Supplementary File 6: Narrative synthesis and additional Tables for Chapter 2, Results: Prosigna

Prosigna is based on a Risk Of Recurrence (ROR) score called ROR-PT, which incorporates the PAM50 gene signature, a weighting for a proliferation score (P, a subset of the 50 genes) and information on tumour size (T). Nodal status is then used when converting the score into a risk category. The commercial Prosigna test uses the nCounter system to analyse ROR-PT. Other research-based versions of ROR-PT exist, for example using qRT-PCR. This assessment includes all studies assessing ROR-PT, whether or not they use the formal Prosigna test. However, studies assessing other versions of the ROR score, such as ROR-S (subtype) or ROR-T/ROR-C (subtype and tumour size) or ROR-P (subtype and proliferation score), are excluded. Studies assessing ROR-PT via whole-transcriptome microarray (in silico studies) are summarised in Appendix 5.

Within this section, the test is referred to as ROR-PT since this covers both Prosigna and other versions of ROR-PT that do not use the nCounter system (but are equivalent to Prosigna in terms of incorporation of PAM50 gene signature and clinical factors).

Development: Prosigna

The PAM50 gene signature was developed and validated by Parker *et al.*[1] (2009) using microarray and qRT-PCR. Risk of recurrence (ROR) models were trained on 141 node-negative (LN0), untreated patients from the Netherlands Cancer Institute (NKI; van de Vijver, 2002),[2] which was also part of the first validation cohort for MammaPrint. ROR models tested included ROR-S and ROR-T. Validation in untreated LN0 patients showed that both ROR-S and ROR-T statistically significantly improved prognosis over clinico-pathologic variables, and that ROR-T statistically significantly improved prognosis over ROR-S. This study is not discussed further as it did not include ROR-PT.

Use of Prosigna (ROR-PT) via the nCounter system was developed and validated by in the British Columbia cohort by Wallden *et al*. (2015), which is included in this section.[3]

Prognostic performance: Prosigna

Study designs: Prosigna prognostic performance

Eight data sets were used to assess the prognostic performance of ROR-PT (Table 1). These included six reanalyses of RCTs (TransATAC,[4, 5] ABCSG-8,[6, 7] CALGB 9741,[8] NCIC MA.21,[9] GEICAM 9906[10, 11] and NCIC MA.12[12]) and two retrospective analyses of

prospective cohorts (the Danish Breast Cancer Cooperative Group [DBCG] cohort[13-16] and two analyses of the British Columbia cohort[3, 17]).

Patients: Prosigna prognostic performance

Two analyses of RCTs (TransATAC[4, 5] and ABCSG-8[6, 7]) included patients who were ER+ HER2-, a mix of LN0 and LN+, and received only endocrine treatment (no chemotherapy). Conversely, the other four analyses of RCTs[8-12] included higher-risk patients who received adjuvant chemotherapy; more patients in these trials were node-positive (LN+), and not all were ER+ HER2- (Table 1).

For TransATAC, two sets of data are presented in the updated analysis via personal communication.[5] The "full dataset" refers to data on all 855 patients with ROR-PT data available, while the "reduced dataset" refers to 774 patients with data for all four in-scope tests analysed in TransATAC. In this report, data for the "full dataset" is used where available; if not available than the "reduced dataset" is used. Both datasets gave very similar results.

The two retrospective analyses of prospective cohorts[3, 13-17] included patients who were mostly ER+ HER2-, a mix of LN0 and LN+, and received only endocrine treatment (no chemotherapy).

Tests and comparators: Prosigna prognostic performance

Four analyses of RCTs[4-9] and two analyses of prospective cohorts[3, 13-16] measured ROR-PT using the nCounter device, while two analyses of RCTs[10-12] and one of a prospective cohort[17] used qRT-PCR (Table 1). The cut-offs used to define risk groups varied across studies, while some analyses assessed ROR-PT as a continuous score (see Table 1 for details).

Some data sets were also used to evaluate other in-scope tests as follows (see Appendix 5 on comparing tests). TransATAC was used to evaluate Oncotype DX, EndoPredict and IHC4. The GEICAM 9906 analysis,[10, 11] as well as a pooled analysis of ABCSG-6 and 8,[18-20] were used to evaluate EndoPredict.

