#3548; Gene-expression pathways and dynamics during neoadjuvant chemo-free therapy predict pathologic complete response in ER+/HER2+ breast cancer (BC)

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Background and aim

- In the NA-PHER2 study we assess the association between biological pathways with pathological complete response (pCR) and Ki67 down-regulation in patients with HER2+ and ER+ (> 10%) breast cancer undergoing neoadjuvant dual HER2 block (trastuzumab+pertuzumab), palbociclib +/- fulvestrant **(Figure 1**)
  - Fulv cohort (n=30, dual block, palbociclib and fulvestrant)[1]
  - NoFulv cohort (n=28, dual block and palbociclib) was enrolled after completing Fulv cohort[2]

Figure 1. Scheme of treatment regimens

Patients with early and locally advanced HER2-positive AND ER-positive breast cancer Chemo-naïve.

→ **Fulv Cohort** → **HPPaF x 6 three-weekly cycles** (§) Herceptin+Pertuzumab+Palbociclib+FLV

→ **NoFulv Cohort** → **HPPa x 6 three-weekly cycles** (§) Herceptin+Pertuzumab+Palbociclib

H = Trastuzumab, 8 mg/kg on first dose, 6 mg/kg thereafter x 6;
P = Pertuzumab, 840 on first dose, 420 mg thereafter x 6;
Pa = Palbociclib, 125 mg po q.d. x 21 q. 4 weeks x 5;
F = Fulvestrant (FLV) administrated intra-muscle at the dose of 500 mg every 4 weeks x 5 with an additional 500 mg dose given two weeks after the initial dose.

(§) The total duration of neoadjuvant palbociclib (5 cycles every 4 weeks) and fulvestrant (5 administration every 4 weeks plus the additional dose given two weeks after the initial dose) was selected to match as closely as possible the total duration of the six planned 3-weekly administration of trastuzumab and pertuzumab.

Methods

- We assessed RNA-seq on core-biopsies obtained pre-treatment [n=53/58 (91.4%)], at week 2 [n=49/58 (84.5%)], and on residual disease at surgery [SX, n=42/45 (93.3%)].
- We investigated curated gene sets from Hallmark (n=50), KEGG (n=186) and Reactome (n=1520) at the single sample level.
- In the overall population and in each cohort, we primarily assessed association of gene sets (biomarkers) with pCR or Ki67 down-regulation (centrally evaluated) at week 2 and at SX.
- Significance defined by Benjamini-Hochberg adjusted p-value < 0.05.

Results

- In the biomarker population, pCR rate was 22.6% (28.6% and 16.0% in Fulv cohort and in NoFulv cohort, respectively) (Figure2).
- High pre-treatment expression of interferons, allograft rejection, IL6/JAK/STAT3 and IL2/STAT5 signaling were associated with pCR, whereas NOTCH signaling and glycerophospholipid biosynthesis with residual disease (Figure 3).
- In Fulv cohort, high expression of metabolic pathways (i.e., reactive oxygen species, fatty acid metabolism) and PI3K/AKT/mTOR signaling were associated with pCR (Figure 3).
- At week 2, most of these pathways were also predictive of pCR (Figure 4A), with the addition of trafficking and processing of endosomal toll-like receptors (TLR, p=4.0E-06, adj p=0.006), with a similar effect in Fulv and NoFulv cohorts (p=0.001 and p=0.0006, respectively) (Figure 4B).
- Pre-treatment signatures were poorly predictive of Ki67 dynamic. Among tumor with low Ki67 (Ki67<10%) at week 2, 71% had a rebound at SX (Ki67≥10%) (Figure 5A). High pre-treatment NOTCH signaling was predictive of rebound (Figure 5B).

Figure 2. Rate of pCR status in the overall population and by cohort.

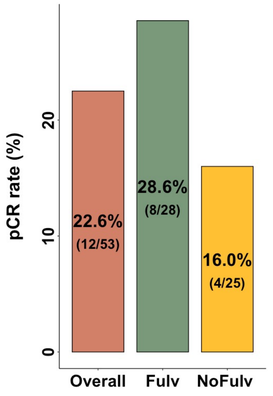


Figure 3. Baseline gene set scores significantly up-regulated (red) and down-regulated (blue) in patients achieving pCR compared to RD patients.

