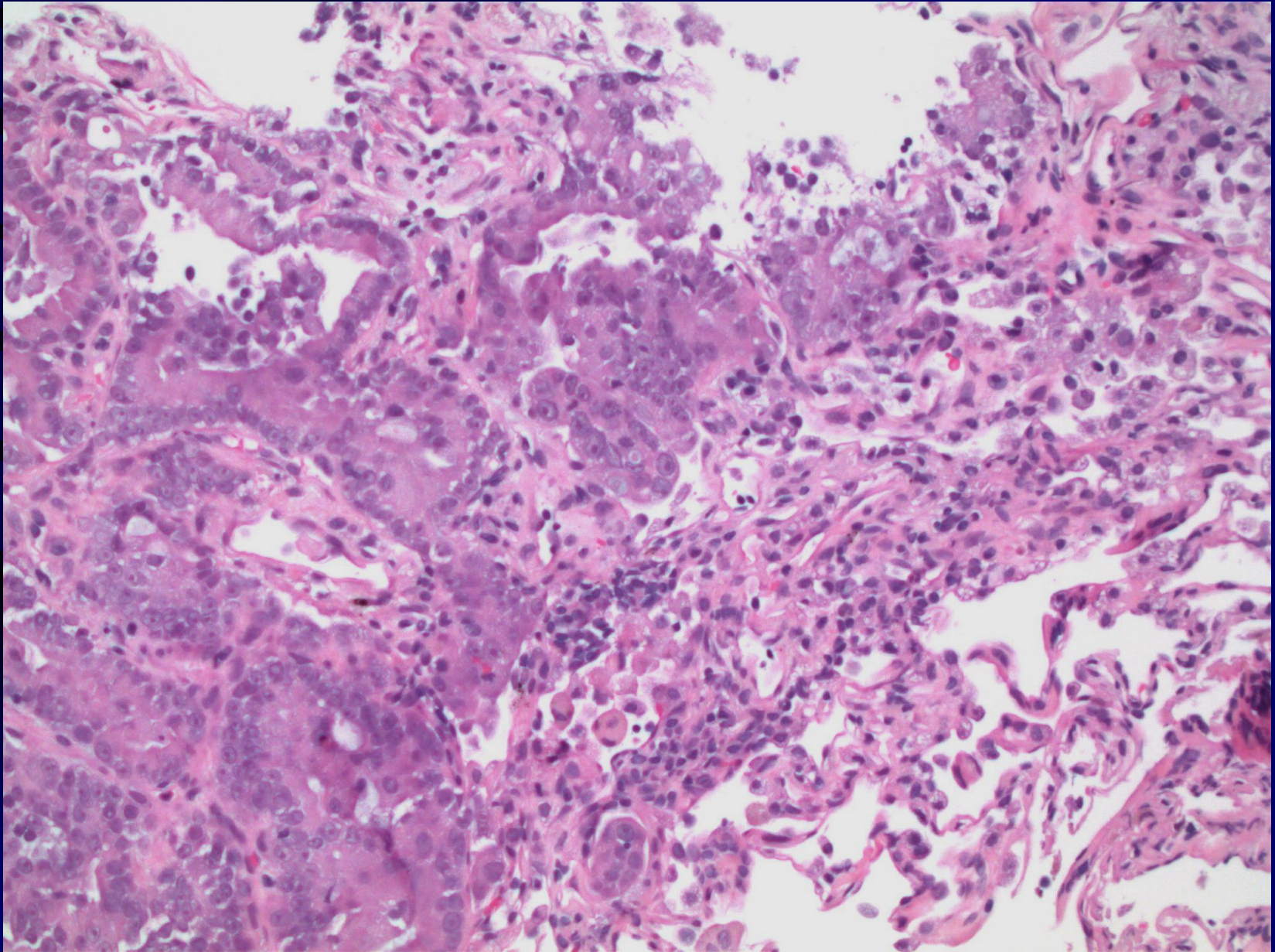


Lung-SCCA-core-TPS <1%

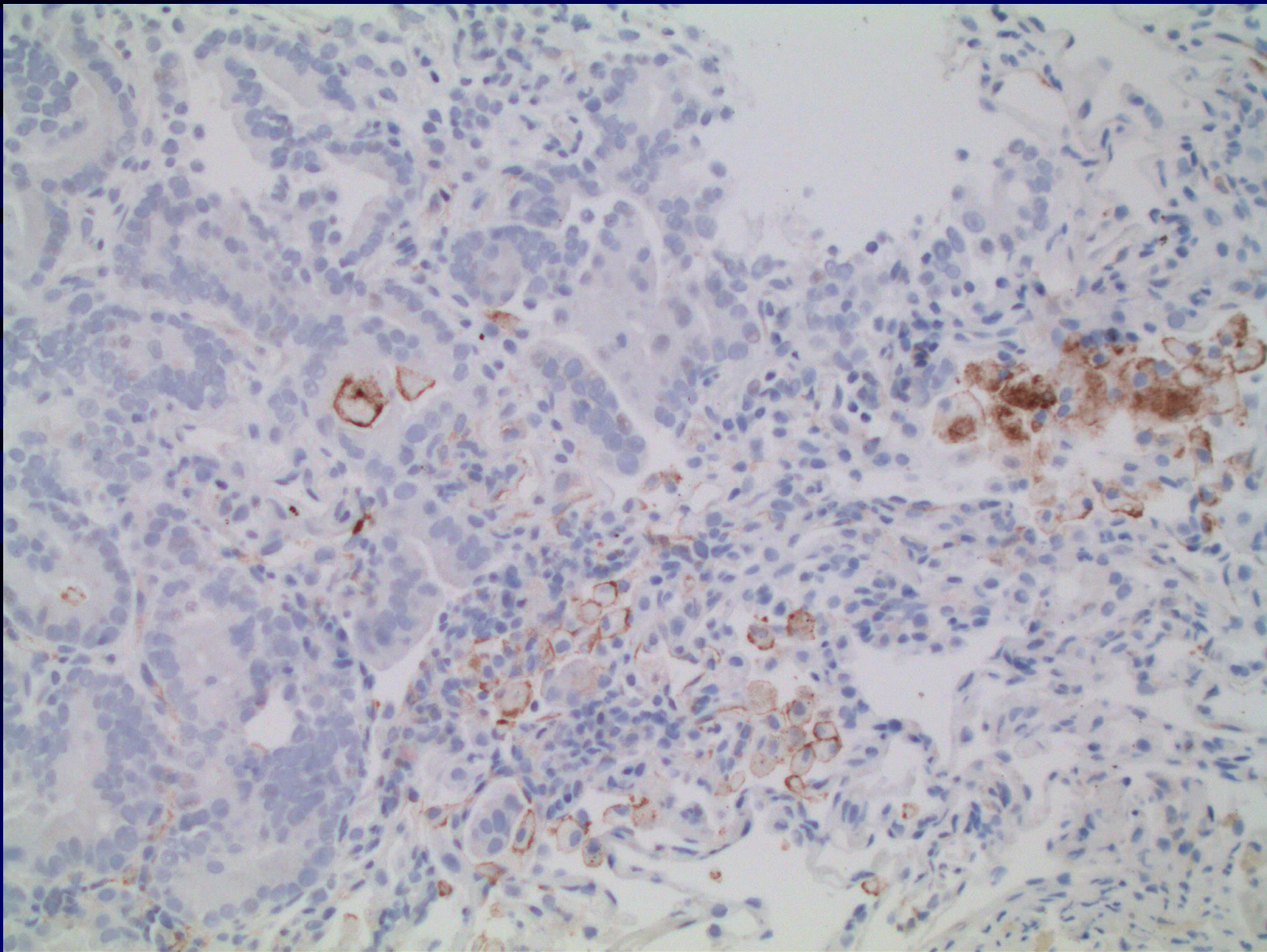
Intratumoral immune cells also reactive



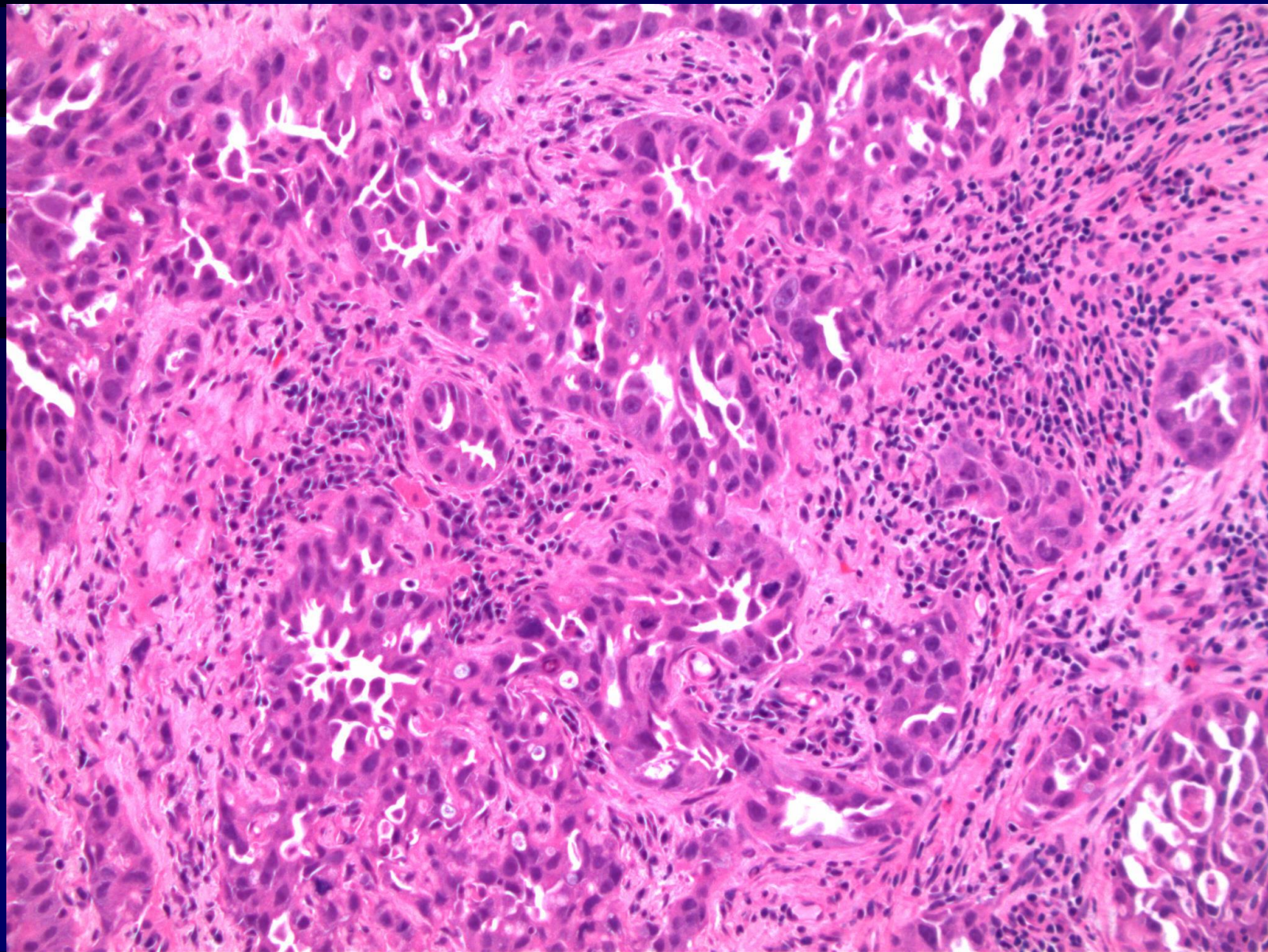
Lung-SCCA-core-TPS 0%



