RNA-based determination of ESR1 and HER2 expression and response to neoadjuvant chemotherapy

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**Background:** Hormone and human epidermal growth factor receptor 2 (HER2) receptors are the most important breast cancer biomarkers, and additional objective and quantitative test methods such as messenger RNA (mRNA)-based quantitative analysis are urgently needed. In this study, we investigated the clinical validity of RT-PCR-based evaluation of estrogen receptor (ESR1) and HER2 mRNA expression.

**Patients and methods:** A total of 1050 core biopsies from two retrospective (GeparTrio, GeparQuattro) and one prospective (PREDICT) neoadjuvant studies were evaluated by quantitative RT-PCR for ESR1 and HER2.

**Results:** ESR1 mRNA was significantly predictive for reduced response to neoadjuvant chemotherapy in univariate and multivariate analysis in all three cohorts. The complete pathologically documented response (pathological complete response, pCR) rate for ESR1+/HER2− tumors was 7.3%, 8.0% and 8.6%; for ESR1−/HER2− tumors it was 34.4%, 33.7% and 37.3% in GeparTrio, GeparQuattro and PREDICT, respectively (P < 0.001 in each cohort). In the Kaplan–Meier analysis in GeparTrio patients with ESR1+/HER2− tumors had the best prognosis, compared with ESR1−/HER2− and ESR1−/HER2+ tumors [disease-free survival (DFS): P < 0.0005, overall survival (OS): P < 0.0005].

**Conclusions:** Our results suggest that mRNA levels of ESR1 and HER2 predict response to neoadjuvant chemotherapy and are significantly associated with long-term outcome. As an additional option to standard immunohistochemistry and gene-array-based analysis, quantitative RT-PCR analysis might be useful for determination of the receptor status in breast cancer.

**Key words:** breast cancer, chemotherapy neoadjuvant, estrogen receptor, HER-2

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**introduction**

Hormone and human epidermal growth factor receptor 2 (HER2) receptors are central breast cancer biomarkers and constitute the dominant biological determinants of breast cancer [1–3]. Currently, these markers are routinely evaluated for each patient by immunohistochemistry.

Major limitations of immunohistochemistry are the inter-observer and technical variability as well as the inability to generate quantitative data. An American Society of Clinical Oncology / American College of Pathologists analysis has stated that the immunohistochemical assessment of hormone and HER2 receptors is inaccurate in up to 20% [4, 5].

The evaluation of messenger RNA (mRNA) markers by quantitative RT-qPCR might be an interesting additional option. Several retrospective evaluations suggest that RNA-based determination of hormone receptors, HER2 as well as other markers is generally feasible [6–11]. However, the current guidelines have emphasized that it would be essential to link the RNA markers directly to the clinical outcome before this new technology can be used as a diagnostic test [4].

In the NEOpredict translational research program, we have validated an RNA-based approach for assessment of the receptor status by evaluation of ESR1 and HER2 mRNAs using predefined cut-offs [13, 12] in a total of 1050 tumor samples from three neoadjuvant multicenter studies. As clinical end-points we use chemotherapy response and outcome after anthracycline–taxane-containing neoadjuvant chemotherapy.

**patients and methods**

**clinical studies**

The bases for the sequential-validation strategy were three sequential neoadjuvant clinical multicenter studies by the German Breast Group and the Arbeitsgemeinschaft Gynäkologische Onkologie Breast Study Group (consort diagram, Figure 1A–C). The Ethics committee approval was obtained for all centers participating in the clinical studies and from the institutional review board of the Charité hospital. All samples were formalin-fixed paraffin-embedded (FFPE) pretherapeutic core biopsies collected at baseline, before randomization, with written informed consent.

**geparTrio study**

As a first cohort 262 core biopsies from patients treated with six cycles of docetaxel (Sanofi-Aventis, Frankfurt, Germany) (Taxotere), doxorubicin (Adriamycin) and cyclophosphamide (TAC), within the GeparTrio (NCT00544765) [13, 14] or the GeparTrio Pilot [15] study, were used. For the current investigation, the inclusion criteria were six cycles of TAC irrespective of response after the initial two cycles of TAC, available pCR data, tissue samples with at least 30% tumor tissue, successful RNA isolation. The expression of HER2 mRNA had been analyzed in a previous project in 278 samples from GeparTrio with a focus on concordance between central and local pathology [16]. Based on the available tissue for further PCR analyses, 262 of those samples were included in the present study.