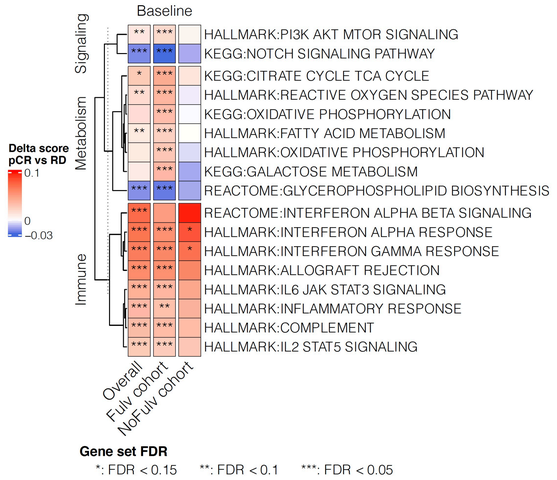


Figure 4. A) Heatmap showing differential expression between pCR and RD patients evaluated at week 2 of gene sets shown in Figure 3. B) Boxplot showing the distribution of the REACTOME:TRAFFICKING AND PROCESSING OF ENDOSOMAL TLR gene set scores evaluated at week 2 in pCR and RD patients.

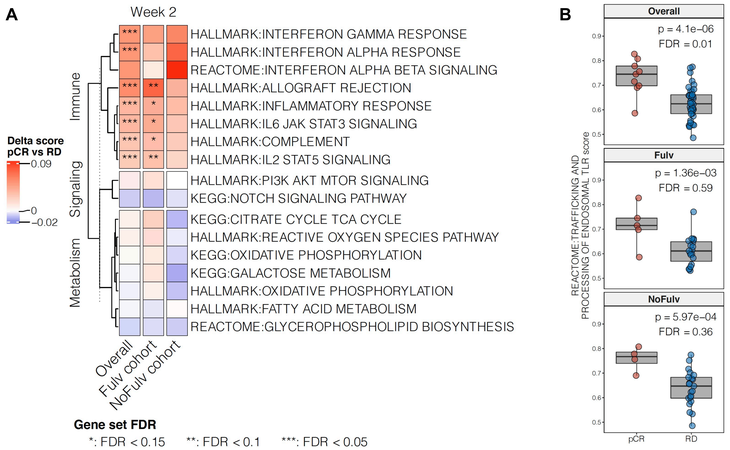
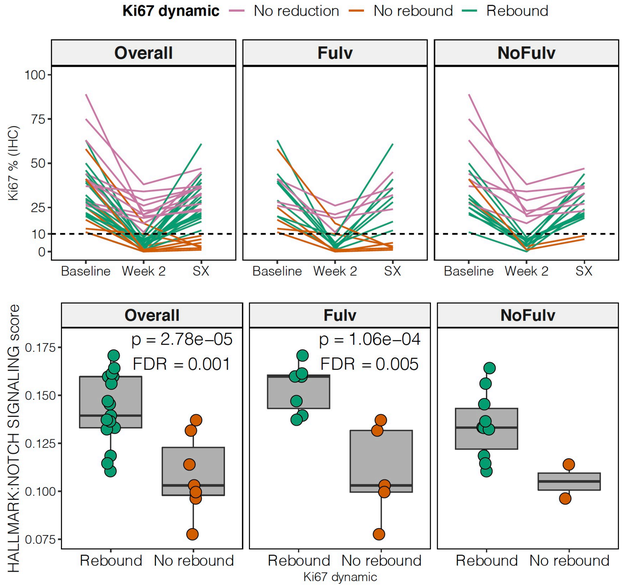


Figure 5. A) Dynamic of Ki67 evaluated by IHC at each time point for the same set of patients. Patients were classified in different groups according to Ki67 levels at week 2 and surgery (cut-point = 10). **B)** Boxplot showing the distribution of the HALLMARK:NOTCH SIGNALING gene set scores evaluated at baseline in patients with and without Ki67 rebound at surgery



Conclusions

- In ER+/HER2+ BC, pre- and on-treatment expression of interferon, JAK/STAT and TLR-related signatures were associated with pCR. Metabolic pathways emerged as associated with both sensitivity and resistance in Fulvestrant cohort.
- NOTCH signaling was associated with lower pCR rate and Ki67 rebound at SX, indicating a significant role of this pathway in resistance.

References
[1] Gianni L, et al. *Lancet Oncol* 2018; [2] Gianni L ASCO 2018

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