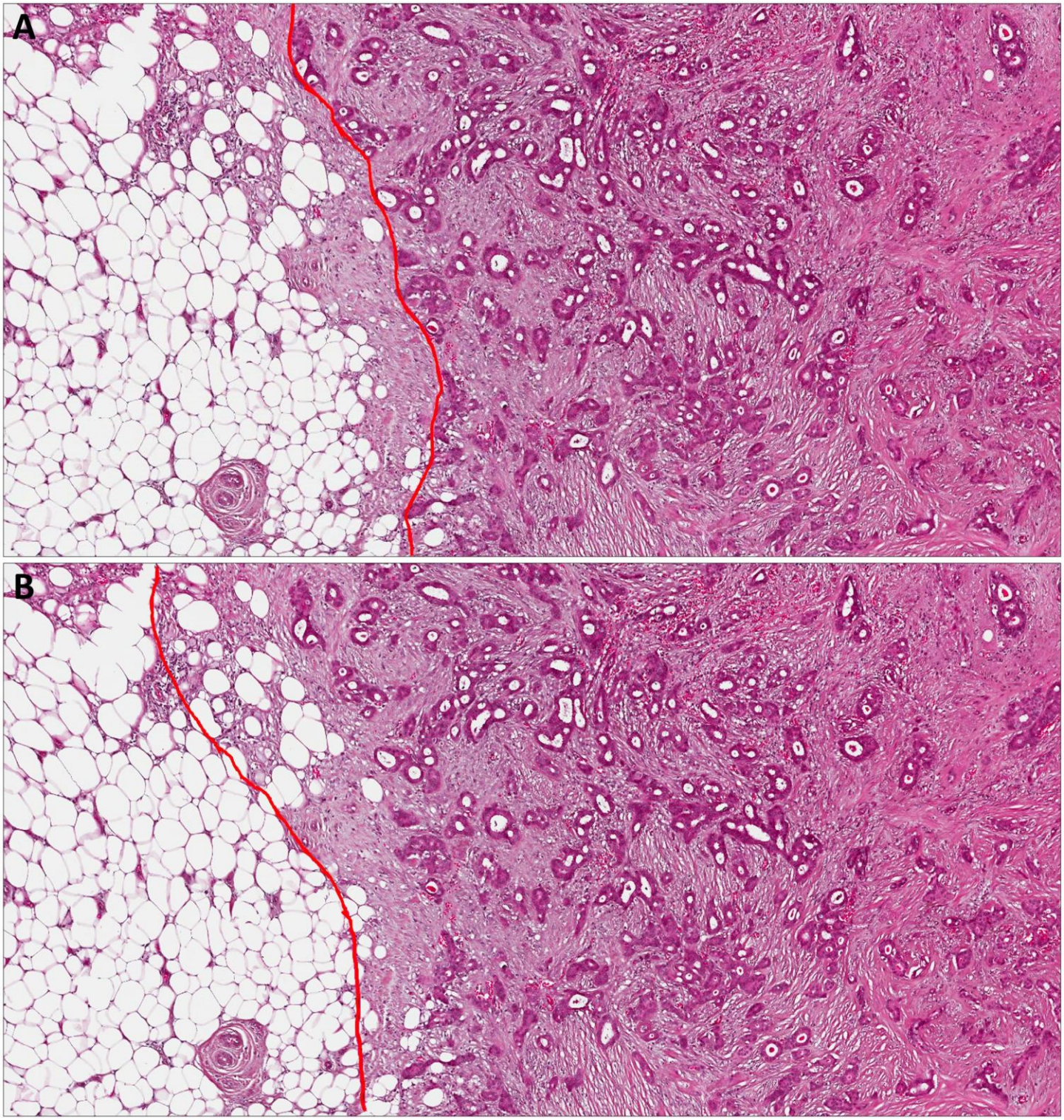
Supplementary Figure 1

Uncertain precise location of the tumour border as an example of an open question when assessing TILs. The tumour border can be the lateral edge of invasive carcinoma cells (A) or as including the peripheral desmoplastic/fibroinflammatory reaction (B).

Supplementary information 1: list of questions given to experts pertinent for TILs assessment in breast cancer

Supplementary information 2: Tutorial on standardized evaluation of TILs in breast cancer for daily clinical and research practice of clinical trial setting

Supplementary information 3: Open questions in the assessments of TILs


A
B

S1 Supplementary Table 1

QUESTIONS
Could you detail the methodology you have used in your study?
At what microscope magnification did you score the slides?
Is slide thickness important?
Did you assess the TIL's in zones with crush-artefacts?
Were the slides scored individually or by two pathologists together or independently?
In the case of full sections, how many slides did you asses per patient?
Did you assess interslide heterogeneity of TIL's in order to define the number of slides to read per patient?
Did you assess tertiary lymphoid structures (TLS)?
If yes, what were the criteria you used to define these TLS?
Did you assess the number of the TLS?
Did you asses the location within a tumor of these TLS?
How did you cope with heterogeneity of TIL-infiltration within a slide?
Did you limit yourself to lymphocytes and plasma cells or did you take other cells also into account, for example neutrophils?
Did you consider the stromal and the intra-tumoral (=within tumor nests) compartment as separate or together?
Did you take all TIL's into account or were the TIL's around 1. DCIS-, 2. normal ducts or 3. areas of necrosis not taken into account?
Could you detail the total number of patients analyzed, the BC subtype in which the TIL's were analyzed and whether treatment was received or not?
Could you detail whether the scoring was performed on core biopsies or full sections?
Could you detail whether and how reproducibility was assessed between the pathologists who scored the slides?
Did you perform a pilot-study in order to assess concordance between the pathologists on the to be used methodology before starting to read the slides for your study?
Could you detail what level of concordance between the pathologists was considered as being acceptable?

