Long-term follow-up of early stage breast cancer patients with results of MammaPrint®, Oncotype DX® and MammoStrat® risk classification assays

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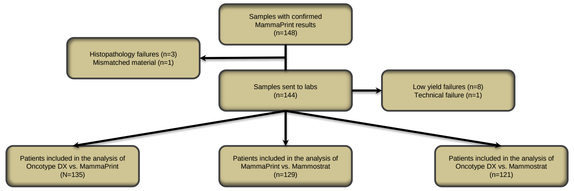
BACKGROUND & OBJECTIVES

The use of genomic tests for the prediction of breast cancer recurrence and the need for chemotherapy is becoming more common. MammaPrint® (**MP**, Agendia NV) is a 70-gene microarray assay designed to assess the 10-year risk of recurrence in an untreated population that was not selected for ER/HER2 results. The Onco*type* DX® Recurrence Score® (**RS**, Genomic Health, Inc.) is a 21-gene RT-PCR assay that is clinically validated to predict the 10-year risk of distant recurrence in ER+ patients treated with Tamoxifen. MammoStrat® (**MS**, Clarient, Inc.) is an IHC assay that uses 5 antibodies and has been validated in a similar population as RS. Several recent reports show that these assays classify patients differently with significant discordances for all risk groups (Shivers, et al., SABCS 2013; Dendduluri, et al., ASCO Breast 2011; Poulet, et al., SABCS 2012; Schneider, et al., ASCO 2013). The present study is an analysis of long-term follow-up in a cohort of patients (Shivers, et al., SABCS 2013) who have results for all three of these risk-stratifying assays side by side in the same samples.

METHODS

Patients with ER+ early-stage breast cancer with an MP result obtained as part of their routine clinical care were identified at the University of South Florida (USF, N=65) and Morton Plant Hospital (N=83). Slides and/or blocks were cut and de-identified at USF and sent to Genomic Health and Clarient for blinded testing. Clinicopathological features were also reviewed by 3 breast pathologists.



RESULTS

148 patients with an MP result had tissue available to send for RS and MS assays. These patients had a median age of 62 years; median tumor size 1.8 cm; 9% low grade, 59% intermediate grade and 32% high grade. In our previous analysis of this study, of 148 patients with MP results, 53% were low risk and 47% were high risk. Of 135 samples that yielded enough RNA to produce an RS result, 53% were low risk, 26% were intermediate risk and 21% were high risk. Of 129 samples that yielded an MS result, 44% were low risk, 28% were moderate risk and 28% were high risk. Of 121 patients with results for all 3 assays, only 22% were concordant for low risk and 9% were concordant for high risk across all 3 assays. Overall, 30% of cases showed a major discordance such as low risk for one assay and high risk for another. After median follow-up of 54 months, 9 patients have had a distant metastasis and/or 10 patients have died (13 patients total; Table 1). One patient who had bone metastasis and died had been classified as low risk by all 3 assays. Three patients with distant metastases had a major discordance between assays, with two high risk and one low risk result. Eight patients were classified as high or intermediate/ moderate risk by all 3 assays.

Figure 1a. MammaPrint vs. Oncotype DX Results.

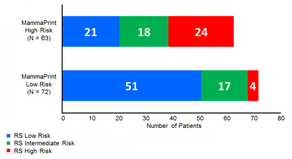
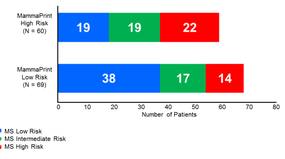
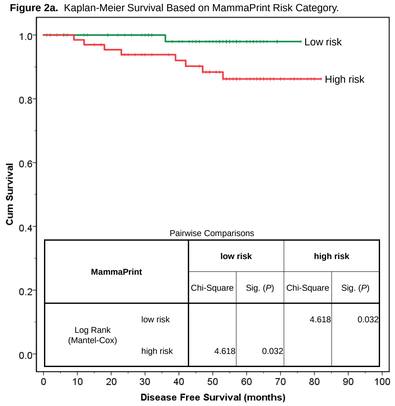


Figure 1b. MammaPrint vs. Mammostrat Results.



RESULTS, Cont.

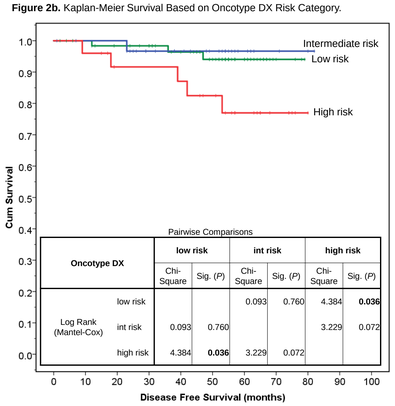
Figure 2a. Kaplan-Meier Survival Based on MammaPrint Risk Category.



