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Original Research

Ten-year follow-up of the observational RASTER study, prospective evaluation of the 70-gene signature in ER-positive, HER2-negative, node-negative, early breast cancer

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MammaPrint;
70-gene signature;
Early breast cancer;
Node-negative;
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HER2-negative;
Gene expression profile;
Prognostic;
Observational;
Prospective

Abstract ***Introduction:*** Prognostic gene expression signatures can be used in combination with classical clinicopathological factors to guide adjuvant chemotherapy decisions in ER-positive, HER2-negative breast cancer. However, long-term outcome data after introduction of genomic testing in the treatment decision-making process are limited.
Methods: In the prospective RASTER study, the tumours of 427 patients with cTanyN0M0 breast cancer were tested to assess the 70-gene signature (MammaPrint). The results were provided to their treating physician to be incorporated in the decision-making on adjuvant systemic therapy. Here, we report the long-term outcome of the 310 patients with ER-positive, HER2-negative tumours by clinical and genomic risk categories at a median follow-up of 10.3 years.
Results: Among the clinically high-risk patients, 45 (49%) were classified as genomically low risk. In this subgroup, at 10 years, distant recurrence free interval (DRFI) was similar between patients treated with (95.7% [95% CI 87.7–100]) and without (95.5% [95% CI 87.1–100]) chemotherapy. Within the group of clinically low-risk patients, 56 (26%) were classified as genomically high risk. Within the clinically low-risk group, beyond 5 years, a difference emerged between the genomically high- and low-risk subgroup resulting in a 10-year DRFI of 84.3% (95% CI 74.8–95.0) and 93.4% (95% CI 89.5–97.5), respectively. Interestingly, genomic ultralow-risk patients have a 10-year DRFI of 96.7% (95% CI 90.5–100), largely (79%) without systemic therapy.
Conclusions: These data confirm that clinically high-risk, genomically low-risk tumours have an excellent outcome in the real-world setting of shared decision-making. Together with the updated results of the MINDACT trial, these data support the use of the MammaPrint, in ER-positive, HER2-negative, node-negative, clinically high-risk breast cancer patients.
Registry: ISRCTN71917916

1. Introduction

The prognosis of early stage breast cancer has improved impressively with the introduction of (neo) adjuvant chemotherapy and endocrine therapy [1–3]. However, as chemotherapy is associated with short- and long-term toxicities that can substantially impact a patient's quality of life, overtreatment remains an important concern [4]. A critical factor in the decision to treat a patient with (neo)adjuvant chemotherapy is the risk of disease recurrence, which can be estimated using various prognostic models taking into account clinical and pathological factors [5,6]. In addition, several gene expression signatures have been developed that aim to distinguish patients with high-risk tumours who need chemotherapy, from patients with lower-risk tumours who can safely forgo chemotherapy without compromising their long-term outcome [7–11]. Three large prospectively randomised clinical trials, MINDACT, TAILORx, and RxPONDER, have shown that the 70-gene prognosis signature (MammaPrint, Agendia, Amsterdam, the Netherlands) and the 21-gene recurrence score (Oncotype DX, Exact Sciences, Madison, WI, USA) both are able to identify a patient population in which omitting chemotherapy does not meaningfully affect disease recurrence risk after a median follow-up of 8–9 years [12–14].

The use of these gene expression signatures has been adopted in various clinical guidelines for patients with ER-positive, HER2-negative tumours, who are candidates for (neo)adjuvant chemotherapy based on their clinicopathological characteristics [6,15–17]. However, the risk of disease recurrence for ER-positive, HER2-negative breast cancer is approximately stable over time up to at least 20 years after diagnosis [18]. Therefore, evidence concerning the impact on long-term outcome of incorporation of genomic risk classification in the treatment decision-making process is clearly warranted. We here report outcomes for the ER-positive, HER2-negative subgroup in the RASTER study after a median follow-up of 10.3 years. The observational RASTER study is a prospective clinical study evaluating the 70-gene signature and aimed to assess the feasibility of the implementation of MammaPrint as a diagnostic test in a series of unselected patients with node-negative breast cancer in community hospitals [19,20]. The RASTER study, as a precursor to the randomised MINDACT trial, did not dictate treatment based on MammaPrint results, but rather allowed the treating physician to incorporate the genomic risk classification into the adjuvant treatment decision. Therefore, this

study provides important insights on the association between MammaPrint classification and outcome in the context of shared decision-making in daily clinical practice. To our knowledge, the RASTER study is the first trial to prospectively evaluate the 70-gene signature in node-negative breast cancer at 10 years after diagnosis.

2. Methods

2.1. Study population

The design of the RASTER study (ISRCTN71917916) has been published previously [19]. In short, the RASTER study was a prospective observational study, which enrolled female patients from 16 community hospitals with clinical unilateral TanyN0 breast cancer. Initially, patients had to be under the age of 61 at the time of diagnosis. After a protocol amendment in 2004, only patients aged 55 or younger could enrol. The current analysis focusses on patients with ER-positive, HER2-negative disease, excluding patients with HER2-positive (3+ with immunohistochemistry (IHC) or 2+ and HER2 amplification on in situ hybridisation) or ER-negative (<10%) tumours based on central pathology revision. The RASTER study was approved by the Medical Ethical Committee of the Netherlands Cancer institute, and patients provided written informed consent for additional analysis and data collection concerning clinical outcome.