Which error margin between 2 pathologists was considered as acceptable? Please include the reasons hereof.
Could you detail how you determined the cut-off for a lymphocyte predominant subtype?
Was this predefined? If yes, explain how.
Was this lymphocyte predominant cut-off different according to subtype?
Do you use the same lymphocyte predominant cut-off for both cores and full sections?
Did you use alternative methods for assessing TIL's, like digital imaging purposes or immunohistochemistry? If IHC was used, please state the epitopes you have stained.
Any idea whether pre-analytical variables, for example fixation time affects the TIL-evaluation? Is this important to know?
How would you suggest validating a morphological biomarker?
Is performing a RING-study useful to assess interlaboratory-concordance?
If yes, how would you suggest organizing this?
What are your thoughts on performing a meta-analysis of all studies done so far, both in the neoadjuvant as in the adjuvant setting?

S2 Supplementary table 2 Open questions in the assessment of Tumor Infiltrating Lymphocytes (TILs)

- Which is the exact margin of the tumor border, the desmoplastic reaction or the tumor cell infiltrates? (Supplementary Figure 1)
- Should the lymphocytes in normal lobules and around DCIS-foci located in between invasive tumor nests be taken into account?
- Can tertiary lymphoid structures (TLS) be characterized merely based on morphology?
- Should TLS be taken into account outside the defined tumor border? Do they reflect a specific type of tumor-immune interaction?
- When there is a gradient between regions with high TILs trailing off into a region of lower TILs, how can this regional heterogeneity best be assessed?
- Can the methodology used for TIL-assessment in primary tumors also be used for metastatic lesions?

Standardized evaluation of Tumor-Infiltating Lymphocytes (TILs) in Breast Cancer for daily clinical and research practice or clinical trial setting

A tutorial prepared by the International Working Group for TILs in Breast Cancer - 2014

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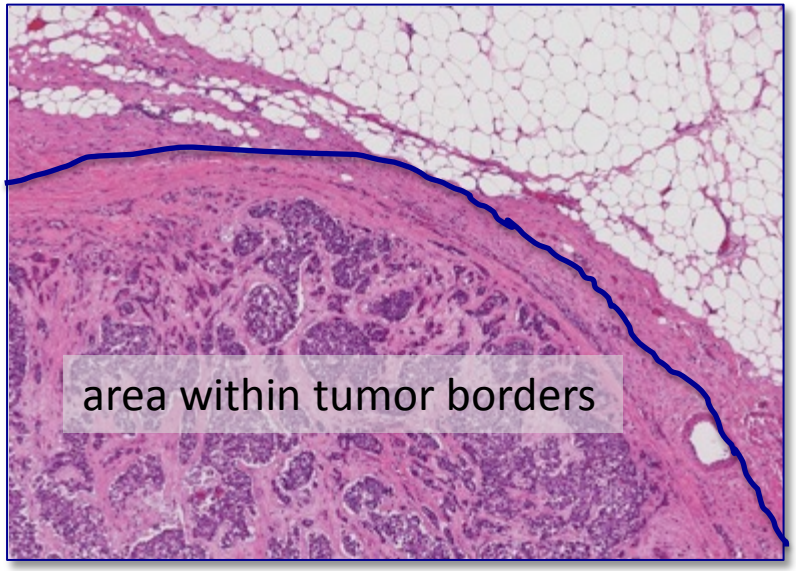
Sandra Demaria

Aim of this tutorial

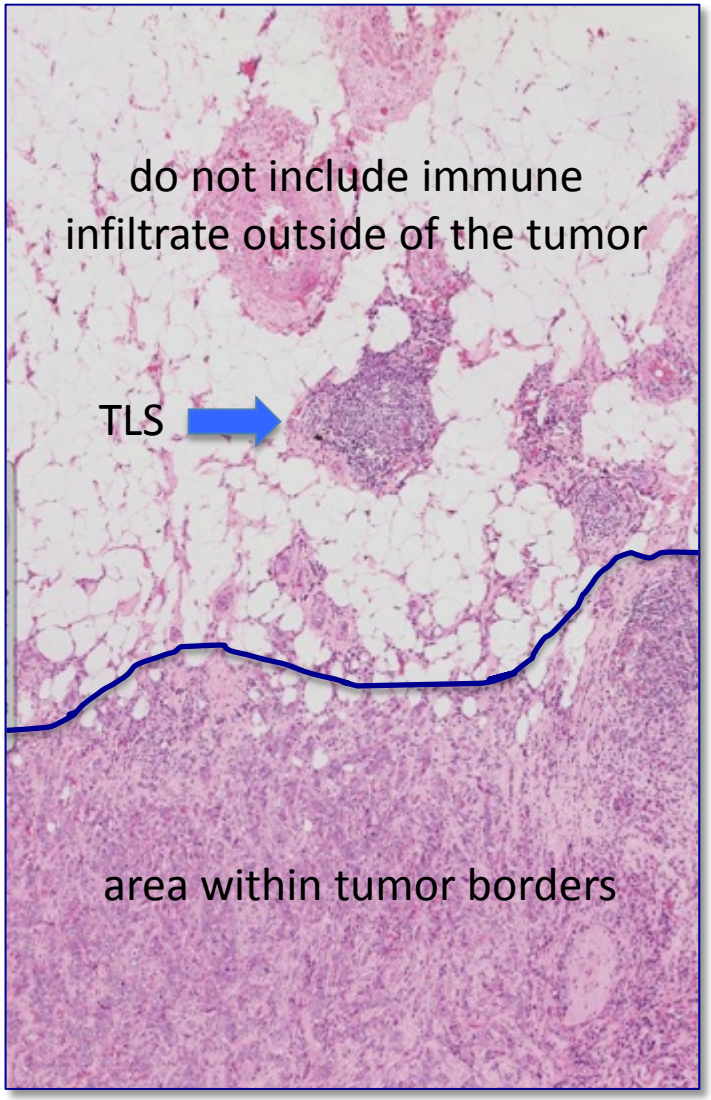
- To provide a guideline to pathologists for the standardized evaluation of tumor-infiltrating lymphocytes based on H&E slides of core biopsies or tumor resections.
- Please consult the manuscript for more specific details.

