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LETTER TO THE JOURNAL

HER2 and HER3 expression during neoadjuvant treatment of HER2-negative early breast cancer: potential for biomarker-driven sequencing of T-DXd and HER3-DXd

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With the development of novel antibody-drug conjugates (ADC) such as T-DXd (trastuzumab deruxtecan) and HER3-DXd (patritumab deruxtecan), global tumor cell targeting has become possible beyond the human epidermal growth factor receptor (HER) 2-positive setting [1, 2]. Both drugs offer promising options for individualized treatment targeting HER2 and HER3 expression, potentially even in tumors which are currently considered "HER2-negative". Relatively little is known about the efficacy of HER3-DXd in tumors with low HER3 expression, except for data from one recent study investigating its efficacy across different HER3 expression levels [3].

The DESTINY-Breast04 trial (NCT03734029) demonstrated that T-DXd-treated patients with HER2-low expressing metastatic breast cancer had significantly longer progression-free and overall survival than those who were treated with the physician's choice of chemotherapy [4]. It is therefore important to understand whether neoadjuvant systemic therapy is able to induce or up-regulate HER2 and/or HER3 protein expression – raising the hope that neoadjuvant chemotherapy (NACT)

Abbreviations: ADC, antibody-drug conjugates; cN stage, clinical nodes stage; cT stage, clinical tumor stage; ER, estrogen receptor; HER, human epidermal growth factor receptor; HER3-DXd, patritumab deruxtecan; MUC1, Mucin-1; NACT, neoadjuvant chemotherapy; NET, neoadjuvant endocrine therapy; pCR, pathologic complete remission; PR, progesterone receptor; SoC, standard-of-care; T-DXd, trastuzumab deruxtecan.

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and neoadjuvant endocrine therapy (NET) could be used to "prime" tumor cells for subsequent HER-targeting by adjuvant systemic therapy in case of non- pathologic complete remission (pCR). Therefore, we investigated the dynamics of HER2 and HER3 expression in HER2 non-amplified breast cancer by retrospectively analyzing the immunohistochemical HER2 and HER3 protein expression in pre- and post-treatment tumor samples, treated with neoadjuvant systemic chemo- and endocrine therapy, from the prospectively randomized ABCSG 34 trial.

The trial design, inclusion criteria, and main clinical results of this trial were reported previously [5]. Briefly, in ABCSG 34, 400 pre- and post-menopausal women with HER2-negative early breast cancer received either standard-of-care (SoC) NACT (n = 311) or NET (n = 98), with or without the Mucin-1 (MUC1) directed vaccine tecemotide (Supplementary Methods). Immunohistochemical data on HER2 and HER3 expression were available from paired pre- and post-treatment samples of 183 of these patients (Supplementary Figure S1), which did not significantly differ from the overall study population regarding clinical-pathological parameters (Supplementary Table S1).

In tumors that had been subjected to SoC NACT, HER2 expression was detected at baseline in 57/134 (42.5%) tumors, with low expression (1+) in 39.6%, and equivocal expression (2+) in 3.0% of cases. HER2 expression in the post-treatment surgical samples was detected in 68/134 (50.7%) tumors, with a HER2 score of 1+ in 43.3%, and a HER2 score of 2+ in 7.5% of tumor samples (p = 0.050 for marginal homogeneity). This corresponds to an increase of HER2 from baseline to surgery in 34/134 (25.4%; 95% CI, 18.8% to 33.4%) tumors, and a decrease in response to SoC NACT in 19/134 (14.2%; 95% CI, 9.3% to 21.1%) tumors (Supplementary Table S2).

In the 58 NET-treated tumors, baseline HER2 expression was observed in 21/58 (36.2%) samples, all of which showed weak expression (1+). HER2 expression in post-treatment samples was detected in 42/58 (72.4%) samples, with low protein expression in 35/58 (60.3%), and equivocal expression in 7/58 (12.1%) cases. This corresponds to a significant difference in HER2 expression levels between baseline and post-treatment ($p < 0.001$). An up-regulation of HER2 expression from baseline to surgery was seen in 30/58 (51.7%; 95% CI, 39.2% to 64.1%) cases, a decrease in response to SoC NET in only 3/58 (5.2%; 95% CI, 1.8 to 14.1%) cases (Supplementary Table S3).

Overall, when HER3 expression in pre- and post-treatment samples were compared, we found significant differences in protein expression ($p < 0.001$ for marginal homogeneity) with an increase in HER3 expression in 29/185 (15.7%; 95% CI, 11.1% to 21.6%), and a decrease in HER3 expression in 62/185 (33.5%; 95% CI, 27.1 to 40.6%) samples.