Quality assessment: Prosigna prognostic performance

All data sets reported here were validation studies (Table 2). All analyses excluded some patients recruited to the original trial or cohort. Blinding of test assessors to outcomes was reported in five analyses. All used standardised outcomes.

Results: Prosigna prognostic performance

Distribution of patients by risk group

Some studies reported the percentages of patients categorised into each risk group by ROR-PT (Table 18 of the main report). For LN0 patients, the percentages categorised as low-risk were reported in two analyses: 55% in TransATAC[5] and 48% in ABCSG-8.[6, 7] Among LN+ patients, far fewer patients were categorised as low-risk: 8% in TransATAC;[5] 4% in ABCSG-8;[6, 7] 19% in GEICAM 9906;[10, 11] and 25% in DBCG.[14] The percentage of patients categorised as intermediate-risk was 30%[5] and 32%[6, 7] in LN0 patients and ranged from 27% to 56% in LN+ patients.[5-7, 10, 11, 14]

Prognostic performance: unadjusted analyses

This section reports unadjusted analyses. Adjusted analyses, which show whether the test has prognostic value over clinicopathological variables, are reported in the section "Additional prognostic value".

For LN0 patients, ROR-PT was statistically significantly prognostic for DRFS/DFMS/DRFI in all three data sets (TransATAC,[5] ABCSG-8,[6, 7] DBCG[14]), with the proportion of patients having 10-year DRFS/DFMS/DRFI in the low-risk groups being 97.4% (TransATAC[5]), 96.5% (ABCSG-8[6, 7]) and 95.1% (DBCG[14]). HRs and p-values between groups are reported in many differing formats and timepoints so are summarised in Table 18 of the main report rather than in the text. ROR-PT was also statistically significantly prognostic for late (5-15-year) recurrence in the one study reporting this (Table 18 of the main report).[6, 7]

For LN+ patients, ROR-PT was statistically significantly prognostic for 10-year DRFS/DFMS/DRFI in all four data sets (TransATAC,[5] ABCSG-8,[6, 7] DBCG[14] and GEICAM 9906[10, 11]), with the proportion of patients having 10-year DRFS/DFMS/DRFI in the low-risk groups being 100.0% (TransATAC[5]), 100.0% (ABCSG-8[6, 7]) and 92% (GEICAM 9906[10, 11]), or 95.1% in the combined low/intermediate-risk groups (DBCG[14]). ROR-PT was also statistically significantly prognostic for late (5-10-year) recurrence in the two studies reporting this (Table 18 of the main report).[6, 7, 14, 15]

In terms of other outcomes (Table 3 and Table 4), ROR-PT was statistically significantly prognostic for 10-year overall survival in LN0 and LN+ patients in TransATAC;[5] for relapse-free survival (RFS) and breast cancer specific survival in LN0 patients in the British Columbia cohort;[17] and for RFS in CALGB 9741;[8] but not for RFS in NCIC MA.21.[9] ROR-PT was also statistically significantly prognostic in both pre- and post-menopausal patients

(CALGB 9741[8]) and in ductal and lobular breast cancer patients (DBCG, Laenkholm *et al*., 2016[16]).

Additional prognostic value

This section reports adjusted analyses, which indicate the additional prognostic value of Prosigna over clinicopathological factors. The clinicopathological factors adjusted for vary from study to study, and are detailed in the footnotes to the tables.

Likelihood ratios: The TransATAC analysis[5] reports a reduced dataset of patients where data for all four in-scope tests are available. Additional prognostic value was assessed via increases in likelihood ratio χ^2 for 10-year DRFI, for ROR-PT plus NPI or CTS, over NPI or CTS alone (Table 19 of the main report). Increases in likelihood ratio χ^2 were statistically significant for LN0 patients: 23.71 ($p<0.0001$) over CTS and 25.54 ($p<0.0001$) over NPI, but borderline statistically significant for LN+ patients: 4.39 ($p=0.04$) over CTS and 2.71 ($p=0.09$) over NPI (Table 19 in the main report). In ABCSG-8,[6] likelihood ratios also showed a statistically significant increase for ROR-PT over the Clinical Linear Predictor (same variables as CTS) in LN0 patients ($p<0.0001$) and LN+ patients ($p=0.0002$). Similar results were found for other outcomes (Table 5).

