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REVIEW ARTICLE

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The multigene classifiers 95GC/42GC/155GC for precision medicine in ER-positive HER2-negative early breast cancer

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Abstract

In clinical decision-making, to decide the indication for adjuvant chemotherapy for estrogen receptor-positive (ER+), human epidermal growth factor receptor-2-negative (HER2−), and node-negative (n0) breast cancer patients, the accurate estimation of recurrence risk is essential. Unfortunately, conventional prognostic factors, such as tumor size, histological grade and ER, progesterone receptor (PR), and HER2 status as well as Ki67 index, are not sufficiently accurate for such estimation. Therefore, several multigene assays (MGAs) based on the mRNA expression analysis of multiple genes in tumor tissue have been developed to better predict patient prognosis. These assays include Oncotype DX, MammaPrint, PAM50, GGI, EndoPredict, and BCI. We developed Curebest™ 95-Gene Classifier Breast (95GC) classifier, which is unique in that mRNA expression data of all 20 000 human genes are secondarily obtainable, as the 95GC assay is performed using Affymetrix microarray. This can capture mRNA expression of not only 95 genes but also every gene at once, and such gene expression data can be utilized by the other MGAs that we have developed, such as the 155GC, which is used for the prognostic prediction of ER+/HER2− breast cancer patients treated with neoadjuvant chemotherapy. We also developed the 42GC for predicting late recurrence in ER+/HER2− breast cancer patients. In this mini-review, our recent attempt at the development of various MGAs, which is expected to facilitate the implementation of precision medicine in ER+/HER2− breast cancer patients, is presented with a special emphasis on 95GC.

KEYWORDS

breast cancer, DNA microarray, ER-positive, prognostic prediction

1 | INTRODUCTION

Estrogen receptor-positive (ER+), epidermal growth factor 2-negative (HER2−), and node-negative (n0) breast cancers account for approximately half of invasive breast cancer in Japanese women.[1] As the report that individuals with invasive breast cancer measuring more than 1 cm derive a significant benefit from adjuvant chemotherapy regardless of nodal and ER status was published in 2000

Abbreviations: CS, chemosensitivity; DRFS, distant recurrence-free survival; ER, estrogen receptor; FF, fresh-frozen; FFPE, formalin-fixed and paraffin-embedded; GC, gene classifier; HER2, human epidermal growth factor receptor 2; MGA, multigene assay; NAC, neoadjuvant chemotherapy; pCR, pathological complete response; PR, progesterone receptor; TAM, tamoxifen.

(NIH), most patients with ER+/HER2−/n0 breast cancer have been treated with adjuvant chemotherapy.[2] However, ER+/HER2−/n0 breast cancer has a less aggressive phenotype compared with other subtypes, and approximately 85% of patients with this type of breast cancer may not experience recurrence even if they are treated with adjuvant hormone therapy alone.[3] This indicates that a high proportion of ER+/HER2−/n0 breast cancer patients are overtreated with unnecessary adjuvant chemotherapy. Therefore, to avoid overtreatment with chemotherapy, many studies have focused on the development of a prognostic indicator that can select patients who are at a very low risk of recurrence and not require adjuvant chemotherapy.

Although conventional clinicopathological parameters, including ER, PR, and HER2 status as well as the Ki67 index, are clinically useful for predicting prognosis, their accuracy and reproducibility are not sufficient to reliably select patients who do not require adjuvant chemotherapy. Therefore, prognostic indicators based on multigene assays (MGA), which measure the mRNA expression of multiple genes in tumor tissue, have been developed and implemented in clinical practice for ER+/HER−/n0 breast cancer patients. Although several MGAs, including Oncotype DX, MammaPrint, and PAM50, have been developed, the most extensively investigated MGA is Oncotype DX, which is a RT-PCR-based mRNA expression analysis of 21 genes.[3] Recently, the TAILORx trial prospectively showed that Oncotype DX can select patients with ER+/HER2−/n0 breast cancer who, when treated with adjuvant hormone therapy, are at a very low risk for recurrence and therefore do not need adjuvant chemotherapy.[4]

Since 2007, we have also studied the ability of MGAs to predict recurrence in patients with ER+/HER2−/n0 breast cancer. Rather than using RT-PCR for selected genes, such as in Oncotype DX, we adopted the Affymetrix DNA microarray for analysis of mRNA expression as this assay can simultaneously measure the mRNA expression of the whole genome in tumor tissue. The expectation is that such a comprehensive whole-gene expression analysis would be useful for the construction of recurrence prediction models not only for ER+/HER2−/n0 breast cancer patients treated with adjuvant hormone therapy alone, but also for those with other types of breast cancer and for the construction of models that predict chemotherapeutic response. Moreover, the availability of public gene expression datasets obtained by Affymetrix microarrays in a large number of patients with long term follow-up provides an excellent opportunity for constructing a prognostic model based on gene expression.