Baseline HER3 expression in NACT-treated tumors was detected in 125/127 (98.4%) cases. Expression was weak (1+) in 11/127 (8.7%), moderate (2+) in 52/127 (40.9%), and high (3+) in 62/127 (48.8%) cases (For representative examples, see Supplementary Figure S2). Within the post-treatment surgical samples, HER3 expression was detected in 118/127 (92.9%) tumors, with weak expression (1+) in 18/127 (14.2%), moderate expression (2+) in 42/127 (33.1%), and high expression (3+) in 58/127 (45.7%) cases, resulting in statistically significant different marginal distributions (p = 0.019). This corresponds to an increase of HER3 protein expression from baseline to surgery in 23/127 (18.1%; 95% CI, 12.4% to 25.7%), and a decrease in response to SoC NACT in 39/127 (30.7%; 95% CI, 23.4 to 39.2%) tumors (Supplementary Table S4).

In the 58 NET-treated tumors, baseline HER3 expression was observed in all 58 (100%) cases, with weak expression (1+) in 4/58 (6.9%), moderate expression (2+) in 12/58 (20.7%), and high expression (3+) in 42/58 (72.4%) cases. After 6 months of aromatase inhibitor treatment, HER3 expression was detected in 57/58 (98.3%) surgical samples, with low expression (1+) in 7/58 (12.1%), moderate expression (2+) in 25/58 (43.1%), and high expression (3+) in 25/58 (43.1%) cases. This corresponds to a highly significant alteration in HER3 expression ($p < 0.001$ for marginal homogeneity) with an up-regulation of HER3 expression from baseline to surgery in 6/58 (10.3%; 95% CI, 4.8% to 20.8%), and a decrease in response to NET in 23/58 cases (39.7%; 95% CI, 28.1 to 52.5%, Supplementary Table S5).

We found a weak correlation between pre-therapy HER2 and HER3 protein expression (r = 0.26, $p < 0.001$; Spearman Correlation Coefficient), estrogen receptor (ER) expression (r = 0.21, p = 0.004), and progesterone receptor (PR) expression (r = 0.17, p = 0.020), respectively. No statistically significant correlation was detected between pre-therapy HER2 and Ki67, cT (clinical tumor) stages, or cN (clinical node) stages. Pre-treatment HER3 expression levels were moderately correlated with ER expression (r = 0.51, $p < 0.001$), weakly correlated with PR expression (r = 0.27, $p < 0.001$), and inversely correlated with Ki67 (r = -0.25, $p < 0.001$). No significant correlation, however, was detected between pre-therapy HER3 expression and neither cT nor cN stages (Supplementary Figure S3).

Understanding the prevalence, magnitude, and kinetics of HER2 and HER3 expression during systemic treatment is critical for the optimization of HER-targeting strategies. In our study, we observed HER3 expression in almost all baseline breast cancer samples. And HER3 protein expression remained high after NACT or NET. These results suggest that HER3 protein expression represents a potential therapy target and is potentially up-regulated in

response to neoadjuvant SoC systemic therapy. Currently, however, HER2 expression kinetics in response to neoadjuvant treatment is clinically more relevant, since T-DXd in HER2 overexpressing tumors within the post-neoadjuvant setting is under investigation in the ongoing DESTINY-Breast05 (NCT04622319) trial. An up-regulation of HER2 through neoadjuvant systemic therapy could render more tumors potential targets for adjuvant T-DXd-based treatment strategies. Our results concurrently suggest that this may be possible, particularly if NET is being used.

In the next step, the kinetics of HER expression in response to anti-HER2 or anti-HER3 treatments need to be investigated. This can only be appropriately addressed in prospective, neoadjuvant clinical trials, which include neo-adjuvant T-DXd and HER3-DXd treatment. Clinical insights derived from such trials might ultimately enable us to offer optimal treatment sequences in a biological setting which, until now, has to be considered HER-therapy refractory.

AUTHOR CONTRIBUTIONS

CFS, SWJ, MF, and MG devised the study concept and analyzed the data. CFS, SWJ, and MF generated and analyzed data. CFS, SWJ, MF, MG, and DH wrote the manuscript. UMH, CMM, DE, MB, AP, GP, SKS, CS, MR, EP, RG, ZBH, CD, GGS, MS, FF, RB, AS, JML, DS, MS, CF, LS, SE critically reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

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CONSENT FOR PUBLICATION

All co-authors declare their consent for the publication of the present article in its current form.

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DATA AVAILABILITY STATEMENT

The clinical data can be shared upon approval of the analysis proposal by the Steering Committee and sponsor of the ABCSG 34 study, and after a data-sharing agreement has been signed. Please contact the corresponding author for more information.

ETHICS STATEMENT

The ABCSG 34 study as well as the research project were conducted according to the principles of the Declaration of Helsinki and the ICH Guidelines, and ethical approvals by the respective appropriate Ethics Committees were obtained as required. Informed consent signed by patients enrolled in the ABCSG 34 study included permission for the future research use of biological samples. For the research project, biological samples from patients with valid informed consent obtained during the conduct of the ABCSG 34 study were eligible for testing without obtaining further patient consent.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.