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Original Research

Prognostic value of the 6-gene OncoMasTR test in hormone receptor–positive HER2-negative early-stage breast cancer: Comparative analysis with standard clinicopathological factors☆

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KEYWORDS
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Prognostic biomarker;
Recurrence score

Abstract ***Aim:*** The aim of the study was to assess the prognostic performance of a 6-gene molecular score (OncoMasTR Molecular Score [OMm]) and a composite risk score (OncoMasTR Risk Score [OM]) and to conduct a within-patient comparison against four routinely used molecular and clinicopathological risk assessment tools: Oncotype DX Recurrence Score, Ki67, Nottingham Prognostic Index and Clinical Risk Category, based on the modified Adjuvant! Online definition and three risk factors: patient age, tumour size and grade.
Methods: Biospecimens and clinicopathological information for 404 Irish women also previously enrolled in the Trial Assigning Individualized Options for Treatment [Rx] were provided by 11 participating hospitals, as the primary objective of an independent translational study. Gene expression measured via RT-qPCR was used to calculate OMm and OM. The prognostic value for distant recurrence-free survival (DRFS) and invasive disease-free survival (IDFS) was assessed using Cox proportional hazards models and Kaplan-Meier analysis. All statistical tests were two-sided ones.
Results: OMm and OM (both with likelihood ratio statistic [LRS] $P < 0.001$; C indexes = 0.84 and 0.85, respectively) were more prognostic for DRFS and provided significant additional prognostic information to all other assessment tools/factors assessed (all LRS $P \leq 0.002$). In addition, the OM correctly classified more patients with distant recurrences (DRs) into the high-risk category than other risk classification tools. Similar results were observed for IDFS.
Discussion: Both OncoMasTR scores were significantly prognostic for DRFS and IDFS and provided additional prognostic information to the molecular and clinicopathological risk factors/tools assessed. OM was also the most accurate risk classification tool for identifying DR. A concise 6-gene signature with superior risk stratification was shown to increase prognosis reliability, which may help clinicians optimise treatment decisions.

1. Introduction

Breast cancer is the most frequently occurring cancer in women globally, with more than 2.2 million women diagnosed in 2020 [1]. Approximately 80% of breast cancer diagnoses are hormone receptor–positive [2], for which the decision on whether to offer cytotoxic adjuvant chemotherapy is influenced by the risk profile of each individual patient [3,4]. For hormone receptor–positive, human epidermal growth factor receptor 2 (HER2)-negative early-stage breast cancer, a patient's risk profile may be determined by clinicopathological risk factors such as patient age, tumour size and grade and lymph node (LN) involvement. The Nottingham Prognostic Index (NPI) [5,6], which combines tumour size, grade and LN involvement, classifies early and locally advanced breast cancer cases into three or more prognostic groups. In addition, the expression of the proliferation marker Ki67 can be used to estimate tumour progression, with high Ki67 antigen expression associated with poor prognosis [7,8]. Clinically, however, the application of Ki67 information in cancer treatment optimisation has been limited by its widely varying cut-off points and the low level of analytical validation [8,9].

More recently, genomic tests such as Oncotype DX Recurrence Score (RS) have aided the identification of patients who are of sufficiently low risk of recurrence that they may safely forego chemotherapy [4,10–15]. Furthermore, evident discordance in risk classification of individual patients with different tests confounds the choice of an optimal molecular-based signature for a given patient [16–18].

The OncoMasTR Molecular Score (OMm), which contains information relating to solely 3 master transcription regulators (MTRs) and 3 reference genes, and its composite OncoMasTR Risk Score (OM), which combines OMm with LN involvement and tumour size and categorises patients as having either low or high risk of recurrence, have been analytically validated [19] and clinically validated in a category B study as significantly prognostic for breast cancer recurrence in patients from the TransATAC cohort [20,21]. Here, in a fully independent translational study, we assessed the prognostic performance of OncoMasTR in 404 Irish patients who were also previously accrued to the 'Trial Assigning Individualized Options for Treatment [Rx]' (TAILORx) trial [12,13,22]. We compared the prognostic performance of OncoMasTR to seven clinically relevant and widely adopted molecular and clinicopathological risk assessment tools/factors: Oncotype DX RS, Ki67, NPI,

clinical risk category (CRC), patient age, tumour size and tumour grade.

2. Materials and methods

2.1. Study design and patients

The Breast Cancer Bank of Tissue (CTRIAL-IE 12–30, NCT02050750) is an exploratory, translational, non-interventional multicentre biobank sponsored by Cancer Trials Ireland (CTI) that aims to identify potential biomarkers. Eligibility required prior registration with the TAILORx trial (CTRIAL-IE (ICORG) 06–31, NCT00310180) [12,13,22], participation in trial arms and having sufficient tumour material available for molecular analysis. Other than the accrual of patients who were also accrued to the Eastern Cooperative Oncology Group trial TAILORx [12,13,22], there was no connection between the two studies, and the analysis performed on the patient samples from CTRIAL-IE 12–30 did not impinge in any way on the TAILORx trial. Hormone receptor–negative or HER2-amplified tumours were excluded from this analysis. Ethical approval for the study was provided by the institutional ethics committees of 11 CTI-affiliated hospitals. The informed consent provided by patients enrolled in the prior TAILORx trial included consent for the use of data and biological samples for future studies, provided the study was ethically approved. Under the study protocol, formalin-fixed paraffin-embedded (FFPE) tissue blocks of the identified eligible patients were retrieved from pathology archives and shipped to the study biobank. A baseline case report form (CRF), which included RS, and follow-up CRFs were completed locally for each patient enrolled and monitored centrally by a translational research coordinator (A.S.) within CTI.

