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High concordance of protein (by IHC), gene (by FISH; HER2 only), and microarray readout (by TargetPrint) of ER, PgR, and HER2: results from the EORTC 10041/BIG 03-04 MINDACT trial

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Background: To investigate the correlation of TargetPrint with local and central immunohistochemistry/fluorescence *in situ* hybridization assessment of estrogen (ER), progesterone (PgR), and human epidermal growth factor receptor 2 (HER2) in the first 800 patients enrolled in the MINDACT trial.

Patients and methods: Data from local ($N = 800$) and central ($N = 626$) assessments of receptor status were collected and compared with TargetPrint results.

Results: For ER, the positive agreement (the percentage of central pathology positive assessments that were also TargetPrint/local laboratory positive) for TargetPrint in comparison to centralized assessment was 98% with a negative agreement (the percentage of central pathology negative assessments that were also TargetPrint/local laboratory negative) of 96%. For PgR, the positive agreement was 83% with a negative agreement of 92%. For HER2, the positive agreement was 75% with a negative agreement of 99%. Even though the local assessment showed higher positive agreement for PgR (89%) and higher positive agreement for HER2 (85%), the range of discordant local versus central assessments were as high as 6.7% for ER, 12.9% for PgR, and 4.3% for HER2.

Conclusion: TargetPrint and local assessment of ER, PgR, and HER2 show high concordance with central assessment in the first 800 MINDACT patients. However, there are concerns about the higher discordance rates for some local sites. TargetPrint can improve the reliability of hormone receptor and HER2 testing for those centers with a lower rate of concordance with the reference laboratory, with the limitation of a positive agreement of 75% for HER2. TargetPrint consequently has important implications for treatment decisions in clinical practice and is a reliable alternative to local assessment for ER.

Clinical Trials number: NCT00433589.

Key words: breast cancer, concordance, FISH, hormone receptor, IHC, TargetPrint

introduction

This study was undertaken to determine the correlation of mRNA readout of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2) by TargetPrint® (commercially available microarray-based test) with immunohistochemistry (IHC)/fluorescence *in situ* hybridization (FISH) assessments determined locally and centrally in the first 800 patients enrolled in the MINDACT trial [1]. Measurement of ER, PgR, and HER2 status in early-stage breast cancer is critical for informing treatment recommendations [2]. Controversy remains about the optimal stratification of patients with indeterminate risk and considerable differences exist among physicians regarding the selection of patients for adjuvant chemotherapy. Accordingly, several guidelines have been issued, e.g. the St Gallen [3], ESMO [4], and NCCN [5].

MINDACT is an international, prospective, randomized, phase III trial investigating the clinical utility of MammaPrint®

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versus standard clinicopathological criteria (Adjuvant! Online) to select patients with breast cancer for adjuvant chemotherapy and has enrolled 6694 patients. The trial's hypothesis is that the molecular assay will outperform the established clinicopathological assessment, reducing the number of patients receiving adjuvant chemotherapy by 10%–15% without impairing long-term outcomes [6, 7]. A pilot phase comprising the first 800 enrolled patients was predefined to ensure the trial's feasibility [1].

We present the results of the preplanned translational research project on the patients from this pilot phase. The aim of this analysis was to investigate the correlation of microarray mRNA readouts with local and central IHC/FISH assessments of ER, PgR, and HER2 status. Interlaboratory variability in ER, PgR, and HER2 assessments remains a major concern worldwide [8] and a more reliable assessment is highly desirable.

patients and methods

Women aged ≥18 years with histologically proven operable invasive breast cancer, 0–3 positive lymph nodes, and a frozen tumor sample containing ≥30% tumor cells were eligible to enroll in MINDACT from February 2007 to July 2011 (closed to accrual). Further eligibility criteria included tumor stage T1–2, or operable T3 and treatment with breast conserving surgery or mastectomy combined with a sentinel node procedure or full axillary clearance. A WHO performance status of 0 or 1 and adequate bone marrow, liver, and renal function were required. Main exclusion criteria included previous or concurrent cancer, previous chemotherapy, endocrine therapy, or radiotherapy, and clinically significant cardiac disease. The protocol was approved by independent ethics committees and medical authorities. All patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines.

