112ª REUNIÓN TERRITORIAL SEAP-COMUNIDAD VALENCIANA

Valencia, 15 Noviembre 2019

Gloria Peiró Cabrera
Servicio de Patología
Hospital General Universitario, Alicante

Determinación de PD-L1 en Cáncer de Mama

IMpassion130 study design

Key IMpassion130 eligibility criteria[a]:

- Metastatic or inoperable, locally advanced TNBC
  - Histologically documented[b]
- No prior therapy for advanced TNBC
  - Prior chemotherapy in the curative setting, including taxanes, allowed if TFI ≥ 12 months
- ECOG PS 0–1

Stratification factors:

- Prior taxane use (yes vs no)
- Liver metastases (yes vs no)
- PD-L1 status on IC (positive [≥1%] vs negative [<1%])[c]

R 1:1

Atezo + nab-P arm:

Atezolizumab 840 mg IV
- On days 1 and 15 of 28-day cycle

+ Nab-paclitaxel 100 mg/m^2 IV
- On days 1, 8 and 15 of 28-day cycle

Double blind; no crossover permitted

Plac + nab-P arm:

Placebo IV
- On days 1 and 15 of 28-day cycle

+ Nab-paclitaxel 100 mg/m^2 IV
- On days 1, 8 and 15 of 28-day cycle

RECIST v1.1 PD or toxicity

- Co-primary endpoints were PFS and OS in the ITT and PD-L1+ populations[d]
  - Key secondary efficacy endpoints (ORR and DOR) and safety were also evaluated

a. Clinical Trials.Gov: NCT02425891. b. Locally evaluated per ASCO–College of American Pathologists (CAP) guidelines. c. Centrally evaluated per VENTANA SP142 IHC assay (double blinded for PD-L1 status). d. Radiological endpoints were investigator assessed (per RECIST v1.1). IC, tumour-infiltrating immune cell; TFI, treatment-free interval.

One of the first and largest phase III trials in TNBC using the ASCO-CAP definition of hormone receptor and HER2 status

Schmid P, et al. IMpassion130. ESMO 2018

Expanding the PD-L1 Testing with VENTANA PD-L1 (SP142) Assay: Role of Immunotherapy in TNBC

19 Oct 2018

Research

JAMA Oncology | Original Investigation

Atezolizumab Plus nab-Paclitaxel in the Treatment of Metastatic Triple-Negative Breast Cancer With 2-Year Survival Follow-up

A Phase 1b Clinical Trial

Sylvia Adams, MD; Jennifer R. Diamond, MD; Erika Hamilton, MD; Paula R. Pohlmann, MD, PhD; Sara M. Tolaney, MD; Ching-Wei Chang, PhD; Wei Zhang, MD; Koho Iizuka, MD; Paul G. Foster, PhD; Luciana Molinero, PhD; Roel Funke, PhD; John Powderly, MD

JAMA Oncol (2018) October 19.

20 Oct 2018

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer

P. Schmid, S. Adams, H.S. Rugo, A. Schneeweiss, C.H. Barrios, H. Iwata, V. Diéras, R. Hegg, S.-A. Im, G. Shaw Wright, V. Henschel, L. Molinero, S.Y. Chui, R. Funke, A. Husain, E.P. Winer, S. Loi, and L.A. Emens, for the IMpassion130 Trial Investigators*

ABSTRACT

New Engl J Med (2018): 2108-2121

13 Nov 2018

Media Release

Roche

FDA grants priority review to Roche's Tecentriq in combination with Abraxane for the initial treatment of people with PD-L1-positive, metastatic triple-negative breast cancer

- **Triple-negative breast cancer is an aggressive disease, with high unmet medical need**
- **If approved, this Tecentriq (atezolizumab) combination would be the first cancer immunotherapy regimen for the treatment of PD-L1-positive, metastatic triple-negative breast cancer**

Basel, 13 November 2018 – Roche (SIX: RO, ROG; OTCQX: RHHBY) today announced the US Food and Drug Administration (FDA) has accepted the company's supplemental Biologics License Application (sBLA) and granted Priority Review for Tecentriq® (atezolizumab) plus chemotherapy (Abraxane® [albumin-bound paclitaxel; *nab*-paclitaxel]) for the initial (first-line) treatment of unresectable locally advanced or metastatic triple-negative breast cancer (TNBC) in people whose disease expresses the PD-L1 protein, as determined by PD-L1 biomarker testing. The FDA is expected to make a decision on approval by 12 March 2019. A Priority Review designation is granted to medicines that the FDA has determined to have the potential to provide significant improvements in the treatment, prevention or diagnosis of a disease.

8 Mar 2019

FDA approves atezolizumab for PD-L1 positive unresectable locally advanced or metastatic triple-negative breast cancer

CM Triple-negativo

- 15%
- Mujeres jóvenes
- Recidivas precoces (3-5ª años)
- Supervivencia 30% (5 años)
- Tratamiento: IQ+QTA vs QTNA➔IQ+QTA

Clasificación molecular en el CM

Año 2000[1]

SUBTIPOS "INTRÍNSECOS"

. Luminal A

. Luminal B/C

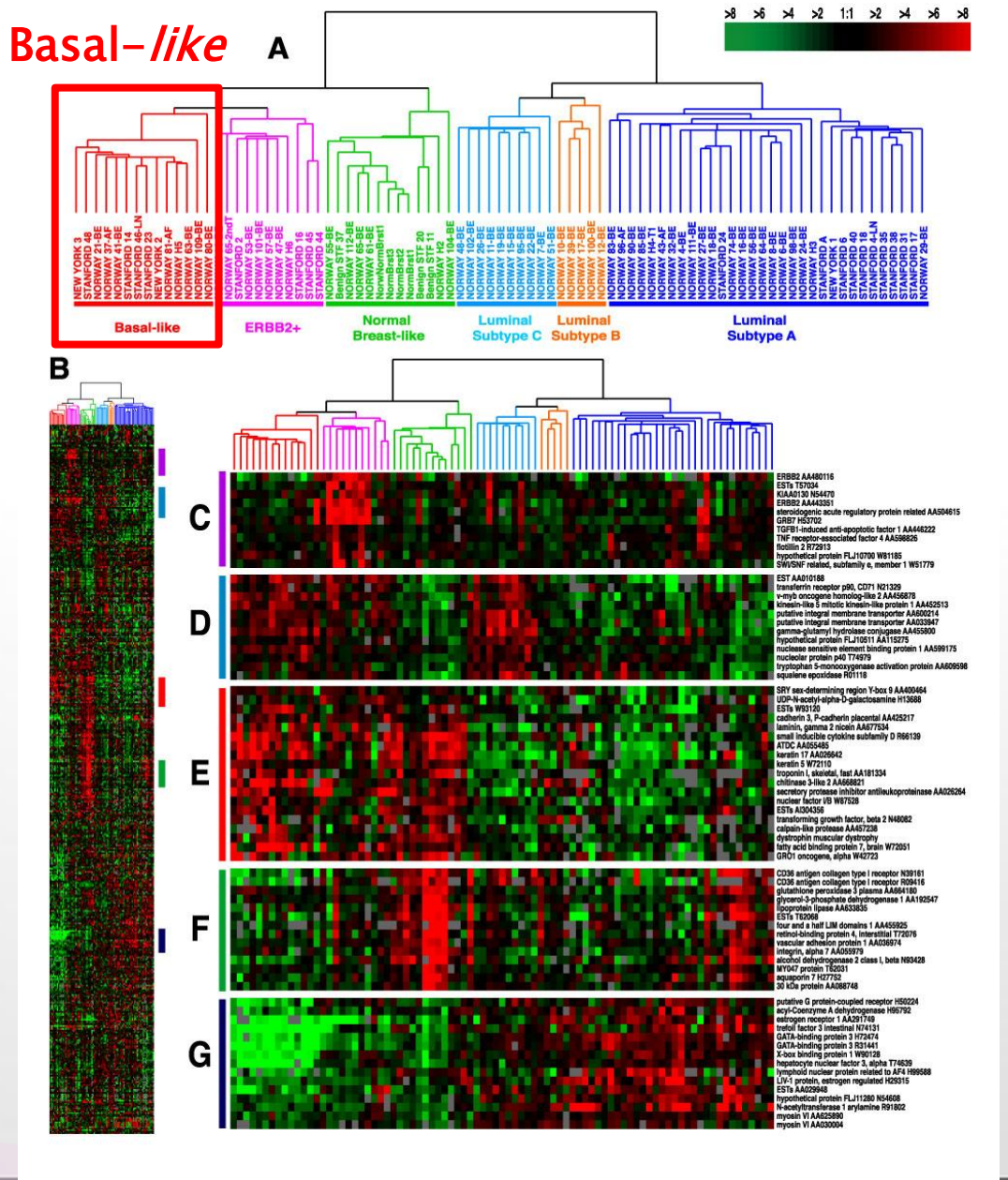
. Normal breast-*like*

. HER2-enriched

. Basal-*like*

Año 2007[2]; 2010[3]

. Claudin-low (3, 4, 7, E-cadherina)



1. Perou et al. Nature 2000; 406: 747-52
2. Herschkowitz JI, Genome Biol 2007, 8:R76
3. Prat et al. *Breast Cancer Res 2010*

CMTN: Subtipos moleculares de expresión génica

Año 2011

- En este estudio recopilaron una extensa serie de perfiles de expresión génica de 587 CMTN

- **Subclasificación en 6 subtipos**

- **HETEROGENEIDAD TUMORAL**

Lemhann et al. *J Clin Invest. 2011*

CMTN: Subtipos moleculares de expresión génica

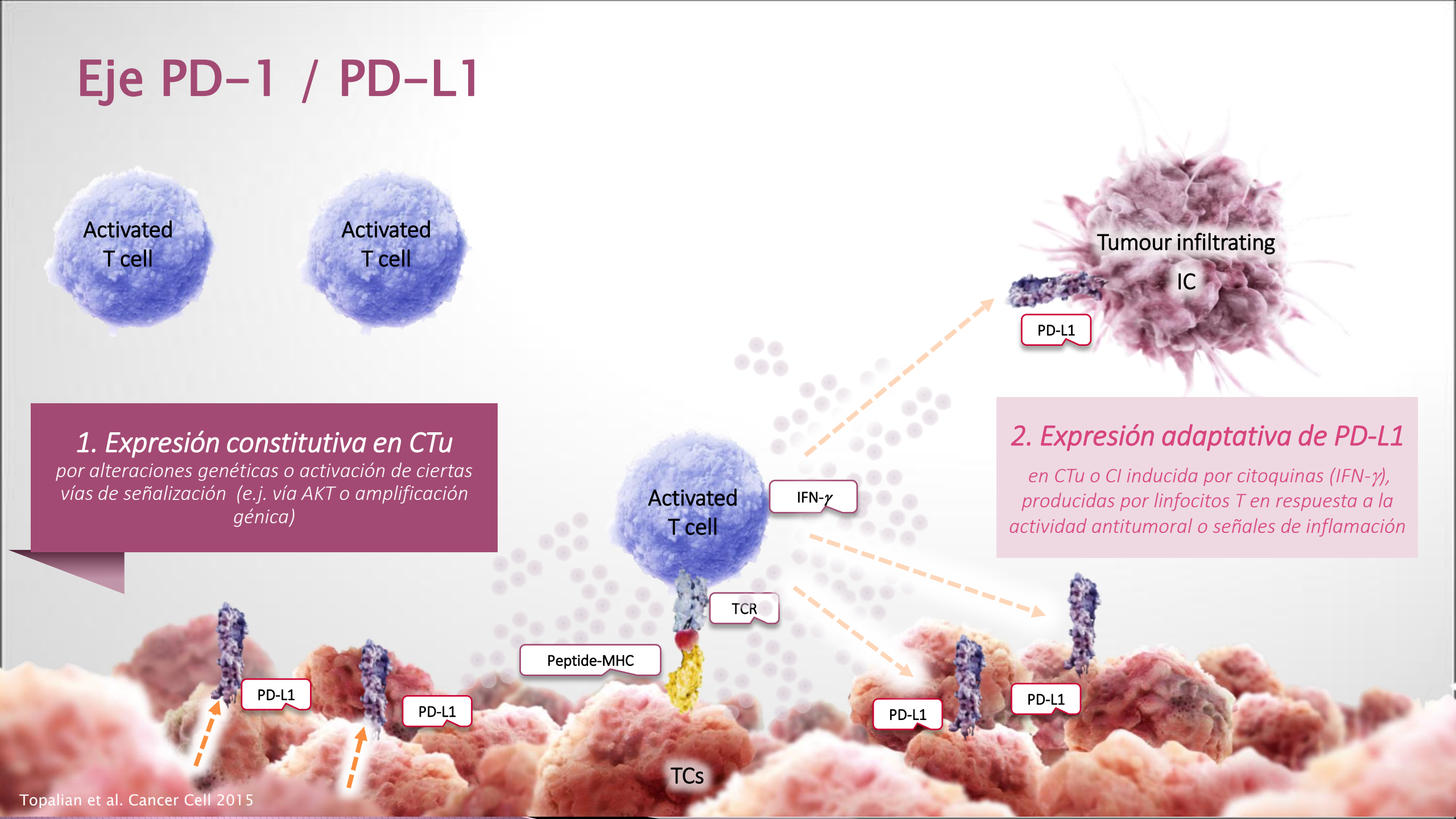
J Natl Cancer Inst. 2019 Oct 29. pii: djz208. doi: 10.1093/jnci/djz208. [Epub ahead of print]

Unraveling triple-negative breast cancer tumor microenvironment heterogeneity: towards an optimized treatment approach.

Bareche Y[1], Buisseret L[1], Gruosso T[2], Girard E[1], Venet D[1], Dupont F[1], Desmedt C[1], Larsimont D[3], Park M[2], Rothé F[1], Stagg J[4], Sotiriou C[1].

- **Basal-like (BL) subtype:** immune "cold" phenotype associated with stromal and metabolism TME signatures
- **Immunomodulator (IM) subtype:** the highest expression of "adaptive immune-related" gene signatures and a fully inflamed spatial pattern
  - ➔ The optimal candidate for immune check-point inhibitors
- **Mesenchymal and Mesenchymal subtype of stem-*like* cells (M and MSL)** and **Luminal Androgen Receptor (LAR) subtype:** "immunosuppressive" phenotype

Eje PD-1 / PD-L1



1. Expresión constitutiva en CTu

por alteraciones genéticas o activación de ciertas vías de señalización (e.j. vía AKT o amplificación génica)

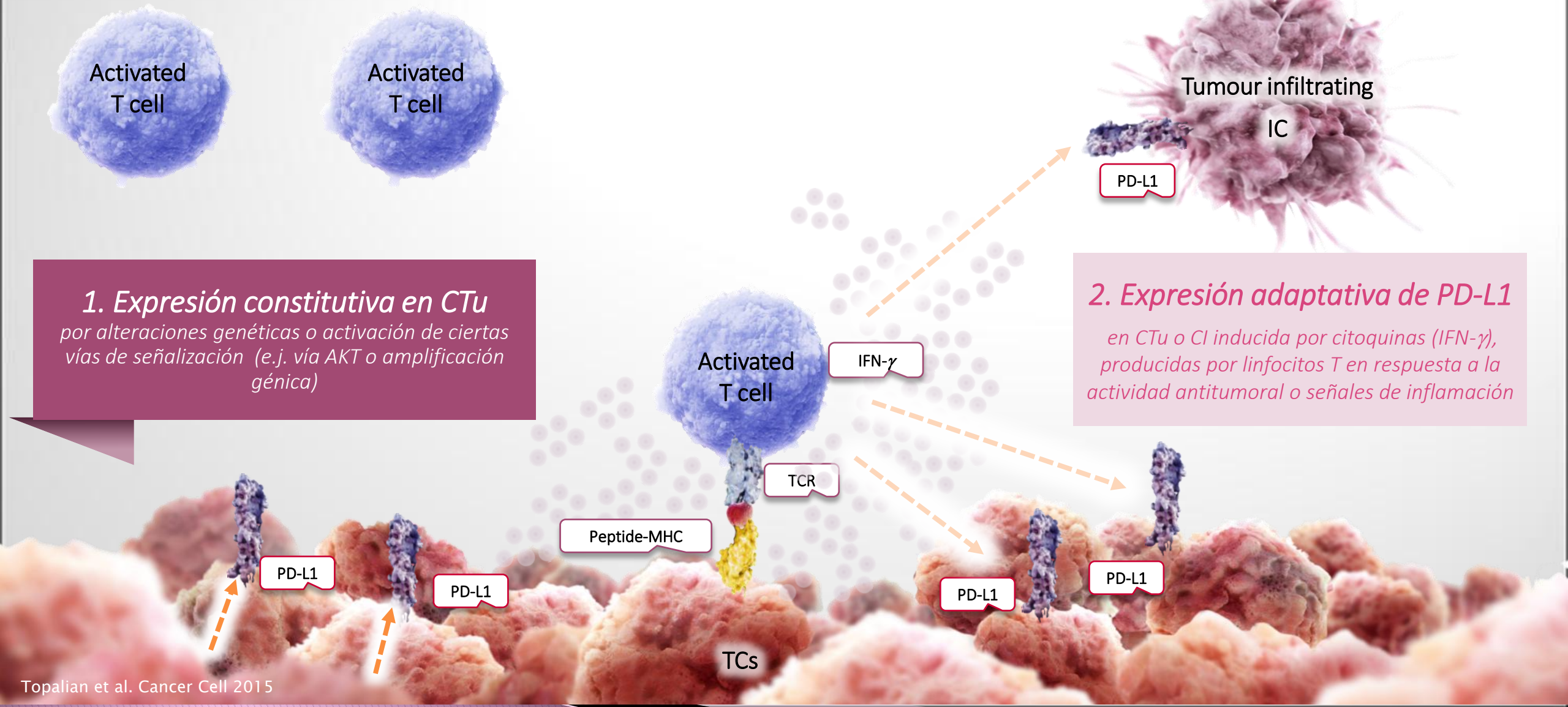
2. Expresión adaptativa de PD-L1

en CTu o CI inducida por citoquinas (IFN-γ), producidas por linfocitos T en respuesta a la actividad antitumoral o señales de inflamación

Topalian et al. Cancer Cell 2015

Eje PD-1 / PD-L1

- **PD-L1 can be expressed on T cells, B cells/plasma cells, NK T cells, activated monocytes, and dendritic cells !!**



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Topalian et al. Cancer Cell 2015

Available commercial PD-L1 IHC assays

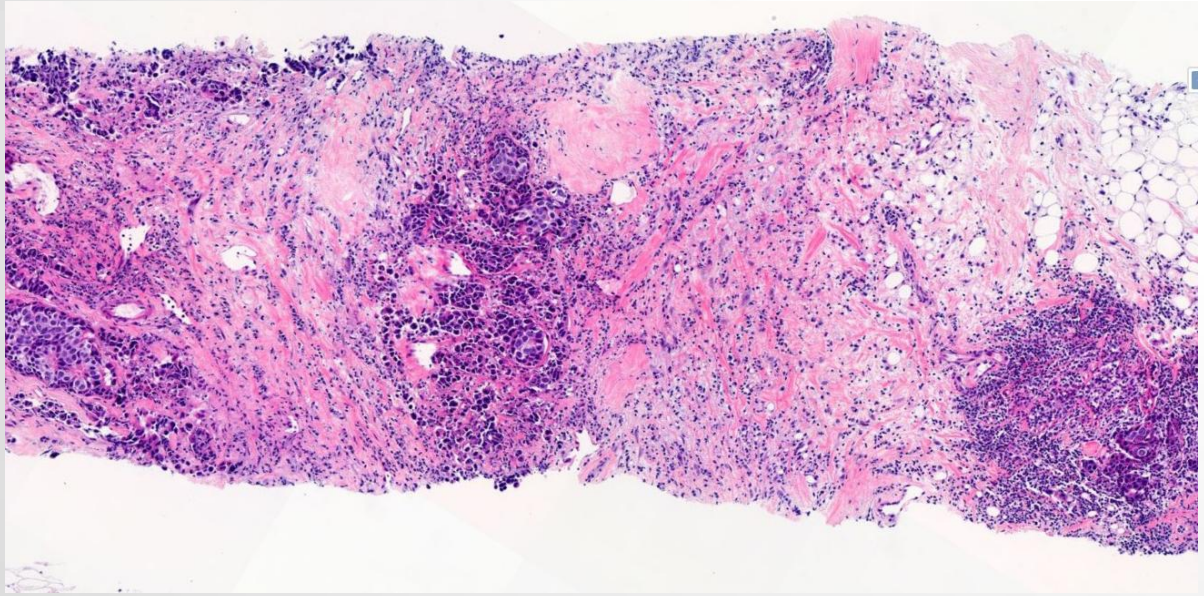
Assay (antibody clone)	VENTANA PD-L1 IHC (SP142) assay[1,2]	VENTANA PD-L1 IHC (SP263) assay[3]	Agilent/Dako PD-L1 IHC (22C3) pharmDx[4,5]	Agilent/Dako PD-L1 IHC (28-8) pharmDx[6]
Anti-PD-1/PD-L1 drug	TECENTRIQ (atezolizumab) – R/G	IMFINZI (durvalumab) – AZ	KEYTRUDA (pembrolizumab) – MSD	OPDIVO (nivolumab) – BMS
Dx manufacturer and platform	VENTANA BenchMark ULTRA	VENTANA BenchMark ULTRA	Dako Link 48 Autostainer	Dako Link 48 Autostainer
Origin	Rabbit monoclonal vs C-terminus	Rabbit monoclonal vs C-terminus	Mouse monoclonal vs N-terminus	Rabbit monoclonal vs N-terminus
Scoring algorithm cell types and cut-off values	▪ NSCLC: TC1/2/3 (≥1%, ≥10%, ≥50% or IC1/2/3 (≥1%, ≥5%, ≥10%) ▪ mUC: IC1/2/3 (≥1%, ≥5%, ≥10%)	▪ NSCLC: TC ≥25% ▪ mUC: TC or IC ≥25%	▪ NSCLC: TC ≥1%, ≥5% and ≥10% ▪ mUC: TC ≥1%, ≥5%	TC <1% TC ≥1% TC ≥5% TC ≥10%

Note, IMpassion130 results are not validated on any assay other than **VENTANA SP142**

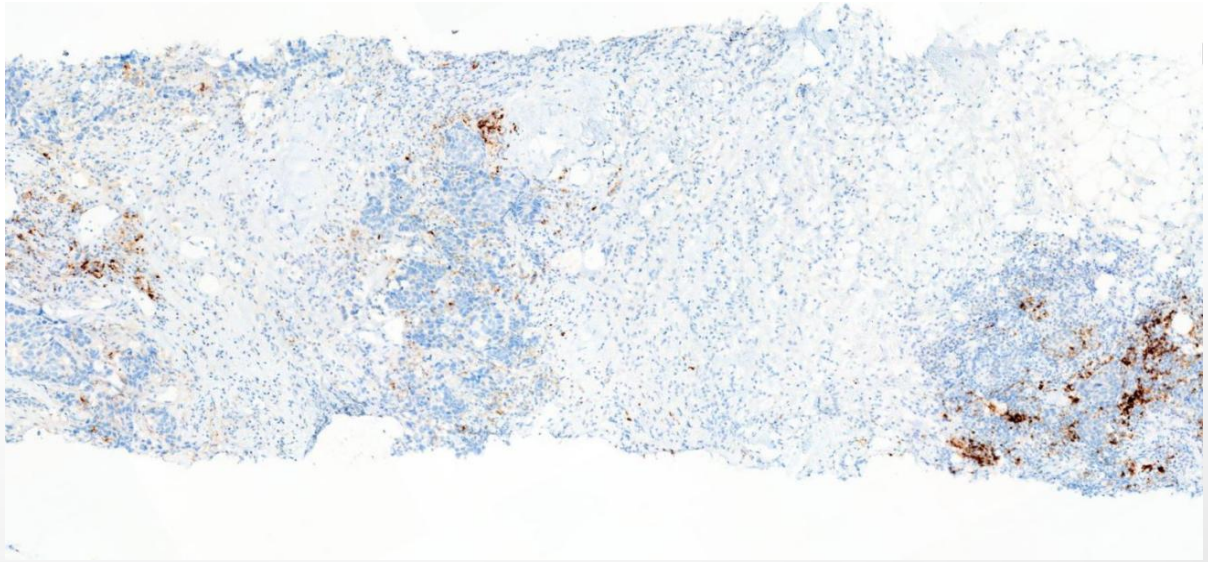
1. Fehrenbacher, et al. Lancet 2016; 2. Rosenberg, et al. Lancet 2016; 2a. ESMO 2018
3. VENTANA PD-L1 (SP263) interpretation guide; 4. Herbst, et al. Lancet 2016; 5. Balar, et al. ESMO 2016 (Abstract LBA32_PR); 6. Borghaei, et al. N Engl J Med 2015

PD-L1 expression in metastatic TNBC in IMpassion130

H&E



VENTANA SP142 PD-L1 (SP142) Assay



- ~40% of cases were PD-L1 positive (immune cell ≥1%)
- Materials: primary tumor (Bx, surgical specimens), mets (LN, others)

Schmid P et al. New Engl J Med (2018): 2108-2121

BARCELONA 2019 ESMO congress

Performance of PD-L1 immunohistochemistry assays in unresectable locally advanced or metastatic triple-negative breast cancer: post hoc analysis of IMpassion130

Hope S. Rugo,[1] Sherene Loi,[2] Sylvia Adams,[3] Peter Schmid,[4] Andreas Schneeweiss,[5] Carlos H. Barrios,[6] Hiroji Iwata,[7] Véronique Diéras,[8] Eric P. Winer,[9] Mark M. Kockx,[10] Dieter Peeters,[10] Stephen Y. Chui,[11] Jennifer C. Lin,[11] Anh Nguyen Duc,[11] Giuseppe Viale,[12] Luciana Molinero,[11] Leisha A. Emens[13]

[1]University of California San Francisco Comprehensive Cancer Center, San Francisco, CA, USA; [2]Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; [3]NYU Langone Medical Center, New York, NY, USA; [4]Barts Cancer Institute, Queen Mary University London, London, UK; [5]University Hospital and German Cancer Research Center Heidelberg, Heidelberg, Germany; [6]Centro de Pesquisa Clínica, HSL, PUCRS, Porto Alegre, Brazil; [7]Aichi Cancer Center Hospital, Nagoya, Japan; [8]Department of Medical Oncology, Centre Eugène Marquis, Rennes, France; [9]Dana-Farber Cancer Institute, Boston, MA, USA; [10]HistoGeneX NV, Antwerp, Belgium; [11]Genentech, Inc., South San Francisco, CA, USA; [12]University of Milan, European Institute of Oncology IRCCS, Milan, Italy; [13]University of Pittsburgh Medical Center Hillman Cancer Center, Pittsburgh, PA, USA

esmo.org

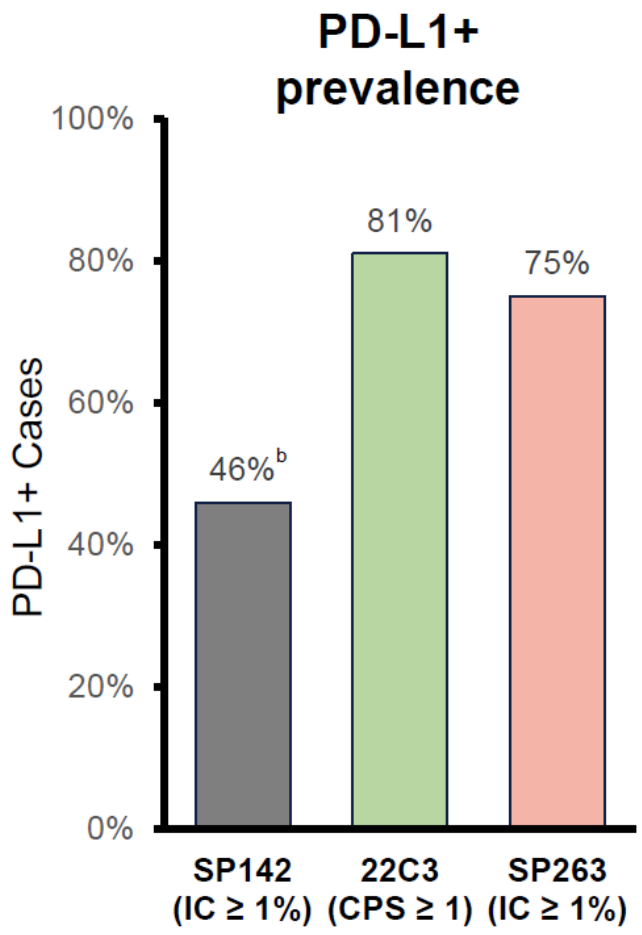
Barcelona, 2019

Background

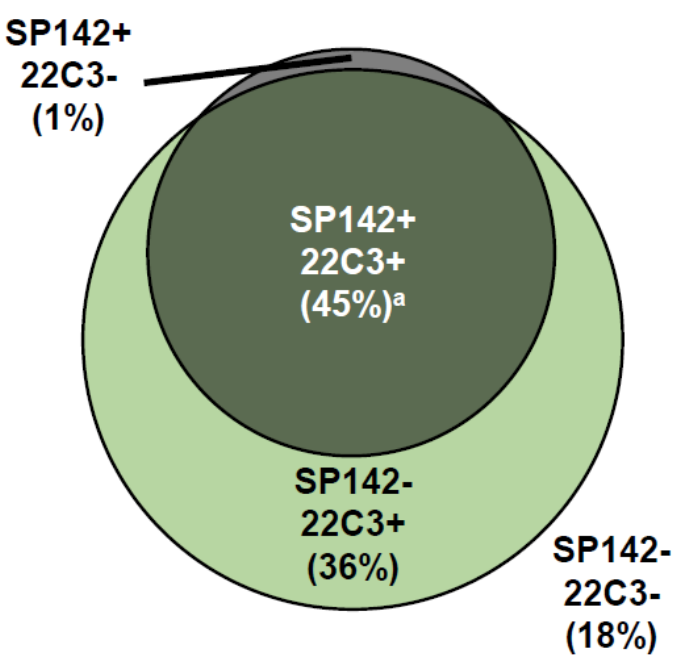
- IMpassion130 is the first Phase III study of cancer immunotherapy in mTNBC to demonstrate clinical benefit in PD-L1+ patients[1,2]
  - Atezolizumab + *nab*-paclitaxel is approved in the United States[3] and European Union[4] and recommended for the treatment of patients with PD-L1 IC+ mTNBC in the NCCN[5] and AGO[6] guidelines
  - PD-L1 expression on IC was evaluated using the VENTANA PD-L1 SP142 IHC assay with a ≥ 1% cutoff[1]
- The SP142 assay has been clinically validated and FDA-approved to identify patients with mTNBC for treatment with atezolizumab + *nab*-paclitaxel[3,4]
  - Dako 22C3[a] and VENTANA SP263 are 2 other commercially available PD-L1 IHC assays approved for non-TNBC indications
- In this exploratory post hoc IMpassion130 substudy, 22C3 and SP263 PD-L1 IHC assays were evaluated for PD-L1 prevalence, analytical concordance with SP142 and estimates of clinical activity

AGO, Arbeitsgemeinschaft Gynäkologische Onkologie; IC, tumour-infiltrating immune cells; IHC, immunohistochemistry; NCCN, National Comprehensive Cancer Network.
[a] Commercialized by Merck to support the use of pembrolizumab.
1. Schmid *New Engl J Med* 2018. 2. Schmid ASCO 2019. 3. TECENTRIQ (atezolizumab) USPI 2019. 4. TECENTRIQ (atezolizumab) SmPC 2019.
5. NCCN Clinical Practice Guidelines. Breast Cancer. V2.2019. 6. AGO Guidelines. Breast. V2019.1.