2.2. Procedures

As in the clinical validation study [21], OMm, ranging from 0 to 100, is a linear combination of the normalised expression of three prognostic genes measured by quantitative reverse transcription polymerase chain reaction (RT-qPCR); OM, ranging from 0 to 10, is a linear combination of OMm, LN involvement and tumour size. A predefined cut-point classified patients into low risk (0 to <5) or high risk (5–10) [21]. Classification of patients who may benefit from chemotherapy by age and RS was conducted as described by Sparano *et al.* [13]. Calculation of Ki67 [23] and NPI [5,6] scores and categories are as detailed in Supplementary Methods. CRC was defined as in the 'Microarray In Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy' (MINDACT) trial [10].

2.3. Study end-points and statistical analysis

The primary and secondary end-points of this study were distant recurrence-free survival (DRFS) and invasive disease-free survival (IDFS), respectively (Supplementary Methods). For the primary and secondary end-points, the prognostic value of the continuous numeric OMm, OM, RS and six additional clinicopathological risk assessment tools/factors was assessed using the likelihood ratio statistic (LRS) and C index from a series of univariable Cox proportional hazards regression models. For DRFS, the additional prognostic value of the OncoMasTR scores and RS to clinicopathological variables was assessed by the change in LRS and C index when the score was added as a second variable to a univariable model consisting of the clinicopathological variable. The same assessment was performed for IDFS, with the exception that the score was added as a third variable to a bivariable model consisting of the clinicopathological variable and chemotherapy treatment. All statistical tests were two-sided ones, and a P value <0.05 was considered statistically significant. For all models, the proportional hazards assumption was verified by Schoenfeld residuals [24].

By design, patients in the published TAILORx trial with a higher RS were assigned to chemotherapy, so it was important to control for potential bias towards decreased or no prognostic effect of the prognostic risk scores. The analysis tested if the OncoMasTR scores were prognostic, but not if they were predictive of chemotherapy benefit. An analysis of the value of OncoMasTR for predicting chemotherapy benefit was not included in the prespecified statistical analysis plan because the relatively small number of events would not provide sufficient power to detect a biomarker by treatment interaction effect. The number of primary end-points of 18 distant recurrences (DRs) in the cohort studied provided an estimated 85% power [24] to detect a true prognostic hazard ratio (HR) of 4 based on a prior clinical validation study of OncoMasTR scores [21]. However, multivariable models of DRFS with more than two variables were not analysed because there were too few DR events to reliably estimate variable effects in larger models. The larger number of secondary end-points of 46 invasive disease (ID) events allowed inclusion of a third variable of chemotherapy effect in models of IDFS analysis. Therefore, all multivariable models of the relationship between IDFS and the prognostic scores were trivariable models that included adjustment for chemotherapy effect. Statistical analysis was carried out by an independent statistician (N.M.R.) using base R (version 3.4.3) [40] and the survival package (version 2.38). The statistical analysis and reporting of results

follow the guidelines of REporting recommendations for tumour MARKer prognostic studies [25].

3. Results

3.1. Sample availability and patient characteristics

A total of 453 FFPE blocks were retrieved from the 11 CTI-affiliated participating hospitals. Of those, 49 were excluded from analysis based on prespecified criteria detailed in Supplementary Fig. S1. The median follow-up duration was 96 months, with an interquartile range of 84–108 months. There were 18 DRs and 46 ID events in the 404 patients analysed.

The characteristics of patients and diseases are shown in Table 1. Distribution of patients in the four treatment arms was similar to that of the 9719 patients reported in the published TAILORx trial [13]. The proportion of patients with a tumour size greater than 2 cm was higher in this study (34% *vs* 25% in the TAILORx trial), as well as the proportion of patients with high-grade tumours (23% *vs* 17%) and patients aged 50 years and younger (36% *vs* 31%).

Table 1
Characteristics of patients and tumours included in this study.

Characteristic	Multicentre Biobank, Ireland (N = 404)		
	OncoMasTR, low *N* (%) 235 (58%)	OncoMasTR, high *N* (%) 169 (42%)	Total *N* (%) 404 (100%)
Age, years			
Median (range)	53 (25–74)	54 (20–75)	54 (20–75)
≤40	11 (5%)	17 (10%)	28 (7%)
41–50	70 (30%)	49 (29%)	119 (29%)
51–60	103 (44%)	60 (36%)	163 (40%)
61–70	46 (20%)	38 (22%)	84 (21%)
71–75	5 (2%)	5 (3%)	10 (2%)
Tumour size, cm			
≤1.0	21 (9%)	3 (2%)	24 (6%)
1.1–2 .0	160 (68%)	83 (49%)	243 (60%)
2.1–3.0	48 (20%)	65 (38%)	113 (28%)
3.1–4.0	5 (2%)	15 (9%)	20 (5%)
≥4.1	1 (0%)	3 (2%)	4 (1%)
Unknown	0 (0%)	0 (0%)	0 (0%)
Tumour grade			
Low	57 (24%)	4 (2%)	61 (15%)
Intermediate	157 (67%)	94 (56%)	251 (62%)
High	21 (9%)	71 (42%)	92 (23%)
Unknown	0 (0%)	0 (0%)	0 (0%)
CRC			
Low	178 (76%)	56 (33%)	234 (58%)
High	57 (24%)	113 (67%)	170 (42%)
Unknown	0 (0%)	0 (0%)	0 (0%)
Ki67% positivity			
Low (<10)	145 (62%)	46 (27%)	191 (47%)
Intermediate (10 to <20)	58 (25%)	61 (36%)	119 (29%)
High (≥20)	32 (14%)	62 (37%)	94 (23%)
Treatment arm (recurrence score/therapy)			
0–10/endocrine	44 (19%)	21 (12%)	65 (16%)
11–25/endocrine	97 (41%)	47 (28%)	144 (36%)
11–25/ chemoendocrine	78 (33%)	43 (25%)	121 (30%)
26–100/ chemoendocrine	16 (7%)	58 (34%)	74 (18%)

