Editorial

T-cell bispecific antibodies to bypass MHC class I loss in breast cancer

In the current issue of *Annals of Oncology*, Messaoudene et al. [1] provide novel and relevant insights on the immune contexture of early breast cancer. The authors showed that human leukocyte antigen (HLA) loss of heterozygosity (LOH) is a frequent event in early breast cancer and is associated with an increase in regulatory T-cell infiltration and high T-cell immunoglobulin and mucin-domain containing-3 expression, minimizing immuno-stimulatory effects of chemotherapy. Notably, T-cell bispecific antibodies (TCBs) bridging CD3 and HER2 or CEACAM5 are able to bypass major histocompatibility complex (MHC) class I loss, partially restoring T-cell functions in metastatic lymph nodes (mLNs).

The immune system plays a crucial role in breast cancer development and progression, strongly influencing disease course. The presence of tumor-infiltrating lymphocytes (TILs) in primary breast cancer has been correlated with a better prognosis, especially in triple negative and HER2+ subtypes [2–4]. TILs can also act as predictive indicators for improved pathological complete response and survival after neoadjuvant chemotherapy (NACT) [5]. In the last years, the presence of tertiary lymphoid structures (TLSs), characterized by an architecture similar to canonical secondary lymphoid organs, in primary organ or in mLNs has been associated with improved outcomes in several malignancies, including breast cancer [6, 7]. Meanwhile, as this evidences has been accumulated, the introduction in clinical practice of immune checkpoint-based treatment radically changed the approach to a broad spectrum of tumors. However, breast cancer has been marginally transformed by this revolution, with some results obtained only in the triple-negative subtype and programmed death-ligand 1 (PD-L1)-positive tumors [8, 9].

The findings provided by Messaoudene et al. [1] have considerable implications, shedding new light on immune escape mechanisms adopted by breast cancer cells. Immune infiltrate presents substantial changes during breast cancer progression moving from primary tumor to lymph nodes. Invasive breast cancer is characterized by an impaired immune response compared with carcinoma *in situ*, mainly related to reduced CD8+ T-cell function and high PD-L1 expression [10]. In the current study, the authors showed that CD8+ T cells markedly increase in primary tumor after exposure to chemotherapy, while this effect is less pronounced in mLNs. Immunogenic chemotherapy is able to switch on effector T cells in primary lesions, while it cannot exert this effect at lymph node level where an immunosuppressive tumor microenvironment (TME) is sustained by T-regulatory cells. However, in the current work, the expression of other immune checkpoint molecules (e.g. PD-L1 and LAG-3), as well as

the TLSs, was not assessed. It would be interesting to know whether there are substantial differences in TLSs between primary tumors and mLNs. Moreover, since the selection of patients for immunotherapy in breast is based on PD-L1 expression, it would be useful to evaluate whether there are differences in terms of PD-L1 expression between primary tumor and mLNs (and possibly also in the metastatic site).

Noteworthy, the authors demonstrate that TCB bridging CD3 and HER2 or CEACAM5 are able to increase CD8+ and NK cell activation and proliferation mainly in lymph nodes. Conversely, primary tumor-resident T cells, already exposed to chemotherapy in the neoadjuvant setting, were not reactivated by the exposure to TCBs. These observations suggest that TCB could have a role in triggering T-cell function before chemotherapy.

The authors also investigated whether MHC class I loss could lead to breast cancer progression, promoting immunosuppression in TME. MHC class I downregulation is a well-known mechanism of immune escape adopted by different tumors, resulting in reduced antigen presentation and so promoting tumor immune evasion [11]. In addition, MHC class I downregulation is recognized as a resistance mechanism to immunotherapy in melanoma and lung cancer [12, 13]. Despite about 40% of breast cancers presenting with MHC class I downregulation, contrasting results have been reported about its predictive and prognostic value [14–16]. The authors reveal a marked MHC class I downregulation in mLNs compared with primary breast cancer, regardless of tumor subtype. Not surprisingly, chemotherapy was able to restore MHC class I expression in mLN. Indeed, chemotherapy administration, inducing an immunogenic cell death, can promote T-cell recruitment and activation in TME, thus increasing IFN-γ levels that facilitate MHC class I upregulation on tumor cells. However, this immunomodulating effect of chemotherapy cannot be exerted when MHC class I loss is related to genetic abnormalities. Interestingly, the authors report that genetic defects in beta-2-microglobulin (B2M), an essential component of MHC class I, are found across all breast cancer subtypes, especially in triple negative. Defective MHC class I antigen processing through deleterious mutations in B2M have been reported as common resistance mechanism to immune checkpoint inhibitor-based immunotherapy [12, 17].

