A novel Laboratory Developed Test for identification of key tumor driving cell signaling pathways in breast cancer: ER, AR, PI3K, MAPK, Hedgehog, TGFβ and Notch

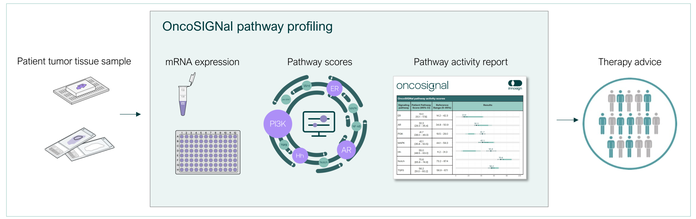
Dianne van Strijp[1], Martijn van Zelst[1], Eveline den Biezen[1], Saskia Vermeer[1], Anke Pierik[1], Jacob Anderson[2], Heather Hocking[2], Robert C. Babkowski, MD[2,3]

1: InnoSIGN B.V., Eindhoven, The Netherlands, 2: InnoSIGN Inc, Mason, OH, USA , 3: Stamford Health, CT, USA

Introduction:

The OncoSIGNal Laboratory Developed Test (LDT) was developed to quantify the functional signal transduction pathway activity levels of 7 key oncogenic signaling pathways (ER, AR, PI3K, MAPK, Hedgehog (Hh), TGFβ and Notch). Expression levels of direct target genes of the respective pathway transcription factors are measured by RT-qPCR. After amplification, computational algorithms calculate the level of pathway activation on a 0-100 scale. Here we present results of the analytical validation of this 7 pathway test.

Methods:



OncoSIGNal Workflow

Sensitivity of all 95 individual qPCR assays was investigated using ivRNA and down to 50 cps of every assay was successfully detected. Linearity and reproducibility of the OncoSIGNal LDT was validated using well characterized non-malignant breast tissue and breast tumor samples (HR+ and TNBC). The minimal amount of tissue required to successfully run the test was based on the expression levels of seven house-keeping genes after testing small amounts (0.35 mm^3) FFPE tissue from 64 breast cancer samples. Repeatability and reproducibility of the test was determined by repeated analysis of the same RNA extract without and with multiple sources of variation, respectively. Accuracy was determined by measuring the same tissue samples (n=40) in two different labs (Eindhoven, NL and Mason, OH). Multiple samples at different locations from the same lesion (9 patients) as well as different lesions (9 patients) from benign breast tissue were analyzed to assess heterogeneity. QC procedures to check for common handling errors are implemented. Thresholds to determine aberrant activity in a tumor sample were determined based on pathway activity scores in reference breast tissue.

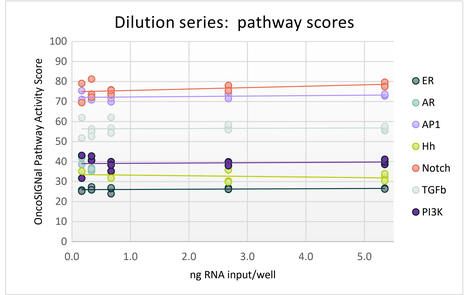
Results:

Validation of the OncoSIGNal LDT for Breast Cancer

Item	Specification	Remarks
Sensitivity qPCR assays	Minimal 50 cps	
Reproducibility qPCR assays (10^3 – 10^6 cps)	Max. +/- 0.5 average Cq-value	
Efficiency qPCR assays (10^3 – 10^6 cps)	90-110%	
Stability qPCR plates at -20°C	Minimal 20 months	
Minimal input	0.35mm^3 tissue or 270ng RNA	
% Valid tests	95%	Extremely poor quality FFPE tissue will not pass QC
Tumor cell percentage input	≥50%	Presence of >50% non-tumor cell RNA may influence the tumor pathway activity score
Validation of signaling pathways	Pathway scores increase after stimulation and decrease after deprivation or inhibition	
Turn Around Time (tissue to result)	<5 hours	
Software QC	QC procedures to check for common handling errors successfully implemented	
Inhibiting factors	Presence of known PCR inhibitors results in a QC fail	e.g. inhibitors such as guanidine and ethanol used in the RNA extraction procedure