CRC (clinical risk category) was defined as in the MINDACT (Microarray in Node-Negative Disease May Avoid Chemotherapy) trial using oestrogen receptor status, tumour grade and tumour size [10].

3.2. Numeric risk scores: univariable and multivariable analyses

In univariable analysis of numeric scores, both OMm and OM were significantly prognostic for DRFS (both with LRS $P < 0.001$; C indexes = 0.84 and 0.85, respectively) and more prognostic than the NPI (LRS $P < 0.001$; C index = 0.74) and RS (LRS $P = 0.004$, C index = 0.73) (Fig. 1A and B). Tumour size in millimetres, tumour grade, Ki67 categories and CRC all offered modest but statistically significant prognostic value. Patient age was not prognostic for DRFS, whether assessed as a continuous variable or dichotomised at 50 years. In bivariable analysis, both OMm and OM scores provided significant additional prognostic value to all of the clinicopathological risk assessment tools/factors assessed (all with LRS $P \leq 0.002$). RS added some additional prognostic value to age (as continuous or dichotomised at 50 years), tumour size, the numeric Ki67 score, NPI category and CRC, but did not provide additional prognostic information to tumour grade, Ki67 category or the numeric NPI. OMm and OM added significant prognostic value to RS (both LRS $P < 0.001$, respectively); however, RS did not add value to OMm or OM (LRS $P = 0.88$ and 0.99, respectively) (Fig. 1C and D).

For IDFS, in univariable analysis of numeric scores, both OMm and OM were significantly prognostic (both with LRS $P < 0.001$; C indexes = 0.68 and 0.70, respectively), followed by tumour size (LRS $P < 0.001$; C index = 0.68), NPI (LRS $P < 0.001$; C index = 0.66) and Ki67 (LRS $P = 0.001$; C index = 0.63). Tumour grade, CRC and age (dichotomised at 50 years) also offered modest but statistically significant prognostic value. RS (LRS $P = 0.06$; C index = 0.55) and age (continuous) were not prognostic for IDFS (Fig. 2A and B). In trivariable analysis wherein analyses were additionally adjusted for chemotherapy, both OncoMasTR scores again provided significant additional prognostic value to all of the clinicopathological risk assessment tools/factors (all with LRS $P \leq 0.002$). RS did not provide significant additional prognostic information to any clinicopathological risk assessment tools/factors assessed (all with LRS $P \geq 0.08$). OMm and OM added significant prognostic value to RS (both with LRS $P < 0.001$); however, RS did not add value to OMm or OM (LRS $P = 0.73$ and 0.51, respectively) (Fig. 2C and D).

