Validation of the prognosis of patients with ER-positive, HER2-negative and node-negative invasive breast cancer classified as low risk by Curebest™ 95GC Breast in a multi-institutional registry study

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Abstract. Curebest™ 95GC breast (95GC) is a multigene classifier we developed for the prognostic prediction of patients with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative and node-negative (ER+/HER2-/n0) invasive breast cancer treated with adjuvant endocrine therapy alone. The aim of the preset study was to evaluate the clinical utility of 95GC in a multiinstitutional registry study. Patients (n=215) with $ER^+/HER2^-$/n0 invasive breast cancer who had undergone the 95GC assay in seven hospitals were consecutively recruited in the registry study at various postoperative times. At recruitment, no patients had disease recurrences and were prospectively followed up for a median of 62 (range, 6-91) postoperative months. Of the 124 patients classified as 95GC low risk, 118 received adjuvant endocrine therapy alone and six received adjuvant chemo-endocrine therapy. Only two patients developed distant recurrences, and the 5-year distant recurrence-free survival (DRFS) was as high as 98.0%. Of the 91 patients classified as 95GC high risk, 81 received adjuvant chemo-endocrine therapy and 10 received adjuvant endocrine therapy alone. A total of four of these patients developed distant recurrences (5-year DRFS=95.5%). Among the 95GC high-risk patients, prognosis was significantly improved for the 81 treated with adjuvant chemo-endocrine therapy compared with for the 77 (historical controls) treated with adjuvant endocrine therapy alone (P=0.0002; hazard ratio, 0.24). Compared with the St. Gallen 2013 guideline, a significant de-escalation from 73.1% (155/212) to 40.6% (86/212) in adjuvant chemotherapy was achieved. The excellent prognosis of patients with $ER^+/HER2^-$/n0 invasive breast cancer classified as 95GC low

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Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; DRFS, distant recurrence-free survival; TAM, Tamoxifen; pCR, pathological complete response; GC, gene classifier; MGC, multigene classifier; FFPE, formalin fixed and paraffin embedded; FF, fresh-frozen; NAC, neo-adjuvant chemotherapy; ODX, Oncotype DX; BGA, between-group analysis

Key words: Curebest 95GC breast, breast cancer, prognosis, registry study

risk could be validated in the present registry study, indicating that 95GC is useful for safe de-escalation of adjuvant chemotherapy in patients with $ER^+/HER2^-$/n0 invasive breast cancer.

Introduction

Breast cancer is the most common cancer in women, and its incidence is increasing in many parts of the world including Japan. There were about 2.2 million new cases of breast cancer, and estimated 685,000 women died from breast cancer in 2020 worldwide (1). A further development of treatments is essential for improving the patient outcome. Since breast cancer is a heterogeneous disease consisting of various subtypes which show a different response to the various treatments and lead to the different clinical outcome, it is important to implement the precision medicine where treatment is individually conducted according to the subtype. Breast cancer subtyping has been done by clinical tumor features and histology with immunohistochemical examination (2). However, in order to improve the accuracy of subtyping so that it will reflect the biological characteristics of tumors (malignancy and response to treatment etc.) more precisely and thus it will be more useful for precision medicine, many multigene profiling assays have been developed which include intrinsic subtyping (3,4) and multigene classifiers (MGCs) for early-stage hormone receptor-positive and human epidermal growth factor receptor 2 (HER2)-negative (HR+/HER2-) breast cancer as mentioned below.

Considering the clinical importance of prognostic prediction, especially in guiding adjuvant systemic therapy, many MGCs have been developed for early-stage HR+/HER2- breast cancer. Among them, Oncotype DX (ODX) has the best evidence that is outstanding. Large-scale prospective studies (5-7) have proven that Oncotype DX is useful in determining the indications for adjuvant chemotherapy in patients with HR+/HER2-/node negative (n0) and HR+/HER2-/nodes 1-3-positive breast cancer and is now widely used in daily practice.

We have also been developing the 'Curebest 95GC breast (95GC)' MGC through an approach different from ODX. The ODX approach includes 16 genes selected from 250 candidate genes on the basis of their prognostic ability (8), but we constructed 95GC by taking advantage of the public datasets with comprehensive gene expression (DNA microarray) data on primary estrogen receptor (ER)+ breast cancer and its prognosis. First, the genes related to recurrence were extracted, and a prognostic prediction model (95GC) was developed using between-group analysis (9). The 95GC model has been confirmed to be useful in the prognostic prediction of ER+/HER2/n0 breast cancer through retrospective studies (10-13). Since the 95GC assay uses an Affymetrix DNA microarray, the comprehensive gene expression data are simultaneously obtained and can be used for determining other MGCs as well as development of a new MGC.