Data from local ($N = 800$) and central ($N = 626$; European Institute of Oncology, Milan, Italy) assessments of ER, PgR, and HER2 status were collected and compared with microarray readout (TargetPrint) provided by Agendia (Amsterdam, The Netherlands). Baseline characteristics are shown in Table 1.

data availability

At the time of analysis, central pathology results were unavailable for 174 patients due to delayed submission of tumor samples. Among the 626 patients with central pathology results, 15 had incomplete data (7 for ER and PgR, 15 for HER2) and 3 equivocal HER2 IHC and FISH. For local pathological laboratory assessments, two patients had a missing PgR assessment and 28 had unknown HER2 status. TargetPrint readout was available for all 800 patients (Table 1).

immunohistochemistry and FISH

In the central laboratory, ER and PgR status were assessed on formalin-fixed paraffin-embedded tissue blocks by IHC using the ER/PgR PharmDX kit (Dako, Glostrup, Denmark). Tumors were classified as ER- or PgR-positive when ≥1% invasive tumor cells showed definite nuclear staining, irrespective of staining intensity [2]. HER2 expression was evaluated with the HercepTest kit (Dako) and scored as 0, 1+, 2+, or 3+, according to the FDA scoring system. Tumors scored as 2+ or 3+ were re-tested with FISH using the PathVysion HER2 DNA probe kit (Vysis-Abbott, Chicago, IL). Cases were considered HER2-positive if scored 3+ by IHC and/or amplified by FISH (ratio ≥2).

Details of the assays and protocols for ER, PgR, and HER2 status assessment used in local centers were not available, but according to the MINDACT protocol, ER/PgR-positive disease was defined as a tumor with ≥1% immunoreactive cells, an Allred score >2, or a biochemical protein concentration ≥10 fmol/mg; HER2 status was determined according to local policies.

Table 1. Baseline characteristics of the first 800 patients enrolled in the MINDACT trial (adapted from Rutgers et al. [1], with permission, with ER, PgR, and HER2 status updated at the time of analysis)

Characteristic	Number of patients (%)		
Age, years			
<50	264 (33.0)		
≥50	536 (67.0)		
Tumor size, cm			
≤2	601 (75.1)		
>2–5	195 (24.4)		
>5	4 (0.5)		
Lymph node status			
Negative	794 (99.3)		
1–3 node positive	4 (0.5)		
Positive before amendment	1 (0.1)		
Missing	1 (0.1)		
Histological grade			
1	169 (21.1)		
2	361 (45.1)		
3	266 (33.3)		
Undefined	4 (0.5)		
ER status (local)			
Positive	674 (84.3)		
Negative	126 (15.8)		
PgR status (local)			
Positive	570 (71.3)		
Negative	228 (28.5)		
Unknown	2 (0.3)		
HER2 status (local)			
Negative	680 (85.0)		
Positive	92 (11.5)		
Unknown	28 (3.5)		
ER + PgR status (local)			
ER positive – PgR positive	562 (70.3)		
ER positive – PgR negative	110 (13.8)		
ER negative – PgR positive	8 (1.0)		
ER negative – PgR negative	118 (14.8)		
Unknown	2 (0.3)		
Data availability	Central IHC/FISH	Local IHC/FISH	TargetPrint
ER	$N = 619$	$N = 800$	$N = 800$
PgR	$N = 619$	$N = 798$	$N = 800$
HER2	$N = 608$	$N = 772$	$N = 800$

ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization.

Table 2. Assessment of ER, PgR, and HER2 status by local versus central pathology (immunohistochemistry/fluorescence *in situ* hybridization) and TargetPrint versus central and local pathology

	ER (central pathology)			
	Positive ($N = 537$), *n* (%)	Negative ($N = 82$), *n* (%)	Missing ($N = 181$), *n* (%)	Total ($N = 800$), *n* (%)
ER (local site)				
Positive	524 (97.6)	2 (2.4)	148 (81.8)	674 (84.3)
Negative	13 (2.4)	80 (97.6)	33 (18.2)	126 (15.8)

	PgR (central pathology)			
	Positive ($N = 490$), *n* (%)	Negative ($N = 129$), *n* (%)	Missing ($N = 181$), *n* (%)	Total ($N = 800$), *n* (%)
PgR (local site)				
Positive	438 (89.4)	12 (9.3)	120 (66.3)	570 (71.3)
Negative	52 (10.6)	116 (89.9)	60 (33.1)	228 (28.5)
Missing	0 (0.0)	1 (0.8)	1 (0.6)	2 (0.3)

