**Immunoscopying breast cancer: TILs remember what they target**

A fundamental ‘dogma’ of tumor immunology and of cancer immunosurveillance in particular is that cancer cells express antigens that differentiate them from their nontransformed counterparts. Tumor antigens, like HER2, are overexpressed normal proteins and therefore are subject to immunological tolerance. Immune system controls not only tumor ‘burden’ (quantity) but also tumor ‘quality’ (immunogenicity) [1, 2]. The tumors formed in mice that lack an intact immune system are, as a group, more immunogenic (classified as ‘unedited’) than similar tumors derived from immunocompetent mice (termed ‘edited’). As a consequence of constant immune selection, pressure placed on genetically unstable tumor cells held in equilibrium, tumor cell variants will be selected. They are no longer recognized by adaptive immunity (antigen loss variants or tumors cells that develop defects in antigen processing or presentation). They become insensitive to immune effector mechanisms, or induce an immunosuppressive state within the tumor microenvironment (tolerance). These tumor cells may then enter the escape phase, in which their outgrowth is no longer blocked by immunity. These tumor cells can re-emerge after adjuvant therapy to cause metastatic disease. Is breast cancer immunogenic?

The results presented by Loi et al. [3] in the present issue of *Annals* provide support of this hypothesis. A prospective–retrospective study was conducted using samples from the FinHER adjuvant trial to validate prognostic importance of tumor-infiltrating lymphocytes (TILs) in triple-negative breast cancer (TNBC) and also to investigate associations with trastuzumab benefit in HER2-overexpressing disease [4].

Authors observed a significant association with a good prognosis in the TNBC and not in luminal or HER2+ subtypes. They also observed that there was a statistically significant interaction between TILs as a continuous variable and trastuzumab treatment. For the primary end point of distant disease-free survival, each 10% increment in lymphocytic infiltrate was associated with an 18% reduction in the relative risk of distant recurrence in patients who received trastuzumab in addition to their chemotherapy. Several aspects of this report deserve careful review. The FinHER2 study enrolled 1010 patients with early-stage breast cancer, and the investigators evaluated TILs by hematoxylin/eosin in ∼92% of the cases. Of those, 134 with triple-negative disease and 209 with HER2-amplified breast cancer were included in this analyses; the number of events was 35 and 49 for TNBC and HER2+ disease, respectively. Moreover, the 209 HER2+ patients included were divided into trastuzumab and non-trastuzumab arms. Thus, the number of events for the analysis of prediction of benefit to trastuzumab is very limited. The authors did not describe the specific number of events for the trastuzumab and non-trastuzumab group (or the numbers per group who were allocated to the different quartiles), numbers that are relevant to help place the data in perspective. Another important fact to point out is that the 0.77 concordance between the two pathologists who reviewed the percentage of TILs in the stromal component may not be adequate enough and something to try to optimize in future studies [3].

Previous observations demonstrated the prognostic role of TILs in TNBC [5, 6]. TNBC are poorly differentiated tumor with high genetic instability and very high heterogeneity. This heterogeneity enhances the ‘danger signals’ and select clone variants that could be more antigenic or, in other words, that could more strongly stimulate a host immune antitumor response. Better prognosis in patients with TNBC and higher TILs is also the result of an ‘immunoediting’ process induced by chemotherapy. Zitvogel et al. [7] has evaluated in cellular and animal models the emerging concept that the response to chemotherapy is at least partly dependent on an immunological reaction against those tumor cells that are dying during the chemotherapy [7]. One of the mechanisms whereby chemotherapy can stimulate the immune system to recognize and destroy malignant cells is commonly known as immunogenic cell death (ICD). Cancer cells dying to ICD are *de facto* converted into an anticancer vaccine and as such elicit an adaptive immune response [7].