Step 1: Define area for TILs evaluation

- Only TILs within the borders of the invasive tumors are evaluated
- The invasive edge is included in the evaluation, but not reported separately
- Immune infiltrates outside of the tumor borders, e.g. in adjacent normal tissue or DCIS are not included



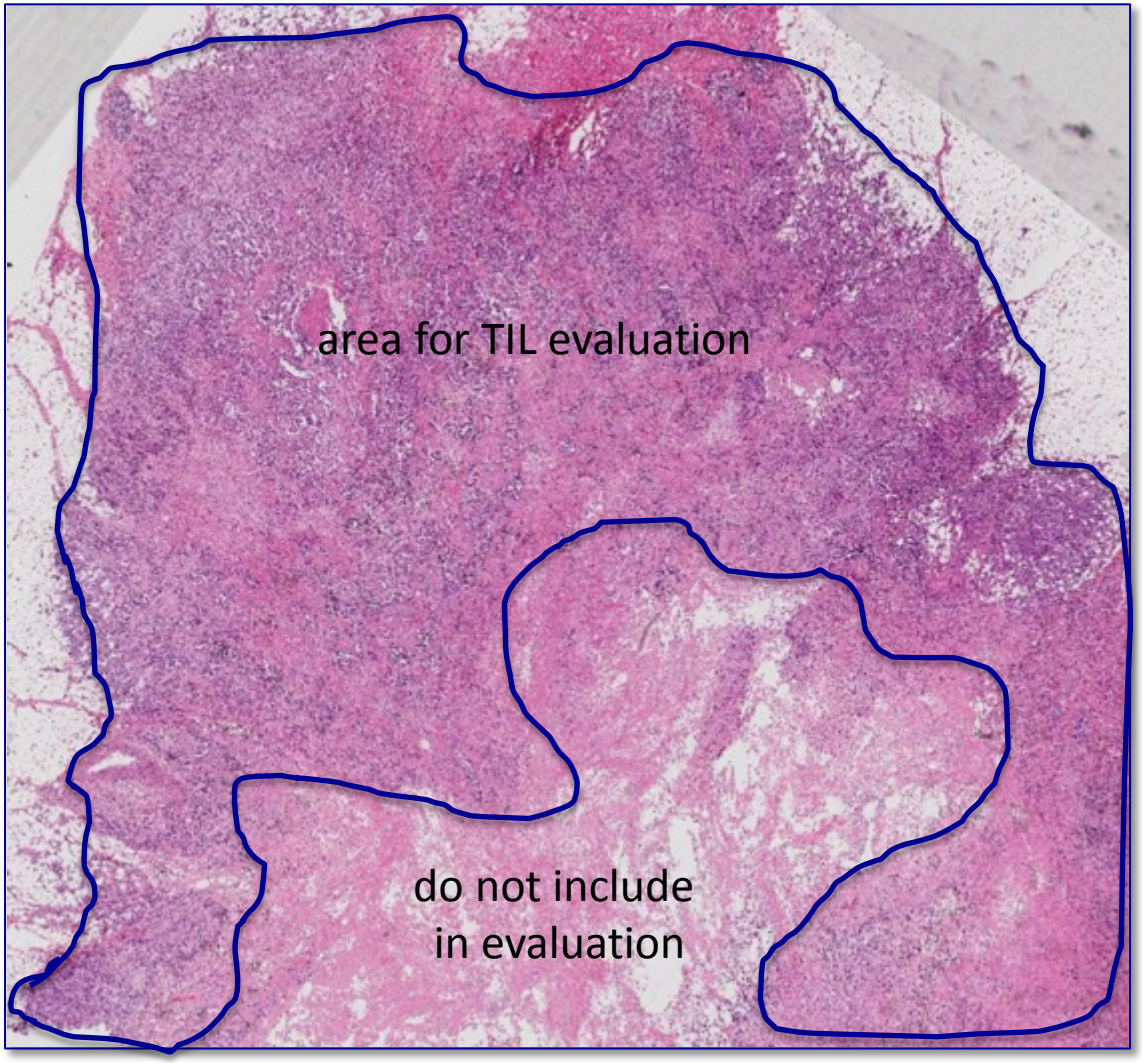
