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Original Research

Comparison of StemPrintER with Oncotype DX Recurrence Score for predicting risk of breast cancer distant recurrence after endocrine therapy

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Abstract ***Objective:*** Molecular tests predicting the risk of distant recurrence (DR) can be used to assist therapy decision-making in oestrogen receptor–positive (ER+) and human epidermal growth factor receptor 2–negative (HER2-) breast cancer patients after considerations of standard clinical markers. The Oncotype DX Recurrence Score (RS) is a widespread tool used for this purpose. Here, we compared the RS with the StemPrintER Risk Score (SPRS), a novel genomic predictor with a unique biological basis in its ability to measure the expression of cancer stemness genes.

Materials and methods: We benchmarked the SPRS *vs.* RS, alone or in combination with clinicopathological variables expressed by the Clinical Treatment Score (CTS), for the prognostication of DR in a retrospective cohort of 776 postmenopausal patients with ER+/HER2- breast cancer enrolled in the translational arm of the randomised Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial. Likelihood ratio (LR) with χ^2 test and C-index were used to assess prognostic performance for the entire ten-year follow-up period and in early (0–5 years) and late (5–10 years) intervals.

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Results: In all patients, the SPRS provided significantly more prognostic information than the RS for ten-year DR prognostication (C-index = 0.688, LR-χ^2 = 33.4 *vs.* C-index = 0.641, LR-χ^2 = 22.1) and for late (5–10 years) DR prognostication (C-index = 0.689, LR-χ^2 = 18.8 *vs.* C-index = 0.571, LR-χ^2 = 4.7). The SPRS also provided more prognostic information than the RS when added to the CTS in all patients (CTS + SPRS: LR-$\Delta\chi^2$ = 14.9; CTS + RS: LR-$\Delta\chi^2$ = 9.7) and in node-negative patients (CTS + SPRS: LR-$\Delta\chi^2$ = 11.7; CTS + RS: LR-$\Delta\chi^2$ = 6.6).

Conclusions: In postmenopausal ER+/HER2- breast cancer patients, SPRS provided more prognostic information than RS for DR when used alone or in combination with the CTS. The SPRS could therefore potentially identify high-risk patients, who might benefit from aggressive treatments, from low-risk patients who might safely avoid adjuvant chemotherapy or prolongation of endocrine therapy.

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1. Introduction

Oestrogen receptor–positive (ER+) breast cancers (BCs) display a high level of molecular heterogeneity and variability in clinical outcome [1,2]. This heterogeneity makes prognosis and therapy response often difficult to predict using the standard clinicopathological features of the tumour. Although the overall prognosis for this group of patients is good, a substantial proportion (>20%) will experience distant recurrence (DR) in the first ten years after surgery [3]. Of these recurrent cases, many will occur after an extended period of apparent remission, confounding the clinical management of these patients [3]. For ER + patients who also have a negative HER2 status (HER2-), the standard of care is five years of endocrine therapy with the addition of adjuvant chemotherapy in those patients deemed at high risk of DR after consideration of clinicopathological parameters [1,2]. The prolongation of endocrine therapy beyond the standard five years is also recommended to reduce the risk of late DR in higher-risk patients [4,5]. However, relying solely on standard clinicopathological parameters for the prediction of DR risk can lead to over- or under-treatment for a significant proportion of ER+/HER2- patients.

Multigene expression assays provide prognostic information beyond that obtained from clinicopathological parameters, and their use to guide decisions on the administration of adjuvant chemotherapy in ER+/HER2- patients is endorsed by several international guidelines [6–8]. The most widely used test in the clinical practice is the Oncotype DX 21-gene recurrence score (RS) [9].

The StemPrintER Risk Score (SPRS) is a recently developed multigene prognostic test that analyses the cancer stem cell characteristics of individual tumours by measuring the expression of a 20-gene signature derived from the transcriptional profile of human normal mammary stem cells [10]. The expression of these twenty genes is quantified by quantitative real-time polymerase chain reaction (RT-qPCR) on RNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue samples and normalised to four housekeeping genes [11]. In a retrospective analysis of a consecutive cohort of 2,453 patients with BC collected from 1997 to 2000 at the European Institute of Oncology, Milan, Italy (the IEO BC 97-00 cohort), the SPRS was prognostic for both early (0–5 years) and late (5–10 years) DR, independently of all other clinicopathological parameters [11].

The aim of this study was to perform a benchmark comparison of the SPRS and RS in the TransATAC cohort, the translational sub-study of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial [12]. The ultimate goal was to increase the clinical level of evidence of the SPRS as a prognostic tool for individualised management of postmenopausal women with ER+/HER2- primary BC.

2. Materials and methods

2.1. Study design and patients

The study cohort consisted of patients who had participated in the TransATAC trial, which compared efficacy and safety of five years of adjuvant hormonal therapy with anastrozole *vs.* tamoxifen in postmenopausal women with early-stage, operable ER+/HER2- BC. Detailed aspects of the study design, its conduct, ethical issues and overall results have been published elsewhere [13]. A total of 915 RNA extracts with sufficient residual RNA for SPRS analysis were available for this study. After the exclusion of RNA samples from women treated with chemotherapy, with ER-negative and/or HER2+ tumours (based on central assessment), with four or more positive lymph nodes, and without RS or SPRS data, 776 patients were available for the comparative analysis (Fig. 1 and Table 1 for patient characteristics).