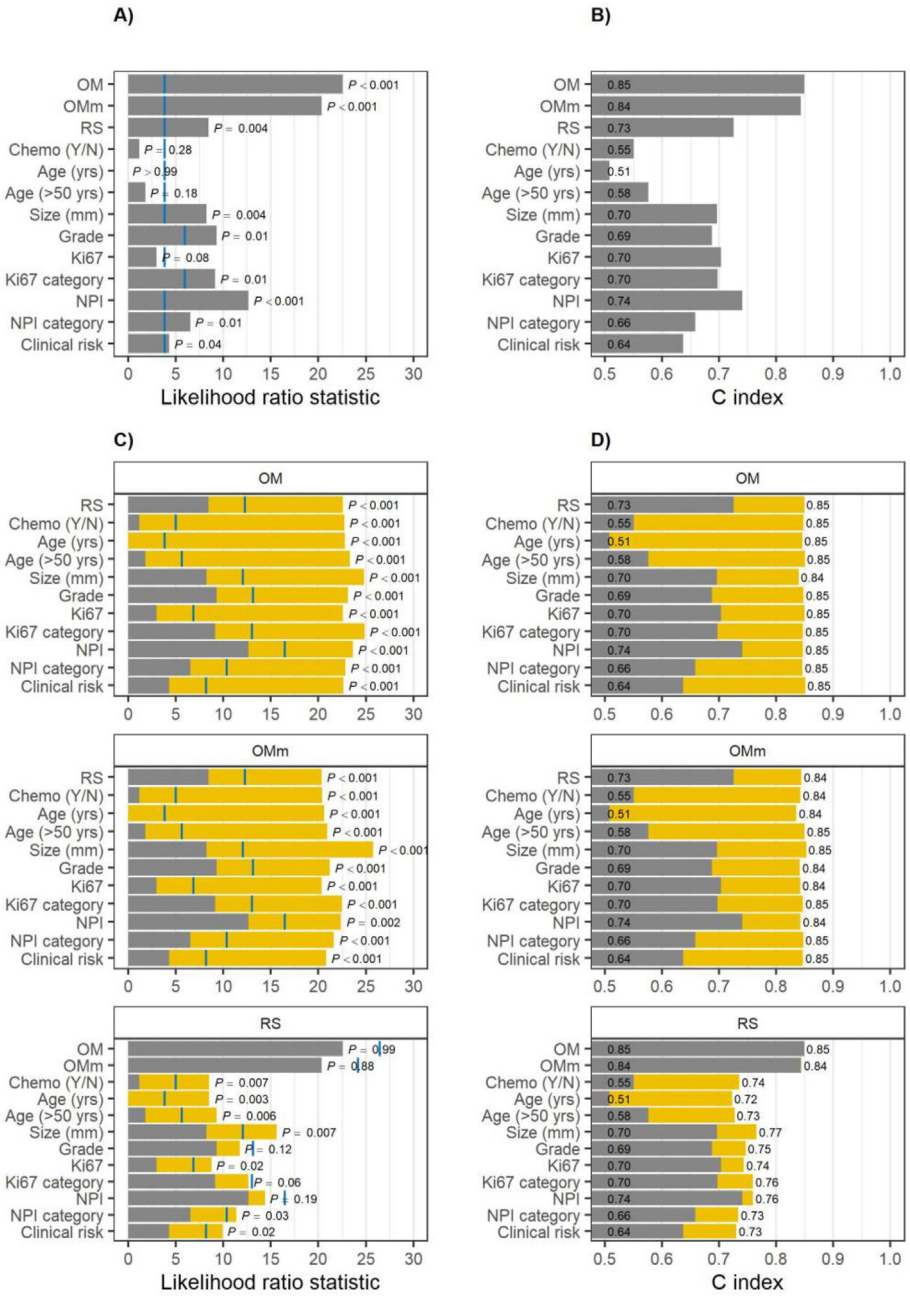

Fig. 1. **Prognostic value of OncoMasTR Risk Score (OM), OncoMasTR Molecular Score (OMm), Recurrence Score (RS) and clinicopathological risk assessment tools/factors for DR ($N = 404$ patients, 18 DR).** **(A)** Univariable prognostic value estimated by the likelihood ratio statistic (LRS). The blue line is the LRS value for significance at $P < 0.05$ (3.84 for variables represented by one term; 5.99 for variables represented by two terms). **(B)** Univariable prognostic value estimated by the C index. **(C)** Bivariable prognostic value estimated by the LRS. The yellow portion of each horizontal bar shows the increase in LRS when OM, OMm or RS is added as a second variable to a univariable model consisting of the clinicopathological factor/tool. The blue line is the increase in the LRS value for significant additional prognostic value at $P < 0.05$ (3.84, as each numeric risk score is represented by one term). P values are for the additional prognostic value of the risk score to the clinicopathological variable, measured by the increase in the LRS between the univariable and bivariable model. **(D)** Bivariable prognostic value estimated by the C index. The yellow portion of each horizontal bar shows the increase in the C index when OM, OMm or RS is added as a second variable to a univariable model consisting of the clinicopathological factor/tool. LRS and C indexes were estimated using Cox proportional hazards regression models. Ki67 percentage positivity was used to categorise patients into low-risk (<10%), intermediate-risk (10% to <20%) or high-risk (≥20%) groups. The Nottingham Prognostic Index (NPI) was categorised as good (≤3.4), moderate (>3.4 and ≤5.4) or poor (>5.4) prognosis. Clinical risk category was defined as in the MINDACT trial using oestrogen receptor status, tumour grade and tumour size [10].