PD-L1 IHC assays: prevalence and analytical concordance

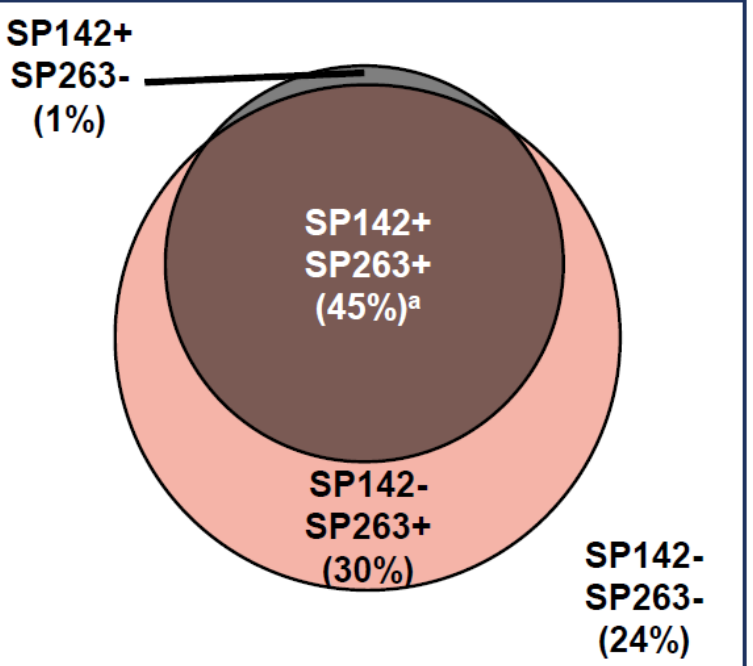


SP142 (IC 1%) and 22C3 (CPS 1)



OPA[c]	64%
PPA	98%
NPA	34%

SP142 (IC 1%) and SP263 (IC 1%)



OPA[c]	69%
PPA	98%
NPA	45%

NPA, negative percentage agreement; OPA, overall percentage agreement; PPA, positive percentage agreement.
[a] > 97% of SP142+ samples included in 22C3+ or SP263+ samples. [b] Compared with 41% in ITT (Schmid, *New Engl J Med* 2018).
[c] ≥ 90% OPA, PPA ***and*** NPA required for analytical concordance.

Rugo et al. Abstract 6571
IMpassion130 PD-L1 IHC
https://bit.ly/30OmOqz

… IMpassion130: mTNBC

- The clinical benefit in 22C3+ and SP263+ subgroups was driven by the SP142+ subgroup
  - The SP142 assay identified patients with the smallest HR point estimates, and longest median PFS and OS from atezolizumab + *nab*-paclitaxel

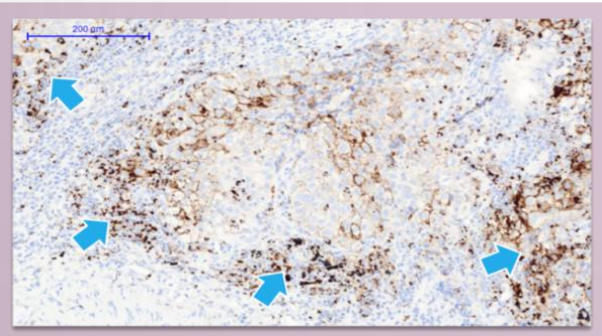
Rugo et al. A-6571; ESMO, Barcelona 2019

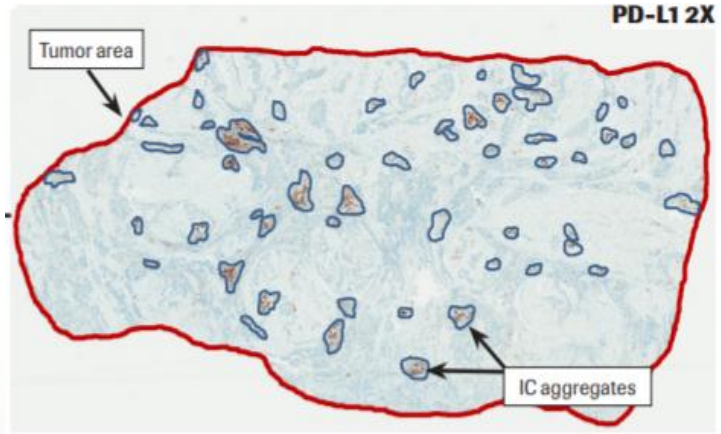
Pathologists require specific training to score ICs

SP142 is designed to enhance visual contrast of IC staining, offer a straightforward scoring algorithm, and to be easily trainable and reproducible

Testing for PD-L1 is needed in order to determine eligibility for atezolizumab + nab-paclitaxel

SP142

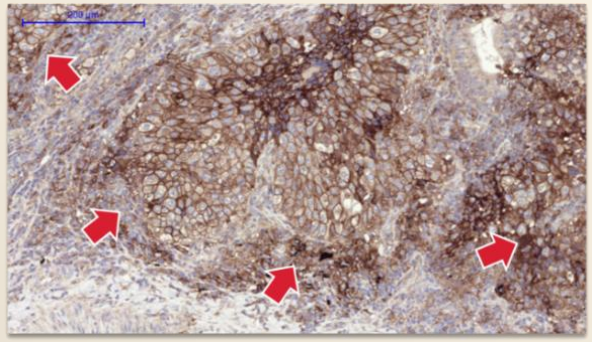


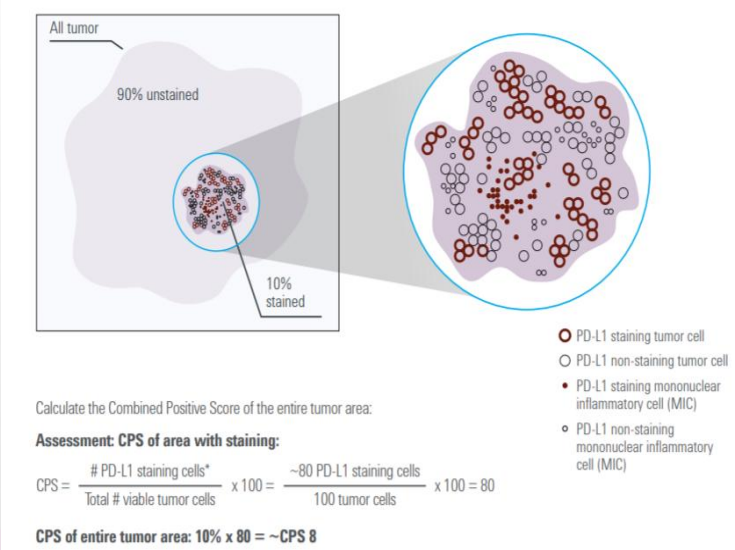


ICs are scored as the proportion of tumour area that is occupied by PD-L1 staining ICs of any intensity

Any IC staining, irrespective of cell type or localization is included

22c3





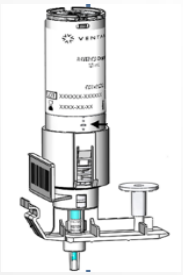
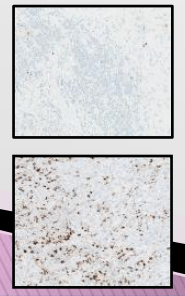
CPS is the number of PD-L1 stained cells (TCs, lymphocytes, macrophages) divided by the total number of viable TCs multiplied by 100

Objectives

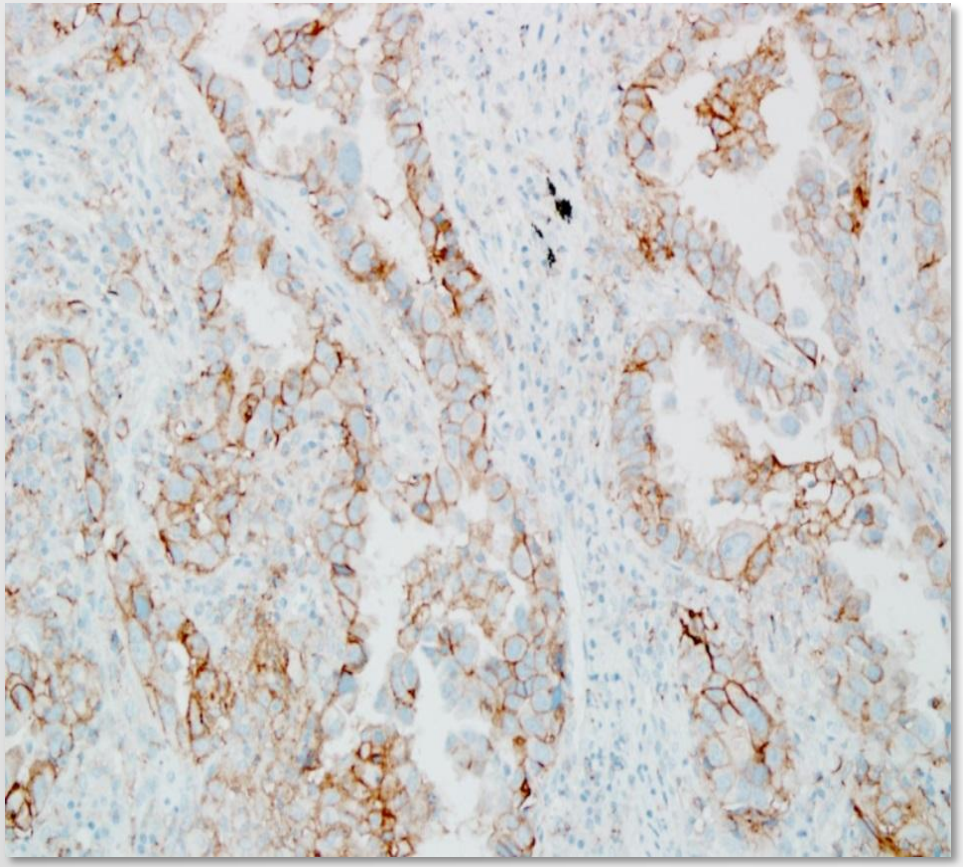
- Overview of PD-L1 SP142 Assay for Triple Negative Breast Cancer (TNBC)
- Evaluation workflow
- Scoring method TNBC
- Scoring challenges and pitfalls

Roche VENTANA PD-L1 (SP142) Assay:
System Approach

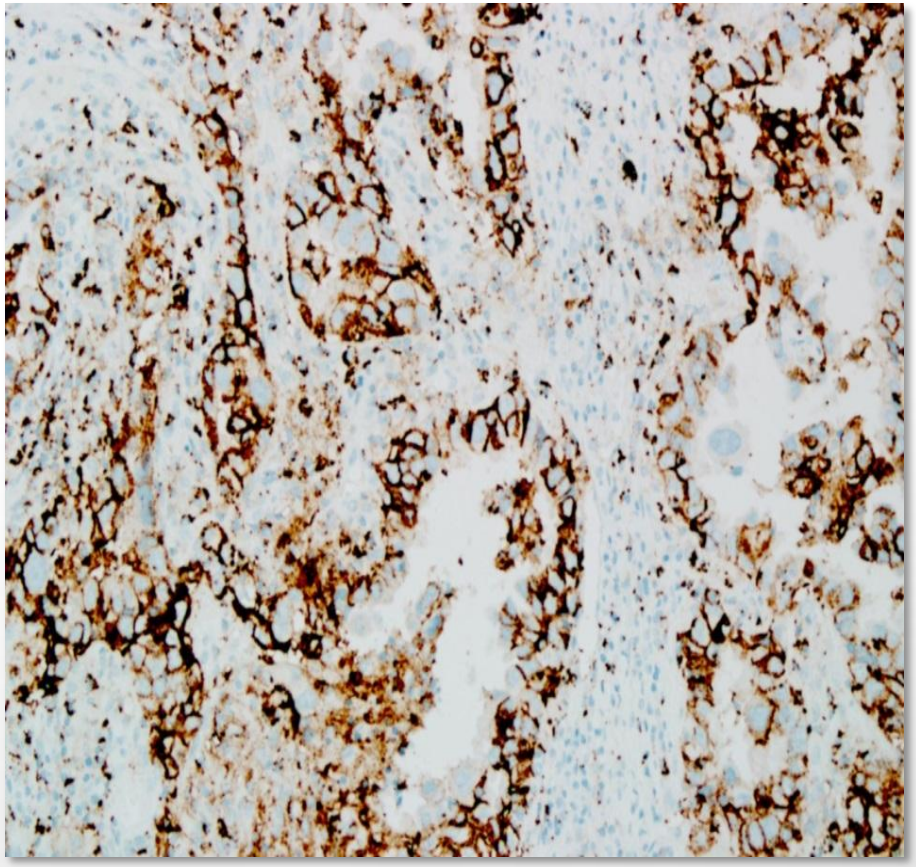
An assay is the entire system – antibody or probe, detection kit, scoring method, system controls and any instrumentation required to perform the assay. The "system" is FDA approved

Reagent Kit		• 50 test dispense of anti-PD-L1 rabbit monoclonal antibody clone SP142 • OptiView DAB Detection w/ Amplification Step • Instructions for Use (package insert)
Staining Platform		• BenchMark ULTRA Instrument • System Controls (slides/tissue) • BenchMark Staining Protocol
Reproducible Results	 IC<1% IC≥1%	• Cell types: Tumor infiltrating immune cells (IC) as well as tumor cells (TC) • Assay Scoring/Interpretation Guide • Pathologist Education Materials

Roche VENTANA PD-L1 (SP142) Assay:
Amplification Effect



Without Amplification



With Amplification

Accepted specimens

- Formalin-fixed and paraffin-embedded tissue
- Archival or fresh tissues from resections, excisions, and biopsies
- Primary and metastatic sites
- Adequacy: at least 50 viable tumor cells

Presence of tumor associated stroma is essential for scoring IC

Unaccepted specimens

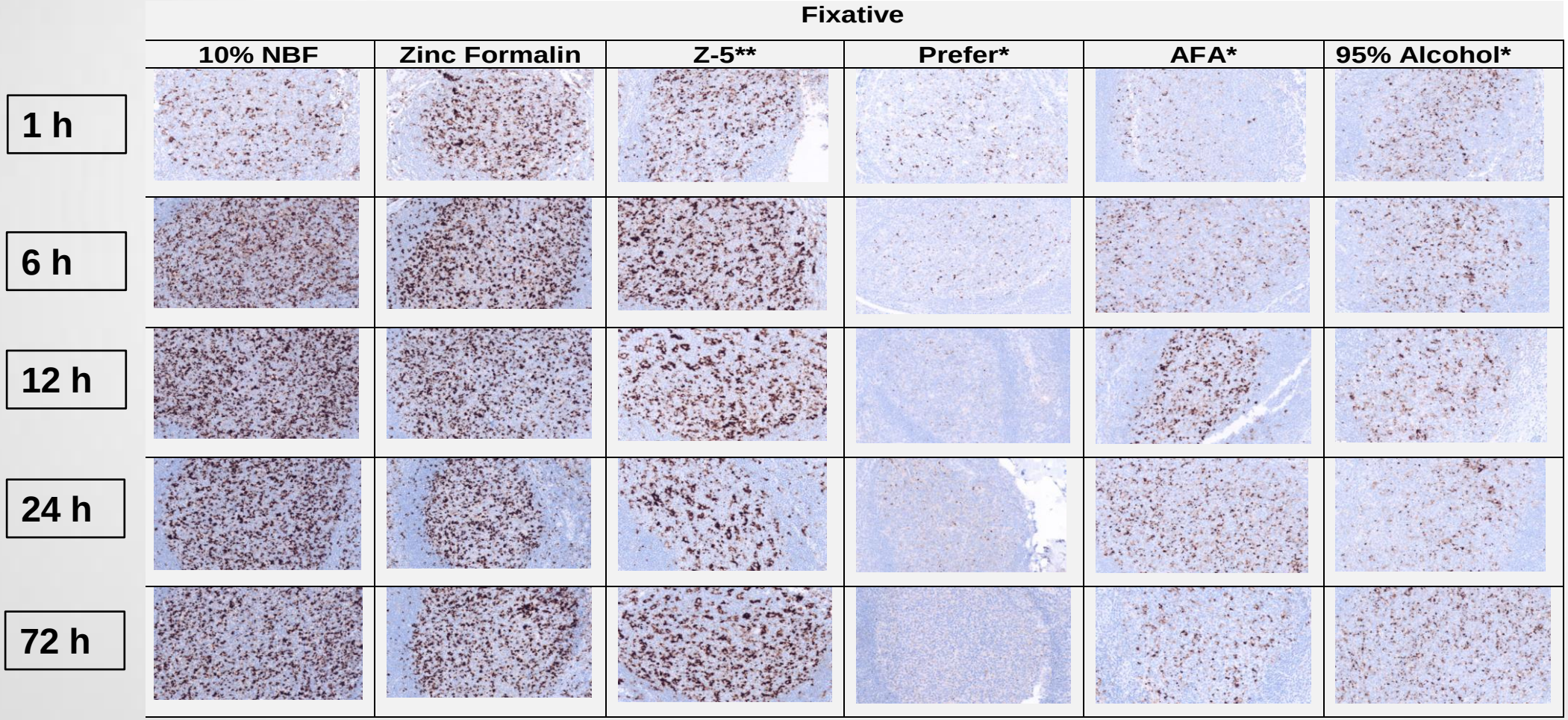
- Cytology samples
- Decalcified metastatic bone lesions

NB: These samples are not acceptable due to lack of validation studies

Pre-analytical variables

- Fixative Types: 10% NBF
- Tissue thickness: 4-6 microns
- Cut slide stability: 2 months

Pre-analytical variables: Fixation studies

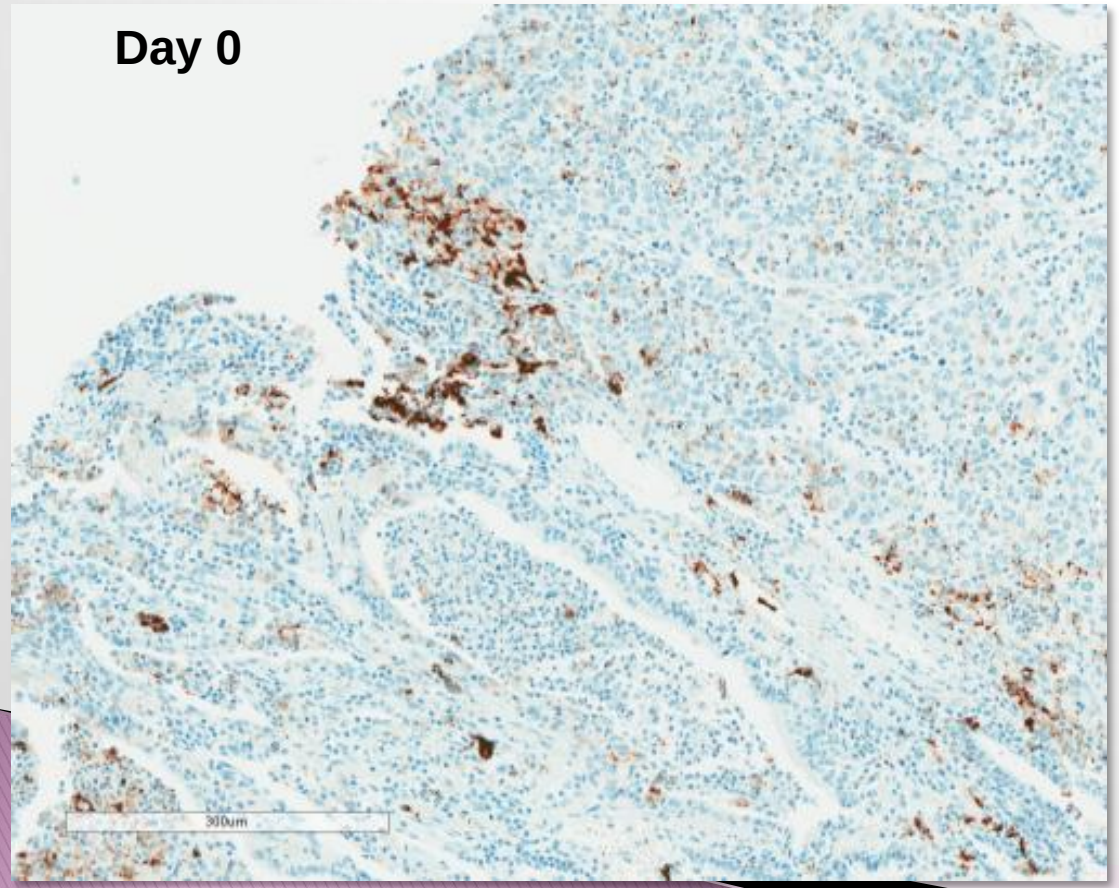


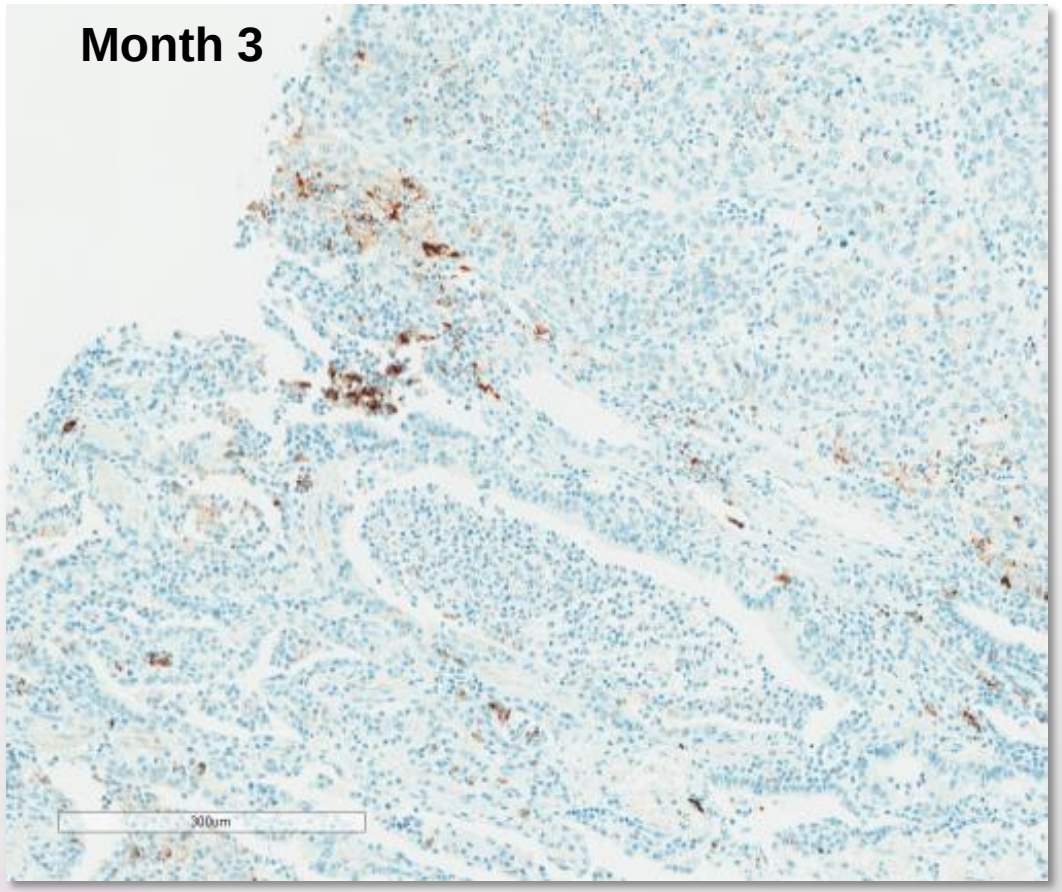
- Ventana recommends fixation in **10% NBF** for **6-72 hours**. <6h fixation is not recommended
- **Zinc Formalin** demonstrates comparable staining to 10% NBF
- ****Z-5** fixative demonstrates inconsistent staining with 10% NBF, and is <u>not recommended</u>
- ***Prefer** and **Alcohol** Fixatives including **AFA** (weaker staining) are <u>not recommended</u>
- [a] (10x magnification)

*Ventana Data on File

Pre-analytical variables – Cont.

- Tissues should be sectioned at 4-6 micron thickness
- Cut slides stored at room temperature should not be used if >2 months

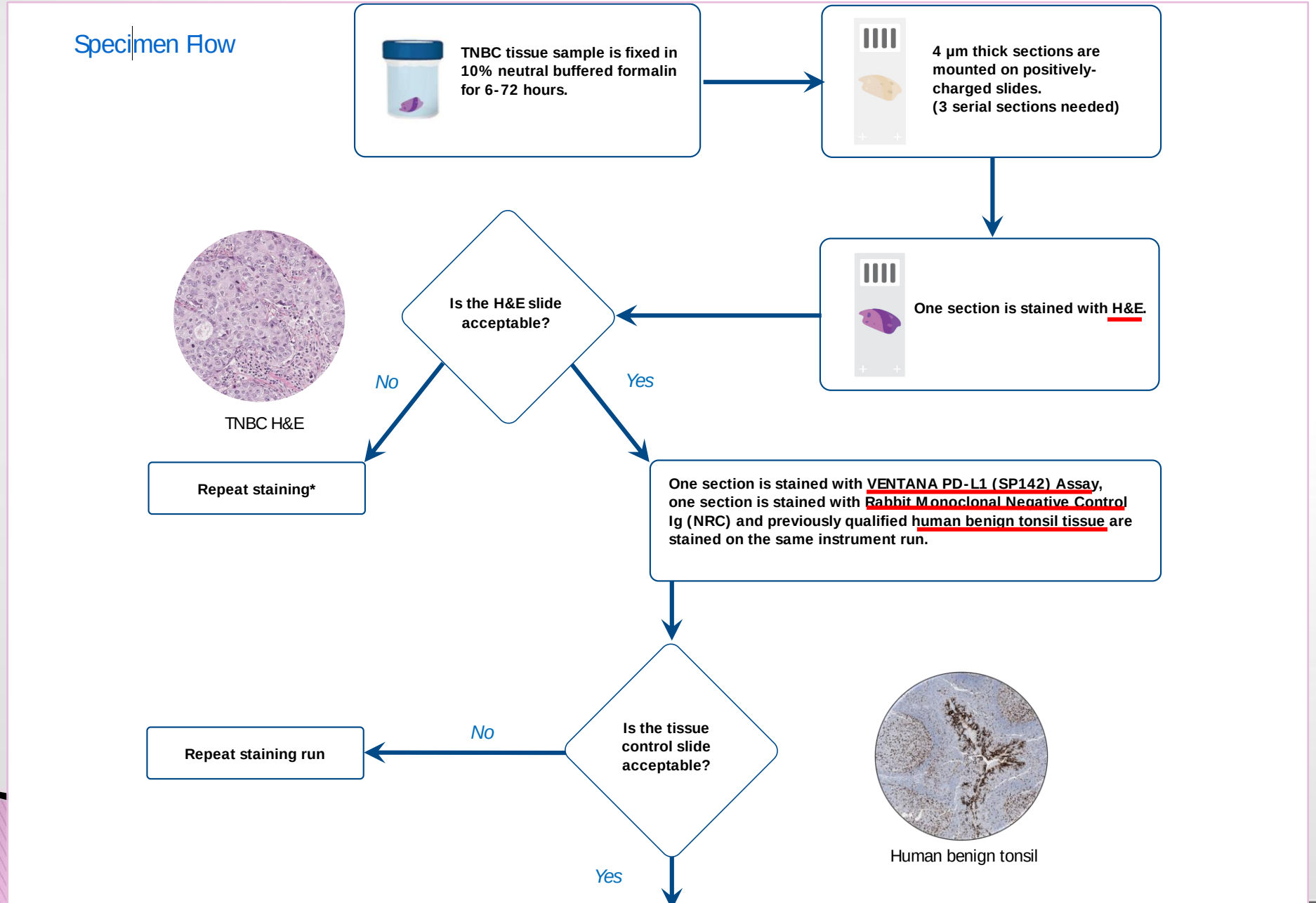




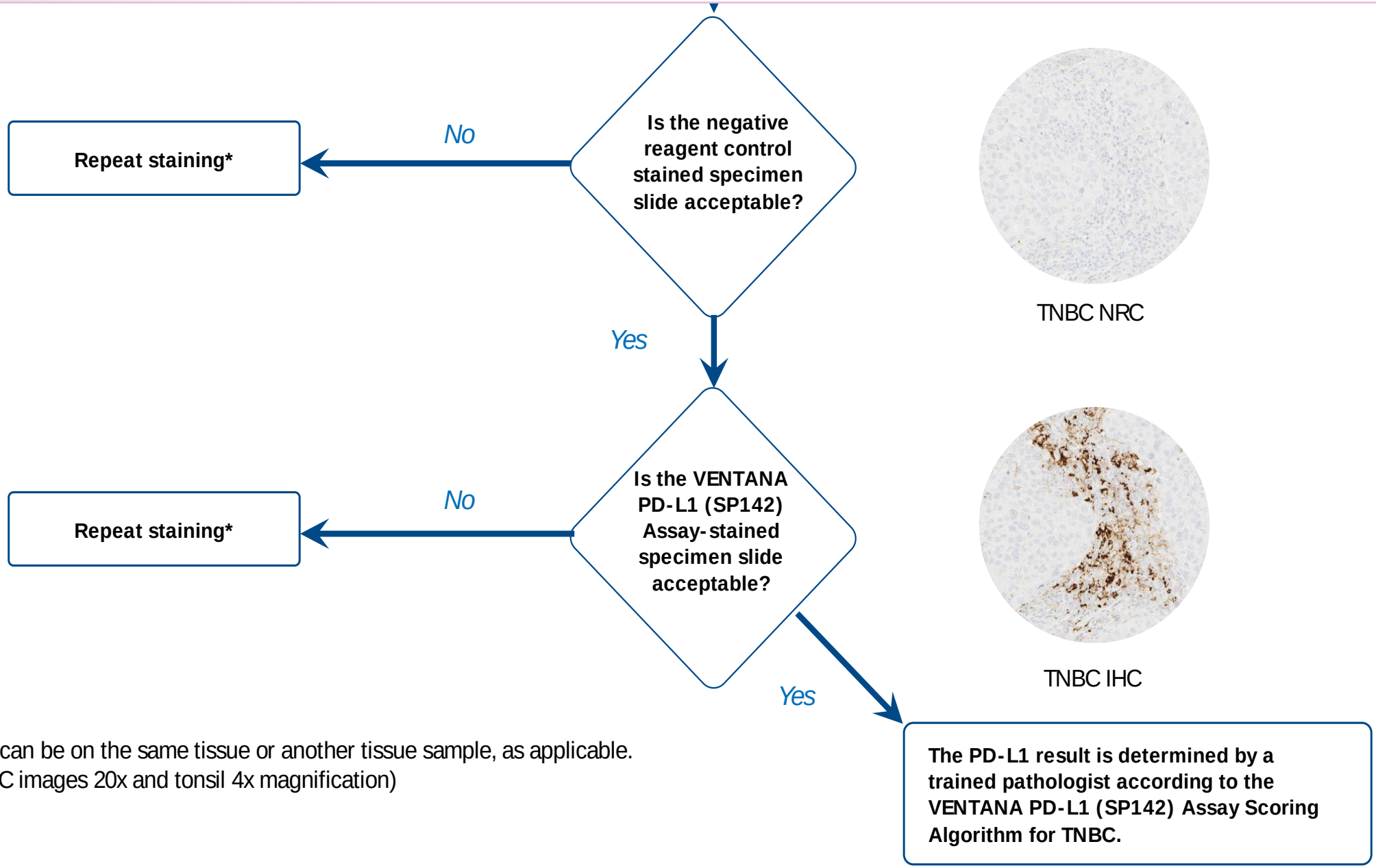
Objectives

- Overview of PD-L1 SP142 assay for Triple Negative Breast Cancer (TNBC)
- **Evaluation workflow**
- Scoring method TNBC
- Scoring challenges and pitfalls

Evaluation workflow (1)



Evaluation workflow (2)

