Evaluation of Tumor Infiltrating Lymphocytes (TILs) in Pleural Malignant Mesothelioma (PMM)

Guidelines for TILs assessment from the "International Immuno-Oncology Biomarker Working Group"

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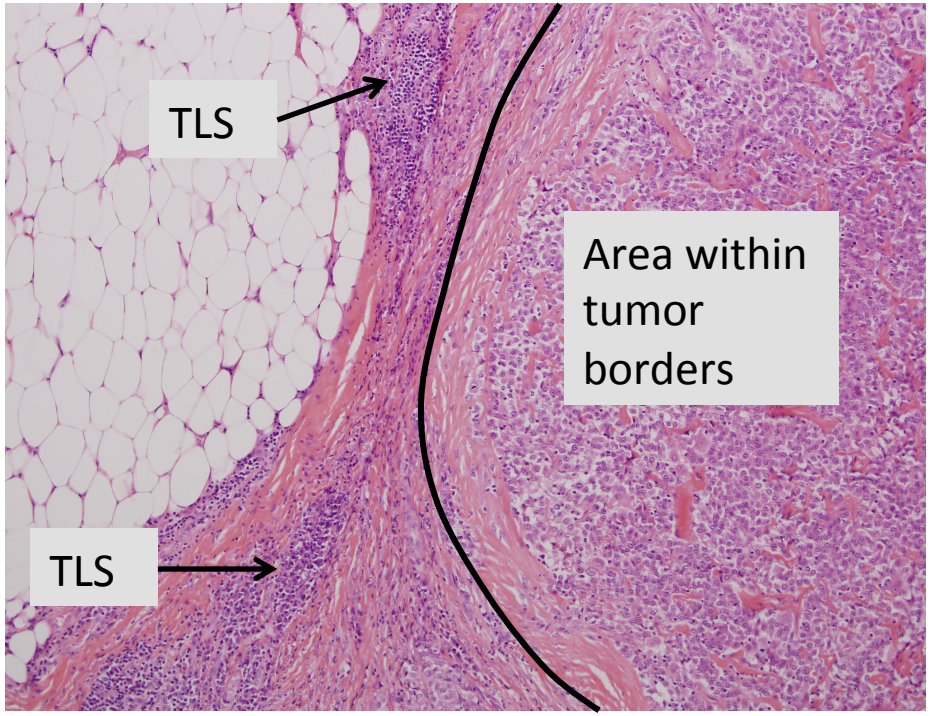
Aim of this tutorial

- To provide an outline of an exploratory method we used to assess tumor infiltrating lymphocytes in pleurodesis biopsy and resection specimens of PMM
- We assessed stromal TILs because these were far more prominent in our cases than intra-tumoral TILs, and stromal TILs have been shown to be of more relevance in some solid organ tumors e.g. breast cancer
- We separately assessed tertiary lymphoid structures outside the tumor, mostly seen along the interface between the parietal pleura and subpleural fat

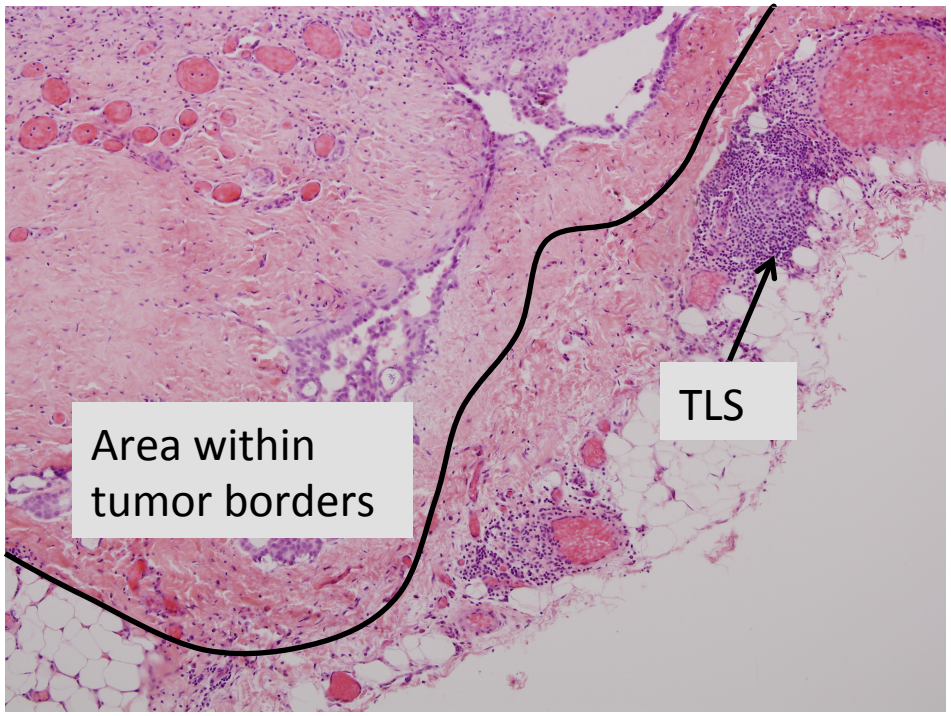
Step 1: Define the area for TILs assessment