	HER2 (central pathology)				
	Negative ($N = 537$), *n* (%)	Positive ($N = 71$), *n* (%)	IHC2 + FISH equivocal ($N = 3$), *n* (%)	Missing ($N = 189$), *n* (%)	Total ($N = 800$), *n* (%)
HER2 (local site)					
Negative	506 (94.2)	10 (14.1)	3 (100.0)	161 (85.2)	680 (85.0)
Positive	12 (2.2)	57 (80.3)	0 (0.0)	23 (12.2)	92 (11.5)
Missing	19 (3.5)	4 (5.6)	0	5 (2.6)	28 (3.5)

	ER (central pathology)			
	Positive ($N = 537$), *n* (%)	Negative ($N = 82$), *n* (%)	Missing ($N = 181$), *n* (%)	Total ($N = 800$), *n* (%)
ER (TargetPrint)				
Positive	525 (97.8)	3 (3.7)	144 (79.6)	672 (84.0)
Negative	12 (2.2)	79 (96.3)	37 (20.4)	128 (16.0)

	PgR (central pathology)			
	Positive ($N = 490$), *n* (%)	Negative ($N = 129$), *n* (%)	Missing ($N = 181$), *n* (%)	Total ($N = 800$), *n* (%)
PgR (TargetPrint)				
Positive	408 (83.3)	11 (8.5)	104 (57.5)	523 (65.4)
Negative	82 (16.7)	118 (91.5)	77 (42.5)	277 (34.6)

	HER2 (central pathology)				
	Negative ($N = 537$), *n* (%)	Positive ($N = 71$), *n* (%)	IHC2 + FISH equivocal ($N = 3$), *n* (%)	Missing ($N = 189$), *n* (%)	Total ($N = 800$), *n* (%)
HER2 (TargetPrint)					
Negative	532 (99.1)	18 (25.4)	3 (100.0)	168 (88.9)	721 (90.1)
Positive	5 (0.9)	53 (74.6)	0 (0.0)	21 (11.1)	79 (9.9)

	ER (local site)		
	Positive ($N = 674$), *n* (%)	Negative ($N = 126$), *n* (%)	Total ($N = 800$), *n* (%)
ER (TargetPrint)			
Positive	661 (98.1)	11 (8.7)	672 (84.0)
Negative	13 (1.9)	115 (91.3)	128 (16.0)

	PgR (local site)			
	Positive ($N = 570$), *n* (%)	Negative ($N = 228$), *n* (%)	Missing ($N = 2$), *n* (%)	Total ($N = 800$), *n* (%)
PgR (TargetPrint)				
Positive	490 (86.0)	33 (14.5)	0 (0.0)	523 (65.4)
Negative	80 (14.0)	195 (85.5)	2 (100.0)	277 (34.6)

	HER2 (local site)			
	Negative ($N = 680$), *n* (%)	Positive ($N = 92$), *n* (%)	Missing ($N = 28$), *n* (%)	Total ($N = 800$), *n* (%)
HER2 (TargetPrint)				
Negative	677 (99.6)	20 (21.7)	24 (85.7)	721 (90.1)
Positive	3 (0.4)	72 (78.3)	4 (14.3)	79 (9.9)

ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor.

microarray readout by targetprint

Gene-expression data for ER, PgR, and HER2 (blinded) were obtained by TargetPrint on frozen samples; tumors were considered as receptor-positive or -negative using previously determined and validated thresholds [9]. RNA isolation, labeling, and hybridization were carried out as described previously [10]. Fluorescence intensities on scanned images were quantified and normalized using Feature Extraction software (Agilent Technologies, Santa Clara, CA).

statistical analysis

Statistical calculations were conducted using SAS® 9.2 (SAS Institute, Inc., Cary, NC). Statistics summarizing the agreement between TargetPrint and local pathology versus central pathology included: positive agreement (percentage of central pathology positives that were TargetPrint/local laboratory positive) and negative agreement [11]; positive (PPV) and negative (NPV) predictive value (PPV: percentage of TargetPrint/local positives that are central pathology positive); percentage of concordance; and Cohen's κ coefficient [12]. To test for a difference between centers in the amount of discordance, a contingency table was constructed with the 51 centers in rows and the number of concordances and discordances in columns (data not shown). A two-sided Fisher's exact test was carried out (5% significance level). Patient data were omitted if one of the two assessments were missing in the comparison.

results

Table 2 cross tabulates local assessments and TargetPrint results versus central assessments and the TargetPrint results versus local assessments. (No statistical trend was seen for local lab-TargetPrint concordance versus percentage missing central pathology assessments, data not shown.)