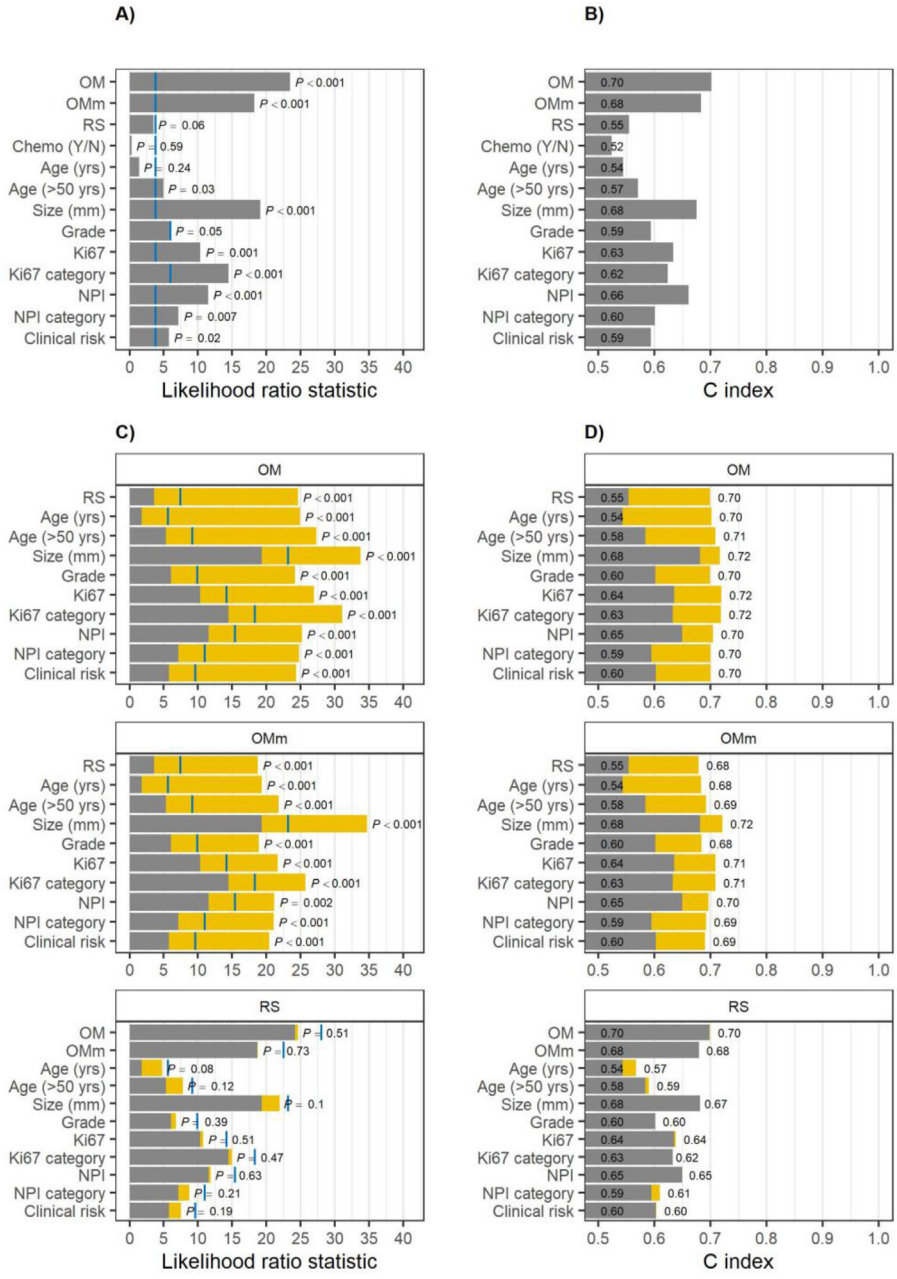

Fig. 2. **Prognostic value of OncoMasTR Risk Score (OM), OncoMasTR Molecular Score (OMm), Recurrence Score (RS) and clinicopathological risk assessment tools/factors for invasive disease ($N = 404$ patients, 46 invasive disease events).** **(A)** Univariable prognostic value estimated by the likelihood ratio statistic (LRS). The blue line is the LRS value for significance at $P < 0.05$ (3.84 for variables represented by one term; 5.99 for variables represented by two terms). **(B)** Univariable prognostic value estimated by the C index. **(C)** Trivariable prognostic value estimated by the LRS. The yellow portion of each horizontal bar shows the increase in LRS when OM, OMm or RS is added as a third variable to a bivariable model consisting of the clinicopathological factor/tool and chemotherapy treatment (yes or no). The blue line is the increase in the LRS value for significant additional prognostic value at $P < 0.05$ (3.84, as each numeric risk score is represented by one term). P values are for the additional prognostic value of the risk score to the clinicopathological variable and chemotherapy treatment, measured by the increase in the LRS between the bivariable and trivariable model. **(D)** Trivariable prognostic value estimated by the C index. The yellow portion of each horizontal bar shows the increase in the C index when OM, OMm or RS is added as a third variable to a bivariable model consisting of the clinicopathological factor/tool and chemotherapy treatment (yes or no).

