

Verification of RT-PCR gene expression assays for biomarker research within the HER2/EGFR pathway

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Personalized Healthcare and predictive diagnostics

People react differently to medications

One group of patients may benefit fully from treatment, while others experience unwanted side effects



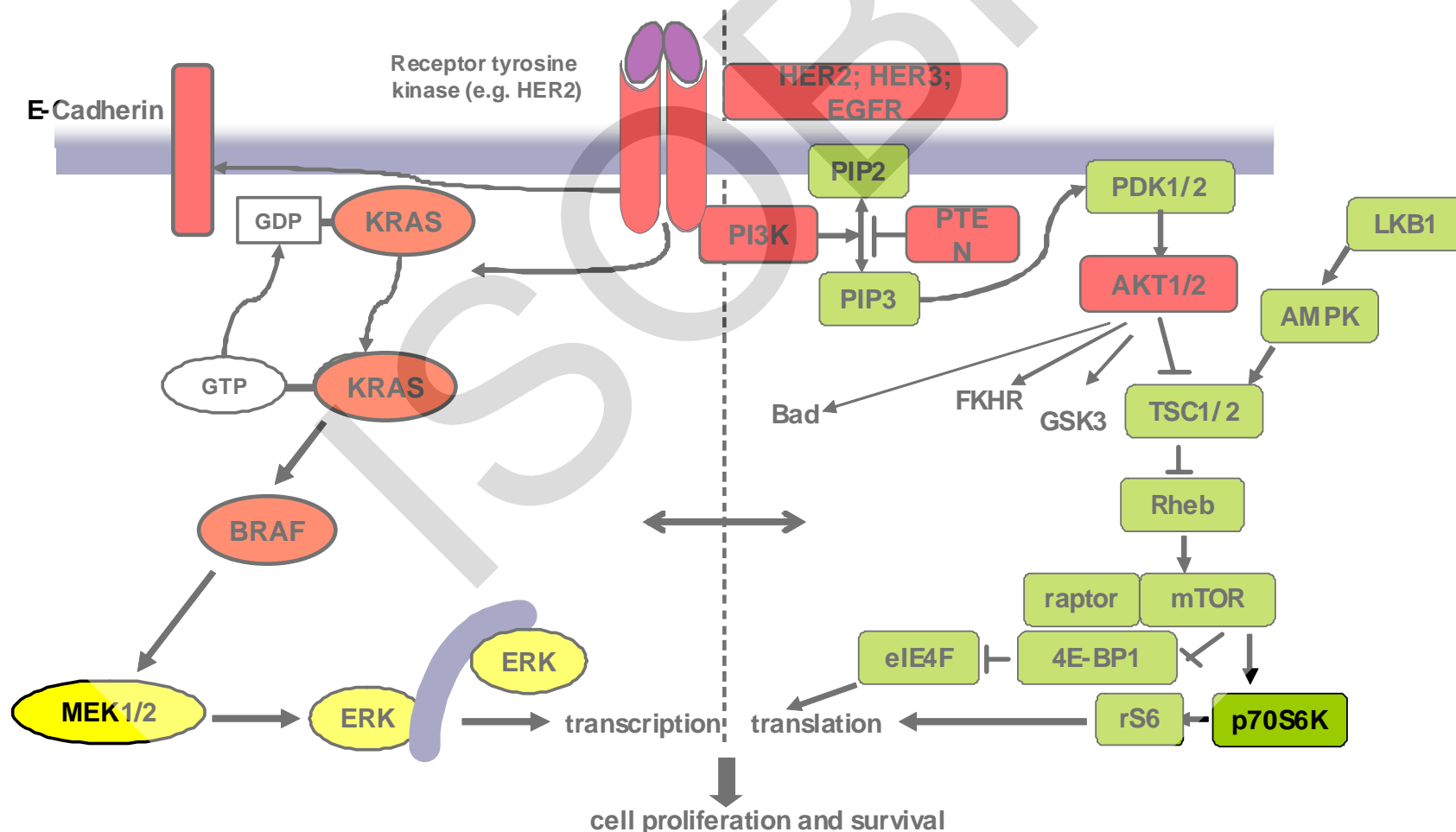
- ◆ *Today, 20-75% of patients do not receive effective treatment*
- ◆ *More than 100,000 patients die from adverse drug reactions in the US*