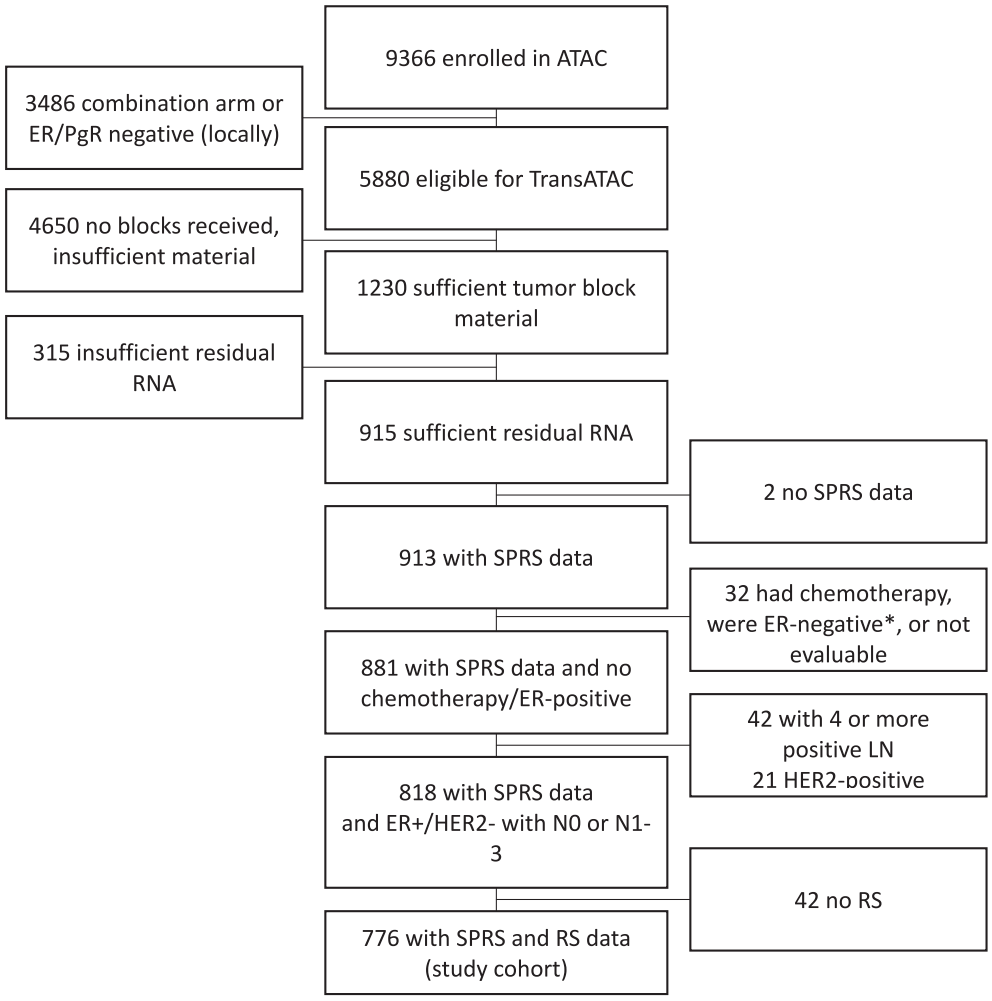

Fig. 1. **CONSORT diagram showing the derivation of the study cohort from the ATAC trial**. SPRS, StemPrintER Risk Score; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; LN, lymph node; N0, lymph node-negative; N1-3, 1 to 3 positive lymph nodes; RS, Oncotype DX Recurrence Score; ∗, based on central assessment; ATAC, Arimidex, Tamoxifen, Alone or in Combination.

2.2. SPRS test

The SPRS is a novel genomic predictor that stratifies BCs based on their degree of molecular resemblance to a 'stem cell-like phenotype', as identified by a set of 20 genes (*H2AFZ, CDK1, EXOSC4, PHLDA2, APOBEC3B, EIF4EBP1, SFN, PHB, EPB41L5, RACGAP1, MRPS23, TOP2A, H2AFJ, NOL3, MIEN1, CENPW, LY6E, ALYREF, MMP1, NDUFB10*) derived from transcriptional profiling of normal human mammary stem cells [10]. Expression levels of these genes were determined by RT-qPCR on total mRNA extracted from FFPE samples. RT-qPCR reactions were performed with an in-house, custom-designed TaqMan® Array, as previously described [11]. Briefly, each target gene was assayed in triplicate and average Cq (AVG Cq) values were calculated and normalised using four reference genes (*HPRT1*, *GAPDH*, *GUSB* and *TBP*) to compensate for possible variations in the expression of single reference genes and in RNA integrity due to tissue fixation. Based on the distribution of the reference genes, we applied the Tukey's interquartile rule for outliers to identify poor-quality RT-qPCR data [14]. All RNA samples were of sufficiently good quality to be included in the statistical analyses.

