Abstract. Despite the progress of tailored therapeutic strategies in patients with breast cancer, there is an unmet medical need for additional biomarkers that would guide therapy, including the administration of targeted agents. It has been demonstrated that the presence of tumor-infiltrating lymphocytes (TILs) is associated with prognosis in patients with early breast cancer. Moreover, TIL counts were shown to predict outcome of neoadjuvant chemotherapy. The neoadjuvant setting is increasingly used to assess the efficacy of new systemic therapies, and TILs are promising as a biomarker reflecting the immune response to tumor. Future studies should investigate on the integration of TILs as predictive biomarkers in patients treated with targeted agents.

Breast cancer is the most common malignant disorder in women (1). The decrease in mortality rates observed in recent decades despite the rising incidence of this tumor type is due to both early diagnosis and improved therapy, including better systemic treatments. In fact, most patients with early breast cancer are currently cured, and even in patients with metastatic disease, the availability of new treatment options results in significant prolongation of survival.

It is increasingly recognized that rather than a single disease entity, breast cancer presents as a spectrum of malignant disorders affecting the same organ (2-5). The diversity of tumors affecting the breast is being increasingly reflected in the diversification of therapeutic approaches that are based on appropriate biomarkers. Current strategies of medical management of breast cancer are directed principally by the presence and expression of hormone receptors (estrogen receptor and progesterone receptor) and human epidermal growth factor receptor (HER)-2. However, despite this progress of tailored therapeutic strategies in patients with breast cancer, there is an unmet medical need for additional biomarkers that would guide therapy, including administration of targeted agents.

Laboratory medicine plays an increasingly important role in the management of patients with cancer (6). It is now well-recognized that the immune system plays an important role in disease outcome in patients affected by cancer. However, the assessment of immune response to the tumor in individual patients is difficult (7). Malignant tumors are associated with changes of peripheral blood lymphocyte phenotype and function (8, 9). It is, however, evident that changes detected in the peripheral blood may not necessarily reflect the situation in the tumor microenvironment. On the other hand, most evidence suggests that the principal events determining the outcome of the host–tumor relation occur at the tumor site (7). Historically, a number of parameters have been assessed as biomarkers of the host immune response in the tumor microenvironment (10-13). The presence of tumor-infiltrating lymphocytes (TILs) has been recognized as a biomarker of anti-tumor immune response across a wide range of tumors. The presence of TILs has been associated with improved prognosis in epithelial ovarian carcinoma (14, 15), endometrial cancer (16-20), and also breast cancer (21-24).
In the late 1980s and 1990s, TILs were studied as potential effectors of adoptive immunotherapy. TILs were isolated from tumor tissue, cultured in vitro and expanded for subsequent autologous administration. It was demonstrated that these expanded TILs, including TILs obtained from patients with breast cancer, exhibited cytolytic activity against tumor cells that was sometimes restricted to autologous tumor cells (25, 26). The results with administration of TILs in some tumor types, e.g. malignant melanoma, were quite promising (27). However, the preparation and expansion of TILs was very demanding from the perspective of logistics and was not feasible in most patients who would otherwise be candidates for therapy. Moreover, the administration of TILs was usually combined with high-dose interleukin-2 therapy that was associated with significant toxicity. The availability of the source of TILs, i.e. fresh autologous tumor tissue, was the most serious limitation to this approach. For patients with breast cancer, the steady influx of new active drugs, including targeted agents, represented an alternative to experimental immunotherapeutic strategies using TILs, and the study of adoptive immunotherapy with TILs was restricted to tumors for which there were few other treatment options, e.g. metastatic malignant melanoma or renal cell carcinoma (28, 29).

The Biology of Tumor-infiltrating Leukocytes in Breast Cancer

It has been known for decades that breast tumors are associated with significant infiltration by leukocytes. The leukocytic infiltrate consists of different cell populations, including lymphocytes, macrophages, polymorphonuclear leukocytes, dendritic cells or mast cells. These leukocyte populations are responsible for innate as well as adaptive immune responses. Among leukocyte populations infiltrating the tumors, TILs have been studied most extensively.