In this mini-review, we describe our accomplishments since 2007 in the development of MGAs useful for the prediction of recurrence in ER+/HER2−/n0 breast cancer patients treated with adjuvant hormone therapy alone (95-gene classifier [GC]) as well as in ER+/HER2− breast cancer patients treated with neoadjuvant chemotherapy (NAC; 155GC). We have also developed MGAs for predicting late recurrence in ER+/HER2− breast cancer patients (42GC). The outline of these MGAs is summarized in Table S1.

2 | Curebest™ 95GC (95GC) FOR PROGNOSTIC PREDICTION IN ER+/HER2−/n0 BREAST CANCER

2.1 | Development of the 95GC

The 95GC assay was developed using public datasets (GSE2034, GSE2990, GSE4922, GSE6532, GSE7390, GSE9195) that included 549 ER+/n0 breast cancer patients who were treated with adjuvant hormone therapy alone or no adjuvant therapy. One marked feature is that 95 gene markers were selected from comprehensive genetic information downloaded from a public database to create a practical test. The advantage of this method is that many cases can be examined at a low cost, but the disadvantage is that it requires an advanced mathematical method. The differentially expressed genes between breast tumors with and without recurrence were selected, and the prognostic prediction model was constructed in accordance with a between-group analysis. Then, the model was optimized by cross-validation, and finally, the 95GC test based on the expression profile of the 95 genes was constructed.[5] The results of the 95GC assay in the validation cohort consisting of the 257 ER+/HER2−/n0

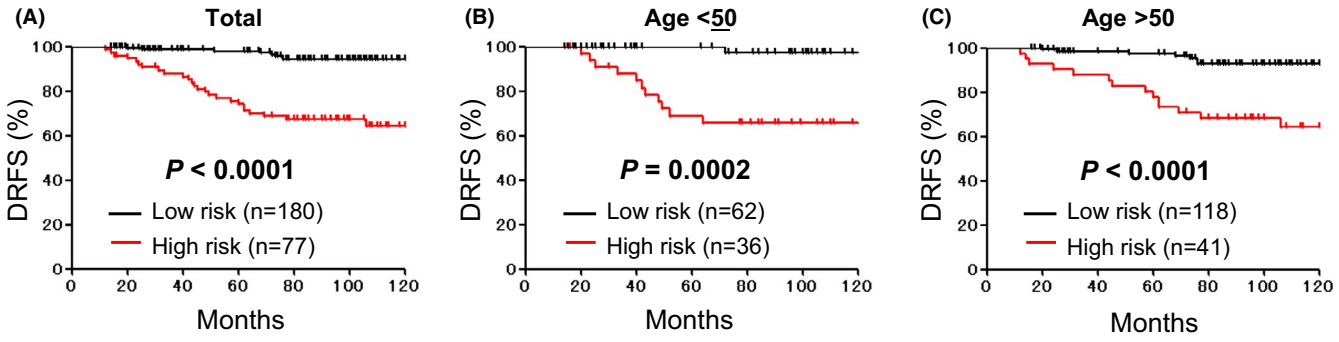

FIGURE 1 Recurrence-free survival curves for risk groups classified by 95GC. Recurrence-free survival curves of 257 breast cancer patients (ER-positive, HER2-negative, and node-negative stage I and stage II breast cancer patients treated with adjuvant hormone therapy alone in our hospital) who were classified into 95GC low-risk and high-risk groups as an independent validation set (A). The patients were divided into 2 subgroups based on age (y), ie, ≤50 (B) and >50 (C). Notably, patients in the high-risk group were more likely to be in the ≤50 group (B) than in the >50 group (C). The 257 patients analyzed in this figure included 105 patients in Figure 1 from reference.[5] DRFS, distant recurrence-free survival

Japanese breast cancer patients who were treated with adjuvant hormone therapy alone are shown in Figure 1A. The low-risk group showed an excellent 10-y DRFS (94.1%), which was highly significantly (P <.0001) better than that of the high-risk group. Multivariate analysis showed that the 95GC is a highly significant (P <.0001) and independent prognostic factor (Table S2). Importantly, as many as 70% of the patients could be classified into the low-risk group and could forgo adjuvant chemotherapy (Figure 1A).

