Validation of the OncoMasTR Risk Score in Estrogen Receptor-Positive/HER2-Negative Patients: a TransATAC study

Richard Buus[1,2], Ivana Sestak[3], Stephen Barron[4], Tony Loughman[4], Bozena Fender[4], Cesar Lopez Ruiz[4], Peter Dynoodt[4], Chan-Ju Angel Wang[4], Des O'Leary[4], William M. Gallagher[4,5], Mitch Dowsett[1,2], Jack Cuzick[3]

1. The Breast Cancer Now Toby Robins Research Centre at The Institute of Cancer Research, London, UK

2. Ralph Lauren Centre for Breast Cancer Research, Royal Marsden Hospital, London, UK

3. Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London, UK

4. OncoMark Limited, Dublin, Ireland

5. UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland

Running Title: OncoMasTR is Prognostic in ER+/HER2- Breast Cancer

Corresponding author:

Richard Buus

The Breast Cancer Now Toby Robins Research Centre at The Institute of Cancer Research, Fulham Road, London, SW3 6JB, UK

Tel: +44 207 808 2619; Fax: +44 207 808 2808

E-mail: richard.buus@icr.ac.uk

Conflict of interest statement:

IS declares speaker's fee from Myriad Genetics and NanoString Technologies. SB is employed by OncoMark Ltd. TL is employed by OncoMark Ltd. BF is employed by OncoMark Ltd. CLR is employed by OncoMark Ltd. PD is employed by OncoMark Ltd. CAW is employed by OncoMark Ltd. DOL is employed by OncoMark Ltd, declared leadership, ownership and advisory role to OncoMark Ltd. WMG is employed by OncoMark Ltd, declared leadership, ownership, expenses claim and advisory role to OncoMark Ltd and received honoraria from Carrick Therapeutics. MD received honoraria from Myriad Genetics and Roche, is a paid adviser to Radius Health and Orion Pharma GmbH, and receives research from Pfizer and Radius Health. JC has received research grants from AstraZeneca, Myriad Genetics, Memorial Sloan Kettering, Qiagen, Beckton Dickinson, Genera, Aventis Pharma, honoraria from Merck, Roche,Qiagen, Myriad Genetics, and is on the speaker's bureau for Beckton Dickinson and Hologic. All remaining authors declared no conflict of interest.

Translational relevance

In this study we report the validation of the OncoMasTR Risk Score for ER+/HER2- primary breast cancer in 646 postmenopausal patients treated with 5 years' tamoxifen or anastrozole. The OncoMasTR Risk Score combines the expression of three master transcription regulators (MTRs) with nodal status and tumor size. The MTRs (FOXM1, PTTG1 and ZNF367) regulate previously known sets of prognostic

genes and have well-characterised functional roles in several aspects of cancer. The signature categorises patients into the clinically actionable low and high-risk groups. We found that the prognostic information from the OncoMasTR Risk Score was more accurate than that from the Oncotype DX Recurrence Score, the most widely used prognostic signature in ER+ breast cancer.

Abstract

PURPOSE: To test the validity of OncoMasTR Molecular Score (OMm), OMclin1 and OncoMasTR Risk Score (OMclin2) prognostic scores for prediction of distant recurrence (DR) in ER-positive/HER2-negative breast cancer treated with 5 years' endocrine therapy only and compare their performance with the Oncotype DX Recurrence Score (RS).

EXPERIMENTAL DESIGN: OMm incorporates three Master Transcription Regulator genes. OMclin1 combines OMm, tumor size, grade, nodal status; OMclin2 incorporates OMm, tumor size, nodal status. OMclin1 and OMclin2 were evaluated for 646 postmenopausal patients with ER-positive/HER2-negative primary breast cancer with 0-3 involved lymph nodes in TransATAC. Patients were randomised to 5 years' anastrozole or tamoxifen without chemotherapy. RS was available in all cases. We used likelihood ratio-χ^2, C-index, Kaplan-Meier analyses to assess prognostic information.

RESULTS: OMm, OMclin1 and OMclin2 were highly prognostic for prediction of DR in years 0-10 among all patients (LRχ^2=25.4, 48.7, 45.0, respectively, all P<0.001; C-index=0.67, 0.71, 0.71, respectively), compared to RS (LRχ2=18.8, P<0.001; C-index=0.63). All three scores provided significant additional prognostic value beyond Clinical Treatment Score, Nottingham Prognostic Index, Ki67. OMclin1 and OMclin2 categorised 190 and 267 node-negative patients into low-risk group (DR rates: 2.9%, 4.9%, respectively). In comparison, RS categorised 296 node-negative patients as low-risk, 128 patients as intermediate-risk (DR rate: 6.6%, 17.3%, respectively).

CONCLUSIONS: OMm, OMclin1 and OMclin2 were highly prognostic for early and late DR in women with early-stage ER-positive breast cancer receiving 5 years'

endocrine therapy. In TransATAC OMclin1 and the OncoMasTR Risk Score (OMclin2) were superior to RS in identifying patients at increased risk of DR.

Introduction

Over 80% of primary breast cancers are estrogen receptor (ER) positive (1). After surgery, women with ER-positive disease typically receive five years of endocrine therapy which markedly improves prognosis (2). A subset of patients, however, will remain at high risk of relapse if treated with endocrine therapy alone and identifying these is a major challenge in the management of breast cancer (3). Several prognostic gene signatures have been developed to assess residual risk after surgery and to guide treatment decisions including the 21-gene Oncotype DX Recurrence Score (RS), the intrinsic subtype-based Prosigna PAM50 Risk of Recurrence (ROR) score, the Breast Cancer Index (BCI) combining the molecular grade index with a two-gene ratio, the 12-gene EndoPredict (EPclin) and the 70-gene MammaPrint Score (4-8).

While all these signatures provide prognostic information on breast cancer recurrence, there is little overlap between the genes. This suggests that there may be upstream coregulation by other genes that are more fundamentally associated with breast cancer recurrence. The OncoMasTR prognostic gene signature was derived by identifying transcriptional components that regulate the genes contained within existing prognostic signatures. A novel bioinformatic approach (ARACNe: Algorithm for the Reconstruction of Accurate Cellular Networks) identified a shared network of ten master transcriptional regulators (MTRs) underpinning two existing prognostic gene signatures (9): the 231 genes from which 70-gene MammaPrint was derived (8) and the 207 genes from which the 97-gene Genomic Grade Index was derived (10) (Supplementary Figure S1). Chromatin immunoprecipitation (ChIP) studies showed that the MTRs bind, and directly regulate, the promoters of a set of proliferation-associated genes, many of which are highly enriched in breast cancer

prognostic signatures. In addition, MTRs were found to be prognostic at both mRNA and protein levels (11).