Pairwise Comparisons

MammaPrint		low risk		high risk	
		Chi-Square	Sig. (*P*)	Chi-Square	Sig. (*P*)
Log Rank (Mantel-Cox)	low risk			4.618	0.032
	high risk	4.618	0.032		

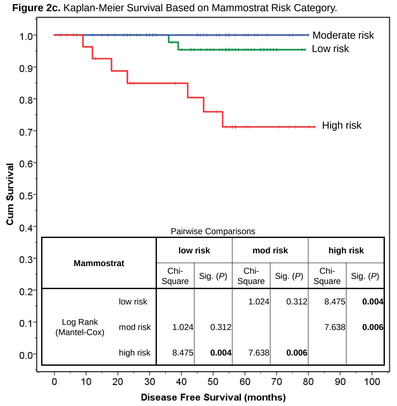
Figure 2b. Kaplan-Meier Survival Based on Oncotype DX Risk Category.



Pairwise Comparisons

Oncotype DX		low risk		int risk		high risk	
		Chi-Square	Sig. (*P*)	Chi-Square	Sig. (*P*)	Chi-Square	Sig. (*P*)
Log Rank (Mantel-Cox)	low risk			0.093	0.760	4.384	**0.036**
	int risk	0.093	0.760			3.229	0.072
	high risk	4.384	**0.036**	3.229	0.072		

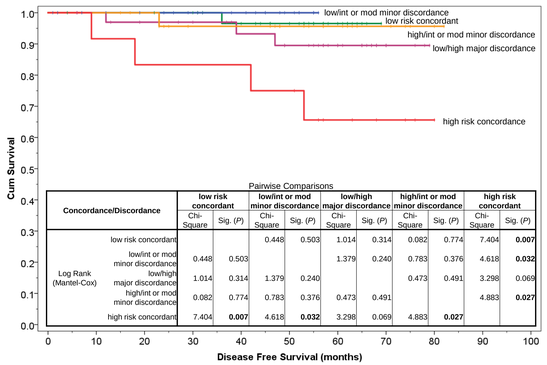
Figure 2c. Kaplan-Meier Survival Based on Mammostrat Risk Category.



Pairwise Comparisons

Mammostrat		low risk		mod risk		high risk	
		Chi-Square	Sig. (*P*)	Chi-Square	Sig. (*P*)	Chi-Square	Sig. (*P*)
Log Rank (Mantel-Cox)	low risk			1.024	0.312	8.475	**0.004**
	mod risk	1.024	0.312			7.638	**0.006**
	high risk	8.475	**0.004**	7.638	**0.006**		

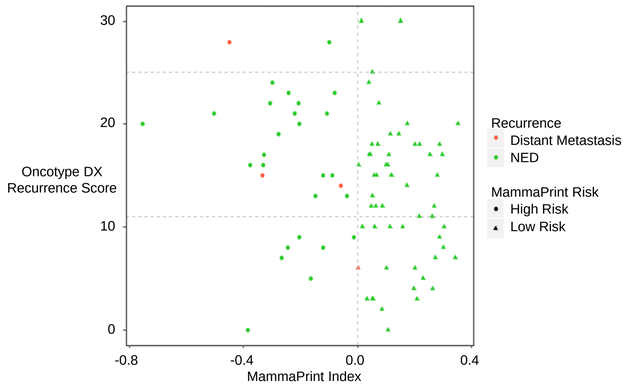
Figure 3. Kaplan-Meier Survival Based on Risk Assay Concordance/Discordance.



Pairwise Comparisons

Concordance/Discordance		low risk concordant		low/int or mod minor discordance		low/high major discordance		high/int or mod minor discordance		high risk concordant	
		Chi-Square	Sig. (*P*)	Chi-Square	Sig. (*P*)	Chi-Square	Sig. (*P*)	Chi-Square	Sig. (*P*)	Chi-Square	Sig. (*P*)
Log Rank (Mantel-Cox)	low risk concordant			0.448	0.503	1.014	0.314	0.082	0.774	7.404	**0.007**
	low/int or mod minor discordance	0.448	0.503			1.379	0.240	0.783	0.376	4.618	**0.032**
	low/high major discordance	1.014	0.314	1.379	0.240			0.473	0.491	3.298	0.069
	high/int or mod minor discordance	0.082	0.774	0.783	0.376	0.473	0.491			4.883	**0.027**
	high risk concordant	7.404	**0.007**	4.618	**0.032**	3.298	0.069	4.883	**0.027**		

Figure 4. MammaPrint Index vs. Oncotype DX Recurrence Score, Intermediate Range Results



RESULTS, Cont.

Table 1. Patient Characteristics for Those Who Had a Distant Metastasis or Died.