The genes included in 95GC are related to cell proliferation, transcription, and apoptosis (9), and interestingly, there is no overlap of the classifier genes between 95GC and ODX, indicating that a combination of the two MGCs may improve prediction accuracy. In fact, we reported that 95GC could further classify the ODX intermediate risk group into the low-risk and high-risk groups (10,13), suggesting that the combination of ODX and 95GC enables a more detailed prognostic prediction and subsequently a more personalized treatment.

In this paper, to validate the prognostic prediction ability of 95GC, we report the results of a multiinstitutional registry study on the prognosis of patients with (ER+/HER2-/n0) invasive breast cancer who underwent the 95GC assay.

Materials and methods

Patients

Registry study: Every patient (n=215) with ER+/HER2-/n0 invasive breast cancer who had undergone the 95GC assay in seven hospitals between December 2014 and March 2019 were consecutively recruited in this registry study conducted by the Japanese Association for Theranostics at various postoperative times. These patients were treated with breast-conserving surgery followed by radiation therapy or mastectomy. As an adjuvant systemic therapy, endocrine therapy alone or chemo-endocrine therapy was administered based on the physician's discretion and patient's preference for treatment. No patients had disease recurrence at the time of recruitment, and thereafter the patients were prospectively followed up with a median of 62 (range, 6-91) postoperative months (Fig. 1).

Historical control: The historical control group included the 77 patients with ER+/HER2-/n0 invasive breast cancer all of which were classified into the high-risk group by 95GC. The controls were treated with breast-conserving surgery followed by radiation therapy or mastectomy and with adjuvant endocrine therapy alone in Osaka University Hospital from 1995 to 2017 with a median follow-up period of 87 (range, 12-190) months from the surgery. This historical control group is composed of the same patients as previously reported (11). The registry study has been approved by the Ethics Committees of All Participating Hospitals, and the historical control study has been approved by the Ethics Committee of Osaka University Hospital.

95GC assay. A tumor sample (4 mm in diameter x10 mm in depth) was taken from the primary tumor using a biopsy punch, after surgical resection, stored in RNAlater® solution (4˚C), and sent to the Sysmex company. Hematoxylin and eosin section was created from both sides of the sample to confirm the presence of cancer cells (tumor cellularity ≥10%). Next, all gene expressions underwent microarray analysis (Affymetrix U133 plus 2.0; Thermo Fisher Scientific, Waltham, MA). The actual method of the assay, the high/low-risk determination method using a 95GC-dedicated algorithm, and the calculation method of the 95GC recurrence score are the same as previously reported (9,13,14). In some cases in the registry study and all of the historical controls, 95GC was assayed using frozen (-80˚C) tumor samples (11).

Histological examination. ER, progesterone receptor (PR), and Ki67 were assessed by immunohistochemistry and HER2 was assessed by fluorescence in situ hybridization and/or immunohistochemistry in local hospitals/laboratories. The

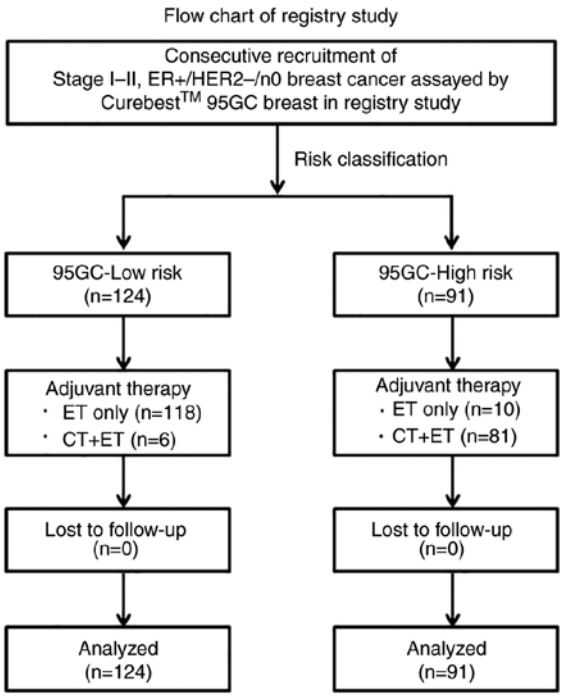

Figure 1. Flow chart of registry study. Every patient with ER$^+$/HER2$^-$/n0 invasive breast cancer who had undergone the 95GC assay in seven hospitals was consecutively recruited in the present registry study. 95GC, Curebest 95GC Breast™; CT, chemotherapy; ET, endocrine therapy; ER, estrogen receptor; n0, pathologically lymph node negative.