Table 3. Positive and negative agreement and predictive value for local and TargetPrint assessment of ER, PgR, and HER2 versus central pathology

Positive and negative agreement, % (*n*/*N*)		
Local versus central pathology		
	Positive agreement	Negative agreement
ER	97.6 (524/537)	97.6 (80/82)
PgR	89.4 (438/490)	90.6 (116/128)
HER2	85.1 (57/67)	97.7 (506/518)
TargetPrint versus central pathology		
	Positive agreement	Negative agreement
ER	97.8 (525/537)	96.3 (79/82)
PgR	83.3 (408/490)	91.5 (118/129)
HER2	74.7 (53/71)	99.1 (532/537)
Positive and negative predictive value		
Local versus central pathology		
	PPV	NPV
ER	99.6 (524/526)	86.0 (80/93)
PgR	97.3 (438/450)	69.0 (116/168)
HER2	82.6 (57/69)	98.1 (506/516)
TargetPrint versus central pathology		
	PPV	NPV
ER	99.4 (525/528)	86.8 (79/91)
PgR	97.4 (408/419)	59.0 (118/200)
HER2	91.4 (53/58)	96.7 (532/550)

ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor; NPV, negative predictive value; PPV, positive predictive value.

positive and negative agreement, PPV and NPV

Of the 537 central ER-positive cases, 525 (98%) were TargetPrint ER-positive, well above the set target of 90%. For PgR, the positive agreement was 83%, which is below the desirable 90% and also below the positive agreement for local assessment (89%). For the negative agreement, the target of 95% was attained for ER (96%); while for PgR, this was slightly lower (92%). For PgR, the negative agreement was also <95% for local assessment (91%). For HER2, the positive agreement for TargetPrint (75%) was quite low versus that for local assessment (85%). For PPV and NPV, there were strong similarities in performance for TargetPrint and local assessment of ER compared with central pathology. For PgR, local IHC assessment was more in line with central assessment compared with the TargetPrint results. The PPV for HER2 status by TargetPrint was higher than that for local assessment (Table 3).

Data for ER and PgR using 10% invasive tumor cells as cutoff are provided in supplementary Table S1, available at *Annals of Oncology* online.

overall agreement

The percentage of concordant assessments and κ coefficients were used to summarize the overall agreement. Comparison of local with central assessments (Table 4) indicated highly similar results for ER (98% concordance; $\kappa = 0.90$), good concordance for HER2 (96%; $\kappa = 0.82$), and somewhat lower concordance for PgR (90%; $\kappa = 0.72$). Comparison of central assessment with TargetPrint (Table 4) indicated a highly similar overall performance, with a concordance of 98% ($\kappa = 0.90$) for ER, 96% for HER2 ($\kappa = 0.80$), and lower concordance for PgR (85%; $\kappa = 0.62$).

range of discordance for local assessment

The percentage of discordant assessments was calculated for each site separately. Only those centers with ≥30 assessments for that receptor were taken into account. The range of discordance was 1.6%–6.7% for ER (eight centers), 5.7%–12.9% for PgR (eight centers), and 0%–4.3% for HER2 (six centers). Fisher's exact test (on all centers) was carried out to see whether there was a significant difference in the level of discordance with central pathology between centers (ER, $P = 0.4$; PgR, $P = 0.03$; HER2, $P = 0.36$). This was the case for PgR. A Cochran–Armitage trend test indicated no trend in discordance versus sample size for ER and HER2, only for PgR (data not shown). However, local centers can work with external and several laboratories. The latter can result in additional heterogeneity within the same local center.

three-way comparison

The scatterplots in Figures 1 and 2 show a three-way comparison of the different assessments. Central IHC assessments for

Table 4. Inter-rater agreement statistics for ER, PgR, and HER2 assessments: local versus central pathology and TargetPrint versus central and local pathology

	% Concordance	95% CI	Cohen's κ coefficient	95% CI	*N*
Local versus central pathology					
ER	97.6	96.4–98.8	0.900	0.851–0.950	619
PgR	89.6	87.2–92.1	0.717	0.653–0.781	618
HER2	96.2	94.7–97.8	0.817	0.743–0.891	585
TargetPrint versus central pathology					
ER	97.6	96.4–98.8	0.899	0.849–0.949	619
PgR	85.0	82.2–87.8	0.621	0.554–0.689	619
HER2	96.2	94.7–97.7	0.801	0.722–0.879	608
TargetPrint versus local pathology					
ER	97.0	95.6–98.1	0.888	0.844–0.932	800
PgR	85.8	83.2–88.2	0.673	0.618–0.728	798
HER2	97.0	95.6–98.1	0.846	0.784–0.907	772

CI, confidence interval; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor.

