San Antonio Breast Cancer Symposium® December 9-12, 2025. #PS3-12-09

# Spatial immune profiling and pathological response after neoadjuvant therapy in early HER2-positive breast cancer

Carla Gullotta[1-3], Sandra Cobo[1-2], Vìctor Albarrán-Fernández[1-2], Carlota Rubio-Perez[1-2] , Patricia Galván[1-2], Angela Aguirre[1-2], Oleguer Castillo[1-2], Paula Blasco[1-2], Esther Sanfeliu[1-2-7], Anabel Martínez- Romero[1-2], Mercedes Marín—Aguilera[8], Benjamin Walbaum[1-4-5], Olga Martínez-Sáez[1-2-6], Milana Bergamino[1-2-6], Maria Vidal[1-2-6], Montserrat Muñoz[1-2-6], Tomas Pascual[1-2-6], Barbara Adamo[1-2-6], Aleix Prat[1-2-8], Fara Brasó-Maristany[1-2-8] , Laura Angelats[1-2-6]

1. Translational Genomics and Targeted Therapies in Solid Tumors, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain. 2. Department of Medical Oncology, Clinic Barcelona Comprehensive Cancer Center, Barcelona, Spain 3. Fondazione Policlinico Universitario Campus Bio-Medico di Roma, Italy
4. Department of Hematology Oncology, Pontificia Universidad Católica de Chile, Santiago, Chile, 5. Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile 6. Facultat de Medicina i Ciencias de la Salut, Universitat de Barcelona, Barcelona, Spain, 7. Pathology Department, Hospital Clínic de Barcelona, Barcelona, Spain, 8 Reveal Genomics, Barcelona, Spain

## Background

- HER2-positive (HER2+) breast cancer is a heterogeneous disease, with variability in tumor architecture, immune infiltration, and protein expression within both the tumor (TU) and the surrounding tumor microenvironment (TME).
- Understanding how the TME influences treatment response is critical.
- This study investigates whether protein expression levels and intratumoral heterogeneity are associated with pathological complete response (pCR) in patients with HER2+ early breast cancer (EBC) treated with neoadjuvant therapy.

## Methods

- 54 HER2+ EBC core biopsies from patients treated with neoadjuvant HER2-targeted chemotherapy at Hospital Clinic of Barcelona (April 2022 – September 2023) were analyzed.
- Protein expression was measured using GeoMX Digital Spatial Profiling in both tumor (PanCK$^+$) and immune (CD45$^+$) compartments.
- For each sample, at least three regions of interest (ROIs) were selected in both TU and TME to ensure spatial coverage and capture intratumoral heterogeneity **(Figure 1).**
- Protein expression data were batch-corrected; within each ROI, the mean expression was calculated to represent the overall protein level, while the coefficient of variation (CV) was computed as a relative measure of variability, serving as a proxy for intraregional heterogeneity **(Figure 1)**.
- The CV (DS/mean) was selected as the preferred metric of heterogeneity since it normalizes variability to the mean, allowing comparison across proteins with different expression magnitudes **(Figure 1).**
- HER2DX and PAM50 data were available from the same sample
- Stromal tumor-infiltrating lymphocytes (TILs) were assessed according to international guidelines.
- Associations between protein metrics, HER2DX scores, TILs, and pCR were tested using Wilcoxon, Kruskal–Wallis, and logistic regression through R software.

## Results

- Clinical and biological features showed general concordance with those reported in a HER2+ neoadjuvant cohort **(Table 1).**
- The HER2DX pCR score was significantly associated with pCR status as a continuous variable **(Figure 2).** This association remained significant across multivariate models that included spatial protein expression metrics and HR status.
- Associations between protein means, TILs and pCR by univariate analysis are illustrated in **Figure 3**. Notably, higher HER2 mean in the tumor compartment correlated positively with pCR (odds ratio [OR] = 1.54, 95% CI: 1.14–2.20, p = 0.008), whereas higher TIM3 mean, a marker of immune exhaustion, was negatively associated with pCR (OR = 0.23, 95% CI: 0.04–0.82, p = 0.042).
- Wilcoxon correlations between mean, CVs of each protein and pCR are shown in **Figure 4.** Notably, it is the CV, rather than the mean expression, that shows a statistically significant positive correlation with pCR status. These associations remained statistically significant in univariate analysis but did not retain significance in multivariate models **(Figure 2)**.