The composition of leukocytes infiltrating breast tumors was dissected in great detail by Gu-Trantien et al. (30). In the leukocytic infiltrate, T-lymphocytes constituted 75% of cells, the proportion of B-lymphocytes was below 20%, monocytes constituted fewer than 10% of cells, and natural killer and natural killer T-cells made up fewer than 5% of all leukocytes. Over 95% of cluster of differentiation antigen (CD)4+ TILs had a memory cell (CD45RO+) phenotype.

TILs may be distinguished into lymphocytes infiltrating the tumor stroma (stromal TILs) and lymphocytes infiltrating the tumor cell islets (thus being in direct contact with tumor cells) called intra-epithelial (intra-tumoral) TILs (31). Marked differences of TIL content have been described in different breast cancer subtypes. Increased lymphocytic infiltration has been reported in tumors with ductal histology, high grade, absence of expression of hormone receptors, or high expression of the proliferation antigen Ki-67 (22-24, 32). High TIL counts are observed in patients with triple-negative breast cancer (32). The presence of TILs has been shown to inversely correlate with the expression of human leukocyte antigen-G (HLA-G) that may be involved in tumor escape (33). The TIL counts were reported to correlate with the presence of breast cancer stem cells and epithelial–mesenchymal transition (32).

Most TILs are T-lymphocytes (30, 34, 35). B-Lympocytes are less numerous in lymphocytic infiltrates of malignant tumors (36). Consequently, less is known about the role of B-lymphocytes (CD20+) as TIL components. The expression of immunoglobulin κC that is produced by plasma cells has been described to be associated with prognosis in patients with breast cancer (37). Compared to peripheral blood lymphocytes, CD3+ TILs were reported to display an activated phenotype with increased expression of activation markers CD69 and human leukocyte antigen DR (HLA-DR) as well as CC chemokine receptor (CCR)4, CCR5 and CXC chemokine receptor 3 (CXCR3) (34). Mulligan et al. described an association between the expression of the chemokine CXCL10, its receptor CXCR3 and lymphocytic infiltrate that included CD4+, CD8+ and regulatory T-lymphocytes expressing forkhead box P3 protein (FOXP3) (38). In an experimental breast cancer model, it was established that the interaction between receptor activator of NF-κB (RANK) and RANK ligand fostered metastatic spread. RANK ligand was produced by regulatory FOXP3+ TILs (39). The presence of FOXP3+ cells within lymphocytic infiltrates surrounding breast tumors was reported by Gobert et al. (35). It has been demonstrated that these cells exhibit immunosuppressive activity in vitro, are recruited through the CCL22/CCR4 interaction, and the presence of FOXP3+ regulatory cells correlated with the presence of CD3+ TILs and dendritic cells. Gu-Trantien et al. reported high expression of CXCL13 chemokine in breast cancer TILs (30).

High endothelial venules (HEV) are responsible for lymphocyte recruitment in peripheral tissues. An association between blood vessels with HEV phenotype and lymphocytic infiltration has also been observed in different malignant tumor types, including breast cancer (40). As outlined below, the high density of HEV was associated with improved disease-free and overall survival. In a subsequent study, high HEV density was associated with lymphotractin-β produced by mature dendritic cells (41). Compared to invasive ductal carcinoma, higher HEV density was observed in ductal carcinoma in situ (DCIS). Progression of DCIS to invasive carcinoma was accompanied by decreased density of HEV, dendritic cells and CD3+ TILs (41).