Example 1



Example 2

Step 1: Define area for TILs evaluation

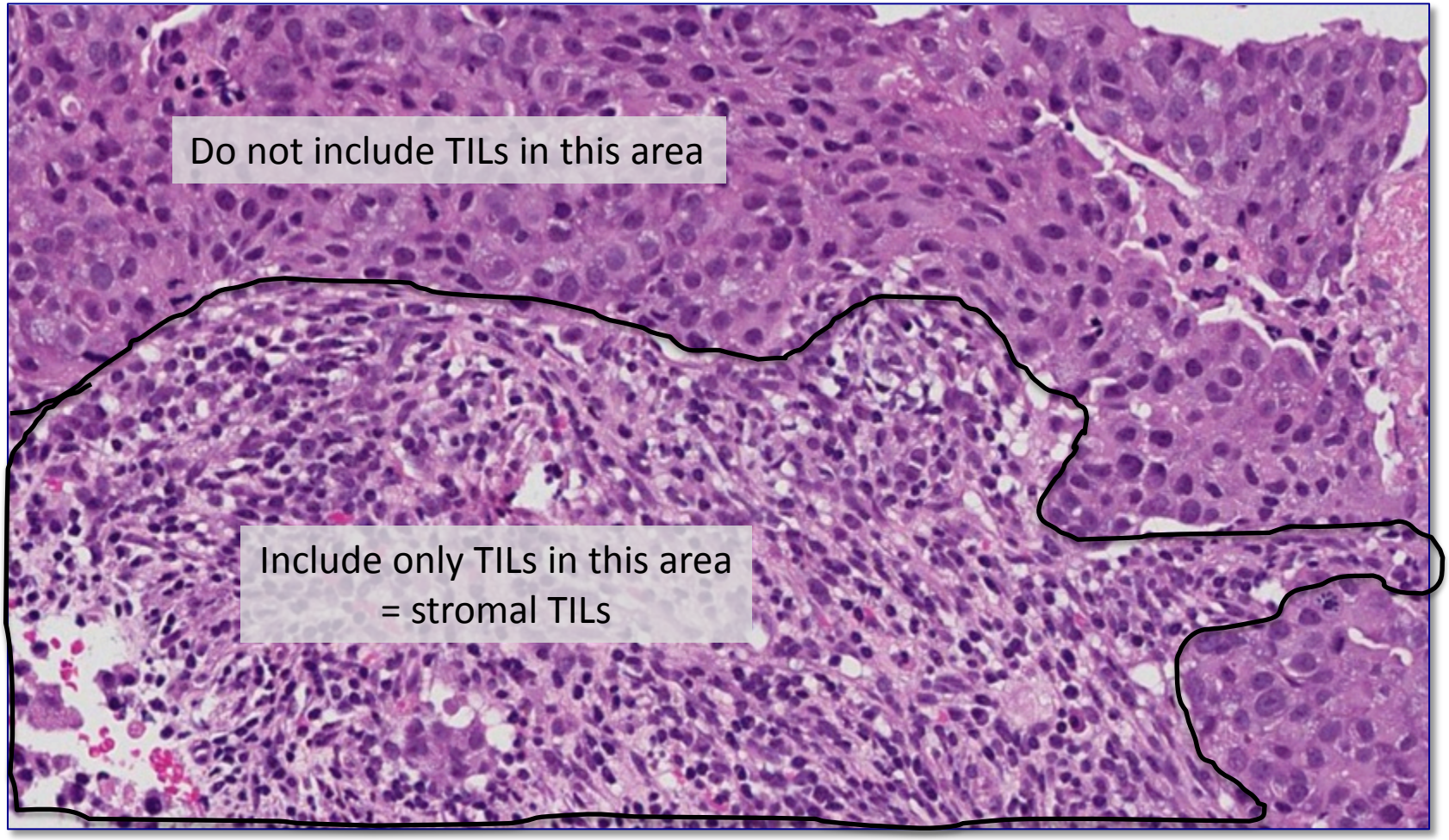
- Large areas of central necrosis or fibrosis are not included in the evaluation



Example 3

Step 2: Focus on stromal TILs

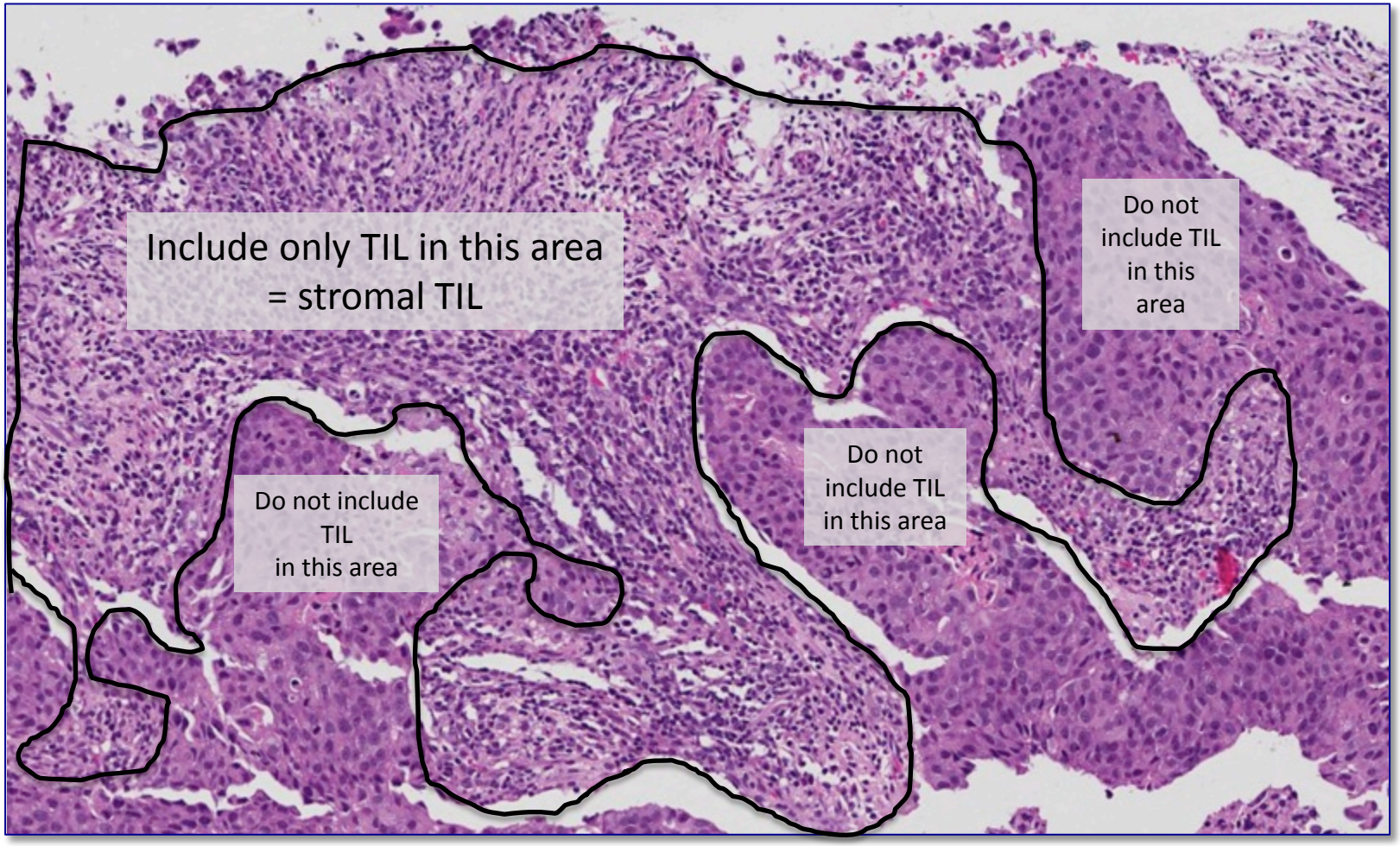
- In the diagnostic setting, only stromal TILs are relevant



