

# Prognostic Utility of HOXB13:IL17BR and Molecular Grade Index for Node-negative Breast Cancer Patients

Laurel A. Habel<sup>1</sup>, Mark G. Erlander<sup>2</sup>, Ninah Achacoso<sup>1</sup>, Xiao-Jun Ma<sup>2</sup>, Dennis Sgroi<sup>3</sup>, Louis Fehrenbacher<sup>4</sup>, Deborah Greenberg<sup>5</sup>, Charles Quesenberry, Jr.<sup>1</sup>

<sup>1</sup> Kaiser Permanente, Oakland, CA; <sup>2</sup> BioTheranostics, San Diego, CA; <sup>3</sup> University of Massachusetts, Boston, MA; <sup>4</sup> Oncology, Kaiser Permanente, Vallejo, CA; <sup>5</sup> Kaiser Permanente, Richmond, CA



Sponsor: BioTheranostics, San Diego, CA

## BACKGROUND

Four studies reported that HOXB13:IL17BR may be prognostic and/or predictive of tamoxifen benefit among early stage breast cancer patients (1-4). More recently, a 5-gene (BUB1B, CENPA, NEK2, RACGAP1, RRM2) molecular grade index (MGI) was reported to predict clinical outcome (5).

## AIMS

To evaluate the performance of these two gene signatures among an independent population of lymph node-negative breast cancer patients from a community hospital setting.

## METHODS

### Design/study population:

Case-control nested within a cohort of 4,964 Kaiser Permanente patients diagnosed with node-negative invasive breast cancer from 1985-94 and not treated with adjuvant chemotherapy.

### Gene selection for HOXB13:IL17BR and MGI

HOXB13 and IL17BR previously identified from microarray analysis of 60 patients with ER+ breast cancer treated with 5 yrs of tamoxifen. From a microarray analysis of 36 breast cancer specimens, a set of 39 genes was identified with increased expression in high-grade tumors. The list of 39 genes was then narrowed to 5, based on functional annotation of the genes, association with clinical outcome, and correlation with tumor grade in an independent sample of 60 patients.

### Gene expression analysis by RT-PCR

Expression of 11 genes (ER, PR, HER2, HOXB13, IL17BR, CHDH, BUB1B, CENPA, NEK2, RACGAP1, RRM2) and 4 normalization genes (ACTB, HMBS, SDHA and UBC) were measured by TaqMan RT-PCR.

### Calculation of gene expression indices

HOXB13:IL17BR and MGI were categorized into low and high groups using pre-specified cutoffs.

### Statistical analysis:

Logistic regression methods were used to estimate the 10-year risk and the relative risk (RR) of breast cancer death associated with pre-specified risk categories based on cross-classification of HOXB13:IL17BR and MGI scores.

## RESULTS

Table 1. Gene lists

HOXB13:IL17BR	MGI
HOXB13	BUB1B
IL17BR	CENPA
	NEK2
	RACGAP1
	RRM2

Table 2 Selected characteristics of the study population

Characteristic	Cases (n = 191)	Controls (n = 417)
<b>Matched variables</b>		
Age at diagnosis (years)		
<40	15 (8%)	20 (5%)
40-49	33 (17%)	94 (23%)
50-59	59 (31%)	113 (27%)
60-74	84 (44%)	190 (46%)
Race/ethnicity		
White, non-Hispanic	146 (76%)	326 (78%)
White, Hispanic	7 (4%)	11 (3%)
Black	19 (10%)	42 (10%)
Asian	19 (10%)	38 (9%)
Surgery year		
1985-1989	127 (66%)	278 (67%)
1990-1994	64 (34%)	139 (33%)
Adjuvant tamoxifen		
No	134 (70%)	281 (67%)
Yes	57 (30%)	136 (33%)
<b>Unmatched variables</b>		
ER status from RT-PCR <sup>1</sup>		
Positive	145 (76%)	370 (89%)
Negative	46 (24%)	47 (11%)
Tumor size (cm)		
≤1	40 (21%)	128 (31%)
1.1-2	84 (44%)	187 (45%)
2.1-4	63 (33%)	96 (23%)
>4	4 (2%)	6 (1%)
Tumor grade (differentiation) <sup>2</sup>		
Well	21 (11%)	123 (29%)
Moderate	79 (41%)	182 (44%)
Poor	91 (48%)	112 (27%)
MGI		
Low risk	64 (34%)	231 (55%)
High risk	127 (66%)	186 (45%)
MGI+HOXB13:IL17BR		
Low risk	64 (34%)	231 (55%)
Intermediate risk	39 (20%)	84 (20%)

<sup>1</sup>Cutoff points based on GHI RT-PCR values: ≤6.5 and >6.5 units.

