Online supplementary material for the paper: Combined pathologic-genomic algorithm for early stage breast cancer improves cost-effective use of the 21-gene recurrence score assay.

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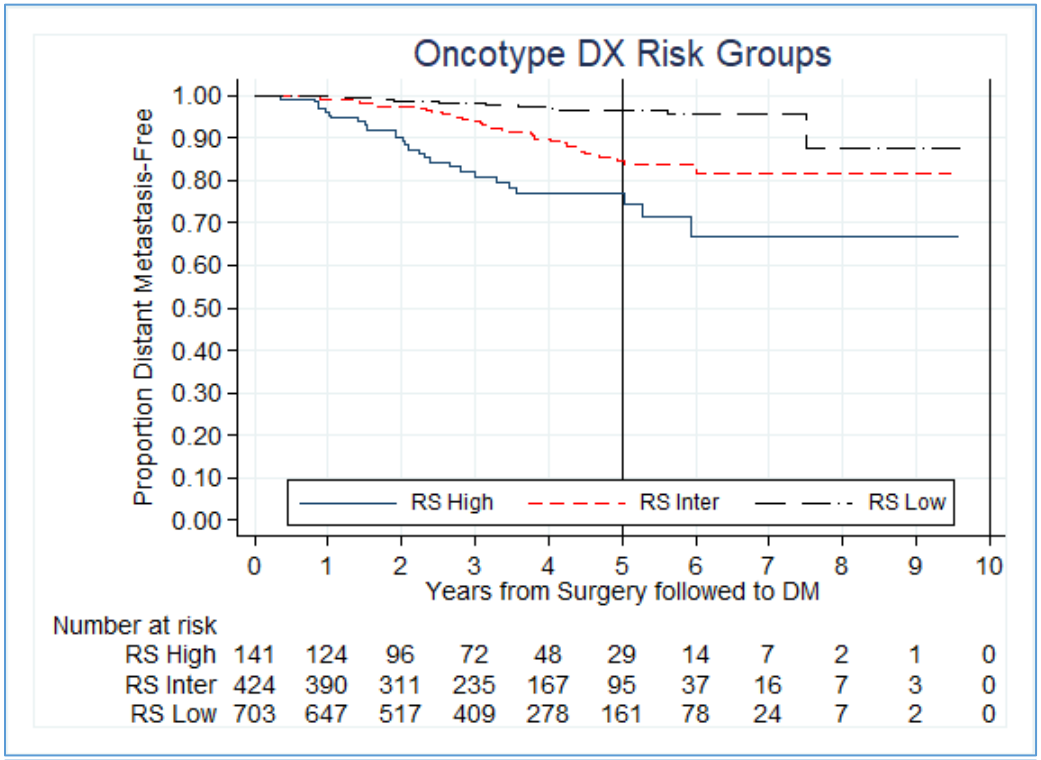
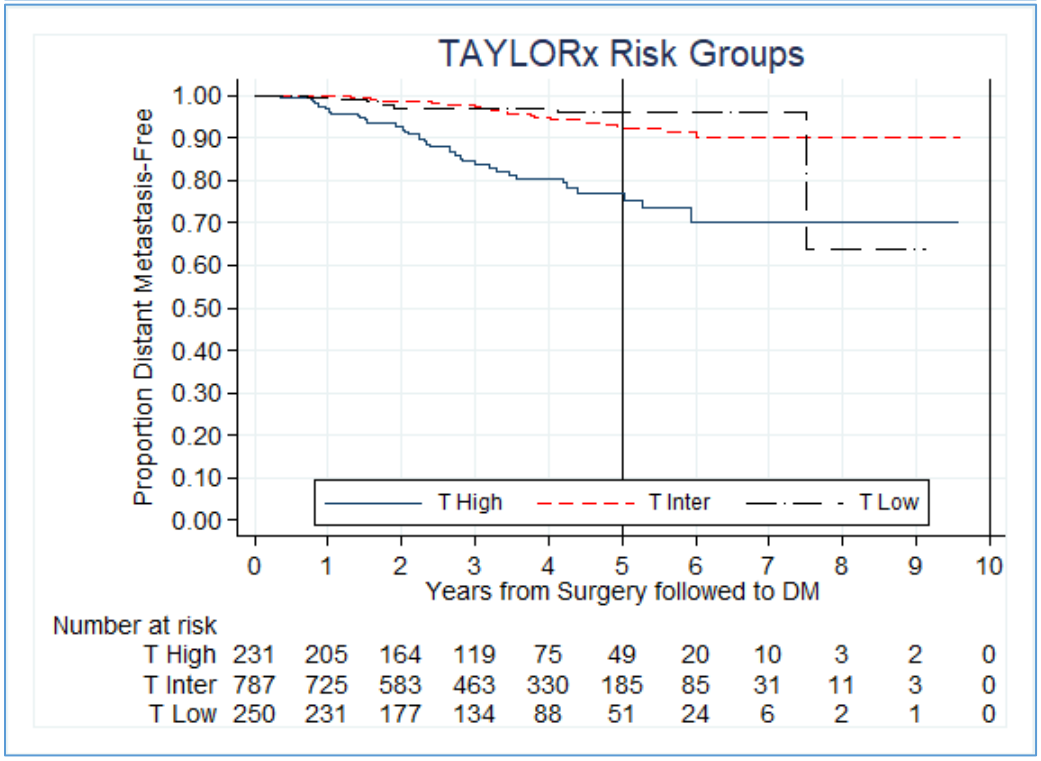
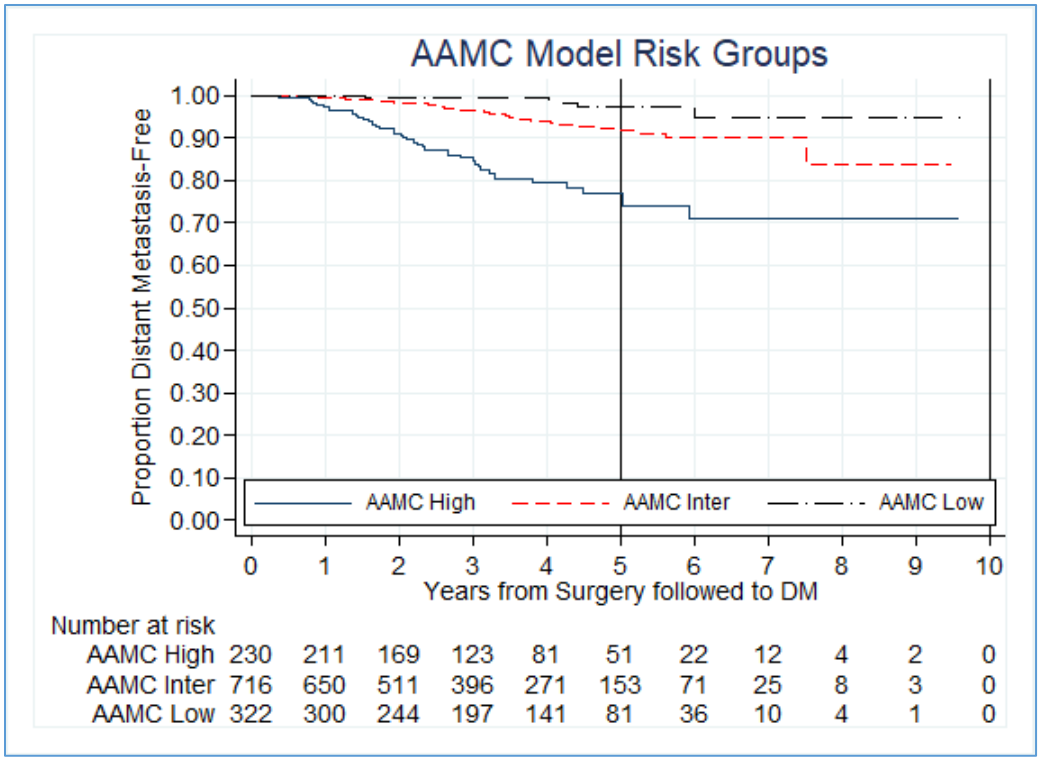
Introduction