## Results

**Figure 1. Schematic overview of the study design**

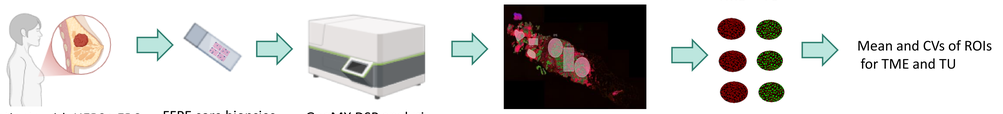



**Table 1. Clinical and biological features**

| Variable | | All (n=54) | no pCR (n=26) | pCR (n=28) |
|---|---|---|---|---|
| Age (median, range) | | 57.9 (29.1–87.7) | 59.2 (34.5–87.7) | 54.7 (29.1–84.0) |
| PAM50 | Basal | 6 (11.1%) | 2 (7.7%) | 4 (14.8%) |
| | Her2 | 23 (42.6%) | 8 (30.8%) | 15 (55.6%) |
| | LumA | 10 (18.5%) | 8 (30.8%) | 2 (7.4%) |
| | LumB | 11 (20.4%) | 8 (30.8%) | 3 (11.1%) |
| | Normal | 3 (5.6%) | 0 | 3 (11.1%) |
| Menopausal status | No | 18 (33.3%) | 6 (23.1%) | 12 (42.9%) |
| | Yes | 36 (66.7%) | 20 (76.9%) | 16 (57.1%) |
| HR status | Neg | 15 (27.8%) | 1 (3.8%) | 14 (50%) |
| | Pos | 39 (72.2%) | 25 (96.2%) | 14 (50%) |
| HER2DX pCR score | High | 16 (31.4%) | 3 (12%) | 13 (50%) |
| | Medium | 14 (27.5%) | 2 (8%) | 12 (46.2%) |
| | Low | 21 (41.2%) | 20 (80%) | 1 (3.8%) |

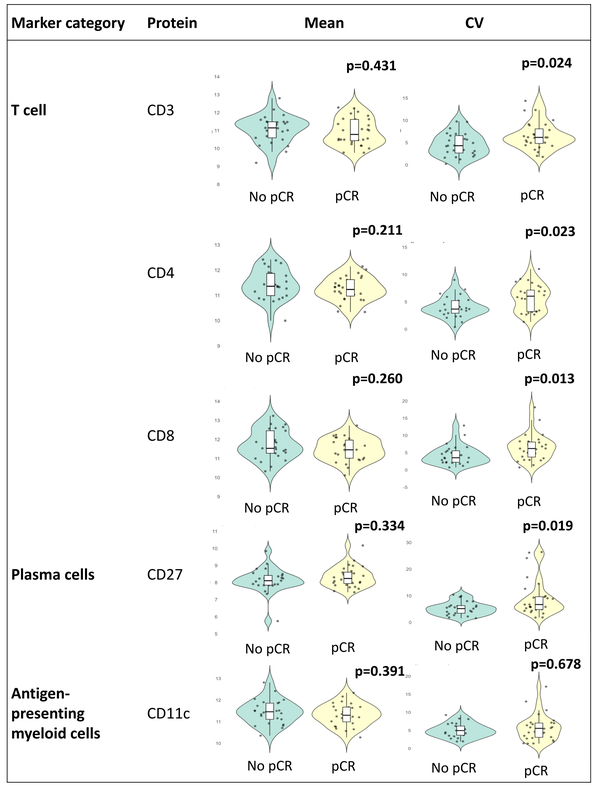
**Figure 2. Univariate and Multivariate analysis of HER2DX pCR score, HR status and immune protein CVs**