- Only stromal TILs within the borders of the tumor are included in the assessment of TILs
- The invasive or pushing edge of the tumor is included in the TILs assessment
- Immune infiltrates i.e. tertiary lymphoid structures (TLS) outside the tumor are not included in the stromal TILs assessment (see later)

Example 1



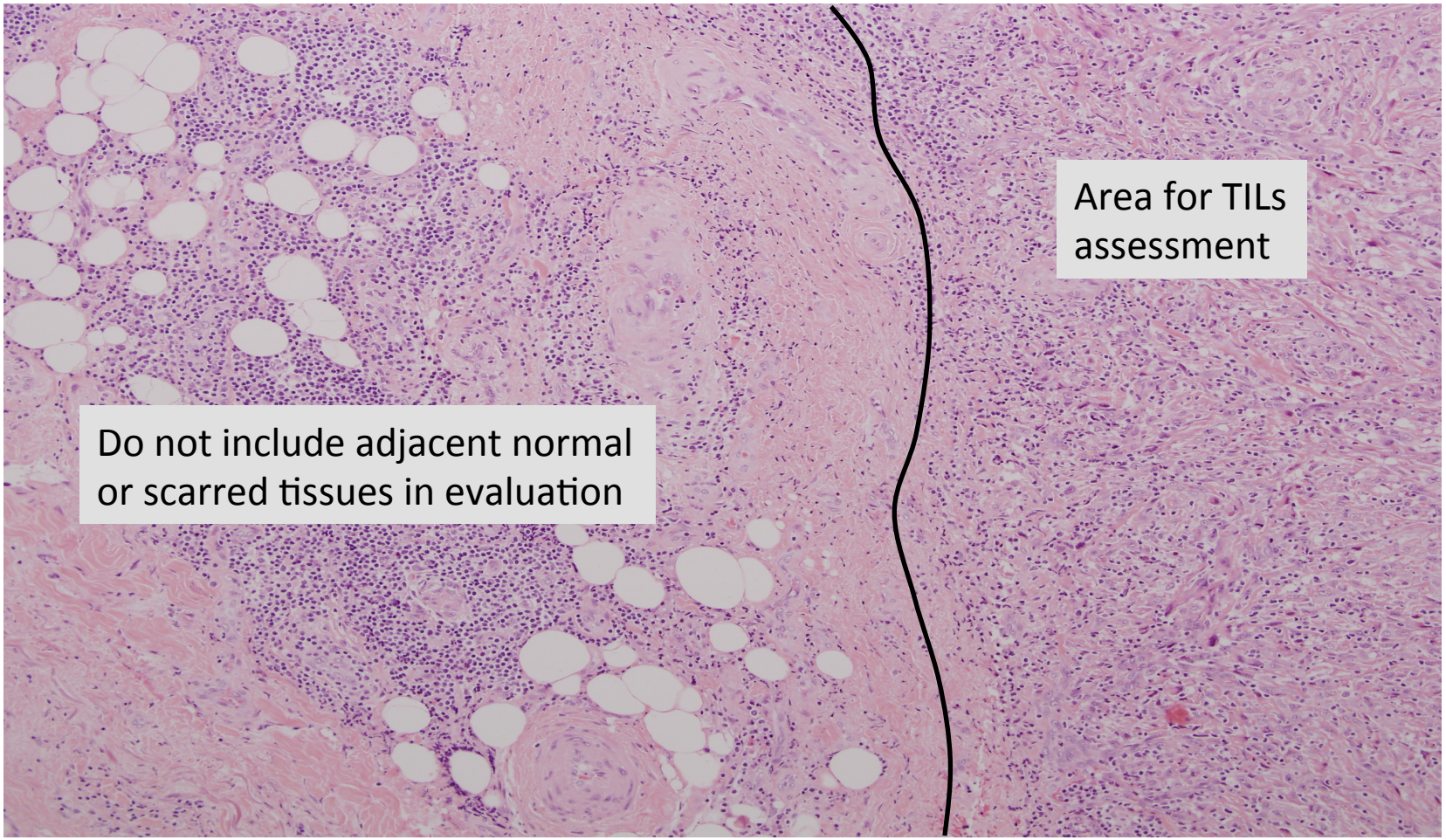
Example 2