This supplement contains information about the group of patients studied in the published paper. The next section displays the Kaplan-Meier curves used in producing the 5-year distant recurrence rates (DRR) reported in the paper. Those graphs are followed by information that is known about the adjuvant therapies given to the patients along with discussion of the context of these data. It then addresses the question of whether the P-G Algorithm, compared to the 21-gene assay, leads to under- or over-treatment of some patients. A discussion of low PR percentage staining as a marker of a poor prognosis is given. These findings support our recommendation that the AAMC Model's definition for low risk be changed to grade 1 and PR >3-5%. Lastly, a table is provided that gives the key characteristics of the patients studied.

Kaplan-Meier Curves Figure S1 shows the Kaplan-Meier curves for freedom from distant metastasis for the four models presented in this paper. The 5-year point is the focus of the results presented.







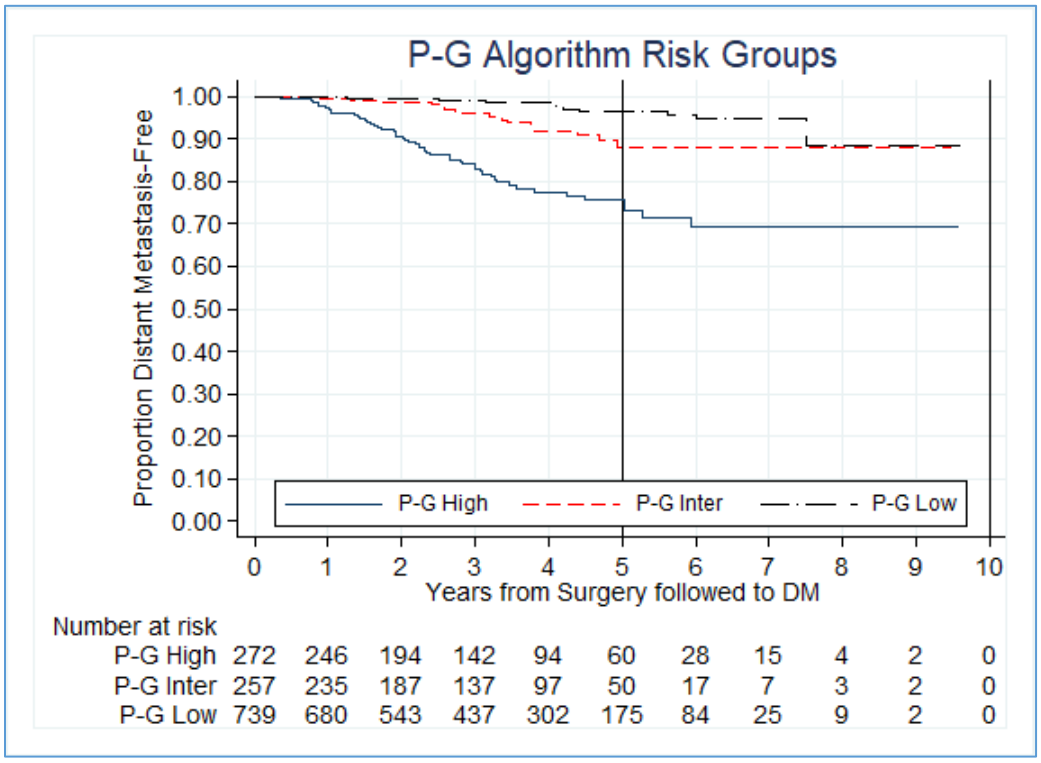


Figure S1. Kaplan-Meier survival curves by model: RS assay, TAILORx, AAMC, and P-G Algorithm. RS= Recurrence Score, T= TAILORx, AAMC= Anne Arundel Medical Center, P-G = Pathologic-Genomic Algorithm.