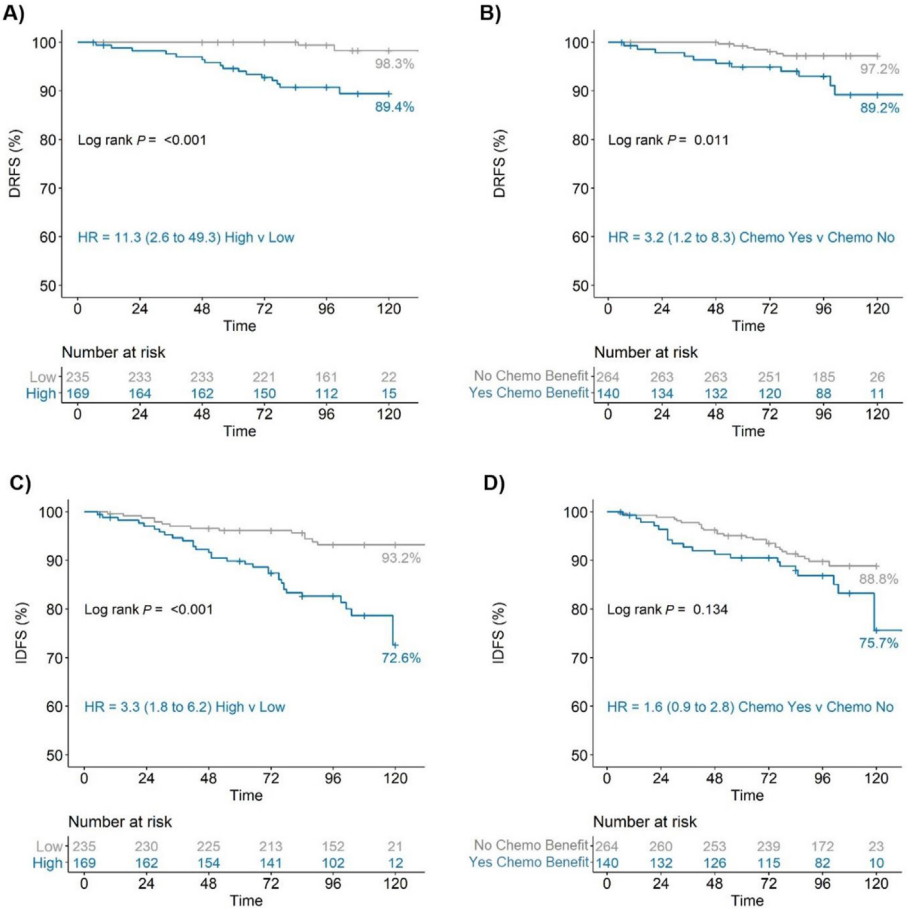

Fig. 3. **Kaplan-Meier curves for distant recurrence-free survival (DRFS) and invasive disease-free survival (IDFS). (A and B)** DRFS with 18 distant recurrence events; **(C and D)** IDFS with 46 invasive disease events. Chemotherapy benefit categories by age and RS: Classification of patients who may benefit from chemotherapy by RS and age was conducted as described by Sparano *et al.* [13]. Women aged ≤50 years with an RS of 16–100 and women aged >50 years with an RS of 26–100 were classified as potentially benefiting from chemotherapy (Chemo Yes), and the rest were classified as potentially not benefiting from chemotherapy (Chemo No). Hazard ratios (HRs) were estimated using univariable Cox proportional hazards regression models.

3.3. Categorical risk score: univariable and multivariable analyses

For DRFS, patients classified as OM high risk were significantly more likely to experience a DR than patients classified as low risk (10.6% *vs* 1.7% DR; HR = 11.3; 95% confidence interval [CI], 2.6 to 49.3; Fig. 3A). The HR was very similar after controlling for the potential bias of chemotherapy treatment (HR = 11.0; 95% CI = 2.5 to 48.3; Supplementary Table S1). When adjusted for each clinicopathological risk factor/tool in a series of bivariable models, the differences in DR rates between OM high- and low-risk patients remained significant, with HRs ranging from 7.1 to 11.4. Patients classified by age and RS as potentially benefiting from chemotherapy [13] were significantly more likely to experience a DR than other patients (10.8% *vs* 2.8% DR; HR = 3.2; 95% CI = 1.2 to 8.3; Fig. 3B). When adjusted for each clinicopathological risk factor/tool in a series of bivariable models, the HR remained significant at 3.0 (95% CI = 1.1 to 8.3). Clinicopathological risk assessment tools differentiating patients more likely to experience a DR were Ki67, NPI and CRC (Supplementary Table S2). Risk factors of age dichotomised at 50 years and tumour grade did not differentiate patients with higher DR risk. Notably, NPI did not classify any DR as poor prognosis.

For IDFS, patients classified as OM high risk were significantly more likely to experience an ID than patients classified as low risk (27.4% *vs* 6.8% ID; HR = 3.3; 95% CI = 1.8 to 6.2; Fig. 3C). When adjusted for chemotherapy status plus each clinicopathological risk factor/tool in a series of trivariable models, the differences in ID rates between OM high- and low-risk patients remained significant, with HRs ranging from 2.5 to 3.3 (Supplementary Table S3). In general, the HRs for ID were lower than those for DR. Patients classified by age and RS as potentially benefiting from chemotherapy [13] were not significantly more likely to experience an ID (24.3% *vs* 11.2% ID; HR = 1.6; 95% CI = 0.9 to 2.8; Fig. 3D).

Clinicopathological risk assessment tools differentiating patients more likely to experience an ID were Ki67, NPI and CRC (Supplementary Table S2). Risk factors of age dichotomised at 50 years and tumour grade did not differentiate patients with higher ID risk.

3.4. Relationship between OncoMasTR and other molecular and clinicopathological risk assessment tools

OM was only moderately correlated with RS (Fig. 4; Pearson's r = 0.45), Ki67 (r = 0.39) or NPI (r = 0.51). When assessing the classification accuracy, OM was the most sensitive risk classifier, identifying the highest number of DR events as high risk (16 DRs of the total 18; sensitivity = 0.89), followed by CRC (12 DRs in the high CRC group; sensitivity = 0.67), chemotherapy benefit categories by age and RS [13] (11 DRs in patients classified as potentially benefitting from chemotherapy; sensitivity = 0.61) and Ki67 (10 DRs in the Ki67 high-risk category; sensitivity = 0.56). The NPI classified all 404 patients as good or moderate prognosis, with 5 and 13 DRs in the good and moderate prognosis groups, respectively (Table 2).

