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Peripheral immune profiling during neoadjuvant HER2-targeted therapy in HER2-positive (HER2+) early breast cancer (EBC): Mass cytometry (CyTOF) analysis from the MARGOT trial (TBCRC052)

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Introduction

- Anti-tumor immune activity impacts outcomes in HER2+ EBC[1], and anti-HER2 antibodies act in part through immune mechanisms such as antibody-dependent cell-mediated cytotoxicity (ADCC)[2].
- Margetuximab is an anti-HER2 antibody engineered to enhance ADCC through increased affinity for FcγRIIIa[3] compared to trastuzumab.
- Early changes in circulating immune populations during anti-HER2 therapy remain poorly characterized and may offer insights into the immune mechanisms of HER2-targeted therapy.

We used mass cytometry (CyTOF) to profile peripheral blood mononuclear cells (PBMCs) at baseline and on-treatment in patients receiving neoadjuvant chemotherapy plus HER2-directed therapy.

Methods & Materials

- The investigator-initiated MARGOT trial (NCT04425018) randomized 171 patients with stage II–III HER2+ EBC 2:1 to receive neoadjuvant TMP (margetuximab/pertuzumab/paclitaxel) or THP (trastuzumab/pertuzumab/paclitaxel) (**Figure 1**).
- The primary endpoint (pCR) was previously reported: TMP showed a numerically higher pCR rate than THP (56% vs 46%), without statistical significance, with a greater delta in HR+ patients (+19%) compared with HR- (-11%)[4].
- This PBMC sub-study included all patients with paired PBMCs at baseline and cycle 2 day 1 (C2D1; 3 weeks on-treatment) from sites meeting quality criteria (QC) before the data cut-off (**Figure 2**).
- A total of 91 patients (THP n=25, TMP n=66) underwent CyTOF profiling at both timepoints using 22 surface and 13 functional markers, identifying 26 immune cell populations (**Figure 3**). For these 91 patients, pCR rates were similar to the overall trial (TMP 52%, THP 48%).
- Descriptive statistics were used to summarize baseline and on-treatment cell type changes (Δ). Associations with pCR were evaluated using logistic regression; on-treatment changes by treatment arm were analyzed using linear regression; associations with HR status were analyzed using Wilcoxon rank sum tests. Multiple testing was addressed using false discovery rate (q-values); raw p-values <0.05 were considered of interest.

We hypothesized that ADCC effector populations (e.g., NK cells, monocytes) would change more with margetuximab, and that evidence of ADCC would correlate with treatment response.

CONCLUSIONS

- **Baseline PBMC profiles showed only modest differences between patients with HR+ and HR- HER2+ early breast cancer.**
- **Baseline circulating CD8 TCM, CD8 TEMRA, and classical monocytes may correlate with response to TMP.**
- **Longitudinal PBMC profiling revealed on-treatment changes after 3 weeks (C2D1), including a decline in classical monocytes in both treatment arms and a decline in a mature NK-cell subset only with TMP, potentially reflecting treatment specific effect on anti-tumor ADCC.**

Figure 1. Study Schema.

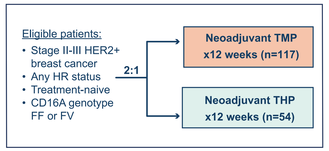


Figure 2. CONSORT diagram.

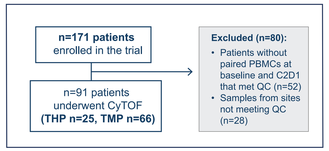
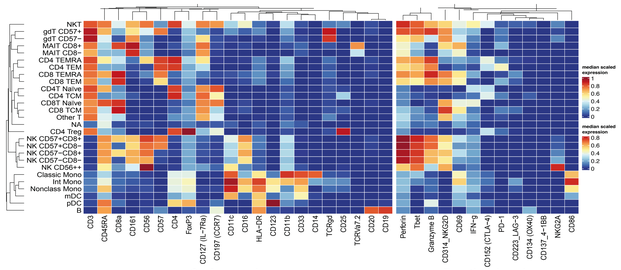


Figure 3. CyTOF panel design and median marker expression and annotation of 26 immune cell clusters



Results

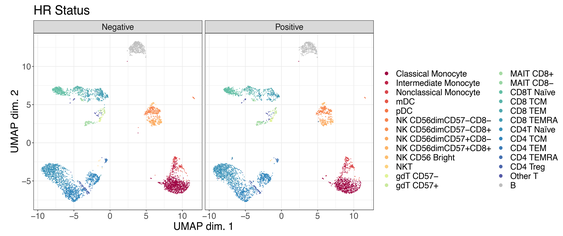
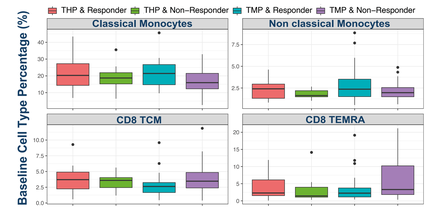


Figure 4. Baseline PBMC composition in early HER2+ breast cancer by HR status.

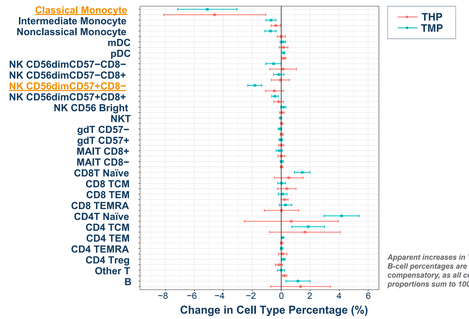
PBMC composition at baseline was comparable between **HR+ (n=56)** and **HR- (n=35)** tumors, with only modest differences such as higher classical monocytes in HR- disease (median 21.4% vs 16.2%; p=0.019, q=0.297).

Figure 5. Baseline immune populations associated with pCR overall and by treatment arm (only raw p-value<0.05 shown).



Baseline cell type percentage	Overall population (n=91)	TMP (n=66)	THP (n=25)
Classical monocytes	OR 1.07 (95% CI 1.01-1.13), p=0.030, q=0.330	OR 1.09 (95% CI 1.01-1.18), p=0.027, q=0.279	
Non classical monocytes	OR 1.45 (95% CI 1.04-2.15), p=0.043, q=0.333		
CD8 TCM		OR 0.75 (95% CI 0.55-0.97), p=0.043, q=0.279	
CD8 TEMRA		OR 0.88 (95% CI 0.77-0.98), p=0.041, q=0.279	

Abbreviations: CD8 TCM - CD8 central memory T cells; CD8 TEMRA - CD8 terminal effector memory RA+ T cells



Apparent increases in T- and B-cell percentages are likely compensatory, as all cell type proportions sum to 100%.

Figure 6. Early on-treatment cell type changes (Δ) by treatment arm.

- After 3 weeks of therapy, classical monocytes showed the largest change across all cell populations in both treatment arms (mean Δ -5.1% [p<0.001, q<0.001] in TMP and -4.6% [p=0.013 q=0.110] in THP).
- A decrease in one mature NK cell subset (CD56dimCD57+CD8a-) was observed only with TMP (TMP: mean Δ -1.83% vs THP: mean Δ -0.47%; mean difference TMP vs THP=-1.36; p=0.002, q=0.052).
- No significant differences were observed between responders and non-responders (*data not shown*).

References: (1) Fernandez-Martinez et al. JAMA Oncol. 2023; (2) Arnould et al. Br J Cancer 2006; (3) Nordstrom et al. Breast Cancer Res 2011; (4) Waks et al. San Antonio Breast Cancer Symposium 2024.

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