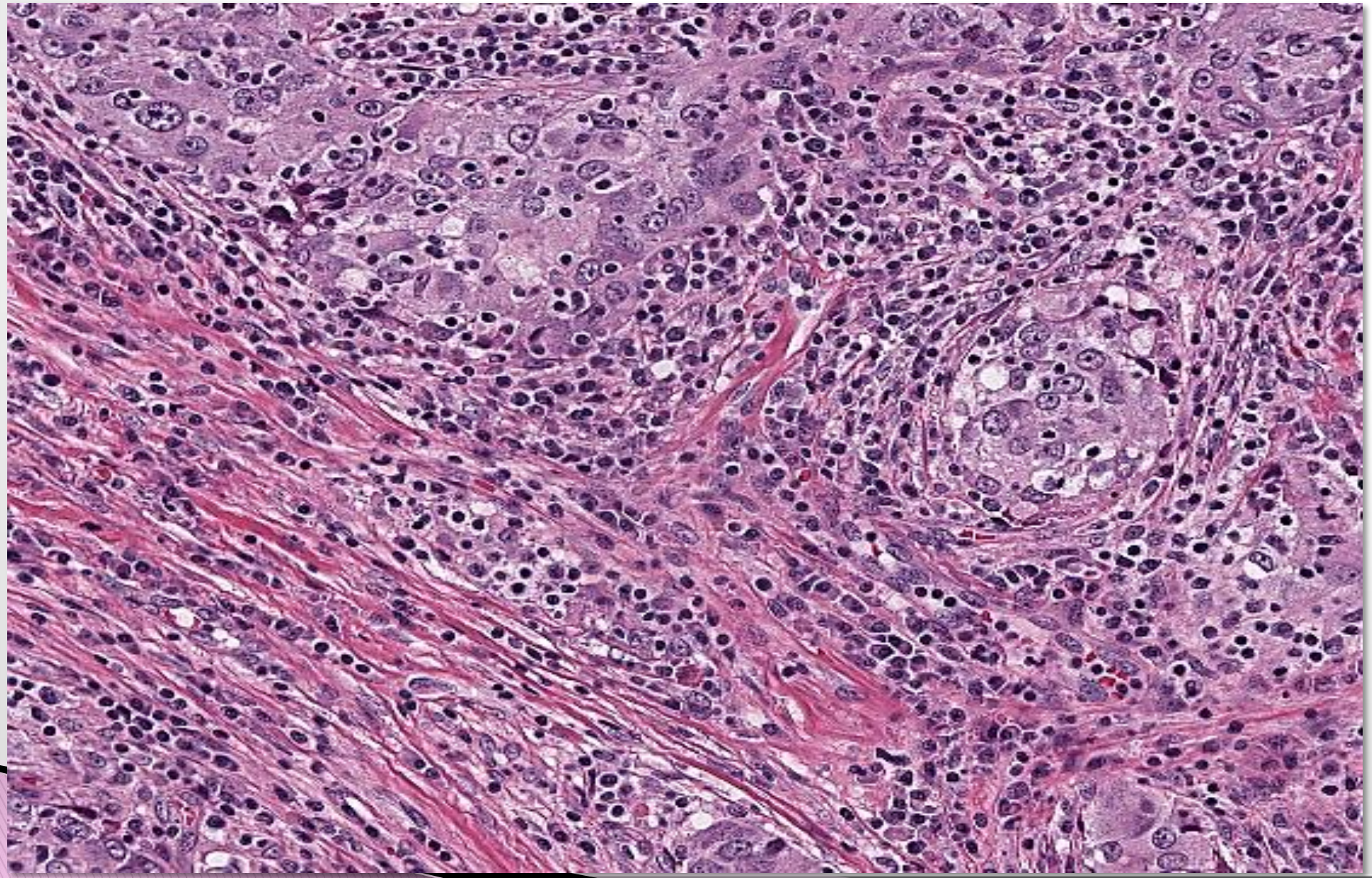


*Repeat can be on the same tissue or another tissue sample, as applicable.
(All TNBC images 20x and tonsil 4x magnification)

– H&E Evaluation

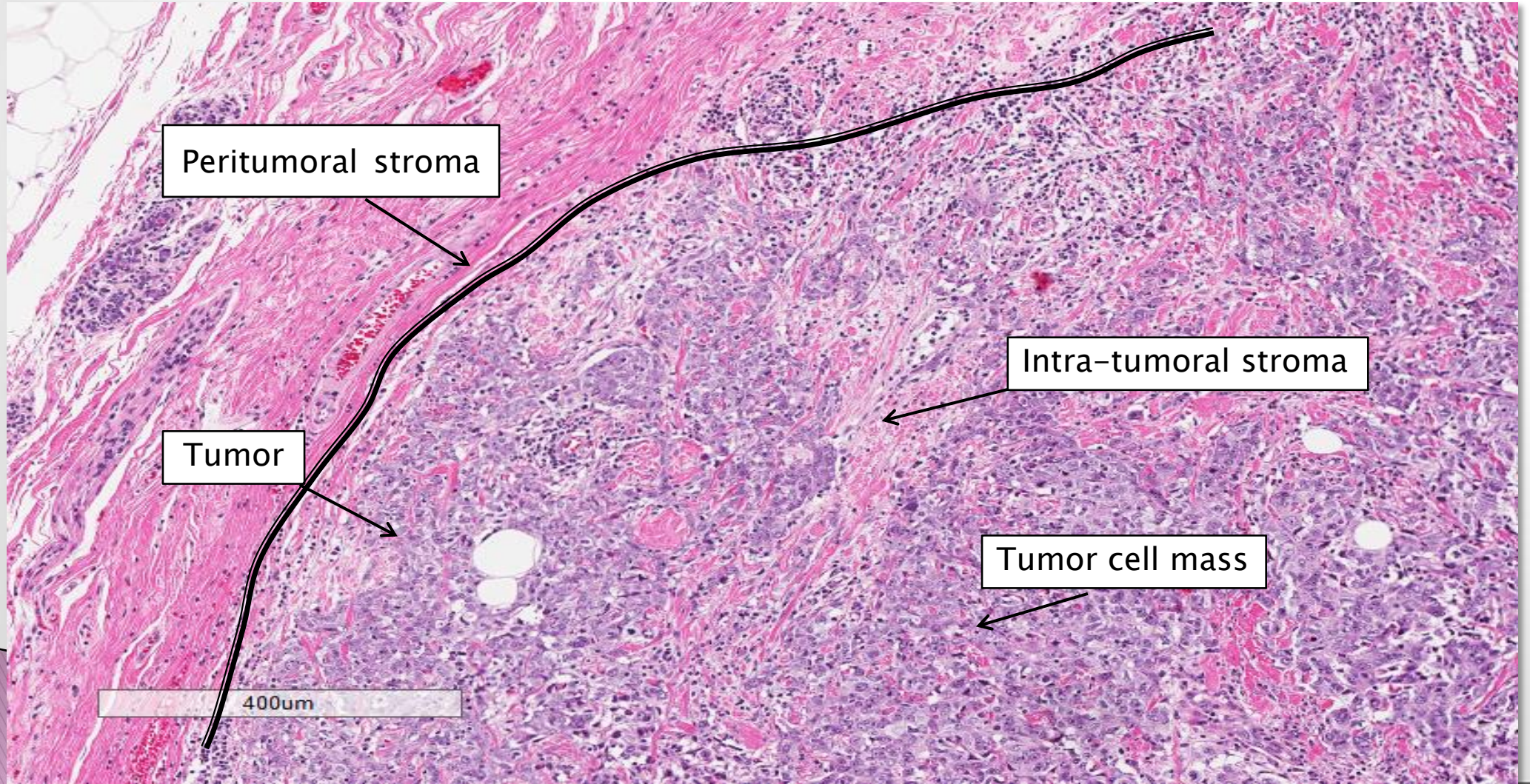
- Tumor Presence and Histology
- Tumor Area
- Tumor Mimics

a. Tumor Presence and Histology

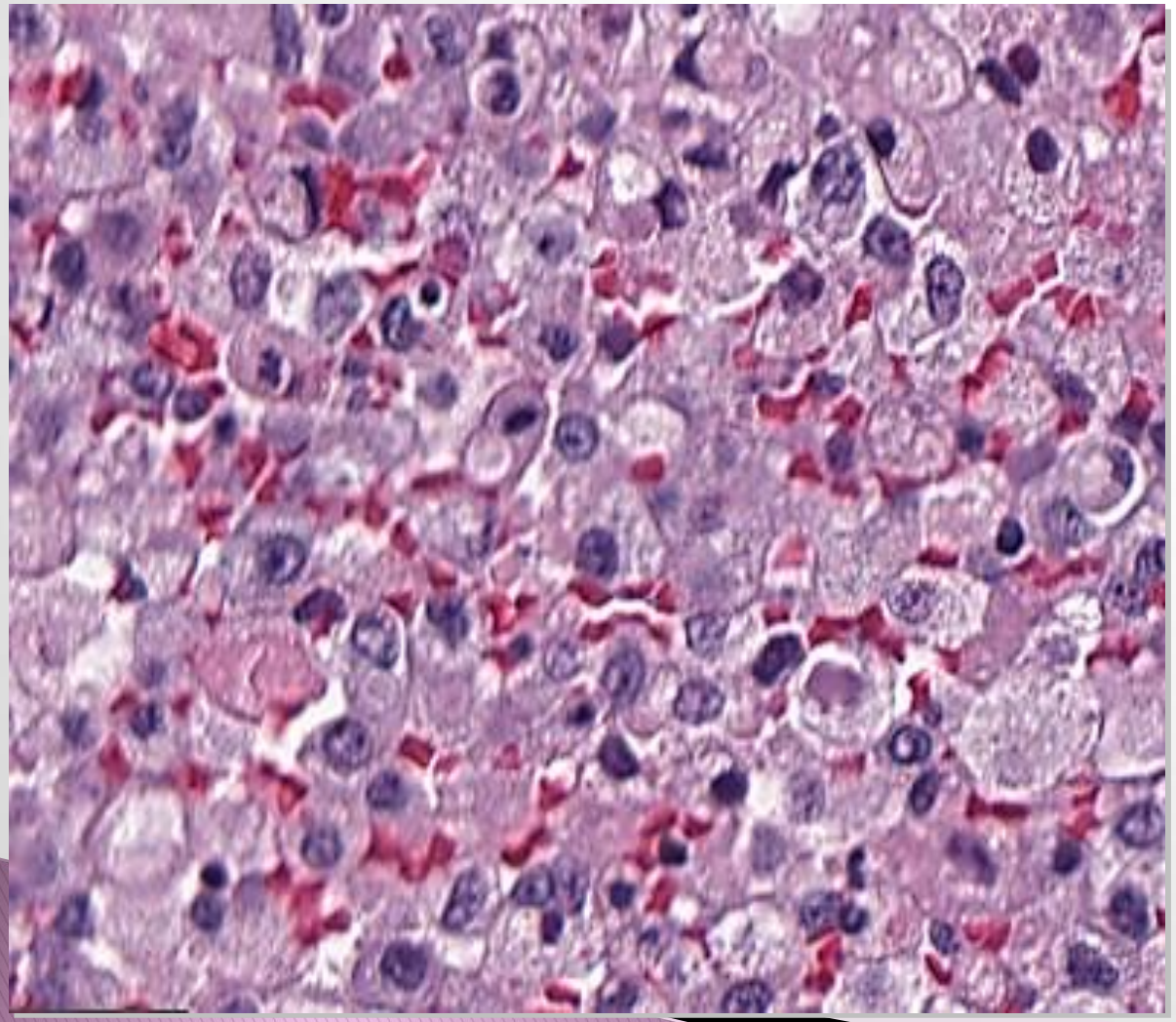


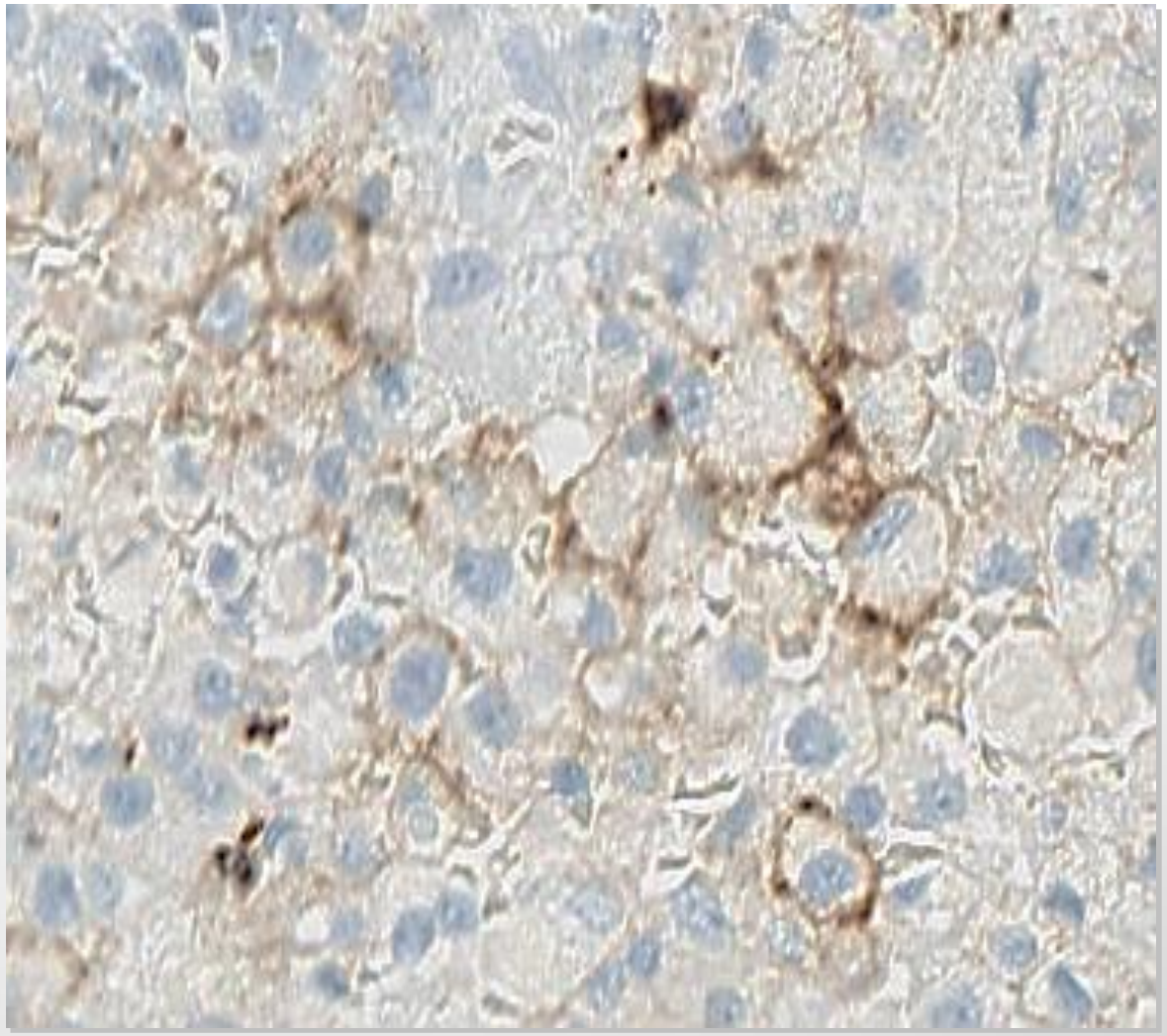
b. Tumor Area

- Area occupied by tumor cells as well as their associated intra-tumoral and peri-tumoral stroma



c. Tumor Mimics (e.g. macrophages)





Tissue Controls

- Used only for monitoring the **correct performance** of processed tissues, test reagents and instruments
  - ➔ not as an aid in determining PD-L1 status for patient samples

- Benign tonsil is an ideal tissue control
  - Contains both positive and negative staining epithelial and immune cells, serving as both a positive and negative tissue control for the VENTANA PD-L1 (SP142) Assay

Negative Reagent Control

- Acceptability
- Artifacts

N P N P

Tonsil tissue stained with PD-L1 (SP142) demonstrates:

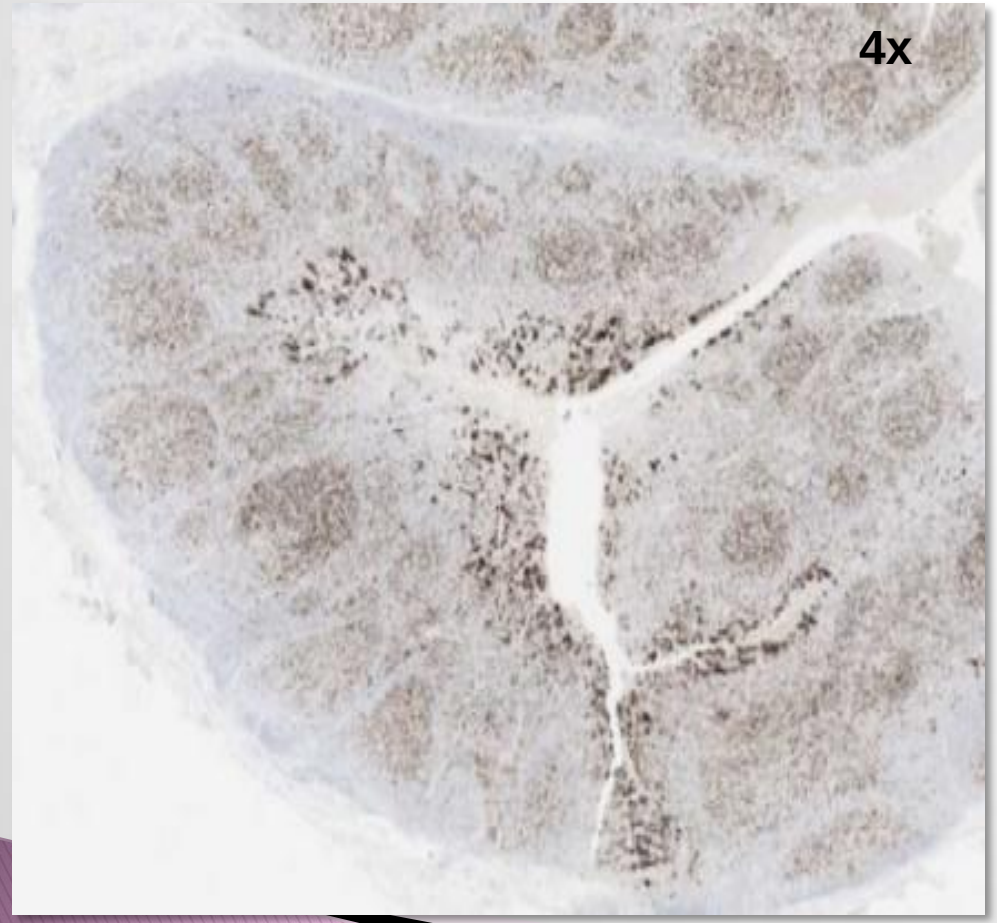
N staining of superficial squamous epithelium

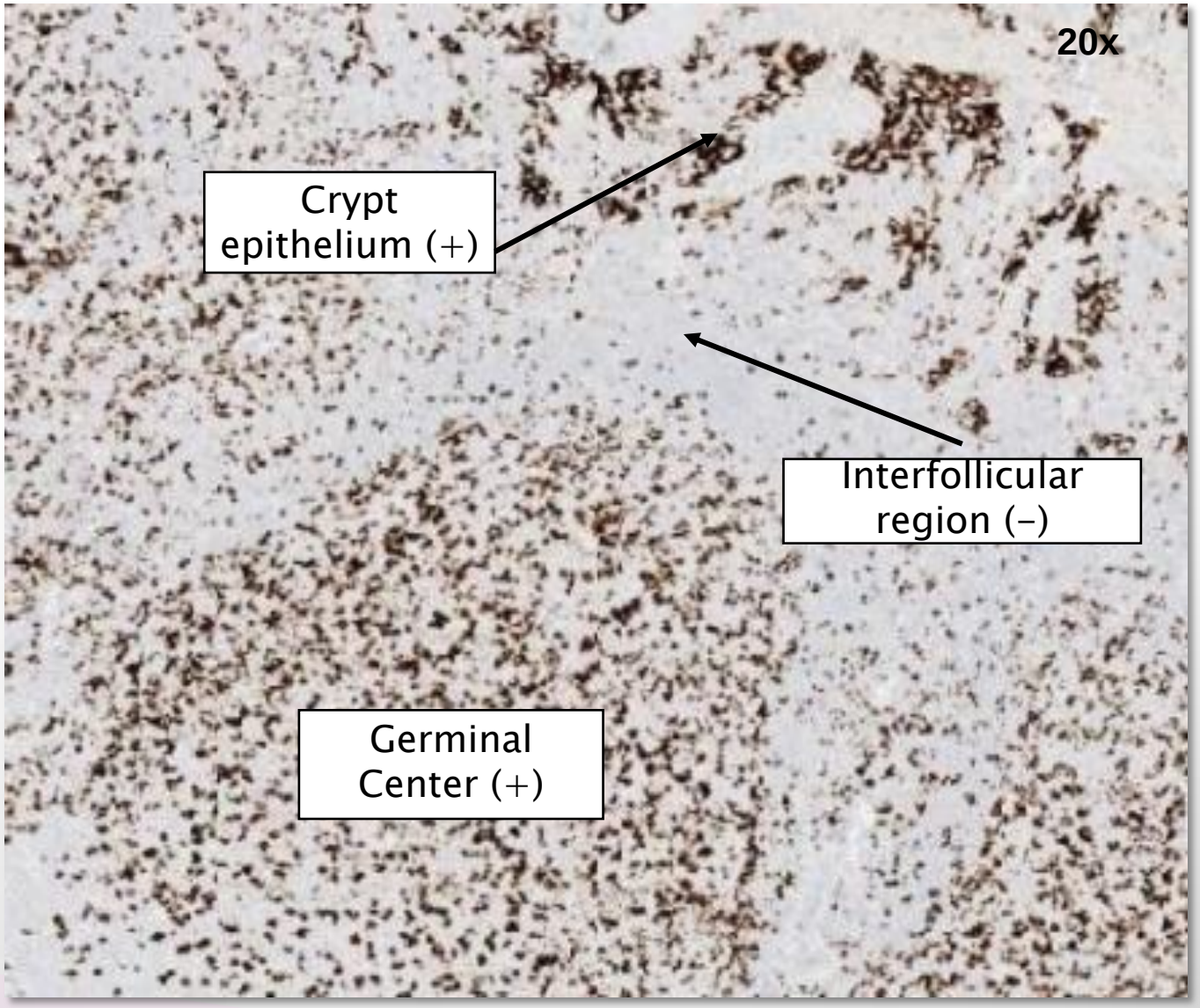
P staining of lymphocytes and macrophages in germinal centers

N staining in interfollicular regions

P staining in the reticulated crypt epithelial cells

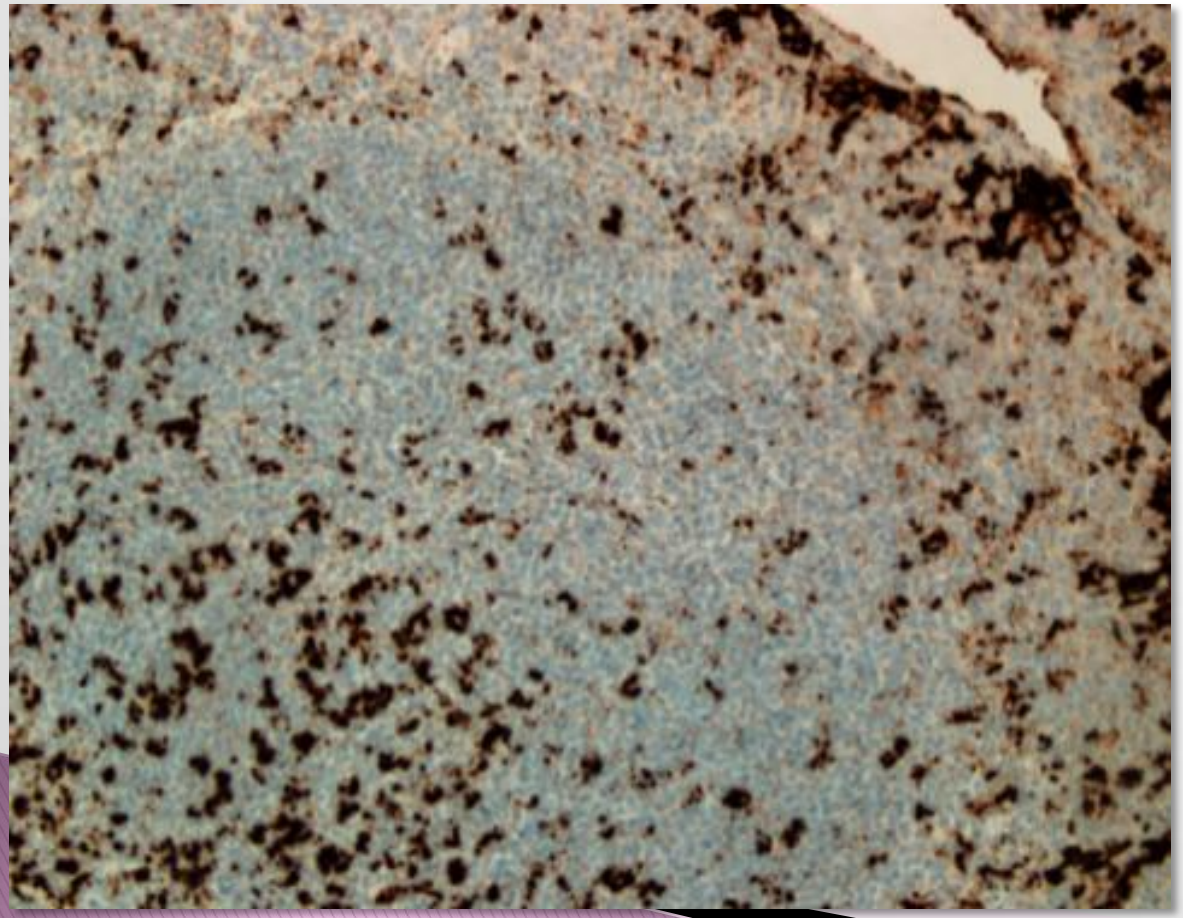
Controls: Tonsil Acceptable



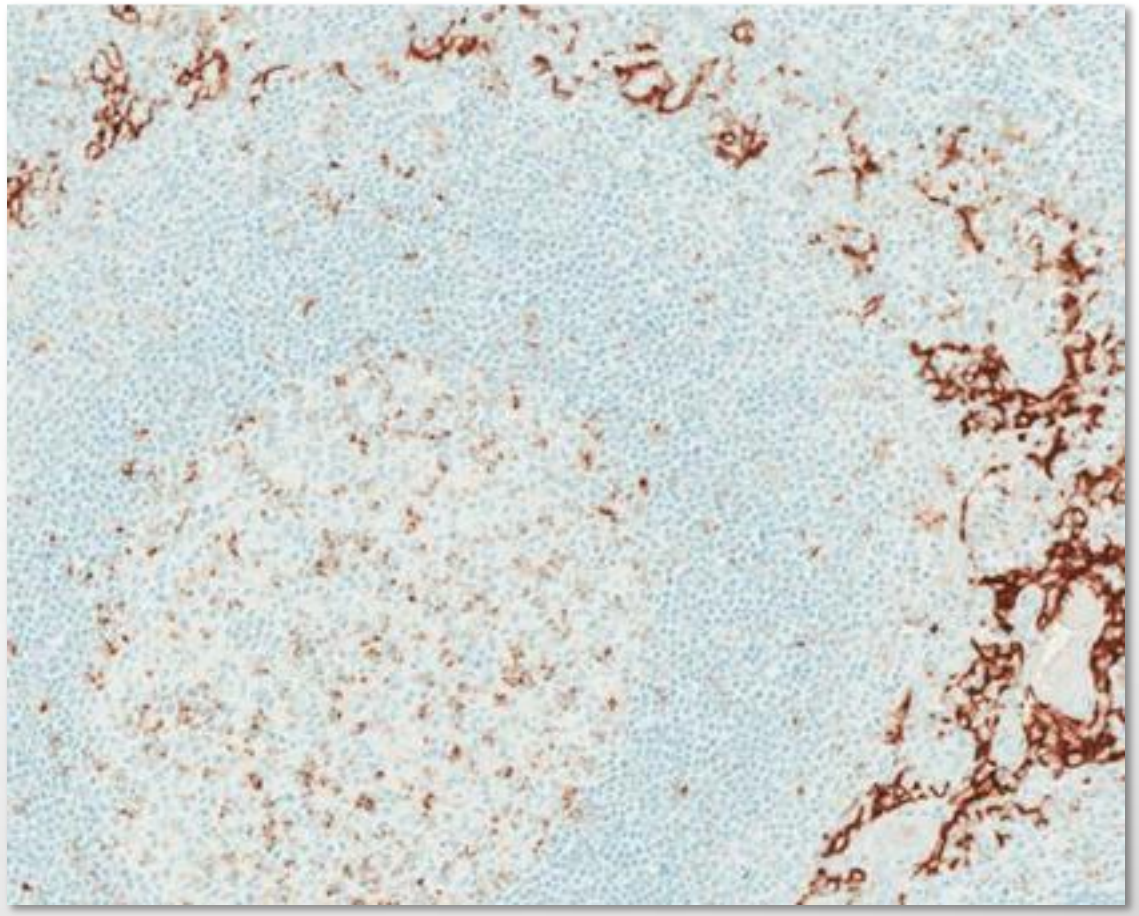


Unacceptable controls

Non-specific background staining



Weak staining in lymphocytes, macrophages and cryptic epithelial cells



Objectives

- Overview of PD-L1 SP142 assay for Triple Negative Breast Cancer (TNBC)
- Evaluation workflow
- **Scoring method TNBC**
- Scoring challenges and pitfalls

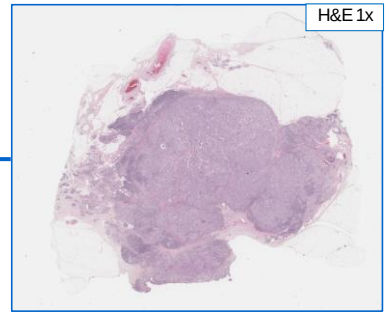
Tool 1: Visual Estimate

Tumor infiltrating IC staining characteristics

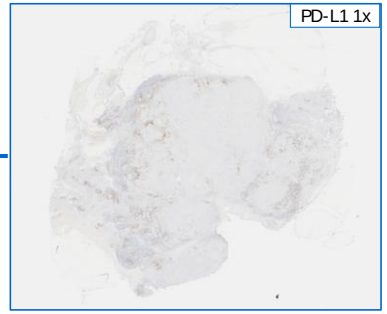
- **Type of cells**
  - Lymphocytes, plasma cells, macrophages, dendritic cells and granulocytes (not in necrotic areas !!)
- **Morphology**
  - Often show **dark brown** punctate or linear staining
  - Distributed as aggregates in stroma intra- and peritumoral
  - Single cell spread among tumor cells can also been seen
- **Distribution**
  - Homogenous or heterogeneous

Approach to the IHC Slide

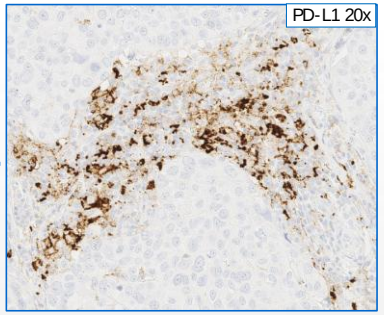
Review the H&E-stained slide for: presence of tumor, necrosis, infiltrating immune cells.



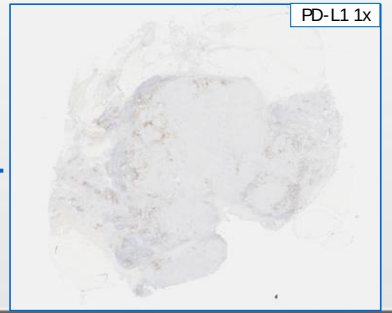
Review VENTANA PD-L1 (SP142) Assay stained slide at low power (2x or 4x): overall staining pattern (IC or TC or both?).