Study ID	Risk Assays*	Molecular Subtype**	Stage at Diagnosis	Hormonal Treatment	Chemotherapy	Months to Dist. Metast.	Site of Metastasis	Months F/U	Vital Status	Disease Status
31300	LLL	luminal	pT2 pN0	TAM		36	bone	46	dead	breast cancer COD
20143	LLL	luminal	unknown					40	dead	other COD
31400	HLH	luminal	pT2 pN0			12	bone	16	dead	breast cancer COD
30600	HLH	luminal	pT2 pN1	AI	AC+T	47	neck	69	alive	AWD
10079	HIH	luminal	pT1c pN0(i-)	AI	capecitabine	23	bone	51	dead	breast cancer COD
10021	HIH	luminal	pT1c pN1a					32	dead	other COD
37700	HHL	luminal	pT2 pN0(i-)			39	site unspecified	49	dead	breast cancer COD
10105	HHM	luminal	pT2 pN0(i-)	AI				59	dead	unknown COD
10048	HHM	luminal	pT1c	TAM, AI				22	dead	unknown COD
36500	HHH	luminal	pT2 pN1	AI	FEC, Tax, Tras	42	bone, lung, liver, adr.	53	dead	breast cancer COD
35600	HHH	luminal	pT1c pN0			53	bone, lung, liver	67	dead	breast cancer COD
10113	HHH	her-2	pT2	TAM, AI, LHRH	Carbo, Tax, Tras	18	liver	23	alive	AWD
10112	HHH	basal	pT2 pN0(i-)	AI	AC+T	9	liver	26	alive	AWD

* Risk category for **MammaPrint**, **Oncotype**, **Mammostrat**, respectively. L=low risk, I=intermediate risk, M=moderate risk, H=high risk.

** Determined by the BluePrint 80-gene microarray assay (Agendia, Inc.)

CONCLUSIONS

1. As previously reported, this direct comparison demonstrates that MammaPrint, Oncotype DX and Mammostrat assays classify significant proportions of patients differently. Major (low-high) and minor (low or high to moderate/intermediate) discordances were observed between all three assays:
   - Between MammaPrint and Oncotype DX assays, we found19% major, 26% minor and 44% overall discordance.
   - Between the Oncotype DX and Mammostrat assays, there was a 12% rate of major discordance.
   - Between MammaPrint and Mammostrat assays we found 26% major, 28% minor and 53% overall discordance.
2. Wide ranges of Recurrence Score values from the Oncotype DX assay and Risk Index values from the Mammostrat assay were observed within each MammaPrint risk group (data not shown).
   - Possible explanations for the observed variation in risk stratification include differences in baseline characteristics of the original study cohorts, differences in tumor biology and/or differences in assay technology.
3. With a median follow-up of 54 months, all 3 assays had a *P* value less than 0.05 for the Cox-Mantel log rank test comparing the survival of patients classified as low risk vs. those classified as high risk
   - For Oncotype DX and Mammostrat, which classify some patients as intermediate or moderate risk, respectively, these risk groups were not significantly different than the low risk or high risk groups (by the log rank test), but their survival curves look more like the low risk groups (see Fig. 2b and 2c)
   - 26% of Oncotype patients were classified intermediate (18-30), (Fig. 1a) when expanded to include up to RS11 (TAILORx trial intermediate range), this percentage increased to 56%, however, the current analysis of TAILORx has not yet reported outcomes data for the randomized intermediate RS sub-cohort. Without these data, physicians cannot exclude or confirm a benefit from chemotherapy in this intermediate population.
   - As seen here, MammaPrint, a binary test, classified Oncotype intermediate patients to 58% low risk and 42% high risk with low or high risk patients at every intermediate recurrence score. (Fig. 4)
   - MammaPrint demonstrated the ability to correctly assign risk in this Oncotype intermediate RS range as indicated by excellent MammaPrint low risk survival. (Fig. 2a)
4. Of the 13 patients who either died or suffered a distant metastasis (Table 1), two were classified as low risk by all 3 assays.
   - One patient developed bone metastasis at 36 months and died of breast cancer at 46 months. The other patient did not recur and apparently died of another cause.
   - MammaPrint classified 11/13 (85%), Oncotype 7/13 (54%) and Mammostrat 8/13 (62%) high risk.
   - Only 4/13 (31%) of these patients were high risk by all 3 assays.
   - When grouped by assay concordance/discordance, only the group that was high risk concordant for all 3 assays was significantly different from the other groups, although the major discordant group (both high and low risk) was not significantly different at *P* = 0.069 (Fig. 3).

This study has important clinical implications since these assays are used to help make treatment decisions. These data demonstrate assays classify a large proportion of patients differently. Some patients, despite being classified as high risk by one or more assays did not receive chemotherapy, underscoring the importance of receiving a clinically accurate and actionable result the first time. Additionally, the current clinical environment does not readily support ordering multiple tests. As seen from these long-term follow-up results, an assay that provides timely, clear and validated results to patients and providers is essential.

ACKNOWLEDGEMENTS

Charles Cox, MD and Peter Blumencranz are on the speakers' bureau for Agendia, Inc. and Dr. Cox is on the Medical Advisory Board for Agendia. Dr. Cox is also the PI of the current study co-sponsored by Agendia, Genomic Health and Clarient (each company provided only genomic testing). All other authors have nothing to disclose.

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