cutoff values were 10% for both ER and PR. The ASCO/CAP 2013 guideline was used to determine HER2.

Statistical analysis. All statistical analyses were performed using R statistical software (version 3.5.1; http://www.r-project.org/). Fisher's exact test was used to compare 2x2 groups. All statistical analyses were two-sided, and $P<0.05$ was considered to be indicative of statistical significance. Distant recurrence-free survival (DRFS) was defined as the time from surgery to distant recurrence or death from any cause, whichever occurred first. DRFS was calculated by the Kaplan-Meier method.

Results

Clinicopathological characteristics of patients with breast cancer recruited in this study according to 95GC category. In total, 215 patients with ER+/HER2-/n0 invasive breast cancer were recruited in this registry study, and 124 were classified into the 95GC low-risk group and 91 into the 95GC high-risk group (Table I). The high-risk group was significantly correlated with high Ki67 ($P<0.001$) and high histological grade ($P<0.001$) and showed a tendency ($P=0.077$) toward larger tumor size.

Effect of 95GC on choice of adjuvant therapy. The adjuvant therapy recommended by the St. Gallen 2013 guideline was compared with the therapy actually given to the patients for the purpose of evaluating the effect of 95GC on the choice of adjuvant therapy (Table II). The guideline recommended adjuvant chemo-endocrine therapy for the 155 patients, of whom 80 patients were in the 95GC high-risk group and 75 were in the 95GC low-risk group. Seventy-five (93.8%) patients in the 95GC high-risk group and four (5.3%) in the 95GC low-risk group were treated with adjuvant chemo-endocrine therapy (Table II). On the other hand, adjuvant endocrine therapy alone was recommended for the 57 patients by the guideline, of whom 10 were in the 95GC high-risk group and 47 were in the 95GC low-risk group. Five (50%) in the 95GC high-risk group and two (4.3%) in the 95GC low-risk group were treated with adjuvant chemo-endocrine therapy. All of the other patients were treated with adjuvant endocrine therapy alone. According to the St. Gallen 2013 guideline, 155 (73.1%) patients were recommended to receive adjuvant chemo-endocrine therapy; however, 86 (40.6%) patients were

Table I. Clinicopathological characteristics of patients recruited in the registry study.

Characteristic	Total	95GC		P-value
		Low	High	
No. of patients	215	124	91	
Age				0.894
≤50	98	57	41	
>50	117	67	50	
Menopausal status				0.786
Premenopausal	111	65	46	
Postmenopausal	104	59	45	
Tumor size				0.077
T1	139	88	51	
T2	74	35	39	
T3	2	1	1	
Histological type[a]				0.502
Invasive ductal	186	108	78	
Special type	28	16	12	
Unknown	1	0	1	
Histological grade				0.001
1	74	59	15	
2	96	54	42	
3	44	11	33	
Estrogen receptor				NA
Positive	215	124	91	
Negative	0	0	0	
Progesterone receptor				0.467
Positive	195	114	81	
Negative	19	9	10	
Unknown	1	1	0	
Ki67 index				0.001
<20%	90	69	21	
≥20%	122	53	69	
Unknown	3	2	1	

[a]Histological classification of breast tumors by the General Rule Committee of the Japanese Breast Cancer Society was referenced (18).

Table II. Effect of 95GC on the choice of adjuvant chemo-endocrine therapy.

	Adjuvant therapy recommended by the St. Gallen Guideline 2013[a]		
95GC risk category	CT + ET	ET	Total
High risk	93.8%[b] (75/80)[c]	50.0% (5/10)	88.9% (80/90)
Low risk	5.3% (4/75)	4.3% (2/47)	4.9% (6/122)
Total	51.0% (79/155)	12.3% (7/57)	40.6% (86/212)

[a]Recommendation of adjuvant systemic therapy was decided according to the St. Gallen Guideline 2013 (2). Three patients lacking Ki67 data were excluded from this analysis. [b]% of patients actually treated with adjuvant chemo-endocrine therapy. [c]Number of patients actually treated with adjuvant chemo-endocrine therapy/total number of patients in each subgroup. CT, adjuvant chemotherapy; ET, adjuvant endocrine therapy.