**geparQuattro study**

As a second validation cohort, we used samples from 474 patients enrolled in the prospective, phase III GeparQuattro trial (NCT 00288002) [17]. The samples from all three chemotherapy arms (study arm A, B, C) were used, all other inclusion criteria were the same as in GeparTrio (Figure 1). A small number (n = 10) of locally HER2 positive cases were included by
mistake which had been treated with trastuzumab. Tumors from ten additional patients were found to be HER2+ by RT-PCR, these tumors had been HER2− by local pathology assessment (and were therefore not treated with trastuzumab), resulting in a total of 20 HER2 positive tumors. It was decided to include these patients in the analysis of RNA-based molecular tumor types.

prospective PREDICT/GeparQuinto study
The PREDICT study was designed as a substudy of GeparQuinto [18–20] for prospective validation of biomarkers in HER2− tumors in the neoadjuvant setting (Figure 1C). Between September 2009 and October 2010, a total of 314 samples were evaluated. The inclusion criteria were (i) HER2− patients who were randomly assigned to setting 1 and did not receive bevacizumab, (ii) available tumor sample, available pCR data. The biomarker analysis was carried out parallel to recruitment of the GeparQuinto study and was completed before the study outcome data were analyzed.

A pathological complete response (pCR) was defined as the pathologically confirmed absence of residual invasive tumor in breast and lymph nodes at the end of chemotherapy (ypT0/Tis, ypN0), based on the histopathological reports, which were centrally reviewed. In GeparTrio survival analysis was carried out with a median follow-up time for disease-free survival (DFS) of 55.43 months (range 1.48–84.76) and for overall survival (OS) of 58.68 months (range 1.48–96.49).

All clinical data, including the immunohistochemical data were extracted from the study database and represent the local assessment. As the current clinical hormone-receptor status is based on the combination of ER and progesterone receptor (PR), we decided to use this combined determination centrally using immunohistochemistry and silver in-situ hybridization, as the testing of HER2 on core biopsies was not fully determined centrally in GeparTrio. HER2 status was based on the immunohistochemistry-based status. For GeparTrio, HER2 status was determined centrally using immunohistochemistry and silver in-situ hybridization, as the testing of HER2 on core biopsies was not fully established at the time the study was conducted.

sample preparation and RNA extraction
From each FFPE tissue block, a 10 µm (GeparTrio and PREDICT samples) or a 5 µm section (GeparQuattro samples) was cut. The presence of at least 30% tumor tissue was evaluated.

The RNA isolation from FFPE sections was carried out using the robotic tissue preparation system and VERSANT tissue preparation reagents (both Siemens Healthcare Diagnostics, Tarrytown) as described earlier [21–23]. Samples were defined to have sufficient RNA if the mean of the Ct values of three reference genes CALM2, OAZ1 and RPL37A was below 33.4. For assessment of DNA contamination, a progestogen-associated endometrial protein gene-specific quantitative PCR without preceding reverse transcription was carried out. The samples were considered to be substantially free of DNA when Ct values >38 were detected.

gene expression analysis using reverse transcription quantitative PCR
Primer and probe sequences of the genes used for determination of molecular tumor types are given in reference [24]. Reference genes were selected by a model-based variance estimation approach [25]. Based on Affymetrix U133A gene expression, datasets from 379 breast cancer tumors (213 surgical specimen; 186 pretherapeutic biopsies), we selected and verified RPL37A, CALM2 and OAZ1 as stably expressed reference genes.

The gene expression was assessed by RT-qPCR using the SuperScript III Platinum One-Step Quantitative RT-PCR System with ROX (Invitrogen, Karlsruhe, Germany) according to manufacturer’s instructions in 384-well plates in an ABI PRISM 7900HT (Applied Biosystems, Darmstadt, Germany) (GeparTrio and GeparQuattro samples) or in 96-well plates in an Agilent MX3005 (Agilent, Böblingen, Germany) (PREDICT samples) for 30 min at 50°C, 2 min at 95°C followed by 40 cycles for 15 s at 95°C and 30 s at 60°C.

All PCR assays were carried out in duplicate in GeparTrio and in triplicate in GeparQuattro and PREDICT. The mean of the Ct values for each gene was calculated.