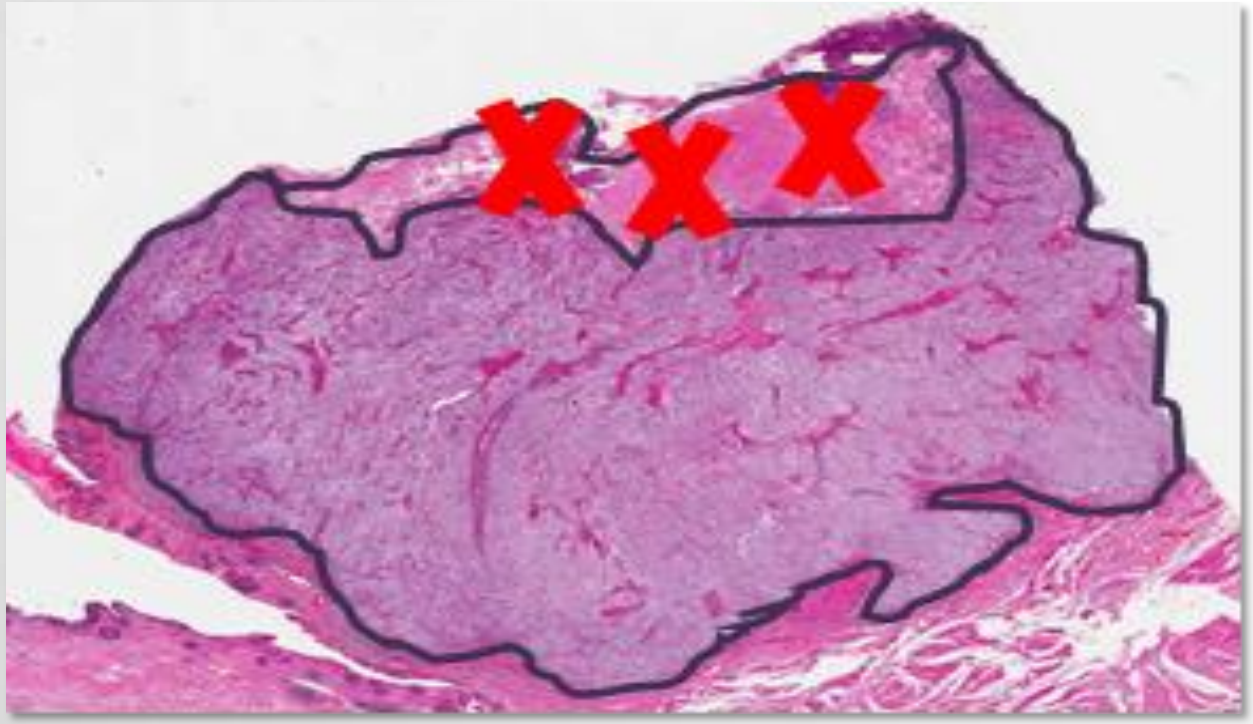
10x or 20x: Examine stroma and tumor cell groups for IC staining. Distinguish IC in the midst of TC staining (if present).



Return to 2x or 4x to visually estimate the VENTANA PD-L1 (SP142) Assay IC %. In this case IC percentage upon visual estimation is ≥ 1%.



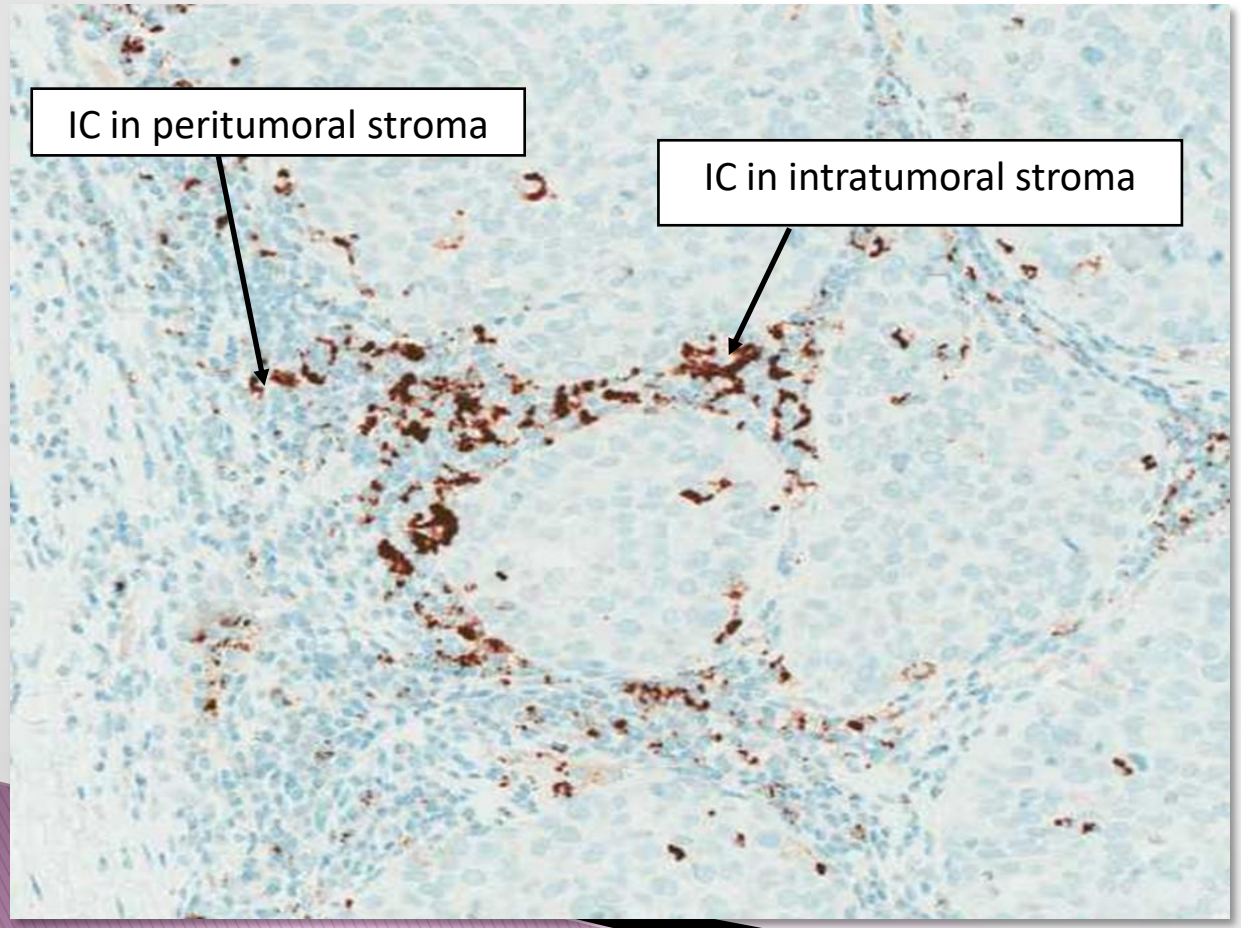
Exclude necrosis from tumor area



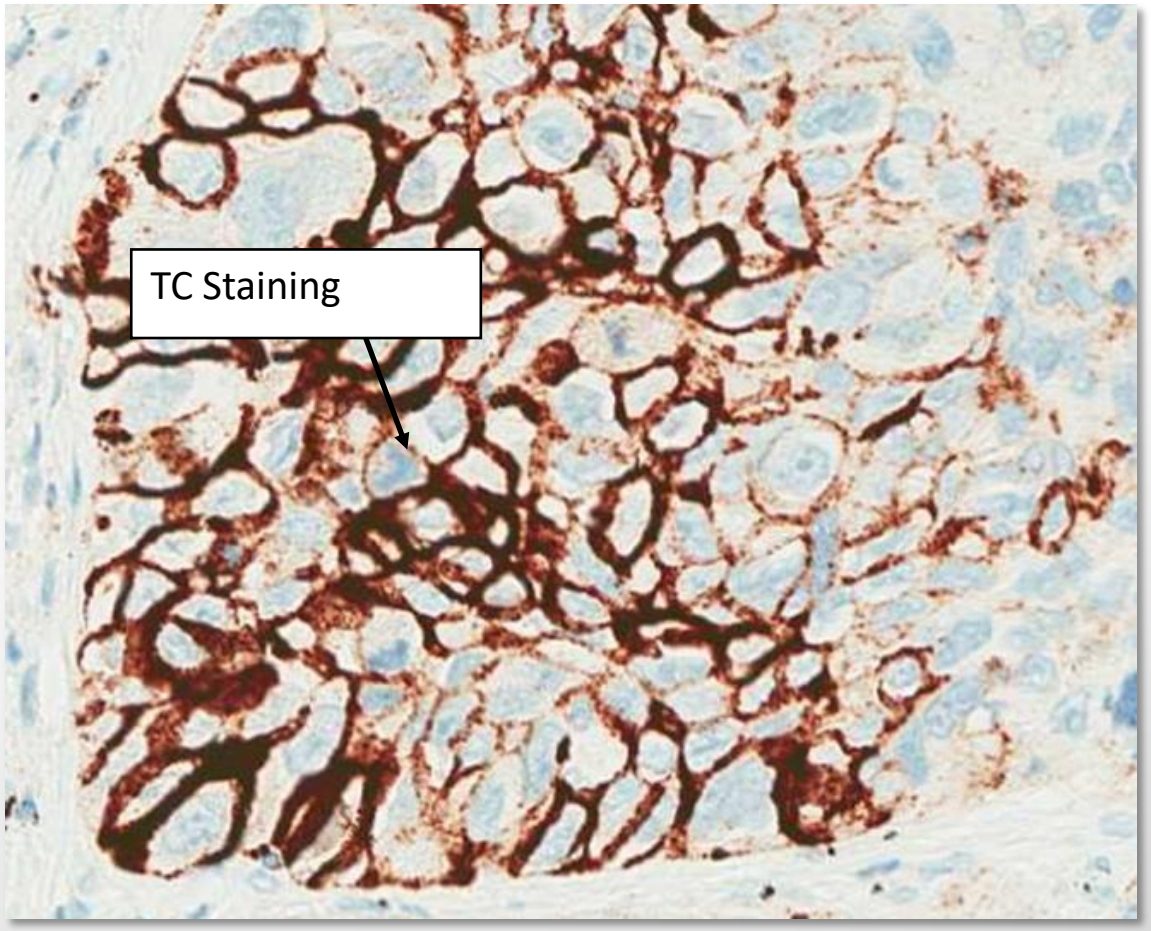
Only IC staining is scored

VENTANA PD-L1 (SP142) Assay: staining in tumor infiltrating immune cells (IC) and tumor cells (TC)

10x



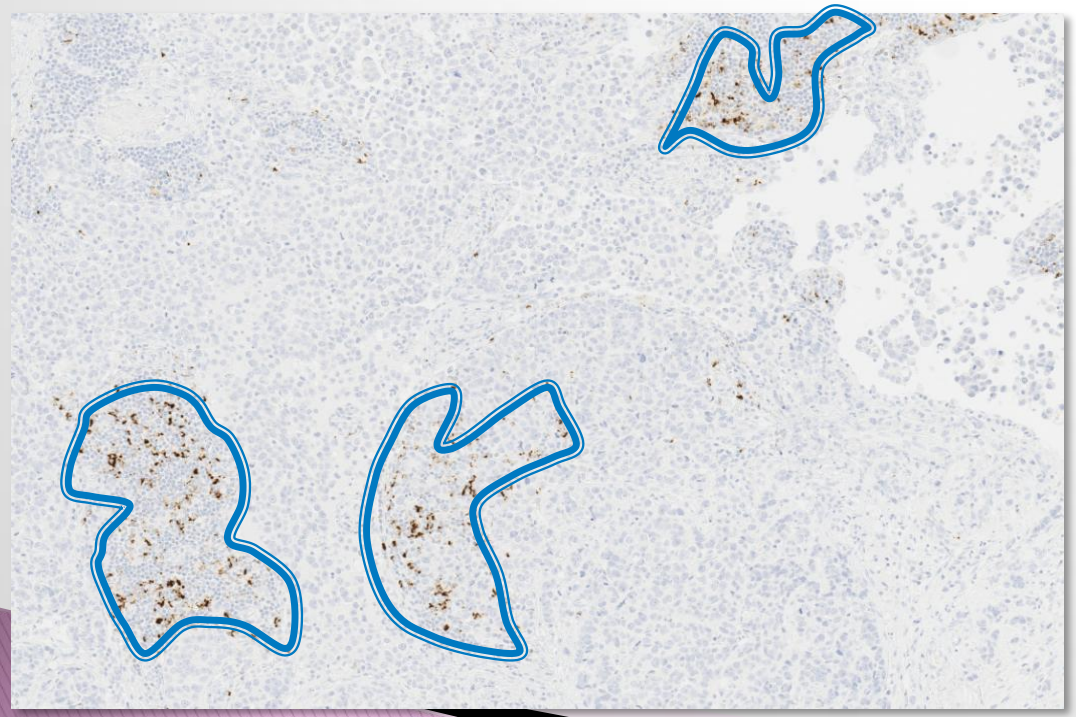
20x

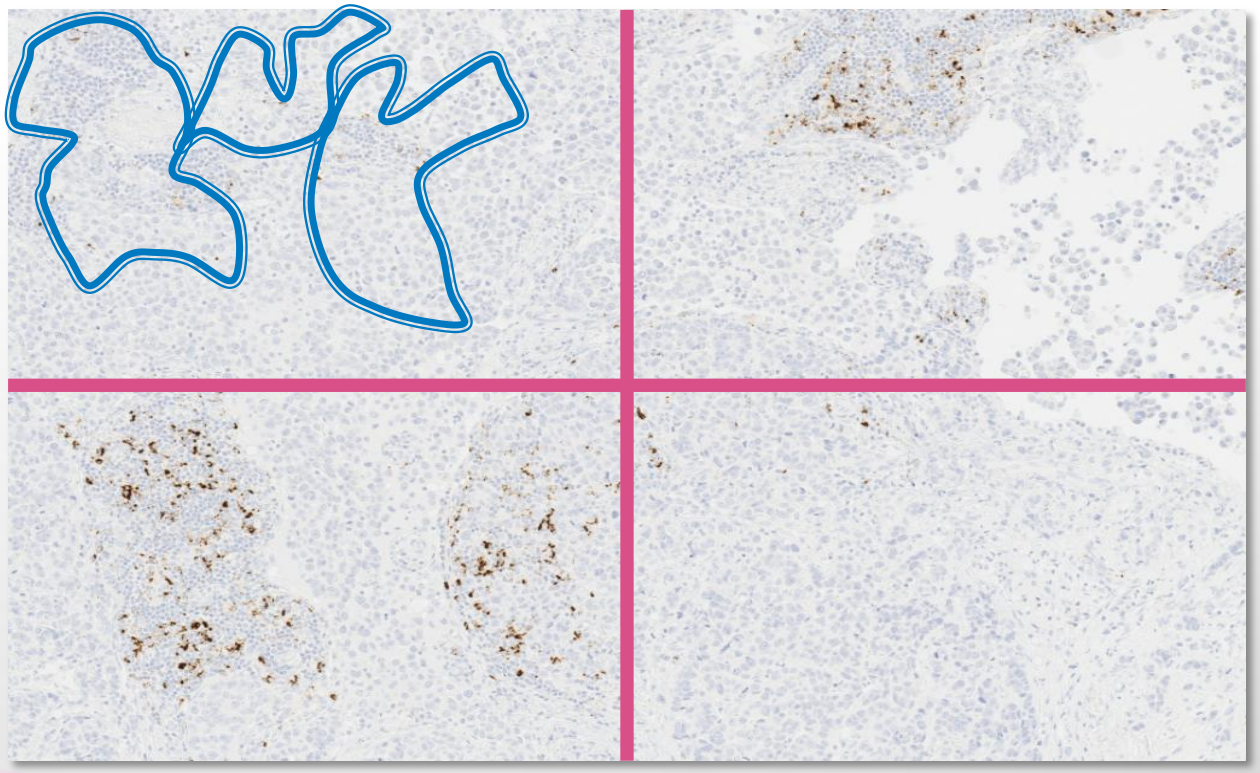


Scoring method – IC aggregates

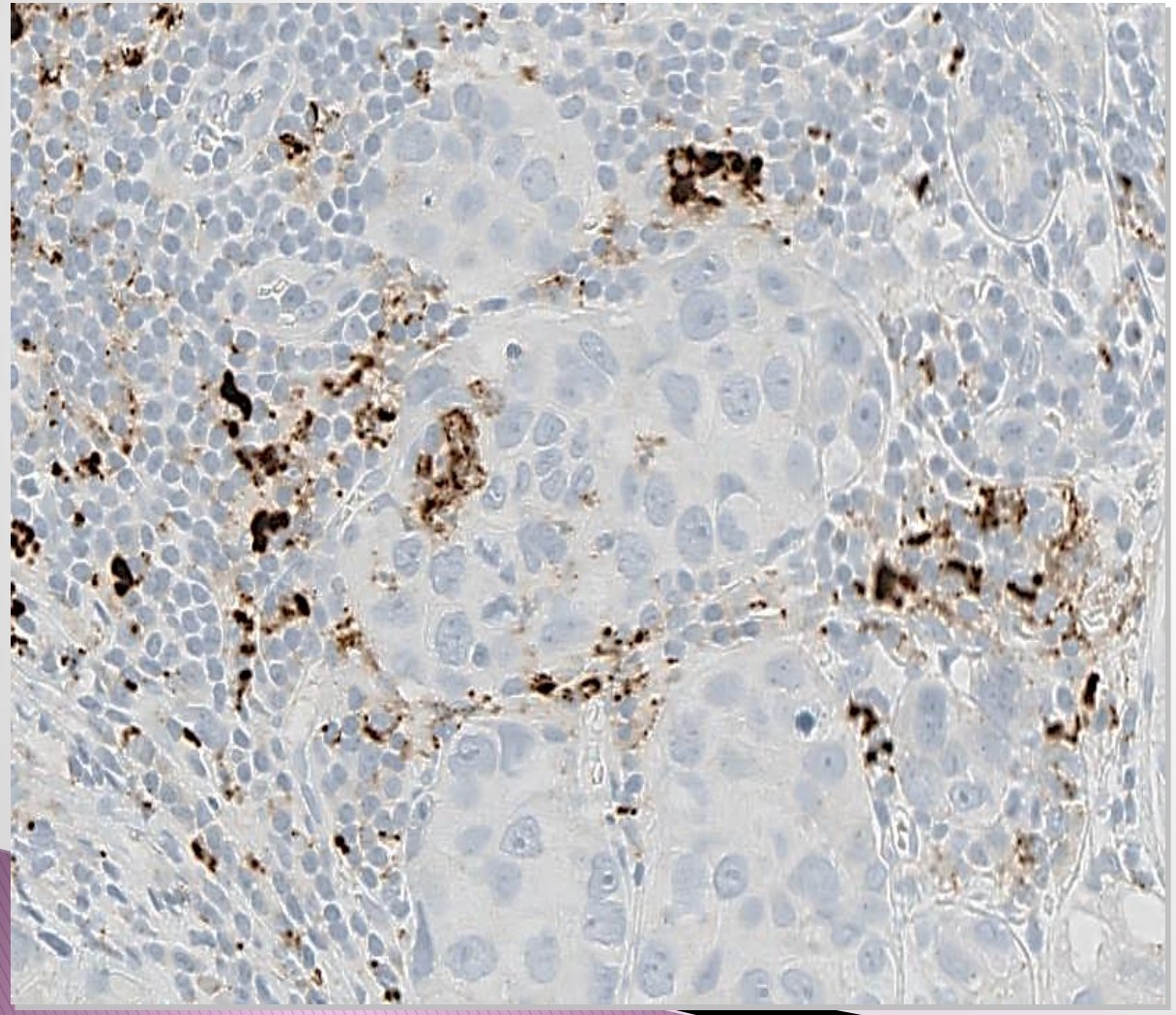
Visually encircle the IC aggregates as closely as possible

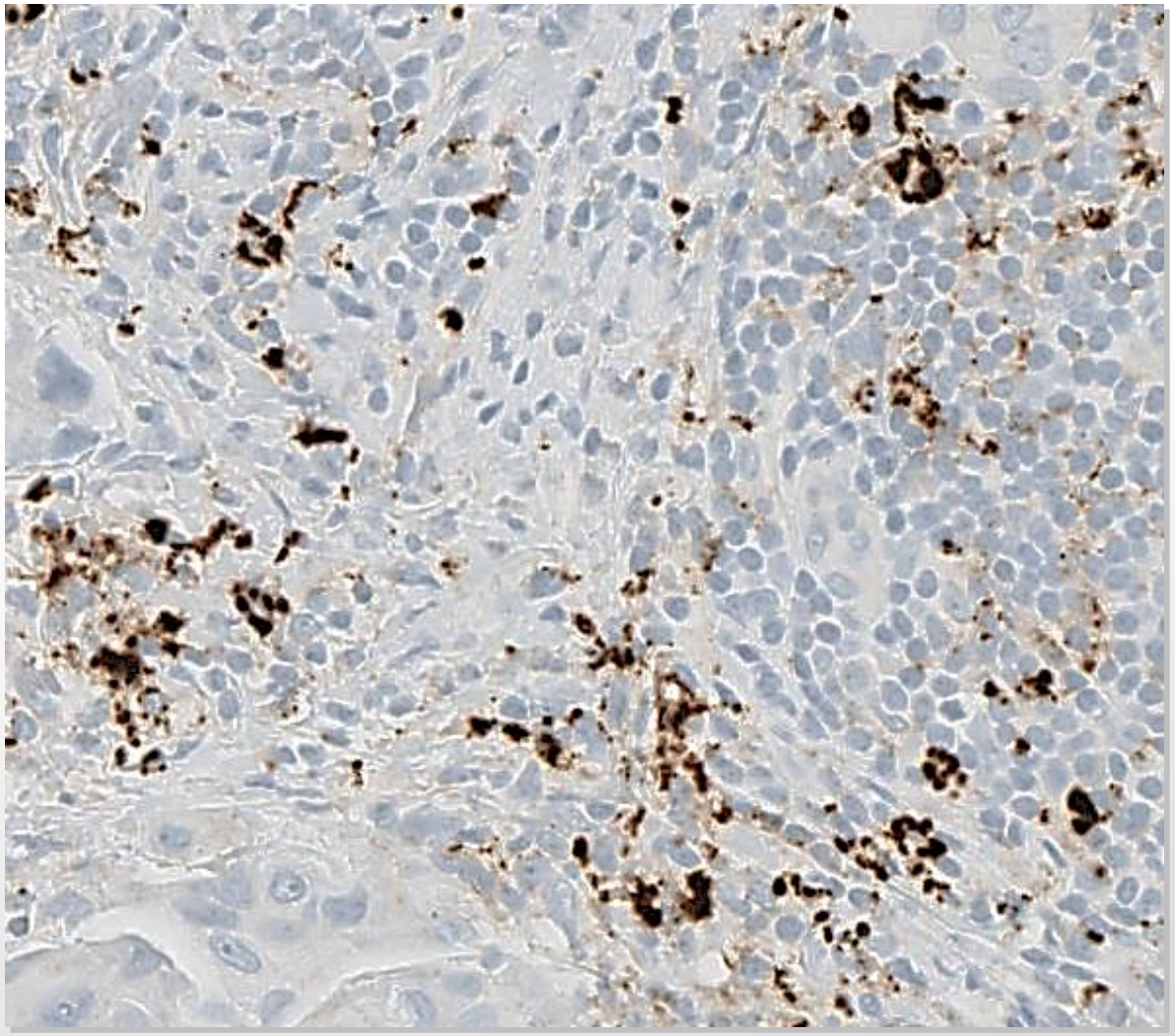
Combine these regions and estimate their combined area in the total tumor area. IC % is ≥1%





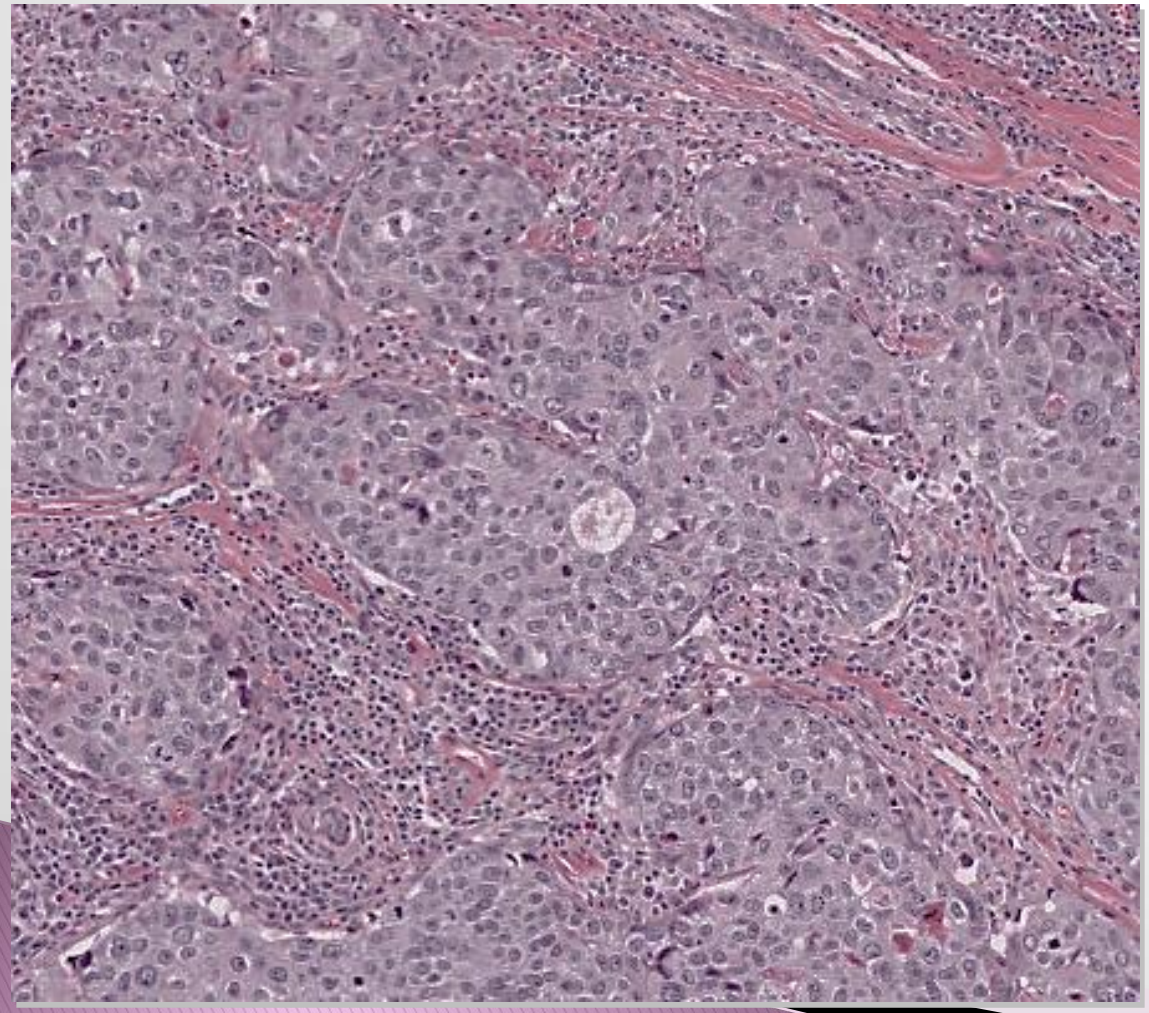
IC Staining Characteristics

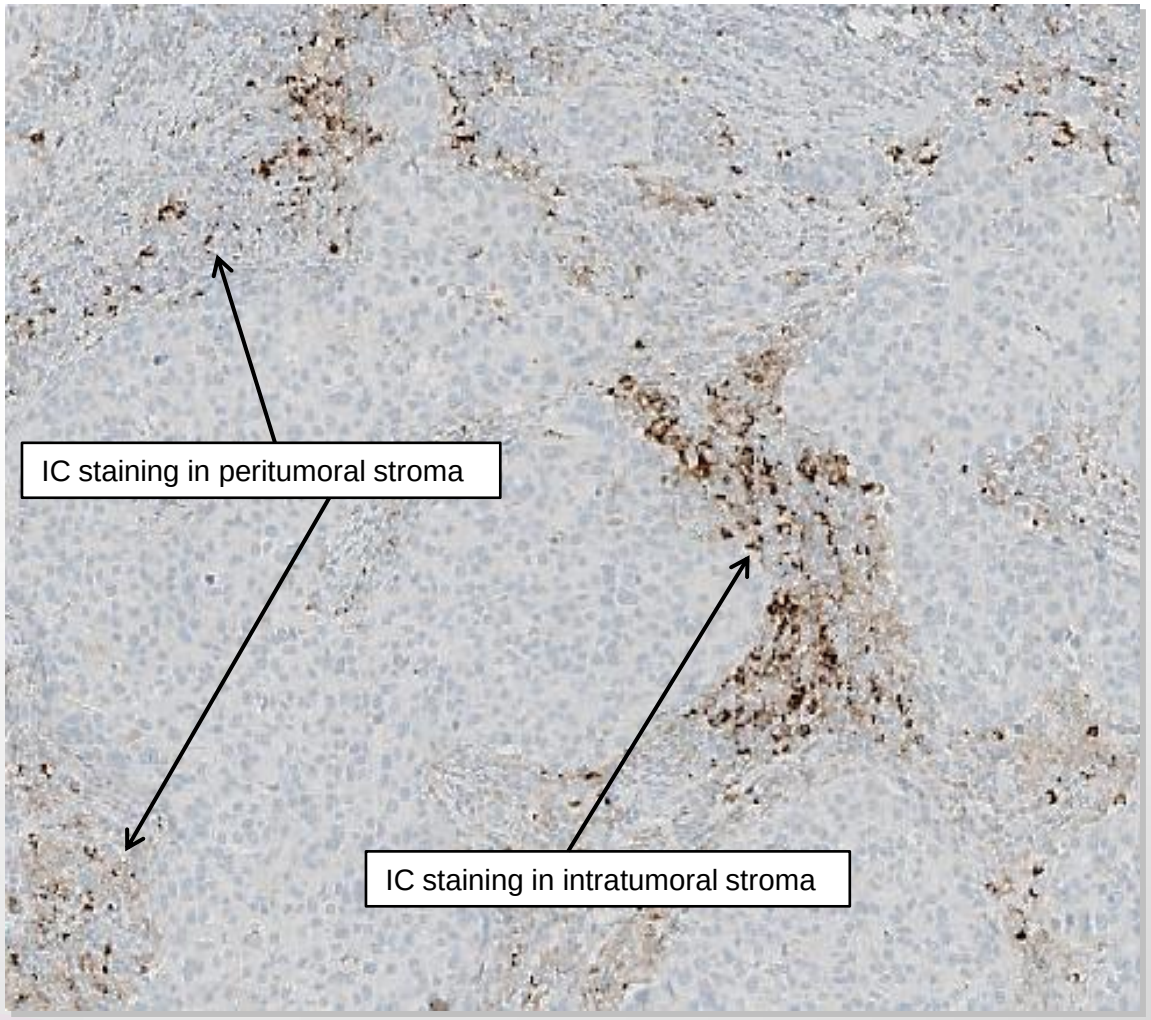




IC staining pattern – Aggregates

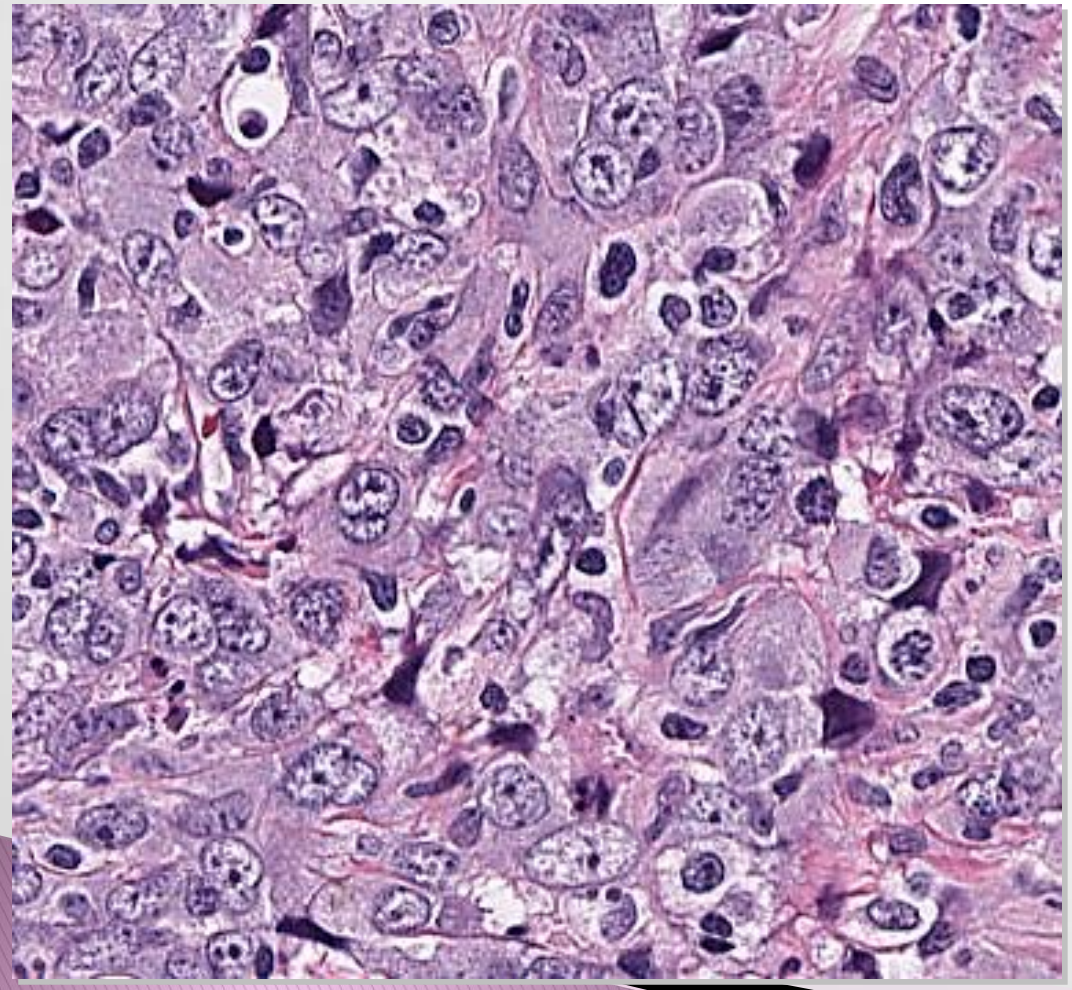
- Often observed as lymphoid aggregates in the intra- or peri-tumoral stroma