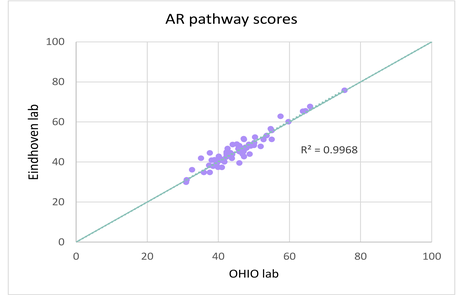
Linearity

Pathway scores show minimal variation when using different amounts of FFPE RNA from the same sample (0.17–5.4 ng per well)



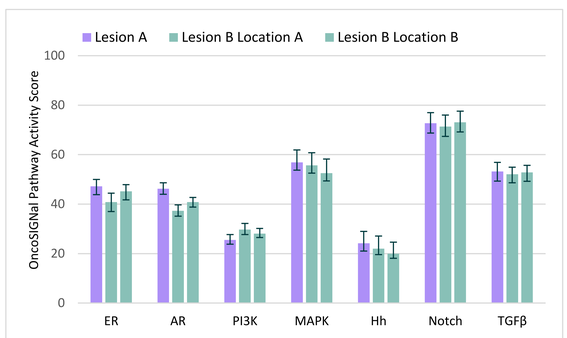
Accuracy

The average difference in pathway score between the labs ranged from 1.5 (AR-see figure) and 3.4 (other pathways), showing high accuracy



Heterogeneity

The average difference in pathway score within a benign lesion ranged between 1.4-4.1 and between lesions of the same patient between 2.1-6.8. An example for a single patient is shown in the figure: 1 sample was analyzed of lesion A and 2 samples (at different locations) of a second lesion (lesion B).
Pathway scores with confidence intervals demonstrate limited heterogeneity.



Repeatability

Repeatability was determined by repeated analysis (n=3) of the same RNA extract (3 different tissue samples in total) minimizing source of variation (same operator, day, reagents & PCR instrument). SD was below 3 points.

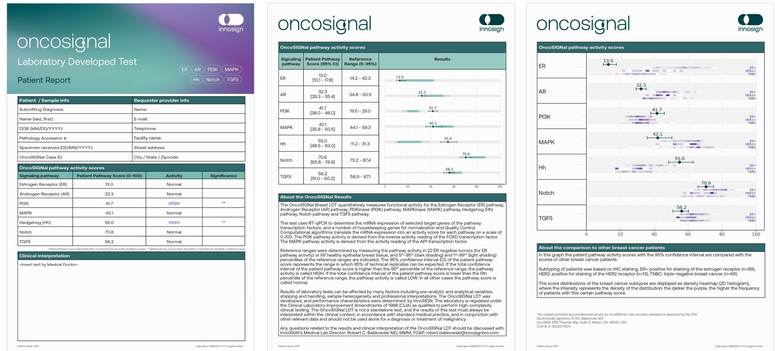
Pathway	ER	AR	PI3K	MAPK	Hh	Notch	TGFβ
Standard deviation repeat	0.70	0.99	2.12	0.57	1.00	2.34	0.97

Reproducibility

Reproducibility was determined by repeated analysis (n=3) of the same RNA extract (3 different tissue samples in total) with multiple sources of variation (different operators, days, reagents & PCR instruments). SD was below 4 points.

Pathway	ER	AR	PI3K	MAPK	Hh	Notch	TGFβ
Standard deviation repeat	1.29	2.59	0.29	3.15	2.54	1.90	1.63

The LDT Patient Report summarizes the activity of the 7 pathways and provides insight into the patient's pathway scores relative to reference ranges and scores of patient cohorts of breast cancer subtypes, ER+, HER2+ and TNBC.
Reference ranges: ER: ER-IHC neg breast tumors, other pathways: healthy breast tissue.



Conclusions:

The OncoSIGNal 7 pathway LDT (ER, AR, PI3K, MAPK, Hh, TGFβ and Notch) is a robust and reliable test to quantify signal transduction pathway activity. By comparing with reference ranges from breast tissue, the test can be used to identify tumor driving pathways. This opens new options for selection of targeted therapies for breast cancer patients.

Corresponding author: Eveline.den.Biezen@innosignbio.com *DOI: both corresponding and first author are InnoSIGN employees*

Poster ST103
AMP 2023