2.2. Genomic and clinical risk profile

For all patients, a tumour sample was collected in RNAlater at the time of surgery and subsequently frozen. mRNA was extracted and gene expression was assessed using a 1.9k Agilent microarray (k070675) at the Agendia Laboratories, Amsterdam, the Netherlands [19,21,22]. This was an earlier version of the microarray used in the MINDACT study [12]. A patient's MammaPrint risk classification was returned to the treating physician who could take it into account in their (shared) treatment decision, i.e., treatment was not dictated by the study protocol. More information on the shared-decision-making process and the impact of clinical guidelines, genomic risk, and patient preference can be found in the baseline RASTER manuscript [19]. Retrospectively, we also assessed formalin-fixed, paraffin-embedded (FFPE) tissue of these tumours using a novel version of the MammaPrint assay (FDA k141142) [23]. These results were not returned to the treating physician. The ultra-low classification was determined based on the MammaPrint index using the established cut-off of 0.355 [24].

Clinical risk at the time of inclusion was based on the Dutch (CBO) guideline of 2002 and 2004, using age, tumour grade, and tumour size to classify node negative breast cancer into *high*- or *low*-clinical risk [25,26]. These guidelines are not used anymore in the Netherlands and were never used internationally. It was therefore decided to use the clinical risk stratification criteria used within the MINDACT trial for the analyses presented in this paper. The following tumours were thus defined as clinical low risk: Bloom and Richard grade 1 and pT $\leq$ 30 mm; grade 2 and pT $\leq$ 20 mm; pT $<$ 10 mm independent of grade. All other node-negative tumours were defined as clinical high risk. The concordance between the CBO guideline at the time of inclusion and the criteria as used within the MINDACT trial was 94.8% (294/310, see Suppl. Table 1). We performed a sensitivity analysis

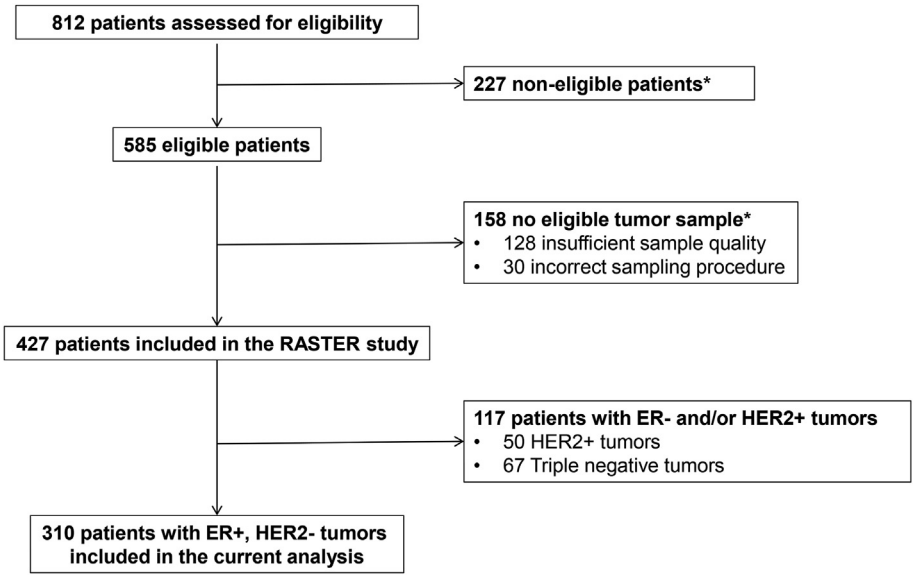


Fig. 1. **CONSORT diagram**. *A detailed breakdown of reasons for patient ineligibility and sample quality/sampling issues has previously been described in Bueno de Mesquita *et al.* [19].

Table 1
Patient and tumour characteristics.