Biological difference of ER+/HER2− breast tumors between premenopausal and postmenopausal women has been suggested, ie, luminal B tumors, which are biologically more aggressive compared with luminal A tumors, are reported to be more frequent in premenopausal compared with postmenopausal patients.[6] Then, we carried out a subgroup analysis in accordance with the age (≤50 or >50) to clarify the prognostic significance of 95GC in accordance with the menopausal status (Figure 1B,C). In both subgroups, 95GC could separate patients into low-risk and high-risk groups with statistical significance, and notably, a proportion of the patients in the high-risk group were more likely to be in the ≤50 subgroup than in the >50 subgroup (P =.062). These results seem to indicate that adjuvant chemotherapy can be safely omitted, irrespective of the menopausal status, from the ER+/HER2−/n0 breast cancer patients who are at a low-risk by 95GC. Typical adjuvant hormone therapy for these low-risk patients would be aromatase inhibitor for postmenopausal women and tamoxifen ± LH-RH agonist for premenopausal women.

Recently, we developed the 95GC recurrence risk score (^{95GC}RS).[7] The ^{95GC}RS, which ranges from 0 to 100, correlated well with distant recurrence (Figure 2A). Breast cancer patients with a ^{95GC}RS ≤50 had a significantly low recurrence rate, whereas those with a ^{95GC}RS >50 had a high recurrence rate. In addition, the recurrence rate increased in proportion to the ^{95GC}RS. Compared with binary results (high-risk vs. low-risk groups), information on recurrence risk using the ^{95GC}RS for individual patients could enable better decision-making in a clinical setting with regard to adjuvant chemotherapy. We also demonstrated a gradual increase in pathological complete response (pCR) after NAC in proportion to the ^{95GC}RS, which indicates greater chemosensitivity in breast cancers with a high ^{95GC}RS (Figure 2B). Altogether, these results suggest the marked ability of the 95GC to categorize patients at a high risk of relapse who would likely benefit from adjuvant chemotherapy. In this study, it was also demonstrated that the 95GC is applicable to FFPE tissues.[7] A significantly high correlation coefficient (R = .92) for the ^{95GC}RS was demonstrated between FF (fresh-frozen) and FFPE tissues, as was a markedly high concordance rate (94.6%) between the high-risk and low-risk groups, which demonstrates the potential use of the 95GC for FFPE tissues.

2.2 | Combination of the 95GC and the 21GC

TAILORx is therefore far the largest prospective study that has clearly shown that Oncotype DX can select low-risk ER+/HER2−/n0 patients that may forgo adjuvant chemotherapy.[4] Although Oncotype DX is determined by the RT-PCR of 21 genes, Balázs Győrffy et al have shown that this assay can be reproduced by 21GC using the Affymetrix microarray data through a website (http://www.recurrenceonline.com/). Therefore, using this website, patients in the independent validation set (Japanese cohort and European and American public datasets (GSE17705, GSE12093, GSE26971), n = 679) were classified by the 21GC into 2 groups, ie, the low/intermediate-risk (recurrence score [RS] = 0-25) and the high-risk group (RS > 25) (Figure 3A) in accordance with the recently proposed cut-off value.[4] Patients in the low-risk/intermediate-risk group showed a significantly (P <.0001) better prognosis compared with those in the high-risk group (Figure 3A) and, interestingly, patients in each group could be further classified into low-risk and high-risk groups by the 95GC (Figure 3B). In addition, we reported that 95GC might be useful for a more accurate prediction of prognosis in the 21GC intermediate-risk group (RS = 18-30)[8] and Fujii T. et al have recently reported the similar observation in the 21GC intermediate-risk group (11-25).[9] These findings indicate the possibility that the combination of 95GC and 21GC would be beneficial for a more accurate selection of patients with an excellent prognosis who can forgo adjuvant chemotherapy, especially in the 21GC intermediate-risk group.

