Leiden University Medical Center

E. Blok[1,2], M. A. Inda[3], A. Charehbili[1,2], E. den Biezen[3], S. Fruytier[1], E. Meershoek-Klein Kranenbarg[1], A. van Brussel[3], S. Linn[4], J. Kroep[2], W. Verhaegh[3], C. van de Velde[1], A. van de Stolpe[3], P. Kuppen[1]

1. Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands
2. Department of Medical Oncology, Leiden University Medical Center, Leiden, The Netherlands
3. Philips Research, Eindhoven, The Netherlands
4. Department of Medical Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands

ER-pathway activity as a predictive biomarker for neo-adjuvant endocrine therapy: Results of the TEAM IIA trial

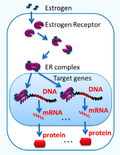
Introduction

Response to endocrine therapy depends on the presence of an active tumor-driving ER signalling pathway and, in the case of treatment with aromatase inhibitors (AI), also on aromatase-induced estradiol as the pathway activating ligand.

Conventional nuclear staining for ER is not necessarily indicative of an estradiol-activated ER signalling pathway. We evaluated a recently described diagnostic computational model which identifies ER-pathway activity based on tissue-derived target gene mRNA levels, for its clinical utility to predict neoadjuvant AI response in ER positive breast cancer patients.

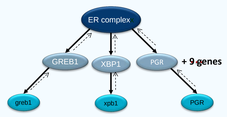
Estrogen Receptor pathway model

We have built a Bayesian network model of the ER transcriptional program, which interprets the pathway target genes' mRNA levels (from Affymetrix HG-U133Plus2.0 arrays) and infers a probability that the ER-pathway is active in a certain sample [1].

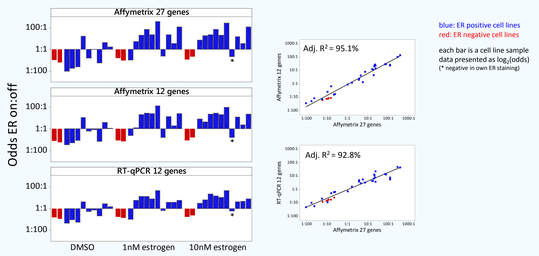


RT-qPCR model

To be able to measure ER-pathway activity in paraffin-embedded (FFPE) samples we translated the original Affymetrix based model to be used with RT-qPCR data, using 12 most discriminating target genes and 4 reference genes.



Agreement between microarray and RT-qPCR based models



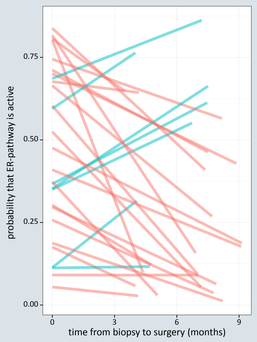
blue: ER positive cell lines
red: ER negative cell lines

each bar is a cell line sample
data presented as $\log_2$(odds)
(* negative in own ER staining)

[1] W. Verhaegh et al. Selection of personalized patient therapy through the use of knowledge-based computational models that identify tumor-driving signal transduction pathways. Cancer Res. 2014 Jun 1;74(11):2936-45.

Results

ER-pathway activity significantly decreased during therapy



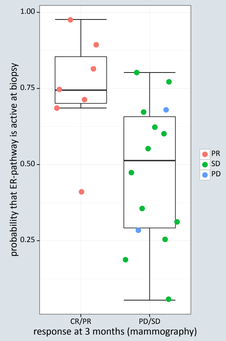
Paired t-test

sample size	28
mean decrease	0.16
2-sided p-value	0.003

Probability that ER-pathway is active

	Mean	Std. Dev.
at biopsy	0.47	0.25
at resection	0.30	0.27

Baseline ER-pathway activity in biopsy predicted therapy outcome after 3 months, based on mammography



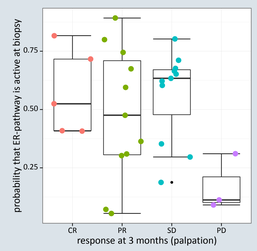
Probability that ER-pathway is active

	responder	non responder
sample size	6	12
mean activity	0.71	0.44
std. dev	0.17	0.24

1-sided t-test p-value = 0.007

When therapy was continued up to 6 months, no correlation was found, suggesting that other factors influence overall outcome of neo-adjuvant therapy.

Baseline ER-pathway activity significantly predicted progressive disease, assessed by palpation, at 3 months …



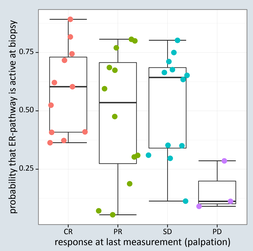
3 months

	CR	PR	SD	PD
sample size	5	11	11	3
mean activity	0.58	0.48	0.56	0.17
std. dev	0.19	0.29	0.19	0.12

1-sided t-test p-value
CR/PR/SD vs PD = 0.006

CR: complete remission, PR: partial remission, SD: stable disease, PD: progressive disease

… and at the end of therapy



End of therapy

	CR	PR	SD	PD
sample size	11	12	12	3
mean activity	0.59	0.48	0.53	0.16
std. dev	0.19	0.28	0.23	0.11

1-sided t-test p-value
CR/PR/SD vs PD = 0.004

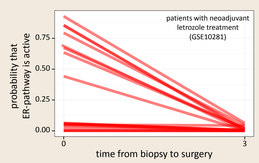
CR: complete remission, PR: partial remission, SD: stable disease, PD: progressive disease

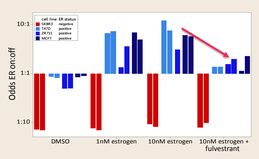
Correlation with Ki67

Pre- and post-therapy ER activity was also correlated to the change in Ki-67, expressed as remaining activity after therapy. A weak correlation (R^2=0.12) was observed between pre-treatment activity and percentage of remaining Ki-67 activity, suggesting a higher decrease in Ki-67 for patients with a higher baseline ER-pathway activity. No correlation was observed between post-treatment ER-pathway activity and decrease in Ki-67 (R^2=0.02)

Earlier studies

These results are in line with earlier explorative studies in which the original Affymetrix model was applied to a publicly available cohort of breast cancer patients treated with letrozole neoadjuvantly (left) and when applying the PCR model to breast cancer cell lines treated with fulvestrant (right, data presented as $\log_2$(odds), each bar is a sample).





Conclusions

- The significantly lower average baseline activity in patients with progressive disease indicates that low ER-pathway activity could be used to predict low response rates.
- This is supported by the observation that all progressive disease cases at end of therapy had low baseline ER activity.
- Furthermore, baseline activity particularly predicted early radiological response based on mammography.

These preliminary results indicate that this ER-pathway activity computational model could be able to predict response to endocrine neoadjuvant therapy. For further use, the model will be optimized and prospectively validated in an independent study.

Materials and Methods

Patient samples

Tumor tissue from pre-treatment biopsies and post-treatment resection material was collected from patients with early breast cancer (>2 cm and >50% ER expression) participating in the TEAM-IIA trial, who were treated with neoadjuvant exemestane for 3 to 6 months, with mean treatment duration of 174 days, ranging from 86 to 288 days.

Inference of ER activity

Using Laser Capture Microdissection (LCM), tumor cells were isolated and the probability of ER-pathway activity was assessed with RT-qPCR. In total, 105 FFPE samples were analyzed (49 biopsies + 50 resection cases, of which 28 were matched). In a preliminary analysis, results were correlated with clinical response based on palpation and mammography.

Acknowledgments

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