Knowledge of the Study Group's Adjuvant Therapies

Knowledge of the adjuvant therapies received by study patients was not central to the thesis of the paper. The source of information about the adjuvant therapies is the MD Anderson's Cancer Registry, and as such there are inherent limitations. For patients receiving chemotherapy within the MD Anderson system, documentation of receipt of chemotherapy indicates that the patient received adjuvant chemotherapy and completed at least the majority of the intended treatment. For patients receiving adjuvant treatment outside of MD Anderson, documentation of receipt of chemotherapy indicates that the Cancer Registry learned that the patient started chemotherapy, but does not indicate that the majority of the course was completed. The data on receipt of adjuvant hormonal therapy is more problematic. The Cancer Registry records only a single entry, yes or no, for receipt of hormonal therapy. Receipt of adjuvant therapy is recorded if the patient started therapy; duration and compliance are not documented. For patients treated outside of the MD Anderson system, data on both chemotherapy and hormonal therapy are more likely to be incomplete. Recognizing these limitations, the known data on adjuvant chemotherapy (Table S1) and adjuvant hormonal therapy (Table S2) are presented below.

Table S1: RS Risk Groups by Adjuvant Chemo Treatment Status

Count Row %	N	Y	
RS High	28 19.86%	113 80.14%	141
RS Inter	222 54.01%	189 45.99%	411
RS Low	681 96.87%	22 3.13%	703
	931	324	1255

28/141=19.9%

Table S2: RS Risk Groups by Adjuvant Hormone Treatment Status

Count Row %	N	Y	
RS High	36 25.53%	105 74.47%	141
RS Inter	61 14.39%	363 85.61%	424
RS Low	67 9.53%	636 90.47%	703
	164	1104	1268

164/1268=12.9% (67+61)/(703+424)=11.3%

Characteristics of the 21-gene Low and High Risk patients that recurred.

Table S3 below is an expanded version of Table 2 in the paper with the additional columns reporting whether the database records a patient began chemotherapy ("Y") or hormone therapy ("Y"). Table S4 gives more information about the 21-gene high risk group than is reported in the section entitled "Cases Defined as High Risk" of the paper.

Table S3. Characteristics of all cases defined as Low Risk by RS assay criteria which experienced distant metastasis. The TAILORx low-risk cases are above the bold line. Low PR and high grade cases are highlighted.

RS	AAMC Risk	ER%	PR%	Grade	T Stage	Years to Metastasis	Adj Chemo	Adj Hormone
1	Unknown	95	Negative (<10)	1	T1c	1.5	N	Y
6	High	100	100	3	T1c	1.7	N	Y
9	Intermediate	96	96	2	T3	1.7	N	Y
9	Intermediate	96	40	2	T1b	0.7	N	Y
9	High	85	75	3	T2	1.9	Y	Y
9	Intermediate	98	50	2	T2	7.5	N	Y
10	Intermediate	95	60	2	T1c	4.1	N	N
10	High	100	70	3	T2	1.1	N	Y
11	Intermediate	90	90	2	T1c	2.5	N	Y
13	Intermediate	100	50	2	T1b	1.3	N	Y
13	Intermediate	95	95	2	T1c	2.8	N	Y
14	Intermediate	75	75	2	T2	5.6	N	Y
14	Intermediate	90	60	2	T1c	3.6	N	N
15	Intermediate	90	2	2	T1b	3.1	N	Y
15	Intermediate	100	30	2	T1c	4.0	N	Y
17	High	80	80	3	T1c	2.2	N	Y
17	High	100	5	3	T1c	3.3	N	Y

Table S4. Characteristics of all cases defined as High Risk by TAILORx criteria which experienced distant metastasis. Low PR, low ER, and low grade cases are highlighted. The RS High-risk cases are below the bold line.