	All		Clinical low risk				Clinical high risk			
			MammaPrint							
			Low		High		Low		High	
	N	%	N	%	N	%	N	%	N	%
Total	310	100	162	52.3	56	18.1	45	14.5	47	15.2
Age										
≤35	14	4.5	5	3.1	3	5.4	2	4.4	4	8.5
36–45	82	26.5	33	20.4	17	30.4	14	31.1	18	38.3
46–55	185	59.7	107	66.0	31	55.4	27	60.0	20	42.6
≥56	29	9.4	17	10.5	5	8.9	2	4.4	5	10.6
Tumour size										
≤10 mm	47	15.2	35	21.6	12	21.4	–		–	
11–20 mm	187	60.3	117	72.2	41	73.2	14	31.1	15	31.9
21–30 mm	61	19.7	10	6.2	3	5.4	23	51.1	25	53.2
≥31 mm	15	4.8	–		–		8	17.8	7	14.9
Histological grade										
1	101	32.6	80	49.4	18	32.1	2	4.4	1	2.1
2	154	49.7	82	50.6	35	62.5	22	48.9	15	31.9
3	55	17.7	0	0.0	3	5.4	21	46.7	31	66.0
Histological subtype										
Ductal	253	81.6	125	77.2	53	94.6	35	77.8	40	85.1
Lobular	45	14.5	26	16.0	3	5.4	10	22.2	6	12.8
Other	12	3.9	11	6.8	0	0.0	0	0.0	1	2.1
ER expression										
ER >50%	286	94.7	151	95.6	52	96.3	41	93.2	42	91.3
ER ≤ 50%	16	5.3	7	4.4	2	3.7	3	6.8	4	8.7
Missing	8		4		2		1		1	
PgR expression										
PgR ≥10%	248	81.8	129	81.1	45	83.3	40	90.9	34	73.9
PgR <10%	55	18.2	30	18.9	9	16.7	4	9.1	12	26.1
Missing	7		3		2		1		1	
Adjuvant therapy										
None	155	50.0	140	86.4	4	7.1	10	22.2	1	2.1
Endocrine therapy (ET)	52	16.8	14	8.6	21	37.5	12	26.7	5	10.6
Chemotherapy (CT)	5	1.6	0	0.0	2	3.6	1	2.2	2	4.3
CT and ET	97	31.1	8	4.9	29	51.8	22	48.9	39	83.0

Table 2
Clinicopathological factors associated with chemotherapy use.

Clinical risk		Low				High			
MammaPrint result		Low		High		Low		High	
Chemotherapy		No	Yes	No	Yes	No	Yes	No	Yes
Total		154 (95%)	8 (5%)	25 (45%)	31 (55%)	22 (49%)	23 (51%)	6 (13%)	41 (87%)
Age (years)	**≤35**	3 (60%)	2 (40%)	0 (0%)	3 (100%)	0 (0%)	2 (100%)	0 (0%)	4 (100%
	36–45	30 (91%)	3 (9%)	5 (29%)	12 (71%)	6 (43%)	8 (57%)	3 (17%)	15 (83%)
	46–55	104 (97%)	3 (3%)	15 (48%)	16 (51%)	14 (52%)	13 (48%)	2 (10%)	18 (90%)
	≥56	17 (100%)	0 (0%)	5 (100%)	0 (0%)	2 (100%)	0 (0%)	1 (20%)	4 (80%)
Bloom and Richardson Grade	**1**	77 (96%)	3 (4%)	8 (44%)	10 (56%)	0 (0%)	2 (100%)	0 (0%)	1 (100%)
	2	77 (94%)	5 (6%)	15 (43%)	20 (57%)	11 (50%)	11 (50%)	2 (13%)	13 (87%)
	3	0	0	2 (67%)	1 (33%)	11 (52%)	10 (48%)	4 (13%)	27 (87%)
Tumour size (mm)	**≤10**	35 (100%)	0 (0%)	7 (58%)	5 (42%)	–	–	–	–
	11–20	112 (96%)	5 (4%)	18 (44%)	23 (56%)	10 (71%)	4 (21%)	4 (27%)	11 (73%)
	21–30	7 (70%)	3 (30%)	0 (0%)	3 (100%)	12 (52%)	11 (48%)	2 (8%)	23 (92%)
	≥31	–	–	–	–	0 (0%)	8 (100%)	0 (0%)	7 (100%)
Histology	**Ductal**	121 (97%)	4 (3%)	23 (43%)	30 (57%)	17 (49%)	18 (51%)	4 (10%)	36 (90%)
	Lobular	23 (89%)	3 (12%)	2 (67%)	1 (33%)	5 (50%)	5 (50%)	2 (33%)	4 (67%)
	Other	10 (91%)	1 (9%)	0	0	0	0	0 (0%)	1 (100%)
PgR expression	<10%	30 (100%)	0 (0%)	2 (22%)	7 (78%)	2 (50%)	2 (50%)	1 (8%)	12 (92%)
	≥10%	124 (94%)	8 (6%)	22 (48%)	24 (52%)	20 (49%)	21 (51%)	5 (15%)	29 (85%)