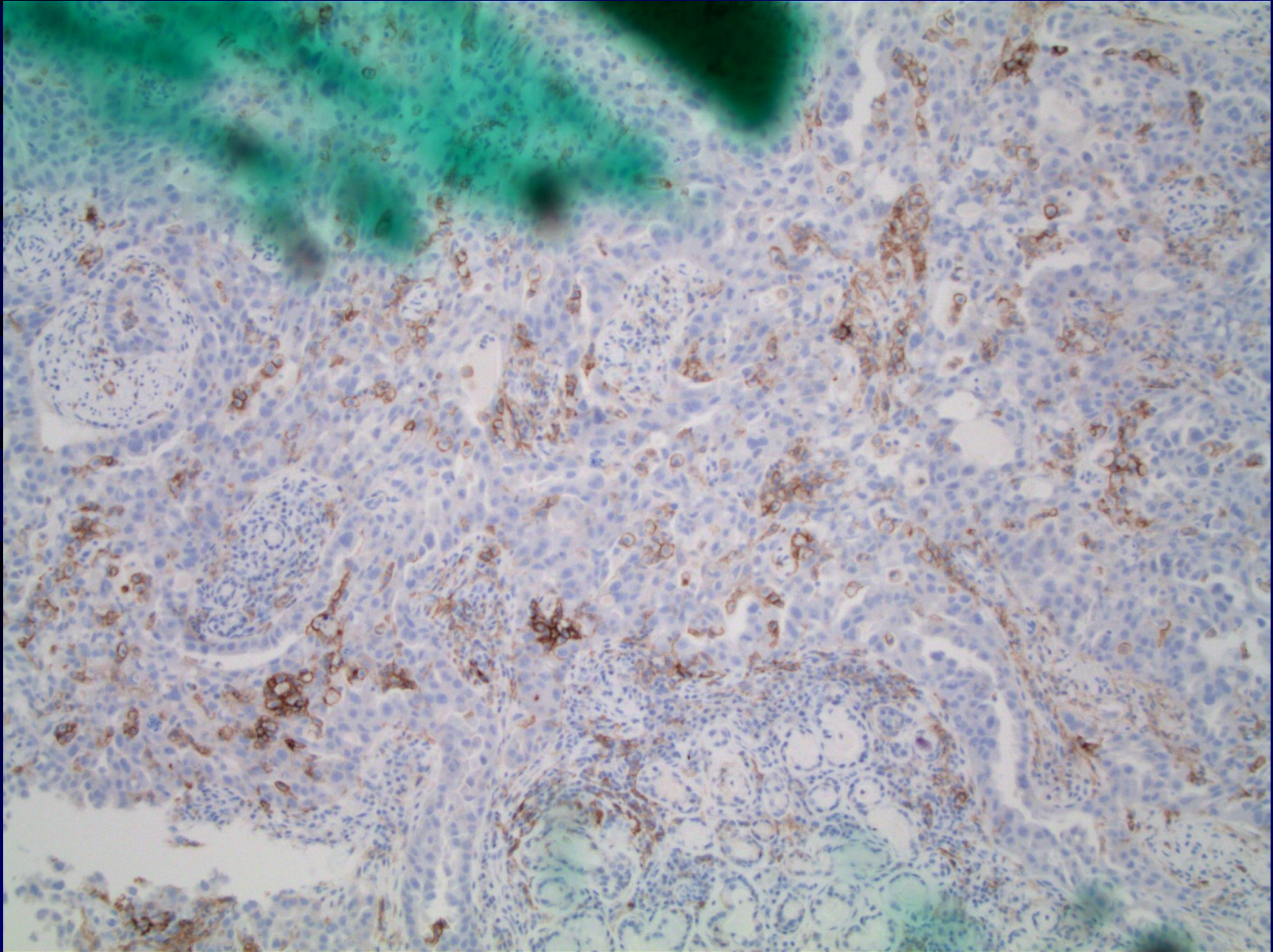
Lung-SCCA-core-TPS <1% Intratumoral immune cells also reactive



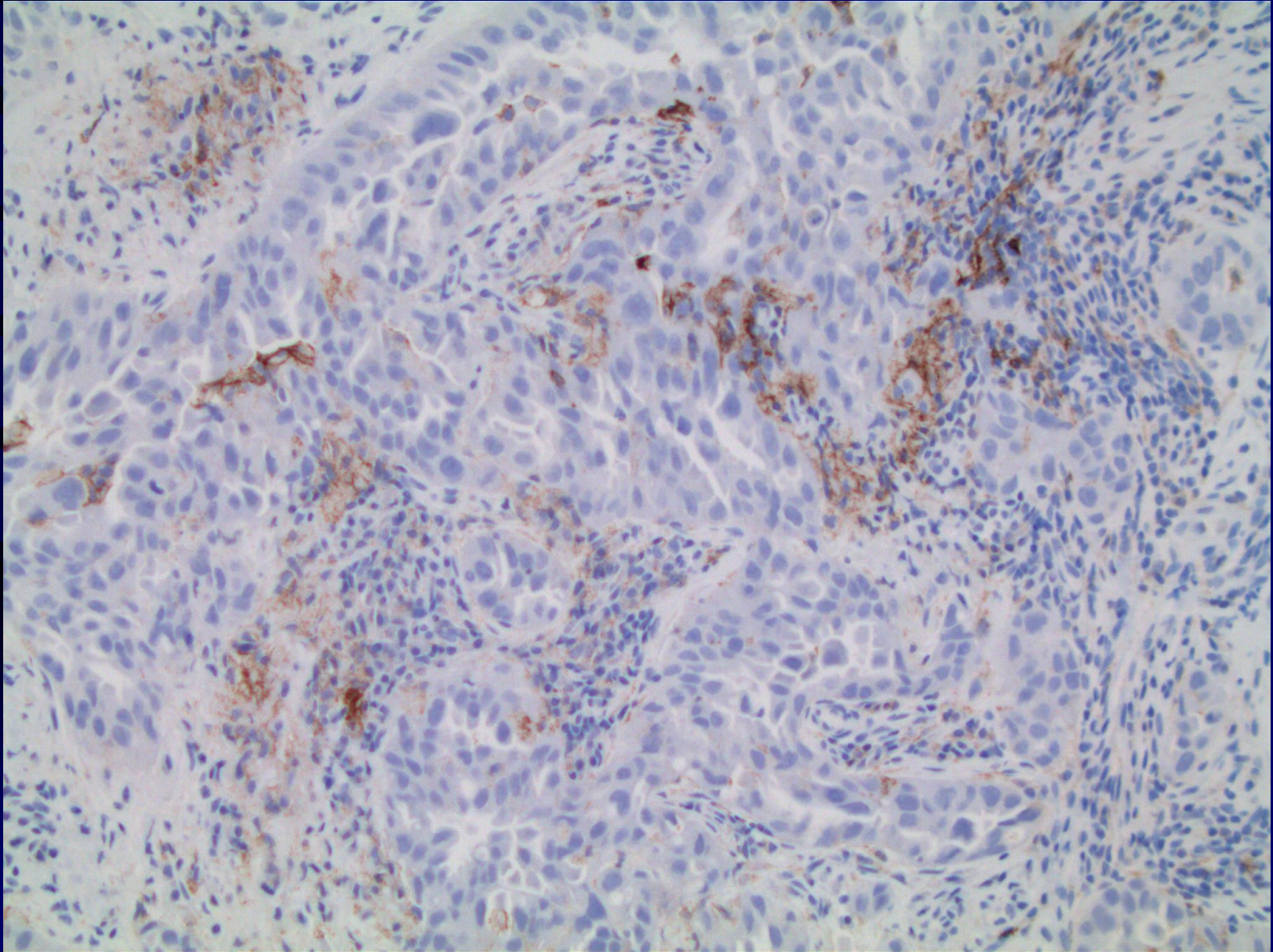
Lung-adenoca-TBbx Intratumoral immune cells