Normalised data were processed for statistical analysis, according to a risk model described in earlier studies [11]. A predefined SPRS cut-off of 56.3, corresponding to a ten-year DR cumulative incidence of 10.7% [11], was used for a two-class stratification into low- *vs.* high-DR-risk groups. This value represents the median of the continuous risk score obtained by the ridge penalised Cox regression model in a training set of ER+/HER2- BC patients from the IEO-97-00 cohort, which stratified patients of a validation set from the same cohort into a low- and high-risk category with a ten-year DR cumulative incidence of 6.0% and 22.4%, respectively [11]. Researchers involved in RT-qPCR analysis of samples and in the final assignment of individual patients to a SPRS risk group were blinded to clinical outcome data.

The calibration of the SPRS was tested by comparing observed with expected DR events in the cohort divided

Table 1
Patient characteristics.

Variable	N = 776
Age (years), median (IQR)	63.6 (58.0–70.8)
Tumour size (mm), median (IQR)	18 (13–23)
Node-negative (N0)	594 (76.6%)
Node-positive (N1-3)	182 (23.4%)
Low grade (G1)	226 (29.1%)
Intermediate grade (G2)	427 (55.0%)
High grade (G3)	123 (15.9%)
Anastrozole	383 (49.4%)
Tamoxifen	393 (50.6%)
DR events	94 (12.1%)
SPRS, median (IQR)	54.5 (47.1–62.8)

IQR, interquartile range; SPRS, StemPrintER Risk Score; DR, distant recurrence.

into quintiles. Expected events were derived for each patient from the baseline hazard in the TransATAC dataset. The baseline hazard was adjusted for patients with recurrences in 5–10 years and those who died of non-BC causes in 5–10 years. Results show a good calibration for the SPRS with an observed *vs*. expected ratio of 0.89 (95% confidence interval [CI] = 0.72–1.09, p = 0.26) (Supplementary Fig. 1). In the fourth quintile, the number of events predicted by the SPRS in the TransATAC cohort was slightly underestimated compared to the number of observed events in this cohort; however, the overall calibration was good and there was no significant difference between observed and expected ten-year DR risk.

2.3. Clinical Treatment Score

The Clinical Treatment Score (CTS) is an algorithm that integrates the prognostic information provided by nodal status, grade, tumour size, age and endocrine treatment and was developed in the TransATAC study [15].

2.4. RS test

The RS was determined by Genomic Health who were blinded to clinical outcome data. Patient stratification by the RS was based on predefined, original RS cut-offs according to the TAILORx trial; low<11, intermediate 11–26, and high >26 to identify low-, intermediate- and high-risk groups, respectively [16]. The number of patients and the ten-year DR risk for each RS category are shown in Supplementary Table 1; hazards ratio values and Kaplan–Meier analyses for ten-year DR rate from the comparison among the different RS categories are shown in Supplementary Fig. 2 for all patients, as well as N0 and N1-3 patients. Based on the similar prognostic behaviour of the RS low and intermediate categories and in compliance with the current international guidelines for the use of RS for chemotherapy response prediction in women aged >50 years [17], the low and intermediate RS categories were grouped together to create a RS low + intermediate (<26) category.

2.5. Statistical analysis

The primary end-point was time to DR. DR was defined as metastatic disease, excluding contralateral disease, as well as locoregional and ipsilateral recurrences. Death from other causes before DR was treated as a censoring event. We assessed overall DR by censoring follow-up of all patients at ten years after diagnosis.

To assess the prognostic performance of the tests (SPRS, RS and CTS), continuous risk scores were normalised to have unit variance. We used two methods to assess the overall discriminative ability of the single signatures (RS, SPRS and CTS) and of their combination: (a) changes in likelihood ratio (LR) values (LR-$\Delta\chi^2$) obtained from Cox regression were used to estimate the improvement in DR prognostication when a signature is added to another risk model; (b) the concordance index (C-index), a generalisation of the area under the receiver operating characteristic curve (AUC) for time-to-event data. Increasing C-index values from 0.5 to 1.0 indicate improved prognostication (1 corresponding to the best model, and 0.5 to a random model). Hazard ratios and associated 95% CIs (statistical significance assessed by CI not crossing 1) were estimated from Cox models and provide an estimate of the overall prognostic performance of each test used alone. Kaplan–Meier curves were used to plot the cumulative incidence of DR in the various risk groups using predefined cut-offs described above for the RS and SPRS.

All statistical analyses were performed with STATA, version 15.1 (College Station, Texas, USA).

3. Results

3.1. Cohort characteristics

The study cohort was comprised of 776 ER+/HER2-, chemonaïve patients with node-negative disease (N0) or 1 to 3 positive lymph nodes (N1-3), for whom both SPRS and RS data were available (Fig. 1). The median age of patients at diagnosis was 64 years (Table 1). A total of 94 DR events were recorded within the ten-year median follow-up period, 56 among the 594 women with negative lymph nodes (N0) and 38 among the 182 women with 1–3 positive lymph nodes (N1-3).

