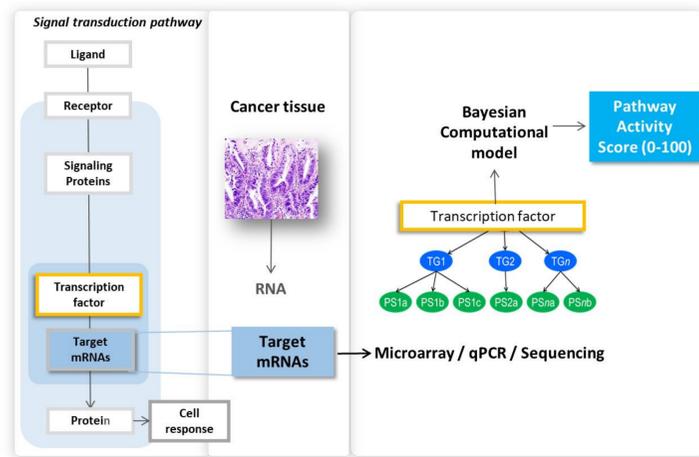


Androgen receptor pathway activity and the ratio between androgen and estrogen receptor pathway activity in breast cancer subtypes

Background

Androgen receptor (AR) immunohistochemistry staining in breast cancer has revealed frequent AR expression in all breast cancer subtypes. In ER staining positive tumors, positive AR staining seems to relate to better prognosis while in ER staining negative tumors, positive AR staining seems to be related to worse outcomes.¹ Therefore it has been suggested that AR presence could be either tumor suppressive or tumor promoting. The AR/ER protein ratio has been suggested as a biomarker to define clinical outcome². However, AR and ER protein expression are not necessarily associated with a functionally active signaling pathway. Therefore we investigated the ratio between functional AR and ER pathway activities as this may have an advantage in predicting outcome and to guide therapy choice.

Test for quantitative measurement of signal transduction pathway activity



RNA-based tests* have been developed for androgen (AR) and estrogen receptor (ER), Hedgehog (HH), Wnt, TGFβ, Notch, NFκB, PI3K, and MAPK pathway activities. They can be performed simultaneously on a single tissue or cell culture sample using Affymetrix HG-U133 Plus 2.0 microarray, qPCR or RNA-sequencing and provide quantitative pathway activity scores (PAS) expressed on a 0-100 scale.^{3,4}

References

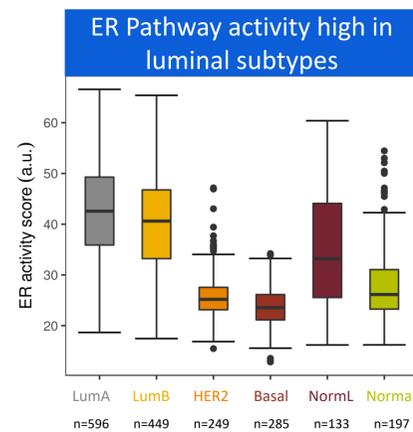
¹ Venema CM *et al.* (2019) *Pharmacol Ther.* 200:135-147
² Bronte G *et al.* (2019), *J Oncol.* 2019:1393505
³ Verhaegh W *et al.* (2014) *Cancer Res.* 74(11):2936-2945
⁴ van de Stolpe *et al.* (2019) *Sci Rep.* 9(1):1603
⁵ van Ooijen *et al.* (2018) *Am J Pathol.* 189(9):1956-1972

⁶ Perou CM *et al.* (2000) *Nature* 406:747-752
⁷ Inda MA *et al.* (2019) *Mol. Cancer. Therap*
doi: 10.1158/1535-7163.MCT-19-0318
⁸ van Boxtel W *et al.* (2019) *Int. J. Cancer*, in press

Breast cancer patient samples

- Affymetrix expression microarray data from multiple clinical breast cancer studies were used: GSE12276, GSE21653, GSE20685, GSE42568, GSE6532, GSE9195, GSE45827, GSE7307, GSE10780, GSE17907, GSE21422, GSE5764, GSE31192, GSE54002 and E-MTAB-365
- Intrinsic subtyping performed as described earlier⁵ and in line with Perou's classification⁶
- Total of **1712 breast cancer samples** and **197 normal tissue samples** (normal + normal adjacent), suitable for signal transduction pathway analysis

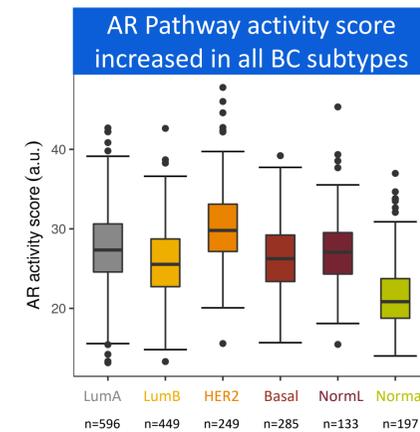
Patient-specific ER and AR pathway activity



- High ER-PAS in luminal BC subtypes
- Low ER-PAS in basal and HER2 subtypes

Range:

- Luminal BC subtypes show large range in ER pathway activity, indicating patient-specific pathway activity
- A few normal samples show relatively high ER pathway activity. This may be related to the fact that tissue is "normal adjacent" and not necessarily "normal".

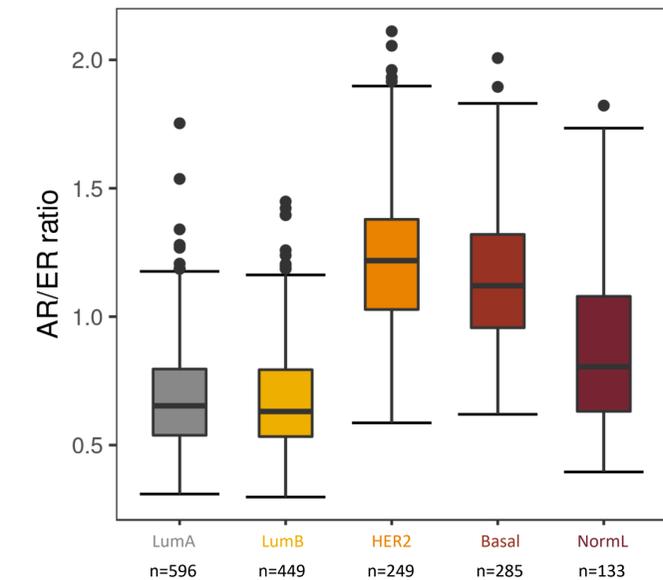


- Increased AR-PAS in all breast cancer subtypes
- Highest AR-PAS in HER2-enriched subtypes

Range:

- Large range in all BC subtypes, indicating patient-specific AR pathway activity

Average AR/ER pathway activity ratio is low in luminal BC and high in more aggressive breast cancer subtypes



- Low AR/ER – PAS ratio in luminal subtypes
- High AR/ER – PAS ratio in HER2 and basal subtypes

Range:

- Large range in all subtypes, indicating patient specific AR/ER pathway activity ratio

Discussion

- AR/ER pathway activity ratio varies with breast cancer subtype; similar to what has been published on the AR/ER protein staining ratio^{1,2}
- However, ER and AR protein expression are a prerequisite but not sufficient to determine functional pathway activity^{7,8}
- Functional AR/ER pathway activity ratio may be a superior measure as compared to AR/ER protein staining in exploring the role of the AR signaling pathway in breast cancer in relation to the possible effect of AR targeted therapy

Future studies are needed to further investigate the value of the AR/ER pathway activity ratio as biomarker to guide targeted therapy with AR modulators in breast cancer patients.