<sup>2</sup>Bloom-Richardson grading criteria, pathologist 1.

ER, estrogen receptor; RT-PCR, reverse transcription polymerase chain reaction.

Table 3 Distributions of tumor characteristics by categories of MGI and MGI+HOXB13:IL17BR for 191 cases and 417 controls

	HOXB13:IL17BR		MGI		MGI+HOXB13:IL17BR		
	Low (n = 277)	High (n = 331)	Low (n = 295)	High (n = 313)	Low (n = 295)	Inter (n = 123)	High (n = 190)
<b>Tumor size (cm)</b>							
≤1 (n = 168)	54%	46%	61%	39%	61%	17%	23%
1.1-2 (n = 271)	45%	55%	48%	52%	48%	21%	31%
2.1-4 (n = 159)	40%	60%	36%	64%	36%	24%	40%
>4 (n = 10)	10%	90%	60%	40%	60%	0%	40%
<b>Tumor grade p</b>							
Well (n = 144)	63%	37%	78%	22%	78%	12%	10%
Moderate (n = 261)	48%	52%	61%	39%	61%	20%	19%
Poor (n = 203)	30%	70%	12%	88%	12%	26%	62%
<b>ER</b>							
Negative (n = 93)	19%	81%	17%	83%	17%	18%	65%
Positive (n = 515)	50%	50%	54%	46%	54%	21%	25%
<b>PR</b>							
Negative (n = 167)	20%	80%	27%	73%	27%	17%	56%
Positive (n = 441)	55%	45%	57%	43%	57%	22%	22%
<b>HER2</b>							
Negative (n = 534)	50%	50%	51%	49%	51%	21%	28%
Positive (n = 74)	15%	85%	30%	70%	30%	12%	58%

Table 4 Relative risks associated with MGI and MGI+HOXB13:IL17BR among ER-positive patients, stratified by treatment with tamoxifen

	Cases		Controls		RR <sup>1</sup> (95% CI)		RR <sup>2</sup> (95% CI)	
	n	%	n	%				
<b>Tamoxifen treated (49 cases and 112 controls)</b>								
HOXB13:IL17BR								
Low risk	16	33%	55	49%	1.0	ref	1.0	ref
High risk	33	67%	57	51%	2.3	1.1 – 4.8	2.0	0.9 – 4.5
MGI								
Low risk	15	31%	62	55%	1.0	ref	1.0	ref
High risk	34	69%	50	45%	2.8	1.3 – 6.2	2.0	0.8 – 4.9
MGI+HOXB13:IL17BR								
Low risk	15	31%	62	55%	1.0	ref	1.0	ref
Intermediate risk	12	24%	27	24%	1.6	0.6 – 4.3	1.1	0.3 – 3.3
High risk	22	45%	23	21%	3.9	1.6 – 9.2	2.9	1.1 – 7.8
<b>Tamoxifen untreated (90 cases and 174 controls)</b>								
HOXB13:IL17BR								
Low risk	38	42%	99	57%	1.0	ref	1.0	ref
High risk	52	58%	75	43%	1.8	1.1 – 3.0	1.4	0.8 – 2.5
MGI								
Low risk	39	43%	115	66%	1.0	ref	1.0	ref
High risk	51	56%	59	34%	2.8	1.6 – 5.1	1.9	0.9 – 4.1
MGI+HOXB13:IL17BR								
Low risk	39	43%	115	66%	1.0	ref	1.0	ref
Intermediate risk	20	22%	27	16%	2.7	1.3 – 5.5	1.9	0.8 – 4.5
High risk	31	34%	32	18%	3.0	1.5 – 5.8	1.9	0.7 – 4.6

<sup>1</sup>Univariate conditional logistic regression models include MGI or MGI+HOXB13:IL17BR

<sup>2</sup>Multivariable conditional logistic regression models include MGI or MGI+HOXB13:IL17BR plus tumor size and grade.