Indoleamine-2,3-dioxygenase (IDO) may be one of the pivotal enzymes determining the outcome of the interaction between the components of leukocytic infiltrate of the
tumors. IDO catalyzes the conversion of tryptophan to kynurenine. Tryptophan depletion may have an inhibitory effect on both tumor cells and lymphocytes. Moreover, kynurenine has a direct cytotoxic activity against tumor cells (42). Thus, depending on other factors, IDO activity may both inhibit or enhance tumor growth and anti-tumor immune responses, and may result in improved as well as worse outcome. Jacquemier et al. reported IDO mRNA overexpression in basal-like breast cancer, specifically in tumors with medullary histology, compared to luminal A tumors (43). The expression of IDO detected by immunohistochemistry was associated with medullary histology and lymphocytic infiltration. IDO expression detected immunohistochemically or at the mRNA level was associated with favorable long-term outcome. Yu et al. described IDO expression by myeloid-derived suppressor cells that positively correlated with FOXP3+ TIL counts (44). Myeloid-derived suppressor cells inhibited the proliferation of T-lymphocytes and induced lymphocyte apoptosis by an IDO-dependent mechanism. The induction of IDO is associated with other biomarkers of immune activation, e.g. neopterin. Neopterin concentration that can be determined in the serum or in urine represents a prognostic biomarker. High neopterin concentrations have been associated with poor prognosis across a spectrum of malignant disorders (45-47), including breast cancer (48). In patients with history of breast cancer, neopterin concentrations have been correlated with peripheral blood lymphocytes or some metabolic parameters associated with the risk factors of atherosclerosis (49, 50).

Methods Used To Detect and Study TILs in Breast Cancer

Most methods currently used to detect TILs in tumor tissues are based on immunohistochemistry. This approach uses paraffin-embedded tumor specimens, and may be utilized in retrospective analyses on archival samples. Commercially-available monoclonal antibodies are usually used to detect different lymphocyte populations. Secondary antibodies and signal amplification systems are utilized for the visualization of lymphocytes that are then quantified using a microscope.

As outlined below, TILs represent an important predictive and prognostic biomarker in patients with breast cancer. From the technological point of view, the methodology is not complicated, and the assessment of TILs is based on routine immunohistochemical demonstration of several lymphocytic surface antigens and may, therefore, be performed in virtually any pathological laboratory. However, compared to other biomarkers currently used in the management of patients with breast cancer (e.g. hormonal receptors or HER2), the determination of TILs places greater demands on the time and especially on the expertise of the pathologist. Consequently, TIL determination has not attained widespread use in routine practice despite strong data supporting the use of this method, and the study of TILs remains limited to experimental studies. It is worth mentioning that the immunohistochemical detection of TILs is unique compared to any other method as it allows for differentiation of subgroups of TILs in individual compartments of the neoplastic tissue (stromal versus intra-epithelial).

For in vitro studies, TILs were obtained by digestion of the mechanically-dissociated tumor tissue with collagenase. After washing and filtration through nylon mesh the lymphocytes were separated by density gradient centrifugation. Subsequently, TIL were expanded in cell culture medium with interleukin-2 (26). Obviously, the phenotypic and functional properties of TILs were affected by prolonged in vivo treatment, and the principal aim of these studies was to evaluate the potential use of these expanded TIL preparations in adoptive immunotherapy.

The cellular composition of the tumor tissue can be inferred from gene expression profiles, and the presence of TILs may also be studied by gene expression analysis using TIL gene signature (51). Lymphocyte gene expression signatures that predict for prognosis have been also identified (30). Similarly, the expression of immunoglobulin κC has been reported to represent a prognostic biomarker in patients with breast cancer (37).

Neoadjuvant Therapy As a Model to Study New Drugs and Tumor Biology

Neoadjuvant (also called primary or induction) systemic therapy is the treatment of choice in patients with inoperable locally-advanced breast cancer. Neoadjuvant therapy was initially used in patients with inoperable locally-advanced tumors. Based on sometimes remarkable responses in patients with locally advanced tumors, the use of neoadjuvant chemotherapy has expanded. Both chemotherapy and hormonal treatment may be administered in the neoadjuvant setting, but cytotoxic chemotherapy is more commonly used because the responses are more extensive and are induced more rapidly with cytotoxics. Targeted-agents may be administered in the neoadjuvant setting in combination with chemotherapy. The combination of chemotherapy with trastuzumab currently represents the standard approach in neoadjuvant chemotherapy of HER2-positive tumors (52). More recently, activity of the combination of bevacizumab with cytotoxic chemotherapy regimens has been demonstrated in patients with HER2-negative tumors (53, 54).