PHC is the use of **gene-based information** to understand each person's **individual requirements** for the **maintenance of their health, prevention of disease, and therapy tailored to their genetic uniqueness**

EGFR/ HER2 signaling pathway

Potential biomarkers for breast cancer

- The HER2/EGFR signaling pathway plays an important role in cancer development
- Different genes being involved in this pathway show genetic defects, influencing the tumor development
- Development of relevant biomarker assays will help to perform more individual therapies for the future



Geneexpression assay development and verification

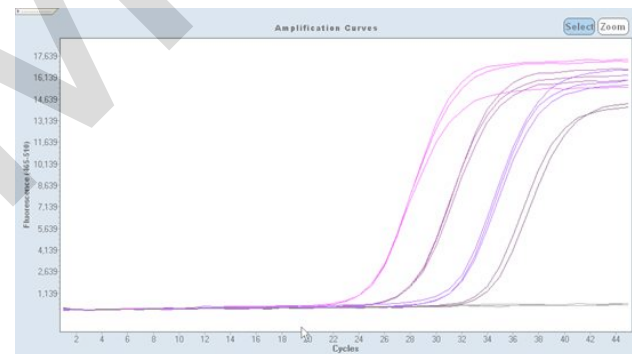
Aim of the project

- Development and verification of geneexpression qRT-PCR assays qualified for biomarker research in breast cancer
 - Technical verification (reproducibility, linearity, robustness) on FFPET (lung, breast, colon)
 - Technical verification of geneexpression assays on a breast cancer collective comprising 106 FFPET samples provided by the Pathology Bonn
 - Correlation of the data with different clinicopathologic patient parameters

Gene expression assay development and verification

- Assay development for following genes:

HER2	AKT1
HER3	AKT2
EGFR	KRAS
PIK3CA	BRAF
PTEN	E-Cadherin



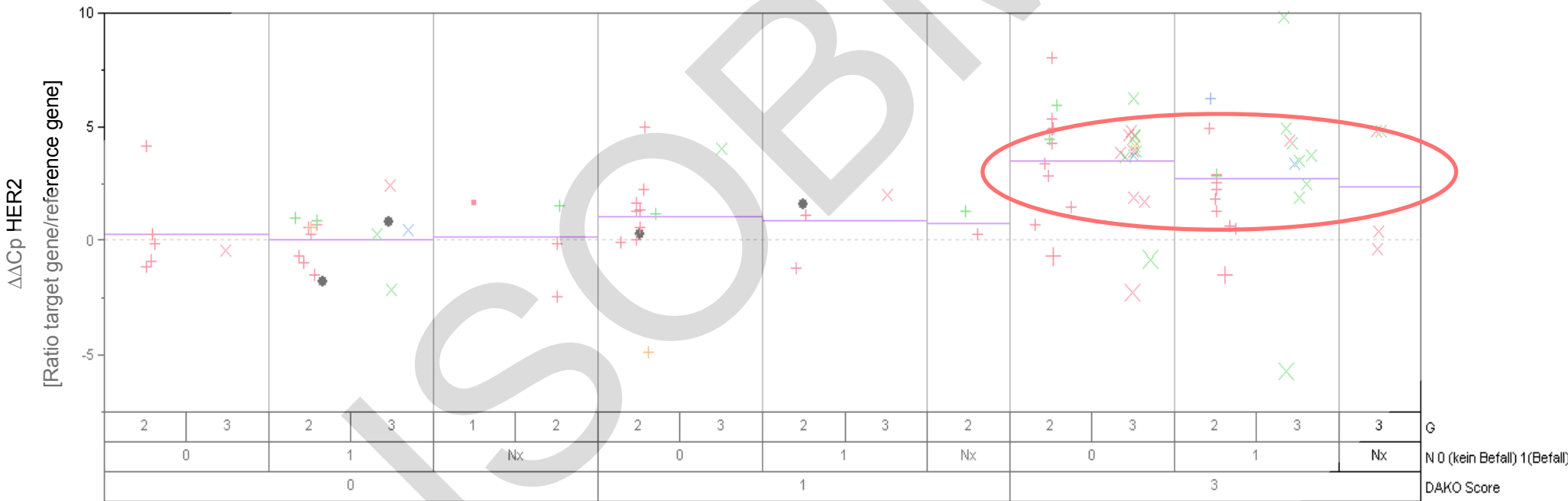
EGFR expression assay; breast cancer FFPE RNA