The OncoMasTR Molecular Score (OMm) was identified as the most prognostic combination of these MTRs, *FOXM1*, *PTTG1* and *ZNF367*, each of which has been demonstrated to play a critical role in cell proliferation and other key features of malignancy (12-15). OMclin1 combines OMm with nodal status, tumor size and grade. OMclin2 (final OncoMasTR Risk Score) is a simpler form of OMclin1 that excludes tumor grade. Both OMclin1 and OMclin2 stratify patients into low and high risk groups.

The main objective of this study was to clinically validate the OMclin1 and OMclin2 prognostic scores in an independent data set (TransATAC) and to compare their performance with that of the Oncotype DX RS. TransATAC, the translational sub-study of the Arimidex, Tamoxifen, Alone or in Combination trial (ATAC) (16) is a large collection of well-characterised samples from postmenopausal patients with ER-positive, HER2-negative primary breast cancer treated with 5 years' of endocrine therapy only. It served as a validation cohort for the Oncotype DX RS, Prosigna PAM50 ROR, BCI and EndoPredict (EPclin) scores (17-20).

Patients and Methods

Study population

Samples were available from TransATAC (16) where RNA was extracted by Genomic Health Inc. (GHI) (17). Eligibility for the current study required hormone

receptor-positive, HER2-negative disease, chemotherapy-naïve, RS available and sufficient residual RNA for OncoMasTR analysis.

Analytic methods

There were sufficient quantities of residual RNA available from 702 patient samples. To establish if RNA extracted by GHI was suitable to obtain reliable OMm scores a pilot study was conducted. From paired tissue sections of 108 patient samples, RNA was extracted using the process validated for the OMm assay and individual gene measurements and OMm scores were compared with that obtained from GHI extracted RNA.

One-hundred to 200ng RNA was used to measure expression of the six genes (the three genes of interest and three reference genes; *GAPDH*, *GUSB*, *TFRC*) constituting OMm by RT-qPCR performed by OncoMark. Data from 14 of the 702 samples did not meet the pre-specified OncoMasTR data quality criteria and were excluded from statistical analyses. All genes were measured in triplicate. The relative expression level of each OMm gene of interest (∆Cq GOI) was calculated as follows: ∆Cq GOI = GeometricMean(Mean(GAPDH triplicates), Mean(GUSB triplicates), Mean(TFRC triplicates)) – Mean(GOI triplicates). The three ∆Cq values were then used to calculate the continuous molecular risk score according to the OMm prognostic algorithm. Thresholds for the numeric score to stratify patients into low and high risk groups were based on sensitivity and specificity in the training cohort. For OMclin1, the threshold was the numeric score value that maximised the sum of sensitivity and specificity (Youden Index). The resulting risk groups had Kaplan-Meier DR rates of 4% and 33% in the training cohort. For OMclin2, the threshold was the numeric score value at which both sensitivity and specificity were 0.7. The resulting risk groups had Kaplan-Meier DR rates of 8% and 36% in the

training cohort. In TransATAC, unscaled OMclin1 and OMclin2 scores ranged between -4.13 to 2.19 and -4.60 to 1.65, respectively. In order to present the scores in a more intuitive, user-friendly way the scores were rescaled to range between 0 and 10 with the following equations: rescaled OMclin1_score = raw score*1.2 + 6.0258; rescaled OMclin2 = raw score*1.2 + 7.0059. In each case the scaling resulted in the high-low risk cut-off having a value of 5. The linear transformations retained the shape of the distribution of the unscaled scores.

These analytic methods were performed by OncoMark blinded to clinico-pathological information and clinical outcome.

Study End Points

The prospectively defined primary endpoint was distant recurrence-free survival (DRFS) defined as the interval from diagnosis until distant recurrence, or death due to breast cancer. Contralateral breast cancer and death due to causes other than breast cancer were censoring events. Death due to breast cancer where a recurrence had not been recorded was treated as an event with the event date being the date of death.

Statistical Analyses

Analyses were performed using 10-year median follow-up outcome data (16) according to a pre-specified statistical analysis plan approved by the Long-term Anastrozole vs Tamoxifen Treatment Effects (LATTE) committee and OncoMark Ltd before data analysis.

Our stepwise primary objectives were to assess whether OMm had statistically significant prognostic information for 10-year DR as a continuous variable

and as a categorical variable. If so, we would test OMclin1 as continuous score and as categorical variable. Secondary analyses included testing the prognostic value of OMm and OMclin1 in early (0-5 years) and late (5-10 years) settings, in patients divided into subgroups by nodal status, and to test if additional prognostic information was provided when added to the clinical treatment score (CTS), Nottingham Prognostic Index (NPI) and Ki67 measured by immunohistochemistry. Subsequently, OMclin2 was added to the analysis plan due to further optimisation of clinico-pathological features and was subjected the same analyses as OMclin1.

Briefly, Cox proportional hazards regression models were fitted and hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated. Likelihood ratio (LR) tests were used for hypothesis testing. As previously reported, the CTS integrated the prognostic information from nodal status, tumor size, histopathologic grade, age and type of endocrine treatment (21). All statistical tests were two-sided, a P value of less than 0.05 was regarded as statistically significant. All statistical analyses were performed with STATA version 13.1 (College Station, TX) at the Queen Mary University of London. This study was approved by the South-East London Research Ethics Committee, and all patients included gave informed consent. This study meets the REMARK recommendations.

Results

Sample availability is shown in the CONSORT diagram (Figure 1). An OncoMasTR Molecular Score was obtained for 688 patients, of whom we are reporting results for node-negative and node 1-3 positive patients in this study (i.e. excluding those with 4 or more positive nodes). For 648 OMm data was available and 646 had data on OMclin1 and OMclin2 (due to missing clinic-pathological data). The characteristics of

this TransATAC cohort are presented in Supplementary Table S1. A total of 88 DRs were recorded within the 10-year median follow-up period. There were 50 DRs in node-negative women (n = 482) and 38 DRs were detected in women with node-positive disease (n = 164).