RS	AAMC Risk	ER%	PR%	Grade	Years to Metastasis	Adj Chemo	Adj Hormone
26	High	100	90	3	2.7	Y	Y
26	Indeterminate	91	25	2	3.2	N	Y
26	Indeterminate	50	20	2	2.7	N	Y
27	High	97	100	3	2.2	Y	Y
27	High	90	60	3	2.3	Y	Y
28	High	80	100	3	0.8	N	N
28	High	100	20	3	1.4	Y	Y
28	High	90	90	3	2.8	Y	Y
28	High	20	80	3	4.3	Y	Y
29	High	91	1	3	0.8	N	N
29	Indeterminate	100	85	2	4.4	Y	Y

30	Low	100	1	1	4.2	Y	Y
31	Low	95	0	1	0.9	N	N
32	High	80	80	3	2.3	N	Y
32	High	67	33	3	1.0	Y	Y
32	Indeterminate	80	1	2	3.5	Y	Y
32	Indeterminate	90	100	2	1.9	Y	Y
32	High	94	35	3	5.9	N	Y
33	High	50	90	3	2.0	Y	Y
34	Inter	95	0	2	1.5	Y	N
34	High	35	40	3	1.4	Y	Y
34	Indeterminate	75	4.5	2	3.6	Y	Y
35	High	81	71	3	1.0	N	Y
35	High	70	20	3	2.1	Y	Y
36	High	10	10	3	0.9	Y	N
36	High	70	0	3	1.9	Y	Y
36	Indeterminate	95	11	2	5.3	Y	Y
36	High	85	30	3	5.0	Y	Y
38	High	90	70	3	0.4	Y	Y
38	Indeterminate	100	4.5	2	1.0	Y	Y
39	High	98	40	3	2.6	N	Y
41	Indeterminate	90	3	2	2.2	Y	Y
41	Indeterminate	100	70	2	2.4	Y	Y
42	High	90	90	3	0.8	Y	Y
45	High	55	4.5	3	3.3	Y	Y
46	High	99	5	3	3.0	Y	Y
46	Indeterminate	90	90	2	2.8	Y	Y
49	High	100	1	3	2.0	Y	Y
56	High	50	0.9	3	1.5	Y	N

The Possibility of Over or Under Treatment When Using the P-G Algorithm Instead of Using RS

There is concern that using the P-G Algorithm, rather than the 21-gene assay alone, could lead to under- or over-treatment. In the study group, no patients were P-G Algorithm low risk but RS high risk. Such cases would likely be very rare in practice. (Table S5).

The P-G Algorithm does categorize more patients as high-risk than the 21-gene assay alone; these additional patients nearly all have grade 3 tumors. We suggest that these additional patients will, in fact, benefit from chemotherapy, as their risk of 5-year distant recurrence exceeds 7%. The Paik *et al*., Figure 3 shows that the 10-year risk of distant recurrence was about 15% for Poorly Differentiated, RS Low risk patients [11]. Since the DRR for the Low risk patient increased almost linearly (as shown in Figure 2), we estimate that the 5-year DRR would be close to 7%.

Of the 272 patients in the P-G Algorithm's high risk group, 52 were RS low risk patients. Of these 52, 5 (5/52=9.6%) experienced a distant recurrence, and 4 out of these 5 did not have a record of receiving adjuvant chemotherapy. We suggest that, with a recurrence rate of 9.6%, the RS low risk patients with

grade 3 tumors (who were thus assigned to the P-G Algorithm's high risk group) were put at risk by not being offered chemotherapy.

There were 79 patients classified as P-G Algorithm high risk and RS intermediate risk. In this group, 19 (19/79=24.1%) experienced a distant recurrence. Only 8 of these 19 are recorded as having received adjuvant chemotherapy.

Of the 88 patients classified as P-G Algorithm low risk and RS intermediate risk there were 4 that had a distant recurrence. Only 1 of the 4 was recorded as having gotten chemotherapy; she was PR=1% and had a RS=30. Two the 4 had PR=1%, which supports the need to change our low risk rule to put very low PR percentage cases into the AAMC intermediate risk group to get a 21-gene test.