ER and PgR were available as integer percentages and for HER2 as five categories. For local IHC, there is only a positive or negative assessment and the TargetPrint results are gene-expression scores on a continuous scale. To improve the visibility of individual observations, random trimmed noise was added to the pathology laboratory assessments.

Figure 1 shows the local assessment of ER, PgR, and HER2 versus the percentage of immunoreactive cells registered by the central laboratory. Figure 2 shows the plots for the TargetPrint results versus central pathology assessment. All the plots illustrate the similarity between the TargetPrint and local laboratory results, as assessed by receptor status determined by central pathology. ER- and PgR-positive cases with a low percentage of immunoreactive cells had the most discordances by both TargetPrint and local assessment.

discussion

Locally and centrally assessed ER, PgR, and HER2 status in the first 800 (626 centrally assessed) MINDACT patient samples indicates a high level of quality for pathology in the local hospitals. Despite the high concordance, there are still concerns about the range of discordance and the false-negative rate of locally assessed ER (13 of 537, 2.4%) and PgR (52 of 490, 10.6%), and the false-positive rate (12 of 69, 17%) for HER2 status. These rates are similar to those observed in other clinical trials (e.g. HERA, ALTTO) and emphasize the need for a continuous effort to harmonize analytical performance and interpretative skills in the assessment of these important biological variables. Importantly, most centers participating in MINDACT had a good-quality pathology department able to comply with the complex study requirements [13]. This may in part explain the substantially high concordance rate between local and central assessments, which is usually lower for less well-performing local laboratories.

TargetPrint assessment of ER and HER2 (and to a lesser extent PgR) status gives results comparable with IHC/FISH and provides an objective and quantitative assessment of tumor-receptor status. Microarray readouts of ER, PgR, and HER2 by TargetPrint were previously shown to be strongly correlated with high-quality IHC/FISH assessment, with concordance rates of 93% ($\kappa = 0.79$) for ER, 83% ($\kappa = 0.65$) for PgR, and 96% for HER2 ($\kappa = 0.88$) [9]. The data indicate that TargetPrint can serve as a reliable alternative to local IHC/FISH.

The positive and negative agreement (>95%) for ER in the current study indicate that TargetPrint is a very stable and reliable assay for this receptor [2]. For the small percentage of discordant samples, a prospective comparison of the two methods will ultimately establish whether mRNA readout for ER is the preferred technique, as suggested by a retrospective analysis [14]. For the majority of the discordant cases, the percentage of immunoreactive cells was quite low, potentially indicating remarkable intratumoral heterogeneity of ER expression. The evaluation of different tumor areas may well be the cause of discordant results in heterogeneous tumors.

PgR concordance was 85%, indicating discordance for ~15% of cases. Compared with ER, the distribution of PgR IHC-positive and discordant cases is somewhat more homogeneously spread across different percentages of immunoreactive cells. Similar to ER, discordance was more likely for low percentages of immunoreactive cells. The concordance for mRNA and IHC assessment of PgR has been shown to be less than that for ER, but mRNA-derived receptor status is more strongly associated with clinical outcome, suggesting that mRNA may be a more reliable method for assessing receptor status [15, 16].

The positive agreement for TargetPrint with IHC/FISH for HER2 is comparable with other mRNA readouts [17, 18], indicating there are differences between the two methods. Intratumor heterogeneity of HER2 status may be one reason for the discordant results, but further research is warranted to determine the suggested appropriateness of mRNA readout for HER2 as alternate approach [19].