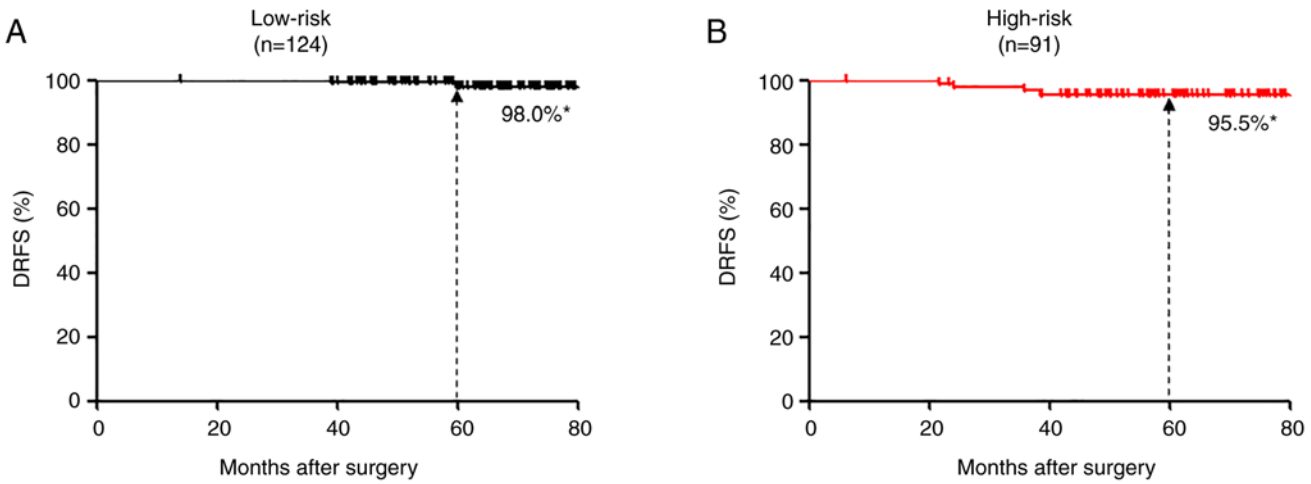

Figure 2. DRFS of patients with breast cancer in the registry study according to the 95GC risk category. (A) 95GC low-risk group (n=124). (B) 95GC high-risk group (n=91). DRFS, distant recurrence-free survival.

treated with adjuvant chemo-endocrine therapy because of the implementation of 95GC.

Prognosis according to 95GC category. The median post-operative follow-up period was 62 (range: 6-91) months, and 123 patients were followed for >5 years. Of the 124 patients in the 95GC low-risk group, 118 received adjuvant endocrine therapy alone and only 6 received adjuvant chemo-endocrine therapy. Only two patients who received adjuvant endocrine therapy alone developed distant recurrences in this group, and the 5-year DRFS was as high as 98.0% (Fig. 2). Of the 91 patients in the 95GC high-risk group, 81 received adjuvant chemo-endocrine therapy and 10 received adjuvant endocrine therapy alone. Four patients developed distant recurrences in this group, resulting in the 5-year DRFS of 95.5% (Fig. 2). The regimens for adjuvant therapy are summarized in Table SI according to the 95GC risk group.

Among all patients classified as 95GC high risk, to estimate the therapeutic benefit of adjuvant chemotherapy, we compared the prognosis of the 81 patients treated with adjuvant chemo-endocrine therapy with that of the 77 patients treated with adjuvant endocrine therapy alone [historical control group (11)]. The patients in these two groups were found to have similar backgrounds (Table III), and the regiments for adjuvant endocrine therapy were also similar between the two groups (Table SII). Prognosis was significantly better for the patients treated with adjuvant chemo-endocrine therapy than for those treated with adjuvant endocrine therapy alone (P=0.0002, HR 0.24) (Fig. 3).