Table S5. P-G Algorithm Risk Groups by RS Assay Risk Groups

Count Total % Col % Row %	RS Assay High	RS Assay Inter	RS Assay Low	
P-G Algorithm High	141 11.12% 100.00% 51.84%	79 6.23% 18.63% 29.04%	52 4.10% 7.40% 19.12%	272 21.45%
P-G Algorithm Inter	0 0.00% 0.00% 0.00%	257 20.27% 60.61% 100.00%	0 0.00% 0.00% 0.00%	257 20.27%
P-G Algorithm Low	0 0.00% 0.00% 0.00%	88 6.94% 20.75% 11.91%	651 51.34% 92.60% 88.09%	739 58.28%
	141 11.12%	424 33.44%	703 55.44%	1268

The Prognostic Significance of PR

The significance of PR has been demonstrated in recent literature [1-5]. The prognostic significance of quantitative PR is also demonstrated in established models such as the IHC4 [6] and the Magee equations for predicting RS scores [7]. In the present study, 22% of recurrences had PR <3%. Chaudhary et al. demonstrated that patients with negative PR were more likely to have higher RS assay scores [8]. The present study found that 18% of RS assay low-risk cases that recurred had PR <10%, suggesting that low PR is important prognostically, regardless of RS score. Clark *et al*. corroborates our assertion that grade 1 PR negative tumors are not low risk tumors. They report that all the case of RS>30 that were grade 1 had low PR values with a median value of 0 [9].

Although the AAMC Model uses negative PR as a risk criterion, the *post hoc* analysis in the present study suggests that not only negative PR, but very low PR, predicts recurrence. For example, among cases that recurred, all those with PR <3% recurred within 5 years, with an average time to distant recurrence of 2.3 years. The significance of very low PR in addition to negative PR may be partially due to inter-

observer variability in PR scoring among pathologists. Cohen *et al.* demonstrated that focal weak expression of a few tumor cells is reported by some pathologists as PR<1%, whereas others report PR≥1% [10].

Based on these findings, we suggest that in the future using the rule that only grade 1 tumors with a PR percent less than 3% (or, for the more risk-adverse, less than 5%) be considered low risk in the AAMC model contained in the P-G Algorithm.

Characteristics of the Study Group

Table S6: Table of characteristics of the studied group of patients.

Characteristic	Number (%)	
Population	1268 (100)	
Mean Age at DX		54.8 years
Tumor Type		
IDC	1029 (81.2)	
IDC&ILC	76 (6.0)	
ILC	127 (10.0)	
Other	36 (2.8)	
Tumor Size		
T1a & mic	29 (2.3)	
T1b	275 (21.7)	
T1c	640 (50.5)	
T2	304 (24.0)	
T3	20 (1.6)	
Grade		
1	337 (26.6)	
2	706 (55.7)	
3	225 (17.7)	

Characteristic	Number (%)	
ER%		
Mean		89.5%
Std Dev		16.6%
ER%<20%	17 (1.3)	
ER%≥20%	1251 (98.7)	
PR%		
Mean		65.4%
Std Dev		36.2%
PR%<3%	122 (9.6)	
PR%≥3%	1146 (90.4)	
HER2		
0	498 (39.3)	
1+	496 (39.1)	
2+ & (FISH Neg)	175 (13.8)	
Negative	99 (7.8)	
Ki67		
Mean		19.3%
Std Dev		17.5%
RS		
RS<11	250 (19.7)	
RS 11-17	453 (35.7)	
RS 18-25	334 (26.3)	
RS 26-30	90 (7.1)	
RS>30	141 (11.1)	
AAMC Model		

Characteristic	Number (%)	
Low Risk	322 (25.4)	
Intermediate	716 (56.5)	
High Risk	230 (18.1)	
P-G Algorithm		
Low Risk	739 (58.3)	
Intermediate	257 (20.3)	
High Risk	272 (21.4)	

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