In order to define potential genetic alterations related to MHC class I downregulation, the authors explored differences in terms of HLA LOH between primary tumor and mLNs. HLA class I genotype has been found to be an early event in lung cancer, promoting and influencing response to immunotherapy [18, 19]. In breast cancer, HLA LOH was more pronounced in mLN as compared with primary tumor region. Notably, tumors with HLA-LOH were less likely to obtain a pathological complete response after NACT (odds ratio 0.27, $P = 0.04$). The administration of

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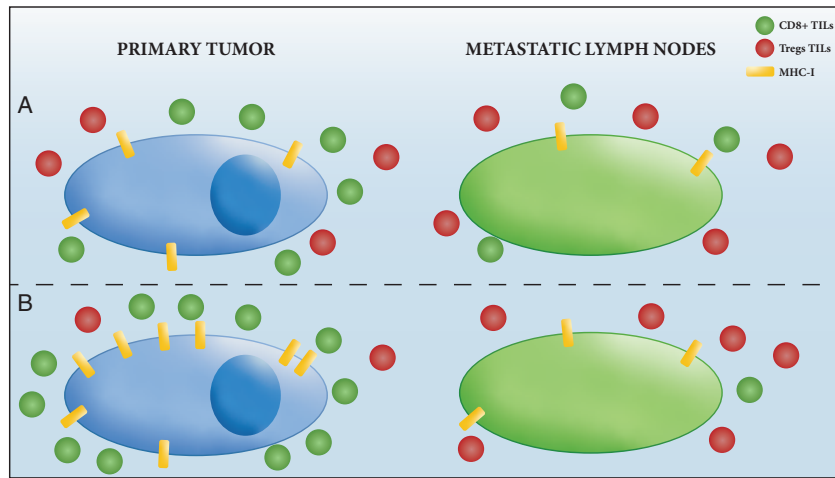
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Figure 1. Breast cancer progression is associated with an increased immunosuppression moving from primary tumor to metastatic lymph nodes (A). Chemotherapy can increase CD8+ T cells in primary breast cancer, while it cannot reverse immunosuppression at lymph node level. Chemotherapy can also partially restore major histocompatibility complex (MHC) class I expression, but with limited effect in case with human leukocyte antigen loss of function (B). TILs, tumor-infiltrating lymphocytes.

TCB targeting antigens such as CEACAM5 or HER2 can restore T-cell effector function, regardless of low target antigen expression related to HLA-LOH.

Is there any potential therapeutic implication for the use of TCB in clinical practice? And, if yes, when and in which setting? Considering that HLA-LOH is predominant in mLN and that NACT is unable to reverse immunosuppression at this level, TCB should act as priming strategy. Therefore, chemotherapy administration should be placed after TCB in order to ensure immunogenic cell death and an antigen release in a 'primed' TME. TCB-based strategies could be implemented in the neoadjuvant setting and afterward as adjuvant treatment. Trials testing NACT strategies represent an ideal *in vivo* laboratory to test novel agents, with the possibility of obtaining baseline biopsy and to reassess both tumor response and TME modifications at established time points. In a clinical scenario, considering that the backbone of NACT is represented by chemotherapy +/- targeted agents (depending on tumor subtype), TCB could be administered before chemotherapy. On the other hand, chemotherapy seems to better target the primary tumor than the mLN, effectively increasing CD8+ T cell at the primary site while rendering CD8+ T cells more immunosuppressed. These observations may favor the implementation of TCB-based therapies also in the adjuvant setting (e.g. in patients not candidates for NACT).

The implementation of new TCB-treatment represents an interesting way to circumvent immune escape in breast cancer. As previously described, MHC class I is a frequent event during breast cancer evolution, rendering chemotherapy and immune checkpoint-based immunotherapy ineffective. TCBs are engineered molecules, consisting of binding sites to the invariant CD3ϵ chain of the T-cell receptor and to a tumor-associated or a tumor-specific antigen, which do not require MHC class I expression to exert their anti-tumor function [20, 21]. One of the principal issues in the TCB development is the lack of well-characterized extracellularly antigens, specifically exposed by tumors and absent in normal tissues. As a result, the majority of TCBs has been developed against antigens, expressed but limited to tumor tissue. For future development, it will be essential to characterize and identify new tumor-associated antigens. Several efforts are being made, representing a relevant challenge in the next years [22].