Based on the results of randomized clinical trials, as well as a meta-analysis indicating that neoadjuvant and adjuvant administration of chemotherapy is equivalent in terms of overall survival (55-58), neoadjuvant chemotherapy is also
being used in patients with initially operable tumors, mostly with the aim of increasing the chance of achieving breast-conserving surgery. Moreover, the neoadjuvant setting is being increasingly used to study the activity of new drugs or new regimens because the primary endpoint of the trial is reached earlier in a neoadjuvant study compared to adjuvant trials or trials in patients with metastatic breast cancer. Virtually all patients treated with neoadjuvant therapy subsequently undergo surgery. The response to systemic therapy, i.e. pathological response, can be assessed with great precision in the surgical resection specimen, and numerous retrospective, as well as prospective, studies have demonstrated that pathological complete response is the most significant prognostic factor in patients with breast cancer treated with neoadjuvant therapy (59-64). Therefore, pathological complete response, meaning the complete disappearance of residual tumor cells, is considered to represent a valid surrogate endpoint for long-term outcomes, including progression-free survival and overall survival, that usually serve as primary end-points in trials in adjuvant or metastatic disease settings. Regulatory authorities are accepting the proof of activity of an experimental agent in neoadjuvant trials with pathological complete response as the primary end-point as the evidence for approval (65), as exemplified for subcutaneous trastuzumab by the HannaH trial (52).

Different classification schemes have been proposed for pathological response assessment. The definitions of pathological complete response also differ, with it being defined either as a complete disappearance of tumor cells or as no residual invasive cancer. The classification of Chevallier is one of the commonly used schemes for pathological complete response evaluation (66). The rate of pathological complete response obviously differs based on the classification used, being more common with the use of less strict criteria (absence of invasive cancer with possible presence of residual in situ component). Most importantly, the pathological complete response rate differs according to the tumor phenotype. While a low pathological complete response rate is observed in patients with hormone receptor-positive, HER2-negative tumors (67-69), markedly higher pathological complete response rates were reported for patients with HER2-positive or triple-negative tumors (70-72). In patients with triple-negative breast cancer, the pathological complete response rate is high, and the prognosis of patients in whom pathological complete response was obtained is excellent. On the other hand, the outcome of patients with triple-negative breast cancer treated with neoadjuvant chemotherapy in whom pathological complete response was not achieved is poor. This contrast between excellent prognosis of patients with pathological complete response and poor prognosis of patient in whom neoadjuvant chemotherapy was less effective is referred to as ‘the triple-negative paradox’ (71).

The pathological complete response rate may also differ according to the particular therapeutic regimen used. In patients with HER2-positive tumors, high pathological complete response rates are observed in patients treated with combination of neoadjuvant chemotherapy and trastuzumab (52, 72, 73). In patients with triple-negative breast cancer, the administration of dose-dense regimens may result in a higher pathological complete response rate (72).

**TILs as Predictors of Response to Neoadjuvant Systemic Treatment in Breast Cancer**

As outlined above, neoadjuvant administration of systemic therapy presents an opportunity for a relatively rapid assessment of therapeutic efficacy of a given regimen. This setting not only allows for evaluation of the predictive role of biomarkers, including TILs, but also enables an assessment of the dynamics of biomarker changes before and after therapy. The administration of systemic chemotherapy to patients with breast cancer is associated with the changes of laboratory parameters indicative of systemic immune activation, including increased concentrations of neopterin (74), or increased numbers of circulating T-lymphocytes (75).