Table 5 Ten-year risk of death in relation to MGI+HOXB13:IL17BR and tumor size and grade among ER+ patients, stratified by treatment

Risk classifier	10-Year risk	
	%	95% CI
<b>Tamoxifen treated (49 cases;112 controls)</b>		
MGI+HOXB13:IL17BR		
Low	3.9	2.2 – 5.5
Intermediate	5.6	2.6 – 8.5
High	15.6	8.7 – 21.9
Tumor size		
≤1 cm	3.9	1.8 – 6.1
1.1-2 cm	6.5	4.1 – 8.9
>2 cm	9.4	5.1 – 13.6
Tumor grade		
Well	2.4	0.8 – 4.
Moderate	7.3	4.8 – 9.7
Poor	10.3	6.3 – 14.1
Tumor size and grade		
≤2 cm and well or ≤1 cm and moderate	3.0	1.3 – 4.7
>2 cm and well, or 1.1-2 cm and moderate, or ≤2 cm and poor	7.1	4.7 – 9.5
>2 cm and moderate/poor	12.2	6.3 – 17.8
<b>Tamoxifen untreated (90 cases;174 controls)</b>		
MGI+HOXB13:IL17BR		
Low	5.9	4.2 – 7.5
Intermediate	15.2	8.3 – 21.5
High	16.5	10.4 – 22.2
Tumor size		
≤1 cm	7.0	4.4 – 9.5
1.1-2 cm	7.8	5.5 – 10.0
>2 cm	13.9	8.8 – 18.7
Tumor grade		
Well	4.6	2.7 – 6.5
Moderate	8.6	6.3 – 10.9
Poor	21.2	13.1 – 28.5
Tumor size and grade		
≤2 cm and well or ≤1 cm and moderate	5.1	3.3 – 6.9
>2 cm and well, or 1.1-2 cm and moderate, or ≤2 cm and poor	10.5	7.7 – 13.3
>2 cm and moderate/poor	15.9	9.6 – 21.7

Table footnotes

## STRENGTHS AND LIMITATIONS

### Strengths

- Population-based design
- Breast cancer treatment and cause of death confirmed by chart review
- Homogeneous patient groups, with respect to treatment, ER, and stage
- Prospectively specified scores and risk categories
- Assay conducted blinded to study endpoints

### Limitations

- Relatively small number of patients treated with tamoxifen
- Small numbers in some risk groups resulting in imprecise risk estimates

## SUMMARY AND CONCLUSIONS

- In this population of lymph-node negative patients not treated with adjuvant chemotherapy, the HOXB13:IL17BR+MGI risk classifier was predictive of risk of breast cancer death.
- HOXB13: IL17BR+MGI was able to identify a subset of patients at low risk of dying of breast cancer at 10 years.
- This low-risk subset included approximately 50% of patients.
- HOXB13: IL17BR+MGI appeared to provide risk information beyond standard prognostic factors.

## REFERENCES

- Ma XJ, Wang Z, Ryan PD *et al.*: A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen. *Cancer Cell* 2004, 5: 607-616.
- Ma XJ, Hilsenbeck SG, Wang W *et al.*: The HOXB13:IL17BR expression index is a prognostic factor in early-stage breast cancer. *J Clin Oncol* 2006, 24: 4611-4619.
- Jansen MP, Sieuwerts AM, Look MP *et al.*: HOXB13-to-IL17BR expression ratio is related with tumor aggressiveness and response to tamoxifen of recurrent breast cancer: a retrospective study. *J Clin Oncol* 2007, 25: 662-668.
- Jerevall PL, Brommesson S, Strand C *et al.*: Exploring the two-gene ratio in breast cancer--independent roles for HOXB13 and IL17BR in prediction of clinical outcome. *Breast Cancer Res Treat* 2008, 107: 225-234.
- Ma XJ, Salunga R, Dahiya S *et al.*: A five-gene molecular grade index and HOXB13:IL17BR are complementary prognostic factors in early stage breast cancer. *Clin Cancer Res* 2008, 14: 2601-2608.