The pilot study demonstrated the suitability of the pre-extracted RNA for OMm analysis (Supplementary Figure S2). Pearson's correlation coefficients for *FOXM1*, *PTTG1* and *ZNF367* were 0.93, 0.81 and 0.80 respectively; for OMm Pearson's correlation coefficient was 0.91.

Univariate analyses of continuous prognostic scores

OMm, OMclin1 and OMclin2 were highly prognostic for the whole population across 10 years, with OMclin1 and OMclin2 providing substantially more information than the molecular OMm score alone (LR-χ^2: OMm = 25.4; OMclin1 = 48.7, OMclin2 = 45.0) (Table 1). OMm was also significantly prognostic in the early and late settings and in node-negative patients however, OMm provided no significant information in the node-positive population. OMclin1 and OMclin2 were significantly prognostic across all sub-populations examined, except for 0-5 years in the node-positive subgroup which was not significant. OMm, OMclin1 and OMclin2 also provided significantly more prognostic information in 0-10 years than RS in all patients (LR-χ^2: RS = 18.8). This was driven by the node-negative group where RS was also inferior. However, in node-positive patients, OMm and RS were equally uninformative. OMclin1 and OMclin2 were also highly prognostic for the prediction of late DR (LRχ2= 25.6 and LRχ2= 25.1, respectively, $P < 0.001$).

C-index statistics calculated for the scores showed superior model fit of OMm, OMclin1 and OMclin2 when compared to RS (C-index: OMm = 0.666; OMclin1 = 0.708; OMclin2 = 0.713; RS = 0.634).

Multivariable analyses of continuous prognostic scores

Multivariable comparisons with CTS are shown in Table 1. Across 10 years in the overall population OMm, OMclin1, OMclin2 and RS all provided significantly more prognostic information beyond that of the CTS, with RS providing the least amount of information (LR-$\Delta\chi^2$: 13.9; 15.8; 15.8 and 10.7 for OMm, OMclin1, OMclin2 and RS, respectively). Similar results were observed in the node-negative subgroup. However, in node-positive patients none of the scores added significant prognostic value to CTS. OMm, OMclin1 and OMclin2 also added significant information to CTS in the early and late settings in the overall population. This was led by their good performance in the node-negative cohort, in contrast to the node-positive group where none of the signatures remained significant when added to CTS. Consistent with the analysis of the continuous scores, Kaplan-Meier analysis of CTS (categorised at the median) vs a CTS+OMclin2 composite score (categorised at the median) showed that CTS+OMclin2 provided better separation than CTS alone in node-negative patients but not in the node-positive group (Supplementary Figure S3).

A similar pattern emerged in the multivariable comparisons with NPI: OMm, OMclin1 and OMclin2 all added significant prognostic information to NPI in all patients across 10 years (LR-$\Delta\chi^2$: 9.4; 11.5 and 13.7 for OMm, OMclin1 and OMclin2, respectively) (Supplementary Table S2). Similar to the comparisons with CTS, no significant added information to NPI was found in the node-positive subgroup analyses. OMm,

OMclin1 and OMclin2 also added significant prognostic information to Ki67 in all patients (LR-$\Delta\chi^2$: 9.4; 30.5 and 27.1 for OMm, OMclin1 and OMclin2, respectively) (Supplementary Table S3). No significant added information was found for OMm in the early setting (LR-$\Delta\chi^2$: 2.5 for OMm) contrary to after five years where it provided additional information (LR-$\Delta\chi^2$: 7.2 for OMm).

Categorical analyses

Using pre-defined cut-offs, the distribution between low and high risk groups for OMclin1 was 219 (33.9%) vs 427 (66.1%) patients and for OMclin2 it was 305 (47.2%) vs 341 (52.8%) patients (Table 2, Figure 2). The mean DR rates at 10 years were 4.0% (2.0-7.9) vs 21.2% (17.3-25.7) for OMclin1 and 5.4% (3.3-8.8) vs 24.3% (19.8-29.7) for OMclin2 in the low and high risk groups, respectively. Greater hazard ratio was found between the high and low risk groups for OMclin1 than for OMclin2: 5.8 (2.8-12.0) vs 4.9 (2.8-8.6) but the difference was not statistically significant. More patients were categorised as low risk by RS (389, 60.2%) than by OMclin1 and OMclin2, however at 9.9% the RS low risk group had substantially greater DR risk than the low risk groups by OMclin1 (4.0%) and OMclin2 (5.4%) (Table 2). We combined the RS intermediate- and high-risk groups to create an RS non-low risk group. OMclin1 and OMclin2 categorised 427 and 341 patients into high risk category compared to 257 in the non-low group of RS. The corresponding DR rates for the three groups were similar at 21.2%, 24.3% and 23.4%, respectively. Figure 3 shows the continuous relationship between OMclin1, OMclin2 scores and 10-year DR risk. OMclin2 corresponds to higher risk than OMclin1 at the cut-off point for risk categorisation.

In women with node-negative disease, OMclin1 identified 39.4% of women as low risk with a 10-year DR risk of 2.9% (1.2-6.8), which was significantly lower compared to those categorised as high risk (10-year DR risk: 17.3% (13.2-22.6); hazard ratio of high risk vs low risk HR = 6.5 (2.6-16.3)). OMclin2 categorised 55.4% of patients as low risk with a 10-year DR rate of 4.9% (2.8-8.5) compared to 19.9% (14.8-26.4) in the high risk group (hazard ratio of high risk vs low risk HR = 4.3 (2.3-8.3)). This compared to 296 (61.4%) low risk patients by RS with a 10-year DR rate of 6.6%. Additionally, we applied the cut-off points for RS used in the TAILORx trial (Tx) to assign patients to treatment (22). In the node-negative group 145, 240 and 97 patients were categorised into the Tx low (RS <11), Tx intermediate (RS 11-25) and Tx high (RS >25) groups, respectively with DR rates of 9.3%, 8.2% and 23.5%, respectively.

In node-positive disease, the hazard ratio for OMclin1 low vs high risk was non-significant at HR = 2.9 (0.9-9.5); however, for OMclin2, the hazard ratio was significant at HR = 4.2 (1.3-13.6).