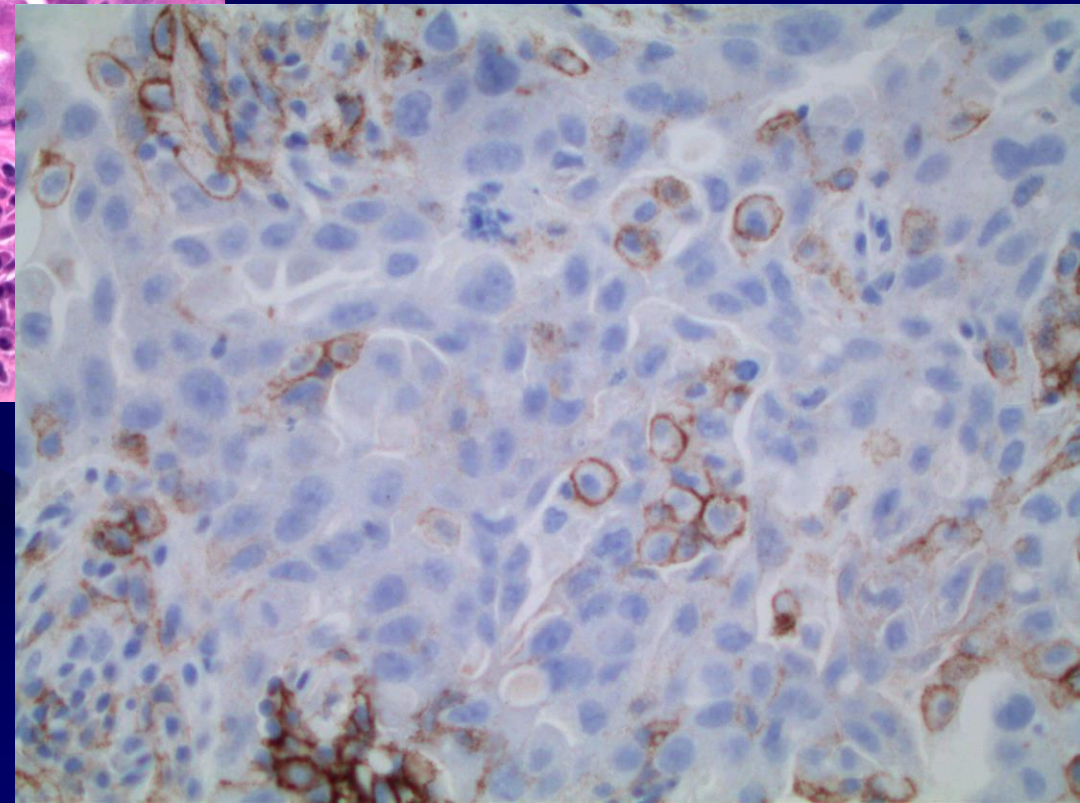
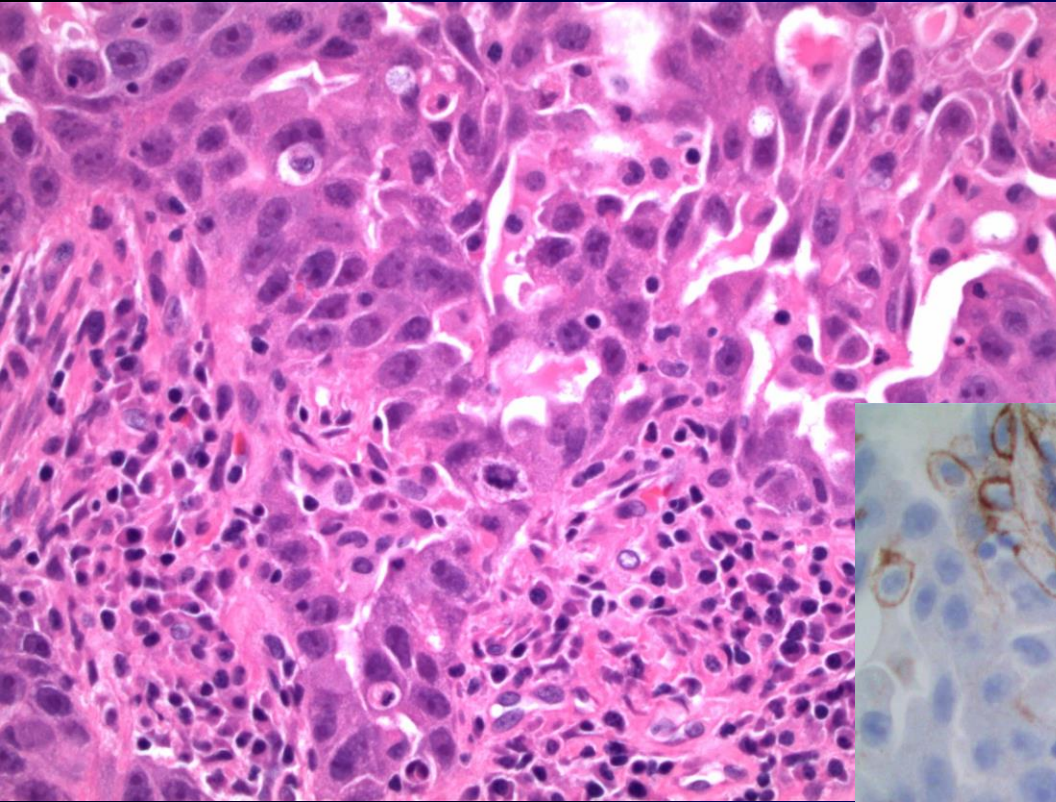
Lung-adenoca-TBbx- ?TPS



Lung-adenoca-TBbx-TPS <1% Intratatumoral immune cells reactive



Lung-adenoca-TBbx-TPS <1% Intratumoral immune cells reactive





Scoring criteria- Dako- PharmDx

For bladder, gastric/GE, cervical cancer:
Combined Proportion Score:

viable tumor + immune cells labeling/ total # viable tumor cells x 100 = 0-100 (100 is the maximum reportable score)

Tumor labeling must be membranous, partial or complete.

Do not score cytoplasmic only, crushed or necrotic cells.

Score of greater than or equal to 1 (≥ 1) is positive.

For H&N SCCA, both TPS and CPS have been used.

What else can we add?

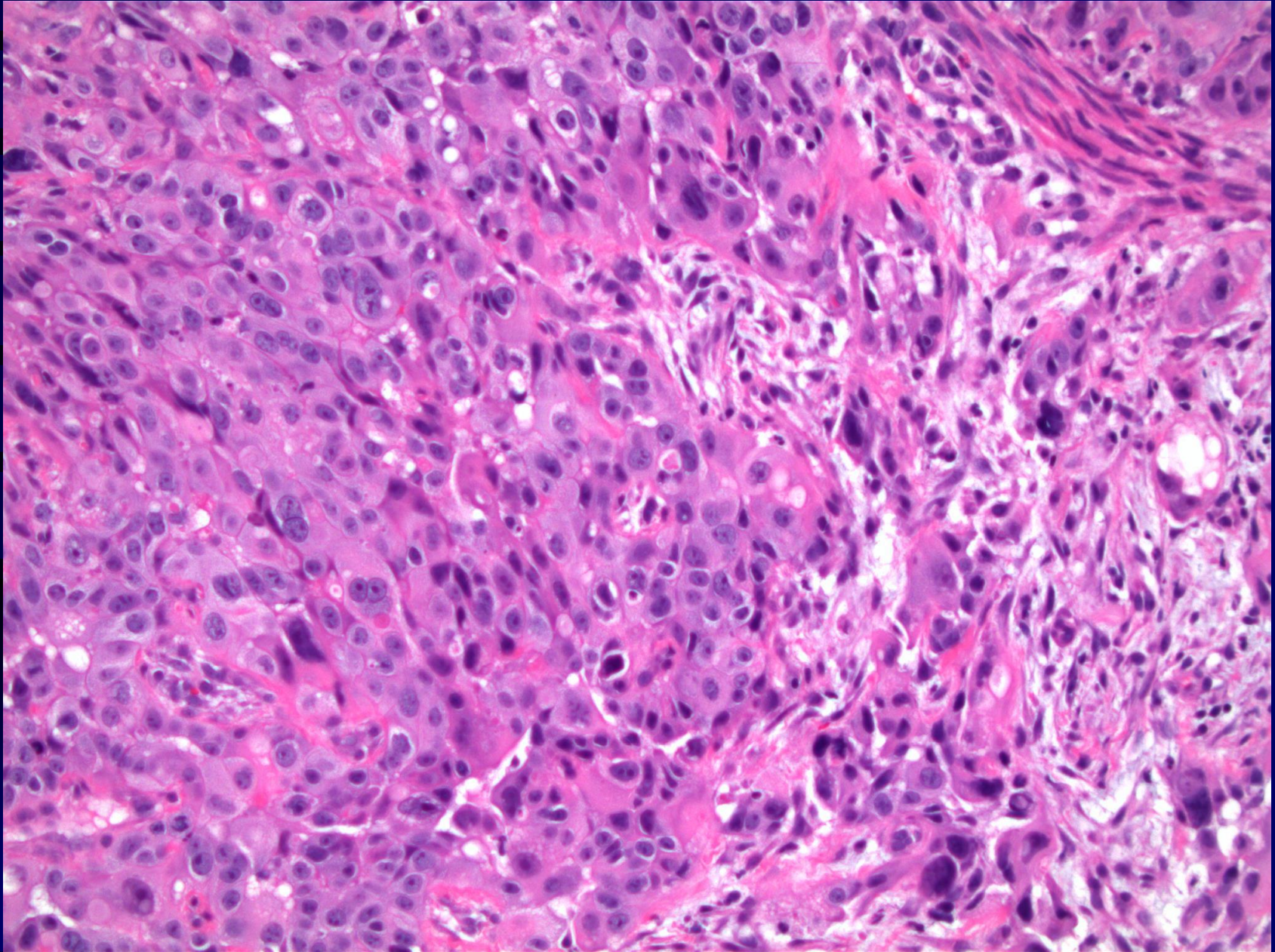
For small cell carcinoma:

Though PD-L1 testing by IHC is not required for treatment in the FDA approval:

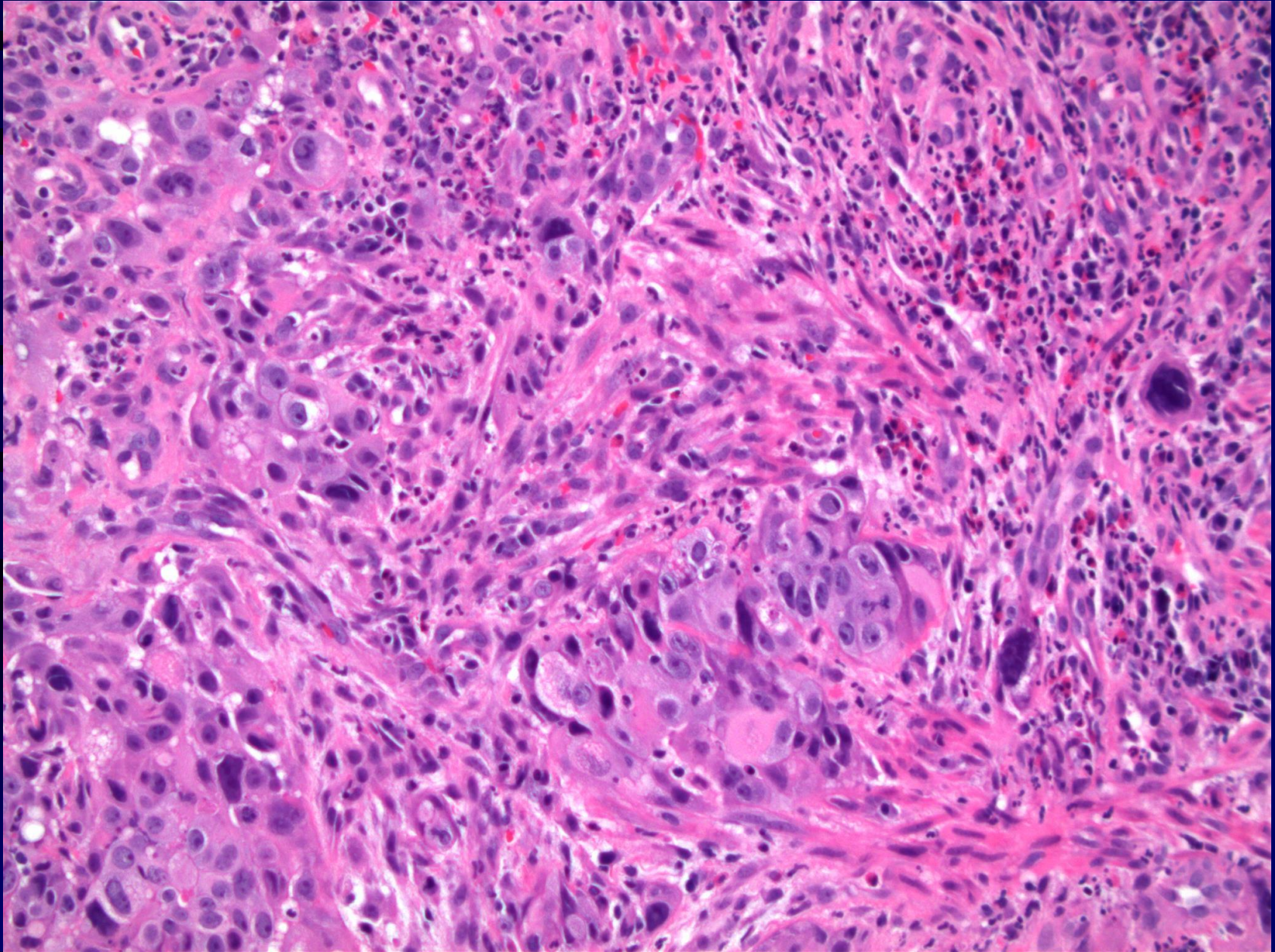
Combined Proportion Score of $>1\%$ or labeling of adjacent tumor stromal inflammatory cells if present.

