Relationship between Tumor Infiltrating Lymphocytes and Progression in Breast Cancer

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\textbf{ABSTRACT}

The immune system is very critical in suppression of cancer development. The composition of breast cancer-infiltrating lymphocytes has been widely investigated so far and it is accepted that the presence of high tumor-infiltrating lymphocytes (TILs) is associated with improved prognoses. The correlation between various TIL subpopulations and experimentally observed responses is still characterized by a certain grade of controversy. Modulating the immune system is promising treatment strategy in breast cancer, particularly in HER2 positive and triple-negative molecular subtypes. However, there is still much to be done to implement immunotherapy in this disease.

\textbf{Keywords:} tumor-infiltrating lymphocytes, breast cancer, immunotherapy, HER2.

\textbf{INTRODUCTION}

Breast cancer is the second leading cause of female cancer-related death worldwide. The role of the immune system in the context of this type of solid tumor has been at the center of a large debate during the last decades.

Tumor infiltrating lymphocytes (TILs) has been observed mostly in a number of presented breast cancer cases. The developing forms of cancers that have a negative status enlargement of the axillary lymph nodes characteristic to a relatively small size of tumor and usually low grade ones are studied by use of TILs. Tumor infiltrating lymphocytes have a negative correlation with the age of a patient and their count is positively associated with the survival period of the affected patients with the presence or absence of estrogen receptors. TILs are the predictive and potential prognostic markers in breast cancer. When a local relatively developed breast cancer is treated with neoadjuvant chemotherapy, any occurrence of tumor infiltrates of lymphocytes is an evident predictor of the response.

T cells account for 75\% of TILs. Among these, CD8\(^+\) T cells represent the class of lymphocytes that correlate better with overall favorable clinical outcomes, usually infiltrating breast lesions in the largest proportion. It has been widely documented that the presence of high rates of infiltrating CD8\(^+\) T cells is associated with overall longer survival rates. Natural killer cells (NK) serve as the first-line defense in association with CD8\(^+\) T lymphocytes. NKs are found to infiltrate breast lesions in a proportion ranging around 5\% of the total lymphocytic population. Recently, it has been observed that low levels of NK cells can be apparently related to more unfavorable clinical outcomes. CD4\(^+\) lymphocytes...
represent a good prognostic factor as well being usually associated with overall better clinical outcomes.

**TILs predictive factors in the neoadjuvant or adjuvant treatment**

The neoadjuvant treatment is commonly considered to be the best model for the evaluation of interactions between anti-cancer drugs, tumor microenvironment and patient’s response to treatment. For breast cancer, the first observation of a positive connection between the presence of TILs and favorable clinical outcomes has been reported by Denkert et al. (2010), who documented a linear correlation between high levels of TILs, especially T cells, and clinical/radiological responses to anthracycline-based neoadjuvant-chemotherapy (NAC) regimens. Since this pivotal study, the predictive role of TILs with regard to the success of NAC has been sustained by a considerable number of studies, often focused on HER2-positive and triple negative breast cancer (TNBC) molecular subtypes. A meta-analysis of studies examining TILs in early stage triple negative breast cancer confirmed that increased infiltrates were associated with a better disease-free survival, distant disease-free survival and overall survival. In Her2-positive early breast cancer treated in a phase III trial, a higher number of TILs was predictive for benefit from trastuzumab treatment. Patients with higher numbers of stromal TILs in their tumors had a significant better survival when treated with trastuzumab compared with those having high TILs but no trastuzumab treatment. In patients with low TILs, addition of trastuzumab in their adjuvant chemotherapy treatment did not affect outcome. These results argue for an important role of antibody-mediated cytotoxic immune response as a mechanism of trastuzumab action in Her2-positive breast cancer.

The presence of TILs in residual disease after NAC is associated with better prognosis in triple negative breast cancer. This suggests that neoadjuvant chemotherapy can convert low TILs tumors in high/intermediate TILs tumors.

