Investigating the role of tumor-infiltrating lymphocytes in advanced HER2-positive breast cancer

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Giant strides in the development of HER2-targeted agents have drastically improved outcomes for patients diagnosed with HER2-amplified breast cancer. Despite this, most patients who develop a recurrence inevitably die from their disease. More recently, anti-tumor adaptive T cell immune responses have been shown to be able to significantly influence both prognosis as well as response and benefit to therapies in early stage disease (1-3). Analyses assessing the role of tumor-infiltrating lymphocytes (TIL) in the setting of advanced HER2-positive breast cancer are only just beginning to emerge.

An extensive recent study by Liu *et al.* (4) assessed the prognostic and predictive value of TILs from 647 patients with metastatic HER2-positive breast cancer from the Canadian Cancer Trials Group MA.31 phase 3 clinical trial. The investigation assessed both percentage TILs by scoring on hematoxylin and eosin (H&E)-stained sections, as well as an assessment of immune constituents [CD8, FOXP3, CD56, and programmed cell death protein 1 (PD-1)] by immunohistochemistry. In the MA.31 clinical trial, patients were randomized 1:1 to receive trastuzumab or lapatinib, in combination with a taxane as first-line therapy in patients with metastatic HER2-positive breast cancer (5). The final analysis confirmed the superiority of the trastuzumab-based approach for progression-free survival (PFS) in this subtype. While lapatinib is now rarely utilized in the first line setting, this secondary analysis examined the prognostic role of immune infiltrates, but was particularly well placed to evaluate lymphocytic infiltrates as a potential predictive biomarker for the benefit of trastuzumab versus lapatinib.

To put this data into context, the recently published secondary analysis of the CLEOPATRA trial is the only other study to evaluate the influence of TILs (as quantified on H&E slides) from 678 patients in the setting of advanced HER2-positive breast cancer (6). Patients enrolled in CLEOPATRA were randomized to the addition of either pertuzumab or placebo to first line therapy with trastuzumab and docetaxel (7). In this study, higher TIL levels were significantly associated with improved overall survival (OS), however there was no significant predictive role for the benefit of the addition of pertuzumab to trastuzumab and docetaxel. While the CLEOPATRA analysis serves as an interesting comparator to the secondary analysis of MA.31, differences between the studies in terms of treatment randomization, number of events, and length of follow-up should be noted and will be described here within.

There are several notable findings from the secondary analysis of MA.31. Even though TIL evaluation occurred exclusively in archival primary tumor samples, the median level of TILs by H&E assessment (5%) is lower than the levels reported in studies of patients with early stage HER2-postive disease (1,3). As higher TIL levels on H&E assessment have been confirmed as an independent positive prognostic factor in early stage disease (1) this is not that surprising, however does reinforce that those patients who eventually do develop incurable advanced disease often have lower levels of pre-existing anti-tumor

immunity at diagnosis. Moreover, in a limited number of paired samples from the CLEOPATRA analysis, TIL levels are shown to be lower in metastatic biopsy samples as compared with archival primaries. This is proposed to be due to a number of mechanisms that develop as a result of evolution to advanced disease including the selection of non-immunogenic clones with immunoediting, and the development of tumor-intrinsic immune-evasive mechanisms or a more suppressive tumor microenvironment (8). Subsequently, TIL levels in freshly obtained metastatic tissue biopsies may provide a better representation of the present immune microenvironment. Furthermore, the lower TIL levels observed in these patients supports the use of therapies that have the potential to induce as well as augment adaptive immune responses, such as trastuzumab and trastuzumab emtansine (9,10).

In the MA.31 study, none of the assessed biomarkers were significantly prognostic for PFS or OS. On the other hand, in the CLEOPATRA study, patients with higher TIL levels had a non-significant trend towards improved PFS, and a statistically significant improvement in OS. This finding was not entirely unexpected. A greater magnitude of benefit for OS as compared with PFS was noted in the CLEOPATRA study itself (median OS benefit of 15.7 months versus median PFS benefit of 6.3 months) (11). A similar phenomenon has also been noted in studies of PD-1 immune checkpoint blockade in non-squamous non-small-cell lung cancer and renal cell carcinoma (12,13). These findings also reinforce OS as the gold standard endpoint for trials assessing the benefit of immunotherapies, as well as the evaluation of immune influences on prognosis.