Phase Ib KEYNOTE-028 Study. JCO; VOLUME 35 •
NUMBER 34 • DECEMBER 1, 2017

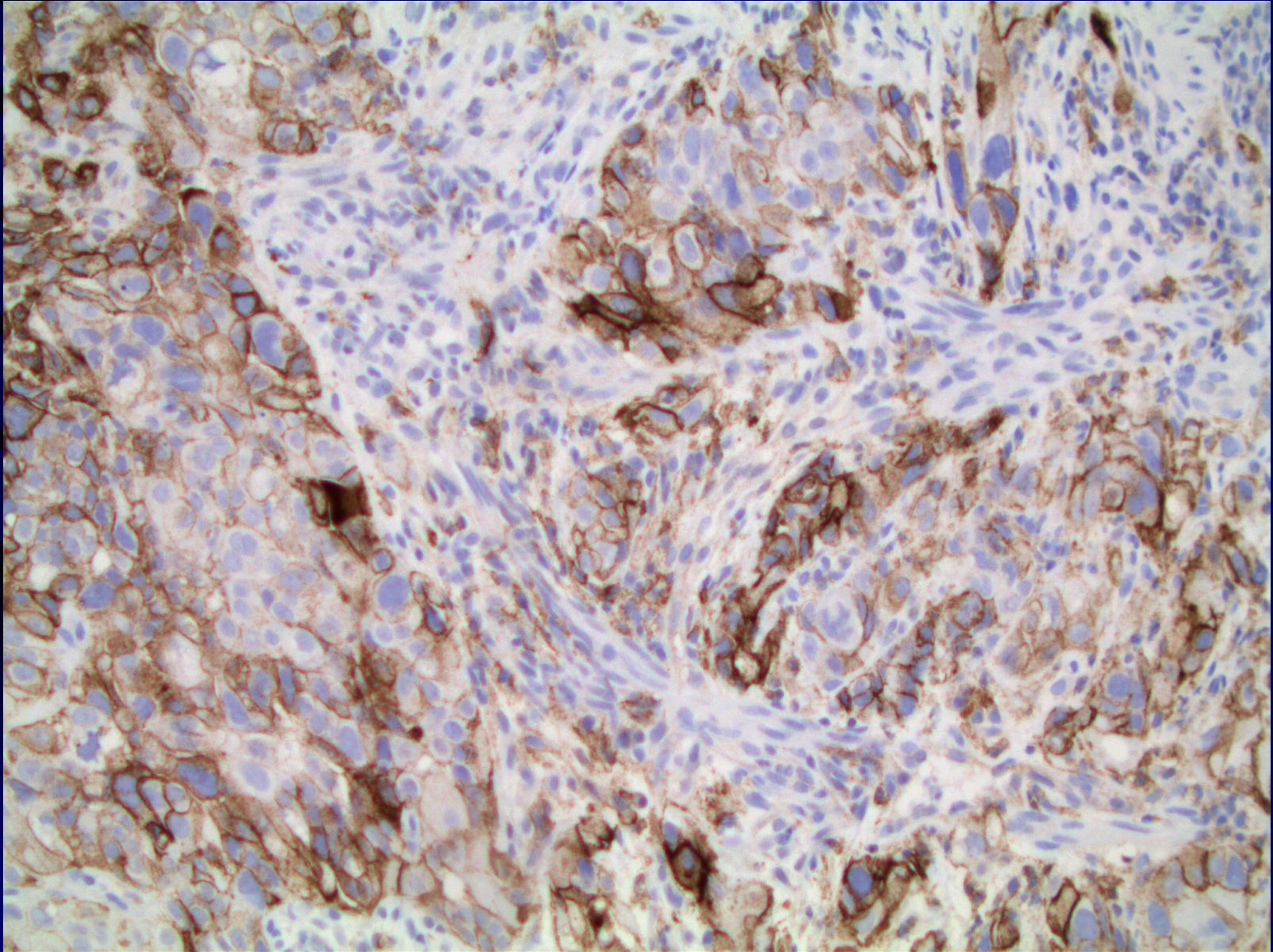
Distal esophageal adenocarcinoma



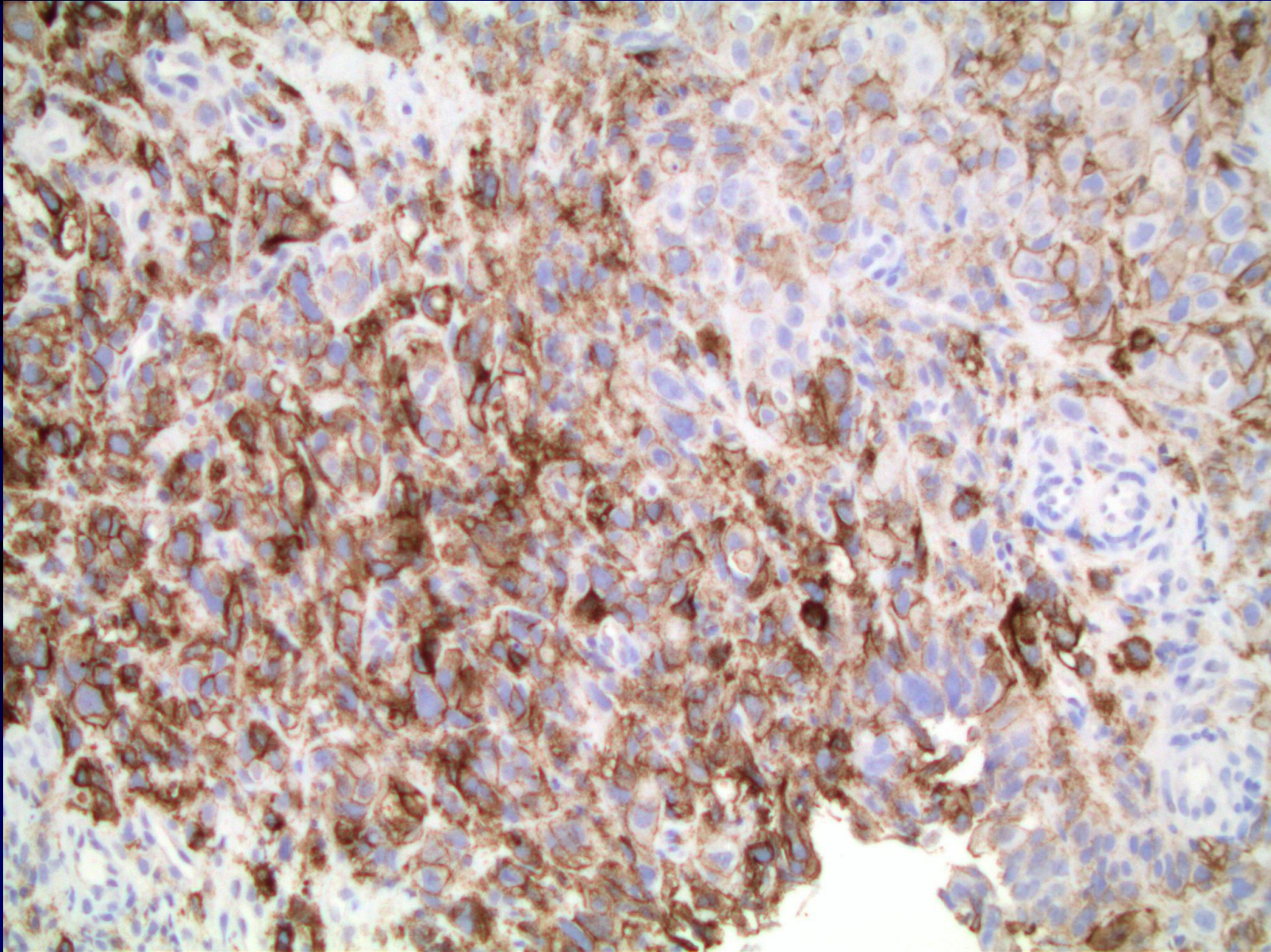
Distal esophageal adenocarcinoma



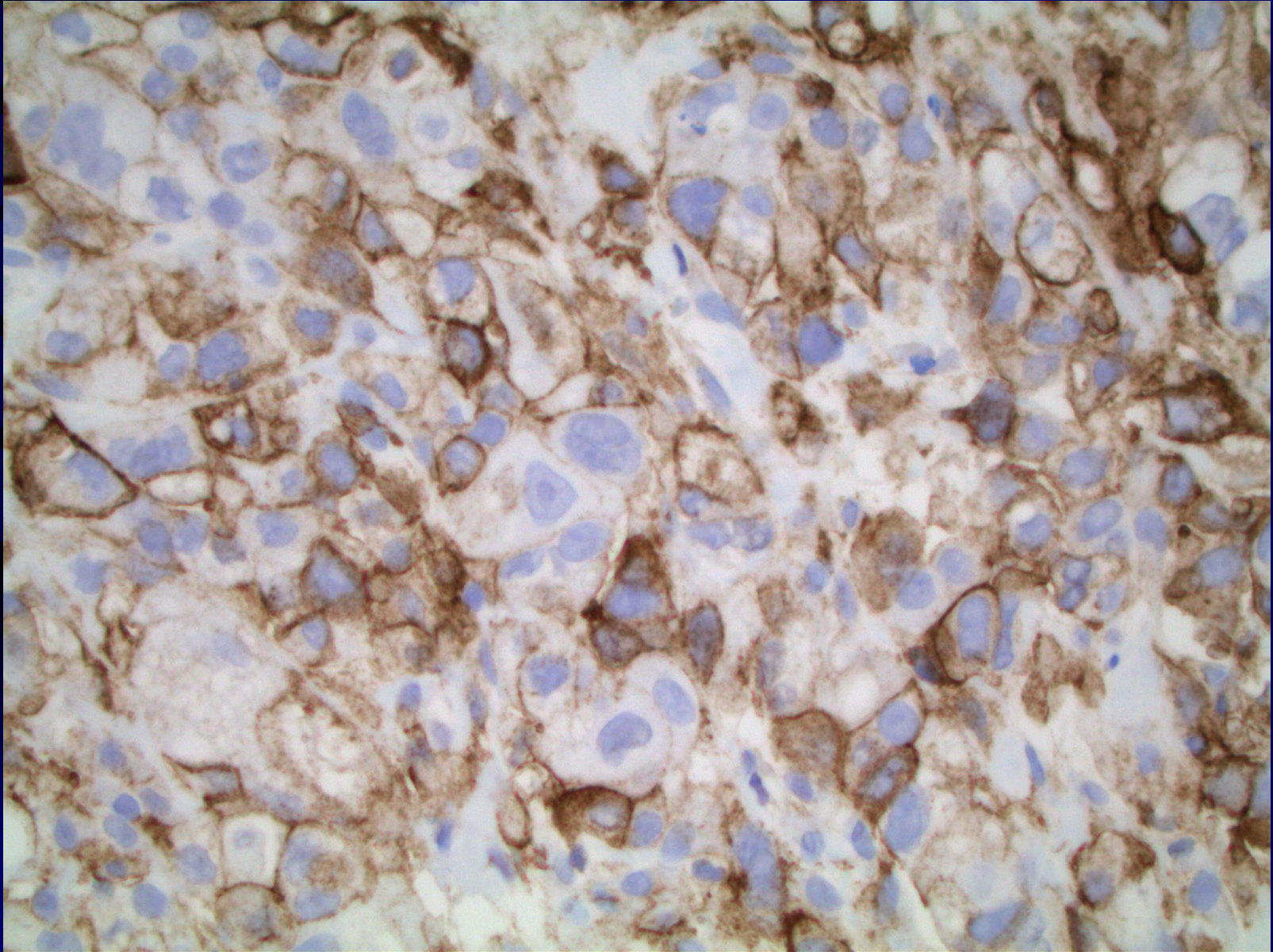
Distal esophageal adeno-CPS 70 Intratatumoral immune cells also reactive