In an early study in a patient cohort of limited size, the numbers of intra-epithelial CD3+ TILs in pre-treatment biopsy were significantly higher in patients who subsequently had pathological complete response (31). Patients who had pathological complete response also had significantly higher dendritic cell (CD83+) counts in pretreatment samples. The potential role of TILs as a biomarker predicting pathological complete response was subsequently confirmed on a much larger cohort of patients (76). In over 1,000 patients enrolled in the GeparDuo and GeparTrio trials, the percentage of tumor epithelial nests containing intratumoral (intra-epithelial) lymphocytes was an independent predictor of pathological complete response in the multivariate analysis (76). The pathological complete response rate was 40% in patients with tumors characterized by high lymphocytic infiltration, but only 7.2% in those with tumors without lymphocytic infiltrate (76). Recently, other reports have confirmed the predictive role of TILs in patients with breast cancer undergoing neodjuvant chemotherapy. In a study in 68 patients treated with anthracycline- and taxane-based regimens, Yamaguchi et al. found the number of TILs to be a significant predictor in pathological complete response in both univariate and multivariate analyses (77).

As mentioned above, the presence of TILs differs according to the breast cancer subtype. Increased presence of TILs has been reported to be associated with ductal histology, high grade, absence of hormone receptor expression and high expression of the proliferation antigen Ki67 (23, 24). Marked lymphocytic infiltration is observed in significant proportion of patients with triple-negative...
breast cancer. As the pathological complete response rate is also high in triple-negative breast cancer (72, 78), some studies have analyzed the association of TILs with pathological response to neoadjuvant chemotherapy in patients with this tumor subtype. Ono et al. observed a statistically significant association between pathological response and TIL score that was evident in patients with triple-negative breast cancer, but not in patients with other tumor types (78). In patients with estrogen receptor-negative tumors, high expression of an eight-gene TIL signature was also reported to be associated with pathological complete response in patients treated with neoadjuvant anthracycline-based regimen (51). High numbers of TILs detected by immunohistochemistry were associated with pathological complete response in patients with both estrogen receptor-negative, HER2-positive and estrogen receptor-negative, HER2-negative tumors (51).

While most of the studies defined TILs as CD3+ lymphocytes, other reports have tried to identify the lymphocytic subsets that comprise TILs. Oda et al. reported that among 180 patients treated with an anthracycline- and taxane-based regimen pathological complete response was significantly associated with high numbers of TILs expressing CD8 or regulatory T-cell phenotype characterized by the expression of FOXP3 (79). The presence of FOXP3+ TILs was an independent predictor of pathological complete response in multivariate analysis. Seo et al. observed higher numbers of CD4+, CD8+ and FOXP3+ TILs in patients who had pathological complete response (32). CD8+ TILs were independent predictors of pathological complete response. Follicular helper T-cell and T-helper 1 gene expression signatures defined by Gu-Trantien et al., as well as the expression of CXCL13 gene, were shown to predict pathological complete response after neoadjuvant chemotherapy (30).

Most recently, the predictive significance of TILs was confirmed prospectively in over 300 patients enrolled in the PREDICT study conducted as part of the GeparQuinto trial (80). Lymphocyte-predominant breast cancer and stromal TILs were independent predictors of pathological complete response in multivariate analysis, while intra-tumoral TILs were associated with pathological complete response only in univariate analysis.

**Dynamics of TILs During Neoadjuvant Therapy and Subsequent Course of Disease**

The availability of tumor samples before and after treatment in patients undergoing neoadjuvant chemotherapy offers a unique opportunity to study changes in leukocyte infiltration associated with therapy. Unfortunately, a major drawback of this strategy is the fact that these changes are difficult to evaluate in the subset of patients that may be regarded as most interesting, i.e., patients who experience pathological complete response, as there is no tumor in surgical resection specimens and consequently TILs cannot be analyzed.