2.3 | The 95GC and chemosensitivity

Breast tumors classified into the high-risk group by Oncotype DX[10] and Mammaprint[11] have been demonstrated to be more chemosensitive

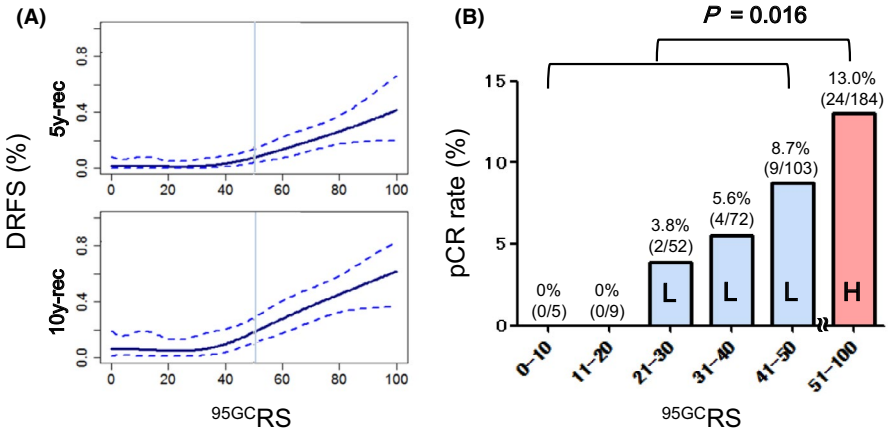

FIGURE 2 95GC recurrence risk score. The 95GC score for the prediction of recurrence risk (A) and response to NAC (neoadjuvant chemotherapy) (B). Recurrence risk is also associated with chemosensitivity. ^{95GC}RS, 95GC recurrence score; DRFS, distant recurrence-free survival; H, high risk by 95GC; L, low risk by 95GC; pCR, pathological complete response. Adapted with permission from Figures 1 and 2 in reference ([7]). Copyright 2019 Spandidos

compared with those classified into the low-risk group. Therefore, the correlation of the recurrence risk with chemosensitivity by the 95GC in ER + breast cancer was also evaluated in patients treated with NAC (Figure 4A). The patients in the high-risk group showed a significantly higher pCR rate than those in the low-risk group, which is consistent with previous reports on Oncotype DX[10] and MammaPrint.[11] These results suggest that breast tumors that are classified into the high-risk group by these MGAs are commonly characterized as highly proliferating tumors more sensitive to chemotherapy.

Figure 4B shows the prognosis of the ER+, HER2−, and node-positive breast cancer patients treated with adjuvant hormone therapy alone (without chemotherapy). The patients could be separated into the low-risk and high-risk groups by the 95GC, and the prognosis of each group was significantly different (P =.0005). Interestingly, the prognosis of patients in the high-risk group improved, but patients in the low-risk and high-risk groups were not significantly different (Figure 4C), when they were treated with NAC and adjuvant hormone therapy, which indicates that patients in the high-risk group were more sensitive to chemotherapy (Figure 4C). Therefore, the treatment of patients considered to be high-risk in accordance with the MGAs with adjuvant chemotherapy is thought to be reasonable from the viewpoint of chemosensitivity.