A possible explanation for the lack of significance for prognosis of the assessed biomarkers in MA.31 is the limitation in statistical power to fully evaluate this. Perhaps the most significant contribution is the randomization to either lapatinib or trastuzumab itself. As trastuzumab is now recognized to have the potential to influence adaptive immune responses (9,10,14) and is recognized as a key component of modern standard-of-care first line therapy, the generalizability of TIL findings in patients randomized to lapatinib is limited. Additionally, with less than half the median follows up time in MA.31 (21.5 months) as compared with CLEOPATRA (50 months), the limited number of PFS events and deaths hampers the ability to find prognostic significance. Analysis after a longer period of follow up, and the accumulation of more events may better define this. Interestingly, the biomarkers assessed by immunohistochemistry were only evaluable in roughly 60% of tumor samples compared with 95% of tumor samples using the more pragmatic method of simple examination of full-face H&E sections described by the International Immuno-Oncology Biomarkers Working Group (15,16). This again presents as a limitation in statistical power, however it is notable that none of the biomarkers showed a significant signal of poor prognosis, including those markers considered to be immunosuppressive (FOXP3 and PD-1).

Perhaps the most interesting finding of the MA.31 secondary analyses is the significantly increased magnitude of benefit of trastuzumab versus lapatinib in patients with low CD8+ TIL counts versus those with high CD8+ TIL counts. As such, low levels of CD8+ TILs were predictive for poorer response to lapatinib versus trastuzumab. The authors postulate that the tumors with low CD8+ TIL counts potentially represent tumors with poor pre-existing immunogenicity and therefore harbor less developed immune-evasive mechanisms. Subsequently, they stand to gain more from trastuzumab based therapy which acts by multiple mechanisms—inhibition of HER2 signalling, enhancement of antibody dependent cell-mediated cytotoxicity, and priming of anti-tumor adaptive T-cell responses (10,14,17). It should be noted however that the subgroup with low CD8+ TIL counts (n=76) comprised much smaller numbers than the subgroup with high CD8+ TIL counts (n=351) using their pre-specified cut-off, and that the interaction was not statistically significant using TIL counts by H&E assessment.

What does this mean for the use of lapatinib in metastatic HER2-positive breast cancer? Seminal trials in the use of dual HER2-antibody blockade with trastuzumab and pertuzumab (7), and for the antibody-drug conjugate T-DM1 (18,19) have resulted in many guidelines recommending the use of lapatinib only after progression in the setting of prior exposure to these antibodies based treatments, at least in the absence of intracranial disease. The benefits of lapatinib may be further reduced in this setting as anti-tumor immunity is often diminished in later stage disease and with extensive disease burden. Lapatinib has also had a poor track record in its ability to be combined with chemotherapy and this may have reduced its efficacy in the registration phase III adjuvant study (20).

Although the MA.31 study is ideal to investigate the role of immune biomarkers in predicting of benefit of trastuzumab versus lapatinib, it does not necessarily address the issue of the differences between a HER2-antibody based approach versus small molecule tyrosine kinase inhibitors in general. A number of new small

molecule tyrosine kinase inhibitors are being developed and have shown promising signs of more potent inhibition with some having very manageable safety profiles (21-24), and may be particularly promising for patients who develop intracranial metastases (25). This is likely, in part due to more potent inhibition of HER2-signalling, as well as improved central nervous system (CNS) penetrance. CNS disease remains the major cause of ongoing morbidity and cause of death for patients with HER2-positive disease, often in the absence of extra-cranial progression. As well as this, ongoing investigation into the interaction of host adaptive immune response and therapeutic benefit to various agents must continue to be addressed in order to optimize precision immune-oncology for patients with HER2-positive disease.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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