In summary, our work has two important implications: (i) the results of the MINDACT trial will not be affected by the fact that risk assessment by clinicopathological factors used local

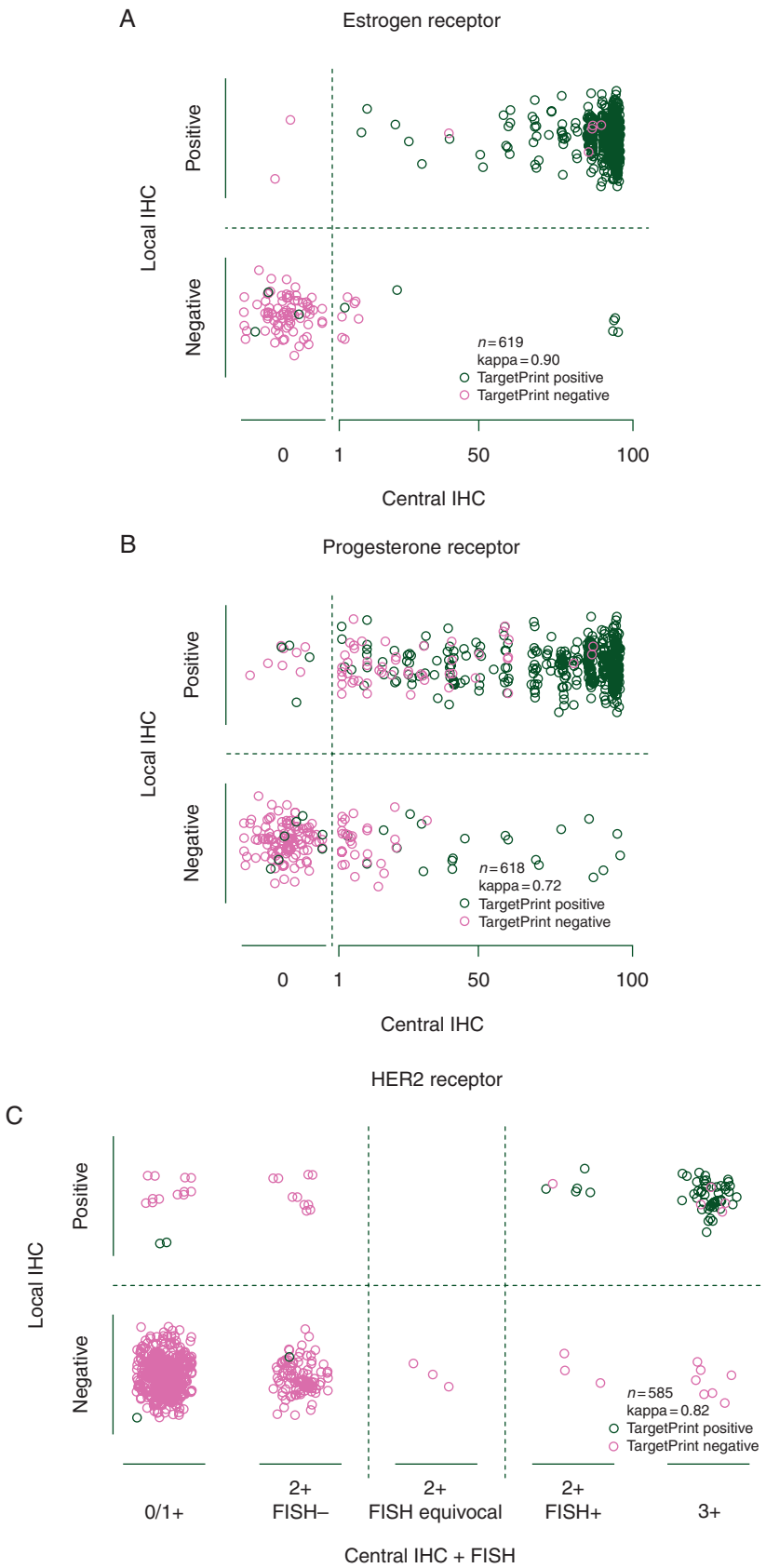

Figure 1. Three-way comparison of local pathology assessment (positive/negative) versus central pathology assessment as integer percentage for ER (A) and PgR (B) and as five categories for HER2 (C). The corresponding TargetPrint assessment is indicated by the color of the dots.