To assure accuracy of the assays, a standardized reference RNA (Stratagene qPCR Human Reference Total RNA, Agilent Technologies, Waldbronn, Germany) was tested for each gene in parallel to the FFPE samples. For exclusion of contamination, no-template-controls were assessed in parallel.

normalization and cut-offs for molecular tumor classification
Normalization was carried out in an identical way in all cohorts using three reference genes. The relative gene expression levels of ESR1 and HER2 are given as ΔCt, whereas ΔΔCt = 20 – [Ct(ESR1) - Ct(HER2)]. The cut-off values were predefined based on the two previous studies including 274 independent breast tumors samples for ESR1 [12] and 167 breast cancer samples for HER2 [6]. Since a different PCR platform was used in the PREDICT study, a constant target-specific shift in ΔCt values between previous and current assay conditions occurred. The cut-offs from the GeparTrio and GeparQuattro studies were therefore transformed by addition of an offset. The offsets for ESR1 and HER2 were predetermined for this study by reassessment of the 167 samples from the previous study using the old and new assay conditions resulting in the cut-offs of 18.6 for HER2 mRNA and 14.5 for ESR1 mRNA. These cut-offs are, therefore, numerically different, but identical with regard to mRNA levels to the previously published cut-offs and the cut-offs used in GeparTrio and GeparQuattro. Variabilities of ESR1 and HER2 PCR assays were published previously [6, 21]. Moreover, we have tested reproducibility in a subset of 25 samples from the PREDICT study in two different laboratories, with high correlations (ESR1 r = 0.998; HER2 r = 0.90) and high concordance of classification (100%) (supplementary Figure S1, available at Annals of Oncology online).

statistical analysis
Statistical analysis was carried out using MATLAB 7.5.0 (The MathWorks, Natick, MA), SPSS version 13.0 (SPSS Inc. Chicago, Illinois, USA), GraphPad Prism 4 (GraphPad software, La Jolla, California), SAS 9.2 (SAS Institute Inc., Cary, NC) as well as the R-package. The probability of pCR as a function of gene expression parameters and/or clinical baseline parameters was determined by univariate and multivariate logistic regression analysis. Fisher’s exact test was used to compare pCR rates in subgroups. Concordance between mRNA and immunohistochemistry/in-situ hybridization was assessed with cross-tables and Yule’s Q. Survival analyses were carried out by the Kaplan–Meier and log-rank test. All tests were two-sided with significance levels set at 0.05.

results
baseline clinical data
An overview on the study patients is given in the consort diagram (Figure 1A–C) and in supplementary Table S1, available at Annals of Oncology online. The clinical parameters age, grade, and stage are comparable in the GeparTrio and GeparQuattro cohort. In the PREDICT study the patients were younger, had more G3 tumors and the tumors were smaller than in the other two cohorts. RNA was isolated from a total
of 1093 core biopsies, 1050 of those samples (96.1%) contained sufficient RNA and were included in the study.

quantitative assessment of ESR1 and HER2 mRNA levels as predictive factor for chemotherapy response in the three cohorts

In univariate logistic regression, mRNA expression of ESR1 was predictive as a continuous parameter for a reduced response to neoadjuvant chemotherapy with an odds ratio (OR) of 0.74, 0.76 and 0.78 in the GeparTrio, GeparQuattro and PREDICT (all P-values <0.001) study. In multivariate analysis including established clinicopathological parameters, ESR1 mRNA was still significant in all the three cohorts with an OR of 0.78 (P = 0.003, GeparTrio), 0.76 (P < 0.001, GeparQuattro) and 0.88 (P = 0.050, PREDICT, Table 1). In contrast, HER2 mRNA levels as a continuous parameter were significantly predictive only in the PREDICT study in univariate analysis, and not significant in the other cohorts and in the multivariate analyses (Table 1).

definition of tumor types based on the mRNA levels of ESR1 and HER2

We have defined four mRNA-based subtypes ESR1+/HER2−, ESR1+/HER2+, ESR1−/HER2+ and ESR1−/HER2−, using predefined cut-offs [6, 12] (Figure 2A–C). In the GeparTrio cohort, 58% ESR1+/HER2−, 10% ESR1+/HER2+, 9% ESR1−/HER2+ and 23% ESR1−/HER2− tumors were found (Figure 2A). In the GeparQuattro and the PREDICT study, the distribution of ESR1+/HER2− and ESR1−/HER2− was very similar in both the studies (Figure 2B and C). In the GeparQuattro cohort, only 4.5% of tumor samples were HER2+ (see the methods section). According to the inclusion criteria of the prospective PREDICT study, only patients from the HER2 negative study arms of PREDICT were included and only two (0.6%) were found to be HER2+ by mRNA analysis.