C-indexes (AUC): In ABCSG-8,[6] C-indexes were numerically higher for ROR-PT than for the Clinical Linear Predictor in both LN0 and LN+ patients, but statistical significance levels were not reported. Similarly in the British Columbia analysis by Wallden *et al.* 2015,[3] C-indexes were higher for ROR-PT than for AOL or IHC4+tumour size in LN0 patients, but statistical significance levels were not reported (Table 19 in the main report).

Multivariable Cox models: ABCSG-8[6] and DBCG[13, 14] used multivariable analyses to show that Prosigna was an independent prognostic parameter for 10-year DMFS/DRFS after adjustment for clinical factors across a mix of nodal status (Table 19 in the main report).

Table 1: Characteristics of prognostic studies: Prosigna

Reference(s)	Cohort(s)	N pts	Country	Study design	Test	Details of test	Cut-offs	Other tests	Population	Nodal status	Endo / chemo
Reanalyses of RCTs: LN status mixed											
100% ET monotherapy											
Sestak 2017 (data request)[5] Dowsett 2013[4]	**TransATAC**	855 (full dataset) 774 (reduced dataset)[a]	UK	R-RCT	ROR-PT	FFPE nCounter	LN0: 40; 60 LN1-3: 15; 40	O-DX EPClin IHC4+C	ER+ HER2- Postmeno 100% female	LN0, 78% LN1-3, 22%	All ET No CT
Gnant 2014,[6] Filipits 2014[7]	**ABCSG-8**	1397	Austria	R-RCT	ROR-PT	FFPE nCounter	LN0: 40; 60 LN1-3: 15; 40 LN>3: all high	EPClin (ABCSG-6+8)	ER+ HER2- Postmeno 100% female	LN0, 71%[b] LN1-3, 26%[b] LN>3, 3%[b]	All ET No CT
Variable ET&CT											
Chia 2012[12]	**NCIC MA.12**	398	Canada	R-RCT	ROR-PT	FFPE qRT-PCR	Continuous score		73% HR+ HER2 NR Premeno 100% female Stage I-III	LN0, 25% LN1-3, 55% LN>3, 20%	Some ET (% NR) All CT
Liu 2015[9]	**NCIC MA.21**	1094	Canada + USA	R-RCT	ROR-PT	FFPE nCounter	LN0: 40; 60 LN1-3: 15; 40 LN>3: all high + cont. score		58% ER+ 71% HER2- 31% postmeno 100% female	LN0, 30% LN1-3, 42% LN>3, 28%	58% ET All CT
Reanalyses of RCTs: LN+											
Variable ET&CT											
Liu 2016[8]	**CALGB 9741** (Alliance)	1311	USA	R-RCT	ROR-PT	FFPE nCounter	Continuous score		64% ER+ HER2 NR 51% postmeno 100% female	All LN+ (1-5 nodes, % NR)	ET NR All CT
100% ET&CT											
Martin 2016,[10] 2014[11]	**GEICAM 9906**	555	Spain	R-RCT	ROR-PT (research-based)	qRT-PCR then microarray	LN+: 18; 65	EP; EPClin	ER+ HER2- 46% postmeno Stage II-III 100% female	All LN+ LN1-3, 64% LN>3, 36%	All ET All CT

Reference(s)	Cohort(s)	N pts	Country	Study design	Test	Details of test	Cut-offs	Other tests	Population	Nodal status	Endo / chemo
Retrospective studies: LN status mixed											
100% ET monotherapy											
Ejlertsen 2015[13]; Laenkholm 2015[14], 2015[15], 2016[16]	**DBCG** 2000-2003	2722	Denmark	Retro. analysis of prosp. cohort	ROR-PT	FFPE nCounter	LN0: 40; 60 LN1-3: low 0-40; high >40		HR+ HER2 NR Postmeno 100% female	LN0, 46% LN1-3, 54%	All ET No CT
Nielsen 2010[17]	**British Columbia** 1986-1992	786	Canada	Retro. analysis of prosp. cohort	ROR-PT	FFPE qRT-PCR	Continuous score? (unclear)		ER+ 89% HER2- 96% postmeno 100% F	LN0, 28% LN1-3, 46% LN>3, 19% Missing, 7%	All ET No CT
Retrospective studies: LN0											
100% ET monotherapy											
Wallden 2015[3]	**British Columbia** (years NR)	232	Canada	Retro. analysis of prosp. cohort	ROR-PT	FFPE nCounter	Continuous score		ER+ 91% HER2- 94% postmeno (% female NR)	All LN0	All ET No CT