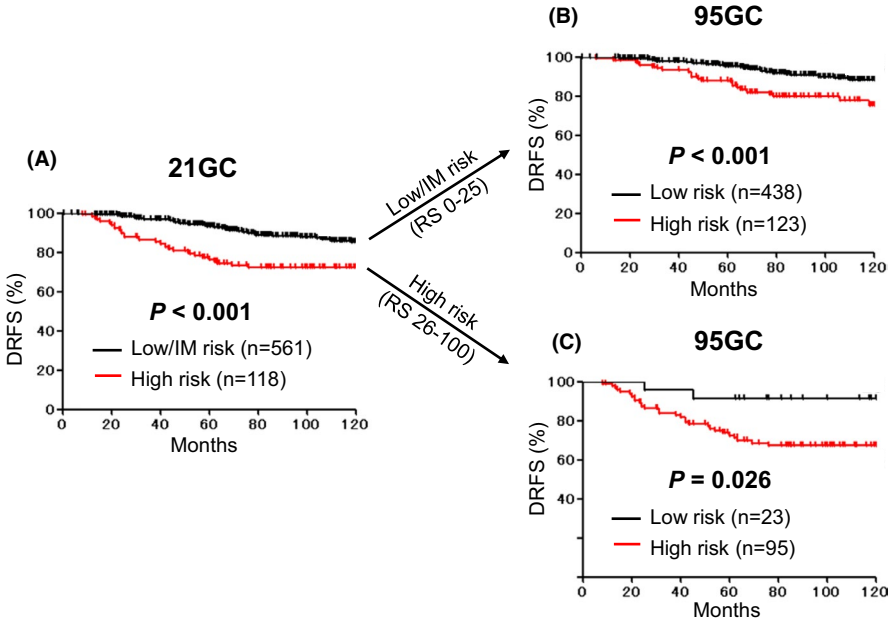

FIGURE 3 Combination of 95GC and 21GC. The independent validation cohort (n = 679) was classified by 21GC into the low-risk/intermediate-risk (RS = 0-25) and high-risk groups (RS = 26-100) (A). The low-risk/intermediate-risk and high-risk groups could be further classified into the low-risk and high-risk groups by 95GC (B, C). The 679 cases include the 459 cases from the original paper ([8]) plus cases in the GSE26971 dataset and our cases that have been uploaded since that time. DRFS, distant recurrence-free survival

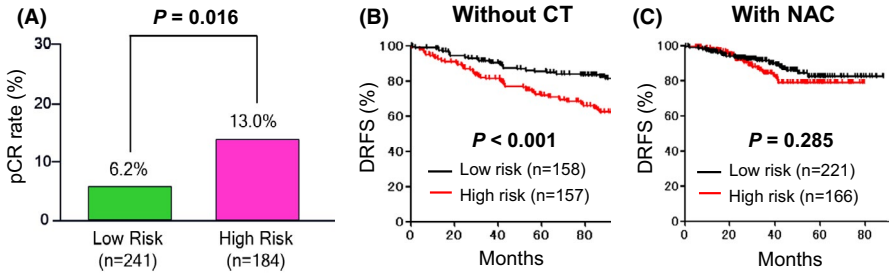

FIGURE 4 Effect of chemotherapy on the 95GC high-risk group. A, A significant difference was observed in the pCR rate between the high-risk/low-risk groups in accordance with 95GC. This analysis was based on 425 patients in our series (Figure 2 in reference [19]) and the GSE25066 dataset. B, A significant difference was observed in the prognosis between the high-risk (red) and low-risk (black) risk groups of patients treated with adjuvant hormone therapy but not chemotherapy. This analysis was based on 315 patients from the GSE6532, GSE9195, GSE17705, GSE26971, and GSE4922 datasets. C, The prognosis of the high-risk group (red) was similar to that of the low-risk group (black), which suggests the improvement in prognosis of the high-risk group due to neoadjuvant chemotherapy (NAC). This analysis was based on 387 patients in our series (Figure 2 in reference [19]) and in the GSE25066 dataset. CT, chemotherapy; DRFS, distant recurrence-free survival; NAC, neoadjuvant chemotherapy; pCR, pathological complete response

3 | THE 42GC FOR THE PREDICTION OF LATE RECURRENCE OF ER+/HER2− BREAST CANCER

Significant numbers of recurrences occur after 5 y (late recurrence) in ER+/HER2− breast cancer patients and that extended hormone therapy is a well-accepted option to prevent recurrence.[12,13] However, the rate of late recurrence is not so high to the point when treating all ER+/HER2− patients with extended hormone therapy would be overtreatment. In practice, tumor size (T) and nodal status (N) of the primary tumors are usually used to estimate the late recurrence risk, and the indication for extended hormone therapy is decided depending on the risk.[14-16] However, such estimation is inaccurate and necessitates the development of a more accurate predictor of late recurrence. Generally, the T and N factors reflect the amount of residual tumor after surgery. Therefore, the T and N factors are markers for both early and late relapse. Instead, we sought to determine a more accurate marker specific for late relapse.

It has been reported that patients at high risk for early recurrence are at low risk for late recurrence and that those at high risk for late recurrence are at low risk for early recurrence,[17] which suggests a biological difference between tumors at high risk for early recurrence and those at high risk for late recurrence. Considering these biological differences between early-recurring and late-recurring tumors, we attempted to develop an MGA for late recurrence.[18] First, we selected genes that were differentially expressed between breast tumor samples that recurred early (less than 4 y after surgery) and those that recurred late (after 4 y) in the training set. The MGA was then optimized using these genes, which resulted in the 42GC, which could separate the patients into the low-risk and high-risk groups in accordance with late recurrence. The 42GC was also evaluated in the validation cohort (Figure 5), and it was shown that patients in the non-late recurrence-like group had a significantly better prognosis compared with those in the late recurrence-like group after 5 y. Interestingly, the former group showed a significantly poorer prognosis within 5 y than the latter group (after 5 y in Figure 5, the prognosis curve was reconstructed by collecting only the relapse-free cases 5 y after surgery).