- Assay format:

- isolation of RNA from tumor tissue slides and adjacent tissue slides of one patient
 - Roche High Pure RNA Purification System
- quantification of target gene expression levels by one-step qRT-PCR using hydrolysis probes
 - Light Cycler™ 480
- relative quantification using two reference genes: MRPL19 and ALAS1
 - Light Cycler™ 480 Software

Correlation of clinical parameters and HER2 expression status

T-N-G, DAKO status

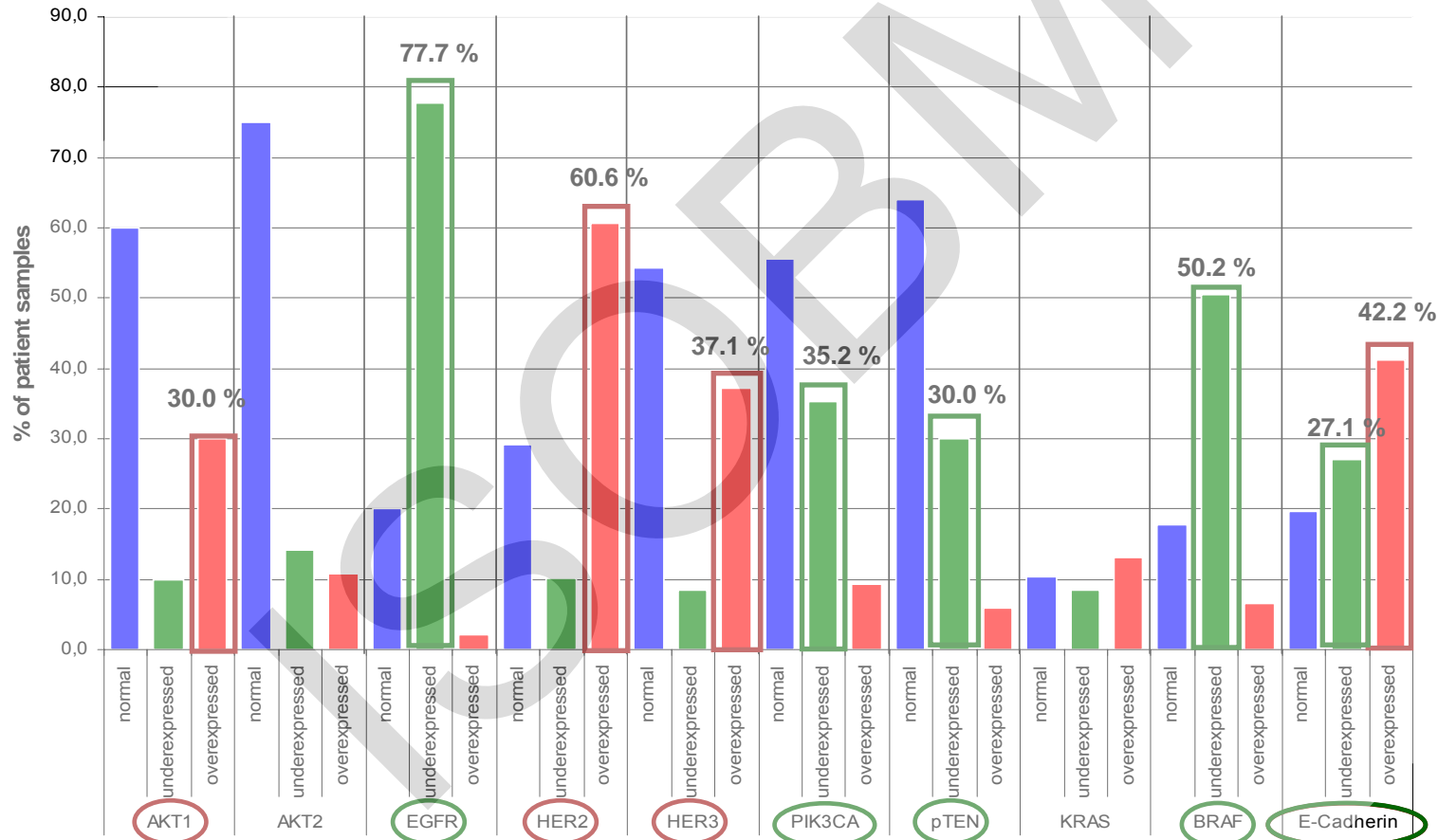


■ $\Delta\Delta C_p$ values of HER2 for 106 patient samples blotted against DAKO score, lymph node state (N; Nx: no data available), tumor grade (G) and tumor size (T; color of + and x)

→ HER2 expression status correlates with DAKO Score but with none of the other parameters