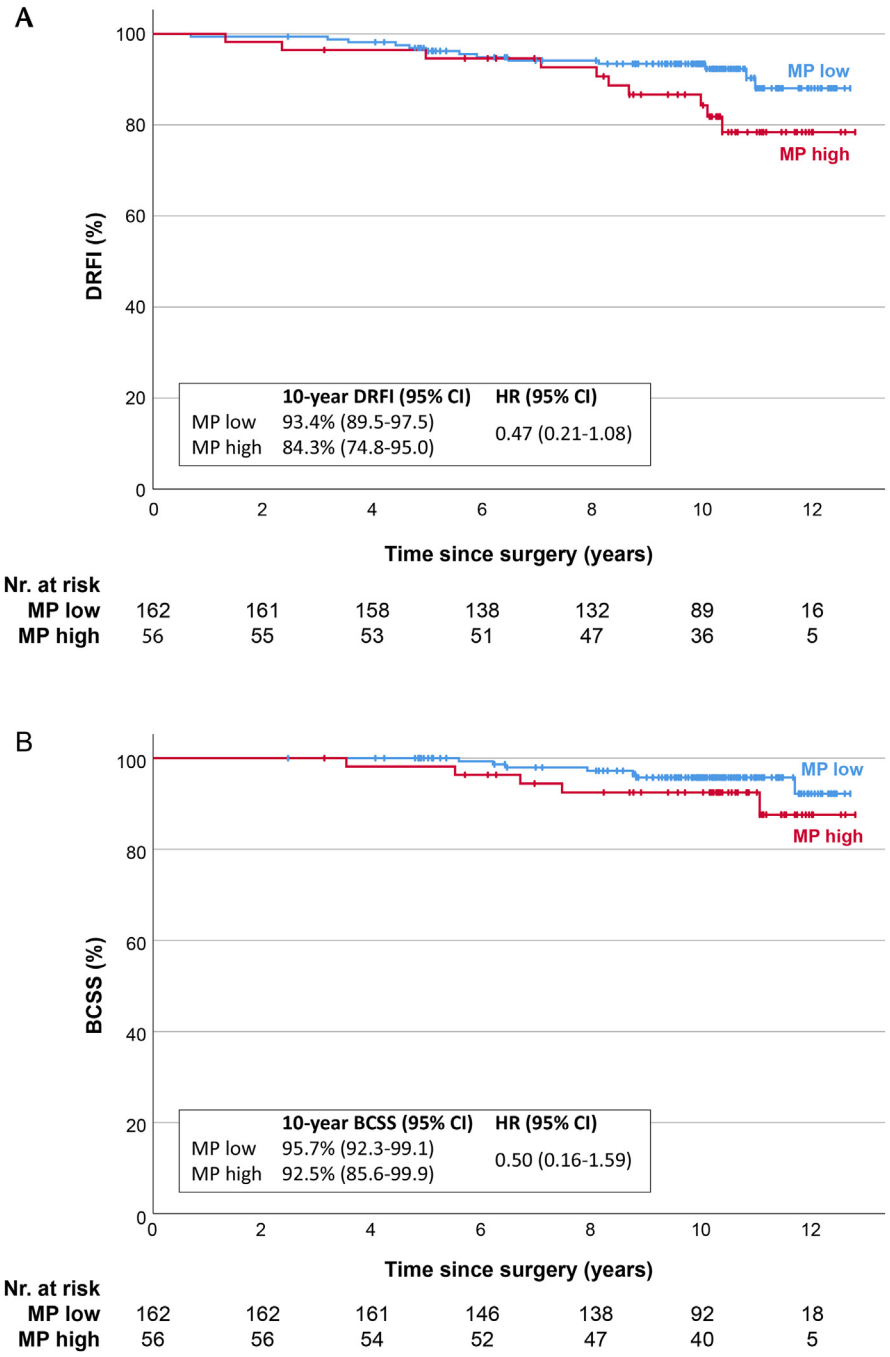
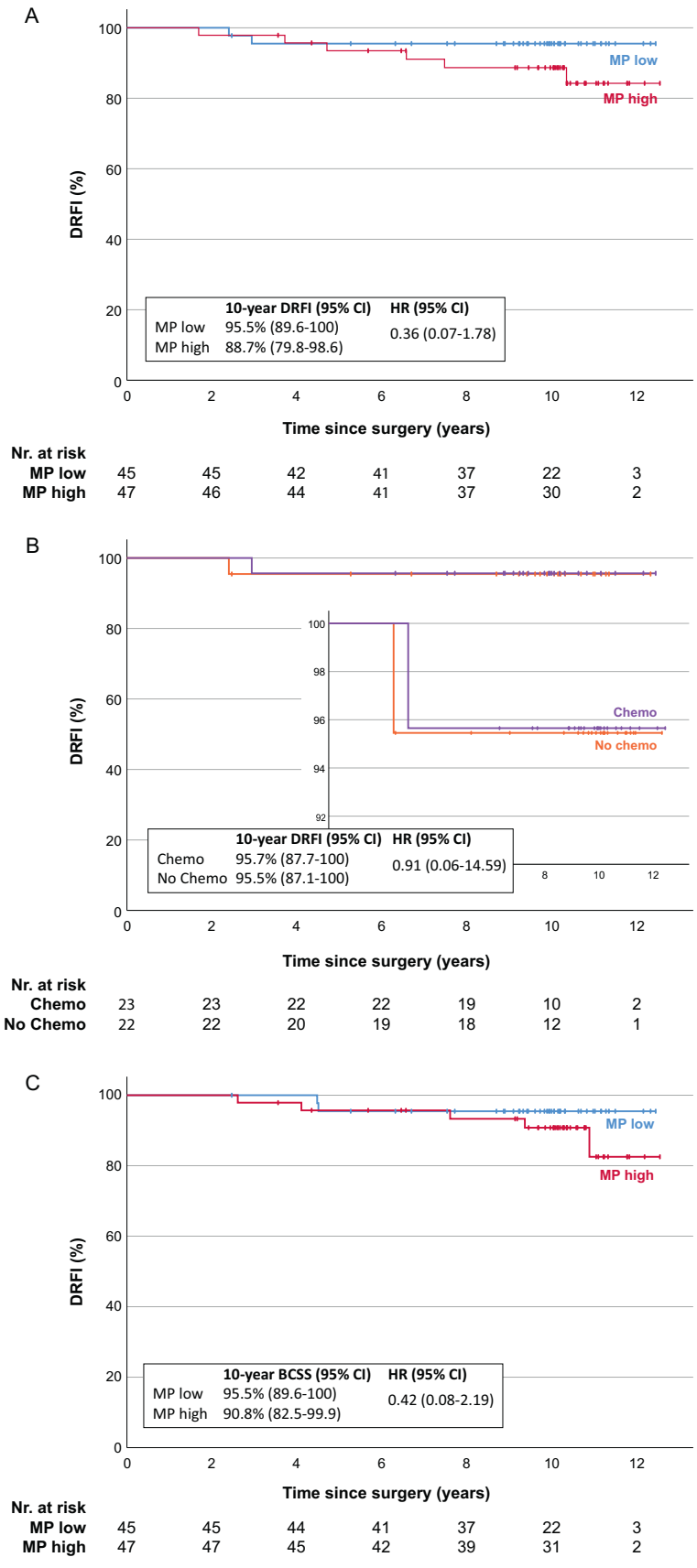