Patient scores by RS plotted against OMm, OMclin1 and OMclin2 scores is presented in Figure 4. Score distribution by nodal status was different for OMclin1 and OMclin2 with a shift of node-positive patients towards higher risk, not seen for RS and OMm. Spearman's rho correlation coefficient was similarly modest across the scores: RS vs OMm (rho = 0.30), RS vs OMclin1 (rho = 0.34) and RS vs OMclin2 (rho = 0.29). Similar correlation coefficients were found in the node-negative subgroups for the three comparisons: RS vs OMm (rho = 0.28), RS vs OMclin1 (rho = 0.34) and RS vs OMclin2 (rho = 0.29).

Discussion

The currently available commercial prognostic signatures for ER-positive breast cancer were trained and discovered using gene expression profiling of breast cancer samples and have generally resulted in panels including a large number of genes. OMm was discovered through querying the dependencies between genes from two well-validated breast cancer prognostic signatures which resulted in the identification of a shared transcriptional network of MTRs upstream of the signatures (9, 11). *FOXM1*, *PTTG1* and *ZNF367* have been demonstrated to play critical roles in tumor progression. The *FOXM1* (Forkhead Box M1) gene encodes a forkhead transcription factor which controls cell proliferation, maintenance of stem cell properties, invasion and metastasis and is associated with poor prognosis in ER-positive patients treated with tamoxifen (12). *PTTG1* (Pituitary Tumor Transforming Gene 1) promotes tumor metastasis through enhancing the proliferation, invasion and metastasis of cancer cells (13). Elevated levels of its protein product, securin, is an independent prognosticator of breast cancer-specific survival even among invasive ductal breast carcinoma with low Ki-67 positivity (14). *ZNF367* (Zinc Finger Protein 367, also known as *ZFF29* and *CDC14B*) is found to be overexpressed in a variety of endocrine cancers. It is reported to inhibit *in vitro* and *in vivo* growth, cellular invasion, migration and adhesion to extracellular proteins, suggesting a protective role by inhibiting cancer progression (15). Thus, biologically, the signature consists of genes that regulate previously known prognostic genes and have identified functional roles in several hallmarks of cancer including cell proliferation, invasion and metastasis. The clinically applicable signature incorporates clinico-pathological information, and categorises patients into clinically actionable low or high risk groups.

In this TransATAC study, we showed that the OncoMasTR Molecular Score (OMm), OMclin1 and the OncoMasTR Risk Score (OMclin2) have statistically significant prognostic ability for distant recurrence in breast cancer patients with ER-positive, HER2-negative disease who received five years' of endocrine therapy. All three scores were significantly prognostic as continuous variables in the early and late settings and in the node-negative groups. However, no substantial prognostic information was found in the node-positive group. This might be at least in part due to the exclusion of patients with 4 or more involved nodes in this validation study and the associated lower number of events in this group. OMclin1 and OMclin2 provided a similar degree of prognostic information and both outperformed the purely molecular OMm score. This finding underlines the prognostic value of clinico-pathological features and the importance of predictors incorporating them for accurate prognostics. The exclusion of grade for OMclin2 did not substantially affect its performance. Comparing the molecular-only scores in the 10-year follow-up period, OMm was found to be moderately superior to RS suggesting that the three MTR genes might be better at capturing key aspects of breast cancer recurrence than the RS algorithm made up of 16 prognostic genes. However, the limited size of the study population and this modest difference means that actual superiority of the OMm should be regarded as uncertain.

To perform a fair comparison of the molecular RS score with OMclin1 and OMclin2, we examined the added prognostic information of these scores to CTS. Both OMclin1 and OMclin2 were found to be modestly superior to RS in the overall and in the node-negative groups in this population; however, none of the three signatures added value to CTS in the node-positive group.

Risk categorisation by OMclin1 and OMclin2 based on pre-defined cut-offs showed a clear separation of low and high risk groups in the overall and node-negative groups. In node-positive patients, OMclin1 showed reduced prognostic performance; however, OMclin2 remained significantly prognostic. Previous data has shown the reduced prognostic power of RS in the late period was partly due to high ER expression being associated with poor prognosis after endocrine treatment ceased at five years, contrary to ER's coefficient in the RS algorithm (23).

Our study has strengths and limitations. Strengths include prospectively defined standardised assays (OMm, OMclin1 and OMclin2) for which data was obtained by personnel blinded to the clinical data and the results of previous assays performed. For this comparison, the same batch of RNA was assayed to measure OMm as was used for RS. Before performing the study, we compared results from GHI-extracted RNA with that of OncoMark-extracted RNA to ensure the RNA samples were suitable for OMm analysis. Our validation cohort is from a large, well-documented prospective randomised clinical trial with long-term follow-up. Limitations include that the patients in TransATAC are from the United Kingdom only and extrapolation of the results to other cohorts may be limited. Our findings are applicable to postmenopausal patients with HER2-negative disease who have not received chemotherapy treatment. CTS was trained in TransATAC and its prognostic performance is marginally better than we would observe in other cohorts. The added information from the molecular scores to this may therefore be somewhat understated. This set of samples is a small subset of the ATAC population but our intention was to make use of this highly annotated group to represent relatively low risk ER-positive disease rather than to represent ATAC *per se*. The prognostic performance of RS in the current study (both univariate and multivariate analyses

with CTS) was lower than that reported previously in the more complete TransATAC cohort (17). Supplementary Table S4 shows the demographic differences between those included in the current study and those that were not included from the earlier study. Of particular note was the difference in performance noted for RS in the two studies. This may be partly explained by our exclusion of HER2+ cases from the current study because contemporary use of molecular signatures is confined to HER2- disease. Also, the node-positive group in the current analysis was restricted to those with 1-3 positive nodes. In addition, because of these eligibility criteria and reduced sample availability, fewer samples were analyzable in the current study compared to the previously published TransATAC studies. This inevitably leads to reduced χ^2 values.

Based on these findings, further validation studies are warranted to assess some key questions such as i) is the performance of the OncoMasTR compared to RS found here confirmed in other cohorts? ii) with sufficient sample size, does OncoMasTR add significant prognostic value to clinical information among lymph node positive patients? iii) is OncoMasTR predictive for therapy benefit? iv) is OncoMasTR prognostic and/or predictive among premenopausal women?