Example 4

Step 2: Focus on stromal TILs

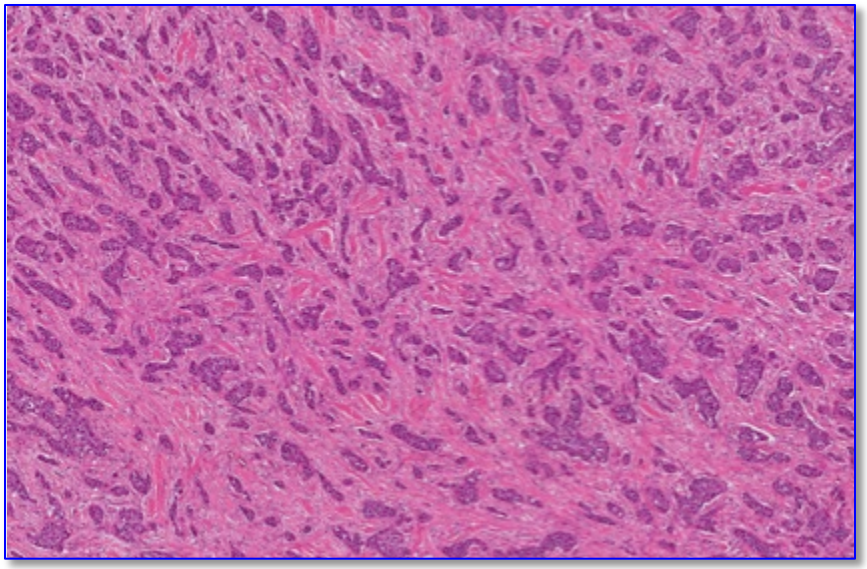
- in the diagnostic setting, only stromal TILs are relevant



Example 5

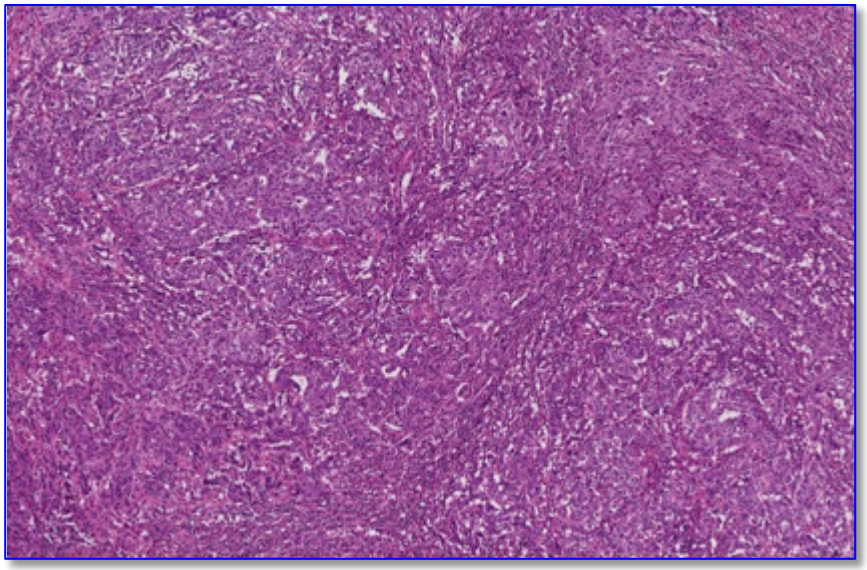
Step 2: Scan tumor at low magnification – focus on the tumor stroma

- Stroma contains predominantly collagenous tissue, few round cells



Example 6

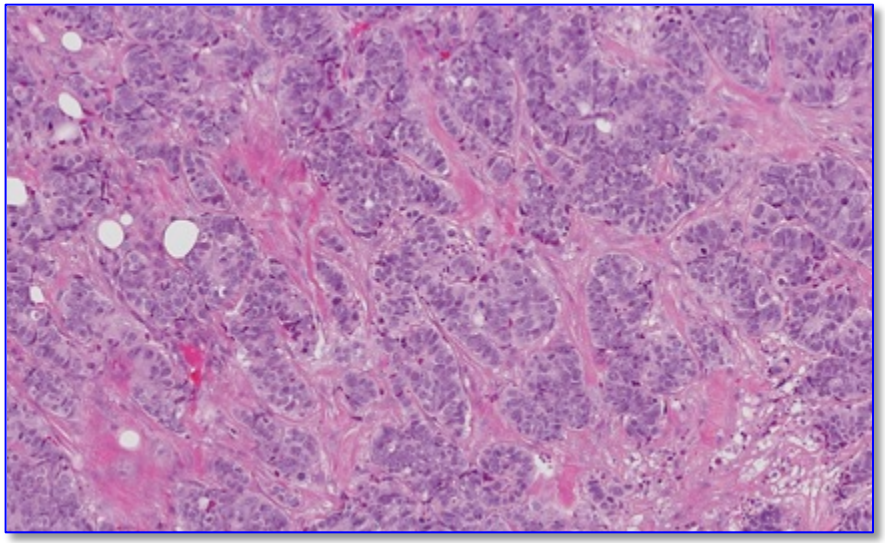
- Stroma contains predominantly round cell infiltrate, collagenous tissue difficult to recognize



