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ER pathway activity score as a predictive biomarker to improve stratification for neo-adjuvant endocrine therapy

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Introduction

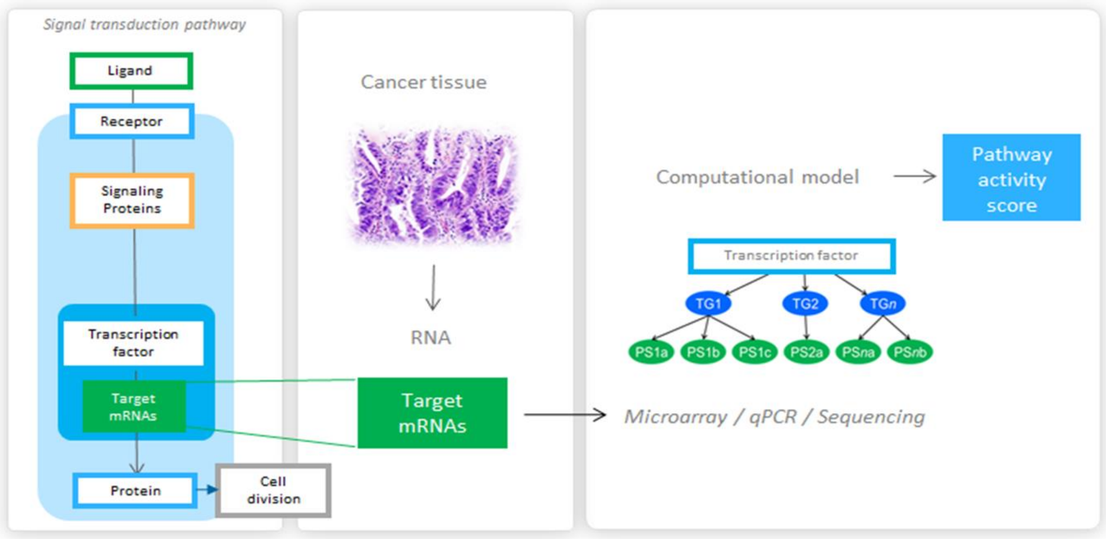
Endocrine therapy for breast cancer patients is currently determined based on positive estrogen receptor (ER) staining measured by immunohistochemistry. However, not all selected patients will respond to this therapy, suggesting that ER staining is not an optimal predictor for therapy response. We previously described a new method to measure functional activity of signal transduction pathways based on a Bayesian inference[1,3] from measurements of mRNA levels (microarray, qPCR) of the specific pathway related target genes[4,5,6]. Initial evaluation indicated that ER pathway activity in primary tumor tissue sample is related to clinical response. In this study this finding is confirmed, making this pathway activity score a candidate biomarker for improved endocrine therapy response prediction.

Conclusion

- **An area-under-curve (AUC= 0.84) analysis showed that ER pathway activity can distinguish responders from non-responders to endocrine therapy, suggesting that ER pathway activity could be used as a predictive biomarker for endocrine therapy response.**
- **Further analysis in an independent patient cohort is planned to validate use of ER pathway analysis to improve selection of patients for neo-adjuvant endocrine therapy.**

Pathway activity measurement method and results

Quantitative measurement of signal transduction pathway activity



By measuring the mRNA expression levels of specific target genes of the pathways a quantitative assessment of pathway activity is performed[3,4,6] Pathway activity is presented on a normalized scale from 0-100 (Pathway activity score).

Pre-treatment activity of ER, AR PI3K, HH, Wnt and TGFβ pathways

Patient	ER_IHC	Clinical Response	ER	AR	PI3K*	HH	Wnt	TGFβ
1	7	CR	78.8	45.8	42.3	31.0	15.7	43.6
2	8	PR	76.5	65.3	34.5	26.3	19.1	56.1
3	8	PR	69.7	34.5	40.9	33.4	19.3	52.9
4	7	PR	61.8	50.1	58.4	35.0	6.9	34.8
5	8	PR	57.6	39.1	39.4	29.0	20.2	60.6
6	8	PR	56.9	46.1	27.3	32.7	15.6	38.6
7	8	PR	55.3	56.4	56.0	30.2	12.9	29.7
8	8	PR	54.3	55.7	50.0	31.2	14.3	41.0
9	8	PR	52.8	43.6	33.5	33.7	5.5	62.0
10	8	PR	52.3	28.5	29.8	33.8	21.3	35.5
11	7	PR	47.7	60.4	29.9	34.1	17.5	57.7
12	8	PR	44.9	47.3	36.6	28.0	10.2	34.6
13	8	PR	40.9	53.4	35.5	33.4	24.0	39.6
14	8	PR	35.9	43.3	62.8	22.9	10.6	38.1
15	7	PR	35.1	43.8	47.1	32.1	16.8	36.8
16	8	PR	32.2	25.3	75.1	29.8	24.9	43.9
17	8	PR	30.7	20.2	58.8	26.1	14.0	26.1
18	8	PR	26.8	24.5	52.8	29.6	14.4	27.1
19	8	PR	24.7	49.4	58.5	30.9	18.7	37.9
20	6	PR	21.1	35.7	45.8	32.1	21.6	38.0
21	8	PR	20.2	22.8	67.0	29.2	5.8	31.4
22	8	PR	7.2	30.1	55.3	39.2	16.8	41.0
23	8	SD	37.4	42.9	50.8	31.1	13.3	35.1
24	8	SD	30.0	30.3	24.9	32.1	23.8	16.3
25	8	PD	45.5	54.2	32.3	38.3	2.9	46.0
26	7	PD	21.4	61.0	53.5	29.7	39.7	29.6
27	8	PD	20.3	52.9	50.1	40.9	27.3	45.7
28	8	PD	13.4	33.0	51.9	35.9	14.0	38.9
29	7	PD	0.4	13.7	75.9	29.1	11.2	33.1