Overexpression and underexpression of target genes

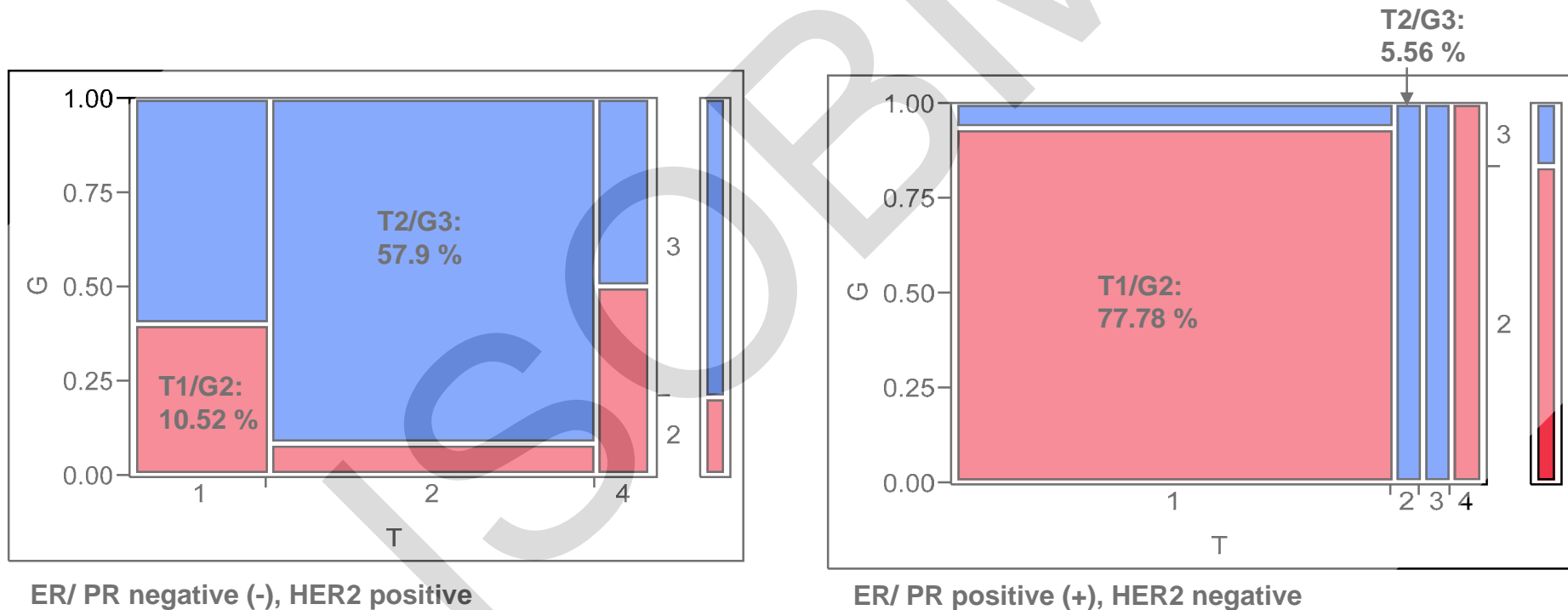
Data analysis



- AKT1, HER2, HER3 and E-Cadherin overexpression in a considerable amount of patient samples
- EGFR, PIK3CA, PTEN, BRAF and E-Cadherin underexpression in a considerable amount of patients

Correlation of clinical parameters and HER2 expression status