Fig. 2. **Clinical outcome for clinically low-risk patients**. Distant recurrence free interval (A) and breast cancer-specific survival (B) for all patients with clinically low-risk breast cancer by genomic risk according to MammaPrint (MP).

Table 3
Main results.

Clinical risk	Genomic risk	No AST n (%)	ET only n (%)	CT ± ET n (%)	Ten-year DRFI (95% CI)	Ten-year BCSS (95% CI)
Low	Low	140 (86%)	14 (9%)	8 (5%)	93.4% (89.5–97.5)	95.7% (92.3–99.1)
Low	High	4 (7%)	21 (38%)	31 (55%)	84.3% (74.8–95.0)	92.5% (85.6–99.9)
High	Low	10 (22%)	12 (27%)	23 (50%)	95.5% (89.6–100)	95.5% (89.6–100)
High	High	1 (2%)	5 (11%)	41 (87%)	88.7% (79.8–98.6)	90.8% (82.5–99.9)

assessing outcome in the main genomic and clinical risk categories including only those patients whose


A
DRFI (%)
MP low
MP high
10-year DRFI (95% CI) HR (95% CI)
MP low 95.5% (89.6-100) 0.36 (0.07-1.78)
MP high 88.7% (79.8-98.6)
Time since surgery (years)
Nr. at risk
MP low 45 45 42 41 37 22 3
MP high 47 46 44 41 37 30 2
B
DRFI (%)
Chemo
No chemo
10-year DRFI (95% CI) HR (95% CI)
Chemo 95.7% (87.7-100) 0.91 (0.06-14.59)
No Chemo 95.5% (87.1-100)
Time since surgery (years)
Nr. at risk
Chemo 23 23 22 22 19 10 2
No Chemo 22 22 20 19 18 12 1
C
DRFI (%)
MP low
MP high
10-year BCSS (95% CI) HR (95% CI)
MP low 95.5% (89.6-100) 0.42 (0.08-2.19)
MP high 90.8% (82.5-99.9)
Time since surgery (years)
Nr. at risk
MP low 45 45 44 41 37 22 3
MP high 47 47 45 42 39 31 2

clinical risk classification matched the original CBO classification.

2.3. Statistics

The primary end-point of this report is distant recurrence free interval (DRFI) defined as a distant breast cancer recurrence or death from breast cancer. As a secondary end-point, breast cancer-specific survival (BCSS) defined as mortality related to breast cancer was assessed. Patients without an event were censored at the date the patient was last known to be alive or without recurrence. Median follow-up time was calculated based on the reversed Kaplan–Meier method. Kaplan–Meier estimates with 95% confidence intervals for DRFI and BCSS were calculated for each clinical and genomic risk group. Subgroup analyses were performed for the most important treatment categories within each risk group. A multivariable model was built using likelihood ratio-based forward stepwise logistic regression taking into account tumour size (continuous), tumour grade, progesterone receptor status (≥10% cut-off for positivity), histology, and genomic risk to assess the factors associated with chemotherapy use. For the clinically high-risk, genomically low-risk subgroup, the effect of chemotherapy use on DRFI was assessed using a univariable Cox model. In addition, we built a series of multivariable Cox models assessing the effect of several potential confounders (tumour size, age, tumour grade, and histology) on the effect of chemotherapy use on DRFI. Data were analysed using SPSS (v27.0.0.0) and R (v4.0.4).

3. Results

3.1. Study population

Between 2004 and 2006, 427 patients with cTanyN0M0 breast cancer were found to be eligible for the RASTER study (Fig. 1) [19,20]. Here, we report an updated analysis of the subgroup of 310 patients with ER-positive, HER2-negative disease, after a median follow-up of 10.3 years (IQR 9.5–11.1).