Example 6

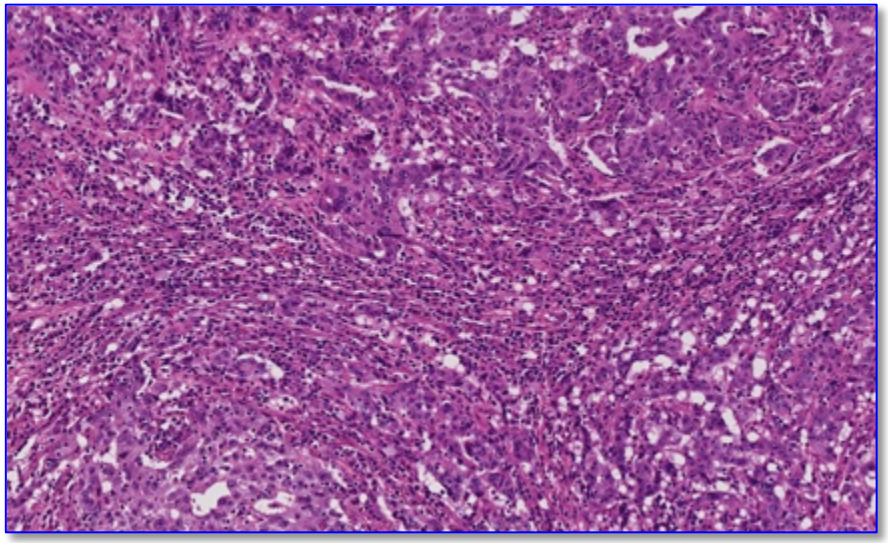
Step 2: Scan tumor at low magnification – focus on the tumor stroma

- Stroma contains predominantly collagenous tissue, few round cells



Example 8

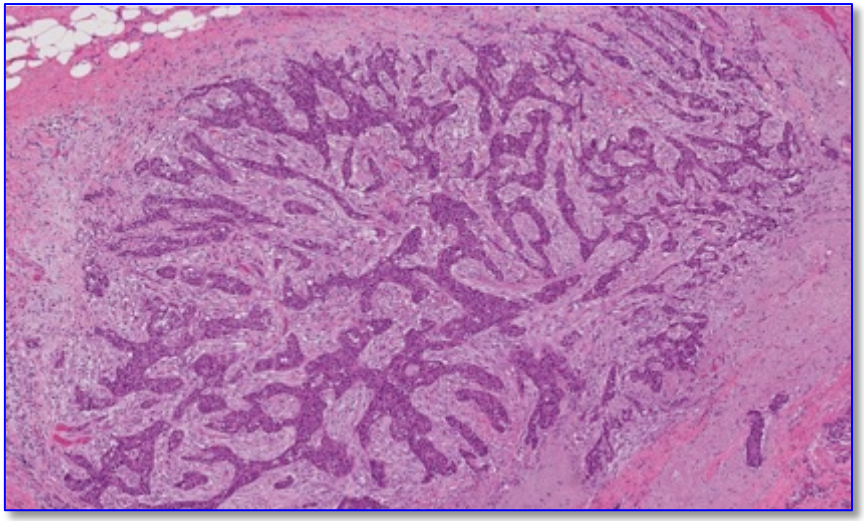
- Stroma contains predominantly round cell infiltrate, collagenous tissue difficult to recognize



Example 9

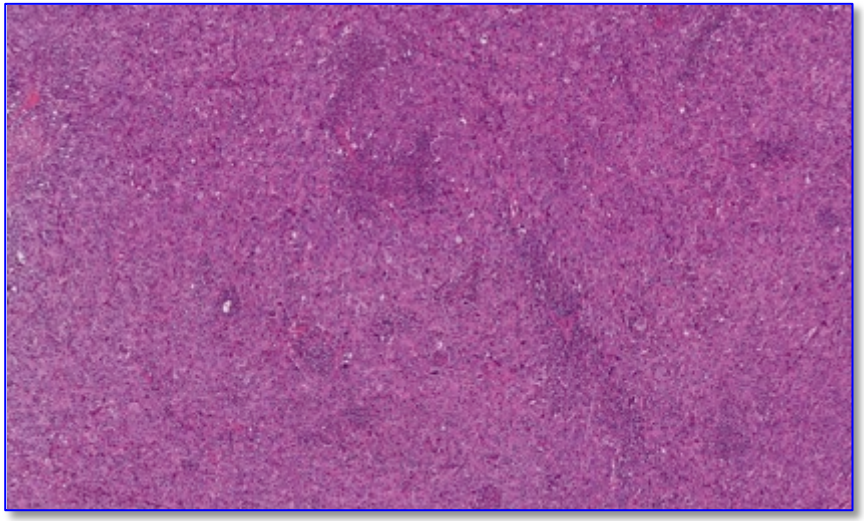
Step 2: Scan tumor at low magnification – focus on the tumor stroma