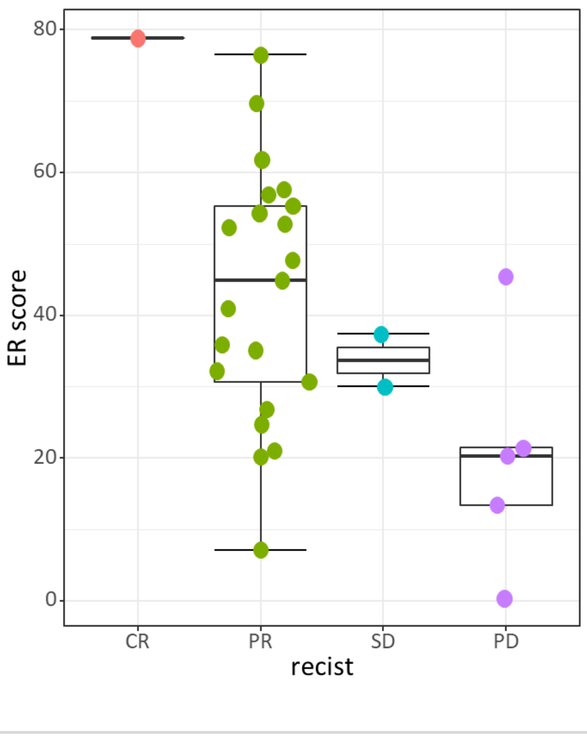
*: The PI3K reading is derived from the inverse activity reading of the FOXO transcription factor. Please be aware that oxidative stress can induce FOXO activity which may inadvertently lead to a low PI3K activity reading.

Pre-treatment pathway activities per patient in relation to clinical response.

- **All samples show high ER-IHC staining (6-8) and no relation to clinical response**
- **Clear relation between ER pathway activity and clinical response.**
- **Differences observed in other pathways (AR, PI3K, TGFb)that may lead to better insight into tumor biology like escape mechanisms**

CR: Complete responder
PR: Partial responder
SD: Stable disease
PD: Progressive disease
ER-IHC: Allred score

Pre-treatment ER pathway activity relates to clinical response to Letrozole therapy (measured at 3 months)

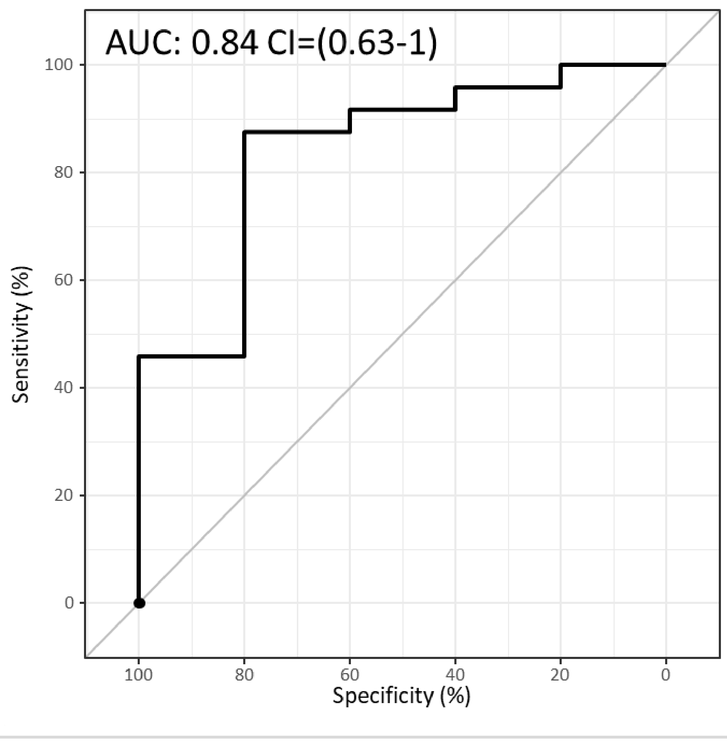


PD (n=5) vs. non-PD (n=24):

T-test p =0.027
Mean PD=20.2, vs non-PD=43.8

Reduced ER pathway activity is associated with higher chance of poor clinical outcome

AUC= 0.84; Responders vs non-responders (PD)



An area-under-curve (AUC) analysis indicate that:

- **Responders and non-responders to endocrine therapy can be distinguished.**
- **ER pathway activity score may be used as predictive biomarker for endocrine therapy response.**

Materials and methods

Study type:	Blinded retrospective study
Patients:	29 post-menopausal patients with primary localized ER-positive breast cancer from Edinburgh Breast Unit[2]
Treatment:	Neo-adjuvant letrozole treatment[2]
Clinical Outcome:	Therapy response assessment by 3D Ultrasound (single physician). Percent volume reduction used between baseline and 3 months post treatment to score, complete responder (CR), partial responder (PR), stable disease (SD) or progressive disease (PD) in line with RECIST classification.
Analysis material:	Isolated RNA from fresh frozen (FF) pre-treatment biopsy samples (from -80^0 C)
Pathway Activity:	Activity scores of ER, AR, PI3K, HH, TGFb and Wnt has been measured by using the pathway specific target gene expression profiles using RT-qPCR followed by Bayesian inference model[1,3-6]. Activity scores expressed on a normalized scale from 0 – 100.
Statistics:	For pathway activity differences t-test was used

References

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Disclosures

Keizer, Inda, Clout and van de Stolpe are regular Philips employees and have nothing else to disclose.
Fernando, Martinez-Peres, Dixon, Sims and Turnbull have nothing to disclose.

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