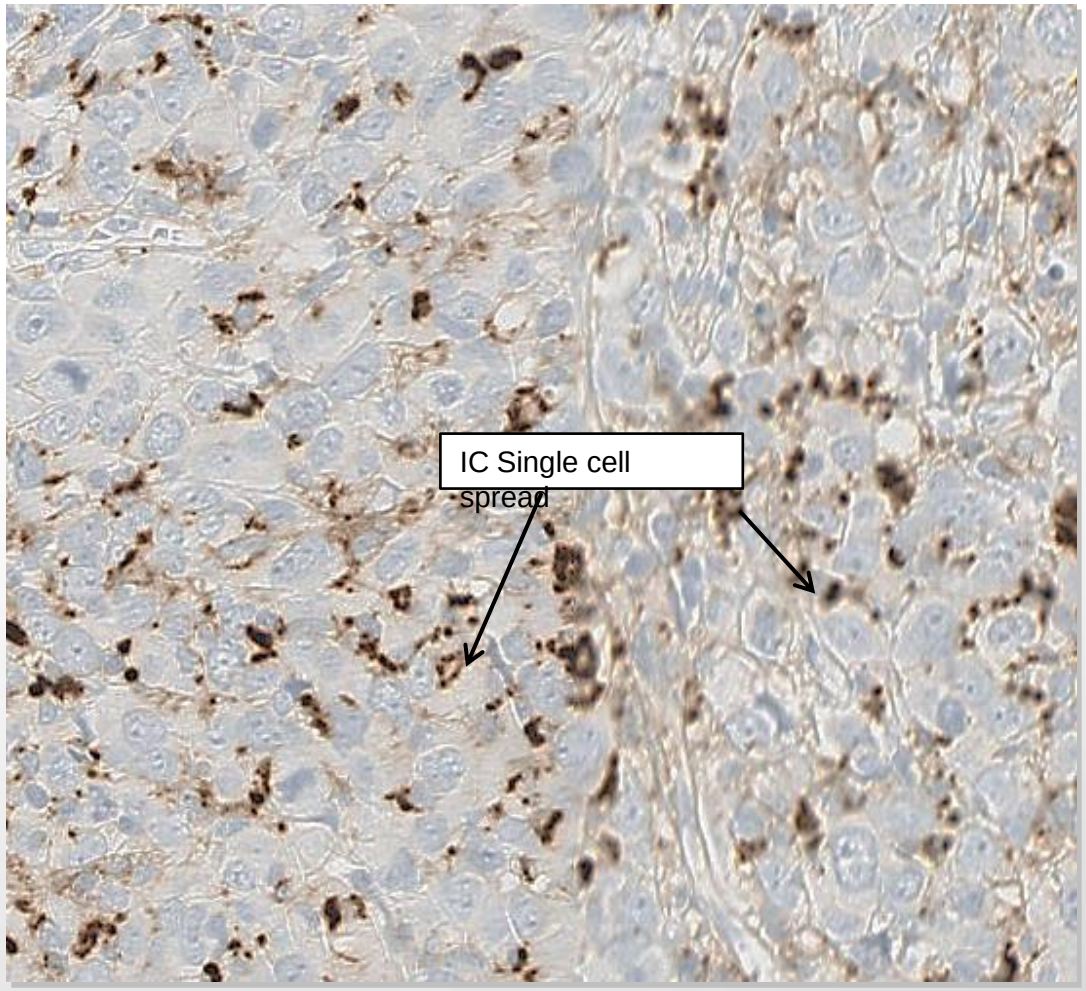




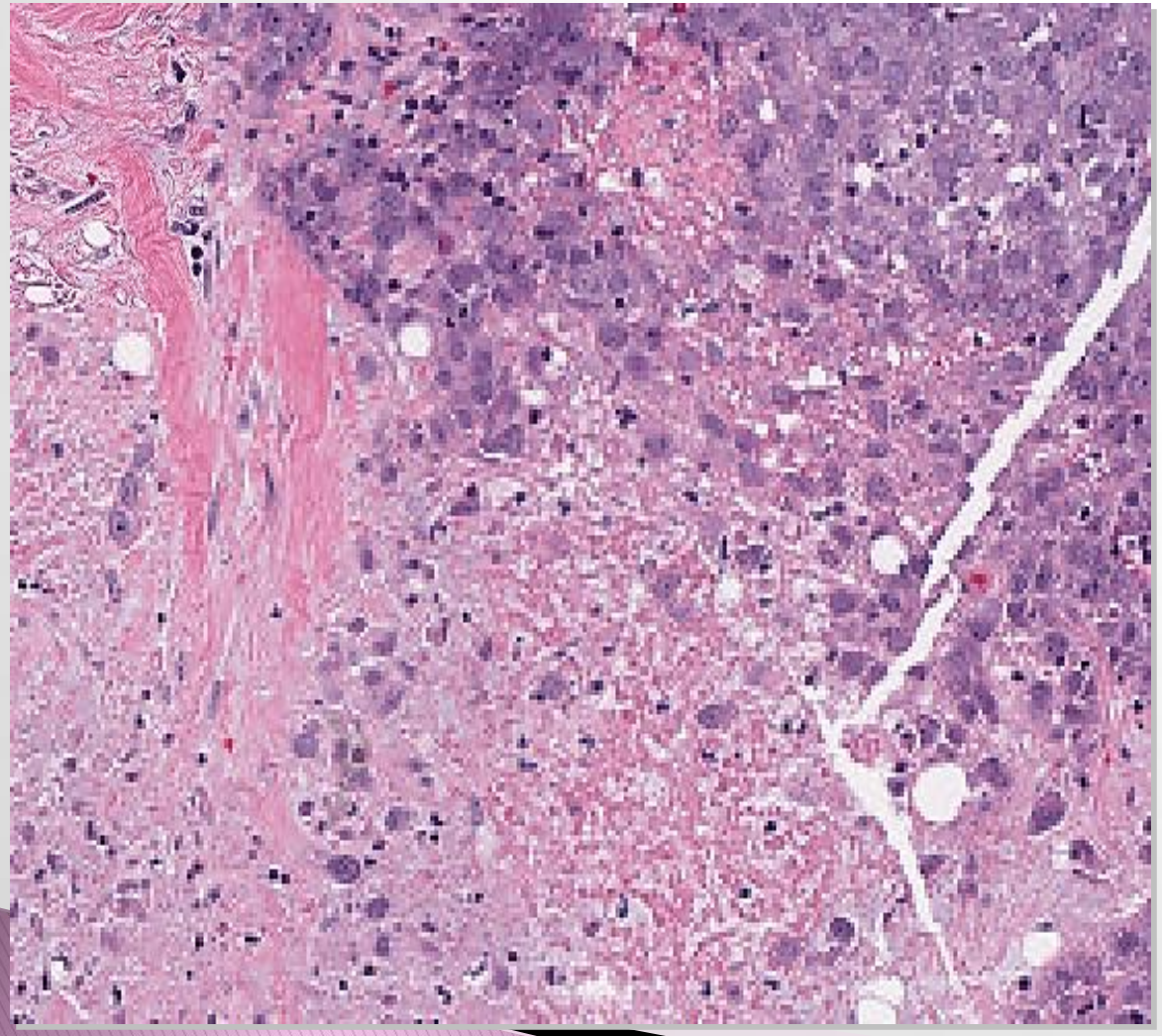
IC staining pattern – Single cell spread

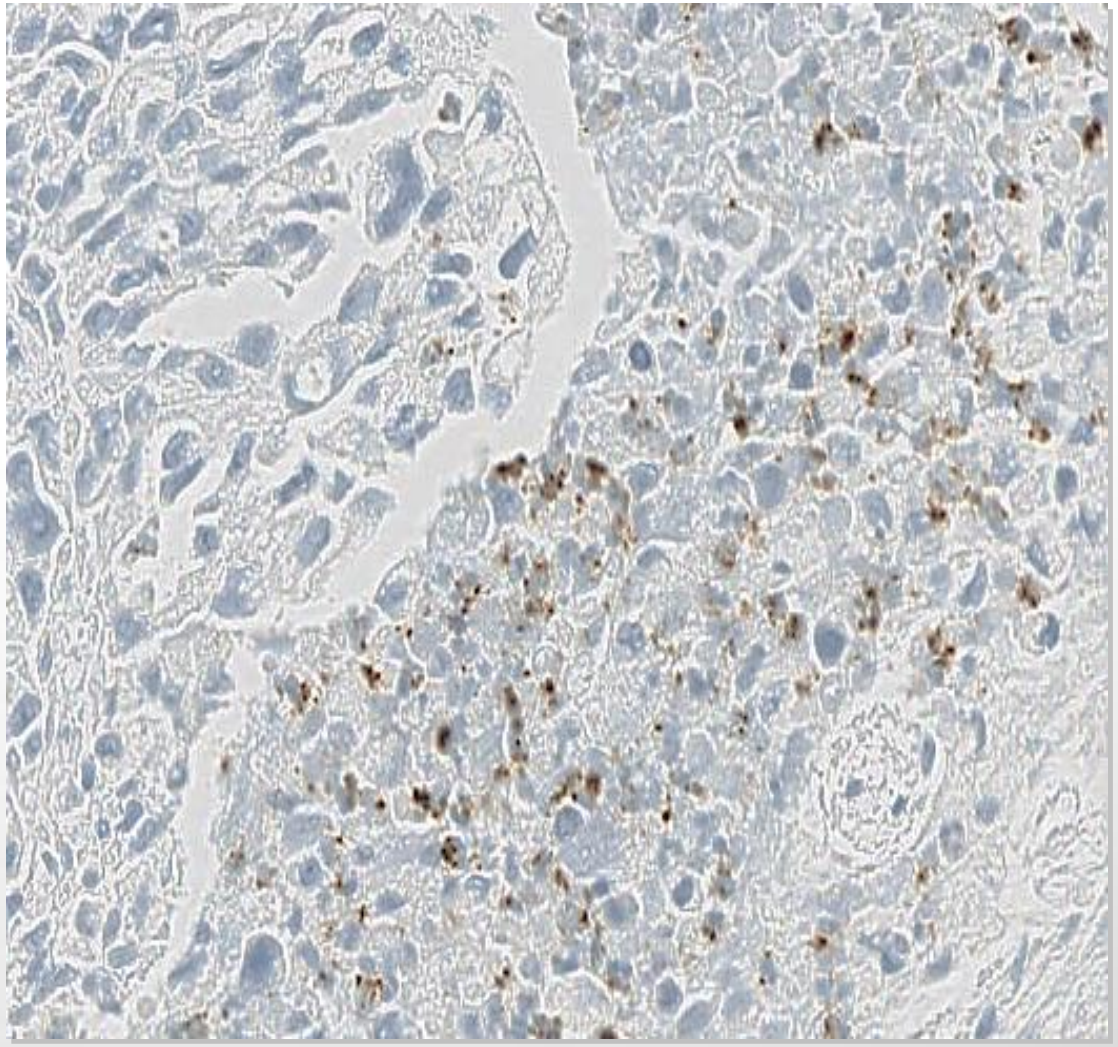
- Occasionally as single cell spread (diffuse) among tumor cells with/without aggregates



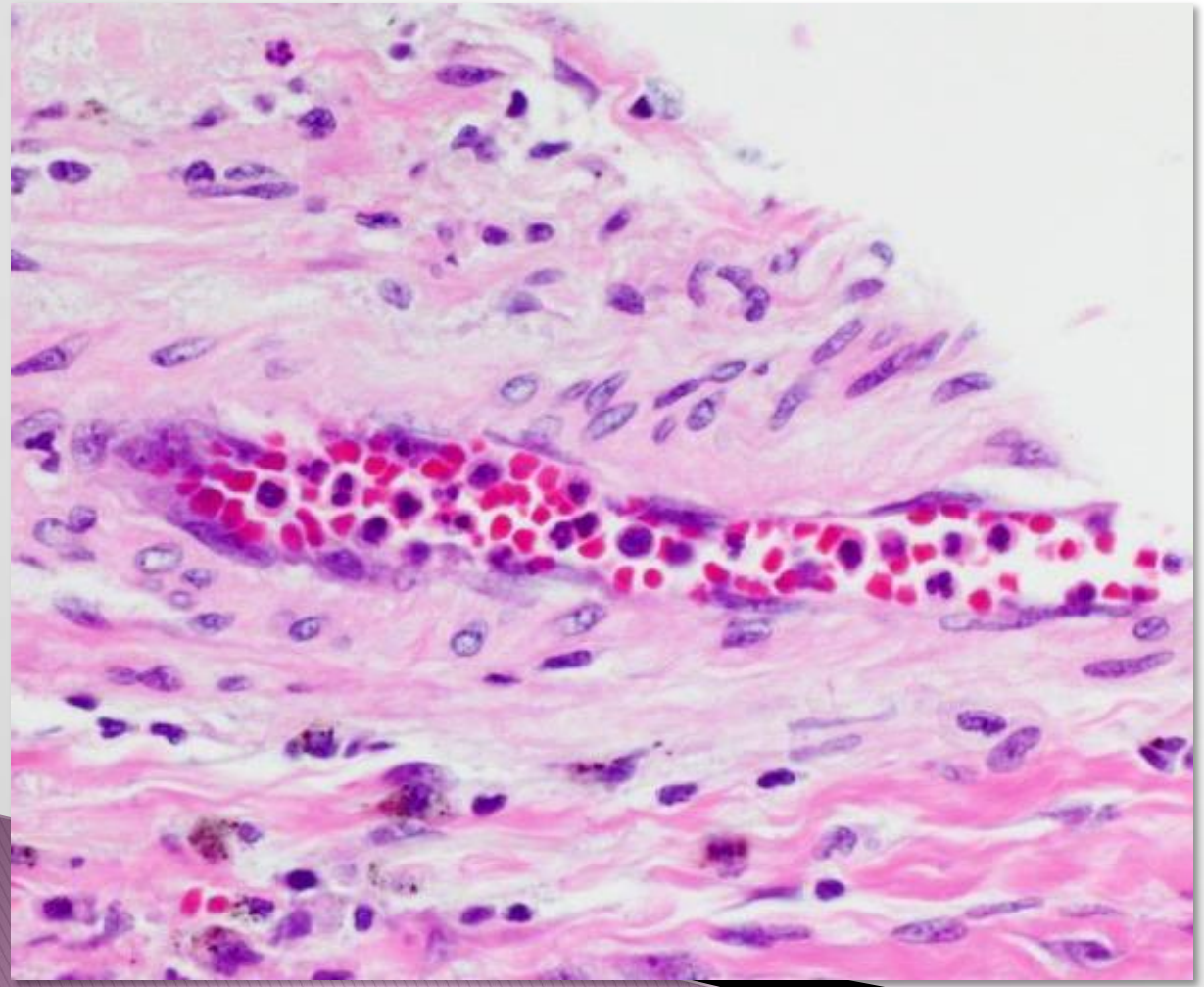


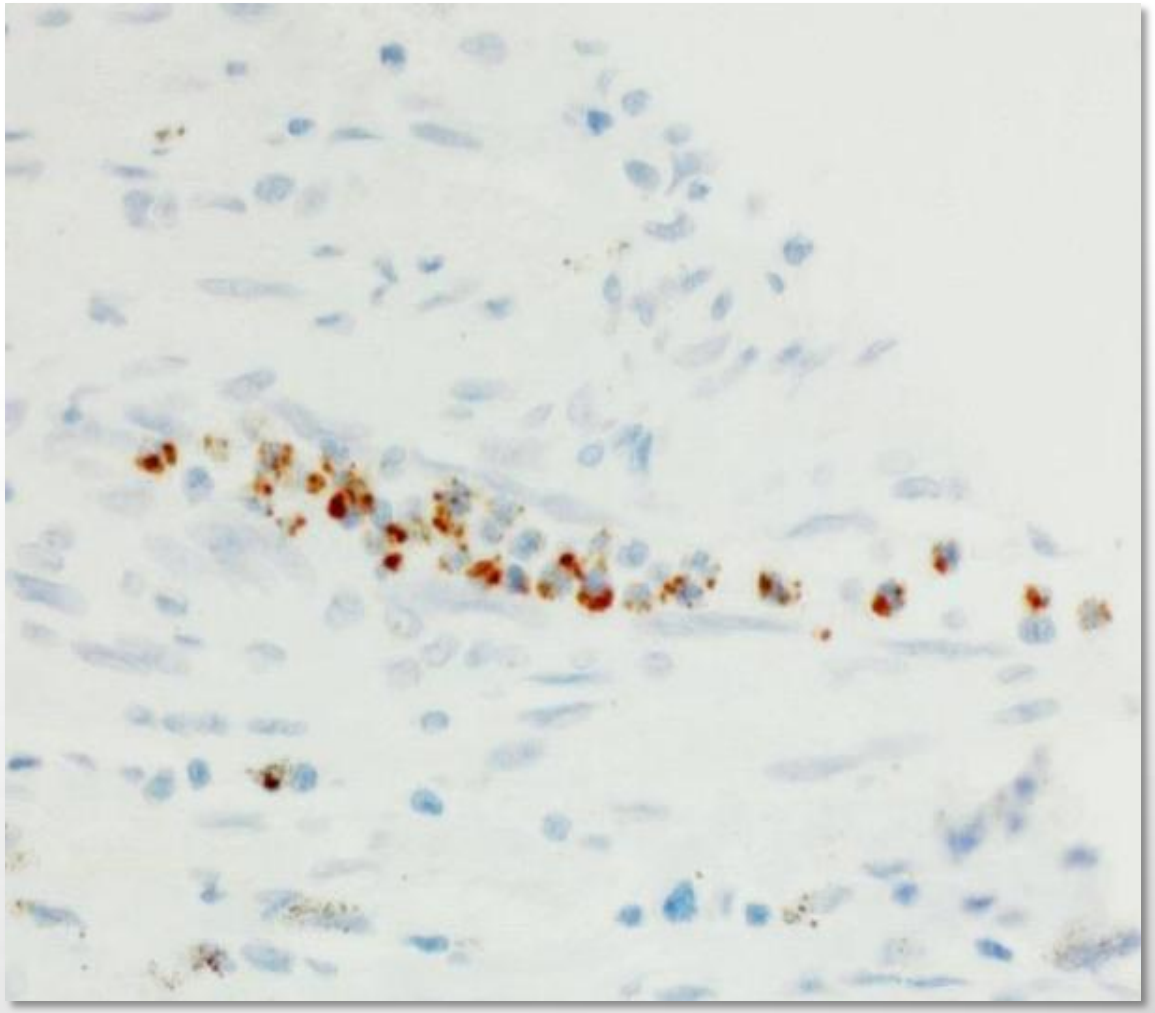
Neutrophils Staining Characteristics



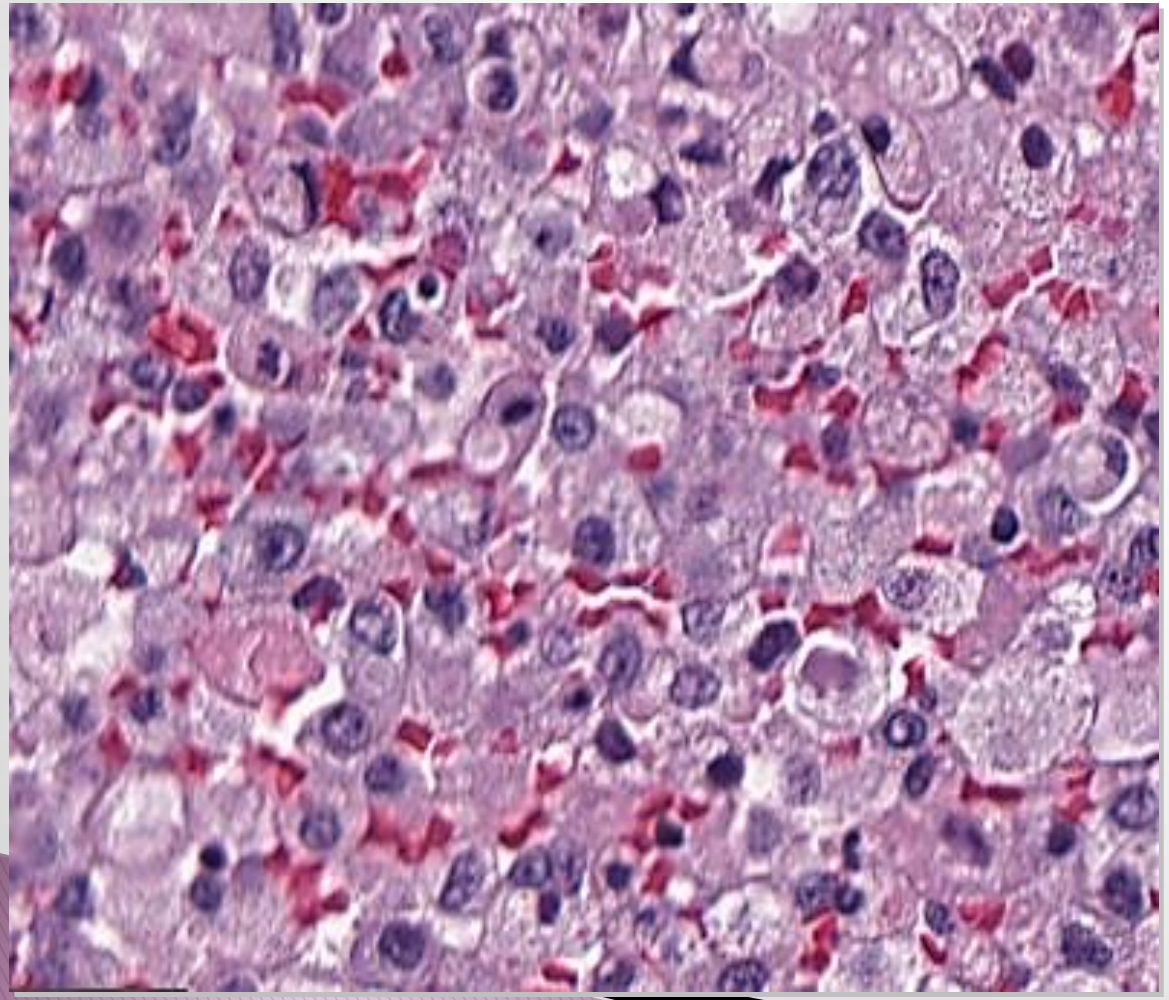


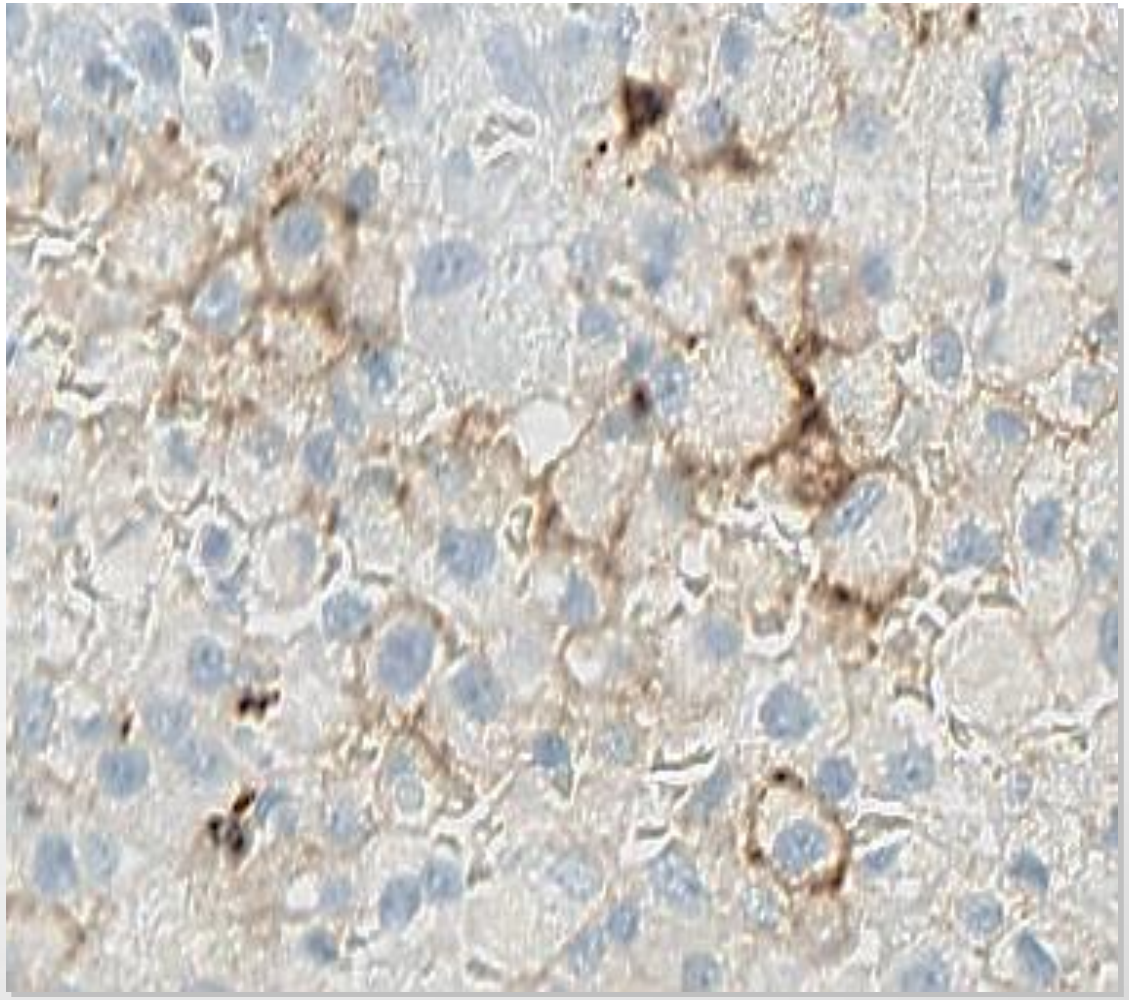
Intravascular IC (e.g. Neutrophils): Do Not Include !!





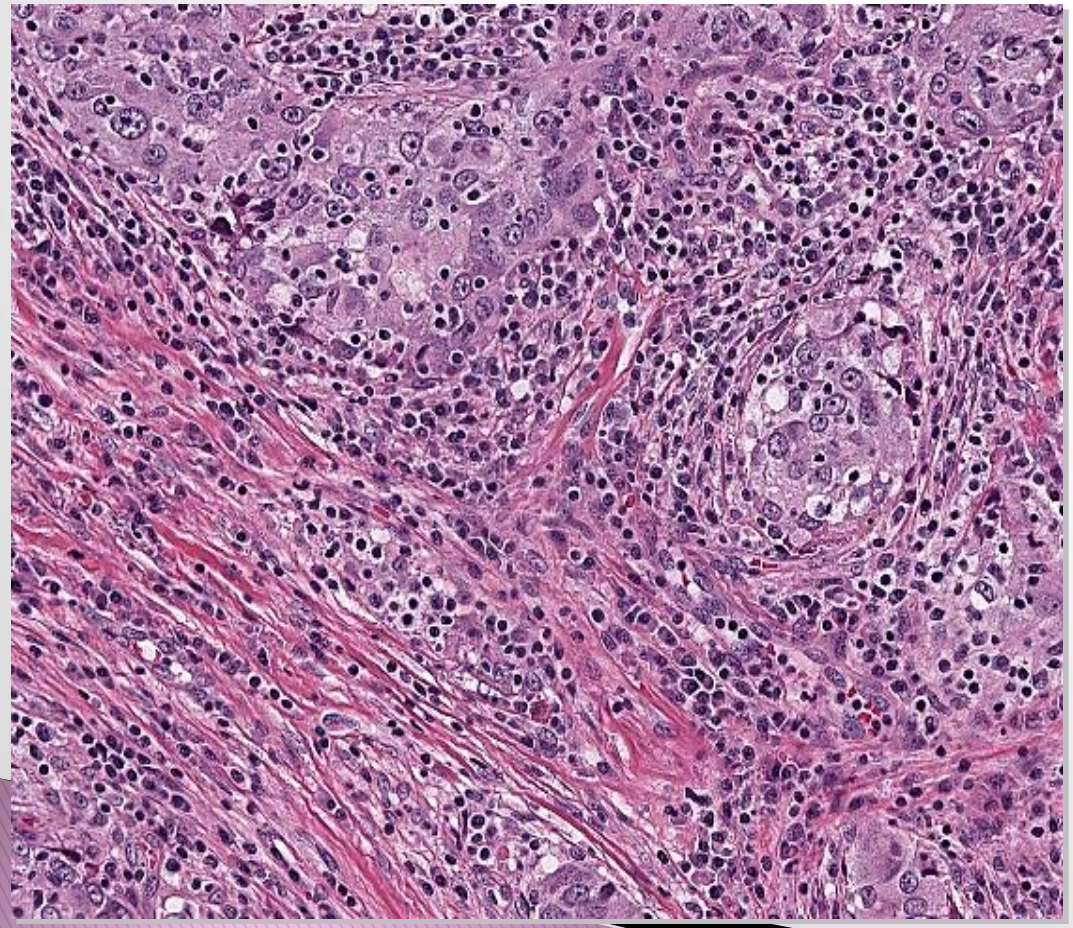
Macrophages Staining Characteristics

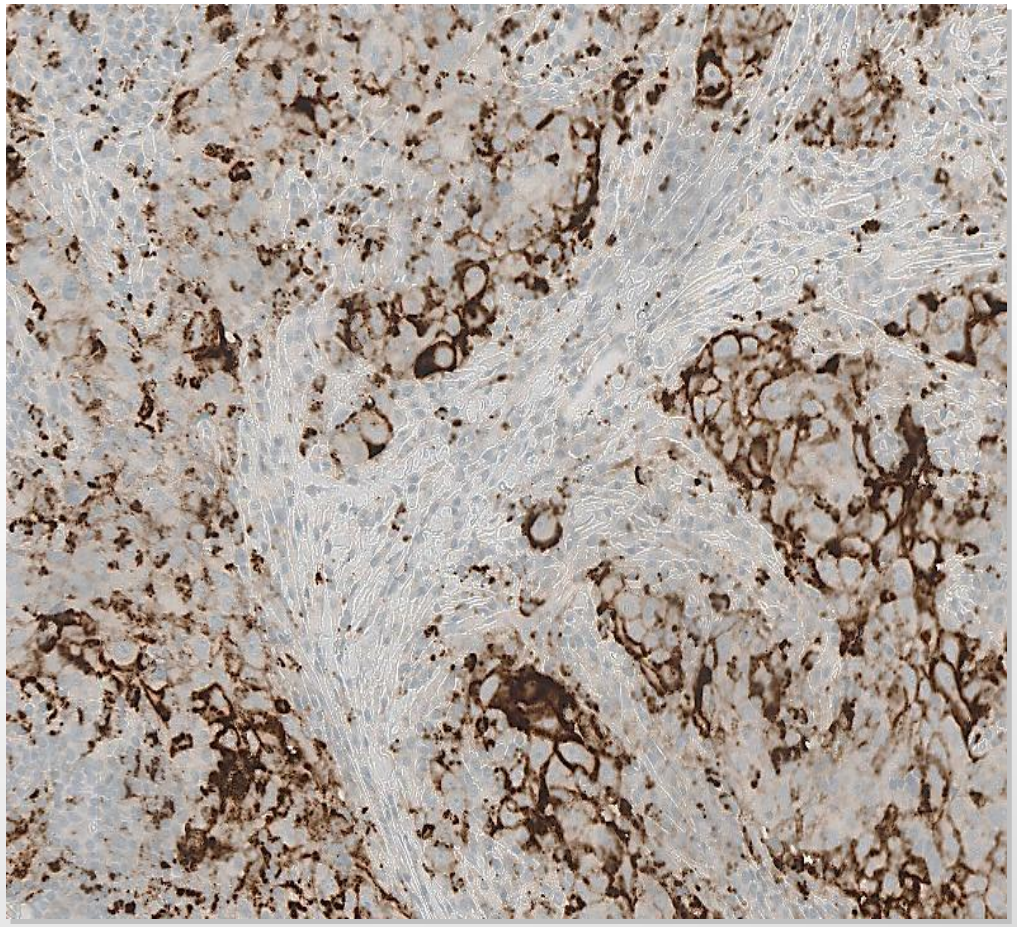




Differentiation of IC from TC staining

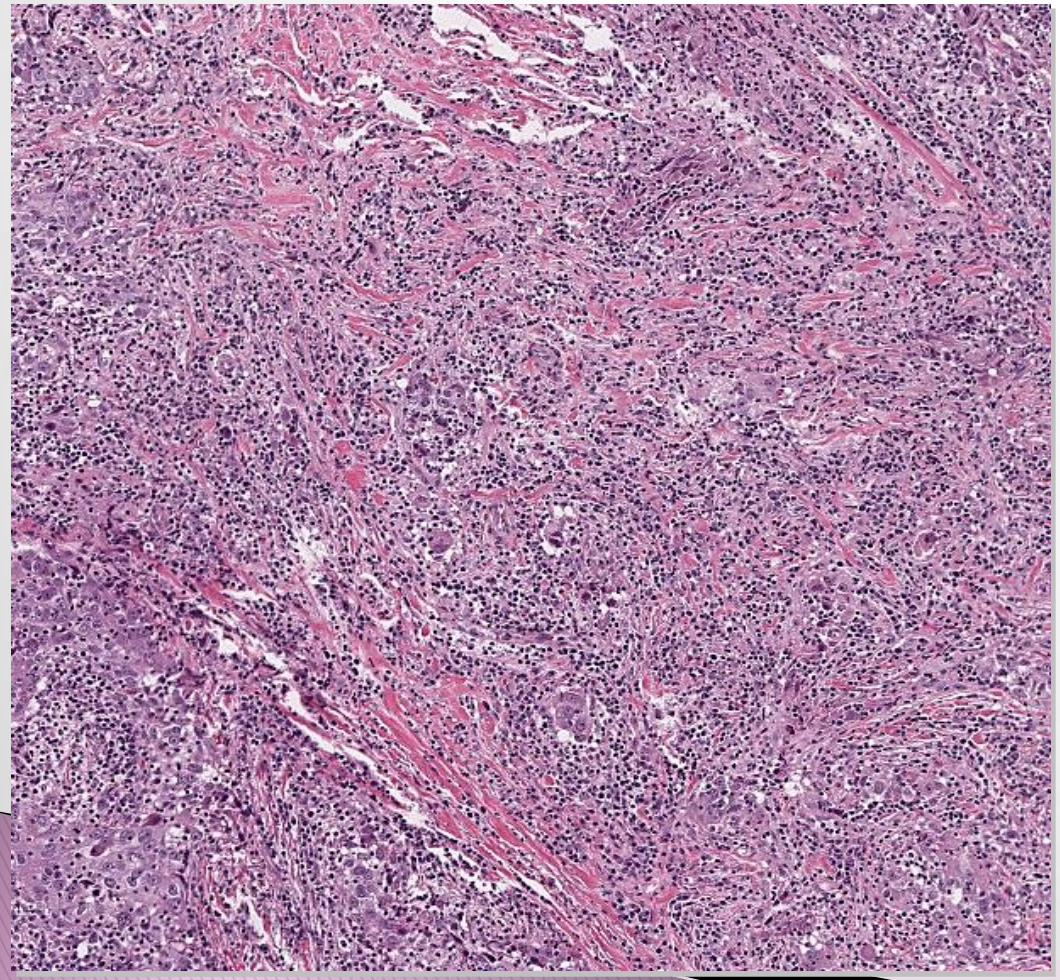
- Review of the corresponding H&E
- High magnification review of PD-L1 slide to define staining characteristics
- Staining pattern and distribution