Step 1: Define the area for TILs assessment

- Large areas of necrosis, fibrosis/scar or adjacent normal tissues are not included in the evaluation

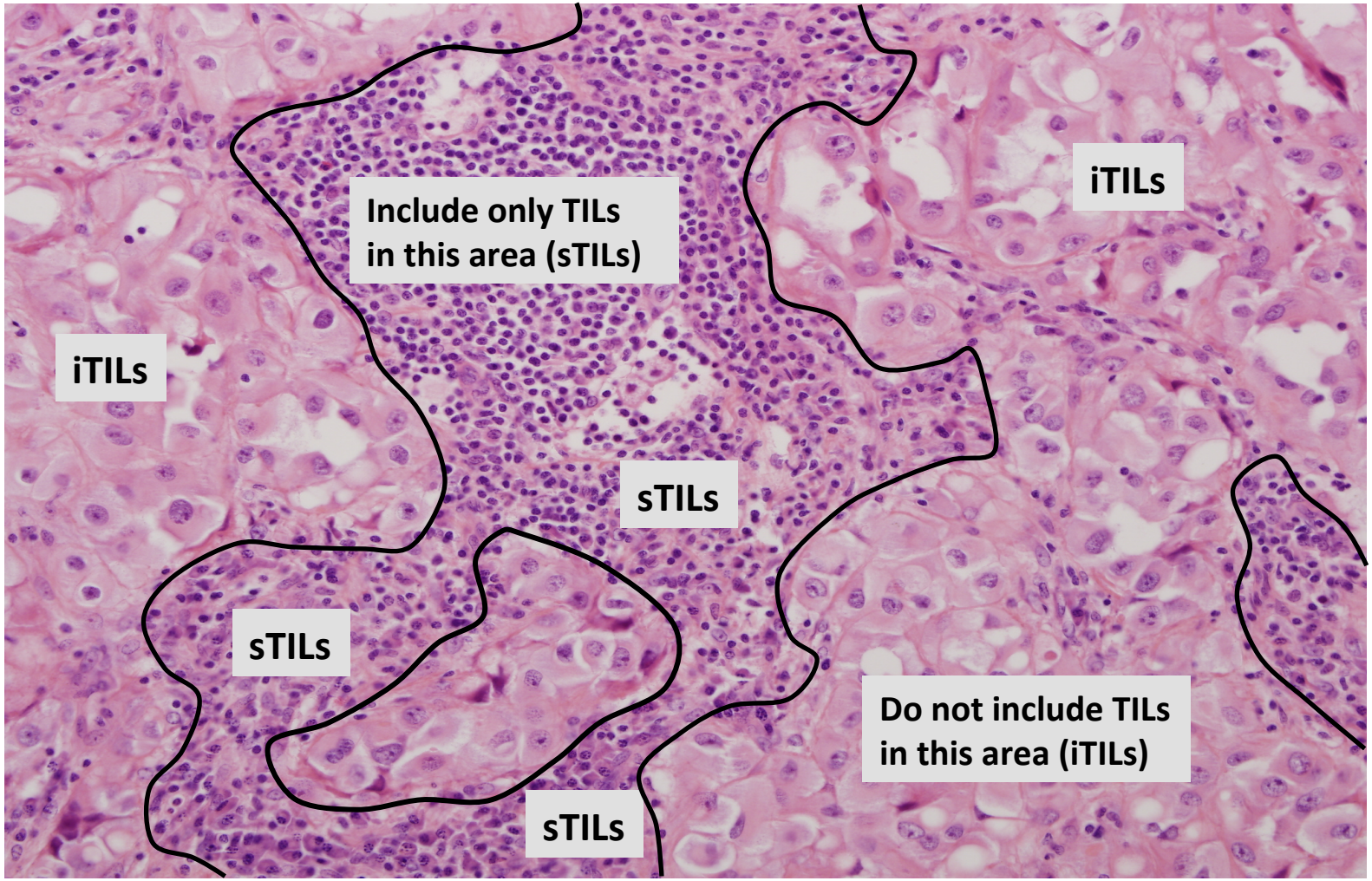
Example 3



Step 2: Focus on the stromal TILs

• Based on literature showing that only stromal TILs are relevant in some solid organ tumors, we focused solely on stromal TILs in PMM