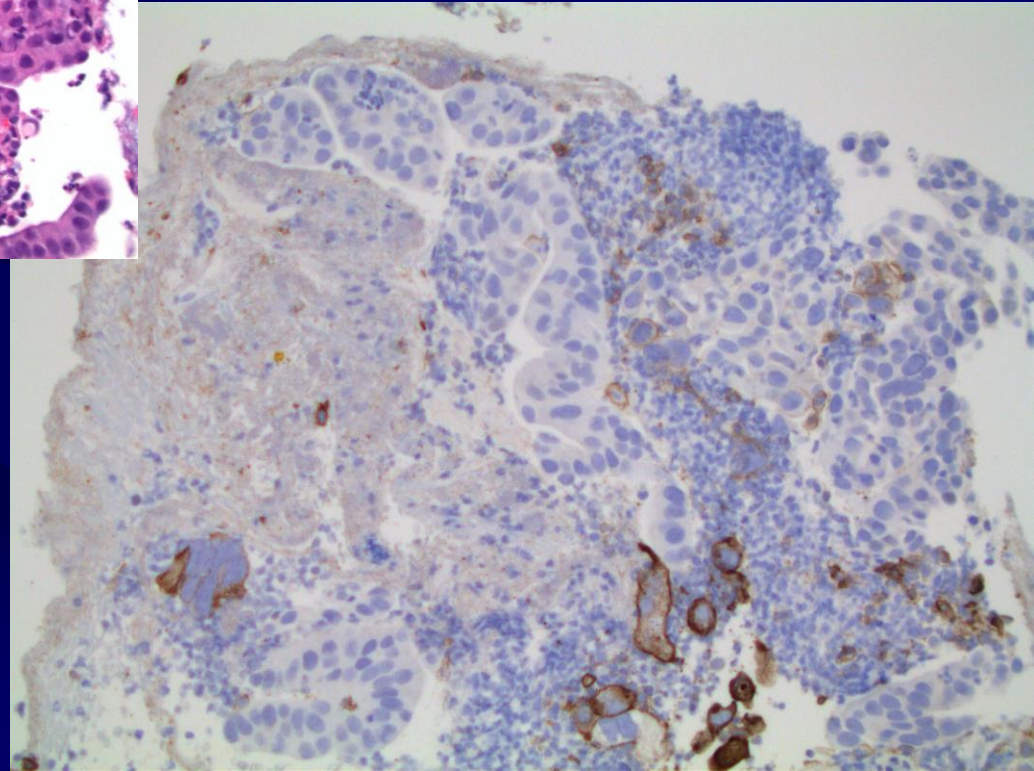
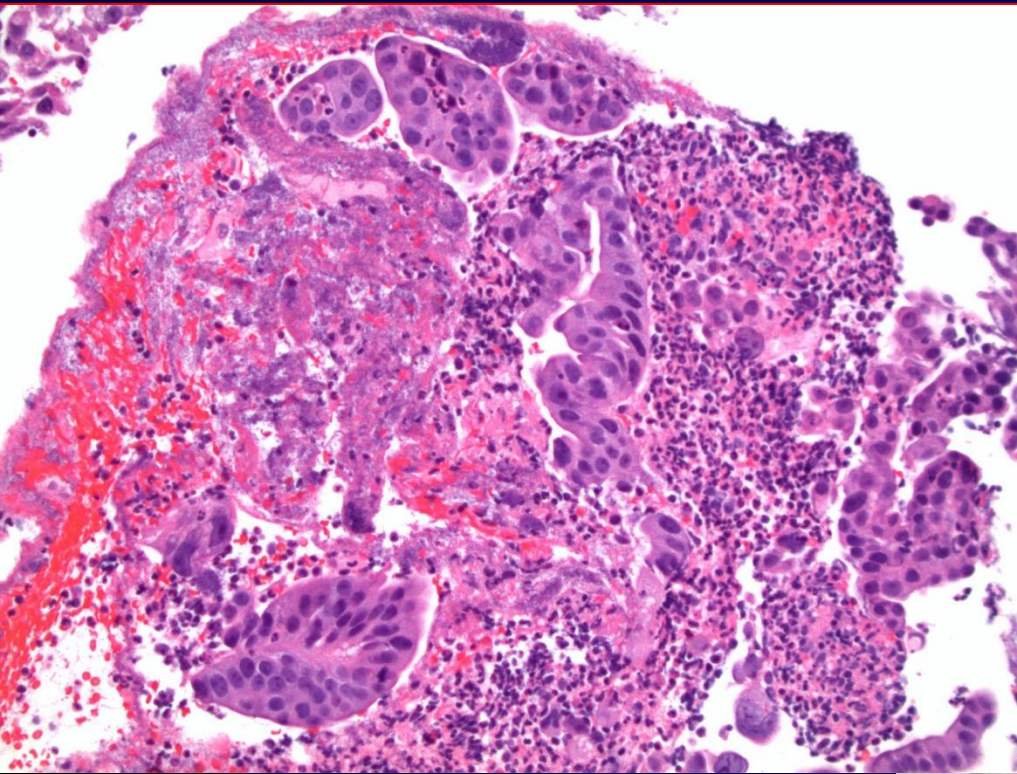
Distal esophageal adeno-CPS 70 Intratatumoral immune cells also reactive