In conclusion, the discovery of significant differences in the immune infiltrate between primary breast cancers and mLNs is important. However, a deeper characterization of TME including presence of TLSs, upregulation of other negative checkpoint molecules (e.g. PD-L1 and LAG-3), and abnormalities in IFN-γ-related genes, could ensure a better understanding of these findings and further research in this way are warranted. Hence, immune checkpoint inhibitors provide scant results in breast cancer, mainly limited to triple-negative tumors harboring PD-L1 expression. The implementation of novel biomarkers that can guide better patient selection represents an urgent need. The suggestion that TCBs might normalize differences between primary tumor, mLNs, and maybe metastatic sites is clinically relevant. Given that immune escape mechanisms tend to increase from primary breast tumor to mLNs, we can speculate that immunosuppression would be even deeper at metastatic sites. Therefore, the development of new therapeutic strategies that can bypass these immune escape mechanisms, such as TCBs and others, is strongly encouraged. The relevant findings of the current work pave the way for further research in this context.

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Disclosure

References

1. Messaoudene M, Mourikis T, Michels J et al. T cell bispecific antibodies in node-positive breast cancer: novel therapeutic avenue for MHC class I loss variants. Ann Oncol 2019; 30: doi.org/10.1093/annonc/mdXXXX.

2. Loi S, Sirtaine N, Piette F et al. 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. JCO 2013; 31(7): 860–867.

3. Dieci MV, Mathieu MC, Guarneri V et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in two phase III randomized adjuvant breast cancer trials. Ann Oncol 2015; 26(8): 1698–1704.

4. Loi S, Drubay D, Adams S et al. Tumor-infiltrating lymphocytes and prognosis: a pooled individual patient analysis of early-stage triple-negative breast cancers. J Clin Oncol 2019; 37(7): JCO1801010:

5. de Melo Gagliato D, Cortes J, Curigliano G et al. Tumor-infiltrating lymphocytes in breast cancer and implications for clinical practice. Biochim Biophys Acta Rev Cancer 2017; 1868(2): 527–537.

6. Sautes-Fridman C, Lawand M, Giraldo NA et al. Tertiary lymphoid structures in cancers: prognostic value, regulation, and manipulation for therapeutic intervention. Front Immunol 2016; 7: 407.

7. Solinas C, Garaud S, De Silva P et al. Immune checkpoint molecules on tumor-infiltrating lymphocytes and their association with tertiary lymphoid structures in human breast cancer. Front Immunol 2017; 8: 1412.

8. Schmid P, Adams S, Rugo HS et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2018; 379(22): 2108–2121.

9. Adams S, Schmid P, Rugo HS et al. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort A of the phase 2 KEYNOTE-086 study. Ann Oncol 2018.

10. Gil Del Alcazar CR, Huh SJ, Ekram MB et al. Immune escape in breast cancer during in situ to invasive carcinoma transition. Cancer Discov 2017; 7(10): 1098–1115.

11. Campoli M, Ferrone S. HLA antigen changes in malignant cells: epigenetic mechanisms and biologic significance. Oncogene 2008; 27(45): 5869–5885.

12. Zaretsky JM, Garcia-Diaz A, Shin DS et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med 2016; 375(9): 819–829.

13. Gettinger S, Choi J, Hastings K et al. Impaired HLA class I antigen processing and presentation as a mechanism of acquired resistance to immune checkpoint inhibitors in lung cancer. Cancer Discov 2017; 7(12): 1420–1435.

14. Madjd Z, Spendlove I, Pinder SE et al. Total loss of MHC class I is an independent indicator of good prognosis in breast cancer. Int J Cancer 2005; 117(2): 248–255.

15. de Kruijf EM, van Nes JG, Sajet A et al. The predictive value of HLA class I tumor cell expression and presence of intratumoral Tregs for chemotherapy in patients with early breast cancer. Clin Cancer Res 2010; 16(4): 1272–1280.

16. Kaneko K, Ishigami S, Kijima Y et al. Clinical implication of HLA class I expression in breast cancer. BMC Cancer 2011; 11: 454.

17. Le DT, Durham JN, Smith KN et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017; 357(6349): 409–413.

18. Chowell D, Morris LGT, Grigg CM et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. Science 2018; 359(6375): 582–587.

19. McGranahan N, Rosenthal R, Hiley CT et al. Allele-specific HLA loss and immune escape in lung cancer evolution. Cell 2017; 171(6): 1259–1271 e1211.

20. Baeuerle PA, Reinhardt C. Bispecific T-cell engaging antibodies for cancer therapy. Cancer Res 2009; 69(12): 4941–4944.

21. Dahlen E, Veitonmaki N, Norlen P. Bispecific antibodies in cancer immunotherapy. Ther Adv Vaccines Immunother 2018; 6: 3–17.

22. Rius Ruiz I, Vicario R, Morancho B et al. p95HER2-T cell bispecific antibody for breast cancer treatment. Sci Transl Med 2018; 10.