There were 218/310 (70%) patients classified as clinically low-risk, of whom 162/218 (74%) had a genomically low-risk tumour and 56/218 (26%) a genomically high-risk tumour (Table 1). Out of the 92/310 (30%) patients classified as clinically high risk, 45/92 (49%) and 47/92 (51%) had a genomically low- and high-risk tumour, respectively. Overall, genomically high-risk tumours were more often grade 3 (34/103, 33% compared to 21/207, 10% of low-risk tumours) and more often seen in younger patients (42/103, 41% of patients with high-risk tumours was age ≤45 compared to 54/207, 26% of those with low-risk tumours). Invasive lobular tumours were less likely to be classified as genomically high risk than invasive ductal tumours (9/45, 20% versus 93/253, 37%).

3.2. Clinically low-risk patients: treatment and outcome

Of the 218 clinically low-risk patients, 144/218 (66%) received no systemic therapy, whereas 35/218 (16%) received endocrine therapy alone and 39/218 (18%) received chemotherapy with (n = 38) or without (n = 1) endocrine therapy (Table 1). Within the group treated with endocrine therapy, 48/73 (66%) were age ≤50. For 37 of these patients, data on ovarian ablation were available, of whom 26/37 (70%) received a GnRH agonist or underwent an ovariectomy (Suppl. Table 2). There was a clear difference in treatments received between the genomic low- and high-risk groups: in the genomic low-risk group, 140/162 (86%) received no systemic therapy, 14/162 (9%) received endocrine therapy alone, and 8/162 (5%) received chemotherapy and endocrine therapy. In contrast, in the genomic high-risk group, 4/56 (7%), 21/56 (38%), and 31/56 (55%) received no systemic therapy, endocrine therapy alone or endocrine therapy, and chemotherapy, respectively. Within both genomic risk groups, patients who were younger or who had a larger tumour were more likely to receive chemotherapy (Table 2). In a multivariable analysis, genomic risk, age, and tumour size were significantly associated with chemotherapy use in clinically low-risk patients (Suppl. Table 3).

The 10-year DRFI for the overall clinically low-risk group was 90.9% (95% CI 86.9–95.0). For the clinically/genomically low-risk patients, the 10-year DRFI was 93.4% (95% CI 89.5–97.5), compared to 84.3% (95% CI 74.8–95.0) in the clinically low-risk/genomically high-risk group (Fig. 2A). Interestingly, this difference between the genomic risk groups seems to only arise beyond 5 years, as the 5-year DRFI was 96.2% (95% CI 93.3–99.2) and 94.6% (95% CI 88.9–100) for genomic low and genomic high risk, respectively. This observation mostly seems to be driven by the patients treated with chemotherapy. The 31 patients treated with chemotherapy in the clinically low-risk/genomic high-risk group had a 5-year DRFI of 90.3% (95% CI 80.5–100), whereas the DRFI at 10 years was 78.9% (95% CI 65.2–95.6, Suppl. Fig. 1). In the group of 140 clinically/genomic low-risk patients who did not receive

Fig. 3. **Clinical outcome for clinically high-risk patients**. Distant recurrence free interval (A) for patients with clinically high-risk breast cancer by genomic risk according to MammaPrint (MP), distant recurrence free interval (B) for patients with clinically high-risk, genomically low-risk breast cancer by chemotherapy treatment and breast cancer-specific survival (C) for patients with clinically high-risk breast cancer by genomic risk according to MammaPrint.

any adjuvant systemic therapy, the 10-year DRFI was 93.9% (95% CI 90.0–98.1) (see Table 3).

The 10-year BCSS for the overall clinically low-risk group was 94.8% (95% CI 91.7–98.0). For the clinically/genomic low-risk group, this was 95.7% (95% CI 92.3–99.1) compared to 92.5% (95% CI 85.6–99.9) in the clinically low-risk/genomic high-risk group (Fig. 2B). Within the clinically low-risk/genomic high-risk group, patients treated with chemotherapy had a lower 10-year BCSS (90.1%, 95% CI 80.0–100) compared to patients who did not receive chemotherapy (95.7%, 95% CI 87.7–100). In the group of clinically/genomic low-risk patients who did not receive any adjuvant systemic therapy, the 10-year BCSS was 96.7% (95% CI 93.5–99.9, Suppl. Fig. 2). For both DRFI and BCSS, 10-year survival estimate for the genomic risk groups was very similar in the sensitivity analysis including only patients whose clinical risk classification matched the original CBO classification (Suppl. Fig. 3).