3.2. Prediction of ten-year DR risk

In univariate analysis, both the SPRS and RS, set as continuous variables, provided a statistically significant prognostic value for DR in 0–10 years. The SPRS (HR for change in 1 SD = 1.79, 95% CI = 1.47–2.17; LR-χ^2 = 33.4) proved to be statistically more prognostic than the RS (HR = 1.52, 95% CI = 1.30–1.78; LR-

Table 2
Comparison of DR risk prediction by RS, SPRS and CTS, alone or in combination, in the 776-patient study cohort.

Years 0–10	All patients (N = 776, DR = 94)					Node-negative patients (N = 594, DR = 56)					Node-positive* patients (N = 182, DR = 38)				
	HR (95% CI)	P-value	C-index (95% CI)	P-value	LR-x^2	HR (95% CI)	P-value	C-index (95% CI)	P-value	LR-x^2	HR (95% CI)	P-value	C-index (95% CI)	P-value	LR-x^2
RS	1.52 (1.30–1.78)	<0.001	0.641 (0.578–0.703)	<0.001	22.14	1.58 (1.31–1.91)	<0.001	0.661 (0.576–0.746)	<0.001	18.01	1.40 (1.03–1.91)	0.03	0.594 (0.500–0.688)	<0.001	4.11
SPRS	1.79 (1.47–2.17)	<0.001	0.688 (0.635–0.740)	<0.001	33.36	1.97 (1.53–2.53)	<0.001	0.721 (0.662–0.780)	<0.001	26.33	1.42 (1.05–1.92)	0.02	0.618 (0.524–0.712)	<0.001	5.23
CTS	2.17 (1.81–2.61)	<0.001	0.742 (0.695–0.790)	<0.001	64.77	2.32 (1.77–3.03)	<0.001	0.732 (0.667–0.797)	<0.001	33.92	1.98 (1.40–2.79)	<0.001	0.683 (0.601–0.765)	<0.001	15.51
RS + SPRS			0.708 (0.656–0.760)	<0.001	16.16			0.747 (0.687–0.808)	<0.001	12.26			0.627 (0.534–0.720)	<0.001	2.38
CTS + RS			0.759 (0.715–0.802)	<0.001	9.72			0.751 (0.692–0.809)	<0.001	6.59			0.699 (0.621–0.777)	<0.001	2.62
CTS + SPRS			0.764 (0.722–0.805)	<0.001	14.92			0.772 (0.723–0.822)	<0.001	11.71			0.697 (0.616–0.778)	<0.001	2.91
Years 0–5	**(N = 776, DR = 42)**					**(N = 594, DR = 23)**					**(N = 182, DR = 19)**				
RS	1.69 (1.38–2.07)	<0.001	0.712 (0.630–0.795)	<0.001	19.54	1.81 (1.43–2.29)	<0.001	0.762 (0.651–0.873)	<0.001	16.79	1.50 (0.99–2.26)	0.06	0.627 (0.501–0.752)	<0.001	3.33
SPRS	1.77 (1.33–2.37)	<0.001	0.686 (0.612–0.761)	<0.001	14.55	2.01 (1.35–2.99)	<0.001	0.731 (0.651–0.811)	0.001	11.58	1.39 (0.91–2.12)	0.13	0.612 (0.480–0.744)	<0.001	2.29
Years 5–10	**(N = 692, DR = 52)**					**(N = 539, DR = 33)**					**(N = 153, DR =19)**				
RS	1.34 (1.05–1.71)	0.02	0.571 (0.479–0.662)	<0.001	4.72	1.35 (1.01–1.82)	0.04	0.577 (0.459–0.695)	<0.001	3.50	1.29 (0.80–2.08)	0.30	0.551 (0.400–0.702)	<0.001	0.99
SPRS	1.81 (1.39–2.34)	<0.001	0.689 (0.615–0.763)	<0.001	18.82	1.94 (1.40–2.69)	<0.001	0.713 (0.628–0.798)	<0.001	14.77	1.46 (0.95–2.24)	0.08	0.627 (0.492–0.762)	<0.001	2.97

HR, hazard ratio for change in 1 SD; CTS, Clinical Treatment Score; C-index, concordance indices; *1 to 3 positive nodes. Likelihood ratio (LR) statistical significance >3.84; SPRS, StemPrintER Risk Score; CI, confidence interval; RS, Oncotype DX Recurrence Score.
CTS + RS means RS added to CTS; CTS + SPRS means SPRS added to CTS. The number of patients (N) and distant recurrence (DR) events for each group is indicated.

