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

A tutorial prepared by the International Working Group for TIL in breast cancer - 2014

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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 TIL 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

do not include immune infiltrate outside of the tumor
Step 1: Define area for TIL evaluation

- Large areas of central necrosis or fibrosis are not included in the evaluation.
Step 2: Focus on stromal TIL

- In the diagnostic setting, only stromal TILs are relevant
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Step 2: Scan tumor at low magnification – focus on the tumor stroma

- Stroma contains predominantly collagenous tissue, few round cells
- 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
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Example 8

Example 9
Step 2: Scan tumor at low magnification – focus on the tumor stroma

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

Example 10

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
Step 3: Determine type of inflammatory infiltrate

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

<table>
<thead>
<tr>
<th>Group A: tumor with no/minimal immune cells</th>
<th>Group B: tumor with intermediate / heterogeneous infiltrate</th>
<th>Group C: tumor with high immune infiltrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10% stromal TILs</td>
<td>10-40% stromal TILs</td>
<td>40-90% stromal TILs</td>
</tr>
</tbody>
</table>

For this intermediate group evaluate different areas at higher magnification.

Example 15

Example 16
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 do not form solid aggregates, therefore even with 90-100% stromal TILs there will still be some space between the individual lymphocytes.
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