HSOA Journal of Clinical Immunology and Immunotherapy

Mini Review

Tumor Infiltrating Lymphocytes-The Next Step in Assessing Outcome and Response to Treatment in Patients with Breast Cancer

Robert Wesolowski[1]* and William E Carson III[2]*

[1]*Division of Medical Oncology, B401 Starling Loving Hall, W10th Avenue, Columbus*

[2]*OSU Comprehensive Cancer Center, N911 Doan Hall, 410 West 10th Avenue Columbus*

Abstract

Tumor infiltrating lymphocytes are studied for their potential as new clinically useful prognostic and predictive biomarkers in patients with triple negative and HER-2/neu amplified breast cancer. This area of research could also help guide the development of novel therapeutic approaches for this disease.

Keywords: Breast cancer; Tumor infiltrating lymphocytes; Biomarkers

Editorial

Over the recent years, new results from multiple groups have pointed towards tumor infiltrating lymphocytes (TILs) as prognostic and predictive biomarkers in breast cancer. This is not a new concept. For instance, it has been known since 1940s that a subtype of breast cancer that characteristically exhibits a very large proportion of stromal lymphocytic infiltrate, called medullary carcinoma, has been associated with excellent prognosis after aggressive local therapy in spite of high histologic grade and axillary lymph node metastases [1,2]. However, over the next several decades, a number of studies examined the association between tumor lymphocytic infiltrates and prognosis in more common histologic types of breast cancer and yielded contradictory results [3-6]. The concept of the immune tumor micro-environment's role in influencing breast cancer biology has resurfaced after the recognition that expression of genes associated

*Corresponding author: Robert Wesolowski, Division of Medical Oncology, B401 Starling Loving Hall, W10th Avenue, Columbus, USA, Tel: 614-366-4810; Fax: 614-293-4372; Email: Robert.Wesolowski@osumc.edu

William E Carson III, OSU Comprehensive Cancer Center, N911 Doan Hall, 410 West 10th Avenue Columbus, USA, Tel: 614-293-6306 Fax: 614-293-3465 Email: William.Carson@osumc.edu

with stromal (fibroblast) and immune (macrophage, T-cell) components significantly contribute to the global gene expression landscape within the breast tumors as detected by the use of cDNA based gene expression microarrays [7]. High expression of genes typical for lymphocytes (such as CD8) and low expression of genes typical of myeloid cells (such as CD68) was found to indicate better prognosis, especially in basal like and HER-2/neu positive breast cancer subtypes [8-10].

This has generated a renewed excitement and multiple investigators began to study the potential association between TILs, prognosis and response to systemic therapy for breast cancer. Lymphocytes can be easily detected by analyzing slides of tumor sections stained with hematoxylin and eosin; a simple and inexpensive technique that can be reliably performed by practically any pathologist. By using such simple methodology, Loi and colleagues have demonstrated that a high proportion of TILs (>50%) in tumors of women with operable, triple negative breast cancer enrolled in a large adjuvant chemotherapy trial BIG 2-98, was strongly associated with favorable disease free (p=0.014) and overall (p=0.029) survival [11]. In their analysis, two distinct subsets of lymphocytes were measured, namely (1) stromal TILs (sTILs – mononuclear cells that were present within the tumor stroma but were not in direct contact with invasive carcinoma cells) and (2) intratumoral TILs (iTILs – mononuclear cells that were directly associated with the malignant cells). Their analysis revealed that for every 10% increase in the levels of sTILs there was 17% reduction in the risk of relapse (p=0.025) and death (p=0.023). They also found close association between higher levels of sTILs and iTILs with infiltrating ductal histology (P<0.001 & 0.48 respectively), high histologic grade (both P< .001), hormone receptor negativity (both P<0.001), and increased Ki67 expression (>14%; both P<0.001). These results suggest that perhaps lymphocyte predominance could select a subgroup of patients with favorable prognosis despite having other poor clinical and histologic characteristics. No association was found between TIL levels and outcomes of patients with hormone receptor positive or HER-2/neu amplified breast cancer. In the recently presented confirmatory study, tumor samples of women with operable triple negative breast cancer from two large adjuvant trials (ECOG 2197 and ECOG 1199) were analyzed for the presence of TILs [12]. Again, the investigators found that for every 10% increase in sTILs there was 18% reduction in the risk of distant recurrence and 19% reduction in the risk of death. The presence of iTILs showed a trend towards better outcome but it did not reach statistical significance. On multivariate analysis, high levels of sTILs predicted improved disease free, distant recurrence free and overall survival independently of other poor clinical and histologic characteristics such as tumor size, lymph node metastases or patient age. Similar findings were shown in patients with triple negative and HER-2/neu positive breast cancer enrolled on a phase III, randomized FinHER trial that tested different adjuvant chemotherapy regiments with or without trastuzumab. This analysis also suggested for the first time that higher levels of TILs could be associated with increased trastuzumab benefit in HER-2/neu positive breast cancer [13].