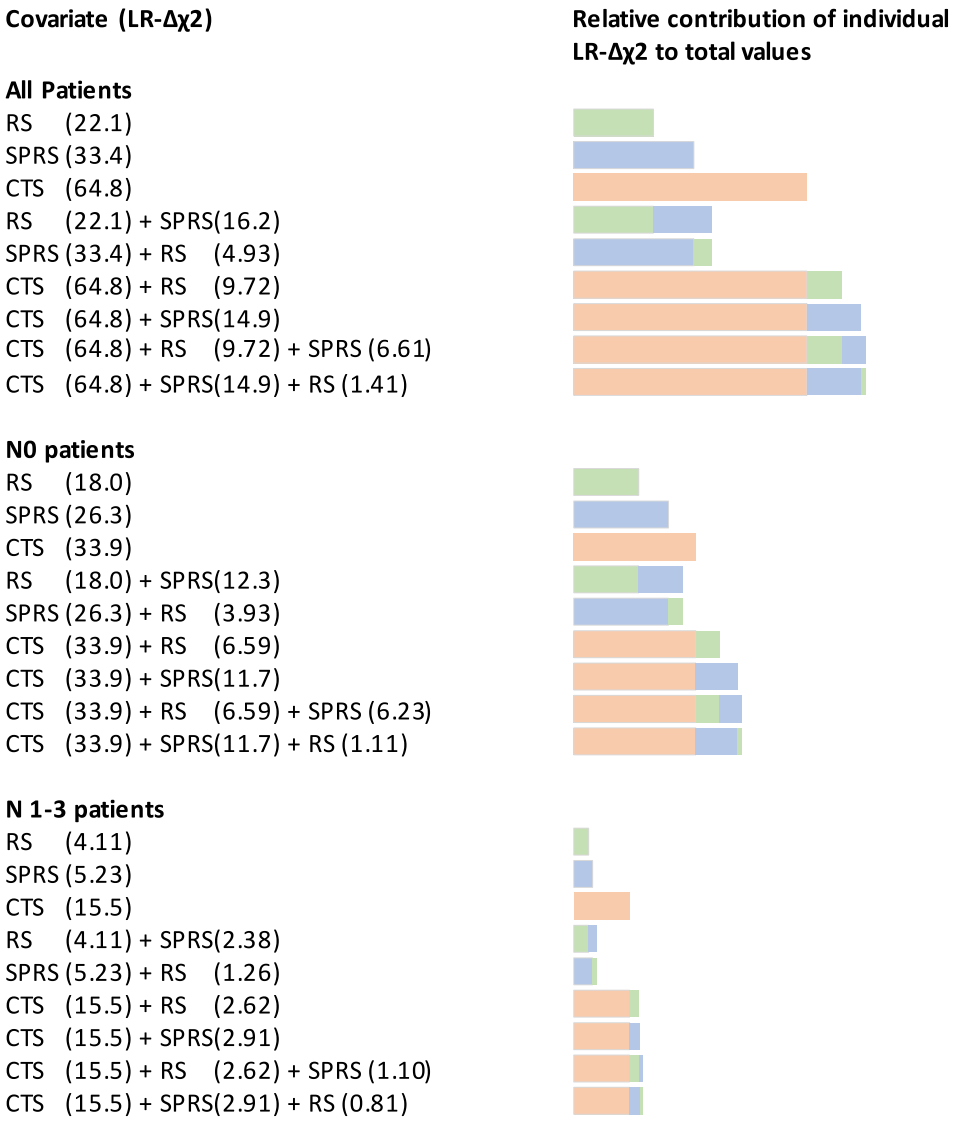

Fig. 2. **Prognostic information provided by the RS, SPRS and CTS, alone or in combination, for ten-year distant recurrence risk.** The likelihood ratio values (LR-Δχ2) are shown in parentheses and represented in the stacked bar charts. LR-Δχ2 >3.84 are statistically significant and denote significant improvement upon the addition of the covariate to the model. Colours in the stacked bars: green, RS; blue, SPRS; orange, CTS. CTS, Clinical Treatment Score; RS, Oncotype DX Recurrence Score; SPRS, StemPrintER Risk Score; LR, likelihood ratio.

χ^2 = 22.1) in all patients (Table 2, Fig. 2). Adding the SPRS to RS or the RS to SPRS significantly improved DR prognostication, but the improvement of add-on SPRS to RS (LR-$\Delta\chi^2$ = 16.2) was far greater than adding the RS to SPRS (LR-$\Delta\chi^2$ = 4.9), indicating that the SPRS covers a substantial amount of the prognostic information provided by the RS.

The CTS alone (HR = 2.17, 95% CI = 1.81–2.61; C-index = 0.742, LR-χ^2 = 64.8) had a statistically higher prognostic value than the RS alone (C-index = 0.641), SPRS alone (C-index = 0.688), or to their combination (C-index = 0.708) (Table 2). Adding the RS to CTS further increased the prognostic information of the model (LR-$\Delta\chi^2$ = 9.7, C-index = 0.759) but to a lesser extent than adding the SPRS to CTS (LR-$\Delta\chi^2$ = 14.9, C-index = 0.764) (Table 2, Fig. 2). Further addition of the RS to CTS + SPRS did not improve prognostication (LR-$\Delta\chi^2$ = 1.4) (Fig. 2).