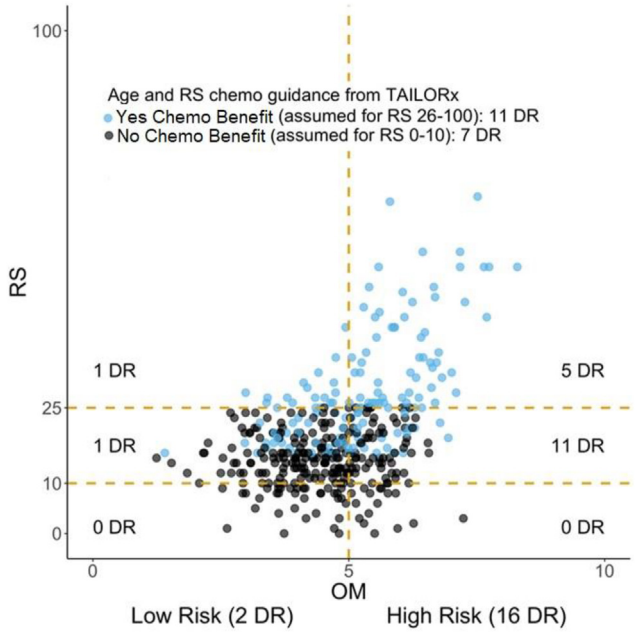

Fig. 4. **Relationship between OncoMasTR Risk Score and Recurrence Score (RS)**. Pearson's correlation coefficient = 0.45. Dashed lines represent the categorical version of the corresponding numeric score. In TAILORx, RS was categorised as low (0–10), intermediate (11–25) or high (26–100) risk. Age and RS chemotherapy benefit category: Classification of patients who may benefit from chemotherapy by age and RS was conducted as described by Sparano *et al.* [13]. Women aged ≤50 years with an RS of 16–100 and women aged >50 years with an RS of 26–100 were classified as potentially benefiting from chemotherapy (Yes Chemo Benefit, blue), and the rest were classified as potentially not benefiting from chemotherapy (No Chemo Benefit, black). DR, distant recurrence; TAILORx, Trial Assigning Individualized Options for Treatment [Rx].

4. Discussion

Investigators of the prospective, randomised TAILORx trial found that among 6711 women with HR-positive, HER2-negative, LN-negative breast cancer and an RS of 11–25, endocrine therapy was not inferior to chemoendocrine therapy [13]. They also found that women in TAILORx with an RS of 26 to 100 treated with chemoendocrine therapy had an estimated DRFS rate of 93% at 5 years, which was an outcome better than that expected with endocrine therapy alone in this population [26]. A further subgroup analysis of TAILORx found that when added to the RS, the CRC provided prognostic information that could be used to identify premenopausal women who could benefit from more effective therapy [27]. Design of this subgroup analysis presented an obvious contradiction to the more clinically relevant question that another prospective study, MINDACT, sought to answer: 'whether a genomic test provides additional prognostic information to the CRC?' [10,27]. As such, we designed this study to answer the latter more clinically relevant question of whether the 6-gene OM signature provides additional prognostic information to commonly used risk assessment tools/factors.

OMm and OM showed greater prognostic value for DRFS and IDFS than the seven molecular and clinicopathological risk assessment tools/factors evaluated, suggesting that expression of the three MTR genes was the strongest predictor of disease recurrence in the cohort of 404 Irish patients studied. OMm and OM provided more additional prognostic information to the six commonly used clinicopathological risk assessment tools/factors than RS. OMm and OM also provided further prognostic information beyond RS [28], but not *vice versa*. This superior prognostic power from just three genes may be attributed to the novel discovery approach of the signature [21,29,30]. Given the lack of commonality between genes contained within currently available genomic tests, despite effectively describing the same phenotype, and studies suggesting that a combination of all signatures improved prediction [16], we previously sought to interrogate the underlying regulatory networks that define early breast cancer prognostic signatures to identify shared features [29]. OncoMasTR was uncovered via use of an innovative bioinformatics technique, Algorithm for the Reconstruction of Accurate Cellular Networks – Master Regulator Analysis [31], to reveal a small panel of MTRs that regulate biomarkers within the 207-gene genomic grade signature [32], the wider 231-gene poor-prognosis signature [33] and a 214-gene core proliferation signature [29]. The MTRs identified via this bioinformatic transcriptional

Table 2
Sensitivity and specificity of risk classifiers to identify distant recurrences (N = 404 patients, 18 distant recurrences).

Risk classifier	True positive rate (for predicting distant recurrences)			True negative rate (for predicting distant recurrences)		
	Category	Patients, N (%)	Sensitivity	Category	Patients, N (%)	Specificity
OncoMasTR	High	169 (42%)	16/18 = 0.89	Low	235 (58%)	233/386 = 0.60
RS, Sparano *et al.* [12]	Intermediate (11–25)	265 (66%)	12/18 = 0.67	Low	65 (16%)	65/386 = 0.17
	High (26–100)	74 (18%)	6/18 = 0.33	Intermediate (11–25)	265 (66%)	253/386 = 0.66
	Intermediate + high	339 (84%)	18/18 = 1.00	Low + intermediate	330 (82%)	318/386 = 0.82
Age and RS chemotherapy benefit category, Sparano *et al.* [13]	Yes Chemo Benefit	140 (35%)	11/18 = 0.61	No Chemo Benefit	264 (65%)	257/386 = 0.67
Ki67	Intermediate	119 (29%)	4/18 = 0.22	Low	191 (47%)	187/386 = 0.48
	High	94 (23%)	10/18 = 0.56	Intermediate	119 (29%)	115/386 = 0.30
	Intermediate + high	213 (53%)	14/18 = 0.78	Low + intermediate	310 (77%)	302/386 = 0.78
Nottingham Prognostic Index	Moderate	171 (42%)	13/18 = 0.72	Good	233 (58%)	228/386 = 0.59
	Poor	0 (0%)		Moderate	171 (42%)	158/386 = 0.41
				Good + Moderate	404 (100%)	386/386 = 1.00
Clinical risk category	High	170 (42%)	12/18 = 0.67	Low	234 (58%)	228/386 = 0.59