| Variable | N | Univariate | p | Multivariate | p |
|---|---|---|---|---|---|
| HER2DX pCR score 10-unit increase | 51 | 1.90 (1.43, 2.77) | <0.001 | 3.23 (1.71, 9.64) | 0.004 |
| HR status NEG | 15 | Reference | | Reference | |
| POS | 38 | 0.04 (0.00,0.24) | 0.003 | 2.04 (0.05, 69.69) | 0.685 |
| CD3 CV | 53 | 1.30 (1.06, 1.67) | 0.022 | 0.65 (0.26, 1.32) | 0.260 |
| CD4 CV | 53 | 1.41 (1.09, 1.90) | 0.015 | 1.59 (0.78, 3.87) | 0.231 |
| CD8 CV | 53 | 1.24 (1.03, 1.54) | 0.038 | 1.09 (0.46, 2.42) | 0.839 |
| CD27 CV | 53 | 1.24 (1.06, 1.55) | 0.030 | 2.18 (1.26, 6.34) | 0.042 |
| CD11C CV | 53 | 1.09 (0.90, 1.35) | 0.395 | 0.49 (0.21, 0.92) | 0.054 |

**Figure 3. Association between protein mean, TILs (%) and pCR**

| Variable | N | Odds ratio | p |
|---|---|---|---|
| TILs (%) | 53 | 1.01 (0.99, 1.04) | 0.318 |
| HER2 | 53 | 1.54 (1.14, 2.20) | 0.008 |
| Tim-3 | 30 | 0.23 (0.04, 0.82) | 0.042 |
| ICOS | 53 | 1.96 (0.94, 4.60) | 0.091 |
| CD14 | 30 | 0.31 (0.07, 1.11) | 0.091 |
| STING | 30 | 0.30 (0.06, 1.13) | 0.105 |
| CD45 | 53 | 0.51 (0.21, 1.13) | 0.113 |
| CTLA4 | 53 | 1.78 (0.83, 4.17) | 0.155 |
| CD45RO | 30 | 0.53 (0.20, 1.26) | 0.165 |
| CD4 | 53 | 0.48 (0.16, 1.35) | 0.179 |
| CD8 | 53 | 0.57 (0.24, 1.27) | 0.184 |
| CD27 | 53 | 1.67 (0.72, 4.46) | 0.259 |
| CD80 | 53 | 1.42 (0.76, 2.86) | 0.289 |
| B7-H3 | 30 | 1.58 (0.65, 4.36) | 0.326 |
| Ki-67 | 53 | 1.81 (0.55, 6.53) | 0.339 |
| OX40L | 30 | 0.57 (0.14, 1.91) | 0.375 |
| PanCK | 45 | 0.79 (0.43, 1.38) | 0.425 |
| CD56 | 53 | 1.68 (0.48, 6.43) | 0.426 |
| PD-L1 | 53 | 1.42 (0.59, 3.69) | 0.439 |
| CD3 | 53 | 0.77 (0.37, 1.58) | 0.480 |
| FOXP3 | 30 | 1.43 (0.48, 4.72) | 0.526 |
| 4-1BB | 30 | 0.67 (0.18, 2.29) | 0.528 |
| CD25 | 53 | 1.30 (0.54, 3.30) | 0.562 |
| CD40 | 53 | 1.22 (0.62, 2.47) | 0.564 |
| Beta-2-microglobulin | 53 | 0.73 (0.23, 2.24) | 0.583 |
| PD-1 | 53 | 1.02 (0.34, 3.05) | 0.978 |

**Figure 4. Wilcoxon correlations between mean and CVs of immune proteins and pCR**




## Conclusions

In HER2+ EBC, spatial heterogeneity of immune-related proteins, mainly the presence of T-cell markers, was associated with a higher pCR rate, while expression levels were not, suggesting a role for immune variability within the TME in modulating treatment efficacy. These results also highlight the ability to predict pCR of total HER2 abundance over regional heterogeneity. The HER2DX pCR score remained a robust predictor of response in all statistical analyses and spatial metrics did not outperform or override its predictive value in this cohort.

The presenting author declares no conflicts of interest. Email: Carla.gullotta24@gmail.com