Other studies looked at association between levels of TILs and the likelihood of achieving a complete pathologic response (pCR)

following neo-adjuvant chemotherapy for breast cancer. Complete pathologic response is a surrogate biomarker that is generally considered to indicate a lower likelihood of recurrence and a higher chance of survival [14]. Loi and colleagues analyzed biopsy specimen from 156 patients with HER-2/neu positive breast cancer enrolled in GeparQuatro trial and treated with trastuzumab containing neo-adjuvant chemotherapy [15]. In this study, every 10% increase in the levels of TILs resulted in 16% increase in the likelihood of achieving pCR. Forty seven percent of patients with high levels of TILs achieved pCR in contrast to only 31.7% of patients in the entire cohort. This provided additional support to the notion that TIL levels might correlate with the response to trastuzumab based chemotherapy. In additional analysis, Loi and colleagues also discovered that high expression of immune checkpoints within breast tumors, such as PD1 were associated with higher likelihood of distant disease free survival after trastuzumab chemotherapy. Other groups have found a correlation between levels of TILs and response to carboplatin. In an analysis of 580 diagnostic tumor biopsies obtained from patients on a clinical trial testing neo-adjuvant chemotherapy with or without carboplatin (Gepar Sixto trial), approximately 60% of patients with lymphocyte predominant breast cancer (defined as presence of ≥60% of TILs) achieved pCR compared to pCR rate of 40% in all participants and 34% in patients with low levels of TILs (p<0.0005) [16]. Among patients with high levels of TILs, those who received carboplatin achieved a pCR rate of 74% compared to 46.6% in patients treated without carboplatin. A similar study by Vinayak and colleagues analyzed diagnostic biopsies of patients with triple negative breast cancer treated with neo-adjuvant carboplatin, gemcitabine and a Poly (ADP – Ribose) Polymerase (PARP) inhibitor iniparib [17]. In this analysis, tumors with high levels of TILs were tightly correlated with an immunomodulatory subtype of triple negative breast cancer, based on Vanderbilt classification [18]. There was a strong association between high levels of both sTIL and iTIL and residual tumor burden score (an objective method of assessing pathologic response to neo-adjuvant chemotherapy). However, an association remained statistically significant only for iTILs after adjustments for age, tumor size, N stage, tumor grade and germline BRCA status. For every 10% increase in iTILs there was a 162% increase in the odds of achieving pCR.

What are the implications of these findings to current clinical practice? Perhaps, the results of the above studies could lead to the routine practice of reporting the levels of lymphocytic infiltrates in the pathology reports of patients diagnosed with breast carcinoma (especially in cases of triple negative and HER-2/neu positive disease)? Before that occurs, the methodology of interpreting TILs and the cut off values for lymphocyte predominant breast cancer will need to be standardized. This data will also require future validation by other groups in prospectively designed clinical trials with pre-specified immune correlative endpoints. Regardless, the findings from the above analysis are already providing crucial clues for future clinical research. As an example, these studies suggest that perhaps conventional anti-neoplastic therapy such as carboplatin or trastuzumab could possibly induce anti-tumor responses by modulating tumor immunity [13,16,19-21]. The finding that high expression of immune checkpoints is associated with improved response rates to trastuzumab provides support for studying combinations of trastuzumab and PD1 or CTLA-4 inhibitors. Additionally, since high levels of TILs most common in the immune-modulatory subtype of triple negative breast cancer, it may be reasonable to select such patients for treatment with immune based therapies. Another important issue that is currently being studied is whether predominance of different subtypes of intra-tumoral lymphocytes (such as CD4+ T-helper cells, CD8+ cytotoxic T-lymphocytes or CD25+/FOXP3+ T-regulatory cells) could have differential effect on patient outcome. This requires more complex analyses using immunohistochemical stains, fluorescent microscopy, and flow cytometry or gene expression microarrays. One such method called Cibersort is a gene profiling based assay that can identify 23 leukocyte subsets present in the tumor based on a unique gene expression signature of these cells. By using Cibersort Vinayak *et al.* demonstrated that high levels of activated memory CD4+ memory T-cells were significantly associated with pathologic response to neo-adjuvant carboplatin containing chemotherapy [17]. Another critical question is why some breast cancer patients have high levels of TILs while others don't. Perhaps elucidating the mechanism by which some breast tumors inhibit lymphocyte infiltration may provide clues for new effective therapeutic strategies.