In summary, our study confirmed the independent prognostic ability of OMm, OMclin1 and OMclin2 in postmenopausal patients with ER-positive breast cancer given five years' of endocrine therapy. Furthermore, we showed that based on a modest enhancement of OMm over RS and also on the incorporation of clinical factors OMclin1 and the simpler OncoMasTR Risk Score (OMclin2) were superior in this population to Oncotype DX Recurrence Score in identifying patients at increased risk of distant recurrence. Further study is required to confirm these findings in other cohorts.

Acknowledgments

This work was supported by Breast Cancer Now, working in partnership with Walk the Walk and by the Royal Marsden National Institutes of Health Biomedical Research Centre and Cancer Research UK grant awarded to JC (C569/A16891). WMG was supported by the Irish Cancer Society Collaborative Cancer Research Centre BREAST-PREDICT (CCRC13GAL) and Science Foundation Ireland (SFI) under grant no. 15/IA/3104. OncoMark received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 698630.

References

1. Dodson A, Parry S, Ibrahim M, Bartlett JMS, Pinder S, Dowsett M, et al. Breast cancer biomarkers in clinical testing: analysis of a UK NEQAS ICC & ISH database containing results from 199,300 patients. J Pathol Clin Res 2018.
2. Dowsett M, Forbes JF, Bradley R, Ingle J, Aihara T, Bliss J, et al. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 2015;386:1341-52.
3. Pan H, Gray R, Braybrooke J, Davies C, Taylor C, McGale P, et al. 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med 2017;377:1836-46.
4. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351:2817-26.
5. Nielsen TO, Parker JS, Leung S, Voduc D, Ebbert M, Vickery T, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. Clin Cancer Res 2010;16:5222-32.
6. Jerevall PL, Ma XJ, Li H, Salunga R, Kesty NC, Erlander MG, et al. Prognostic utility of HOXB13:IL17BR and molecular grade index in early-stage breast cancer patients from the Stockholm trial. Br J Cancer 2011;104:1762-9.
7. Filipits M, Rudas M, Jakesz R, Dubsky P, Fitzal F, Singer CF, et al. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. Clin Cancer Res 2011;17:6012-20.
8. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530-6.

9. Moran B, Rahman A, Palonen K, Lanigan FT, Gallagher WM. Master Transcriptional Regulators in Cancer: Discovery via Reverse Engineering Approaches and Subsequent Validation. Cancer Res 2017;77:2186-90.
10. Sotiriou C, Wirapati P, Loi S, Harris A, Fox S, Smeds J, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst 2006;98:262-72.
11. Lanigan F, Brien GL, Fan Y, Madden SF, Jerman E, Maratha A, et al. Delineating transcriptional networks of prognostic gene signatures refines treatment recommendations for lymph node-negative breast cancer patients. FEBS J 2015;282:3455-73.
12. Bergamaschi A, Madak-Erdogan Z, Kim YJ, Choi YL, Lu H, Katzenellenbogen BS. The forkhead transcription factor FOXM1 promotes endocrine resistance and invasiveness in estrogen receptor-positive breast cancer by expansion of stem-like cancer cells. Breast Cancer Res 2014;16:436.
13. Liao YC, Ruan JW, Lua I, Li MH, Chen WL, Wang JR, et al. Overexpressed hPTTG1 promotes breast cancer cell invasion and metastasis by regulating GEF-H1/RhoA signalling. Oncogene 2012;31:3086-97.
14. Talvinen K, Karra H, Hurme S, Nykanen M, Nieminen A, Anttinen J, et al. Securin promotes the identification of favourable outcome in invasive breast cancer. Br J Cancer 2009;101:1005-10.
15. Jain M, Zhang L, Boufraqech M, Liu-Chittenden Y, Bussey K, Demeure MJ, et al. ZNF367 inhibits cancer progression and is targeted by miR-195. PLoS One 2014;9:e101423.
16. Cuzick J, Sestak I, Baum M, Buzdar A, Howell A, Dowsett M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. Lancet Oncol 2010;11:1135-41.
17. Dowsett M, Cuzick J, Wale C, Forbes J, Mallon EA, Salter J, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. J Clin Oncol 2010;28:1829-34.
18. Dowsett M, Sestak I, Lopez-Knowles E, Sidhu K, Dunbier AK, Cowens JW, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. J Clin Oncol 2013;31:2783-90.
19. Sgroi DC, Sestak I, Cuzick J, Zhang Y, Schnabel CA, Schroeder B, et al. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. Lancet Oncol 2013;14:1067-76.
20. Buus R, Sestak I, Kronenwett R, Denkert C, Dubsky P, Krappmann K, et al. Comparison of EndoPredict and EPclin With Oncotype DX Recurrence Score for Prediction of Risk of Distant Recurrence After Endocrine Therapy. J Natl Cancer Inst 2016;108.
21. Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J Clin Oncol 2011;29:4273-8.
22. Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, et al. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. N Engl J Med 2018;379:111-21.
23. Dowsett M, Sestak I, Buus R, Lopez-Knowles E, Mallon E, Howell A, et al. Estrogen Receptor Expression in 21-Gene Recurrence Score Predicts Increased Late Recurrence for Estrogen-Positive/HER2-Negative Breast Cancer. Clin Cancer Res 2015;21:2763-70.