ER/ PR status and T-G status

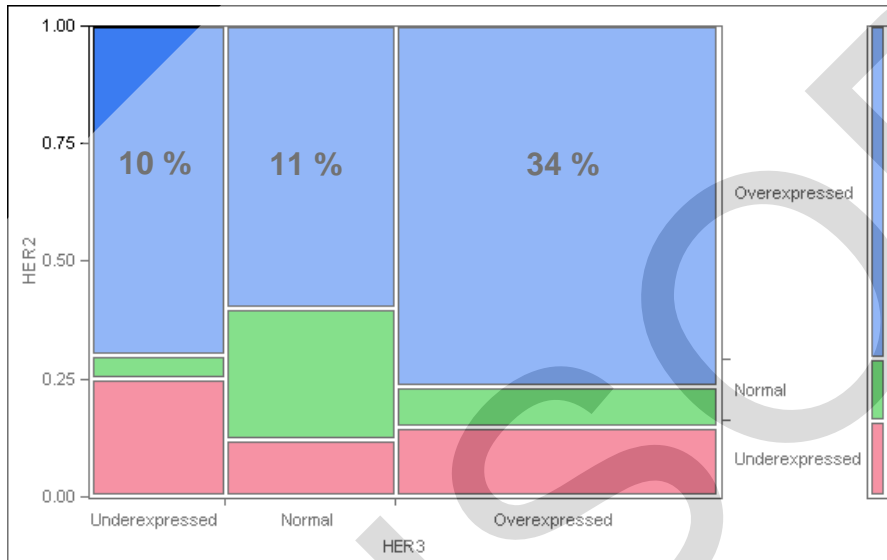


- ER/PR negative, HER2 positive patients have higher T and G values by trend compared to the HER2 negative, ER/ PR positive patients showing lower T and G values by trend

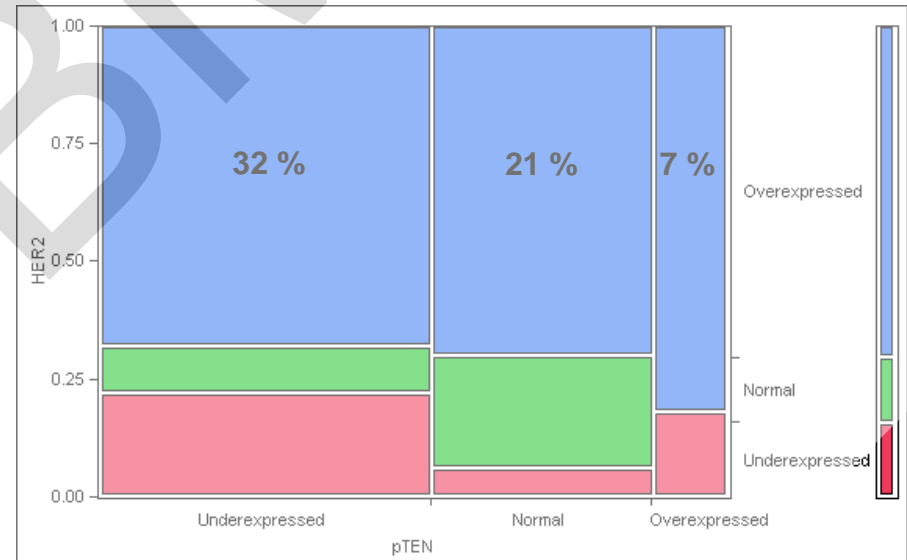
→ confirms other data that HER2 overexpression is correlated with a poor prognosis in breast cancer