ABCSG, Austrian Breast and Colorectal Cancer Study Group; AC/T, doxorubicin, cyclophosphamide + paclitaxel; CEF, dose-intense cyclophosphamide, epirubicin + flurouracil; CT, chemotherapy; DBCG, Danish Breast Cancer Cooperative Group; EC/T, dose-dense, dose-intense epirubicin, cyclophosphamide + paclitaxel; CEF, cyclophosphamide, epirubicin and fluorouracil; CMF, cyclophosphamide, methotrexate and fluorouracil; DC, doxorubicin and cyclophosphamide; ER, oestrogen receptor; ET, endocrine therapy; FEC, 5-Fluorouracil, epirubicin, and cyclophosphamide; FEC-P, FEC + paclitaxel; FFPE, formalin-fixed paraffin-embedded; HER2, human epidermal growth factor receptor 2; HR+, hormone-receptor positive; LN, number of positive nodes; NR, not reported; prosp, prospective; qRT-PCR, quantitative reverse transcription polymerase chain reaction; R-RCT, reanalysis of RCT; retro, retrospective.

[a]Full dataset=all patients with EndoPredict data available; reduced dataset = patients with data for all four in-scope tests analysed in TransATAC

[b]Nodal status for all 1478 patients; NR for 1397 who were HER2-

Table 2: Quality assessment of prognostic studies: Prosigna

Reference(s)	Cohort(s)	Derivation or validation?	Study design appropriate?	All eligible patients included?	Blinding (of test assessors to outcomes)	Outcome definition standardised *or a priori*?	Applicability: Patient Spectrum	Applicability: Test as per decision problem?
Sestak 2017 (data request),[5] Dowsett 2013[4]	**TransATAC**	V	Y, R-RCT, no chemo	N (InT, MS, TF)	Y	Y	Y	Y
Chia 2012[12]	**NCIC MA.12**	V	N, R-RCT, adj chemo	N (InT, MS, TF)	UC	Y	N (27% HR-/unknown, HER2 NR, 20% LN>3)	N (qRT-PCR, continuous score)
Ejlertsen 2015[13]; Laenkholm 2015[14], 2015[15], 2016[16]	**DBCG**	V	Y, prospective cohort, no chemo	N (reason NR)	UC	Y	UC (HER2 NR)	Y
Gnant 2014,[6] Filipits 2014[7]	**ABCSG-8**	V	Y, R-RCT, no chemo	N (InT, MS, TF)	Y	Y	Y (for subgroup analysis)	Y
Liu 2016[8]	**CALGB 9741**	V	N, R-RCT, adj chemo	N (InT, MS, TF)	Y	Y	N (36% ER-, HER2 NR, LN>3 NR)	N (continuous score)
Liu 2015[9]	**NCIC MA.21**	V	N, R-RCT, adj chemo	N (InT, MS, TF)	Y	Y	N (42% ER-, 29% HER2+ / unknown, 28% LN>3)	Y
Martin 2016,[10] 2014[11]	**GEICAM 9906**	V	N, R-RCT, adj chemo	N (reason NR)	Y	Y	N (36% LN>3)	N, Prosigna via qRT-PCR then microarray
Nielsen 2010[17]	**British Columbia**	V	Y, prospective cohort, no chemo	N (InT, TF)	UC	Y	Most (11% HER2+ / missing; 19% LN>3)	No - qRT-PCR, continuous score? (unclear)
Wallden 2015[3]	**British Columbia**	V D (nCounter)	Y, prospective cohort, no chemo	N (InT, TF)	UC	Y	Most (9% HER2+ / missing)	No - continuous score

Y, yes; N, no; UC, unclear
ABCSG, Austrian Breast and Colorectal Cancer Study Group; D, Development; DBCG, Danish Breast Cancer Cooperative Group; InT, insufficient tissue; LN, number of positive nodes; MS, missing samples; qRT-PCR, quantitative reverse transcription polymerase chain reaction; R-RCT, reanalysis of RCT; TF, test failure; V, validation.