The most novel finding in this study was the association between TILs and increased benefit from the addition of trastuzumab to chemotherapy in HER2+ disease, although TILs were reported not to be prognostic for the overall group of patients with HER2-positive disease and there were challenges with pathological concordance and the number of events, as described above [3]. This and other studies support the potential role of an immune biomarker as predictive of therapeutic benefit of trastuzumab. A genomic approach has been used to define biological processes that predict benefit from trastuzumab in terms of relapse-free survival (RFS), and was recently reported by Perez et al. at ASCO 2014 [8]. Among 1282 patients enrolled in the N9831 adjuvant trial (NCT00005970), functional ontology analysis and network modeling were used to identify key biological processes associated with RFS in patients who received chemotherapy alone or chemotherapy plus trastuzumab. Using probes with hazard ratio (HR) *P* < 0.01, 10 of 13 significantly enriched biological processes associated with RFS (HR *P* < 0.01) were linked to immune functions. These 10 processes defined a cohort of 87 immune function genes. Patients defined as immune function positive based on the 87 genes experienced a favorable outcome when treated with T_H (HR = 0.55, *P* = 0.0005). Patients

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who did not exhibit immune function enrichment and were treated with H did not have better RFS than patients with immune function enrichment who were treated with chemotherapy alone (HR = 0.93, P = 0.72). Among patients who received chemotherapy alone, enriched immune function was not associated with increased RFS (HR = 1.01, P = 0.96). These data clearly demonstrate that improved RFS following treatment with adjuvant trastuzumab appears to be associated with a heightened state of immunological function. This observation may define a significant biological process that is linked to the efficacy of HER2-targeted therapy, may provide a means of predicting probability of relapse following adjuvant trastuzumab and suggests possible routes of therapeutic enhancement [8]. Expression of 12 immunologically relevant genes (CXCL9, CCL5, CD8A, CD80, CXCL13, IGKC, CD21, IDO1, PD-1, PDL1, CTLA4, FOXP3) was evaluated by quantitative RT-PCR in 481 core biopsies from the GeparSixto trial (that investigated the addition of carboplatin to a doxorubicin/taxane combination in HER2-positive or triple-negative primary breast cancer; trastuzumab and lapatinib were added for HER2+ disease and bevacizumab for triple-negative disease) [9]. All immune mRNA markers showed a strong positive correlation with each other and with the stromal lymphocyte infiltrate. Expression of immune marker mRNAs in breast cancer is predictive for response to neoadjuvant chemotherapy. In GeparSixto, these immunological parameters can be used in addition to TILs to identify patients with increased response rates to carboplatin [9].

In the report of Loi et al. TILs are associated with increased benefit from the addition of trastuzumab to chemotherapy in HER2+ disease. Trastuzumab mediates some of its therapeutic effect through the recruitment of Fc receptor (FcR)-expressing immune cells such as monocytes and natural killer (NK) cells [10, 11]. Correlative evidence has additionally supported that antibody-dependent cellular cytotoxicity (ADCC) may play a major role in the antitumor effects of monoclonal antibodies. Consistently, an increase of TILs, especially FcR+ cells such as NK cells, has been observed in tumor tissue after trastuzumab treatment [12], and patients responding to trastuzumab had higher in situ infiltration of leukocytes and an increased capacity to mediate ADCC activity [13, 14]. Increased influx of both innate and adaptive immune cells into the tumor microenvironment by a selected immunotherapy further enhances subsequent antibody-induced immunity, leading to increased tumor eradication and resistance to rechallenge.

Which are the clinical implications of all ‘immunome’ data produced in the last years? First, validation of whether TILs are prognostic or predictive in HER2+ breast cancer is needed, preferably in a large population set with appropriate follow-up time. Second, validate immune genomic signatures that may be predictive and prognostic in patients with triple-negative and HER2-positive disease. Third, we support the concept of developing and potentially incorporate an ‘immunoscore’ into traditional classification of breast cancer, thus providing an essential prognostic and potentially predictive tool in the pathology report. Fourth, implement clinical trials for triple-negative and HER2-positive breast cancer in the metastatic setting with drugs that target immune-cell-intrinsic checkpoints. Blockade of one of these checkpoints, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or the programmed death 1 (PD-1) receptor may provide proof of concepts for the activity of an immune-modulation approach in the treatment of a breast cancer.

The immune system remembers what it targets, so once the system is correctly activated, it may mediate a durable tumor response.

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Disclosure

The authors have declared no conflicts of interest.

References