Similar results were observed in N0 patients. In N1-3 patients, the RS, SPRS, and CTS all provided a statistically significant prognostic value for DR during 0–10 years, but neither the RS (LR-$\Delta\chi^2$ = 2.6) nor the SPRS (LR-$\Delta\chi^2$ = 2.9) added statistically significant prognostic information to that provided by the CTS alone (LR-$\Delta\chi^2$ = 15.5). All HRs, C-index and LR-χ^2 values in the N0 and N1-3 groups are shown in Table 2 and Fig. 2.

3.3. Prediction of early (0–5 years) and late (5–10 years) DR risk

In the early follow-up interval (0–5 years), the RS provided slightly more prognostic information than the SPRS for DR risk in all patients (**RS**: HR = 1.69, 95% CI = 1.38–2.07, C-index = 0.712, LR-χ^2 = 19.5 *vs.* **SPRS**: HR = 1.77, 95% CI = 1.33–2.37; C-index = 0.686, LR-χ^2 = 14.6) (Table 2) and in N0 patients (**RS:** HR = 1.81, 95% CI = 1.43–2.29, C-index = 0.762, LR-χ^2 = 16.8 *vs.* **SPRS:** HR = 2.01, 95% CI = 1.35–2.99; C-index = 0.731, LR-χ^2 = 11.6) (Table 2).

In contrast, in the late follow-up interval (5–10 years), the SPRS was superior to the RS for DR prognostication. In all patients, the SPRS was highly prognostic for DR risk (HR = 1.81, 95% CI = 1.39–2.34, C-index = 0.689, LR-χ^2 = 18.8), whereas the RS was relatively weak (HR = 1.34, 95% CI = 1.05–1.71; C-index = 0.571, LR-χ^2 = 4.7) (Table 2). The SPRS remained highly prognostic in the group of N0 patients (HR = 1.94, 95% CI = 1.40–2.69; C-index = 0.713, LR-χ^2 = 14.8), where the RS showed no statistically significant prognostic value (HR = 1.35, 95% CI = 1.01–1.82; C-index = 0.577, LR-χ^2 = 3.5) (Table 2). Neither the SPRS nor the RS provided statistically significant prognostic information in the group of N1-3 patients in the early or late interval (Table 2).

3.4. Risk stratification

The SPRS and RS were compared as categorical variables for ten-year DR prognostication adopting a two-risk class stratification (low and high) for the SPRS, based on a prespecified cut-off of 56.3 [11], and a two-risk class stratification (high >26 and low + intermediate <26) for the RS, in compliance with current international guidelines [17].

Both the SPRS and RS were highly prognostic for DR in all patients (Fig. 3), with a greater absolute separation of the risk of developing a DR between the high- and low-risk groups for SPRS than between the high- and low + intermediate-risk group of RS (**SPRS**, HR = 4.27, 95% CI = 2.67–6.84; **RS**, HR = 2.75, 95% CI = 1.80–4.19) (Fig. 3). Similar results were observed for N0 patients (**SPRS**, HR = 5.59, 95% CI = 2.95–10.58; **RS**, HR = 3.72, 95% CI = 2.19–6.31) (Supplementary Fig. 3). In N1-3 patients, only the SPRS retained statistically significant prognostic value (HR = 2.43, 95% CI = 1.21–4.90) (Supplementary Fig. 4).

Both in all patients and N0 patients, the ten-year DR risk rate in the low-risk category of the SPRS was substantially lower (approximately one half) than in the low + intermediate-risk category of RS (*All patients*: **SPRS**, 5.8%, 95% CI = 3.9–8.7 *vs.* **RS**, 10.9%, 95% CI = 8.5–13.8; *N0 patients*: **SPRS**, 3.8%, 95% CI = 2.2–6.6 *vs.* **RS**, 7.6%, 95% CI = 5.4–10.7) (Fig. 3 and Supplementary Fig. 3), whereas the ten-year DR rate for the high-risk categories of the SPRS and RS was similar (*All patients*: **SPRS**, 23.2%, 95% CI = 18.8–28.4 *vs.* **RS**, 24.8%, 95% CI = 18.3–32.9; *N0 patients*: **SPRS**, 19.9%, 95% CI = 15.2–26.0 *vs.* **RS**, 23.0%, 95% CI = 16.0–32.4) (Fig. 3 and Supplementary Fig. 3).

Concordance between the risk groups of the SPRS (low and high) and RS (low + intermediate and high) is shown in Supplementary Table 2. In all patients, we noted that the ten-year DR rate for women classified as SPRS low/RS low + intermediate (4.8%, CI = 3.0–7.7) or SPRS high/RS low + intermediate (20.7%, CI =

