Tumor-infiltrating lymphocytes in breast cancer
A new predictor for responses to therapy

Yasmin Issa-Nummer1,*, Sibylle Loibl1, Gunter von Minckwitz1, and Carsten Denkert2

1German Breast Group; Neu-Isenburg, Germany; 2Institute of Pathology; Charité-Universitätsmedizin Berlin; Berlin, Germany

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Abbreviations: pCR, pathological complete response; LPBC, lymphocyte predominant breast cancer; TIL, tumor-infiltrating lymphocyte; Treg, regulatory T cell

We have prospectively validated in an independent clinical cohort the finding that elevated amounts of tumor-infiltrating lymphocytes in breast carcinoma tissues predict the response of patients to neoadjuvant chemotherapy. These results suggest that a robust tumor infiltration by T and B cells is a promising biomarker to define patients who might benefit from neoadjuvant chemotherapy.

In breast carcinoma as well as other types of cancer, tailoring therapeutic intervention to individual patients has turned out to constitute a promising approach for maximizing treatment efficacy while minimizing the risk and severity of side effects. In particular, predicting the response of an individual to a given treatment, i.e., distinguishing responders from non-responders can significantly assist therapeutic decisions and therefore improve the clinical benefits obtained from cancer patients.

Neoadjuvant chemotherapy is increasingly being used in breast carcinoma patients, mainly as a means to improve surgical options and long-term outcome upon the achievement of a pathological complete response (pCR). Furthermore, neoadjuvant chemotherapy constitutes a test of sensitivity to chemotherapy in vivo, and tuning the treatment according to early clinical responses has the ability to improve long-term disease outcome.1 Biological specimens from clinical studies based on neoadjuvant chemotherapy are valuable resources for the identification of biological markers that predict response to therapy. The correlation between putative predictive biomarkers, as measured in core biopsies obtained from primary neoplastic lesions before treatment, and disease outcome after treatment provides indeed an excellent basis for the prediction of responses to therapy.

As only a fraction of the patients bearing a specific subtype of breast carcinoma achieve a pCR to therapy, new and/or more reliable biological markers are needed.2 Such biomarkers will facilitate the prediction of response or resistance to conventional chemotherapy or targeted anticancer agents. Tumor-infiltrating lymphocytes (TILs) might constitute a valuable predictive biomarker in this setting. In a previous retrospective study, we were able to demonstrate that patients with breast carcinomas bearing elevated amounts of TILs had a significantly increased pCR rate upon neoadjuvant chemotherapy compared to patients with poorly infiltrated tumors (Fig. 1). In this analysis, tumors exhibiting a particular strong lymphocytic infiltrate, i.e., neoplasms exhibiting more than 60% infiltration by either stromal or intratumoral lymphocytes, were defined as lymphocyte predominant breast cancers (LPBCs).3 Using pre-therapy core biopsies from the GeparQuinto study, we have recently validated these results in a prospectively assessed cohort of breast carcinoma patients treated with neoadjuvant chemotherapy. Patients with LPBCs exhibited a significantly higher rate of pCRs than individuals affected by non-LPBC tumors, i.e., 36.6% vs. 14.3%. Multivariate analyses demonstrated that bearing a LPBC is a statistically significant and independent predictor for pCR to neoadjuvant chemotherapy, being associated with odds ratios of 2.7 and 1.2, respectively. Furthermore, among patients bearing hormone receptor-expressing neoplasms, the pCR rate associated with LPBCs was 28.2%, whereas that associated with non-LPBC lesions was only 8.2%.4

The presence of TILs has been documented in many types of cancer. Moreover, the mutual interactions between TILs and malignant cells are relevant for clinical outcome in various paradigms of anticancer therapy, ranging from conventional chemotherapeutics to monoclonal antibody-based therapeutics. Several studies revealed a synergistic interaction between therapy and components of the immune system, such as TILs, correlates with improved clinical outcomes for patients. Results from large clinical studies have demonstrated an association between the
presence of TILs in early-stage breast carcinomas, high response rates to neoadjuvant chemotherapy and improved prognosis. The composition of TILs, which generally encompass various subtypes of T and B cells, can influence its predictive and/or prognostic impact in patients affected by several tumors including breast carcinoma. A number of distinct mechanisms underlying the interaction between the immune system and malignant cells in response to chemotherapy are known to be capable to recruit TILs to neoplastic lesions thereby potentially mediating beneficial effects. For instance, different chemotherapeutic agents have been described to stimulate antitumor immune responses. Some chemotherapeutic agents such as cyclophosphamide, anthracyclines, and taxanes, stimulate T-cell proliferation, activate natural killer (NK) cells, deplete circulating regulatory T cells (Tregs) and inhibit Treg infiltration into tumor tissue. Furthermore, cell death as induced by some anticancer drugs can stimulate cytotoxic T cells to establish a protracted tumor-specific immune response. Different studies have demonstrated that neoadjuvant chemotherapy stimulate the infiltration of neoplastic lesions by cytotoxic T lymphocytes (CTLs), an event that is generally associated with improved disease outcome. Chemotherapy can also shift a TIL-depleted tumor into a lesion that bears high TIL levels. In addition, B cells can contribute to antitumor immune responses by activating innate immune cells, secreting antibodies specific for tumor-associated antigens, by releasing various cytokines, by triggering the complement cascade, and by operating as antigen-presenting cells to induce tumor-specific CTL responses. These observations support the hypothesis that besides their direct immunostimulatory effects, some chemotherapeutics promote humoral and adaptive tumor-specific immune responses as they trigger the death of cancer cells and hence the release of tumor-associated antigens. In our study, we have evaluated the amounts of TILs without differentiating between

**Figure 1.** Predictive value of tumor-infiltrating lymphocytes in human breast carcinoma. The tumor-infiltrating lymphocytes (TILs) found in the neoplastic lesions of breast carcinoma patients comprise T and B cells. (A) Elevated amounts of TILs predict high rates of pathological complete response (pCR) to neoadjuvant chemotherapy. (B) In contrast, patients with tumors that contain low amounts of TILs exhibited low pCR rates in response to neoadjuvant chemotherapy.
T- and B-cell populations. Further studies are currently ongoing to fill this gap. Different patterns of T and B-cell sub-populations may indeed inhibit or facilitate tumor evasion, depending on several other immunological and tumor-intrinsic parameters.

In summary, our results strongly support the notion that immune responses contribute to the success of chemotherapy. The interactions between TILs and malignant cells are indeed very relevant for the response of cancer patients to therapy. The possibility to exploit such a synergistic interaction should be carefully considered for the design of conceptually new clinical studies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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