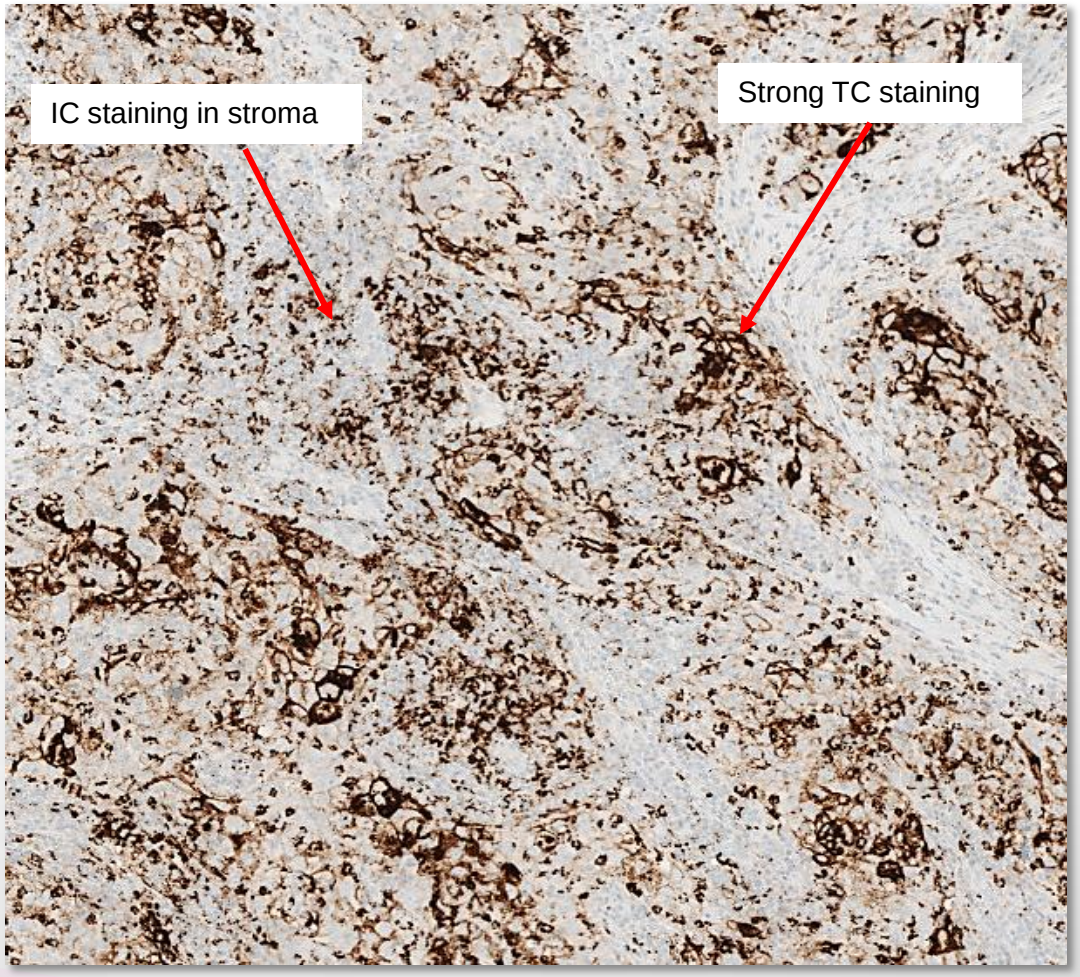




TC and IC

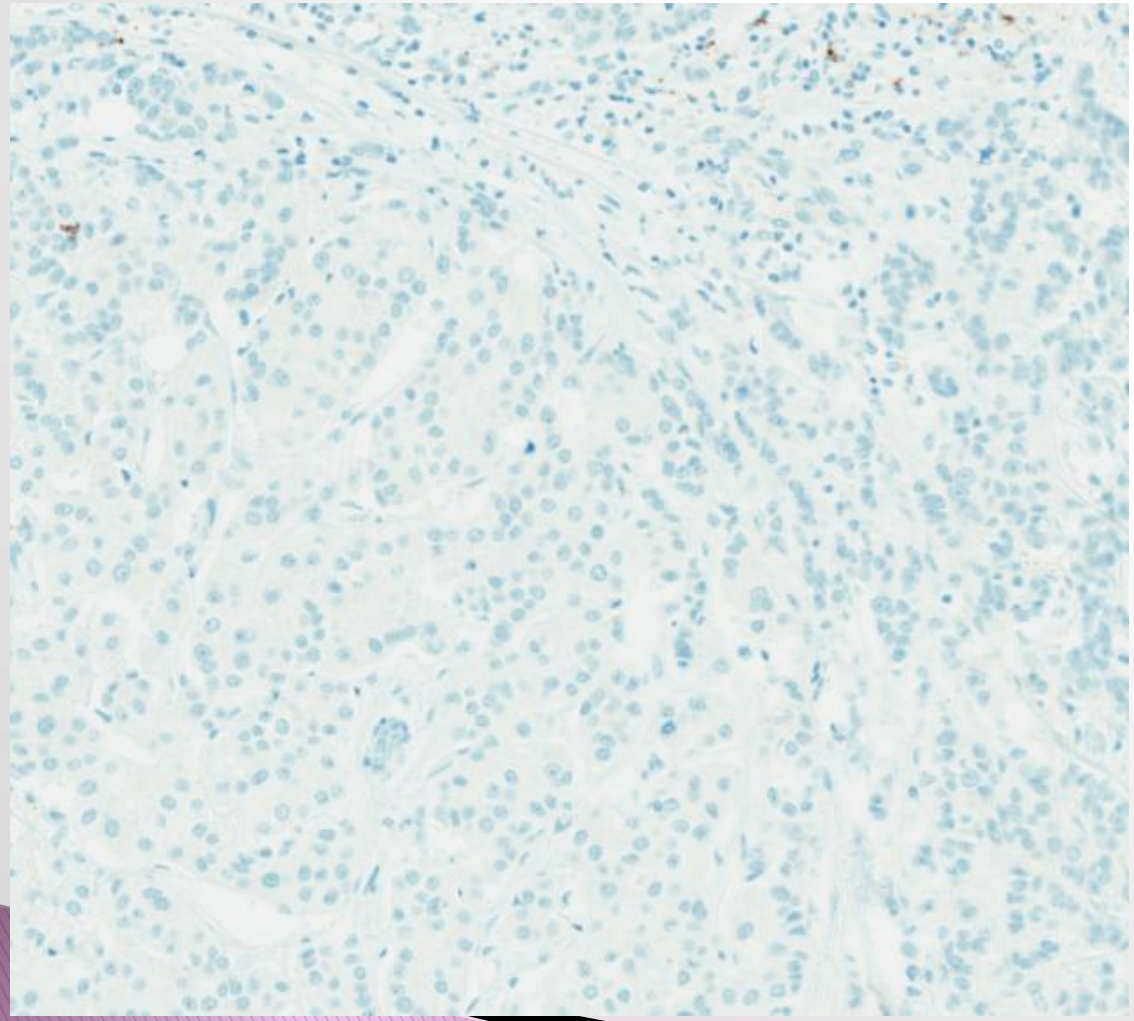
- H&E shows immune cells infiltrating between tumor cells and in stroma
- TC staining can be strong or weak, linear or granular