Compared to resection specimens obtained in chemotherapy-naive patients, the surgical tumor specimens from patients after neoadjuvant chemotherapy are characterized by increased infiltration by myeloid-lineage leukocytes, including neutrophils and mast cells (34). TILs in patients after neoadjuvant chemotherapy are characterized by an activated phenotype reflected in higher CD8+/CD4+ ratio or granzyme B expression. In studies comparing samples before and after administration of neoadjuvant chemotherapy, marked changes in the leukocytic infiltrate of the tumors have been reported. An increase in the numbers of intra-epithelial CD3+, stromal CD3+ or CD56+ TILs and numbers of dendritic cells (defined as CD83+, CD1a+ or S100+ cells) was reported to be accompanied by a decrease of vascular endothelial growth factor (VEGF) expression and lower numbers of CD68+ monocytes (31). In an early study using semi-quantitative assessment, an association between increased TILs and objective response to neoadjuvant paclitaxel was observed (81). Ladoire et al. examined CD3+, CD8+ and FOXP3+ TILs before and after neoadjuvant chemotherapy in 56 patients (82). Pathological complete response was associated with disappearance of FOXP3+ TILs, while the infiltration of surgical resection specimens by CD3+ and CD8+ TIL was stable. In subsequent investigations, the authors extended this observation to study the association between these changes and prognosis (83). High CD8+ and low FOXP3+ TIL counts after neoadjuvant chemotherapy were associated with improved long-term outcomes in patients with breast cancer treated with neoadjuvant chemotherapy. Pathological stage and CD8+/FOXP3+ TIL ratio were the only significant prognostic parameters in multivariate analysis. A scoring system incorporating the pathological stage and CD8+/FOXP3+ TIL ratio was proposed, and patients with limited residual tumor and favorable CD8+/FOXP3+ TIL ratio had long-term relapse-free survival and overall survival approaching 100% (83). An association between disappearance of peri-tumoral FOXP3+ TILs and pathological complete response has also been reported by Liu et al. (84). The persistence of intra-tumoral FOXP3+ TILs in that study was associated with poor prognosis. The changes of FOXP3+ TILs during neoadjuvant chemotherapy were also studied by Aruga et al. (85). A comparison between FOXP3+ TIL counts before and after neoadjuvant chemotherapy was made, and patients with low FOXP3+ TIL counts in both specimens seemed to have a better prognosis.

Cimino-Mathews et al. published a comparison of TILs in the primary tumor and subsequent metastases in 15 patients (36). The CD3+, CD4+, CD8+, FOXP3+ and CD20+ TILs were less abundant in metastatic lesions compared to...
primaries. Specifically, the trend of lower TILs was evident in brain metastases. While CD3+, CD4+, CD8+ and FOXP3+ TIL counts were higher in triple-negative compared to luminal tumors, in metastatic lesions, TIL counts were lower in triple-negative compared to luminal tumors (36). Interestingly, while a CD8+/FOXP3+ TIL ratio of 3 or more in the primary was predictive of better prognosis, in metastases, a CD8+/FOXP3+ TIL ratio of 3 or more was associated with inferior prognosis.

**TIL as Prognostic Biomarker in Patients with Breast Cancer**

The presence of different leukocyte populations in the breast cancer microenvironment may have different prognostic implications. While lymphocytic infiltration of breast cancer is associated with improved prognosis, plasma cell or other inflammatory cell infiltrates herald poor long-term outcome (22). Numerous studies have indicated that the number of TILs is associated with prognosis in patients with early breast cancer. An analysis of more than 2,000 patients enrolled in the Breast International Group 02-08 trial revealed that increasing numbers of intra-tumoral and stromal TILs were associated with improved disease-free survival, as well as overall survival in patients with estrogen receptor-negative, HER2-negative tumors (23). Among 256 patients with estrogen receptor-negative HER2-negative tumors, the 5-year survival rate was 92% in patients with tumors characterized by lymphocytic infiltration of 50% or more (lymphocyte-predominant breast cancer) compared to 71% in patients with less intense lymphocytic infiltration. In a cohort of more than 1,300 patients with breast cancer, higher CD8+ TIL counts were associated with improved prognosis (24). As mentioned above, the density of TILs is associated with the presence of blood vessels displaying an HEV phenotype. High HEV counts were shown to be an independent predictor of improved disease-free and overall survival (40). In another study, the follicular helper T-cell gene expression signature was shown to be prognostic in a large cohort of patients with breast cancer (30).