The pCR rates were different in the four biological subgroups (Figure 3A–C). In ESR1+/HER2− tumors, the pCR rate was 7.3% in GeparTrio, 8.0% in GeparQuattro and 8.6% in PREDICT. ESR1−/HER2− tumors had a pCR rate of 34.4%, 33.7% and 37.3% in GeparTrio, GeparQuattro and PREDICT (P < 0.001 compared with ESR1+/HER2− tumors in all the three cohorts). ESR1−/HER2+ tumors had a particularly high pCR rate of 43.5% (GeparTrio) and 50% (GeparQuattro). In GeparTrio, trastuzumab was not part of the neoadjuvant regimen, in GeparQuattro 50% of patients (10 of 20) in this study cohort had received trastuzumab, which might have contributed to the slightly higher pCR rate.

Table 1. Univariate and multivariate analysis of continuous ESR1 and HER2 mRNA levels for prediction of pCR in the three study cohorts

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariate analysis</th>
<th></th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>GeparTrio cohort</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR1 mRNA level</td>
<td>0.74 (0.66–0.83)</td>
<td>&lt;0.001</td>
<td>0.78 (0.67–0.92)</td>
</tr>
<tr>
<td>HER2 mRNA level</td>
<td>1.05 (0.89–1.24)</td>
<td>ns</td>
<td>1.05 (0.86–1.28)</td>
</tr>
<tr>
<td>Age group (&lt;50 versus ≥50 years)</td>
<td>2.71 (1.41–5.23)</td>
<td>0.003</td>
<td>3.57 (1.51–8.44)</td>
</tr>
<tr>
<td>Tumor type (ductal/other versus lobular)</td>
<td>1.85 (0.62–5.53)</td>
<td>ns</td>
<td>1.24 (0.32–4.90)</td>
</tr>
<tr>
<td>Tumor grade (G3 versus G1–G2)</td>
<td>2.61 (1.29–5.31)</td>
<td>0.008</td>
<td>1.77 (0.72–4.35)</td>
</tr>
<tr>
<td>Tumor stage (cT1-2 versus cT3-4)</td>
<td>3.57 (1.45–8.81)</td>
<td>0.006</td>
<td>6.42 (1.90–21.67)</td>
</tr>
<tr>
<td>Clinical nodal status (cN0 versus cN+)</td>
<td>1.09 (0.58–2.05)</td>
<td>ns</td>
<td>1.56 (0.67–3.60)</td>
</tr>
<tr>
<td>GeparQuattro cohort</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR1 mRNA level</td>
<td>0.76 (0.70–0.82)</td>
<td>&lt;0.001</td>
<td>0.76 (0.69–0.83)</td>
</tr>
<tr>
<td>HER2 mRNA level</td>
<td>0.87 (0.72–1.07)</td>
<td>ns</td>
<td>1.16 (0.96–1.40)</td>
</tr>
<tr>
<td>Age group (&lt;50 versus ≥50 years)</td>
<td>1.33 (0.83–2.13)</td>
<td>ns</td>
<td>1.02 (0.60–1.72)</td>
</tr>
<tr>
<td>Tumor type (ductal/other versus lobular)</td>
<td>3.09 (1.20–7.94)</td>
<td>0.02</td>
<td>1.87 (0.62–5.63)</td>
</tr>
<tr>
<td>Tumor grade (G3 versus G1–G2)</td>
<td>2.28 (1.40–3.71)</td>
<td>0.001</td>
<td>1.17 (0.65–2.08)</td>
</tr>
<tr>
<td>Tumor stage (cT1-2 versus cT3-4)</td>
<td>3.27 (1.72–6.23)</td>
<td>&lt;0.001</td>
<td>2.68 (1.33–5.42)</td>
</tr>
<tr>
<td>Clinical nodal status (cN0 versus cN+)</td>
<td>1.23 (0.77–1.96)</td>
<td>ns</td>
<td>0.95 (0.56–1.61)</td>
</tr>
<tr>
<td>PREDICT cohort</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ESR1 mRNA level</td>
<td>0.78 (0.70–0.86)</td>
<td>&lt;0.001</td>
<td>0.88 (0.77–1.00)</td>
</tr>
<tr>
<td>HER2 mRNA level</td>
<td>0.64 (0.47–0.85)</td>
<td>0.002</td>
<td>0.82 (0.59–1.11)</td>
</tr>
<tr>
<td>Age group (&lt;50 versus ≥50 years)</td>
<td>1.3 (0.74–2.3)</td>
<td>ns</td>
<td>1.2 (0.63–2.2)</td>
</tr>
<tr>
<td>Tumor type (ductal/other versus lobular)</td>
<td>9.2 (1.2–68.9)</td>
<td>0.03</td>
<td>4.8 (0.62–37.5)</td>
</tr>
<tr>
<td>Tumor grade (G3 versus G1–G2)</td>
<td>3.3 (1.8–5.9)</td>
<td>&lt;0.001</td>
<td>1.7 (0.84–3.6)</td>
</tr>
<tr>
<td>Tumor stage (cT1-2 versus cT3-4)</td>
<td>2.3 (0.86–6.0)</td>
<td>ns</td>
<td>1.8 (0.62–4.9)</td>
</tr>
<tr>
<td>Clinical nodal status (cN0 versus cN+)</td>
<td>2.0 (1.1–3.6)</td>
<td>0.02</td>
<td>1.7 (0.92–3.2)</td>
</tr>
</tbody>
</table>

