Impact of neoadjuvant endocrine therapy on tumor transcriptome in patients with early-stage breast cancer from the FLEX trial

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Background

Neoadjuvant Endocrine Therapy (NET) is seldom used in breast cancer management except in patients with several comorbidities or in elderly patients in which chemotherapy is not an option. Clinical response with NET is not typically achieved until after several months of treatment. In the NET setting, reduction of Ki67 (< 10%) after 2-4 weeks has been used as a predictor of positive response, but studies such as ALTERNATE have questioned this association[1]. It remains uncertain whether a single gene or protein can adequately predict outcomes or inform how NET alters a variety of cancer genes and global tumor biology.

Objective: This study evaluated the effect of short-term NET on the tumor genomics of patients with early-stage breast cancer (EBC) by comparing whole transcriptome gene expression changes in matched pre- and post-NET tumor samples.

Methods

Patients: In this single-institution FLEX substudy performed at Johns Hopkins, 30 HR+HER2- EBC patients with matched pre- and post-treatment specimens who received at least two weeks of NET between 2019 – 2021 were included. Premenopausal women and 1 male patient with breast cancer received Tamoxifen (n=10) and postmenopausal women received either Letrozole (n=10) or Exemestane (n=10).

Changes in immunohistochemistry (IHC): For patients with available clinical information, changes in IHC expression levels of Estrogen receptor (ER), Progesterone receptor (PR), and Ki67 between pre- and post-NET samples were quantified using absolute values, and the median percent change was reported, with significance assessed using the Wilcoxon test.

Genomic Testing: The observational FLEX trial (NCT03053193) enrolls patients with EBC who receive standard of care MammaPrint with or without BluePrint testing and consent to clinically annotated full transcriptome data collection. MammaPrint classifies tumors as having a Low Risk or High Risk of distant metastasis. BluePrint together with MammaPrint, classifies tumors as Luminal A-Type (Low Risk), Luminal B-Type (High Risk), HER2-Type, or Basal-Type.

Differential Gene Expression Analysis: Limma R package was used for quantile normalization and differential gene expression analysis. Significant differentially expressed genes (DEGs) had a false discovery rate of <0.05 and >2-fold change. Pathway enrichment analysis was performed using Metascape.

NET induces robust gene expression changes in breast tumors

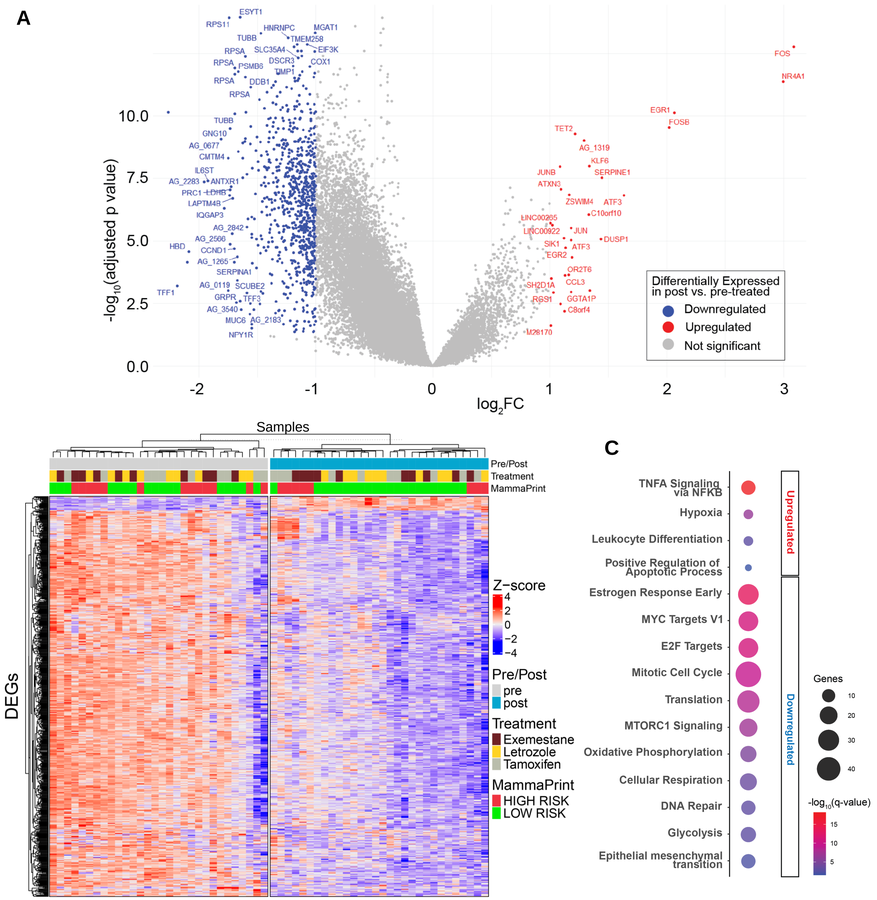

Figure 1: DEGs (red=upregulated; blue=downregulated) detected in post-NET vs. pre-NET samples shown overall in **(A)** volcano plot and **(B)** among each of the 30 patients in a heatmap. **C.** Functional enrichment of DEGs.