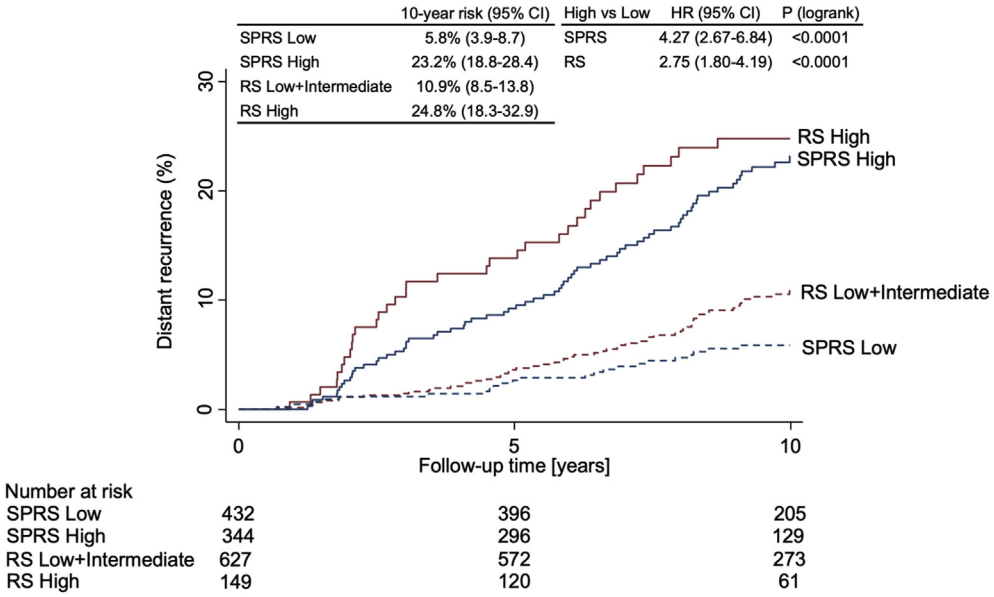

Fig. 3. Kaplan–Meier graph and ten-year distant recurrence risk (%) for pre-specified SPRS and RS cut-offs with RS low and intermediate categories combined, in all patients. RS, Oncotype DX Recurrence Score; SPRS, StemPrintER Risk Score.

15.7–27.1) was inferior or superior, respectively, to that of women classified as RS low + intermediate only (10.9%, CI = 8.5–13.8; see Fig. 3 for the DR rate of this category). Similar results were observed in N0 patients (Supplementary Table 2). A substantial reduction of the DR rate was also observed in N1-3 patients comparing the group of women classified as SPRS low/RS low + intermediate (11.8%, CI = 6.0–22.2) with that of women classified as RS low + intermediate only (21.7%, CI = 15.4–30.0) (Supplementary Table 2).

4. Discussion

StemPrintER is a new RNA-based molecular test for the prognostication of DR in ER+/HER2- BC patients that is unique among multigene assays for BC prognostication in that it interrogates the cancer stem cell characteristics of the primary tumour rather than proliferation and hormone receptor status [11]. Considering the increasingly recognised role of cancer stem cells in driving tumour progression and metastasis [18–20], the implementation of a cancer stem cell–based genomic tool to estimate the individual risk of metastasis could help guide more accurate decision-making on the choice of adjuvant therapy.

The primary objective of this study was to perform an independent validation of the SPRS in postmenopausal women with early-stage, operable ER+/HER2- BC treated with endocrine therapy, using the TransATAC cohort [12], a large patient cohort with long-term follow-up from a registration standard clinical trial, ATAC, with excellent clinical records [13]. We found that SPRS was highly prognostic in all patients and in N0 patients, and moderately prognostic in N1-3 patients, across the ten-year follow-up. When looking at the early *vs.* late follow-up period, the prognostic ability of the continuous SPRS was maintained in all patients and in N0 patients in both the early (0–5) and late (5–10) time intervals. These results are consistent with our previous findings, obtained in a large retrospective consecutive cohort of more than 1,800 ER+/HER2- BC patients, where the SPRS was prognostic for ten-year DR risk, as well as for early (0–5 years) and late (5–10 years) DR, in both premenopausal and postmenopausal patients and in patient subgroups stratified by lymph node status [11]. Therefore, through an independent validation in the TransATAC study, we have further increased the clinical level of evidence of the SPRS as a genomic tool for DR prognostication in postmenopausal women.

The availability in the TransATAC study of prognostic data obtained by the use of CTS, a risk model that integrates prognostic information provided by all the standard clinicopathological parameters, such as nodal status, grade, tumour size, age and endocrine treatment [15], allowed us to establish that the SPRS can add substantial prognostic information to clinicopathological risk factors, in all patients and in N0 patients. Based on these findings, we submit that the SPRS can represent a potential tool for patient risk prognostication after consideration of standard clinical parameters and could therefore be used to complement clinical risk-stratification models currently used by oncologists in the clinical practice for therapy decision-making, such as Predict [21,22].

In the analysis of the prognostic performance of the SPRS in the ATAC cohort, we noted that the ten-year DR rate in the SPRS low-risk groups in all patients (5.8%) and in N0 patients (3.8%) is much lower than the current threshold (10%) empirically considered acceptable by oncologists to spare ER+/HER2- BC patients from chemotherapy. Although this reassures that patients deemed to be at low risk of recurrence based on SPRS testing can safely avoid chemotherapy, the current SPRS cut-off to distinguish high- *vs.* low-risk patients might be too stringent, with the consequent possibility to overestimate as at high-risk women who could instead reasonably avoid aggressive treatments. This will likely require a redefinition of the SPRS cut-off around the 10% DR rate threshold for considering patients for treatment de-escalation.