3.3. Clinically high-risk patients: treatment and outcome

Out of the 92 patients classified as clinically high risk, 11/92 (12%) received no adjuvant systemic therapy, whereas 17/92 (18%) received endocrine therapy alone, 64/92 (70%) received chemotherapy with (n = 60) or without (n = 3) endocrine therapy. Of the patients treated with endocrine therapy, 56/77 (73%) were age ≤50. For 32 of these patients, data on ovarian ablation were available, of which 22/32 (69%) received a GnRH agonist or underwent an ovariectomy (Suppl. Table 2). Clinically high-risk/genomically high-risk patients received on average more treatment, with 1/47 (2%) receiving no adjuvant systemic therapy, 5/47 (11%) receiving endocrine therapy alone, and 41/47 (87%) receiving chemotherapy compared to 10/45 (22%), 12/45 (27%), and 23/45 (50%) for no adjuvant systemic therapy, endocrine therapy alone, and chemotherapy, respectively, in the clinically high-risk/genomic low-risk group. In contrast to the clinically low-risk population, the age of a patient seemed to be a less important factor when deciding on treatment with chemotherapy in clinically high-risk patients (Table 2). In a multivariable analysis, only genomic risk and tumour size were significantly associated with use of chemotherapy in clinically high-risk patients (Suppl. Table 4).

The DRFI for the overall group of patients classified as clinically high risk was 92.1% (95% CI 86.6–97.9). This was 95.5% (95% CI 89.6–100) for the clinically high-risk/genomic low-risk group compared to 88.7% (95% CI 79.8–98.6) for the clinically and genomic high-risk group (Fig. 3A). Within the clinically high-risk/genomic low-risk group, the 10-year DRFI for those who received chemotherapy (n = 23, 95.7% [95% CI 87.7–100]) was similar to those who did not receive chemotherapy (n = 22, 95.5% [95% CI 87.1–100], Fig. 3B). In a univariable analysis, the hazard ratio for chemotherapy was 0.91 (95% CI 0.06–14.59). Multivariable analyses show only minor changes in the hazard ratio for chemotherapy in this subgroup and always towards 1 (see Suppl. Table 2). In the clinically and genomic high-risk group, 10-year DRFI was 92.5% (95% CI 84.7–100) for those who received chemotherapy.

The 10-year BCSS was 92.9% (95% CI 87.6–98.6) for the overall clinically high-risk population. Clinically high-risk/genomic low-risk patients had a 10-year BCSS of 95.5% (95% CI 89.5–100), compared to 90.8% (95% CI 82.5–99.9) in the clinically and genomic high-risk patients (Fig. 3C). Within the clinically high-risk/genomic low-risk group, the 10-year BCSS was similar for those who did and did not receive chemotherapy with 95.7% (95% CI 87.7–100) and 95.2% (95% CI 86.6–100), respectively (Suppl. Fig. 4). The sensitivity analysis in which only patients were included whose clinical risk classification matched the original CBO classification yielded very similar survival estimates for both DRFI and BCSS (Suppl. Fig. 5).

3.4. Post hoc analyses using the MammaPrint read-out on FFPE material

After enrolment for the RASTER study had finished, a version of the MammaPrint assay for FFPE material was developed [23]. As this is the test used in the clinic today, we retrospectively analysed the FFPE material from the RASTER study and were able to obtain results for 257/310 (83%) ER+/HER2-node-negative patients. Outcome data for the FFPE-based genomic risk-groups were very similar to data presented for the original MammaPrint test (Suppl. Figs. 6–8).

We also explored the value of its more recently established ultralow-risk cut-off [24]. Of the 257 tumours with FFPE-based results, 34 (13%) were classified as ultralow risk and 100 (39%) low- but not ultralow risk. Within the clinically low-risk group, patients with a genomically ultralow-risk tumour had a 10-year DRFI of 96.7% (95% CI 90.5–100), compared to 94.6% (95% CI 90.1–99.3) for those with a low- but not ultralow-risk tumour (Suppl. Figs. 9 and 10). In the clinical low-risk/genomically ultralow-risk group, 27/34 (79%) had received no adjuvant systemic therapy, compared to 72/100 (72%) in the clinical low-risk/genomically low-risk group (Suppl. Table 6).

4. Discussion

In this updated analysis of the observational RASTER study, we show that at 10.3 years median follow-up patients with clinical high-risk, genomic low-risk tumours have an excellent outcome, with a 10-year DRFI of more than 95% regardless of chemotherapy use. Even though our study is not randomised, it provides reassurance that

in this real-world setting of shared decision-making, implementation of MammaPrint testing does not compromise long-term clinical outcome. These results are in line with the updated results from the MINDACT trial, which reports only a small difference in distant metastasis-free survival between patients treated with and without chemotherapy in the clinically high-risk, genomically low-risk group at 8 years (92.0%, [95% CI 89.6–93.8] versus 89.4% [95% CI 86.8–91.5]) [12].