Table 3: Prognostic performance of Prosigna: Overall survival

Reference(s)	Cohort(s)	Population	Nodal	Endo /	Test	% pts per group	% OS risk: 0-5yr	% OS risk: 0-10yr	OS: HR (95% CI)

	Design; Country		status	chemo		Low	Int	High	Low	Int	High	Low	Int	High	
LN status mixed															
100% ET monotherapy															
Sestak 2017 (data request)[5] (reduced dataset)[a]	**TransATAC** R-RCT; UK	ER+ HER2- N=774	LN0, 76% LN1-3, 24%	All ET No CT	**ROR-PT** nCounter	43	30.5	26.5	-	-	-	-	-	-	**0-10yr: L vs I:** 1.84 (1.29, 2.61). **L vs H:** 3.42 (2.46, 4.75)
LN0															
100% ET monotherapy															
Sestak 2017 (data request)[5] (full dataset)[a]	**TransATAC** R-RCT; UK	ER+ HER2- N=663	LN0	All ET No CT	**ROR-PT** nCounter	55	30	15	93.7	93.4	84.2	84.4	70.3	54.0	**0-5yr: L vs I:** 1.05 (0.54, 2.10) **L vs H:** 2.57 (1.36, 4.87) **0-10yr: L vs I:** 1.96 (1.34, 2.86) **L vs H:** 3.59 (2.41, 5.35)
LN+															
100% ET monotherapy															
Sestak 2017 (data request)[5] (full dataset)[a]	**TransATAC** R-RCT; UK	ER+ HER2- N=192	LN1-3	All ET No CT	**ROR-PT** nCounter	8	32	60	100.0	88.7	81.7	90.0	72.0	53.1	**0-5yr: L vs I or L vs H:** no events **I vs H:** 1.52 (0.65, 3.54) **0-10yr: L vs I:** 4.75 (0.63, 35.67) **L vs H:** 8.91 (1.23, 64.52)

-, not reported; CI, confidence interval; CT, chemotherapy; ER, oestrogen receptor; ET, endocrine therapy; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; int, intermediate; LN, number of positive nodes; OS, overall survival; R-RCT, reanalysis of RCT.

[a]Full dataset=all patients with EndoPredict data available; reduced dataset = patients with data for all four in-scope tests analysed in TransATAC

Table 4: Prognostic performance of Prosigna: Other outcomes

Reference(s)	Cohort(s) Design; Country	Population	Nodal status	Outcome	Endo / chemo	Test	% pts per group			% risk of outcome per group			HR (95% CI)
							Low	Int	High	Low	Int	High	
LN status mixed													
Variable CT&ET													
Liu 2015[9]	**NCIC MA.21** R-RCT; Canada+USA	58% ER+, 71% HER2- N=1094	LN0, 30% LN1-3, 42% LN>3, 28%	**RFS 8yr**	58% ET **All CT**	**ROR-PT** nCounter	3	18	79	-	-	-	Low/int vs high: 1.27 (0.83. 1.95), p=0.275
LN0													
100% ET monotherapy													
Nielsen 2010[17]	**British Columbia** Cohort; Canada	ER+, 89% HER2- N=222	LN0	**BCSS 10+yr**	All ET No CT	**ROR-PT** qRT-PCR	-	-	-	-	-	-	Between groups: p=0.026 (cut-points unclear)
				RFS 10+yr	All ET No CT	**ROR-PT** qRT-PCR	-	-	-	-	-	-	Between groups: p=0.009 (cut-points unclear)
LN+													
Variable CT&ET													
Liu 2016[8]	**CALGB 9741** R-RCT; USA	64% ER+, HER2 NR N=1311	All LN+ (1-5 nodes, % NR)	**RFS 12.5yr**	ET NR **All CT**	**ROR-PT** nCounter	N/A	N/A	N/A	N/A	N/A	N/A	Per 10-unit change: 1.12 (1.07, 1.18), p<0.0001

-, not reported; BCSS, breast cancer-specific survival; CI, confidence interval; CT, chemotherapy; ER, oestrogen receptor; ET, endocrine therapy; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; int, intermediate; LN, number of positive nodes; RFS, relapse-free survival (locoregional or distant); R-RCT, reanalysis of RCT.