Tables

			CTS		OMm*		OMclin1		OMclin2		OMm + CTS vs CTS		OMclin1 + CTS vs CTS		OMclin2 + CTS vs CTS		RS		RS + CTS vs CTS	
	No. of patients	**No. of DR**	**LR-χ^2**	**P**	**LR-χ^2**	**P**	**LR-χ^2**	**P**	**LR-χ^2**	**P**	**LR-$\Delta\chi^2$**	**P**	**LR-$\Delta\chi^2$**	**P**	**LR-$\Delta\chi^2$**	**P**	**LRχ^2**	**P**	**LR-$\Delta\chi^2$**	**P**
All patients																				
0-10 years	646	88	41.7	<0.001	25.4	<0.001	48.7	<0.001	45.0	<0.001	13.9	<0.001	15.8	<0.001	15.8	<0.001	18.8	<0.001	10.7	0.001
0-5 years	646	39	22.2	<0.001	12.6	<0.001	23.2	<0.001	20.0	<0.001	6.3	0.012	6.3	0.01	5.5	0.02	-	-	-	-
5-10 years	571	49	19.7	<0.001	12.8	<0.001	25.6	<0.001	25.1	<0.001	7.5	0.006	9.5	0.002	10.4	0.001	-	-	-	-
Node-negative patients																				
0-10 years	482	50	23.3	<0.001	23.5	<0.001	31.3	<0.001	30.4	<0.001	12.0	<0.001	12.8	<0.001	13.0	<0.001	15.0	<0.001	7.0	0.008
0-5 years	482	21	14.9	<0.001	14.5	<0.001	19.3	<0.001	16.5	<0.001	7.2	0.007	7.8	0.005	6.2	0.01	-	-	-	-
5-10 years	436	29	9.1	0.003	9.5	0.003	13.0	0.001	14.2	<0.001	4.9	0.03	9.5	0.002	10.4	0.001	-	-	-	-
Node-positive patients																				
0-10 years	164	38	5.6	0.02	3.2	0.07	6.0	0.02	4.3	0.04	2.3	0.13	2.4	0.13	2.1	0.15	3.5	0.06	2.9	0.09
0-5 years	164	18	2.4	0.12	0.7	0.42	1.3	0.27	0.8	0.37	0.3	0.58	0.2	0.67	0.15	0.70	-	-	-	-
5-10 years	135	20	3.2	0.07	2.8	0.09	5.3	0.03	4.0	0.05	2.4	0.12	2.9	0.09	2.6	0.11	-	-	-	-

Table 1. Likelihood (χ^2) for distant recurrence for CTS, OMm, OMclin1, OMclin2 and RS continuous prognostic scores in all patients and subgroups. Likelihood ratio test based on Cox proportional hazard models for univariate and multivariable analyses. Comparisons with RS are presented for the 0-10 years time period only. *OMm was available for 648 patients. DR, distant recurrence; CTS, clinical treatment score; OMm, OncoMasTR Molecular Score; OMclin2, OncoMasTR Risk Score; RS, Recurrence Score; LR, likelihood ratio

	OMclin1		OMclin2		RS						
	low	**high**	**low**	**high**	**low (<18)**	**intermediate (18-31)**	**high (>31)**	**non-low (≥18)**	**Tx low (<11)**	**Tx intermediate (11-25)**	**Tx high (>25)**
All patients											
No. of patients	219	427	305	341	389	177	80	257	188	325	133
10-year DR risk (95% CI)	4.0% (2.0-7.9)	21.2% (17.3-25.7)	5.4% (3.3-8.8)	24.3% (19.8-29.7)	9.9% (7.1-13.7)	21.5% (15.9-28.6)	27.7% (18.7-39.8)	23.4% (18.4-29.4)	12.1% (8.0-18.1)	12.9% (9.5-17.5)	25.5% (18.6-34.3)
Hazard Ratio (95% CI)	reference	5.81 (2.81-12.01)	reference	4.93 (2.83-8.60)	reference	2.55 (1.58-4.10)	3.64 (2.09-6.34)	2.86 (1.85-4.40)	reference	1.08 (0.63-1.86)	2.57 (1.46-4.50)
Node-negative patients											
No. of patients	190	292	267	215	296	128	58	186	145	240	97
10-year DR risk (95% CI)	2.9% (1.2-6.8)	17.3% (13.2-22.6)	4.9% (2.8-8.5)	19.9% (14.8-26.4)	6.6% (4.1-10.5)	17.3% (11.5-25.6)	24.6% (15.0-38.8)	19.6% (14.3-26.5)	9.3% (5.4-15.8)	8.2% (5.1-13.0)	23.5% (15.9-33.8)
Hazard Ratio (95% CI)	reference	6.47 (2.57-16.29)	reference	4.31 (2.25-8.25)	reference	2.96 (1.55-5.66)	4.73 (2.30-9.76)	3.47 (1.93-6.24)	reference	0.84 (0.40-1.76)	3.04 (1.49-6.18)
Node-positive patients											
No. of patients	29	135	38	126	93	49	22	71	43	85	36
10-year DR risk (95% CI)	11.8% (3.9-32.6)	30.0% (22.4-39.4)	8.7% (2.9-24.7)	32.4% (24.3-42.3)	21.3% (13.5-32.6)	31.9% (20.6-47.3)	36.5% (19.0-62.4)	33.3% (23.3-46.1)	22.4% (11.7-40.2)	26.9% (18.1-38.8)	31.3% (18.1-50.6)
Hazard Ratio (95% CI)	reference	2.91 (0.89-9.46)	reference	4.17 (1.28-13.57)	reference	1.93 (0.95-3.90)	2.37 (0.97-5.78)	2.05 (1.07-3.90)	reference	1.35 (0.60-3.08)	1.74 (0.69-4.41)

Table 2. 10-year distant recurrence risk of patients groups as categorised by, OMclin1, OMclin2 and RS. DR, distant recurrence; OMclin2, OncoMasTR Risk Score; RS, Recurrence Score; CI, confidence interval; Tx, TAILORx

Figure legends

Figure 1. CONSORT diagram of the availability of samples for analysis from the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial. ER, estrogen receptor; PgR progesterone receptor; RS, Recurrence Score; QC, quality control; LN, lymph node.

Figure 2. Kaplan-Meier plots for 10 year distant recurrence for OMclin1 and OMclin2 risk groups in all patients, node-negative and node-positive patients. The numbers of patients at risk in each group at various time points are given below each graph. HR, hazard ratio; CI, confidence interval.

Figure 3. Likelihood of distant recurrence as a continuous function of OMclin1 and OMclin2 and 95% confidence interval (dashed lines). Vertical line represents cut-off point for low and high risk categorisation.

