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Comparison of an automated cartridge-based system for mRNA assessment with central immunohistochemistry in the neoadjuvant GeparX trial

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Background

Hormone receptors, HER2 and Ki67 are prognostic values typically determined for breast cancer (BC) outcome and prediction of therapy response. A RT-qPCR based system, the Xpert® Breast Cancer STRAT4*, is used to classify BC tissues regarding their hormone receptor status, HER2 and proliferation via Ki67. We compared mRNA expression analysis of *ESR1*, *PGR*, *ERBB2*, and *Mki67,* in a large clinical trial cohort, by an automated *in-vitro* diagnostic platform, with central immunohistochemistry (IHC).

Patients and Methods

Breast cancer patients from the prospective GBG multicenter trial GeparX (NCT02682693) were included in this biomarker project. We used formalin-fixed paraffin embedded (FFPE) pretherapeutical core biopsies with a tumor content ≥10%. A 4 µm FFPE tissue section was first processed with the Xpert® FFPE Lysis Kit*, the sample lysate was placed in the GeneXpert®IV instrument system in which the purification, amplification and real-time detection took place automatically [1, 2].

OBJECTIVES

- Evaluation of concordance between IHC (ER, PgR, Ki67, HER2) and mRNA score
- Validation of Xpert® Breast Cancer STRAT4 assay in a clinical trial cohort
- Determine new IHC-cutoffs for ER, PR, Ki-67

tumor H&E staining

use tumors with a tumor content ≥10%

transfer macrodissected tissue in a tube

FFPE lysis & protein digestion

load sample chamber

run STRAT4

mRNA STRAT4 dCt score

3 hours

Figure 1: Workflow.

Results

Table 1: Baseline characteristics. ER pos. ≥ 1% IHC; PgR pos. ≥ 10%IHC; HER2 pos. IHC 3+ or IHC 2+ and ISH pos.; Ki67 high > 20% IHC.

Parameter	Parameter value	N (%)
ER (central pathology)	Negative (<1%)	245 (48.7)
	Positive (≥1%)	258 (51.3)
PgR (central pathology)	Negative (<10%)	307 (61.0)
	Positive (≥10%)	196 (39.0)
HER2 status (central pathology)	negative	425 (84.5)
	positive	78 (15.5)
Ki67 (central pathology)	≤20%	82 (16.3)
	>20%	421 (83.7)
Breast cancer subtype	HER2-/HR+	217 (43.1)
	TNBC	208 (41.4)
	HER2+	78 (15.5)
Tumor grading	G1	10 (2.0)
	G2	151 (30.0)
	G3	342 (68.0)

Available for STRAT4 assay **537** → ≥ 10% tumor content in biopsy **509** → valid STRAT4 dCt-Values **503**

Figure 2: CONSORT diagram.