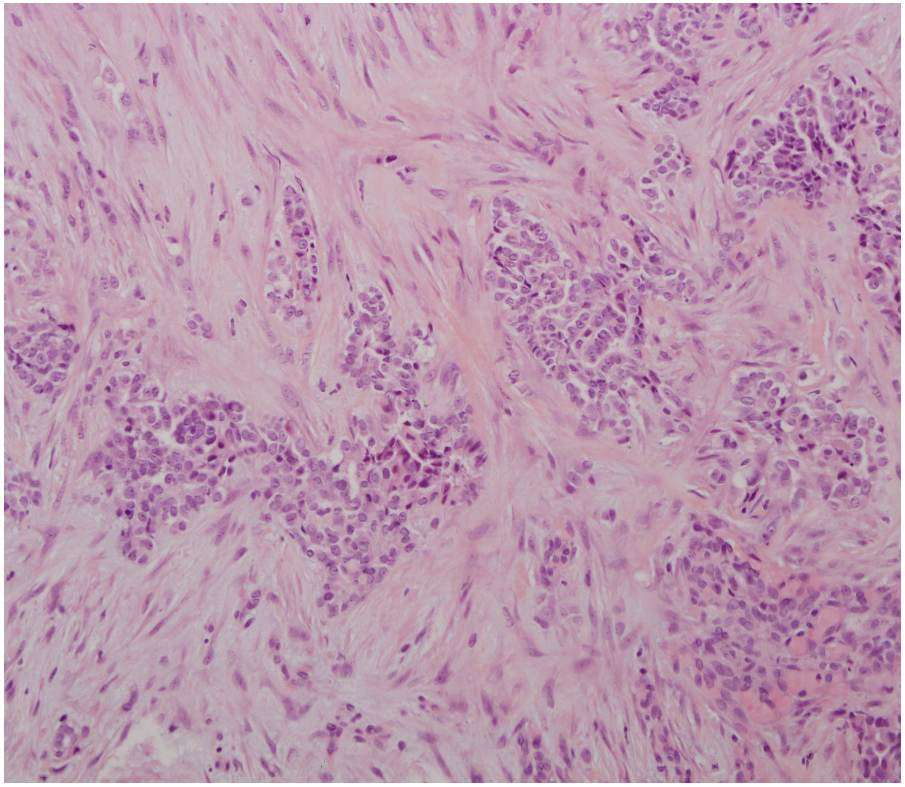
Example 4



Step 2: Scan tumor at low power, identifying the stromal areas within the borders of the tumor

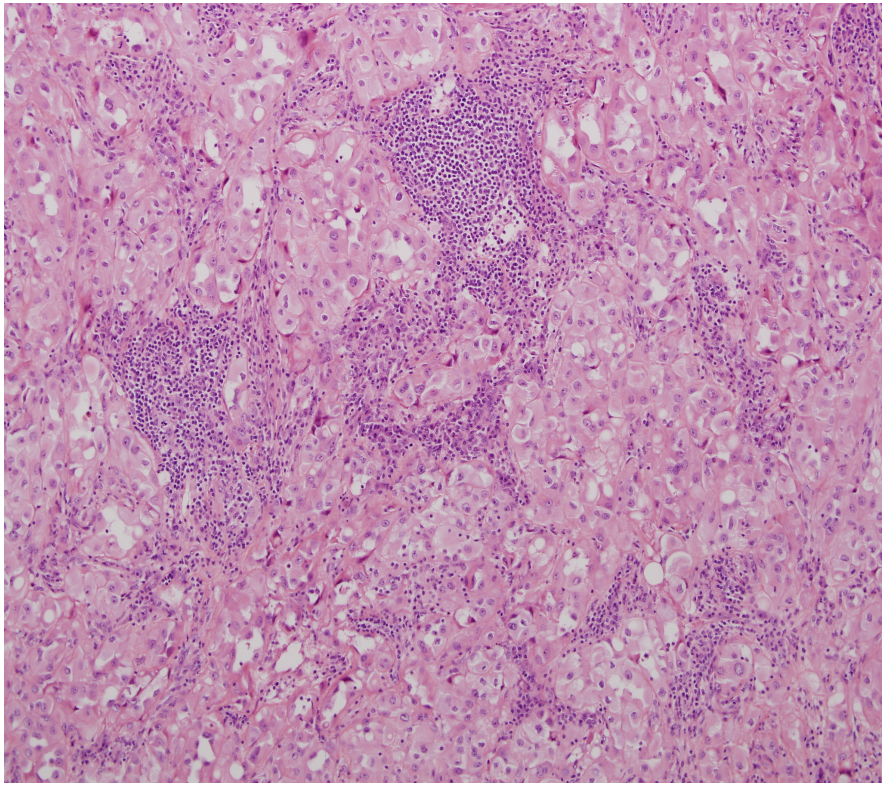
- Stroma contains mostly fibrous tissue with few TILs

