Pathway profiling for prediction of response to neoadjuvant letrozole therapy in ER positive postmenopausal breast cancer; gaining new insights for targeted treatment

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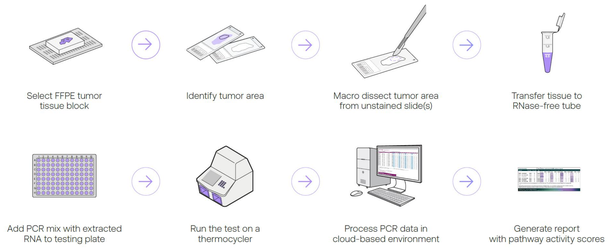
Introduction:

The NEOLBC (NCT03283384) study included postmenopausal patients with hormone receptor positive (ER-IHC ≥50%, PR any), HER2 negative, stage II/III breast cancer. A baseline biopsy was taken prior start to letrozole treatment and after two weeks of treatment. Based on the two weeks Ki67-IHC, patients were randomized (Ki67 ≥1%) to receive either letrozole + ribociclib or standard chemotherapy until surgery. Patients with Ki67 <1% at two weeks continued letrozole monotherapy. Here we describe the signal transduction pathway profiles to better understand the molecular mechanisms of response to neoadjuvant letrozole.

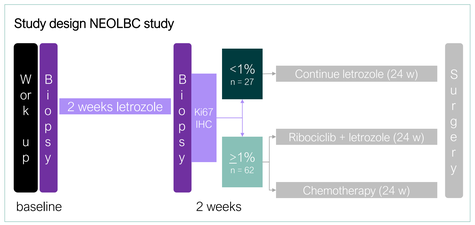
Methods:

From 82 patients of the NEOLBC study, signal transduction pathway activities for ER, AR, MAPK, PI3K, Hh, Notch and TGFβ pathways were measured in baseline and two weeks biopsy samples (≥ 30% epithelial tumor cell content) using the mRNA-based OncoSIGNal pathway activity profiling qPCR test (InnoSIGN BV, The Netherlands):



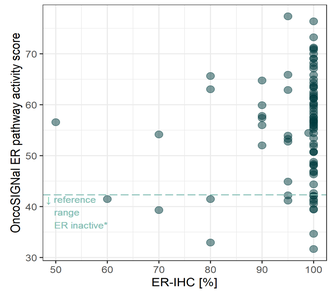
High pathway activity in a tumor sample was concluded when the pathway activity value (incl. confidence interval) for the sample was above the 95th percentile of reference epithelial breast tissue.

The pathway activity profiles of Ki67 <1% (n=27) and Ki67 ≥1% (n=62) groups were compared between baseline and two weeks of treatment.



Results:

Positive ER IHC staining does not relate to ER pathway activity

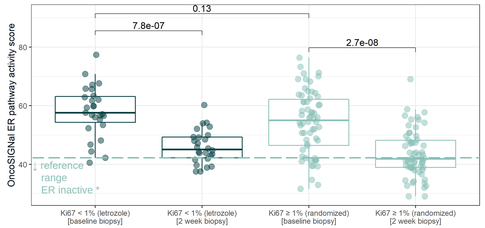


Despite all baseline samples being ≥ 50% ER-IHC staining positive, a large range in ER pathway activity is observed.

20% (18 / 89) of ER-IHC staining positive samples show low ER pathway activity (comparable to triple negative breast cancer), which are not likely to respond to endocrine therapy.

** threshold established based on 22 ER negative samples (independent data obtained outside this study cohort)*

ER pathway activity relates to response to letrozole as measured by Ki67-IHC



		Baseline ER pathway activity	
2 week IHC		elevated	non-elevated
Ki67 <1%	n=27	**89%**	**11%**
Ki67 ≥1%	n=62	76%	24%

89% (73 / 82) of the patients showed a decrease in ER pathway activity (-10.9 ± 8.3) after two weeks of letrozole treatment compared to baseline (p = 7.4e-14 paired Wilcoxon test).

Note: Data not shown here: high baseline ER pathway activity relates to better RECIST outcome observed after 26 weeks in letrozole only arm

The group with Ki67 ≥1% (n=62) after two weeks letrozole treatment showed more often non-elevated ER pathway activity at baseline (24%) compared to the Ki67 <1% group (n=27; 11%).