MammaPrint is associated with endocrine sensitivity

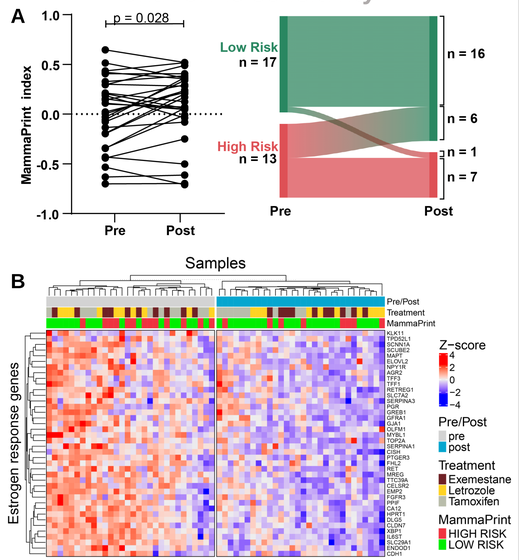

Figure 2: **A.** Change in MammaPrint index and risk following NET. **B.** Downregulated genes associated with estrogen response in post-NET samples

ER, PR, and Ki67 IHC expression following NET

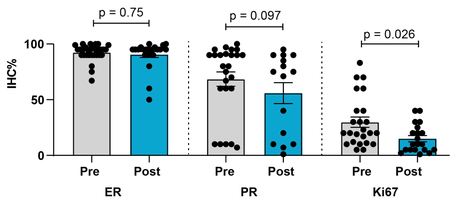

Figure 3: ER, PR and Ki67 expression in pre- vs post-NET samples.

Results

Short-term NET resulted in 774 DEGs detected in breast tumors (Figure 1A-B). A majority of DEGs (748) were downregulated and associated with MYC and MTORC1 signaling, metabolism, cell proliferation, and epithelial mesenchymal transition (Figure 1C). *MGAT1* and *IQGAP3*, which are associated with tumor aggressiveness and metastasis[2,3], were downregulated in post-NET samples (Figure 1A). The 26 upregulated DEGs enriched to terms associated with innate immunity and cell death. *FOS*, *JUN*, and *EGR1*, which are associated with better outcomes, were upregulated following NET[4] (Figure 1A).

Among the 30 patients, there was no change in BluePrint classification (29 Luminal-Type and 1 Basal-Type). However, the average MammaPrint index significantly increased towards a Low Risk score following NET. Indeed, nearly 1 in 4 patients (n=7) had their MammaPrint risk reclassified following NET, with a majority (n=6) changing from High Risk to Low Risk (Figure 2A). This finding is further supported by a downregulation of "Estrogen response – Early" in post-NET samples (Figure 2B).

The median percent change by IHC in matched pre- and post-NET tissue was 2.5% for estrogen receptor (ER) (range: 0-50%; p=0.750), 22% for progesterone receptor (PR) (range: 0-81%; p=0.097), and 9% for Ki67 (range: 0-43%; p=0.026) (Figure 3).

Conclusions

In this study, significant gene expression changes were discovered within a shorter timeframe than when clinical responses are usually observed in the NET setting. This could indicate biological complexity and diverse response pathways, which is better detected by whole transcriptome analysis and MammaPrint than single IHC biomarkers, as evidenced by the downregulation of ER pathway despite no change in ER%. Previous studies indicate that MammaPrint and BluePrint are prognostic of outcome following neoadjuvant chemotherapy[5]. This study suggests that MammaPrint may be helpful in informing post-operative decisions for patients receiving NET and warrants further study. Results from this study should be confirmed using a larger cohort. Future studies will determine the significance of these DEGs and their impact on outcomes and will further define gene expression changes by endocrine therapy type.

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References: 1. Ma *et al*, 2020. ASCO; 2. Lin et al, 2018. AnnalsofOnc; 3. Zavareh et al 2011. CancerRes; 4. Binato et al 2021. SciRep; 5. Chung et al, 2021. SABCS