- Stroma contains predominantly collagenous tissue, few round cells



Example 10

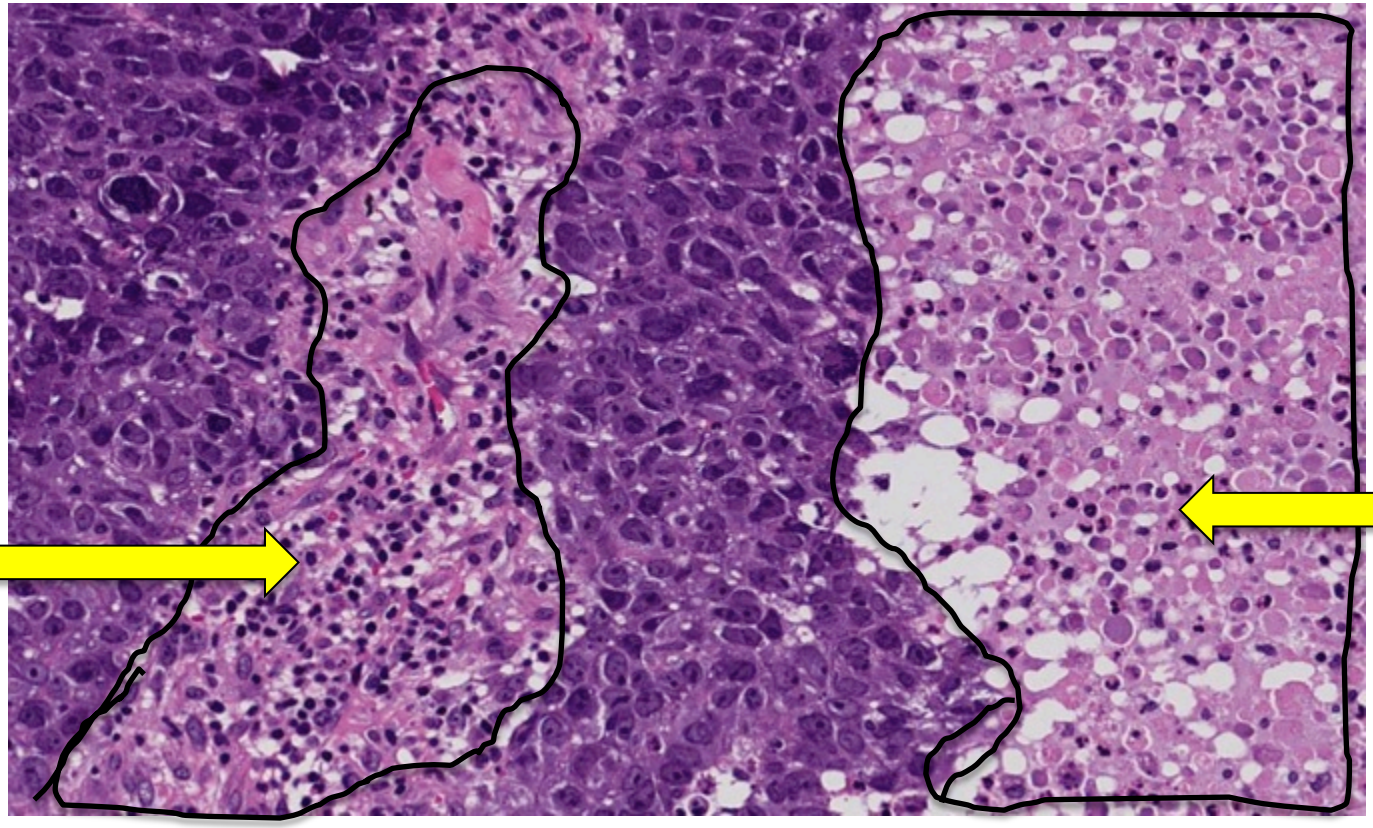
- Stroma contains predominantly round cell infiltrate, collagenous tissue difficult to recognize



Example 11

Step 3: Determine type of inflammatory infiltrate

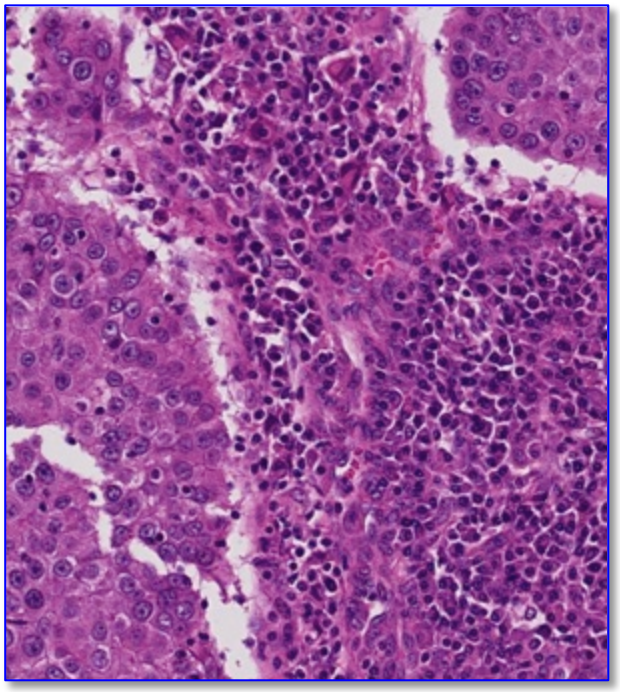
- Include only mononuclear infiltrate (lymphocytes & plasma cells)
- Do not include granulocytic infiltrate in areas of tumor necrosis



Example 12

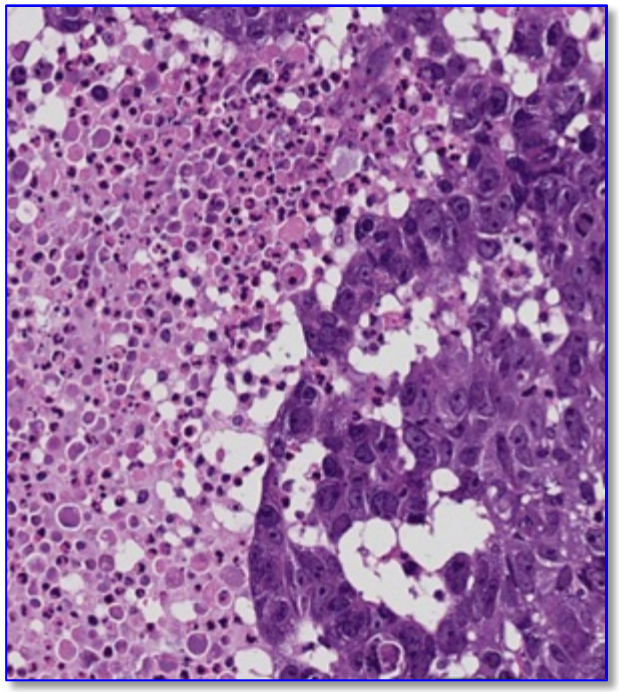
Step 3: Determine type of inflammatory infiltrate