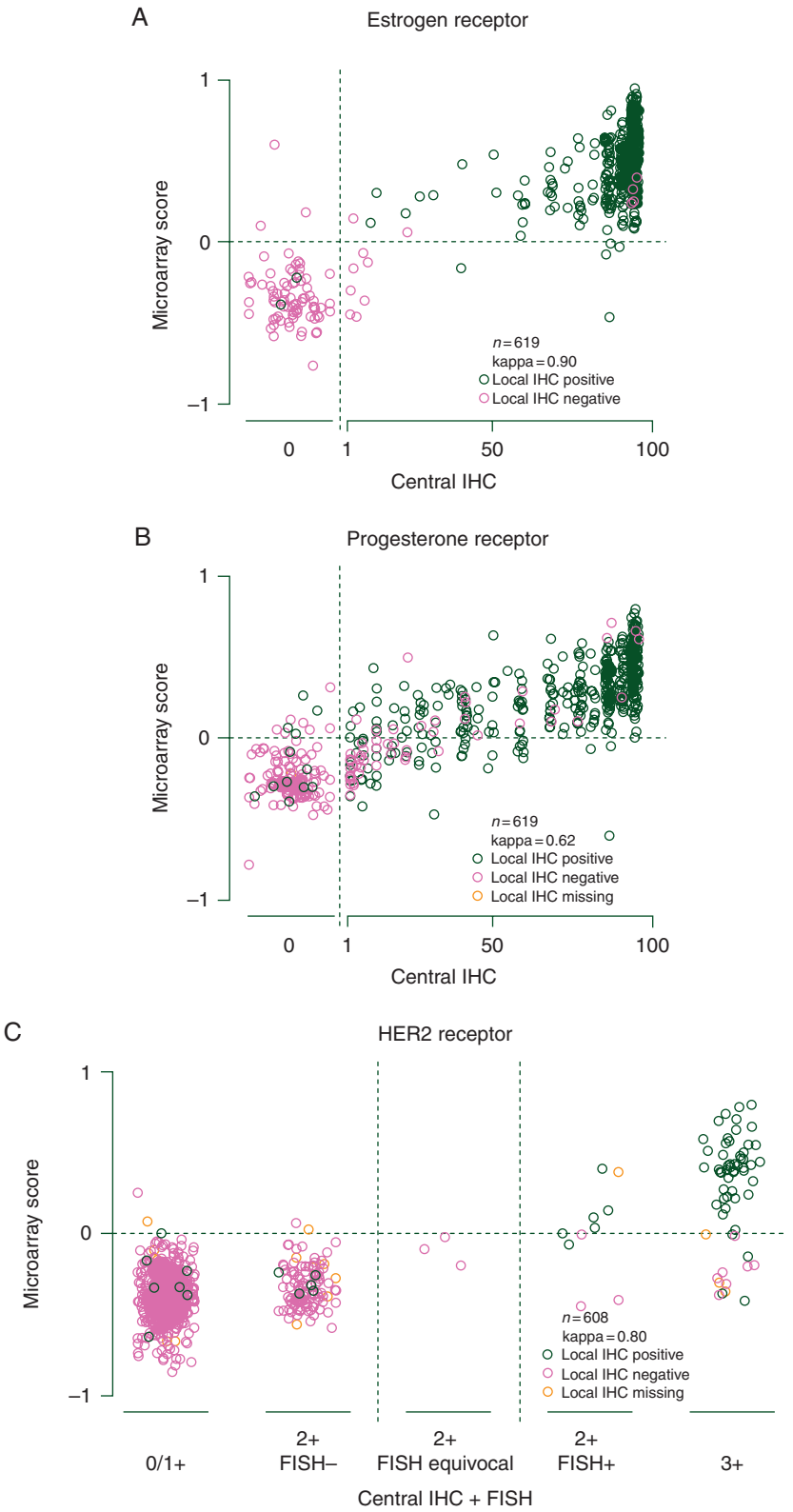

Figure 2. Three-way comparison of gene expression scores on a continuous scale for TargetPrint versus central pathology assessment as integer percentage for ER (A) and PgR (B) and as five categories for HER2 (C). The corresponding local assessment is indicated by the color of the dots.

pathology and risk assessment by MammaPrint® was carried out centrally; (ii) TargetPrint can improve the reliability of hormone receptor and HER2 testing for those centers with a lower concordance rate with the reference laboratory, with the limitation of a positive agreement of 75% for HER2. TargetPrint consequently has important implications for treatment decisions in

routine clinical practice and is a reliable alternative to local assessment for ER.

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disclosure

FdS, LS-S, JvdA, and AG are employees of Agendia. LvV is a founder of Agendia and has stock ownership. All other authors have declared no conflicts of interest.

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