Table 5: Additional prognostic value for other outcomes: Prosigna

Reference(s)	Cohort(s)	Population	Nodal status	Endo / chemo	Outcome	Test or comparator[a]	C-index (AUC)	Increase in C-index (AUC) over CP[a]	Multivariable model (adj. for CP factors[a]): HR (95% CI)
LN status mixed									
Variable CT&ET									
Chia 2012[12]	**NCIC MA.12** R-RCT	73% HR+, N=398	LN0, 25% LN+, 75%	Some ET	**OS 10 yr**	**ROR-PT** qRT-PCR	0.611		

Reference(s)	Cohort(s)	Population	Nodal status	Endo / chemo	Outcome	Test or comparator[a]	C-index (AUC)	Increase in C-index (AUC) over CP[a]	Multivariable model (adj. for CP factors[a]): HR (95% CI)
				All CT	**DFS 10yr**	**ROR-PT** qRT-PCR	0.576		
Liu 2015[9]	**NCIC MA.21** R-RCT	58% ER+, 71% HER2- N=1094	LN0, 30% LN1-3, 42% LN>3, 28%	58% ET All CT	**RFS 8yr**	**ROR-PT** nCounter			**L/I vs H:** 1.98 (0.53, 7.45), p=0.311; **HR (cont score):** 1.01 (1.00, 1.02), p=0.029
LN0									
100% ET monotherapy									
Nielsen 2010[17]	**British Columbia** Cohort	ER+, 89% HER2- N=222	LN0	All ET No CT	**BCSS >10yr**	**ROR-PT** qRT-PCR	0.69	p=0.002 vs AOL p=0.033 vs IHC-T	
						AOL	0.56		
						IHC-T	0.63		
					RFS >10yr	**ROR-PT** qRT-PCR	0.67	p=0.001 vs AOL p=0.047 vs IHC-T	
						AOL	0.57		
						IHC-T	0.62		
Wallden 2015[3]	**British Columbia** Cohort	ER+, 91% HER2- N=232	LN0	All ET No CT	**BCSS (time NR)**	**ROR-PT** nCounter	0.672[b]		
						AOL	0.565[b]		
						IHC-T	0.560[b]		
LN+									
100% ET monotherapy									
Nielsen 2010[17]	**British Columbia** Cohort	ER+, 89% HER2- N=511	LN1-3, 70% LN>3, 30%	All ET No CT	**BCSS >10yr**	**ROR-PT** qRT-PCR	0.62	p=0.59 vs AOL p=0.30 vs IHC-T	
						AOL	0.63		
						IHC-T	0.61		
					RFS >10yr	**ROR-PT** qRT-PCR	0.60	p=0.72 vs AOL p=0.31 vs IHC-T	
						AOL	0.61		
						IHC-T	0.59		

BCSS, breast cancer-specific survival; CI, confidence interval; CP, clinical/pathological; CT, chemotherapy; DFS, disease-free survival; ER, oestrogen receptor; H, high; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; HR+, hormone receptor-positive; I, intermediate; L, low; LN, number of positive nodes; LR, likelihood ratio; NR, not reported; OS, overall survival; RFS, relapse-free survival (locoregional or distant); R-RCT, reanalysis of RCT.

[a]CP factors (ABSCG) = age, grade, nodal status, tumour size, Ki67. CP factors (GEICAM) = age, grade, nodal status, tumour size, treatment, ER, PR, Ki67. CTS (TransATAC) and CLP (ABCSG-8) = age, grade, nodal status, tumour size, treatment. CLP . CP factors (MA.21): not reported which. [b]Estimated from graph.