Chemotherapy benefit categories by age and RS: Classification of patients who may benefit from chemotherapy by age and RS was conducted as described by Sparano *et al.* [13]. Women aged ≤50 years with an RS of 16–100 and women aged >50 years with an RS of 26–100 were classified as potentially benefiting from chemotherapy (Yes Chemo Benefit), and the rest were classified as potentially not benefiting from chemotherapy (No Chemo Benefit). Clinical risk category was defined as in the MINDACT trial [10] using oestrogen receptor status, tumour grade and tumour size. RS, Recurrence Score.

network analysis approach [29,30] were shown to regulate previously known prognostic genes and to possess functional roles covering multiple hallmarks of cancer including proliferation, invasion and metastasis [34–38]. Furthermore, several of the MTRs identified via this analysis were then experimentally confirmed to exert mechanistic interactions with the promoters of proliferation-related genes [29], with a subset of MTRs then used to develop OMm. OM further incorporated tumour size and LN status with the molecular-only score OMm. We found that combining tumour size moderately improved the prognostic power of OMm in the present study. The additional effect of LN status in the OM signature could not be evaluated as all patients studied here were LN negative.

We also found that OM was a more reliable risk stratifier than Ki67, NPI, chemotherapy benefit categories by age and RS [13] and CRC, with a larger HR for high risk and higher sensitivity to correctly identify patients with DR events as high risk. Similar findings were observed for IDFS, with the exception that the chemotherapy benefit categories by age and RS [13] did not differentiate patients with higher ID risk. At an individual patient level, our study highlighted that most risk assessment tools underestimated the DR risk and did not adequately identify high-risk patients who experienced a DR during the follow-up period (Fig. 4 and Table 2). For instance, 7 of 18 patients who experienced DR during the follow-up were classified as potentially not benefitting from chemotherapy based on age and RS. Whether these seven patients who relapsed were indeed unlikely to benefit from chemotherapy remains unclear. OM, in contrast, was particularly strong at capturing DR in the population with an RS of 11–25 and more reliably identified patients at high risk of recurrence (Fig. 4). The reliable identification of truly high-risk patients is of potential clinical significance and requires further testing and confirmation in a wider patient population.

A reported obstacle in accessing molecular risk profiling information is the high cost [39], which is partly due to the molecular method and the large panels of biomarkers analysed. The OncoMasTR test kit was designed to be performed in either local molecular pathology or reference laboratories. It obtained a CE mark in 2018, with a plan in place to submit for US Food and Drug Administration clearance. The concise nature of the 6-gene OncoMasTR Risk Score, together with its superior risk stratification performance and decentralised testing, may prove to be more economical and enable provision of accurate risk assessment to a wider patient population.

Strengths of this study include a cohort of contemporary patients with tissue specimens collected under clinical trial standards, standardised and analytically validated assays for which gene expression data were obtained by personnel blinded to the clinical data, a prospectively defined statistical analysis plan and a study designed to suitably answer a clinically relevant question. Furthermore, the outcome of this study was

supported by the findings from the category B clinical validation study of OncoMasTR scores using the TransATAC samples showing that OncoMasTR provided improved prognostic performance over clinicopathological information and the RS [20,21]. Limitations include a relatively modest cohort size involving only patients based in Ireland and the relatively low number of DR events as a consequence of the cohort size and follow-up time as well as the introduction of potential bias due to the varying treatments offered to patients according to the design of the TAILORx trial. Further studies in the TAILORx trial and other cohorts are required to verify the findings from this study. To conclude, the validated OncoMasTR Risk Score is significantly prognostic for DRFS and IDFS, adds significant prognostic information to routinely used molecular and clinicopathological risk assessment tools/factors and provides accurate identification of truly low-risk patients who may stay recurrence free on standard-of-care endocrine therapy.

CRediT author statement

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Clinical trial registration number

The Breast Cancer Bank of Tissue (CTRIAL-IE 12–30, NCT02050750) is an exploratory, translational, non-interventional multicentre biobank that is independent of and not affiliated with the 'Trial Assigning Individualized Options for Treatment [Rx]' TAILORx trial (CTRIAL-IE (ICORG) 06–31, NCT00310180). There was no direct connection between the two studies, and the analysis performed on the patient samples from CTRIAL-IE 12–30 did not impinge in any way on the TAILORx trial.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: A.P.B. and W.M.G. report a patent issued and licensed to OncoMark Ltd., with all rights transferred to The Provost, Fellows, Foundation Scholars and the other members of the Board, the College of the Holy and Undivided Trinity of Queen Elizabeth near Dublin, and University College Dublin, National University of Ireland, Dublin. D.O'L. and W.M.G. report being employees and shareholders of OncoMark Ltd. J.C., S.B., C.-J.A.W., T.L., P.D., B.F. and C.L.-R. report being employees of OncoMark Ltd. D.P.O'C. reports receiving a travel bursary to part support attendance at ASCO 2019 from OncoMark. All the remaining authors have declared no conflicts of interest.

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Appendix A. Supplementary data

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