The work summarized above represents an exciting example of the ongoing efforts to understand the interactions between breast cancer cells and the host immune system. These efforts could, in the near future, lead us towards developing vital prognostic and predictive biomarkers that would guide the selection of the most effective management strategies for breast cancer and provide support for the development of novel treatments [22].

References

1. Moore OS Jr, Foote FW Jr (1949) The relatively favorable prognosis of medullary carcinoma of the breast. Cancer 2: 635-642.

2. RICHARDSON WW (1956) Medullary carcinoma of the breast; a distinctive tumour type with a relatively good prognosis following radical mastectomy. Br J Cancer 10: 415-423.

3. Horst HA1, Horny HP (1988) Frequency distribution of lymphoreticular infiltrates in invasive carcinoma of the female breast. Cancer Detect Prev 11: 297-301.

4. Aaltomaa S1, Lipponen P, Eskelinen M, Kosma VM, Marin S, et al. (1992) Lymphocyte infiltrates as a prognostic variable in female breast cancer. Eur J Cancer 28A: 859-864.

5. Chin Y1, Janseens J, Vandepitte J, Vandenbrande J, Opdebeek L, et al. (1992) Phenotypic analysis of tumor-infiltrating lymphocytes from human breast cancer. Anticancer Res 12: 1463-1466.

6. Ménard S1, Tomasic G, Casalini P, Balsari A, Pilotti S, et al. (1997) Lymphoid infiltration as a prognostic variable for early-onset breast carcinomas. Clin Cancer Res 3: 817-819.

7. Perou CM1, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, et al. (2000) Molecular portraits of human breast tumours. Nature 406: 747-752.

8. Rody A1, Holtrich U, Pusztai L, Liedtke C, Gaetje R, et al. (2009) T-cell metagene predicts a favorable prognosis in estrogen receptor-negative and HER2-positive breast cancers. Breast Cancer Res 11: R15.

9. DeNardo DG1, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, et al. (2011) Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov 1: 54-67.

10. Mahmoud SM1, Paish EC, Powe DG, Macmillan RD, Grainge MJ, et al. (2011) Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J ClinOncol 29: 1949-1955.

11. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, et al. (2013) Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J ClinOncol 31: 860-867.

12. Adams S1, Gray RJ2, Demaria S2, Goldstein L2, Perez EA2, et al. (2014) Prognostic Value of Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancers From Two Phase III Randomized Adjuvant Breast Cancer Trials: ECOG 2197 and ECOG 1199. J ClinOncol .

13. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, et al. (2014) Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol.

14. Mieog JS1, van der Hage JA, van de Velde CJ (2007) Preoperative chemotherapy for women with operable breast cancer. Cochrane Database Syst-Rev : CD005002.

15. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V et al. (2013) Abstract S1-05: Tumor infiltrating lymphocytes (TILs) indicate trastuzumab benefit in early-stage HER2-positive breast cancer (HER2+ BC). Cancer Res 73: S1-05.

16. Denkert C, Loibl S, Salat C, Sinn BV, Schem C, et al. (2013) Increased tumor-associated lymphocytes predict benefit from addition of carboplatin to neoadjuvant therapy for triple-negative and HER2-positive early breast cancer in the GeparSixtotrial (GBG 66). Cancer Res 73: S1-06.

17. Vinayak H, Gray RJ, Adams S, Jensen KC, Manola J, et al. Association of increased TILs with immunomodulatory (IM) triple-negative breast cancer subtype and response to neo-adjuvant platinum-based therapy in PrECOG 0105. J ClinOncol 32: 5s.

18. Masuda H1, Baggerly KA, Wang Y, Zhang Y, Gonzalez-Angulo AM, et al. (2013) Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. Clin Cancer Res 19: 5533-5540.

19. Wu X1, Feng QM, Wang Y, Shi J, Ge HL, et al. (2010) The immunologic aspects in advanced ovarian cancer patients treated with paclitaxel and carboplatin chemotherapy. Cancer ImmunolImmunother 59: 279-291.

20. Clynes RA1, Towers TL, Presta LG, Ravetch JV (2000) Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med 6: 443-446.

21. McDonnell AM1, Nowak AK, Lake RA (2011) Contribution of the immune system to the chemotherapeutic response. SeminImmunopathol 33: 353-367.

22. Melichar B1, Å tudentova H, KalÃ¡bovÃ¡ H, VitÃ¡skovÃ¡ D, ÄŒermÃ¡kovÃ¡ P, et al. (2014) Predictive and prognostic significance of tumor-infiltrating lymphocytes in patients with breast cancer treated with neoadjuvant systemic therapy. Anticancer Res 34: 1115-1125.