Distal esophageal adeno-CPS 70 Intratatumoral immune cells also reactive

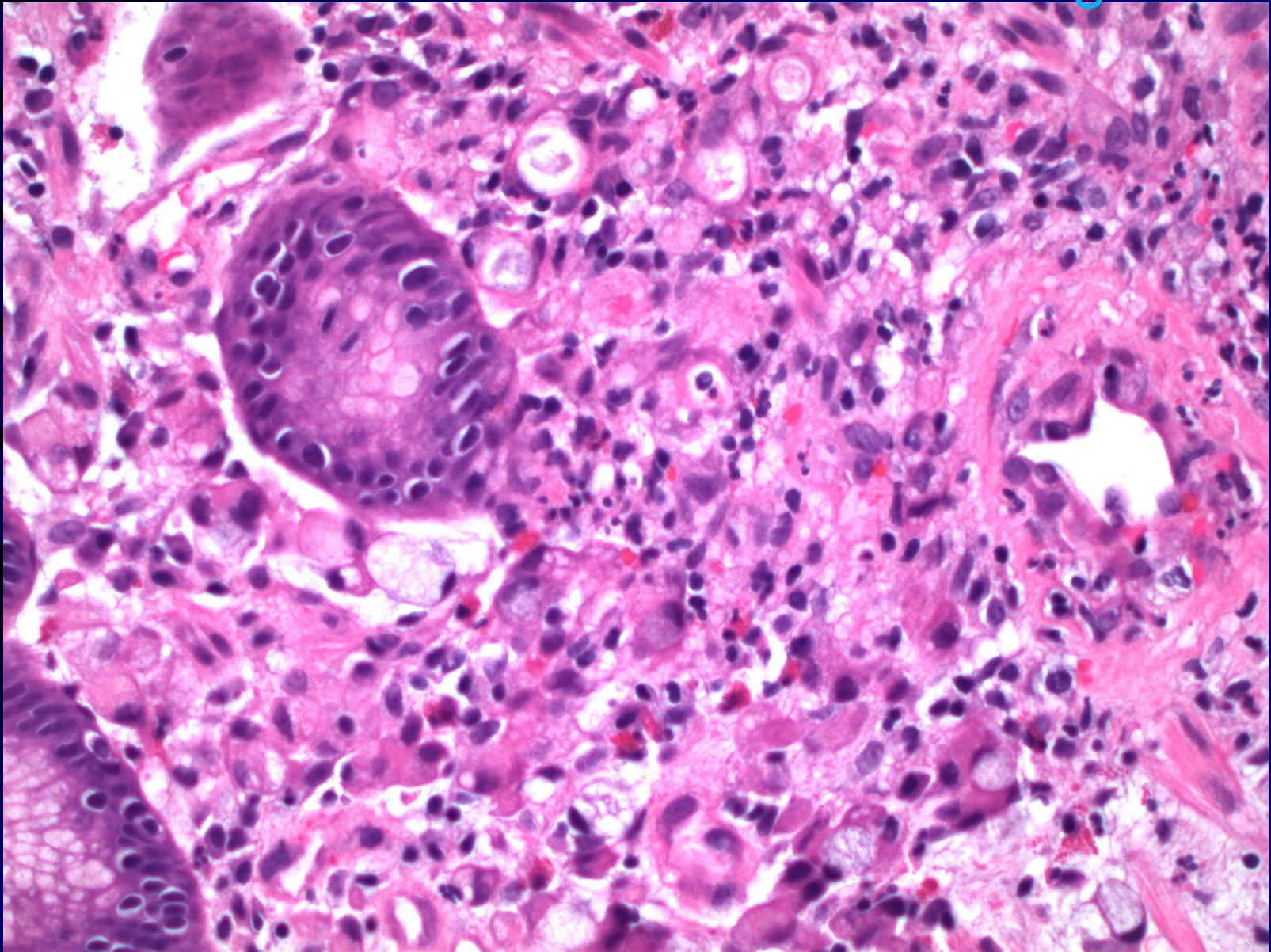


Do not score surface/ non-invasive tumor



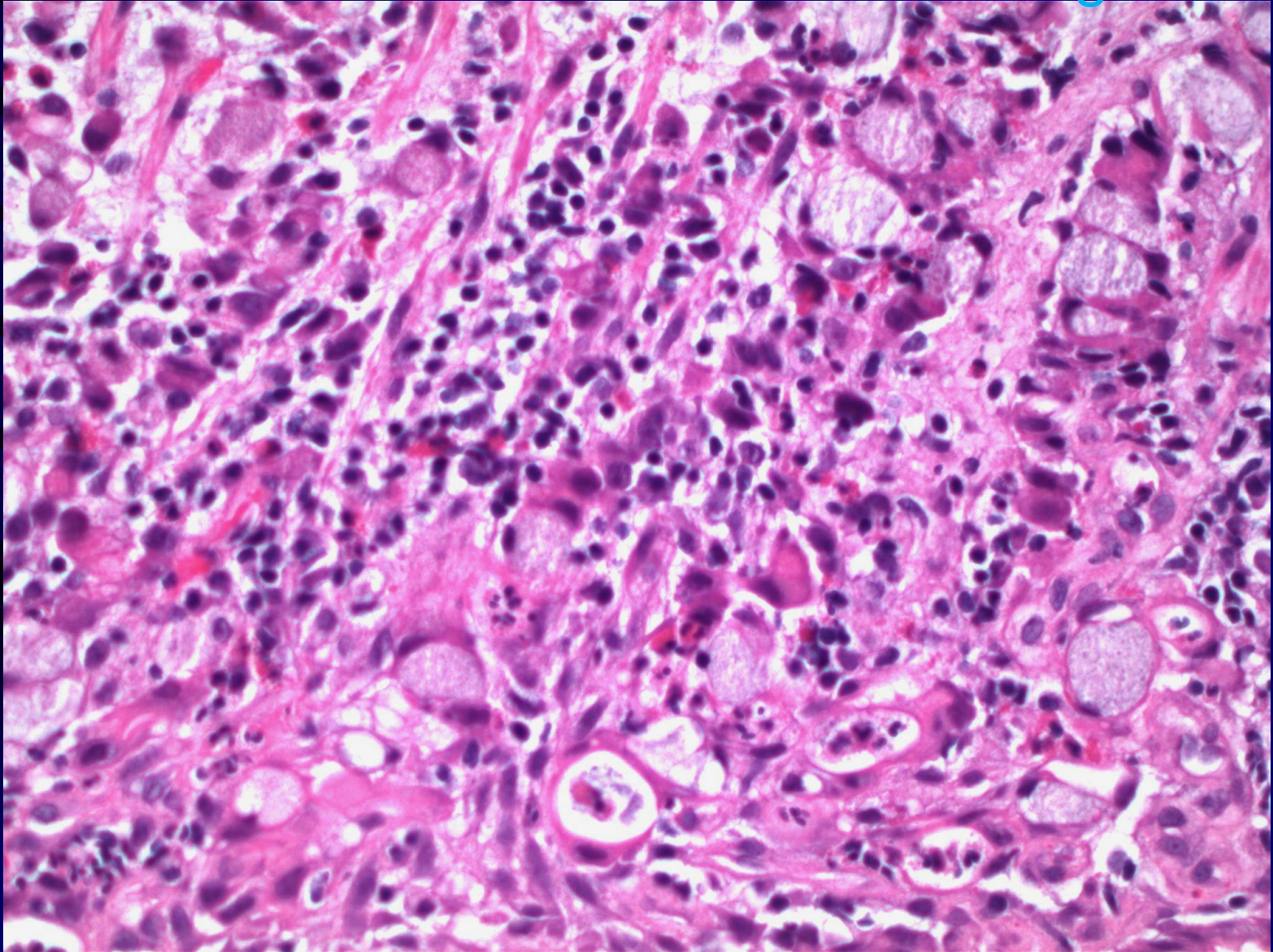
Gastric signet ring ca: CPS 10

Immune cells reactive/ tumor cells negative



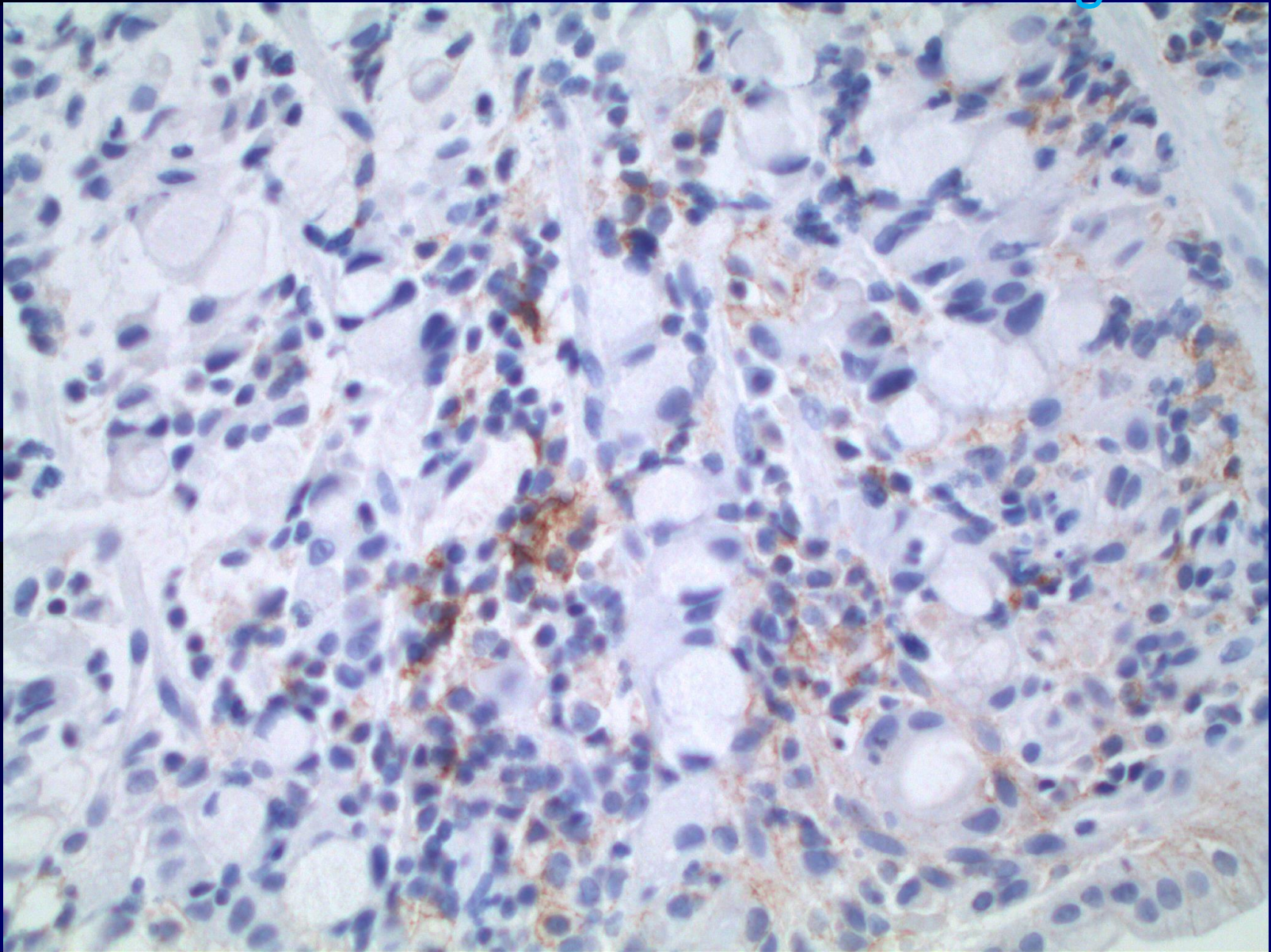
Gastric signet ring ca: CPS 10

Immune cells reactive/ tumor cells negative



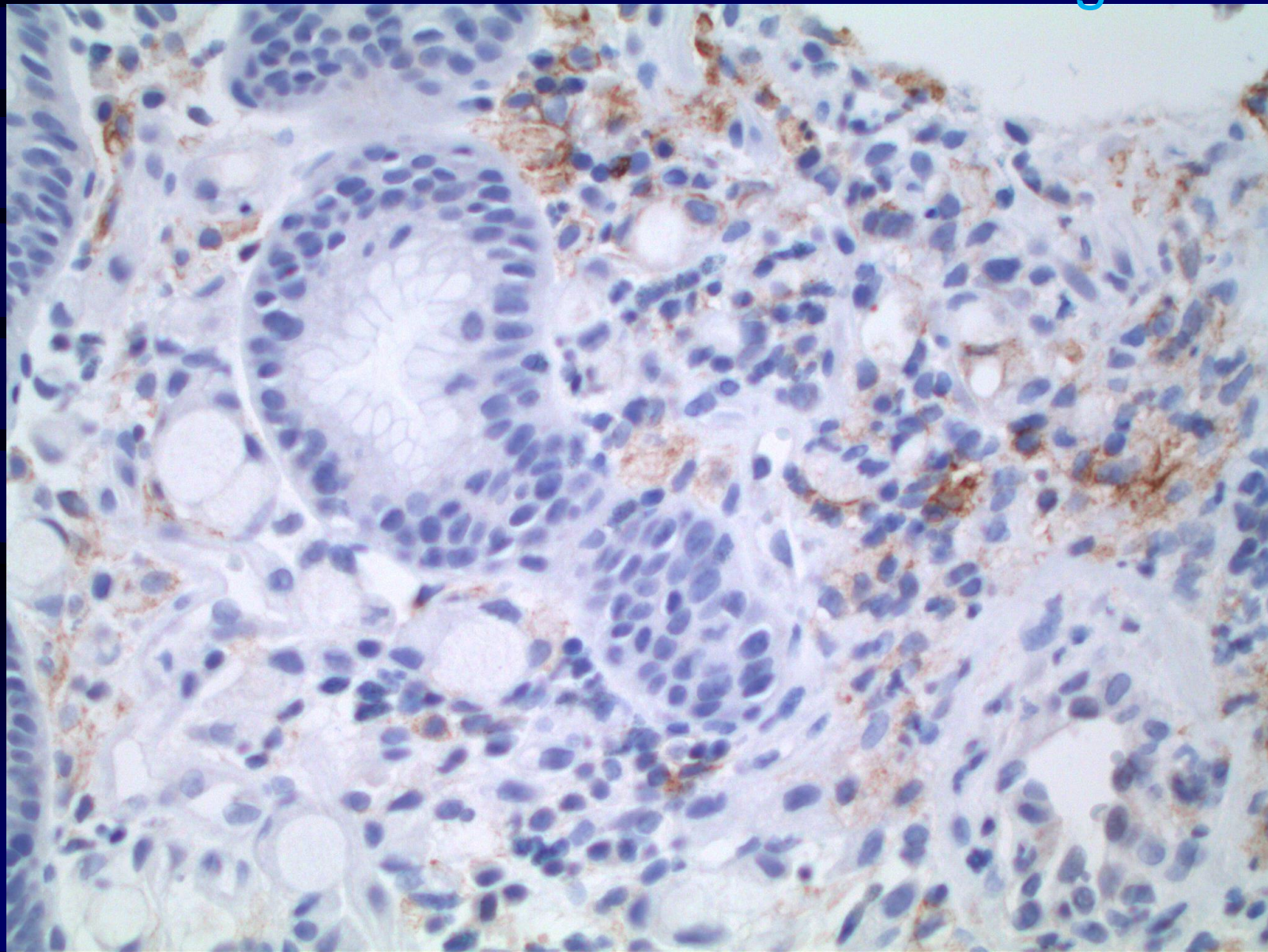
Gastric signet ring ca: CPS 10

Immune cells reactive/ tumor cells negative

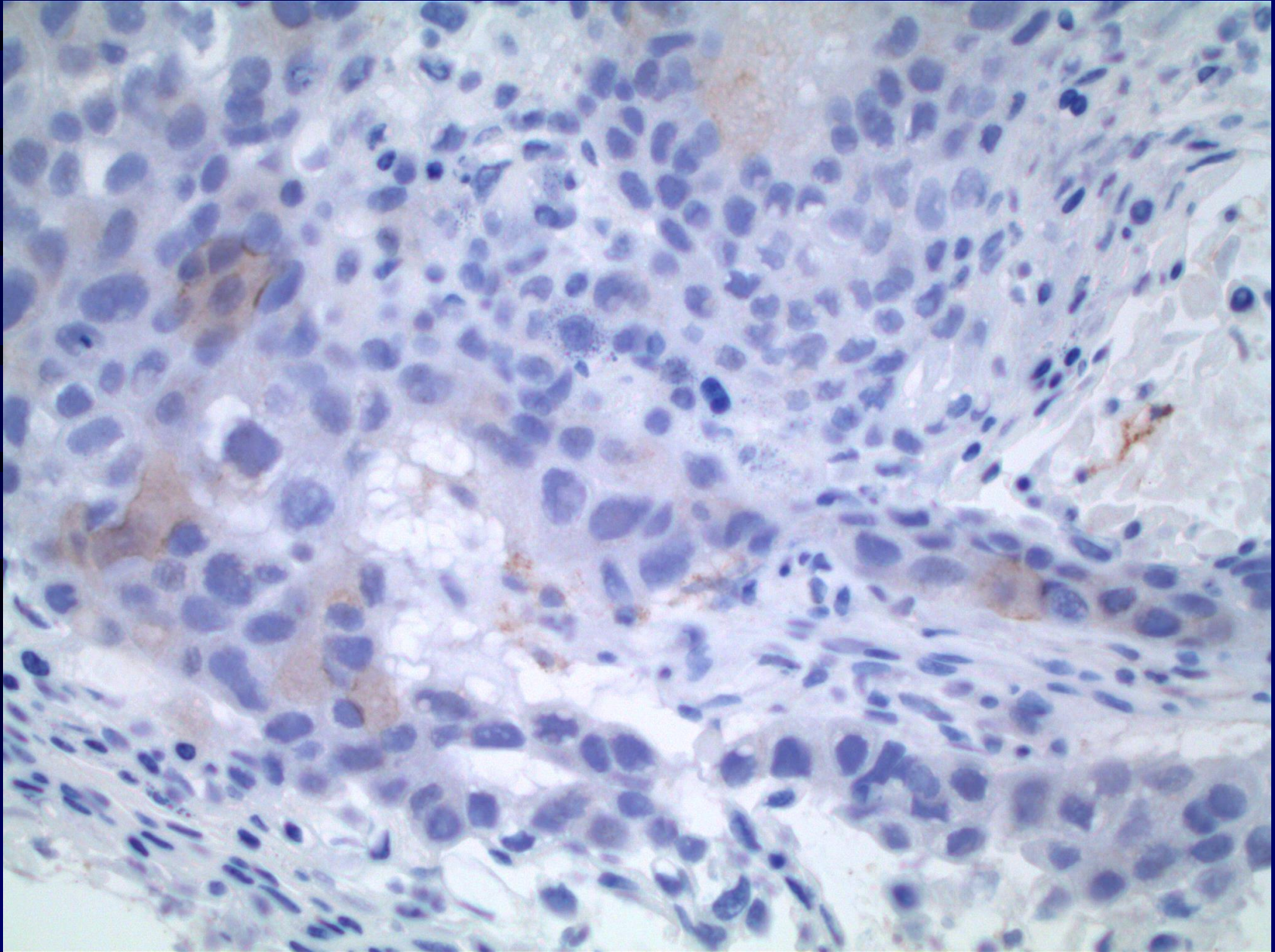


Gastric signet ring ca: CPS 10

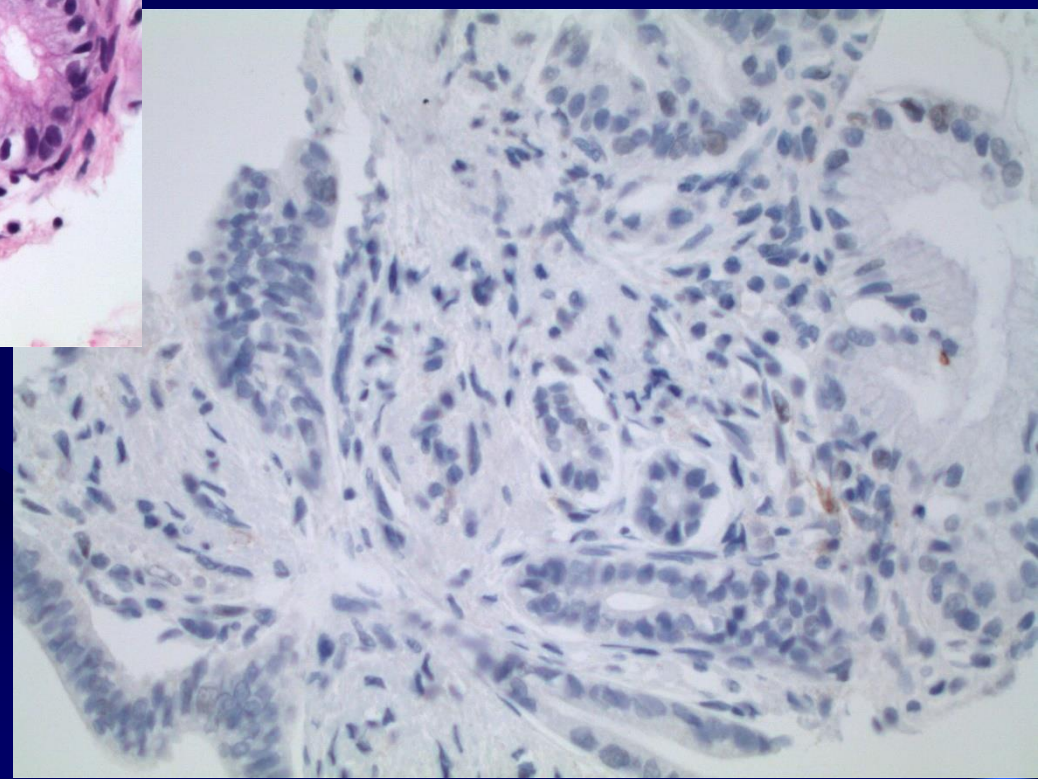
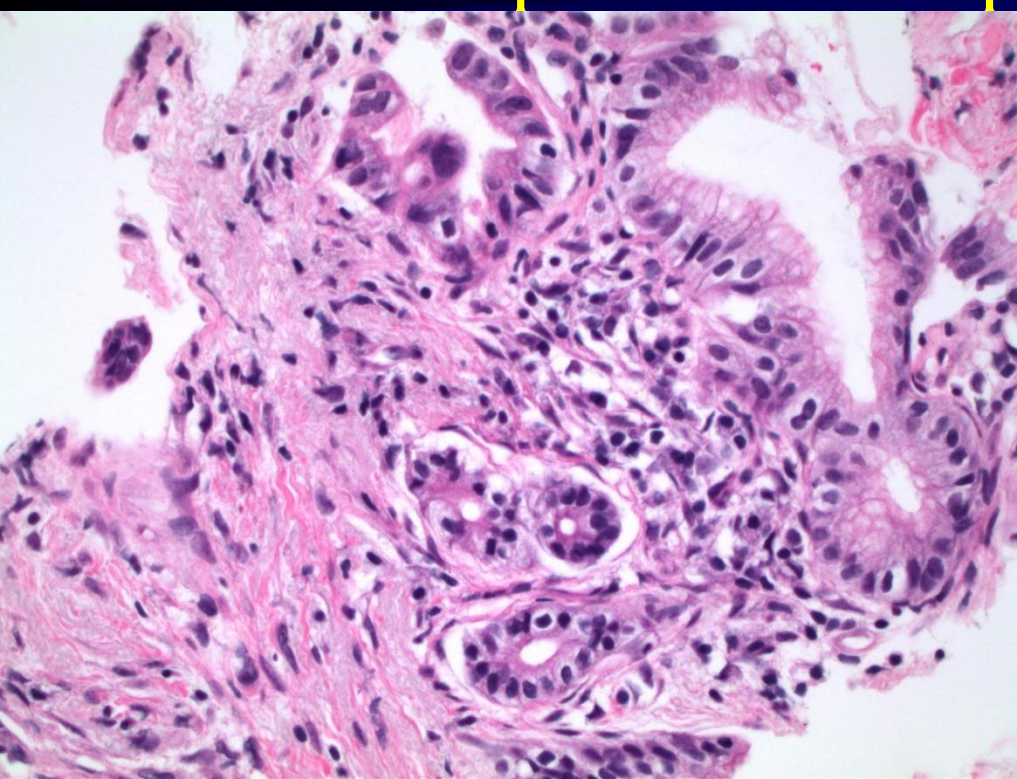
Immune cells reactive/ tumor cells negative



Cytoplasmic blush- do not score



Pancreatic cancer- negative but should not be reported. Not approved by PD-L1 IHC



PD-L1 Testing: Summary

Companion vs complementary diagnostic assays vary by drug and tumor site (i.e. pembro, 22C3 vs nivolumab, 28-8). For some indications there is no specified assay or it states “an FDA approved assay”.

Considering using the assay in the trial that led to the approved indication.

Scoring criteria also vary: TPS, CPS, stromal compartment. Minimum number of viable cells (100).

Most assays on the market are equivalent with exception of SP142.

Ongoing Blueprint studies may allow for testing with one kit that is equivalent.

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THE END