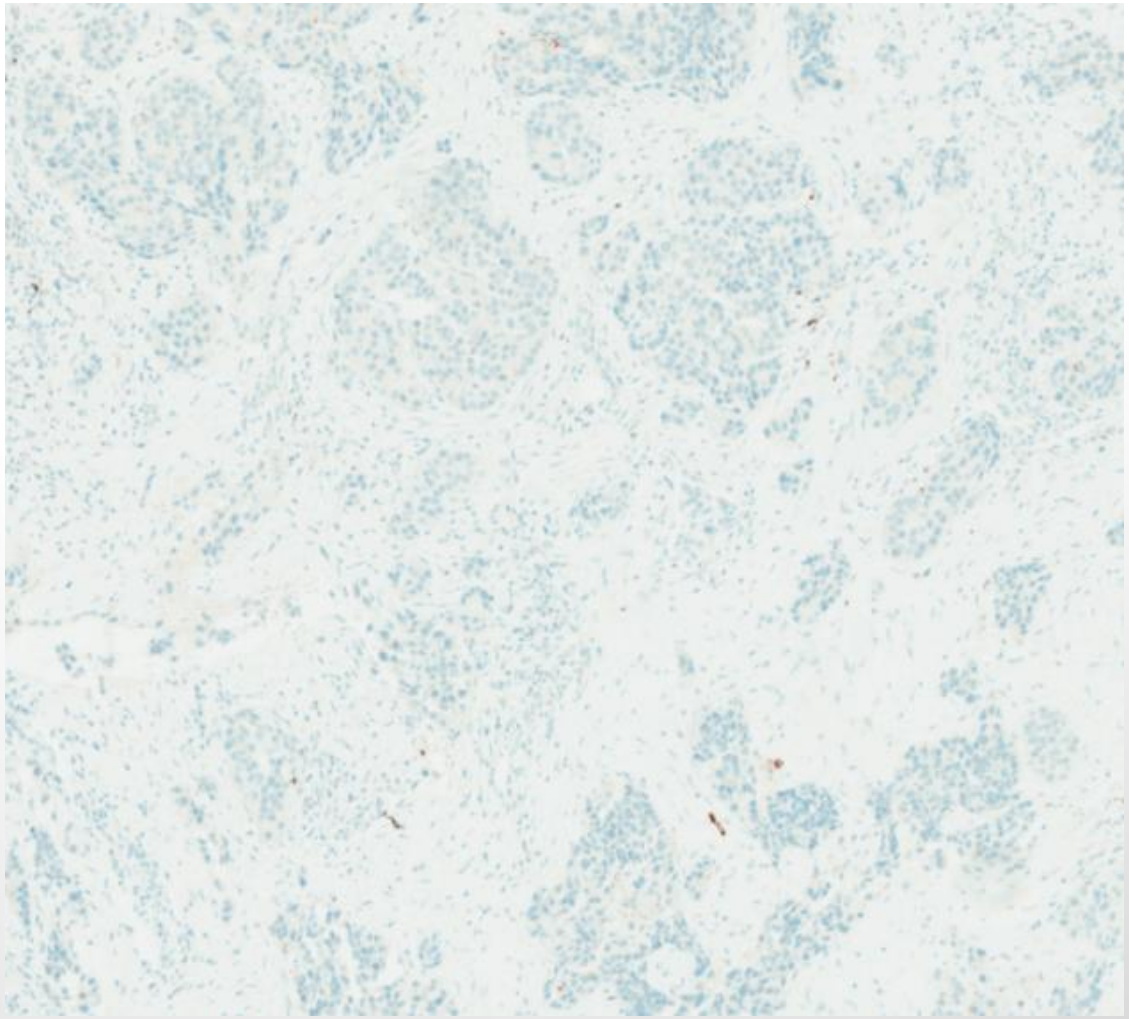




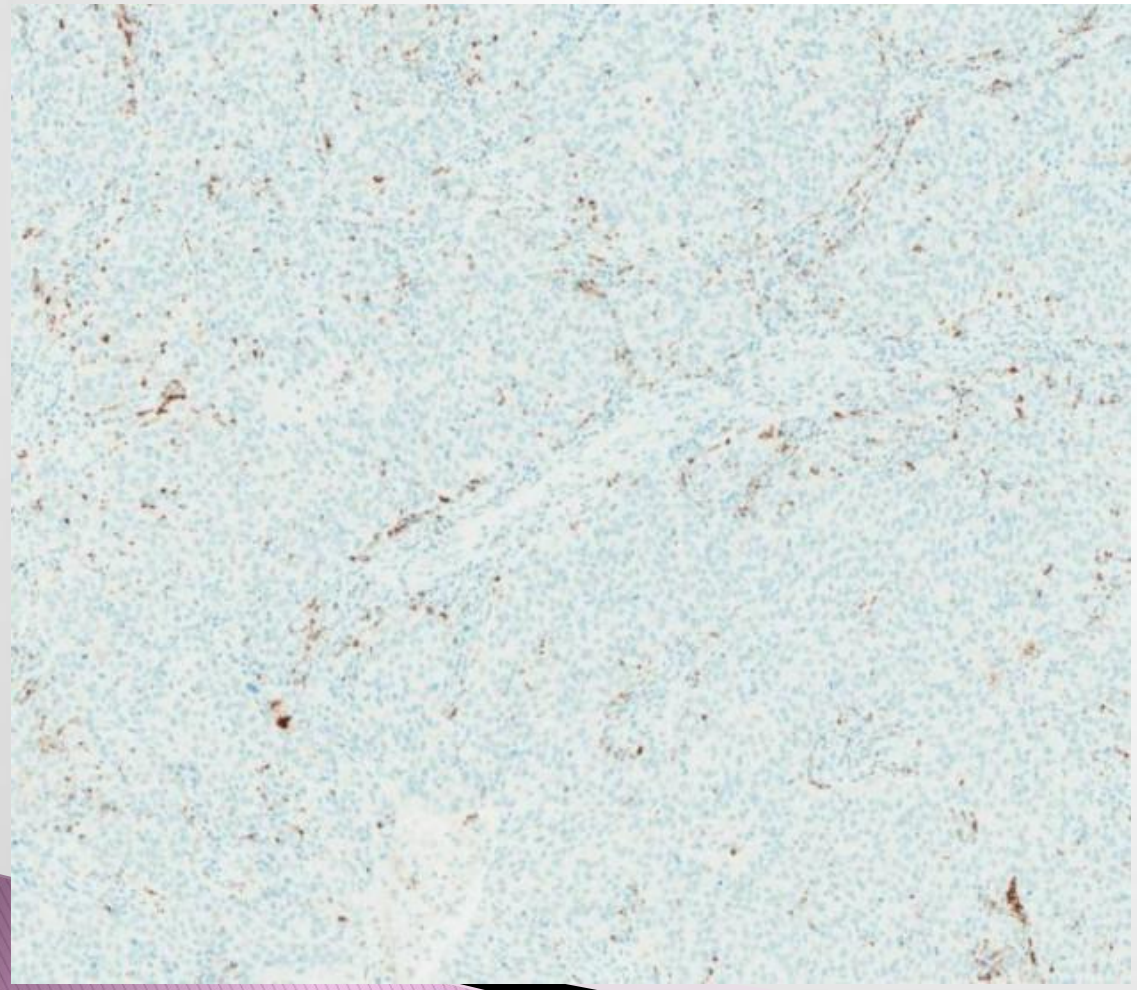
Tool 2 : Reference Images

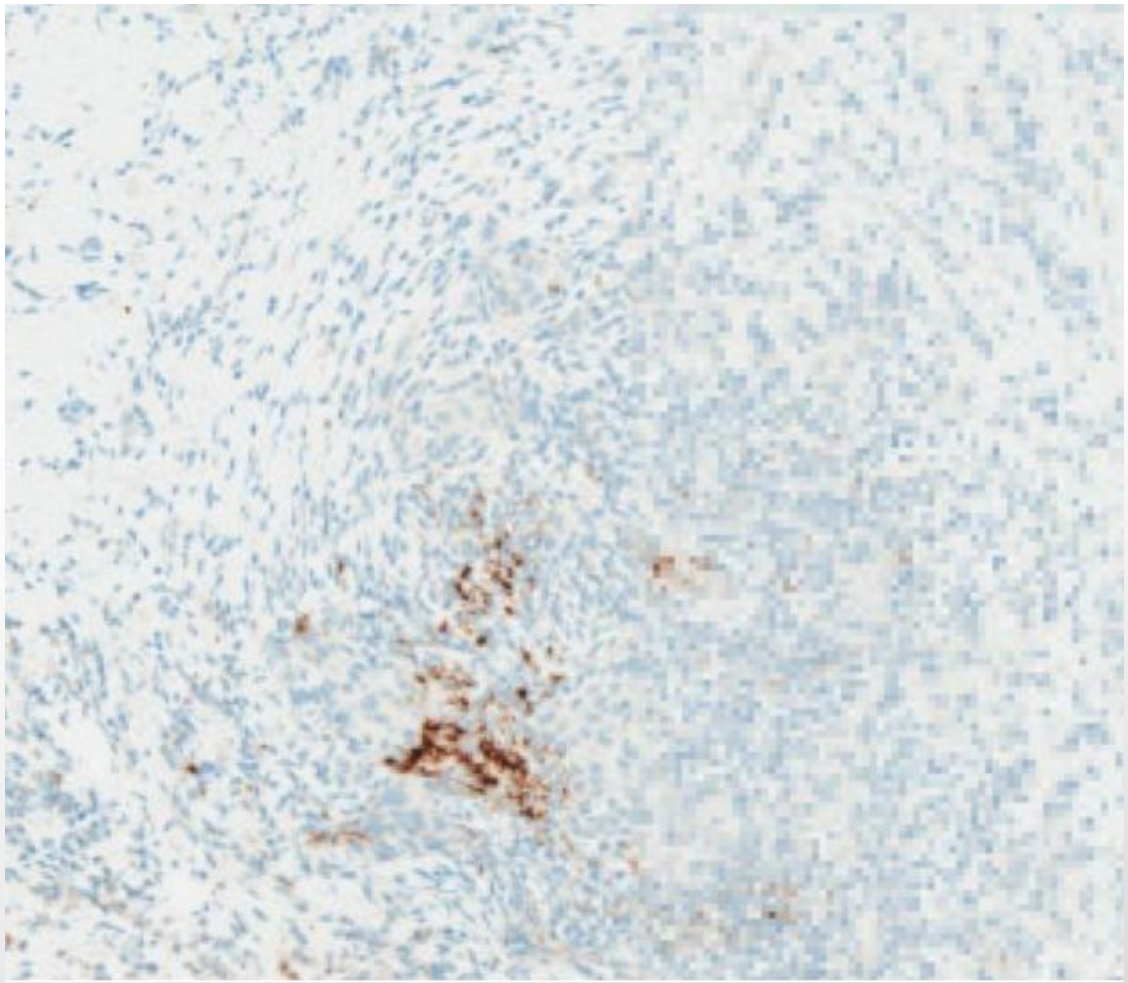
IC Single-Cell Spread Scoring: IC <1%



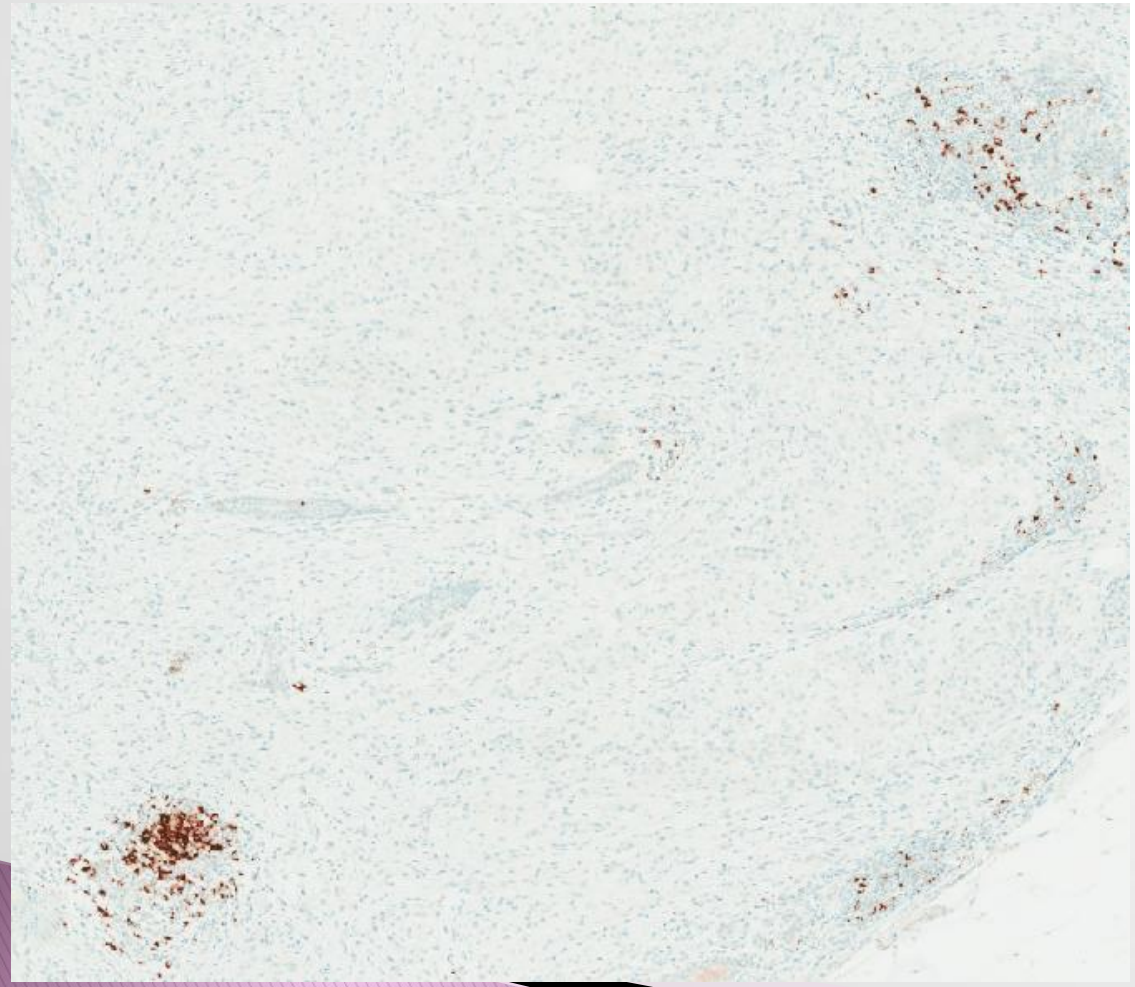


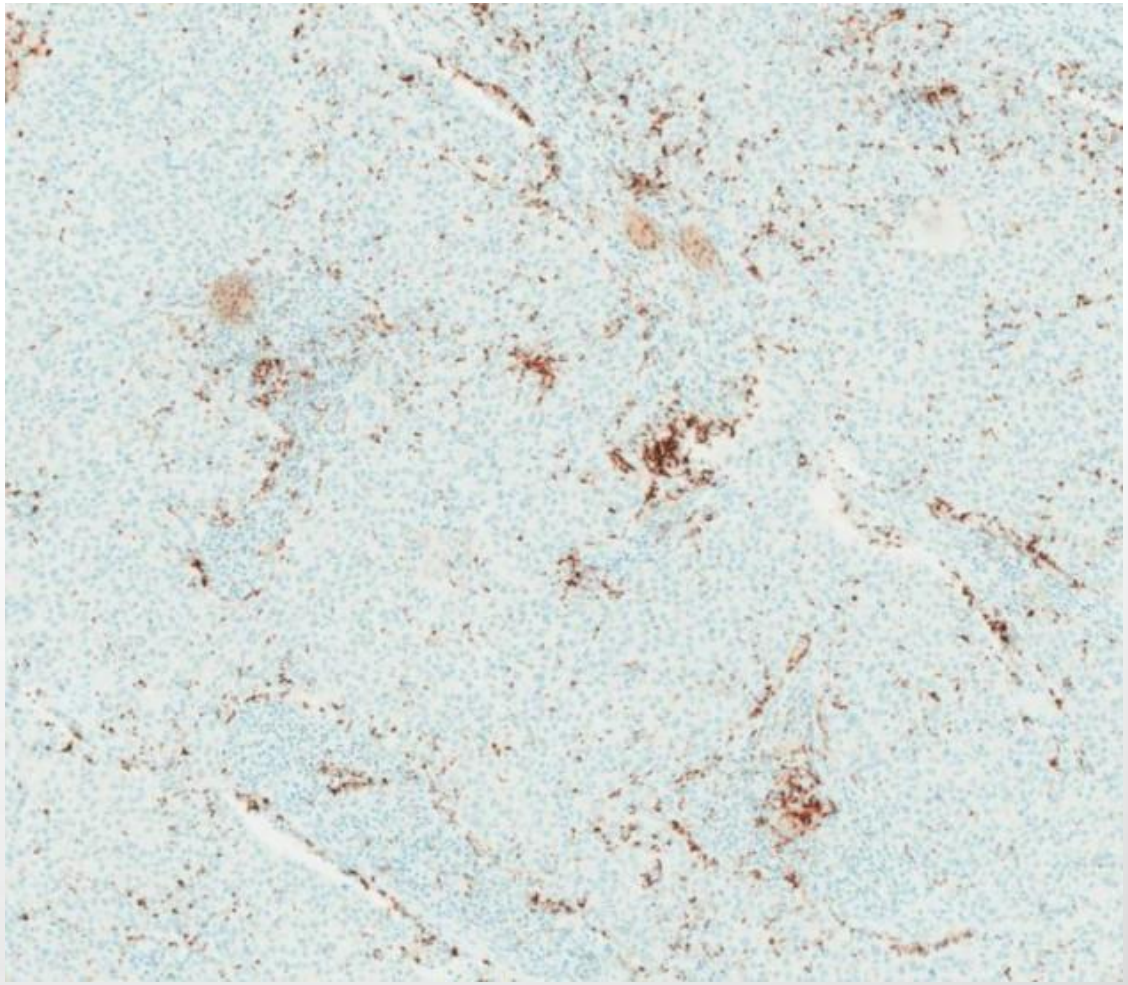
IC Single-Cell Spread Scoring: IC >1%



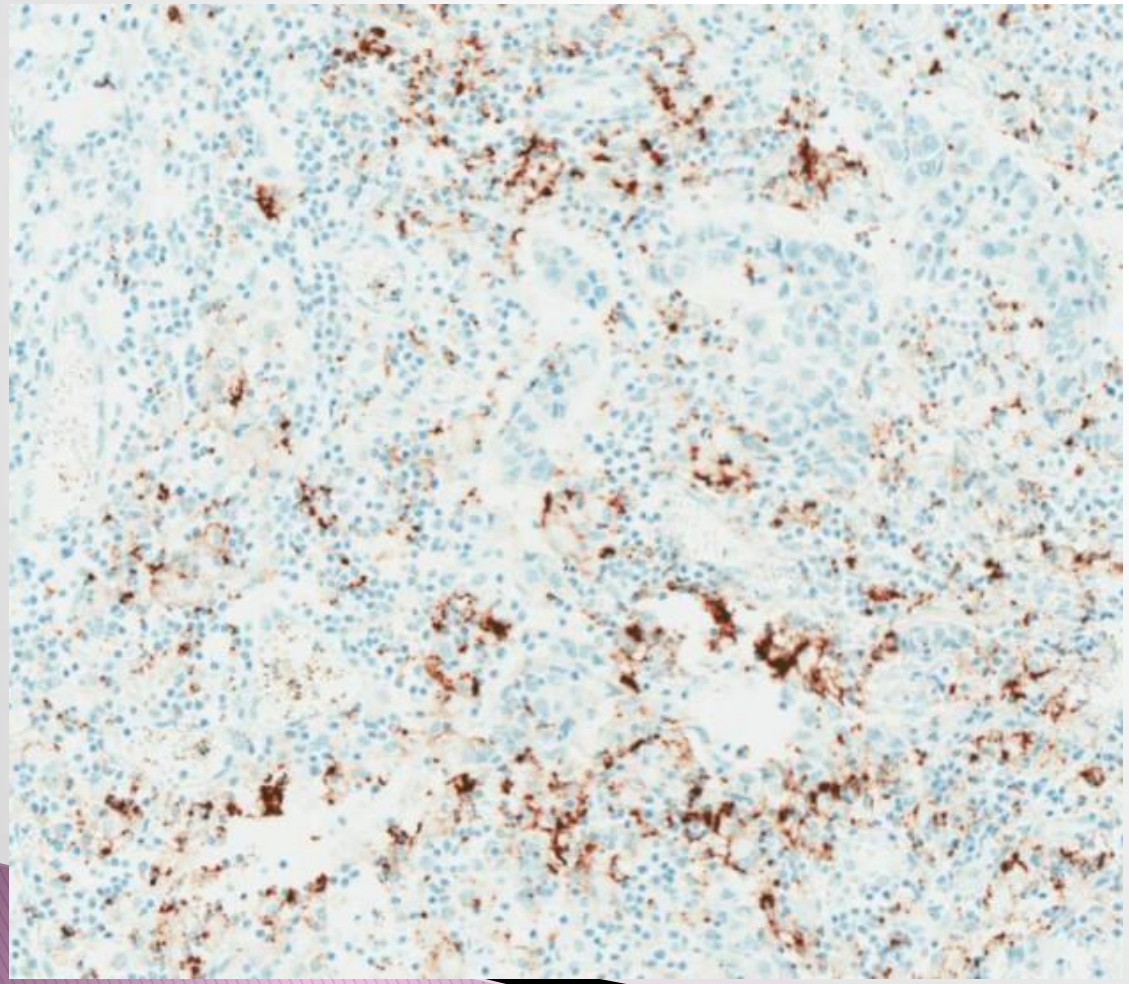


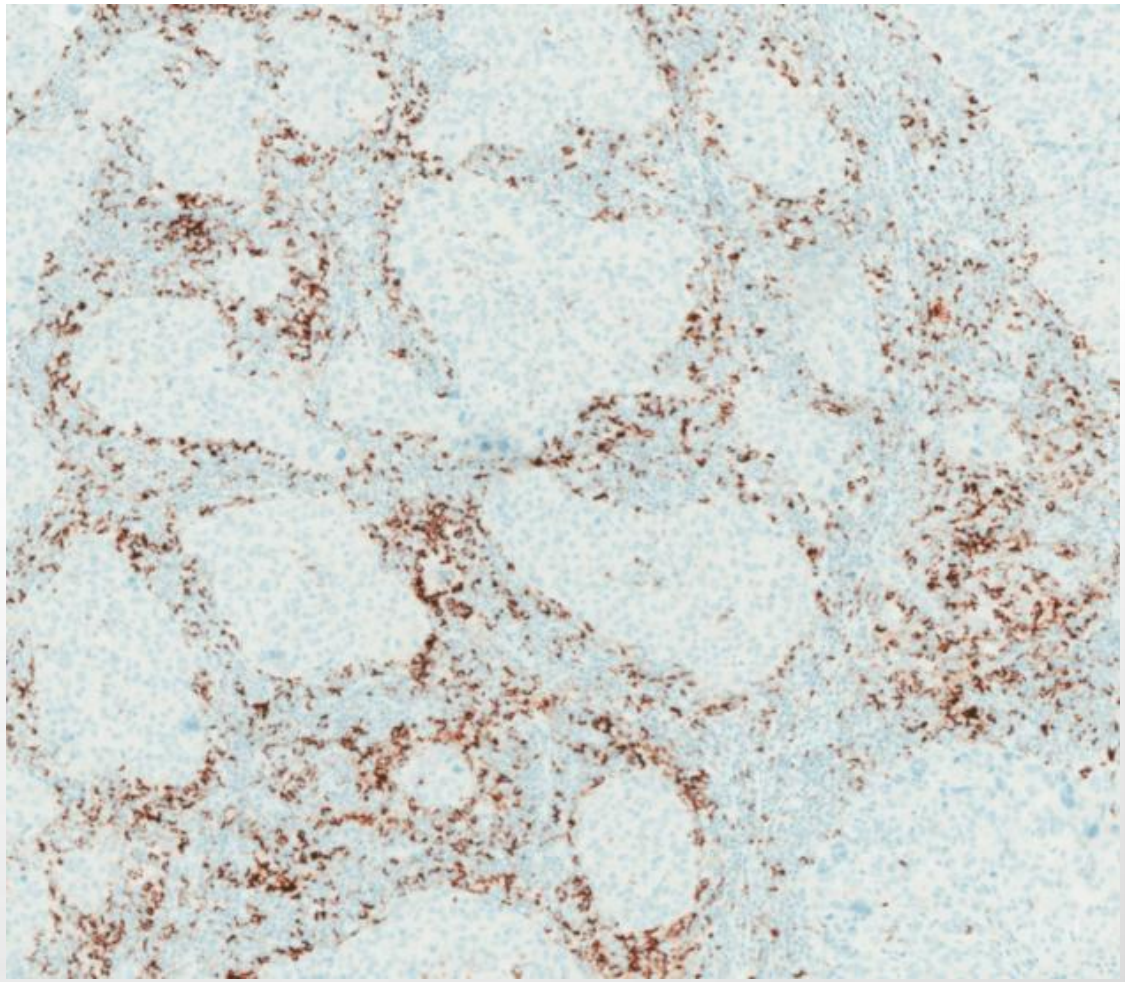
IC Single-Cell Spread Scoring: IC >1%





IC Single-Cell Spread Scoring: IC >1%





VENTANA PD-L1 (SP142) Assay

Scoring Algorithm–TNBC

Staining Criteria/Characteristics	PD-L1 Expression
Absence of any discernible PD-L1 staining OR Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering < 1% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	<1% IC
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 1 % of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	≥1% IC

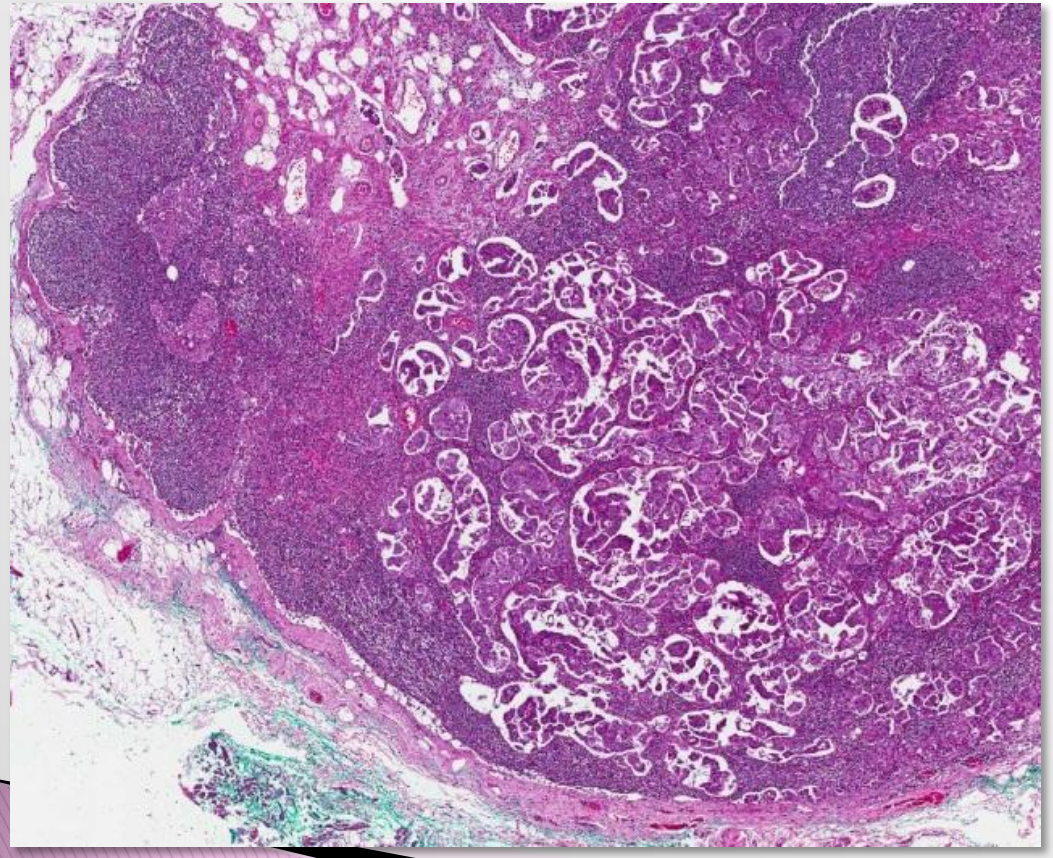
Only IC staining is counted as part of algorithm for TNBC

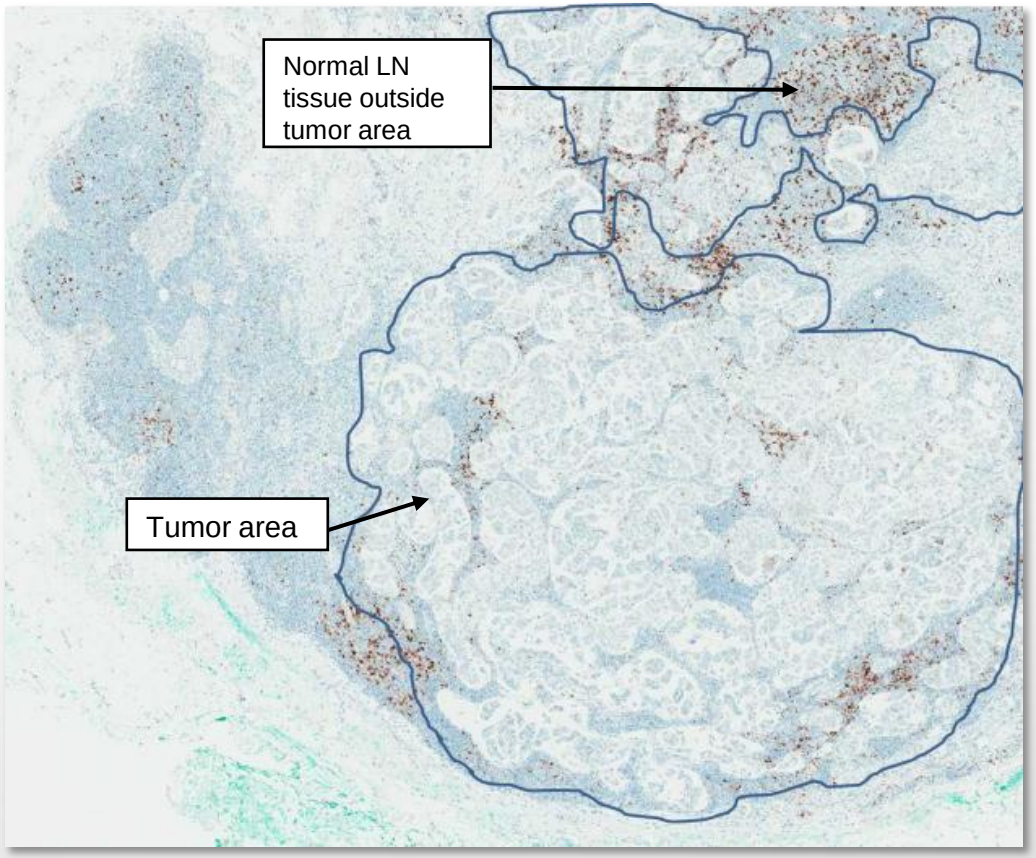
Objectives

- Overview of PD-L1 SP142 assay for Triple Negative Breast Cancer (TNBC)
- Evaluation workflow
- Scoring method TNBC
- **Scoring challenges and pitfalls**

a. Lymph Node Metastasis

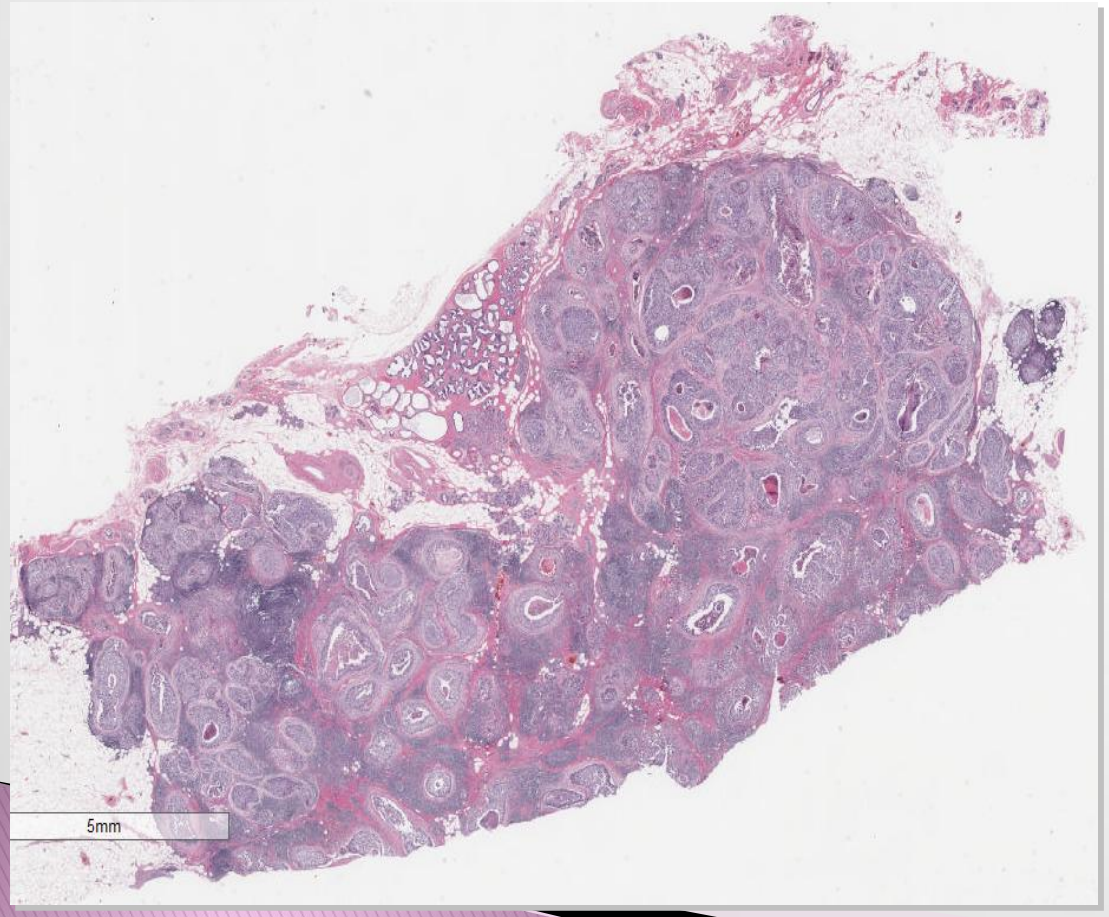
- Method of scoring similar to any other case (include IC in intra-tumoral and contiguous peri-tumoral desmoplastic stroma)

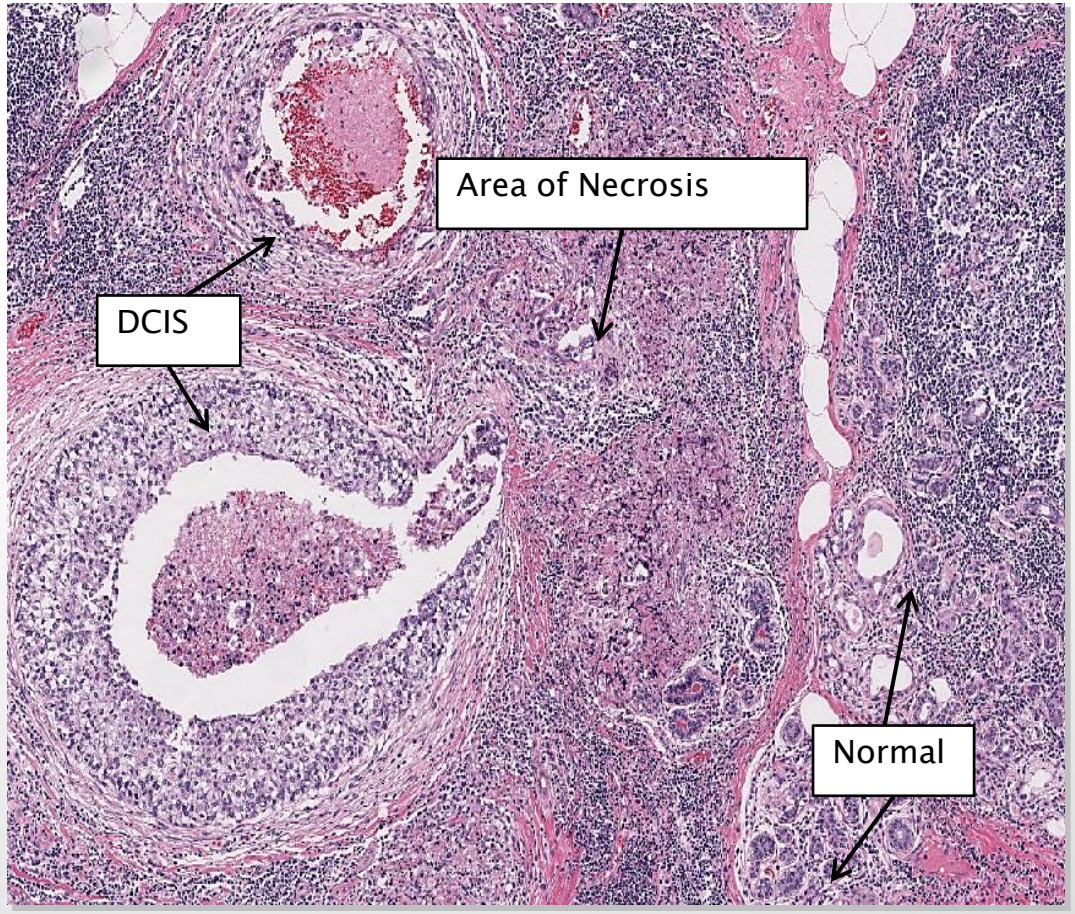




b. In Situ Carcinoma

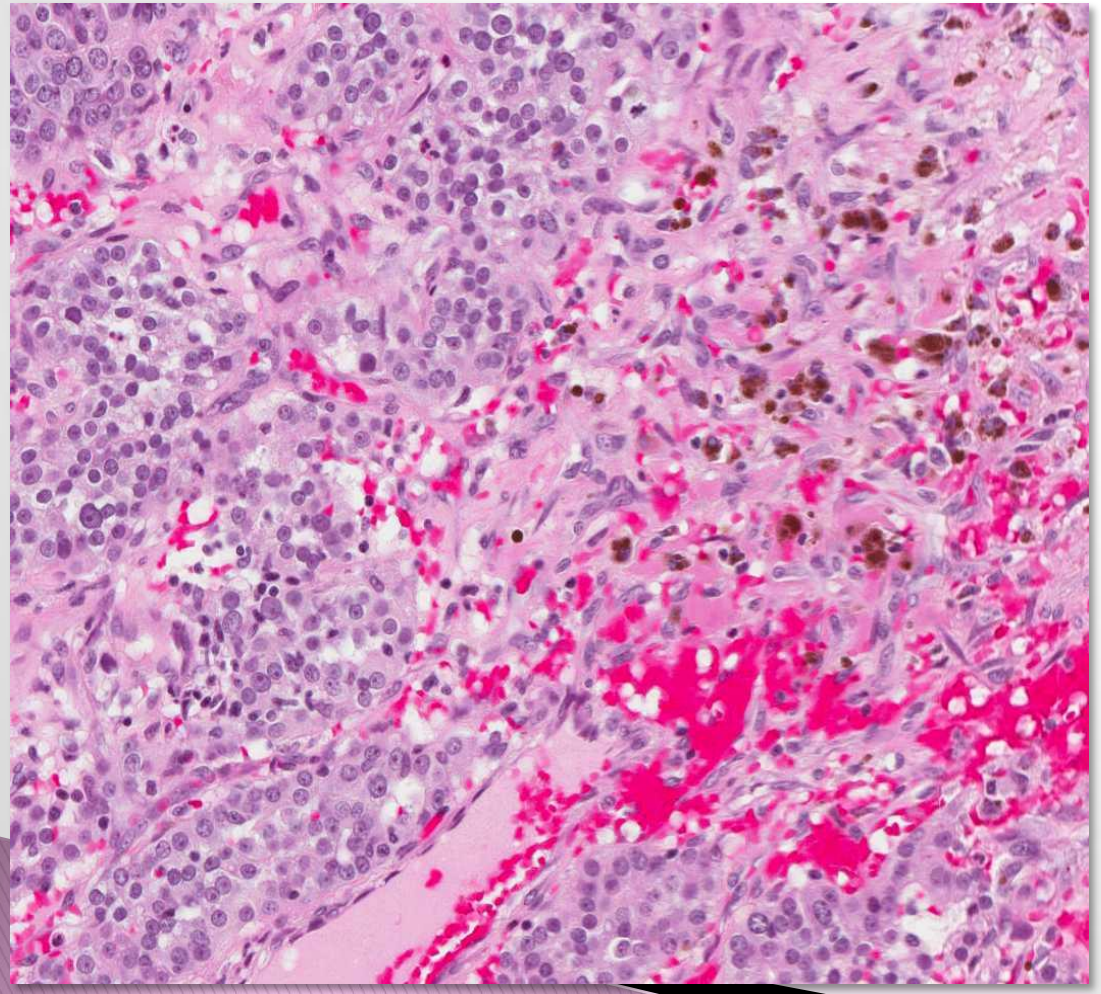
- Score only IC in areas of invasive tumor
- Do not score IC in areas of DCIS or LCIS

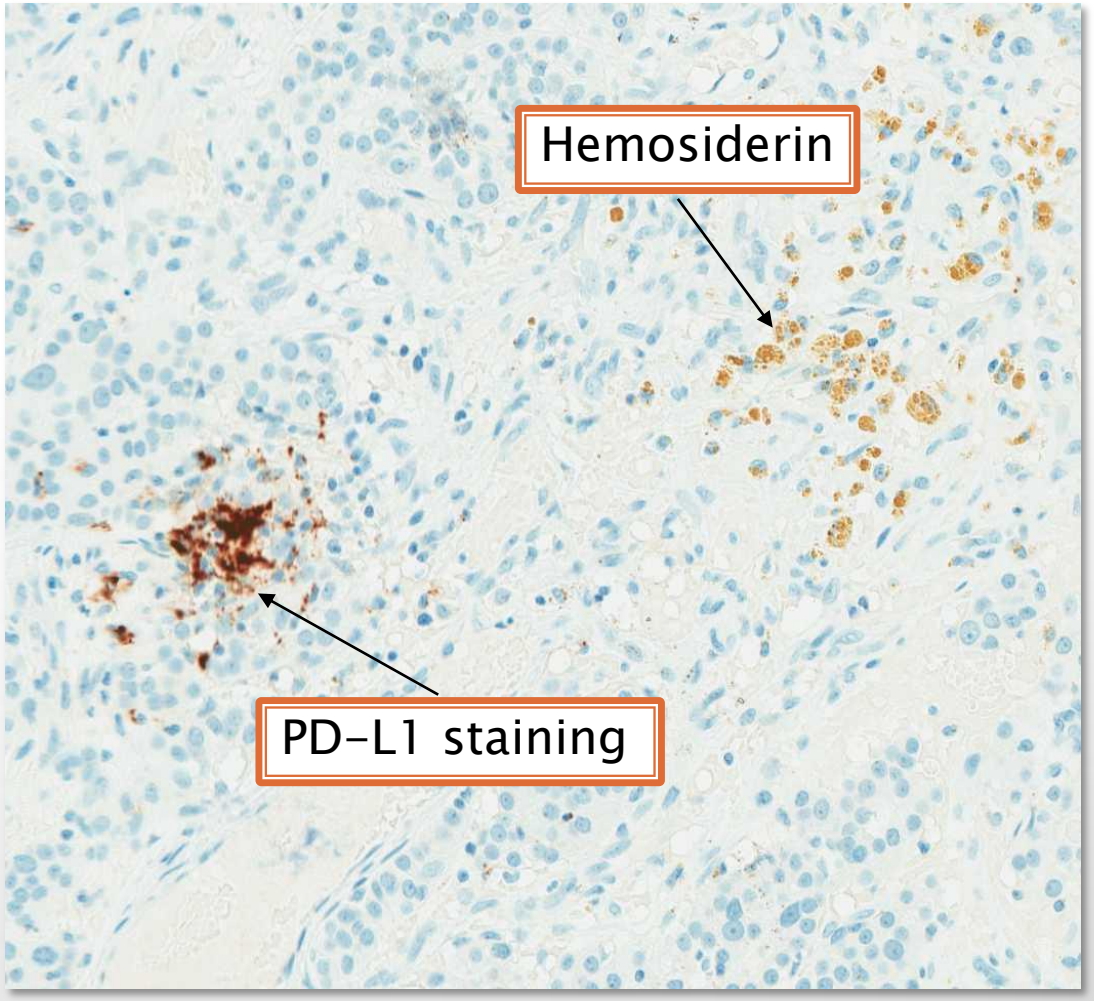




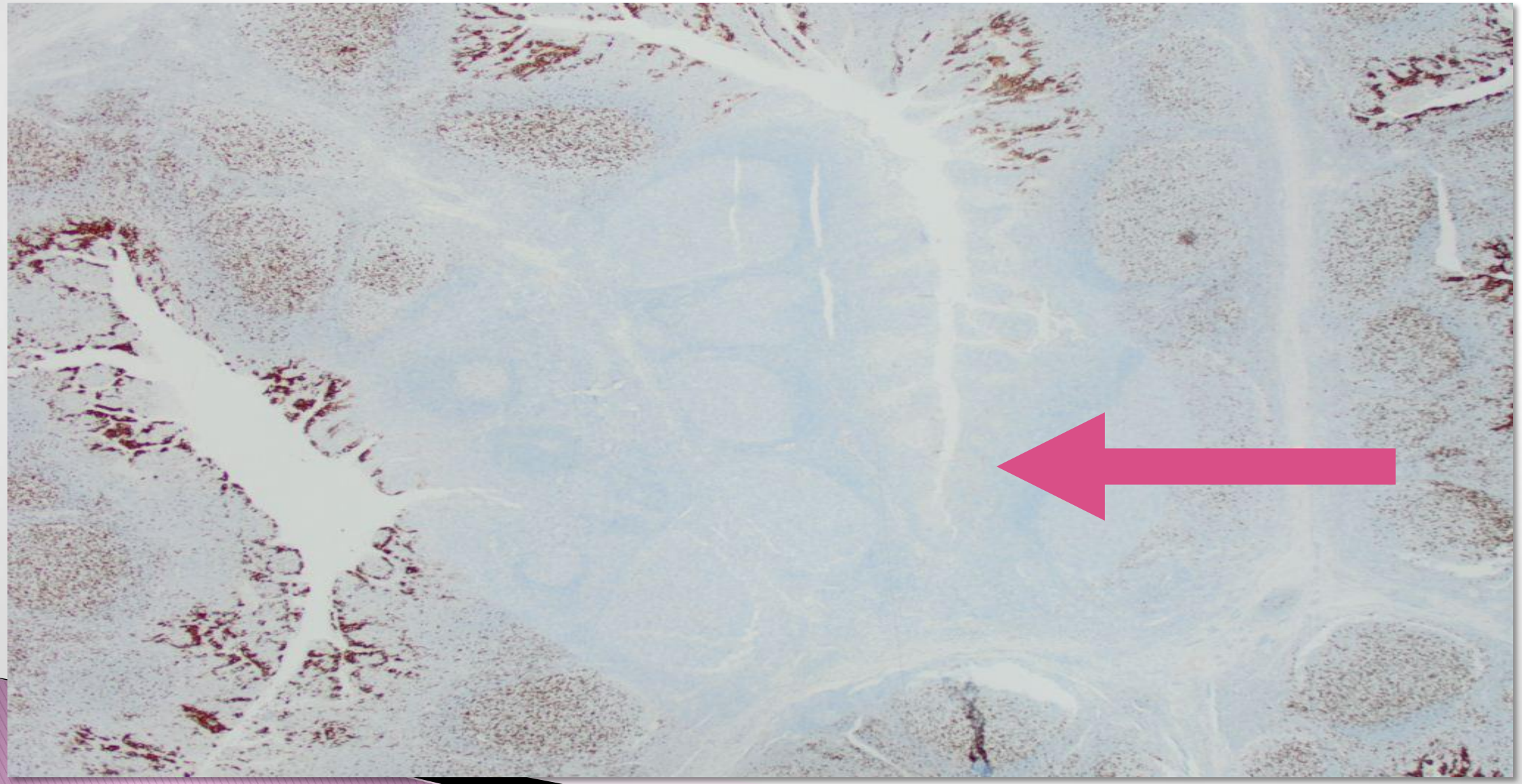
c. Hemosiderin pigment

- Seen on H&E and negative control



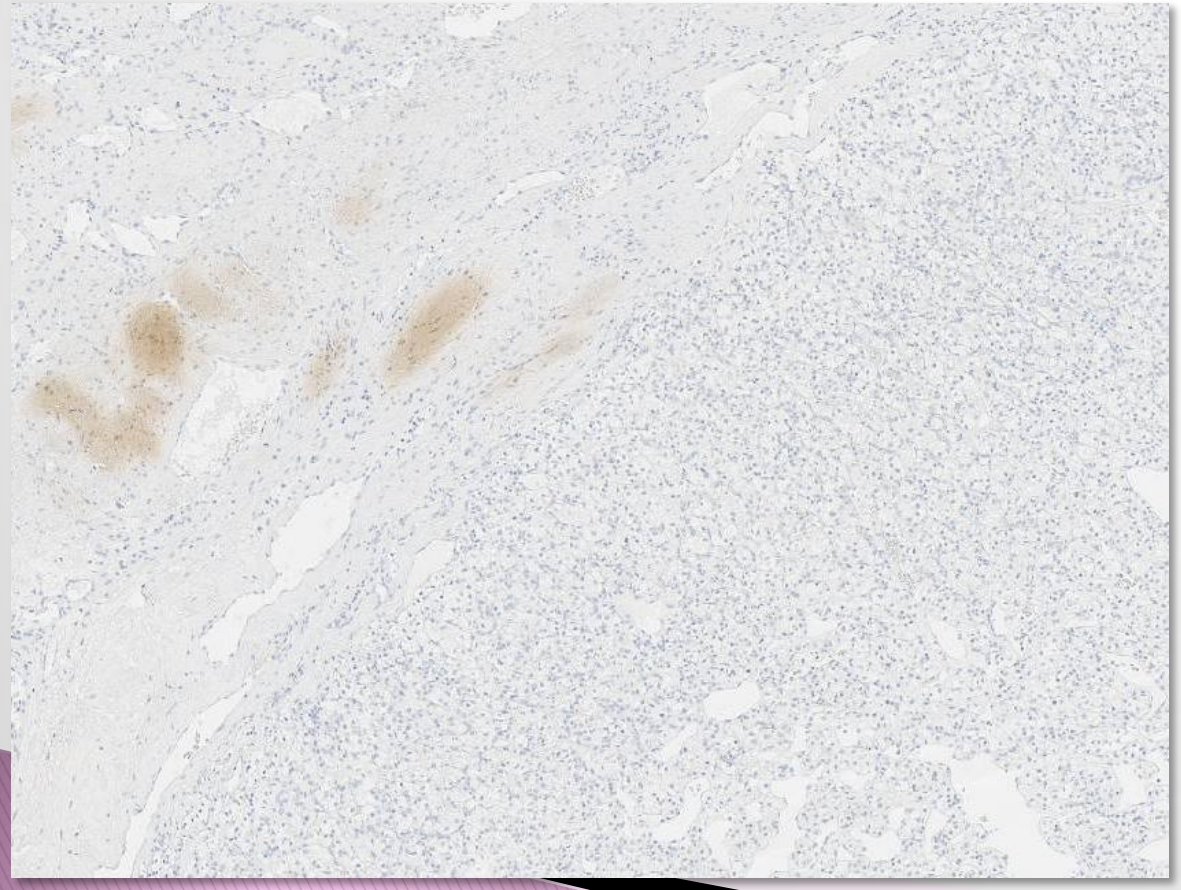


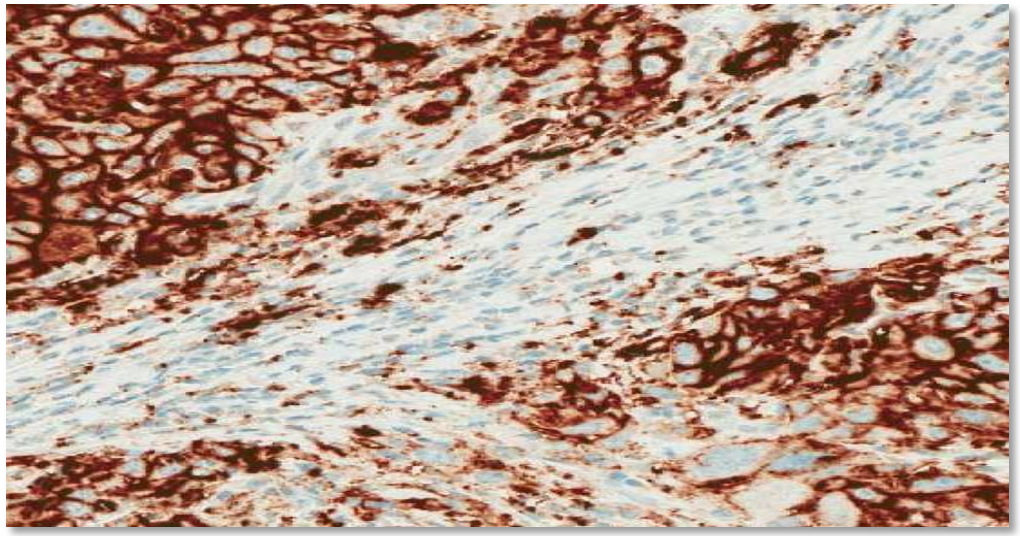
d. Blank spot: Technical artifact

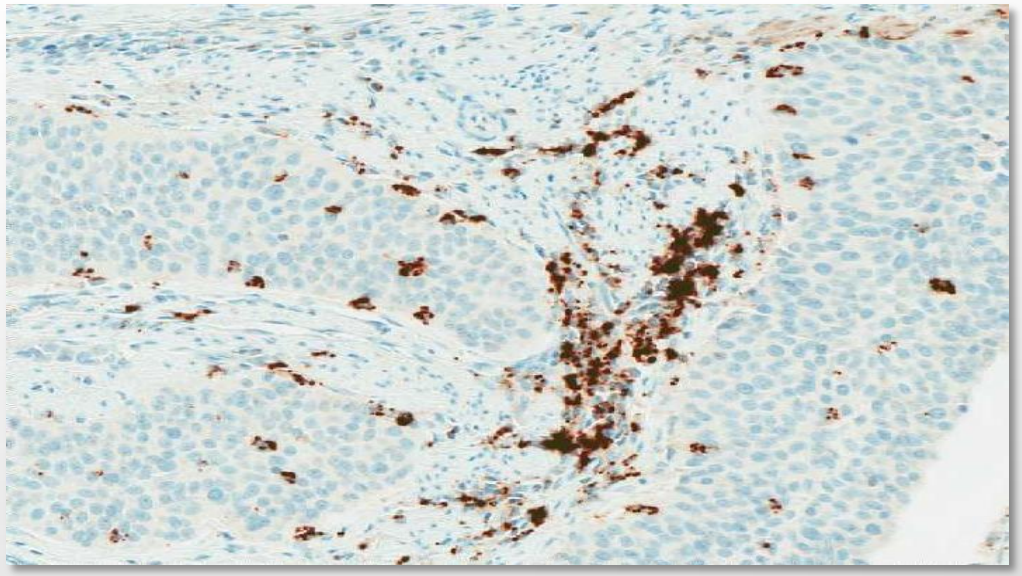


e. DAB spot vs TC / IC staining

- DAB spot are circular brown areas that may be formed during staining procedure. The tissue elements in a DAB spot can show non-specific staining and this should not be mistaken for specific staining

