Four Genes by RT-PCR Predicts Distant Relapse for Women Given Adjuvant Tamoxifen

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Background

1. Many gene classifiers developed recently claim to predict clinical outcome better than currently used clinical factors.

2. Whilst microarray technology offers huge advantages in furthering our knowledge of breast cancer biology, for present-day use, clinically applicable and useful tools may need only the quantitative information that is provided by genes that are already well known to convey prognostic/predictive information in breast cancer: **ESR1, PgR, Her2 and Ki67**.

Material and Methods

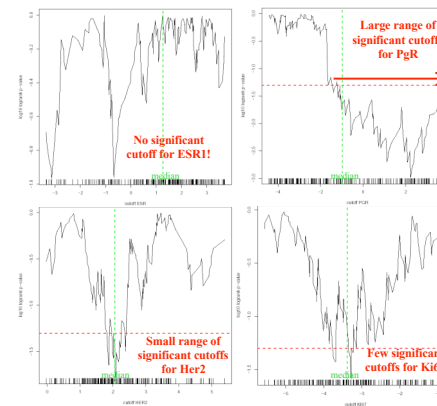*Table 1: Tamoxifen-only treated patient's demographics*

	All patients	OXF	GUY1	GUY2
Number of patients	227	79	77	71
Median Age				
≤ 50 (%)	13 (6%)	3 (4%)	3 (4%)	7 (10%)
> 50 (%)	214 (98%)	74 (96%)	74 (96%)	64 (90%)
Median Tumor Size(cm)				
≤ 2 cm (%)	103 (45%)	34 (43%)	39 (51%)	30 (42%)
> 2 cm (%)	122 (55%)	45 (57%)	36 (47%)	40 (58%)
Unknown	2 (1%)		2 (2%)	
Nodal Status				
N0	111 (49%)	49 (62%)	24 (31%)	38 (54%)
>N0	116 (51%)	29 (38%)	53 (69%)	33 (46%)
Histological Grade				
I	42 (19%)	16 (20%)	13 (17%)	13 (19%)
II	91 (40%)	40 (51%)	33 (43%)	18 (25%)
III	46 (20%)	9 (11%)	15(19%)	22 (31%)
Unknown	48 (21%)	14 (18%)	16 (21%)	18 (25%)
Number of distant metastases	50	26	9	15
Median follow up (years)	8.88	5.74	13.19	8.43

Table 2: RT-PCR primers-performed on frozen samples

100 ng   1 ng   10 pg
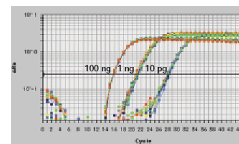
gene	Forward primers	Reverse primers	Size
ESR1	TGTGTGTTTAGAGCTGTGCACCCTAGAAACAAC	GCACCTGCTCATGGGACAA	78
PgR	AATTCCTTTGGAAGGGCTACG	CCAATTGCCTTGATGAGCTCT	84
Her2	TTCAAAGGGACACCTACGGC	GCCTTCTGGTTCACACTGGC	70
Ki67	CAGCCTCTCTTGGGCTTTCTT	GTGTGGTGGTTGGAGGTGC	82

Results

1. **Correlation between Affymetrix and RT-PCR results:**

	Spearman rho	p-value
Her2	0.636	0.000001
PgR	0.758	0.000001
ESR1	0.465	0.000001
Ki67	0.447	0.000001

→ **A highly significant correlation is observed between microarray and RT-PCR values**

2. Cutoff effect on DMFS significance:

No significant cutoff for ESR1!

Large range of significant cutoffs for PgR

Small range of significant cutoffs for Her2

Few significant cutoffs for Ki67

3. Kaplan-Meier using median as a cutoff:
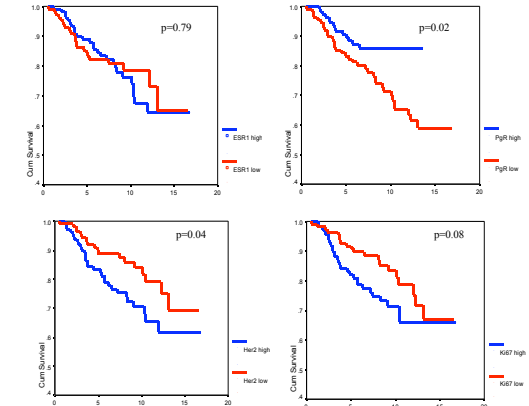
4. Multivariate analysis (backward selection):

	p-value	HR	95.0% CI for HR	
PgR (median)	**0.007**	0.33	0.15	0.74
Her2 (median)	**0.013**	2.41	1.2	4.82
Grade	0.063	1.67	0.97	2.88
Tumor Size	**0.006**	2.84	1.35	5.96

Conclusions & Perspectives

1) A very good correlation was observed between microarray and RT-PCR values regarding these 4 genes.

2) Only PgR and Her2 were significantly associated with DMFS in univariate and multivariate analysis. ESR1 was not associated with DMFS in these Tamoxifen treated patients.

3) Correlation between microarray, RT-PCR and immunohistochemistry data is currently being evaluated.

4) This RT-PCR protocol is being validated on formalin-fixed, paraffin-embedded samples.