- Include only mononuclear infiltrate (lymphocytes & plasma cells)



Example 13

- do not include granulocytic infiltrate in areas of tumor necrosis



Example 14

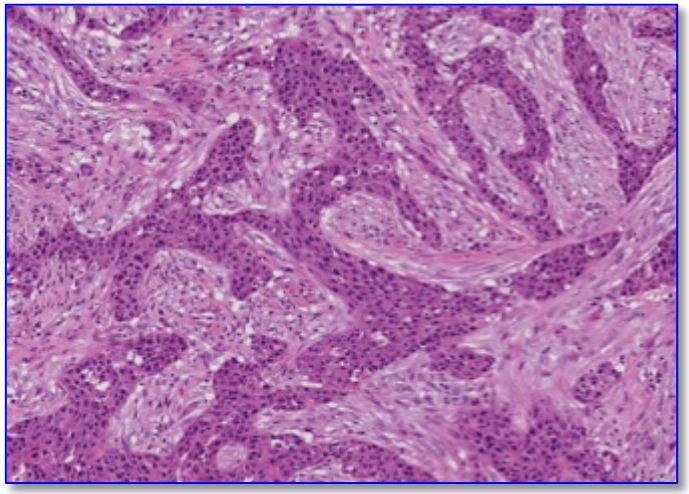
Step 4: As a first approach, include tumor in one of three groups based on low magnification and assess % stromal TILs (continue with Step 5 for percentage)

Group A: tumor with no/minimal immune cells

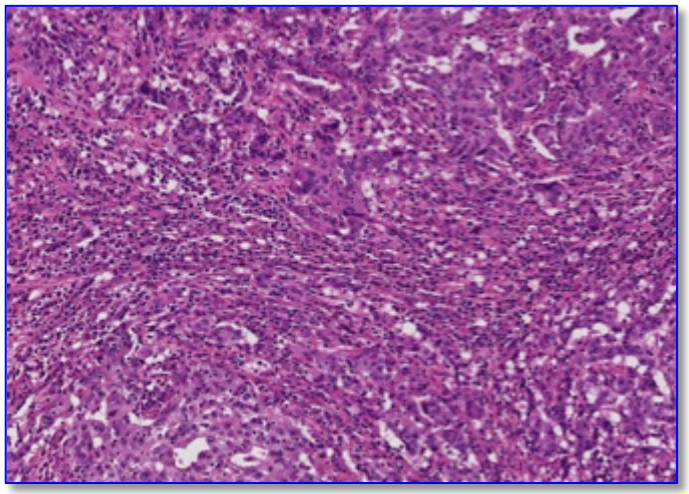
Group B: tumor with intermediate / heterogeneous infiltrate

Group C: tumor with high immune infiltrate

0-10% stromal TILs | 10-40% stromal TILs | 40-90% stromal TILs



For this intermediate group evaluate different areas at higher magnification.

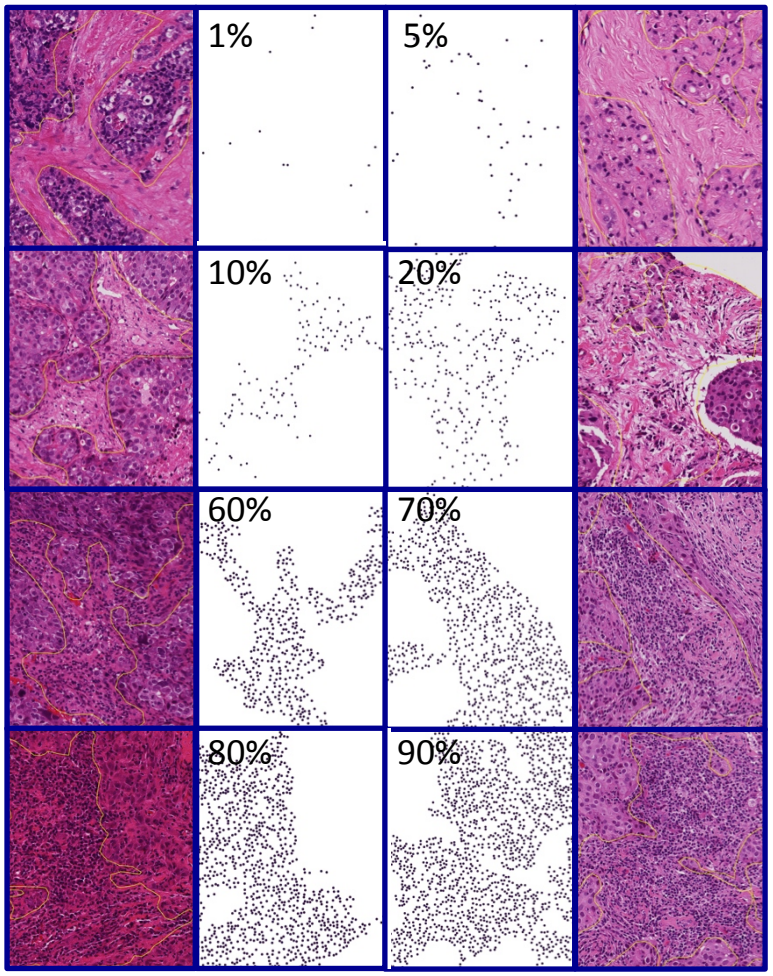


Example 15

Example 16

The denominator used to determine the % stromal TILs is the area of stromal tissue (i.e. area occupied by mononuclear inflammatory cells over total intratumoral stromal area), not the number of stromal cells (i.e. fraction of total stromal nuclei that represent mononuclear inflammatory cell nuclei)

Step 5: Report percentage of stromal lymphocytes



- Report the average of the stromal area, do not focus on hot spots.
- For intermediate group evaluate different areas at higher magnification.
- Please note that lymphocytes to not form solid aggregates, therefore even with 90-100% stromal TILs there will still be some space between the individual lymphocytes.

Please send any questions or comments to:

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