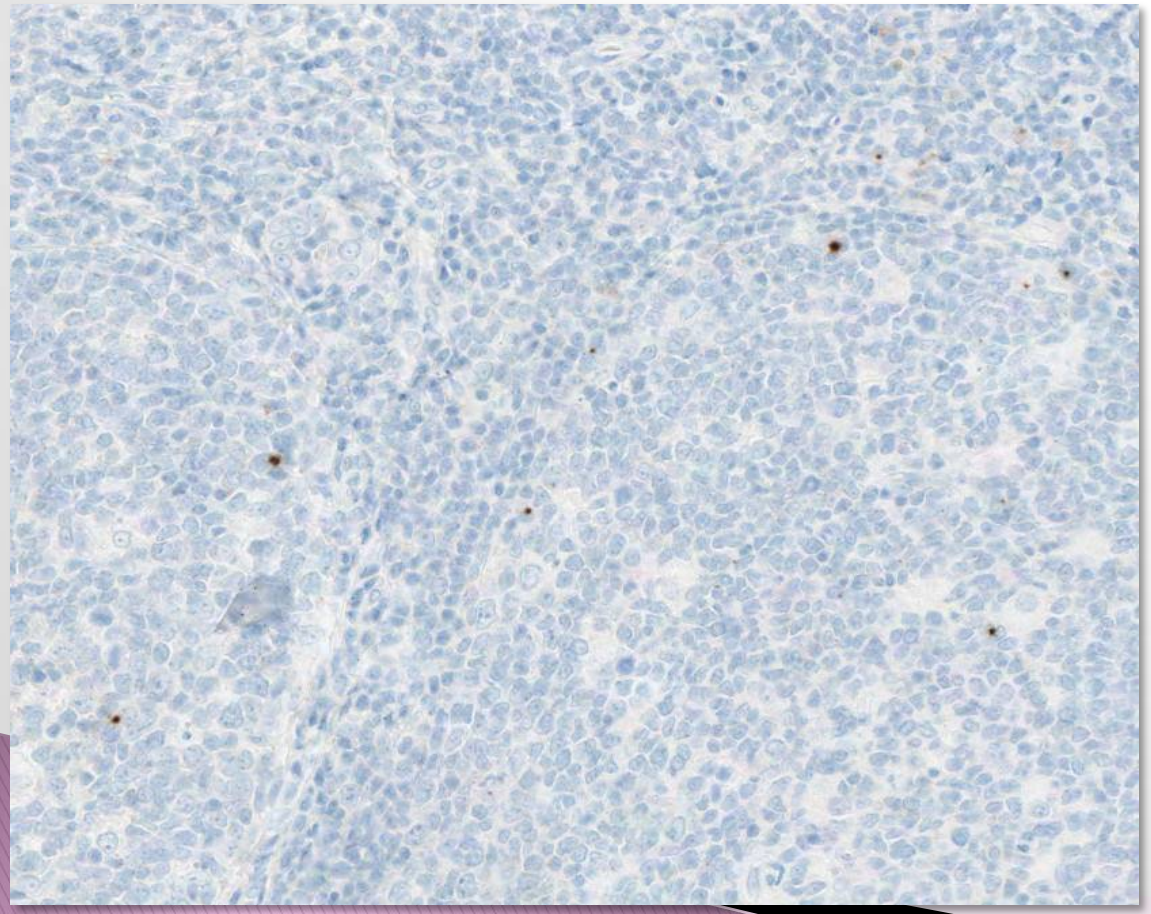


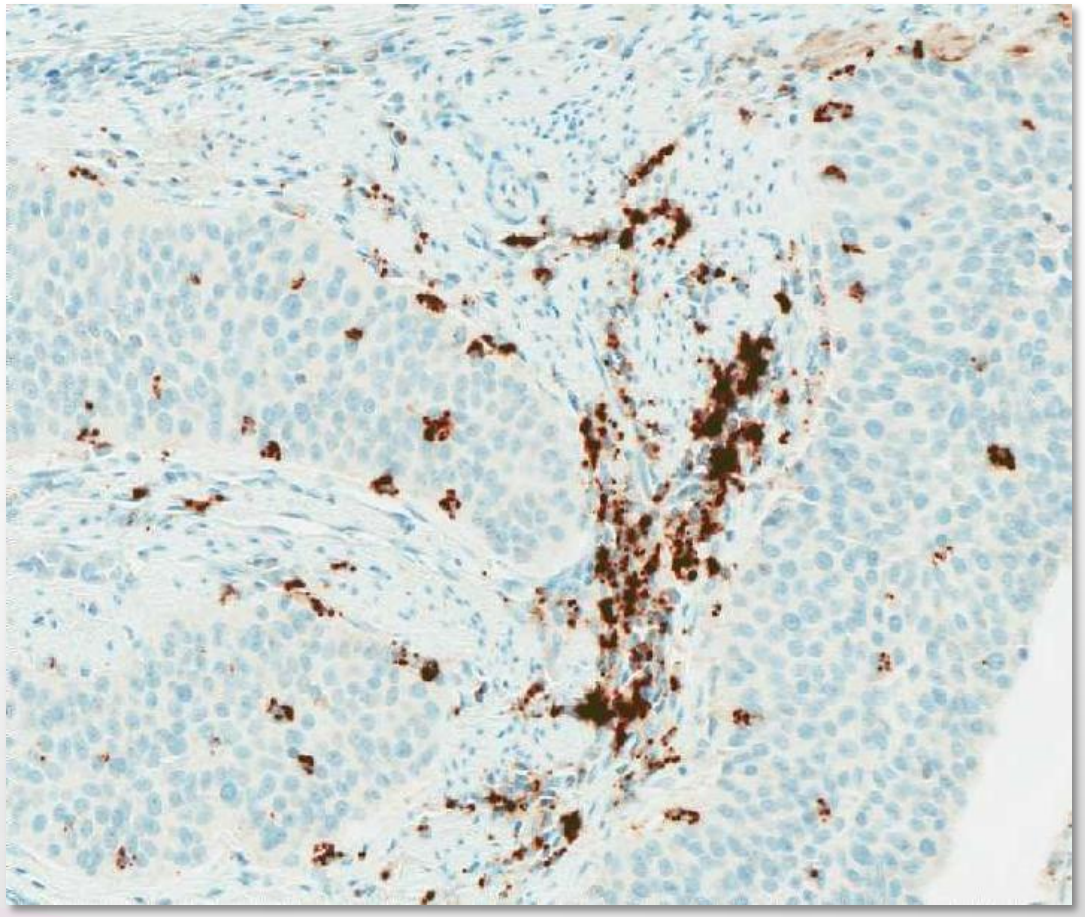




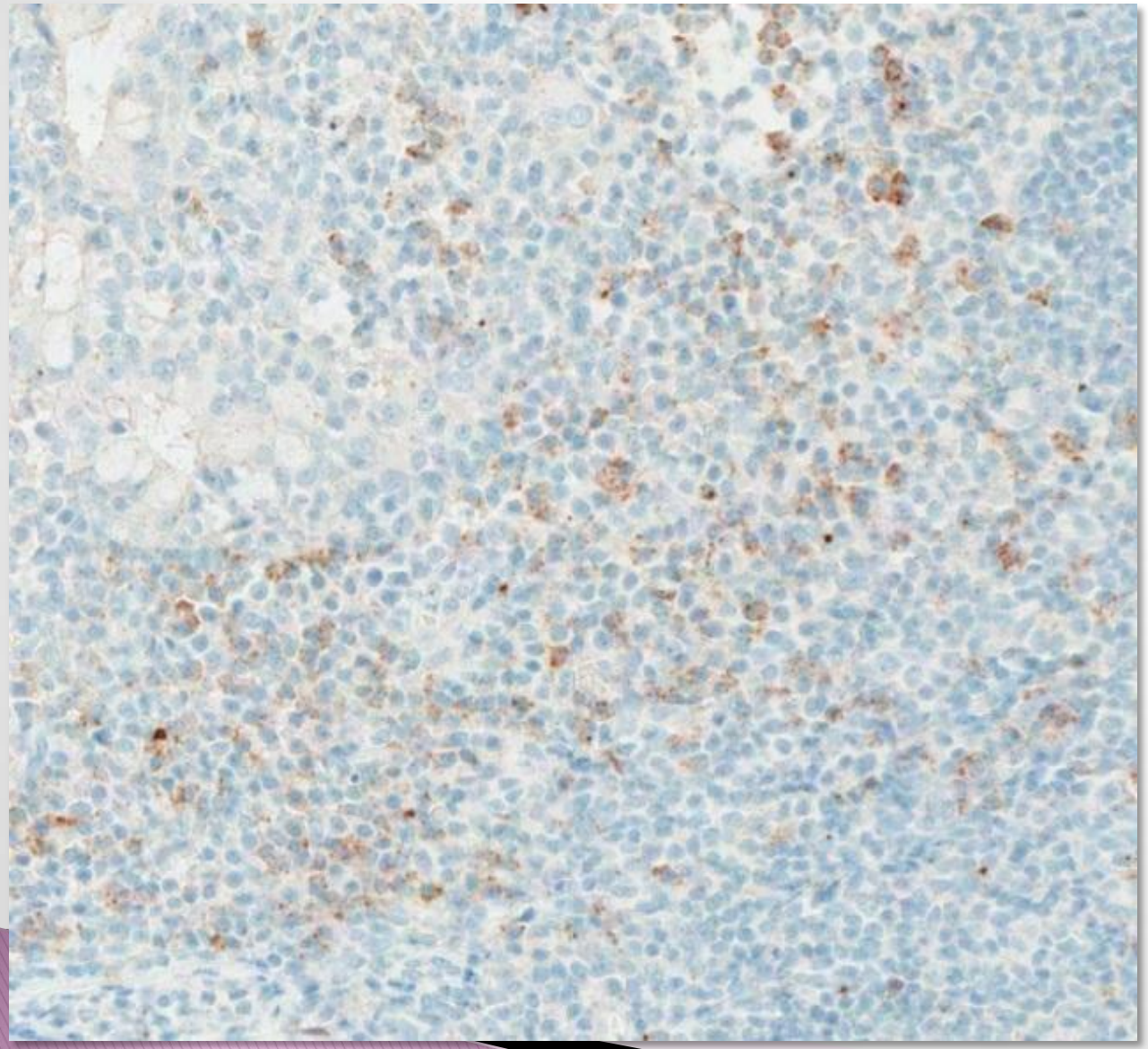
f. Punctate dots vs IC Staining

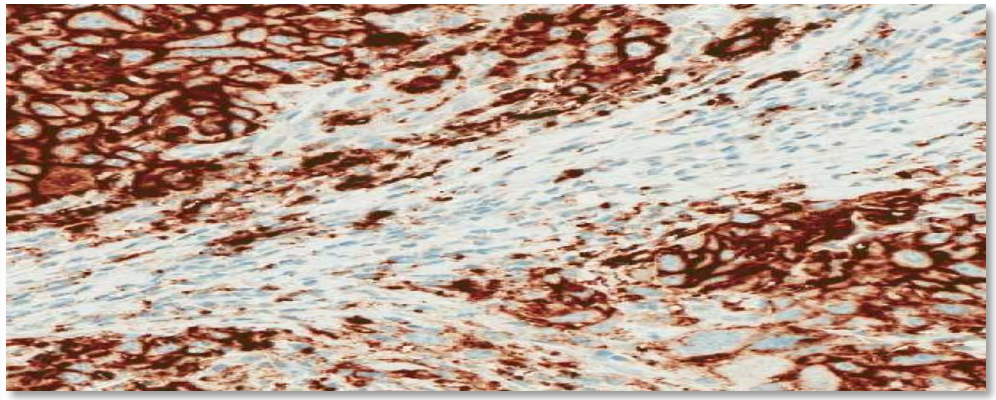
- Punctate dots are non-specific reaction products that are pin point sized and are often seen in tonsil negative control
- These are due to the amplification kit

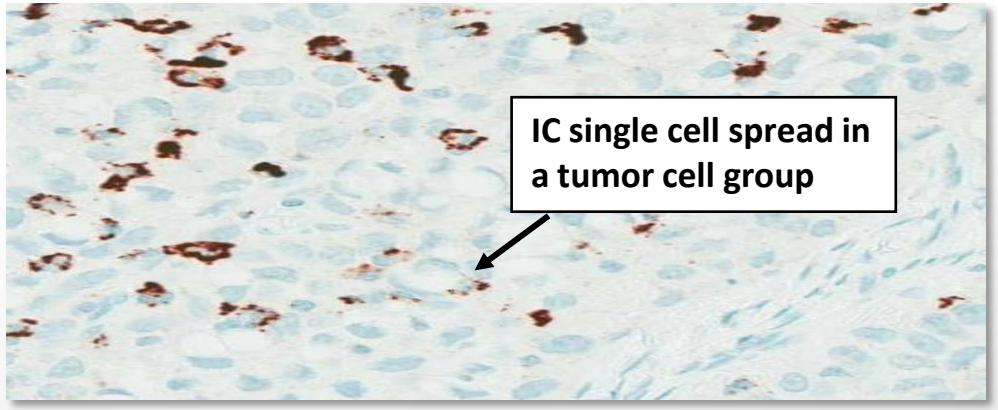


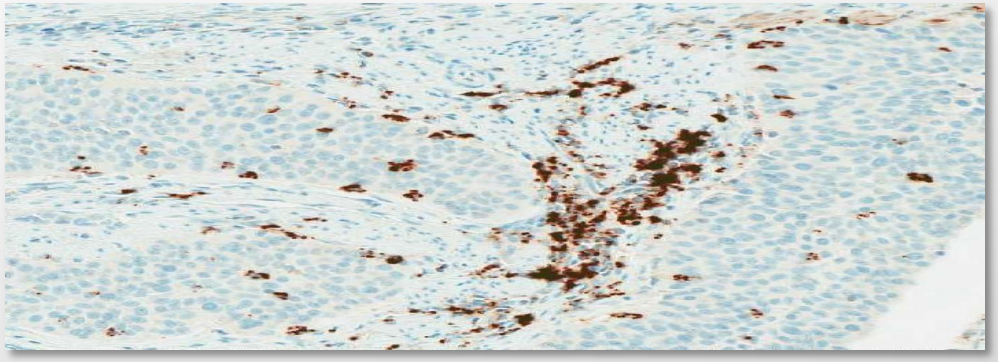


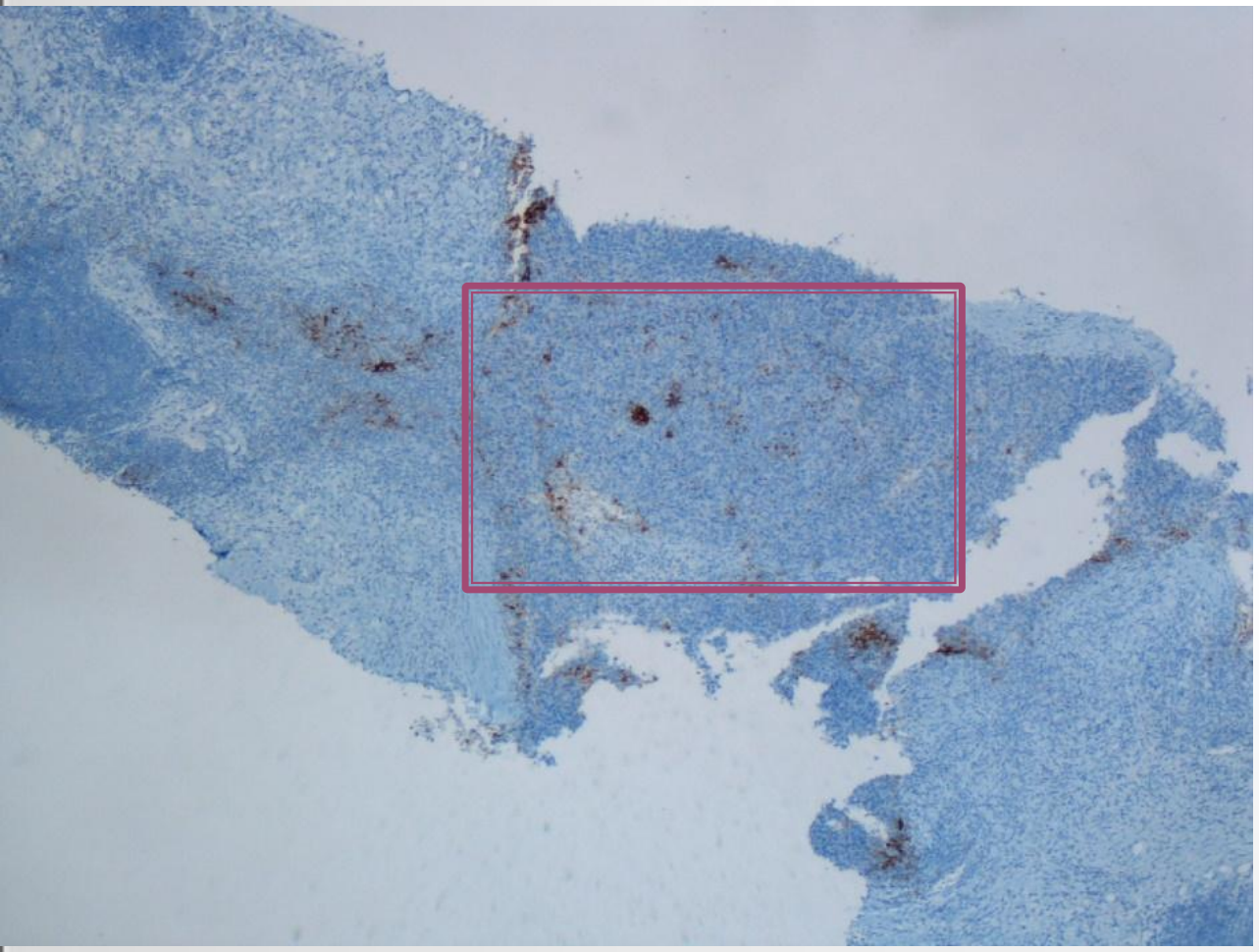
g. Speckling vs IC vs TC



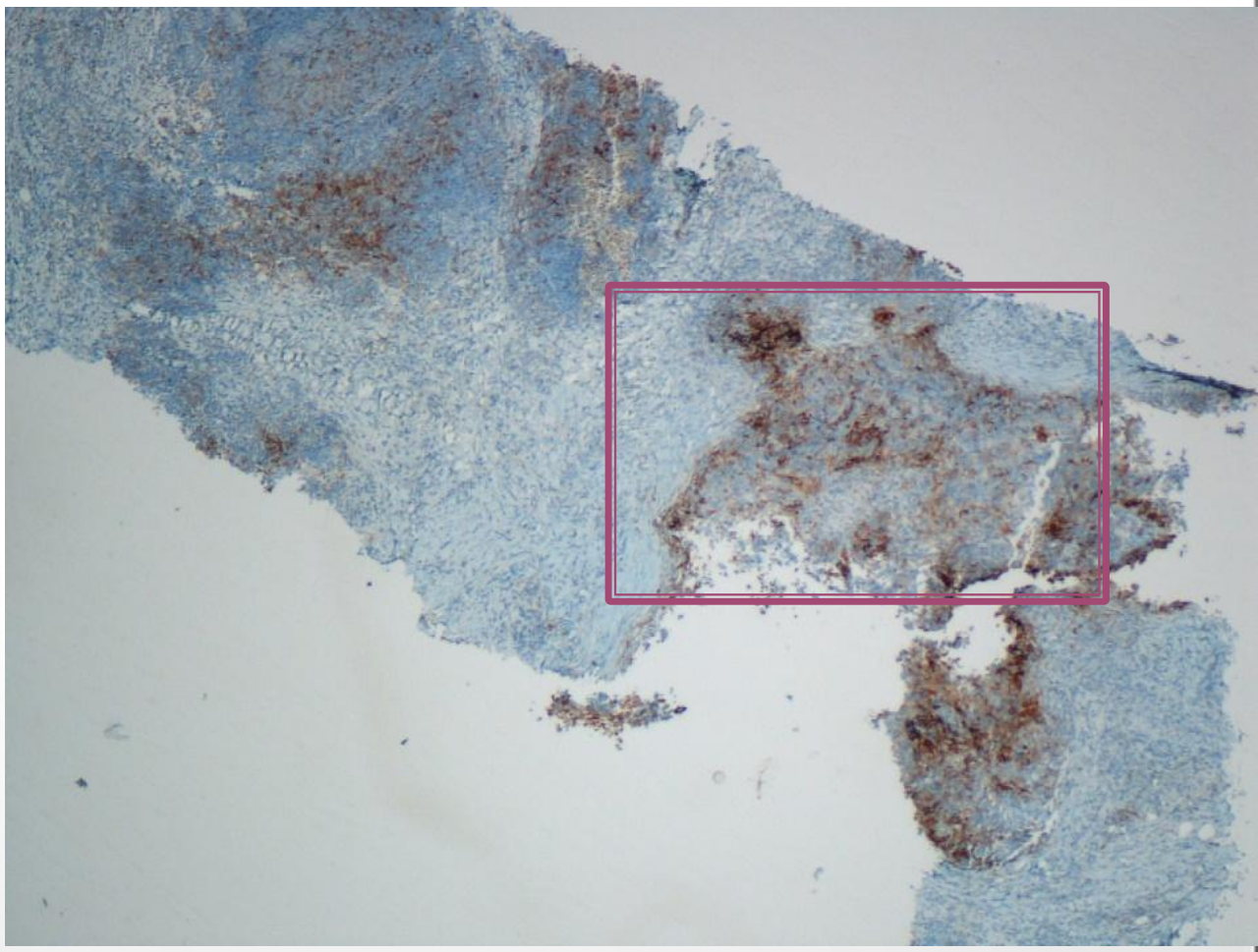




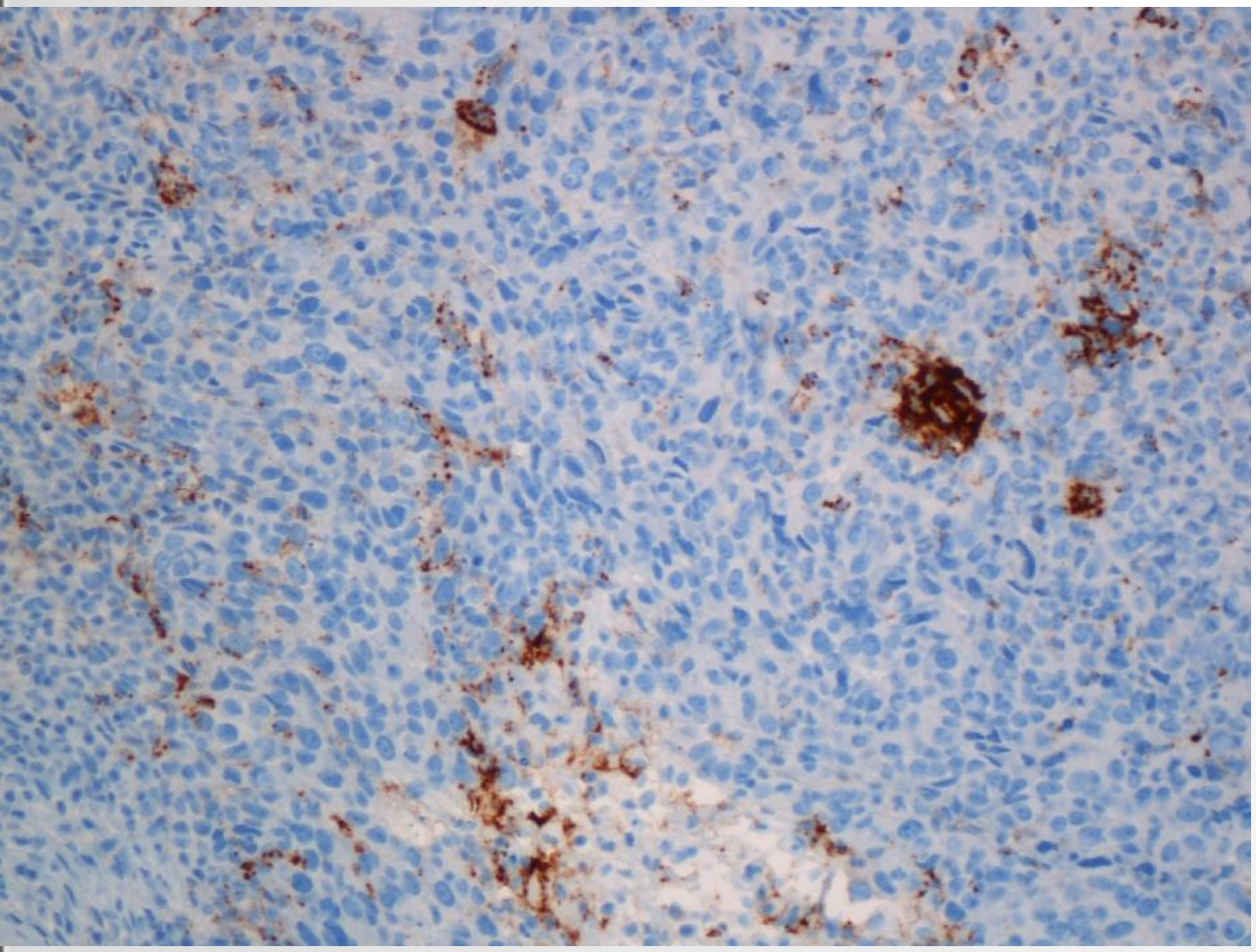




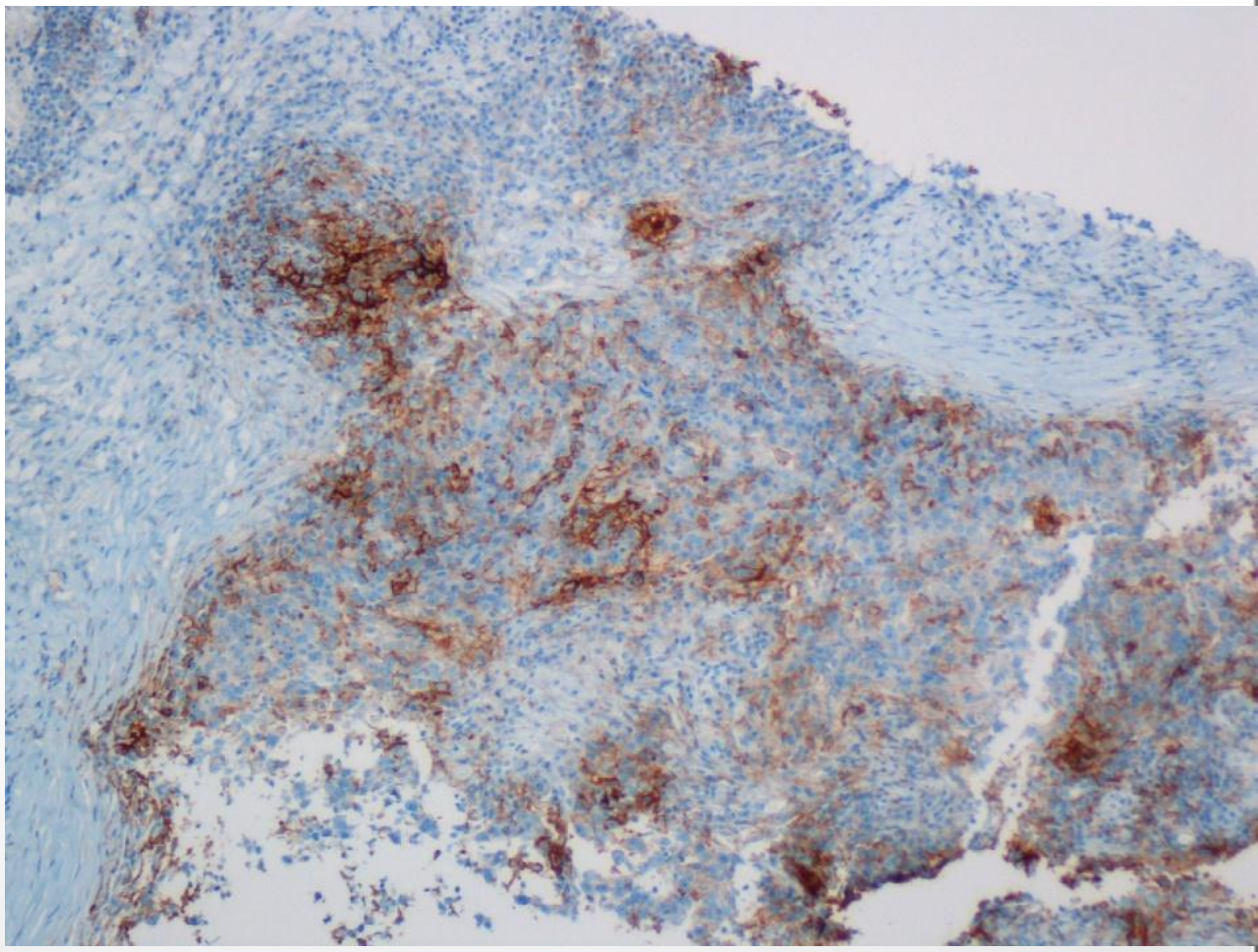
SP142



SP263



SP142



SP263

Muchas Gracias !!