The presence of FOXP3+ cells in lymphocytic infiltrates surrounding the tumor was reported to herald a poor prognosis, while the presence of intra-tumoral FOXP3+ TILs had no prognostic significance (35). West et al. reported an association between increased counts of FOXP3+ TILs and improved prognosis in patients with estrogen-receptor-negative tumors (86). FOXP3+ TILs correlated with CD8+ TILs (86). On the other hand, high γδ TIL counts were predictive of poor prognosis in another study (87). γδ T-lymphocyte represent regulatory population that may be responsible for the induction of tumor tolerance. Similarly, the presence of programmed death (PD)-1+ TILs was reported to be associated with inferior overall survival. The prognostic significance of PD-1+ TILs was observed in patients with luminal B tumors (independent of HER2 expression) and in patients with basal-like subtype (88). PD-1+ TILs were an independent prognostic parameter in multivariate analysis. Flow cytometric studies demonstrated that in breast tumors, PD-1 is expressed primarily on CD4+ TILs.

**TIL and Potential Immunotherapeutic Strategies in Breast Cancer**

Agents currently used in systemic therapy of breast cancer include cytotoxic drugs, hormonal agents and targeted-drugs. Some of these agents may affect immune response. There is, for example, evidence indicating that the activity of trastuzumab may, at least partially, be explained by the induction of antibody-dependent cell-mediated cytotoxicity (89-91). It was demonstrated in patients with HER2-positive metastatic breast cancer treated with trastuzumab that certain polymorphisms of immunoglobulin receptors are associated with increased antibody-dependent cell-mediated cytotoxicity and significantly longer progression-free survival (92). The addition of bevacizumab, a monoclonal antibody against VEGF may also increase activity of neoadjuvant chemotherapy (53, 54). VEGF suppresses immune responses (93), and anti-VEGF therapy has been shown to enhance the antitumor activity of the immune system, but it remains to be determined whether bevacizumab augments antitumor immunity in patients with breast cancer. A number of drugs that act by inducing potent immune response are being introduced into cancer treatment, including ipilimumab, a monoclonal antibody against cytotoxic T-lymphocyte antigen (CTLA)-4, and anti-PD-1 antibodies such as nivolumab (94).

On the other hand, the insight into pathogenic mechanisms may be helpful in identifying new roles for drugs already used in breast cancer. The RANK ligand produced by FOXP3+ TILs and its role in the progression of metastases (39) may define another facet of antitumor activity of the monoclonal antibody against RANK ligand, denosumab. Given the paucity of immunotherapeutic strategies in breast cancer, the role of TILs as biomarkers of immune response to agents targeting the immune system currently remains unknown. On the other hand, based on the data demonstrating the predictive role of TIL in patients treated with neoadjuvant chemotherapy that are summarized here, it is also evident that the immune system plays an important role in the antitumor activity of conventional cytotoxic drugs.

**Conclusion**

Numerous studies have established that the presence of TILs represents a predictive as well as prognostic biomarker in patients with breast cancer. In contrast to other biomarkers that are currently being used in the management of patients
with breast cancer, the concept of TILs seems to defy the
trend of laboratory diagnostics relying on commercialized
and standardized technologies. Although different
populations of lymphocytes were studied and different
methods were used to assess the presence of TILs, several
studies have demonstrated a strong association between high
TIL numbers before the start of neoadjuvant chemotherapy and
pathological complete response at surgery. Given the
prognostic significance of pathological complete response,
it is not surprising that the presence of TILs also determines
the prognosis of patients with early breast cancer in general.
The role of TILs as a predictive biomarker may increase
with the introduction of new targeted agents into therapy.
TIL count is not only a simple biomarker, but the study of
TILs offers unique insights into the role the immune
response plays in malignancy and in response to therapy. In
fact, the data on the role of TILs in patients treated with
neoadjuvant chemotherapy are an important part of
cumulative evidence indicating that the host antitumor
response is one of the principal factors determining the
outcome of malignant disease.

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