*Non-significant.
comparison of ESR1 mRNA status and ER status by immunohistochemistry, the concordance was 90.2%, 90.0% and 93.3% in the three studies (supplementary Table S3, available at Annals of Oncology online). For HER2, the concordance between immunohistochemistry/in situ hybridization and RT-qPCR was 95.8%, 96.6%, and 99.4% in the three studies (supplementary Table S4, available at Annals of Oncology online).

Supplementary Table S5, available at Annals of Oncology online, shows the pCR rates of the groups resulting from the combination of ER immunohistochemistry and mRNA status. Those tumors that were ER+ by both methods had a pCR rate of 9.8% (all the three cohorts combined), and those that were negative by both methods had a pCR rate of 37.8%. Tumors that were negative by immunohistochemistry and positive by mRNA had a low pCR rate of 12.9%, while tumors that were positive by IHC and negative by mRNA analysis had a pCR rate of 23%.

### survival analysis of the RNA-based tumor types

As an additional outcome parameter, we carried out a Kaplan–Meier analysis for DFS and OS in the GeparTrio cohort comparing the RT-qPCR-based and immunohistochemistry-based molecular typing (Figure 4). Patients with ESR1+/HER2− tumors had the best DFS and OS, while patients with ESR1−/HER2− and ESR1−/HER2+ tumors had a comparably poor prognosis (Figure 4, DFS: P < 0.0005, OS: P < 0.0005). The prognostic impact of RT-qPCR-based and IH-based assessment was very similar for the four molecular subtypes. In this cohort that had not been treated with neoadjuvant trastuzumab, patients with ESR1+/HER2+ tumors had a similar DFS as ESR1−/HER2− tumors. For OS, the prognosis of the ESR1+/HER2+ tumors was better than the ESR1−/HER2− tumors.

### discussion

In this study, we have validated mRNA assessment of ESR1 and HER2 for prediction of response to neoadjuvant chemotherapy by analysis of three independent cohorts with a total of 1050 patients. The pCR rates of the individual subtypes are similar to those observed rates using immunohistochemistry in other neoadjuvant studies [26–30].

The introduction of PCR-based molecular typing as an additional method is a promising approach to reduce the inherent variability of immunohistochemistry. It should be noted that both the approaches have their advantages: immunohistochemistry allows a direct visual control, but is only semi-quantitative with a relevant observer-related and instrument-related bias, which might be partly improved by automated analysis platforms. RNA-analysis is quantitative, objective and offers many options for integrated quality control by use of tissue-specific and marker-specific positive and negative controls. Both the approaches depend on an enzymatic reaction as a central element; however, the reaction conditions are more stable in the standardized environment of a quantitative PCR cycler. The PCR analysis should be always linked to a histopathological quality control to ensure an adequate selection of tissue.