In the clinical low-risk subgroup, we observe that beyond 5 years, a difference in outcome emerges between genomically low- and high-risk tumours, resulting in a 10-year DRFI of 93.4% (95% CI 89.5–97.5) and 84.3% (95% CI 74.8–95.0) for low-, and high-risk tumours, respectively. Interestingly, a previous study has suggested that MammaPrint is mostly prognostic in the first 5 years after diagnosis [22]. However, that study was performed in a population with all breast cancer subtypes. As ER-positive, HER2-negative breast cancer, unlike other breast cancer subtypes, is associated with a substantial risk of recurrence beyond 5 years, this might explain why we were able to pick up a difference in DRFI between these two subgroups [18]. The 8-year DMFS data of the MINDACT trial do not show a divergence of the genomically low- and high-risk patients after 5 years for the clinically low-risk group. It should however be noted that due to the endocrine therapy randomisation in the MINDACT trial, most patients in this study likely received at least 7 years of endocrine therapy, while for the RASTER study, the median duration of endocrine therapy was only 5 years. Although we should be careful when interpreting our results due to confounding by indication, they do suggest that MammaPrint might offer prognostic value beyond 5 years in ER-positive, clinically low-risk breast cancer. Further research would be needed to explore if the clinically low-risk, genomically high-risk group could benefit from additional therapy, for example in the form of extended endocrine therapy.

Although generally better tolerated than chemotherapy, endocrine therapy is associated with toxicities that can substantially and persistently affect quality of life [27–30]. Therefore, it is worth exploring whether the recently established MammaPrint ultralow-risk classification can identify a subgroup of patients with such a good prognosis that de-escalation of endocrine therapy is an option [24]. An analysis in the Stockholm tamoxifen trial showed that node-negative, postmenopausal patients with ultralow-risk breast cancers who did not receive any systemic therapy had a 20-year BCSS of 94% [31]. A retrospective analysis of the ultralow-risk tumours in the MINDACT trial found an 8-year DRFI of 97% although most of these patients had been systemically treated [32]. In the RASTER study, we observe a 10-year DRFI of 96.7% (95% CI 90.5–100) in patients with an ultralow-risk tumour of whom 79% did not receive any adjuvant systemic therapy and the majority were pre- or perimenopausal. Together, the excellent long-term outcomes observed in these three retrospective analyses could support shared decision-making on endocrine treatment de-escalation in some clinical contexts.

The RASTER study has some limitations. Due to its observational nature, confounding by indication hampers an unbiased assessment of the prognostic value of MammaPrint. The RASTER study does however provide important data on outcome in a situation where MammaPrint results are combined with clinical risk factors, information on comorbidities and patient preferences to come to a treatment decision. The variety of treatment strategies used within each of the clinical and genomic risk categories highlights that the factors taken into account in treatment decisions go well beyond a binary clinical risk categorisation. This also underscores the need to conduct studies in a real-world setting to assess the value of prognostic gene signatures. Due to the current reimbursement situation for MammaPrint, the number of studies reporting on its effects on treatment decisions is still limited [33–37]. To our knowledge, there is only one other study that reports on disease outcome after implementation of MammaPrint testing in a real-world setting [38]. However, due to the different clinical risk profile and lack of stratification by clinical risk, the results of this study are difficult to compare to ours. A final limitation of our study is that treatment based on the clinical risk categories would be different today, most importantly almost all patients would have received endocrine therapy regardless of risk of recurrence [5,6]. This means that the absolute survival probabilities we report here cannot be generalised to current clinical practice. At the same time, the RASTER study offers a unique opportunity to assess outcome in patients treated without adjuvant systemic therapy, which would today no longer be standard of care.

5. Conclusion and implications

With a median follow-up of over 10 years, we observe that patients with clinical high-risk, genomic low-risk tumours have an excellent outcome regardless of the use of chemotherapy in the shared-decision-making context of the RASTER study. Together with the updated results of the MINDACT trial, these data support the use of the MammaPrint, in ER-positive, HER2-negative, node-negative, clinically high-risk breast cancer patients.

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Ethics

The RASTER study was approved by the Medical Ethical Committee of the Netherlands Cancer institute and patients provided written informed consent for additional analysis and data collection concerning clinical outcome.

Data availability

The data collected for this study can be made available to others in de-identified form in the presence of a data transfer agreement. Requests for data sharing can be made to the corresponding author.

Author contribution

Sonja Vliek: data curation, formal analysis, writing – original draft; **Florentine Hilbers**: data curation, formal analysis, writing – original draft; **Agnes Jager**: supervision, writing – review and editing; **Valesca Retél**: data curation, writing – review and editing; **Jolien Bueno-de-Mesquita**: data curation, writing – review and editing; **Caroline Drukker**: data curation, writing – review and editing; **Sanne Veltkamp**: resources, writing – review and editing; **Anneke Zeillemaker**: resources, writing – review and editing; **Emiel Rutgers**: resources, writing – review and editing; **Harm van Tinteren:** conceptualisation, data curation, formal analysis, writing – review and editing; **Wim van Harten**: conceptualisation, writing – review and editing; **Laura van't Veer**: conceptualisation, supervision, writing – review and editing; **Marc van de Vijver**: conceptualisation, writing – review and editing; **Sabine Linn**: conceptualisation, funding acquisition, resources; supervision, writing – review and editing.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

SCL and VPR have received unrestricted research grants from Agendia outside of the context of this study. LvtV is co-founder, stockholder and part-time employee of Agendia NV.

All remaining authors have declared no conflicts of interest.

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Appendix A. Supplementary data

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