# Changes in ER pathway activity score during neoadjuvant letrozole to assess therapy response and predict disease free survival (DFS) in ER positive breast cancer patients

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## Introduction

**Despite estrogen receptor (ER) positive immunohistochemistry (IHC) staining, some patients do not respond to neoadjuvant endocrine therapy, suggesting that ER staining lacks specificity to predict response. We developed a method to infer a quantitative signal transduction pathway activity score (PAS) from mRNA levels (microarray, qPCR) of pathway-associated transcription factor target genes [1-3]. Initial studies suggest that ER PAS has higher specificity than ER-IHC in predicting endocrine therapy response [4]. In this study, we correlated pre-treatment ER PAS and changes in ER PAS during neoadjuvant letrozole treatment to therapy response and DFS.**

## Conclusions

**This study confirms the potential of ER PAS measured before, during, and after neoadjuvant endocrine therapy to predict and assess therapy response and predict DFS in ER-positive patients:**

- **Progressive disease (PD) may be characterized by low ER and high PI3K* pre-treatment pathway activity score.**
- **Fast and sustained decrease in ER pathway activity score suggests that early monitoring (2 weeks) is possible to predict response.**
- **A high post-treatment ER PAS may be indicative of short DFS.**

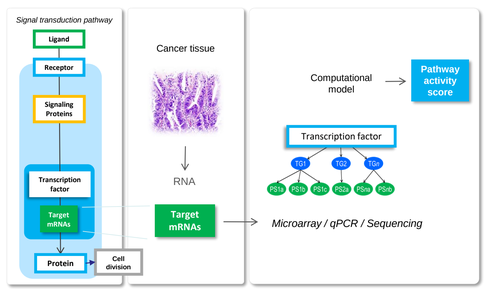
* Readout through FOXO. FOXO inversely related to PI3K in absence of oxidative stress [2].

## Materials and methods

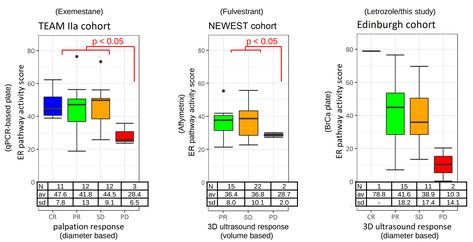
We isolated RNA from fresh frozen tumor samples of 30 ER IHC positive post-menopausal patients with primary localized breast cancer, treated for three months with neoadjuvant letrozole at Edinburgh Western General.

In total 30 pre-treatment, 25 mid-treatment (median 27 days), and 29 post-treatment (median 136 days) samples were analyzed.

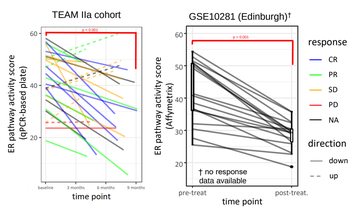
Clinical outcome was assessed (RECIST, n=29) at circa 3 months treatment by 3D ultrasound, with 1 complete (CR), 15 partial responses (PR), 11 stable (SD), and 2 progressive diseases (PD). Using RT-qPCR, target gene expression was measured for ER, androgen receptor, FOXO, Hedgehog, TGFβ and Wnt pathways. PAS were expressed on a normalized scale (0 to 100).




## Previous findings



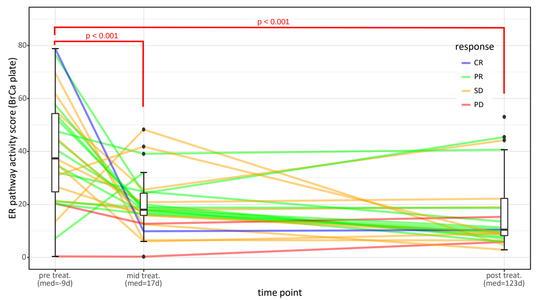

Baseline ER pathway activity score was consistently higher in non-progressive disease compared to progressive disease independent of endocrine therapy choice, sample type (FFPE or FF), and mRNA measuring platform of choice (qPCR, Affymetrix).




- ER pathway activity score (PAS) was significantly reduced during treatment.
- Largest decrease in score patients with higher baseline PAS

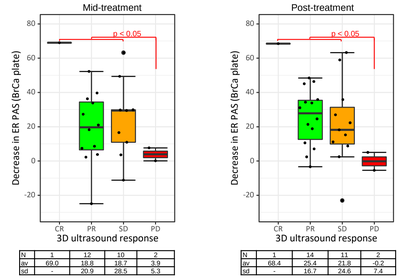
## Results

### ER pathway activity score (PAS) decreased significantly during therapy




- Maximum decrease in ER pathway activity score was already evident at circa 2 weeks treatment.
- Decrease in ER PAS was sustained until the end of treatment.

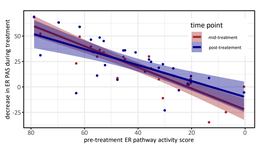
### Decrease in ER PAS was significantly higher in non-PD




| | Mid-treatment | | | |
|---|---|---|---|---|
| N | 1 | 12 | 10 | 2 |
| av | 69.0 | 18.8 | 18.7 | 3.9 |
| sd | - | 20.9 | 28.5 | 5.3 |

| | Post-treatment | | | |
|---|---|---|---|---|
| N | 1 | 14 | 11 | 2 |
| av | 68.4 | 25.4 | 21.8 | -0.2 |
| sd | - | 16.7 | 24.6 | 7.4 |

### A high pre-treatment ER PAS correlated with a high decrease in ER PAS during treatment




Pearson correlation

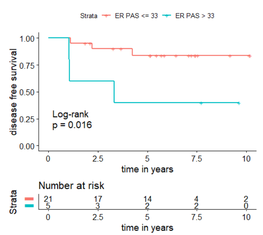
| time point | median treat. time | n | corr (CI) |
|---|---|---|---|
| mid-treatment | 17 days | 26 | 0.88 (0.76, 0.95) |
| post-treatment | 4 months | 29 | 0.69 (0.42, 0.84) |

### Higher post-treatment ER PAS correlated to shorter DFS

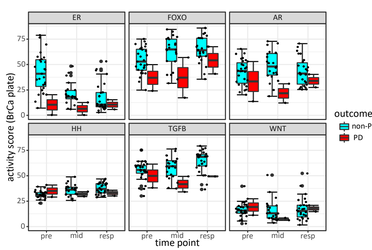
univariate Cox proportional hazards

| Post-treatment ER PAS | HR (CI) | p-value (*) |
|---|---|---|
| continuous (HR relative to an increase of 10 points in score) | 1.8 (1.1, 2.9) | 0.017 |
| dichotomous (score > 33) | 5.8 (1.2, 28.8) | 0.043 |

(*) likelihood ratio test




### Progressive diseases had low ER and low FOXO/high PI3K* baseline pathway activity scores




- PI3K* pathway activity score derived from FOXO. PI3K* is inversely related to FOXO in absence of oxidative stress [2].
- Progressive disease (PD) had low ER and low FOXO/high PI3K* pathway activity score.
- Non-PD typically had a high ER and higher FOXO/lower PI3K* pathway activity score
- Letrozole treatment seemed to influence activity score of other pathways

### References and disclosures

[1] W. Verhaegh et al., Cancer Res. 2014; 74(11):2936-45. [2] Ooijen et al., Am. J. Pathol.; 188(9):1956-72. [3] Stolpe et al., Sci. Rep. 2019; 9(1):1603. [4] Inda et al., Mol. Cancer Ther.; under final revision.

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