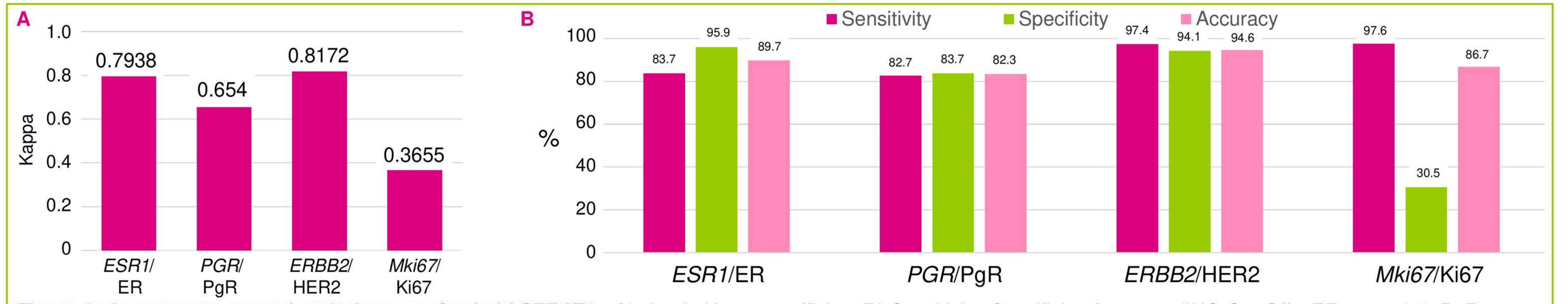


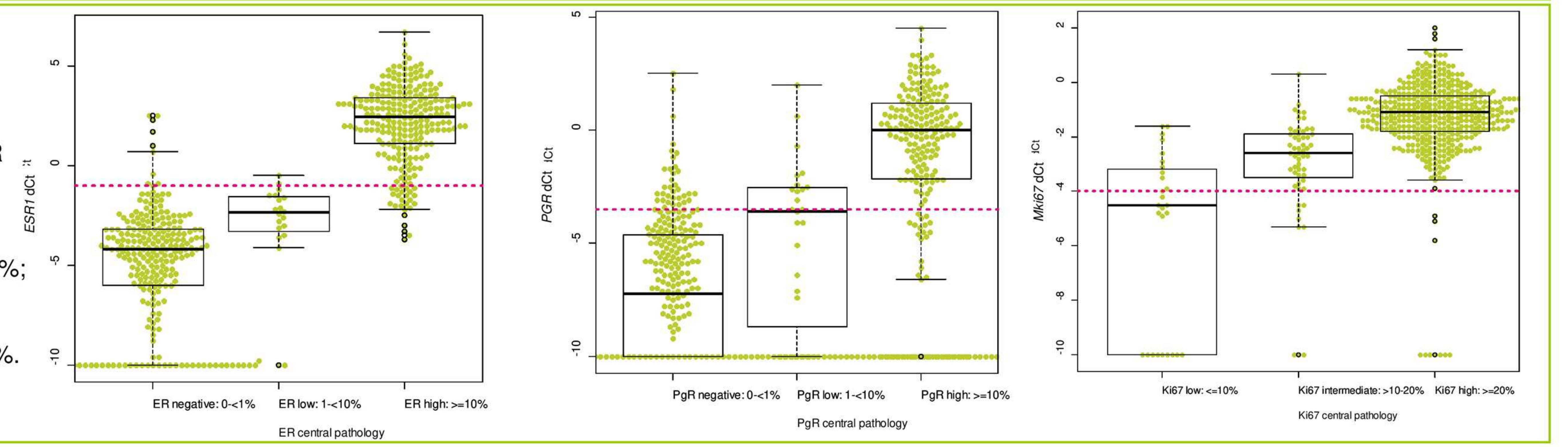
Figure 3: Agreements central pathology vs. Cepheid STRAT4. A) simple Kappa coefficient **B**) Sensitivity, Specificity, Accuracy (IHC-Cut-Offs: ER pos. ≥1%; PgR pos. ≥10%; HER2 pos. IHC 3+ or IHC 2+ and ISH positive; Ki67 high >20%; dCt-Cut-Offs: *ESR1* -1; *PGR* -3.5; *ERBB2* -1; *Mki67* -4.)

Figure 4: Boxplots dCt values vs. central pathology IHC.

ESR1 dCt-Cut-Off = -1, ER IHC-Cut-Off = 1%, 10%;

PGR dCt-Cut-Off = -3.5, PgR IHC-Cut-Off = 1%, 10%;

Mki67 dCt-Cut-Off = -4, Ki67 IHC-Cut-Off 10%, 20%.