Discussion

This is the first multiinstitutional registry study in which the patients were prospectively followed up to investigate the effect of 95GC on the prognosis of patients with ER+/HER2-/n0 breast cancer. Only two of 124 patients at 95GC low-risk had distant recurrences, and their 5-year DRFS was as high as 98.0%. This result is consistent with the previous retrospective studies (10-13), suggesting that 95GC is useful in selecting patients who show an excellent prognosis with adjuvant endocrine therapy alone and thus can forgo adjuvant chemotherapy. In addition, when the therapeutic effect of adjuvant chemotherapy was evaluated in the patients in the 95GC high-risk group, their prognosis was significantly improved by adding adjuvant chemotherapy relative to that of the historical controls treated with adjuvant endocrine therapy alone (Fig. 3). This result is also consistent with our previous observation that 95GC high-risk tumors were more sensitive to neoadjuvant chemotherapy than 95GC low-risk tumors (15).

Table III. Clinicopathological characteristics of patients with invasive breast cancer at 95GC high risk and treated with adjuvant chemo-endocrine therapy or endocrine therapy alone.

Characteristic	Adjuvant therapy		P-value
	Endocrine[a]	Chemo-endocrine	
No. patients	77	81	
Menopausal status			0.867
Premenopausal	37	40	
Postmenopausal	40	41	
Tumor size			0.721
T1	44	44	
T2	33	37	
Histological classification[b]			0.132
Invasive ductal	74	72	
Special type	3	9	
Histological grade			0.163
Grade 1	16	10	
Grade 2 + 3	61	70	
unknown	0	1	
Estrogen receptor			NA
Positive	77	81	
Negative	0	0	
Progesterone receptor			0.685
Positive	61	62	
Negative	16	19	
Human epidermal growth factor receptor 2			NA
Positive	0	0	
Negative	77	81	

[a]Historical control group. [b]The histological classification of breast tumors by the General Rule Committee of the Japanese Breast Cancer Society was referenced (18).

One important requirement for MGC is that it has a significant effect on the de-escalation of adjuvant chemotherapy in patients with ER+/HER2-/n0 breast cancer. Therefore, we evaluated MGC by comparing the frequency of adjuvant chemo-endocrine therapy actually given to the patients with that recommended by the St. Gallen 2013 guideline (2). According to the guideline, 155 (73.1%) patients were recommended to receive adjuvant chemo-endocrine therapy. Actually; however, 86 (40.6%) patients were treated with adjuvant chemo-endocrine therapy because of the implementation of 95GC (Table II), indicating a significant de-escalation in adjuvant chemotherapy from 73.1% to 40.6%. The excellent prognosis of the 95GC low-risk group (n=124), even though it included the 71 (75-4) patients who were recommended to receive adjuvant chemo-endocrine therapy by the guideline but actually were treated with adjuvant endocrine therapy alone, suggests that 95GC is useful in a safe de-escalation of adjuvant chemotherapy.

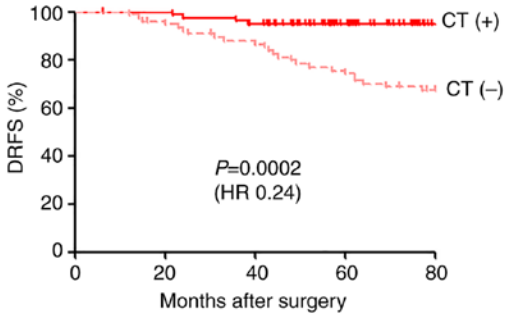

Figure 3. Comparison of the prognoses of patients with breast cancer at 95GC high-risk treated with or without adjuvant chemotherapy. The prognosis of the patients with breast cancer at 95GC high risk and treated with adjuvant chemo-endocrine therapy (n=81) in the present registry study was compared with that of those at 95GC high risk and treated with adjuvant endocrine therapy alone in the historical control group (n=77). CT(+), with chemotherapy; CT (-), without chemotherapy; HR, hazard ratio; DRFS, distant recurrence-free survival.

The TAILORx trial showed no benefit of adding adjuvant chemotherapy to endocrine therapy for HR+/HER2-/n0 breast cancer with ODX recurrence score (RS) of 11-25, but the exploratory analyses indicated that adjuvant chemotherapy was associated with some benefit for women ≤50 years old who had an RS of 16-25 (5,6). In addition, the recent RxPonder trial in patients with HR+/HER2-/n1-3 breast cancer and an ODX RS

≤ 25 showed a significant benefit of adjuvant chemotherapy in premenopausal but not postmenopausal patients (7). Recently, we showed that patients with breast cancer and an ODX RS of 11-25 could be classified into low-risk and high-risk groups by 95GC (10). This further classification by 95GC might be useful since the low-risk group could be treated with adjuvant endocrine therapy alone and the high-risk group could be treated with adjuvant chemo-endocrine therapy or adjuvant endocrine therapy with ovarian suppression. Thus, 95GC can potentially provide a more individualized treatment for patients with breast cancer and an ODX RS ≤ 25 (11).