The use of TransATAC RNA samples previously used in the validation of the Oncotype DX RS [12] also allowed the direct comparison of the prognostic value of SPRS with the most widely used genomic tool for DR prediction in the clinical practice. The SPRS proved to be statistically more prognostic than the RS for DR prognostication over the ten-year period in the entire population, as well as in N0 and N1-3 patients. More clinically relevant, in all patients and in patients with N0 disease, the SPRS provided more independent prognostic information than the RS in addition to the CTS and provided a substantial amount of new prognostic information beyond that provided by a prognostic model combining CTS plus the RS.

Important clinically relevant findings were also derived from the comparison of the performance of the SPRS *vs.* RS at different time intervals. This analysis revealed that, the RS is more prognostic than the SPRS in the early follow-up period in all patients, whereas in the late interval, the SPRS outperformed the RS, whose prognostic value declined to become barely significant in the total patient population. The SPRS was also strongly informative for late DR risk in the N0 subgroup, where the RS completely lost prognostic power. Neither assay reached significance in N1-3 patients in the early or late time interval, an effect likely due to the low number of patients and events available for this subgroup analyses.

We submit that the improved performance of the SPRS compared with the RS in the ten-year follow-up period and, to a greater extent in the late time interval, is likely attributable to its molecular components rooted in

the biology of cancer stem cells. Indeed, cancer stem cells are thought to possess the ability to remain in a dormant state and resist therapies for prolonged periods and, consequently, drive DR even after long periods of apparent remission and response to therapy [23,24]. In contrast, the poor prognostic ability of the RS in the late time interval is likely the consequence of the variable loss of prognostic power of the ER- and proliferation-associated gene modules included in the Oncotype DX assay [25]. In this context, it will be interesting to compare in future studies the SPRS with other second-generation genomic predictors, such as EPclin, ROR and BCI, which have also been shown to outperform the RS for the prediction of the late recurrence [26].

A strength of our study was that the direct comparison of the SPRS with RS was conducted on the same extracts of RNA, thus avoiding any bias resulting from intra-sample variations, and by personnel blinded to both clinical data and results of previous analyses on the performance of the RS. By including only samples with sufficient residual RNA extracts and the absence of any chemotherapy, we might have introduced an unintentional bias in the study cohort favouring the inclusion of patients with larger tumours and lower risk, respectively, in the spectrum of ER+/HER2- BC patients. However, this is unlikely considering that the distribution of high- *vs.* low-risk patients identified by the SPRS in TransATAC is similar to that reported in previous analyses [11].

In summary, through its independent validation in the TransATAC cohort, we have increased the level of evidence of the clinical usefulness of the SPRS as a genomic tool to predict recurrence risk in postmenopausal women with ER+/HER2- BC who have been treated with endocrine therapy only. In particular, we submit that the SPRS could help identify ER+/HER2-patients with negligible metastatic risk, who could benefit from de-escalation of aggressive treatments, in particular chemotherapy or prolongation of endocrine therapy beyond the standard five years of treatment. Our results highlight the potential clinical value of the SPRS in the management of ER+/HER2- BC patients either as a standalone test or in combination with other predictor tools for the personalised treatment of BC patients.

Authors' contributions

Pece, Sestak, Dowsett, and Di Fiore had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Pece and Sestak are co-first authors and contributed equally to this work. Pece, Dowsett, and Di Fiore are co-last authors and contributed equally to this work.

Concept and design: Pece, Sestak, Dowsett, and Di Fiore.

Acquisition, analysis, or interpretation of data: Montani, Tillhon, Freddi, and Disalvatore.

Drafting of the manuscript: Pece, Sestak, and Di Fiore.

Critical revision of the manuscript for important intellectual content: Pece, Sestak, Maisonneuve, Colleoni, Veronesi, Viale, Buus, Cuzick, Dowsett, and Di Fiore.

Statistical analysis: Sestak, Maisonneuve, and Chu.

Obtained funding: Pece, Dowsett, and Di Fiore.

Administrative, technical, or material support: Montani, Tillhon, Freddi, and Disalvatore.

Supervision: Pece, Dowsett, and Di Fiore.

Availability of data

Data will be available according to TransATAC/LATTE's data sharing policy. Requests for specific analyses or data can be submitted via email to j.cuzick@qmul.ac.uk.

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Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pece and Di Fiore report a patent EP/20.06.16/EPA16175354 and a patent EP/20173612.1, both pending to Tiziana Life Sciences (TLS). They also report a research sponsored grant from TLS from June 2014 to June 2018 related to the development of StemPrintER, outside the submitted work which was completely developed with academic funding. Sestak reports personal fees from Myriad Genetics and Pfizer Oncology, outside the submitted work. Viale reports personal fees from Roche Genentech, Daiichi-Sankyo, AstraZeneca, Agilent, Menarini and Ventana, outside the submitted work. Dowsett reports personal fees from Radius, Myriad, Orion, G1, AbbVie, H3 Biomedicine, Lilly, Zentalis, Agilent and Nanostring, outside the submitted work. All other authors declare no other competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2022.01.003.

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