Figure 4. Distribution of prognostic scores. Scatterplot of Recurrence Score with OMm, OMclin1 and OMclin2 for 646 patients. Blue circles indicate node-negative, red circles indicate node-positive patients, dashed line shows cut points for risk stratification. Spearman's rho and p-values are presented.

figure 1

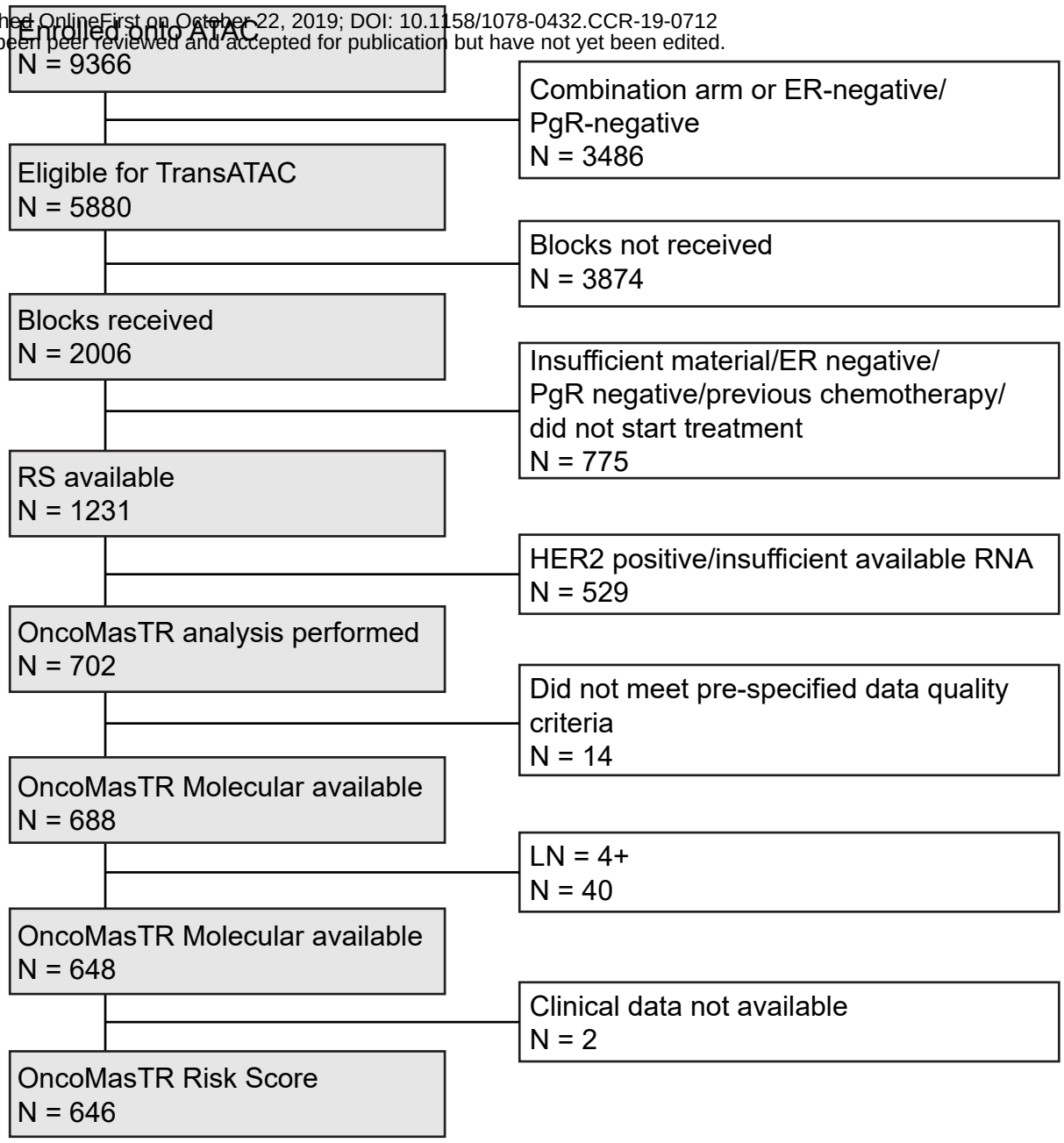


figure 2

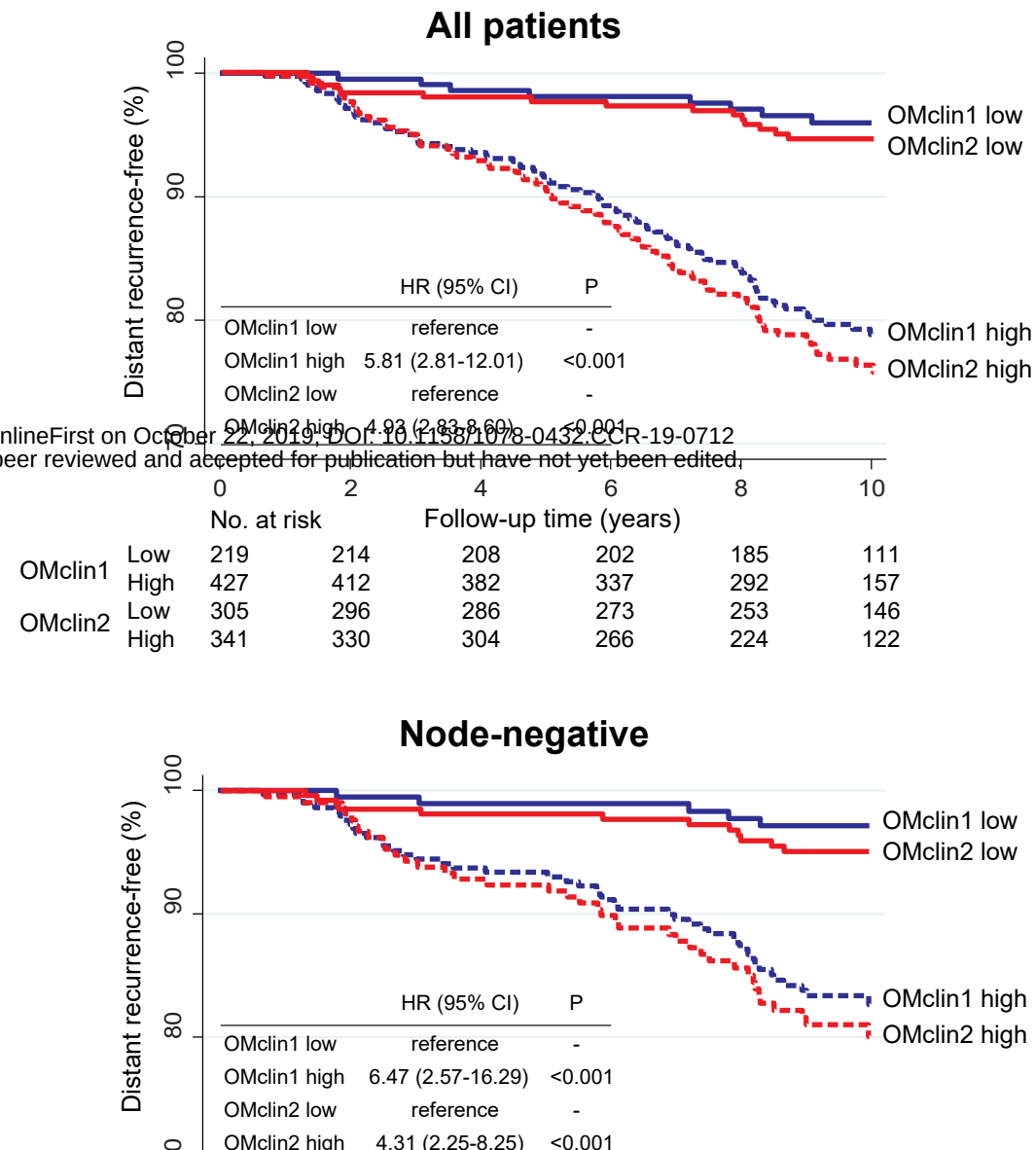


figure 3

Author Manuscript Published OnlineFirst on October 22, 2019; DOI: 10.1158/1078-0432.CCR-19-0712