Since the 95GC assay is performed using microarray, the expression data of all genes are available for each tumor. One advantage of 95GC is that by utilizing such data, it is possible to simultaneously analyze multiple MGCs including those developed for prediction of chemosensitivity (anthracycline/taxane) such as 23GC (16) and 155GC (17). Additional information on chemosensitivity might be helpful in the selection of adjuvant therapeutic regimens. Besides, we are conducting a registry study to collect not only clinical information but also gene expression data (DNA microarray CEL files) from patients subjected to the 95GC assay. We believe that this registry will facilitate not only further validation of 95GC but also the development of a new MGC through the ecosystem proposed in Fig. S1.

One limitation of this study is that it is a retrospective study including a relatively small number of patients who underwent the 95GC assay, which might have introduced survival bias. However, to minimize this bias, we consecutively recruited every patient with ER+/HER2-/n0 breast cancer who underwent the 95GC assay from each institution. Another limitation is that this was an observational study in which the decision on adjuvant therapy was at the physician's discretion and patient's preference for treatment and not according to a protocol. However, the fact that a percentage of the patients who were at 95GC low risk and treated with adjuvant chemo-endocrine therapy accounted for only 4.8% is unlikely to compromise our hypothesis that patients at 95GC low risk will have an excellent prognosis when treated with adjuvant endocrine therapy alone.

In conclusion, the excellent prognosis of patients with ER+/HER2-/n0 invasive breast cancer classified as 95GC low risk could be validated in this registry study, indicating that such patients can forgo adjuvant chemotherapy. However, to establish the clinical utility of 95GC, it would be necessary to conduct a prospective study in a large number of patients with long-term follow-up.

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Availability of data and materials

The raw data analyzed for the current study is not publicly available due to the study protocol, but might be available from the corresponding author on reasonable request under the permission of the Japanese Association for Theranostics.

Authors' contributions

YN conceived and designed the study, acquired and analyzed the data and wrote and edited the original manuscript. RT, KS, MO, SI, HK, YK, KI and MS acquired and analyzed the data and edited the original manuscript. TO, TK, AS, SNa and HT conceived and designed the study and edited the original manuscript. SNo conceived and designed the study, acquired and analyzed the data and wrote and edited the original manuscript. YN and SNo confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The registry study has been approved by the ethics committees of all participating hospitals, and the historical control study has been approved by the Ethics Committee of Osaka University Hospital (approval nos. 19510-3 and 14106-10).

Patient consent for publication

Because this study is a retrospective registry (observational) study carried out by the opt-out method, informed consent was not obtained.

Competing interests

Yasuto Naoi has received research funding from Sysmex and AstraZeneca; he has received honoraria from AstraZeneca, Pfizer, Eli Lilly, and Chugai outside the submitted work; and he holds joint patents with Sysmex including Curebest™ 95GC Breast (JP.5725274.B2) and has received patent royalties outside the submitted work. Kenzo Shimazu has received honoraria from Sysmex and research funding to institution from Sysmex. Tomo Osako received honoraria from Diaceutics and Daiichi Sankyo, and consulting fee from Chiba Cytopathology Laboratory outside the submitted work. Seigo Nakamura/Showa University received a research grant from Sysmex Corporation. Hitoshi Tsuda received research grant from Roche Diagnostics, Goryo Chemical and Scholarship donation from Chugai Pharmaceutical. Kazuhiro Ishihara received lecture fees from Nippon Kayaku, Kyowa Kirin, Daiichi Sankyo and Eisai. Shinzaburo Noguchi has received consulting fees and research funding from Sysmex; he has received consulting fees from AstraZeneca and Nittobo outside the submitted work; he has received honoraria from AstraZeneca, Pfizer, Eli Lilly, and Chugai outside the submitted work; and he holds joint patents with Sysmex including Curebest™ 95GC Breast (JP.5725274.B2) and has received patent royalties outside the submitted work.

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