Our analysis shows that both the methods have a high concordance and are similarly predictive. However, tumors that are ER− by immunohistochemistry and ER+ by RT-PCR have a comparably low pCR rate of 12%. This might suggest
that tumor biology could be better reflected by mRNA analysis; however, the small sample size of the discordant group limits the interpretation.

In our investigation, we have used only ESR1 mRNA expression and have not included the PR. This decision was based on the report of the Early Breast Cancer Trialist’s Collaborative Group that the ER is the only relevant determinant of response to endocrine therapy [1]. The concordance is similar if ESR1 mRNA is either compared with ER immunohistochemistry or compared with HR status (combined ER and PR).

Reproducibility of the RT-PCR assay has been tested with high correlations in a subset of 25 samples from PREDICT. In addition, the variability of the qRT-PCR assays for ESR1 and HER2 used in this study has been reported in two separate publications [6, 21]. For a very similar test, the Endopredict multigene assay that uses the identical platform, we have carried out a proficiency test in seven laboratories with a very good performance (Pearson’s correlation r = 0.994; 100% concordance of classification) [31]. Therefore, RNA-based molecular assays can be used in established molecular pathology laboratories.

The RNA-based approach could be used for additional validations of those tumors that are hormone-receptor negative by immunohistochemistry but that have histological features that are highly suggestive for hormone-receptor positivity, such as tubular, lobular or grade 1 histology. Furthermore, standardized RNA-based molecular assays may serve as an additional standardized entry criterion for clinical studies.

Interestingly, our data suggest that ESR1 expression is predominantly regulated on a continuous scale and that the level of ESR1 expression is linked to the OR for chemotherapy response. In contrast, HER2 is significant for response if the predefined groups are used but it is not significant on a continuous scale. This might lead to the hypothesis that the underlying biological event for HER2 overexpression, the HER2 gene amplification, leads to distinct tumor groups rather than to a continuum of HER2 expression levels. The distribution pattern of HER2 mRNA expression in Figure 2 is suggestive of differences between ESR1+ and ESR1− tumors. In ESR1+ tumors, HER2 mRNA expression was generally higher and a continuum is observed with gradually increasing expression levels. In contrast, in ESR1− tumors there are distinct groups of HER2+ and HER2− tumors. The identical observation has recently been described by Pinhel et al. [32]. This suggests that other pathways, such as the estrogen receptor pathway, might also influence HER2 expression levels [33, 34]. Considering the continuum of HER2 mRNA expression in hormone-receptor positive tumors, it is not clear if the cut-off levels for HER2 that are used in this study will also be valid in the context of trastuzumab therapy, in particular as recent reports have suggested discrepancies between mRNA and immunohistochemical HER2 determination [35]. The GeparTrio trial did not contain trastuzumab therapy and the analyses in GeparQuattro and PREDICT were focused on patients with HER2− tumors. It should be noted as a limitation of the study that the number of HER2+ cases was very low in the GeparQuattro and the PREDICT cohort, so that the high concordance rates between RNA and protein analysis are mainly caused by the large number of HER2 negative cases. Therefore, the role of HER2 mRNA expression should be further evaluated in tumor cohorts that have been treated with trastuzumab, this evaluation is currently ongoing in the HER2+ study arm of the GeparQuattro study.

Taken together, it would be a major improvement for individualized therapy if oncologists and pathologists have the option to choose between different methods for biomarker analysis for the determination of receptor status. Based on the results of our study, it is possible to develop a certified standardized diagnostic test system for RNA-based receptor analysis that could be used for biomarker assessment in the local pathology laboratories. The integration of immunohistochemistry, mRNA-based analysis and clinical parameters could be used to reliably identify those patients who have an increased benefit from neoadjuvant chemotherapy.

Figure 3  Different rates of pathological complete response (pCR) in the molecular tumor types in the GeparTrio (A), GeparQuattro (B), and the PREDICT cohort (C). The molecular tumor types have significantly different response rates to neoadjuvant chemotherapy, which are similar in the three cohorts. (*two-sided Fisher’s test; **chi-square test).
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**disclosure**

CD, RK, CP and MD are shareholders of Sividon Diagnostics. All remaining authors have declared no conflicts of interest.

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