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

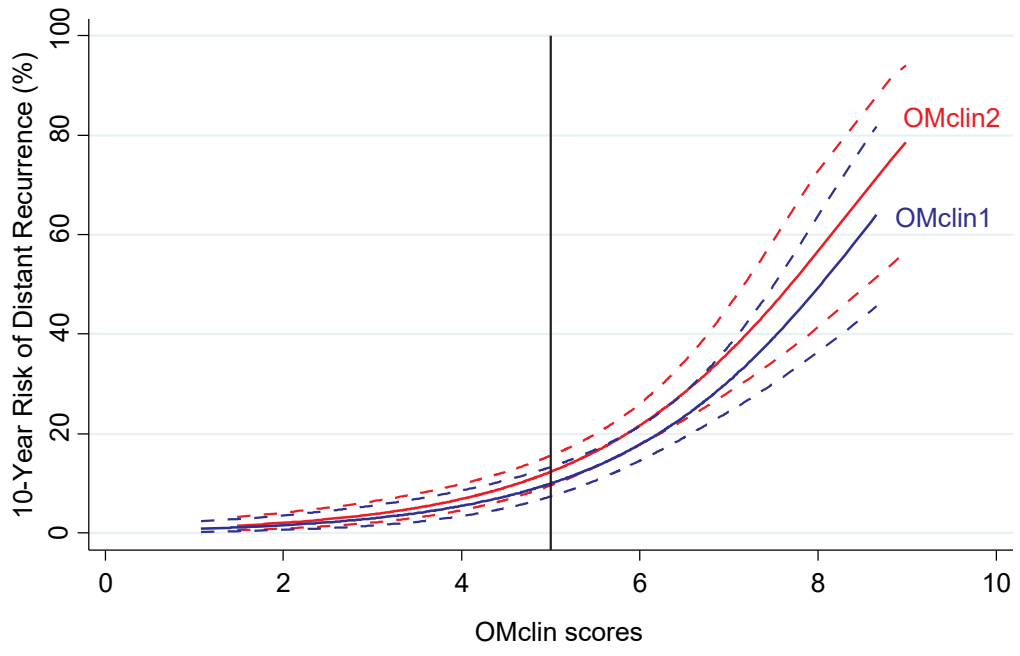
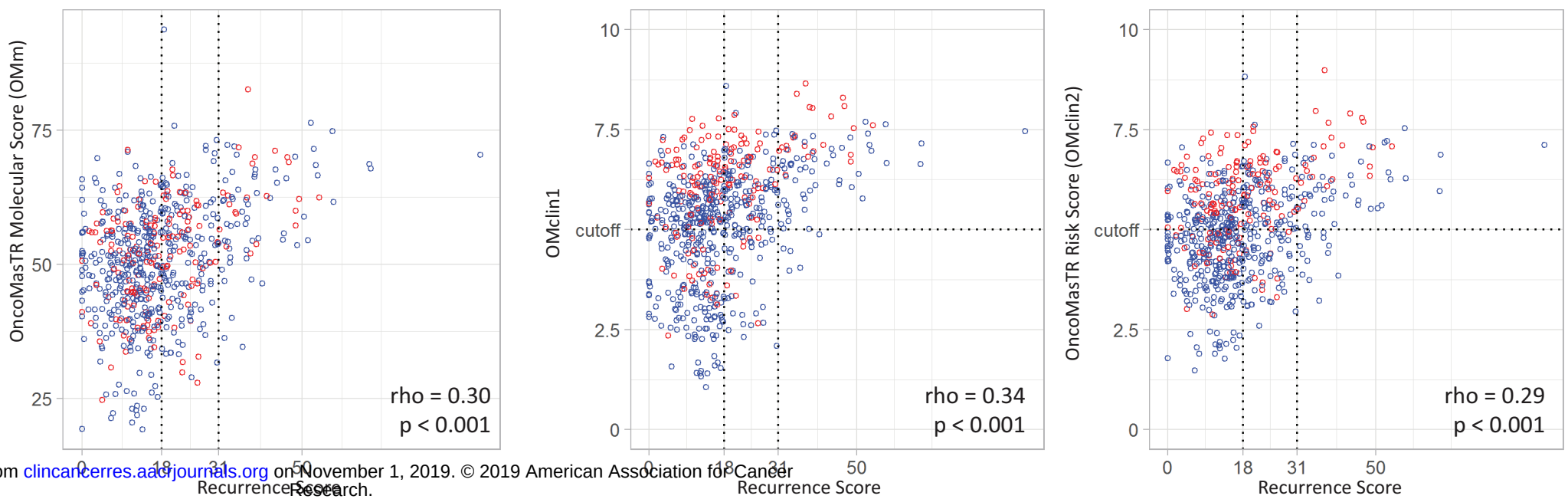


figure 4



AACR American Association for Cancer Research

Clinical Cancer Research

Validation of the OncoMasTR Risk Score in Estrogen Receptor-Positive/HER2-Negative Patients: a TransATAC study

Richard Buus, Ivana Sestak, Stephen Barron, et al.

Clin Cancer Res Published OnlineFirst October 22, 2019.