It is hypothesized that early-recurrent tumors are characterized by a high proliferation rate of residual (disseminated) tumor cells and that late-recurrent tumors are by a long time dormancy of such cells. Therefore, we think that the 42 genes, which were selected from the differentially expressed genes between early- and late-recurrent tumors, reflect such a biological difference although it still remains to be studied. The assay result of 42GC is reported as late recurrence-like (LR) or non-late recurrence-like (NLR), and patients determined as LR is at a high risk for late recurrence and, therefore, are thought to be candidates for extended hormone therapy.

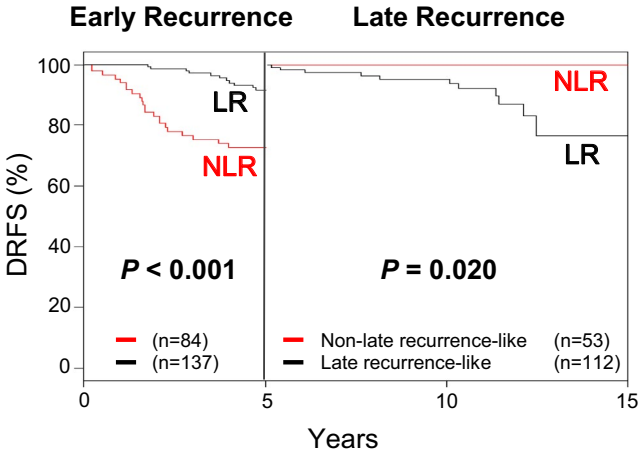

FIGURE 5 Figure shows the 42-gene classifier (42GC) in the independent validation set (n = 221). Prediction of early and late recurrence by 42GC. Distant recurrence-free survival (DRFS) rates were compared between the late recurrence-like (LR) and the non-late recurrence-like (NLR) groups containing patients in the validation set. DRFS, distant recurrence-free survival. Adapted with permission from Figure 2 in reference.[18] Copyright 2018 Springer

4 | THE 155GC FOR THE PREDICTION OF RECURRENCE OF ER+/HER2− BREAST CANCER TREATED WITH NAC

Currently, most patients with ER+/HER2− breast cancer with lymph node metastasis or T3/T4 tumors or both are treated with NAC. Although patients who achieve pCR after NAC exhibit an excellent prognosis, those who do not achieve pCR have a recurrence rate of approximately 30%, which indicates a necessity for additional postoperative adjuvant systemic therapy to improve their prognosis. However, treatment of all patients who do not achieve pCR with additional adjuvant therapy may be overtreatment and, therefore, an accurate prognostic indicator should be developed to distinguish patients at a high risk of recurrence from those who cannot achieve pCR.

Therefore, we attempted to develop an MGA that can classify ER+/HER2− breast cancer patients treated with NAC into low-risk and high-risk groups.[19] RNAs extracted from vacuum-assisted biopsy specimens obtained before NAC were subjected to the Affymetrix microarray assay, and the differentially expressed genes between poor responders (grade 1 by histological assessment)[20] and good responders (grades 2 and 3) were determined in the training set. Optimization of the MGA using these genes by the leave-one-out cross-validation method resulted in the construction of the 155GC assay. The 155GC distinguishes patients who are likely to achieve pCR (g-Responders: High-CS) from those who are unlikely to achieve pCR (g-Non-Responders: Low-CS). Evaluation of the 155GC in the validation set has revealed that this MGA seems to be very useful in the selection of patients