Actionable pathway profiling beyond ER:

In total 18 patients showed low ER pathway activity at baseline (below triple negative threshold of 42,3), despite ≥ 50% IHC. As shown earlier*, ER pathway activity relates to response to letrozole. In this study, 83% (15 / 18) of patients with low ER pathway activity were correctly predicted to have Ki67 ≥ 1% after two weeks letrozole therapy.

When ER pathway activity is low, it is observed that especially PI3K is often elevated (67%), followed by Hh (33%) and MAPK (20%), suggesting other tumor driving pathways instead of ER.

*ESMO Breast 2019/ Berlin/ abstract 411/ Nr 50P).

patient	2 week response	ER ↓	AR	PI3K	MAPK	Hh	Notch	TGFβ
OSST19519	Ki67 ≥ 1%	32 [28-38]		■		■		
OSST19537	Ki67 ≥ 1%	33 [30-39]		■		■		
OSST19698	Ki67 ≥ 1%	35 [32-37]	■	■				
OSST19479	Ki67 ≥ 1%	39 [37-43]		■		■		
OSST19528	Ki67 ≥ 1%	39 [35-50]		■		■		
OSST19708	Ki67 ≥ 1%	40 [36-44]		■				
OSST19490	Ki67 < 1%	41 [39-42]		■	■			
OSST19686	Ki67 ≥ 1%	41 [39-44]		■		■		
OSST19503	Ki67 ≥ 1%	41 [39-45]		■				
OSST19526	Ki67 ≥ 1%	41 [39-43]			■			■
OSST19713	Ki67 ≥ 1%	41 [38-45]	■					
OSST19487	Ki67 ≥ 1%	41 [39-45]						
OSST19682	Ki67 ≥ 1%	42 [40-44]		■				
OSST19754	Ki67 ≥ 1%	42 [39-45]		■				
OSST19494	Ki67 < 1%	42 [40-46]						
OSST19520	Ki67 ≥ 1%	43 [40-45]		■	■			
OSST19521	Ki67 < 1%	44 [41-49]	■					
OSST19539	Ki67 ≥ 1%	45 [40-49]		■	■	■		

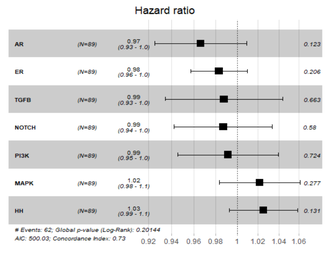
High pathway activity profiles per patient indicated in purple when full measurement (including confidence interval) > 95th percentile of reference range for breast

The Ki67 > 1% group showed more often activation of non-hormonal pathways

	Baseline high pathway activity						
2 week IHC	ER	AR	PI3K	MAPK	Hh	Notch	TGFβ
Ki67 <1%	**89%**	**26%**	33%	19%	30%	0%	4%
Ki67 ≥1%	76%	13%	**47%**	**29%**	**44%**	2%	**13%**

The higher pathway activity of non-hormonal pathways MAPK, Hh, TGFβ and/or PI3K could explain why Ki67 values after two weeks letrozole are still elevated (escape mechanism).

Baseline hormonal pathway activity may predict for response to letrozole after two weeks treatment



Activation of hormonal pathways (both ER and AR) seems to be related to response towards letrozole as measured by Ki67 staining at two weeks.

While MAPK and Hh pathway activation seem to be related to possible escape mechanisms. Interestingly these pathways are also often observed to be activated in TNBC (AACR 2023 poster 6221)

Conclusions:

- Positive ER-IHC staining does not always relate to high ER pathway activity, possibly explaining that not all of these patients respond equally well to ER inhibition therapy.
- Activation of hormonal pathways (ER, but also AR) might be predictive for early (two weeks) response to letrozole as assessed by Ki67 staining. Involvement of non-hormonal pathways is associated with less effective response towards letrozole alone.
- Pathway activity profiling in individual patients can improve insight into underlying hormonal therapy escape mechanisms and may support strategies for alternative treatment options in patients with ER-positive breast cancer.

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