REFERENCES

1. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T*, et al.* Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol* 2009;27:1160-7.
2. van de Vijver MJ, He Y, van't Veer LJ, Dai H, Hart AA, Voskuil DW*, et al.* A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002;347:1999-2009.
3. Wallden B, Storhoff J, Nielsen T, Dowidar N, Schaper C, Ferree S*, et al.* Development and verification of the PAM50-based Prosigna breast cancer gene signature assay. *BMC Med Genomics* 2015;8:54.
4. 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.
5. Sestak I, Dowsett M, Cuzick J. NICE request - TransATAC data analysis. In; 2017.
6. Gnant M, Filipits M, Greil R, Stoeger H, Rudas M, Bago-Horvath Z*, et al.* Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. *Ann Oncol* 2014;25:339-45.
7. Filipits M, Nielsen TO, Rudas M, Greil R, Stoger H, Jakesz R*, et al.* The PAM50 risk-of-recurrence score predicts risk for late distant recurrence after endocrine therapy in postmenopausal women with endocrine-responsive early breast cancer. *Clin Cancer Res* 2014;20:1298-305.
8. Liu MC, Pitcher B, Mardis E, Davies S, Friedman PN, Snider J. PAM50 gene signatures and breast cancer prognosis with adjuvant anthracycline- and taxane-based chemotherapy: correlative analysis of C9741 (Alliance). *Breast Cancer* 2016;2.
9. Liu S, Chapman JA, Burnell MJ, Levine MN, Pritchard KI, Whelan TJ*, et al.* Prognostic and predictive investigation of PAM50 intrinsic subtypes in the NCIC CTG MA.21 Phase III chemotherapy trial. *Breast Cancer Res Treat* 2015;149:439-48.
10. Martin M, Brase JC, Ruiz A, Prat A, Kronenwett R, Calvo L*, et al.* Prognostic ability of EndoPredict compared to research-based versions of the PAM50 risk of recurrence (ROR) scores in node-positive, estrogen receptor-positive, and HER2-negative breast cancer. A GEICAM/9906 sub-study. *Breast Cancer Res Treat* 2016;156:81-9.
11. Martin M, Brase JC, Calvo L, Krappmann K, Ruiz-Borrego M, Fisch K*, et al.* Clinical validation of the EndoPredict test in node-positive, chemotherapy-treated ER+/HER2- breast cancer patients: results from the GEICAM 9906 trial. *Breast Cancer Res* 2014;16:R38.
12. Chia SK, Bramwell VH, Tu D, Shepherd LE, Jiang S, Vickery T*, et al.* A 50-gene intrinsic subtype classifier for prognosis and prediction of benefit from adjuvant tamoxifen. *Clin Cancer Res* 2012;18:4465-72.
13. Ejlertsen B, Jensen MB, Eriksen JO, Kiboll T, Rasmussen BB, Knoop AS*, et al.* Validation of prediction of distant recurrence (DR) by Prosigna (PAM50) in subgroups of a Danish Breast Cancer Cooperative Group (DBCG) cohort of node-positive (N1), hormone receptor positive (HR+), post-menopausal early breast cancer (EBC) patients allocated 5yr of endocrine therapy (ET). *J Clin Oncol Conf* 2015;33.
14. Laenkholm AV, Jensen MB, Eriksen JO, Kiboll T, Rasmussen BB, Knoop AS*, et al.* Prediction of 10yr distant recurrence (DR) using the Prosigna (PAM50) assay in a Danish Breast Cancer Cooperative Group (DBCG) cohort of post-menopausal Danish women with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5yr of endocrine therapy (ET) alone. *J Clin Oncol Conf* 2015;33:Abstract 546.
15. Laenkholm A, Jensen M, Eriksen J. Prediction of late distant recurrence (DR) using the Prosigna (PAM50) test in a Danish Breast Cancer Cooperative Group (DBCG) cohort of postmenopausal women diagnosed with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5yr of endocrine therapy (ET). *J Clin Oncol* 2015;33:Abstract 544.
16. Laenkholm AV, Jensen MB, Buckingham W. Prediction of 10yr distant recurrence (DR) using the Prosigna® (PAM50) assay in histological subgroups of a Danish breast cancer

group (DBCG) cohort of postmenopausal Danish women with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5yr of endocrine therapy (ET) alone. *SABCS* 2016.

17. 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.
18. Dubsky P, Brase JC, Jakesz R, Rudas M, Singer CF, Greil R*, et al.* The EndoPredict score provides prognostic information on late distant metastases in ER+/HER2- breast cancer patients. *Br J Cancer* 2013;109:2959-64.
19. Dubsky P, Filipits M, Jakesz R, Rudas M, Singer CF, Greil R*, et al.* EndoPredict improves the prognostic classification derived from common clinical guidelines in ER-positive, HER2-negative early breast cancer. *Ann Oncol* 2013;24:640-7.
20. Myriad Genetics. EPclin in ABCSG-6+8: Subgroup analyses. 2015.