Example 5



- Stroma contains mostly TILs, with fibrous stroma difficult to identify

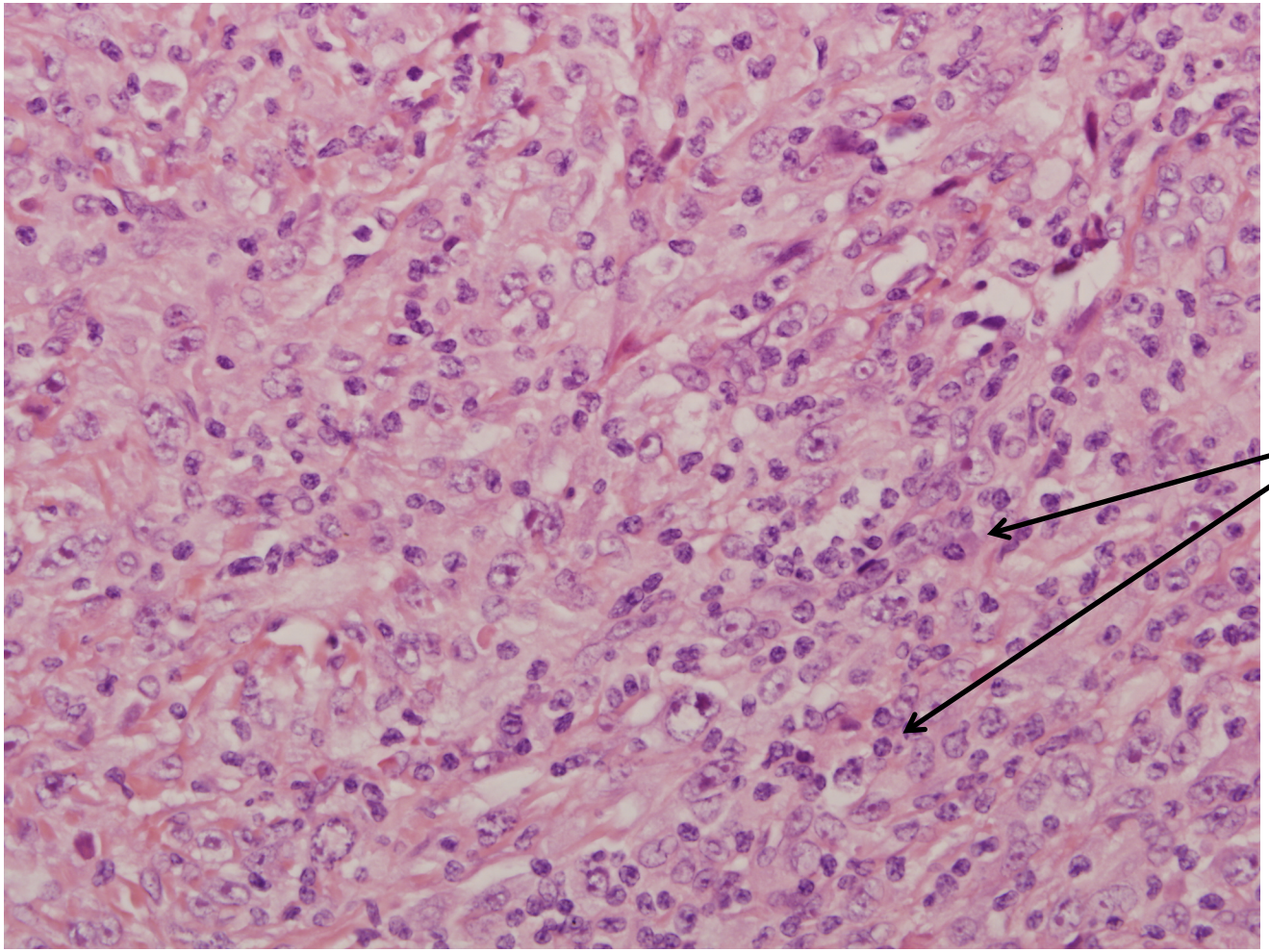
Example 6



Step 3: Determine the type of inflammatory infiltrate

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

Example 7



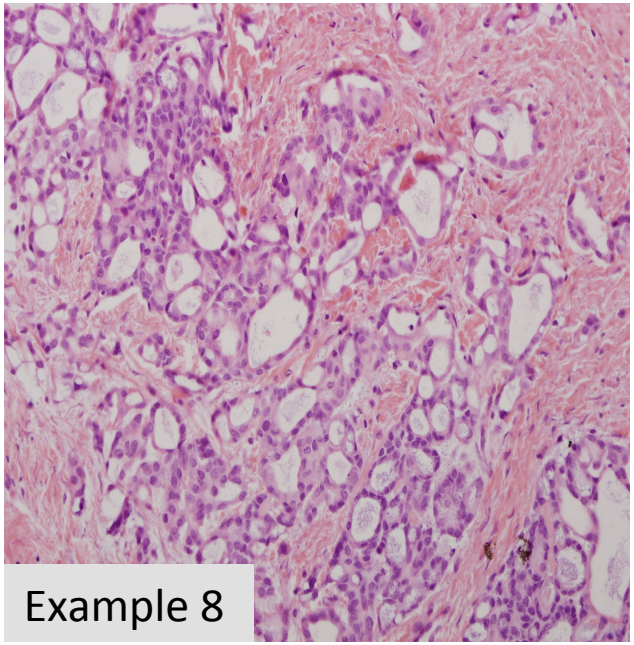
Step 4: To start with, include tumor in one of three groups on low magnification and assess % stromal TILs

Group 1: tumor with no / minimal TILs

Group 2: tumor with Intermediate/heterogeneous TILs

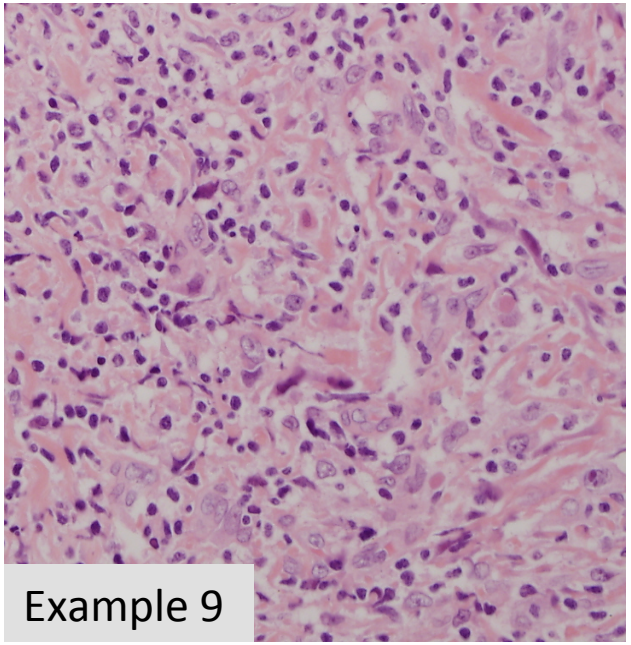
Group 3: tumor with high TILs

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



Example 8

For the intermediate group evaluate different areas at higher magnification



Example 9

The denominator used to determine the % of stromal TILs is the area of stromal tissue (i.e. area occupied by TILs 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: Assessment of TLS

- We assessed simply the presence or absence of tertiary lymphoid structures in the tissues outside the tumor area
- In PMM, these mostly consisted of TLS present along the interface between the parietal pleura and the underlying subpleural fat

Example 10