Correlation of gene expression between different genes

HER2, HER3, PTEN



HER2 blotted against HER3

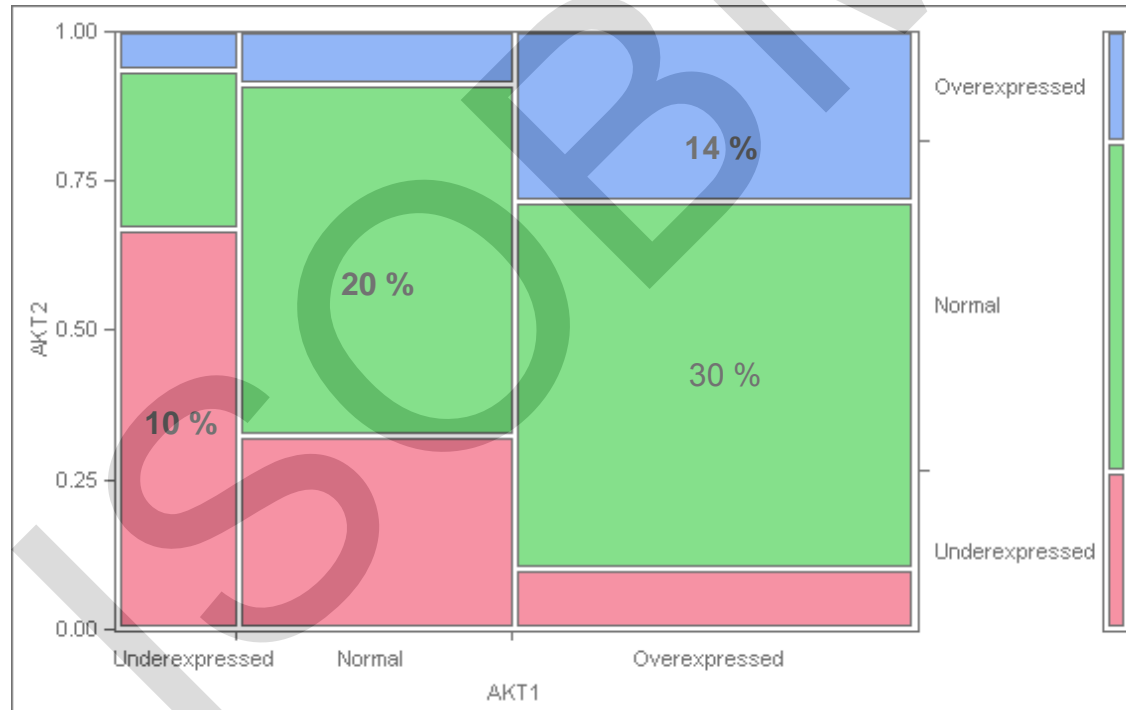


HER2 blotted against PTEN

- HER2 and HER3 expression correlation by trend
→ HER2/ HER3 coexpression has been observed in other studies
- HER2 and PTEN expression correlation (inverse) by trend
→ cancer development may be influenced by HER2 overexpression and PTEN underexpression

Correlation of gene expression between different genes

AKT1 and AKT2



- AKT1 and AKT2 showed a very similar expression level

→ expression status quantification of one of the two genes may give a suggestion about the expression status of the other gene

Conclusions

- Technical verification of ten gene expression assays within the HER2/ EGFR pathway using a mamma collective comprising 106 breast cancer patient samples
- Qualification of ten gene expression assays to be suitable for biomarker research in breast cancer patients
- Data analysis showed that some genes may serve as biomarkers for breast cancer studies
- Further studies on breast cancer have to be performed to validate these trends using these verified assays (HER2, HER3, PTEN, AKT1 assays)

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We Innovate Healthcare