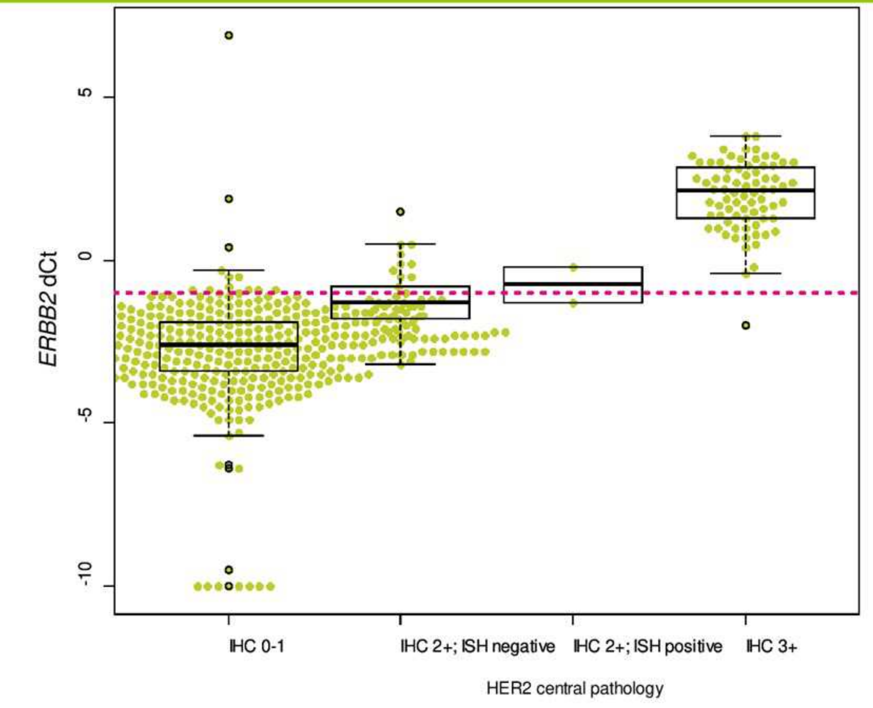


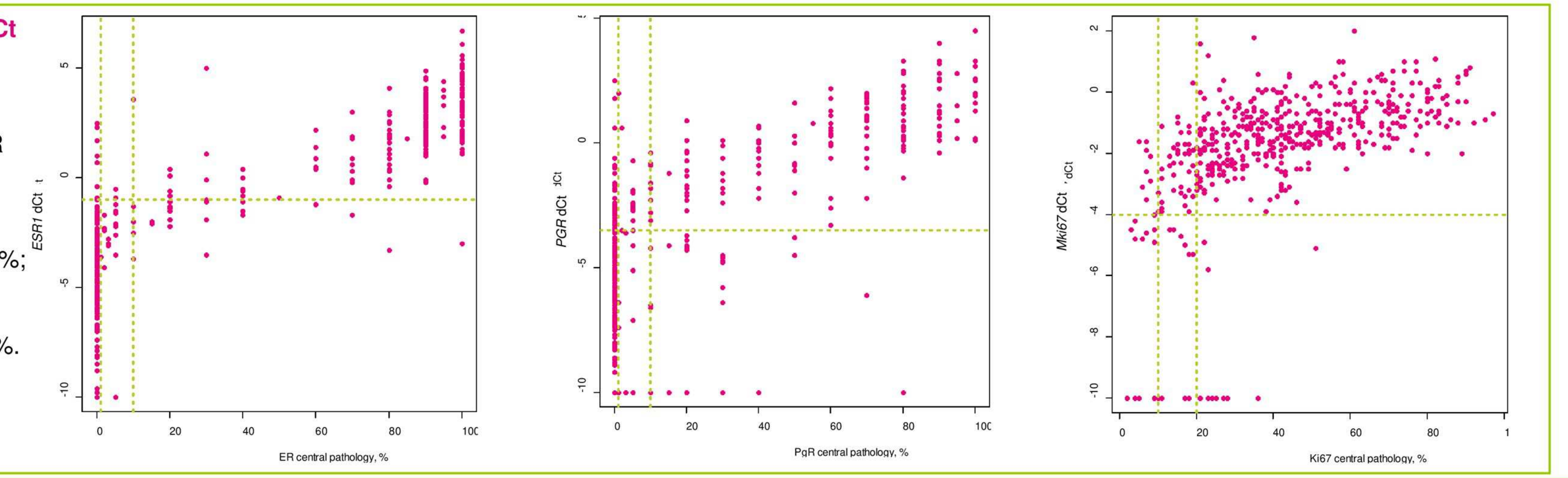
Figure 5: Boxplots dCt values vs. central pathology IHC. *ERBB2* dCt-Cut-Off = -1.

Figure 6: Scatter plots dCt value vs. expression of central pathology IHC.

ESR1 dCt-Cut-Off = -1, ER IHC-Cut-Off = 1%, 10%;

PGR dCt-Cut-Off = -3.5, PgR IHC-Cut-Off = 1%, 10%;

Mki67 dCt-Cut-Off = -4, Ki67 IHC-Cut-Off 10%, 20%.



Summary of Results

503 of 509 (≈99%) measurements of the Xpert® Breast Cancer STRAT4* assay were performed successfully, with valid results. ER, PgR and HER2 revealed a high Kappa coefficient: 0.65 - 0.82, while Ki67 has a slightly lower Kappa with 0.37. Sensitivity, specificity and accuracy are in all cases >80%, except Ki67 specificity (30.5%), which could be explained with a low rate of right negative values (fig. 3).

HER2 measurement delivered the highest number of positive predicted values (94.6 %), followed by ER (89.7%), Ki67 (86.7%) and PgR (82.3%).

Figure 4 – 6 show, that negative mRNA values (NV) are widely distributed across IHC expression: for ER 1 – 100%, PgR 10 – 80%, Ki67 20 – 50%. With 2 events, distributed over IHC2+, ISH pos. and IHC 3+, HER2 measurement delivered the lowest number of false negative values (total NV = 402).

Conclusion

Our results show a high concordance between standardized central IHC and automated mRNA expression analysis for the most important BC biomarkers ER, PgR and HER2. For the continuous proliferation marker Ki67, the concordance is slightly lower. The STRAT4 assay might offer additional option to conventional methods for BC biomarker assessment, in particular in situations where IHC is not feasible. Results show that adjusting IHC cutoffs may be necessary. To determine the clinical validity, additional outcome analyses are necessary.

References

(**1**) Xpert Breast Cancer STRAT4 (CE-IVD*) package insert. 301-4981, Rev. B August 2017. (**2**) Xpert FFPE Lysis Kit (CE-IVD*) package insert. 301-5224, Rev B, March 2017.

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*CE-IVD. In vitro diagnostic device. Not available in all countries. Not available in the U.S.

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