The evaluation of programmed death-ligand (PD-1/PD-L1) expression, in parallel to TIL count and characterization, can be a valid parameter allowing the selection of those patients who might potentially benefit from immunotherapy agents like Pembrolizumab or Atezolizumab.

In one study, Park et al. investigated the correlation between TIL presence and PD-L1 expression in 333 early-stage BC patients. Specifically, the authors focused on CD8+ T and CD4+ T lymphocytes cells and analyzed the eventual correlation between the two populations with regard to PD-L1 expression levels on tumor cells. While no correlation has been documented between CD4+T and PD-L1, CD8+ T cells have been found to be associated with low expression levels of PD-L1. Moreover, the amount of CD8+ T cells has been linearly related to higher infiltration of CD4+T; thus, increased amounts of infiltrating CD8+ T cells have been positively associated with higher rates of CD4+T and, interestingly, with lower expression levels of PD-L1 on tumor cells. Therefore, these data suggest that future neoadjuvant clinical trials aiming to evaluate the anti-tumor activity of anti PD-L1 agents should be performed on patients with low TIL scores and higher tumor PD-L1 expression levels.

**The prognostic value of TILs**

The potential clinical utility of TILs is not officially recommended now as a valid prognostic factor, but several studies have examined TILs independently of immune blockade molecules as prognostic markers influencing breast cancer outcomes in chemotherapy trials.

At the 2015 St Gallen Consensus Conference, the recommendation of TIL evaluation was clearly denied, mainly because standardized procedures for their isolation and characterization are lacking.
and no clinical validation and data reproducibility have been documented so far. In parallel to the publication of the consensus, an international expert team with proven experience in TIL analyses (the International TIL Working Group) developed and published a standardized method which might definitively pose the bases for harmonized and clear TIL determination in the clinical setting for diagnostic purposes. The guidelines stated by the Working Group also take into consideration the standardized guidelines for TIL counts, therefore allowing a better reproducibility of the data, which are being increasingly accrued (Table 1).

### CONCLUSIONS

TILs are one of the best examples of the strict relationship that exists between natural defenses and carcinogenesis and represent a snapshot of the tumor scenario. TILs can be accordingly seen as an unloaded weapon, whose drug-induced reactivation can lead to a restoration of natural anti-cancer defenses, which were once fully operative.

The evaluation of TILs as novel prognostic and therapy-predicting factors should become a routinely performed analysis, with particular regard to the most aggressive breast lesions, such as the triple-negative and HER2-positive molecular subvariants. Moreover, the molecular evaluation based on the detection of TILs along with the PD-L1 expression would guide clinicians into the choice of the most appropriate therapy.

### TABLE 1. Adapted Recommendations for the evaluation of TILs from the 2014 International TILs Working Group

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<th>Step</th>
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| Step 1. Selection of tumor area | TILs to be analyzed must be located:  
• Inside the borders of invasive tumors, including invasive edges  
TIL evaluation must NOT include  
• Adjoining normal tissue or DCIS:  
• Large areas of necrosis/fibrosis |
| Step 2. Definition of stromal TILs | For diagnostic purposes, only stromal TILs must be considered after careful observation. |
| Step 3. Microscopic observation at low magnification | A magnification of ×200/×400 is considered as optimal |
| Step 4. Determination of type of inflammatory infiltrate | • Only infiltrating mononuclear cells must be considered (lymphocytes and plasma cells);  
• Granulocytes located in necrotic areas must NOT be considered |
| Step 5. Assessment of the amount of TILs | Based on low magnification observation, the amount of stromal TILs must be calculated as a percentage over the analyzed stromal area.  
The lesion must be then included in the following three groups:  
• group A (0%–10% stromal TILs) => represent low TILs  
• group B (10%–40% stromal TILs) => represent intermediate TILs  
• group C (40%–90% stromal TILs) => represent high TILs |

### REFERENCES