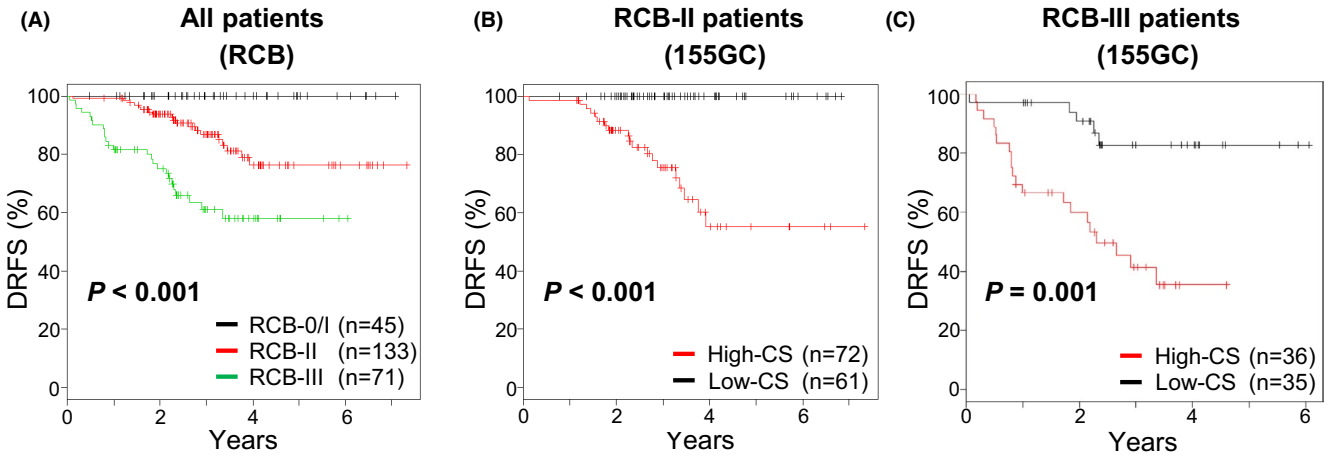

FIGURE 6 Figure shows the 155-gene classifier (155GC) in the independent validation set. Prognostic prediction in accordance with residual cancer burden (RCB) and 155GC. Distant recurrence-free survival (DRFS) rates for (A) all patients in the validation set were compared in terms of the RCB in groups 0/I, II, and III. The DRFS rates were also compared in the low-CS and high-CS groups in the RCB-II group (B) and in the RCB-III group (C). DRFS, distant recurrence-free survival; high-CS, high chemosensitivity (g-Responders); low-CS, low chemosensitivity (g-Non-Responders); RCB, residual cancer burden. Adapted with permission from Figure 4 in reference.[19] Copyright 2015 Elsevier

who are very unlikely to achieve pCR due to the very high negative predictive value of 97.4%. Interestingly, patients classified as g-Non-Responders: Low-CS by the 155GC showed an excellent prognosis compared with those classified as g-Responders: High-CS; this was the case as well as in patients with residual cancer burden (RCBII or RCBIII) in the validation cohort (Figure 6).[19] These results suggest that if ER+/HER2− breast cancer patients with residual cancer burden after NAC are classified into the g-Non-Responders: Low-CS group (ie, low risk of relapse), and they are expected to show an excellent prognosis so that additional systemic adjuvant therapy can be avoided. In contrast, those classified into the g-Responders: High-CS group (ie, high risk of relapse) should be treated with additional systemic adjuvant chemotherapy.

5 | FUTURE PERSPECTIVES OF MGAs

Compared with other MGAs that measure the mRNA expression of a limited number of genes, the 95GC is unique in that the assay is performed using an Affymetrix microarray and, therefore, the gene expression data of not only the 95 genes but also data of all 20 000 human genes can be obtained simultaneously (CEL file CD data). Such data are so valuable that they can be applied to simultaneous analyses of other MGAs, such as the 155GC and the 42GC, as well as to the development of novel MGAs, which would facilitate more comprehensive MGA-guided treatment strategies for ER+/HER2− early breast cancer patients (T1-2N0M0). Such a website-based analysis has already been achieved and will be available for the members soon. Here, the analysis is automatically performed once the CEL file is uploaded from a personal computer to the original membership website after the 95GC test. Then, the 42GC/155GC results can be obtained in minutes. Suggested application of these MGAs to treatment decision for ER+/HER2− primary breast cancer is shown in Figure S1. By using these MGAs, more precise treatment is expected to be implemented in the future, although the clinical validity and utility of these MGAs still remain to be prospectively demonstrated in a future study that includes a larger number of patients. Currently, a prospective registered study on the 95GC is ongoing and will hopefully demonstrate its clinical validity and utility in the prognostic prediction of ER+/HER2−/n0 breast cancer patients.

DISCLOSURE

SN received honoraria and research funding from Sysmex Corporation, and SN and YN hold a patent for Curebest™ 95GC Breast.

ETHICAL STANDARDS

The experiments comply with the current laws of the country in which they were performed.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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