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## CYP2D6 and HOXB13/IL17BR: Combining inherited and tumor gene markers for prediction of tamoxifen resistance

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### Abstract

**Purpose**—Genetic variation in *Cytochrome P450 2D6* (*CYP2D6*) and the gene expression ratio of the homeobox 13 (*HOXB13*) to interleukin-17B receptor (*IL-17BR*) are associated with tamoxifen resistance. We sought to determine the combined effect of inherited (*CYP2D6*) and somatic (*HOXB13/IL17BR*) gene variation in tamoxifen treated breast cancer.

**Experimental Design**—Retrospective analysis of women with node negative breast cancer randomized to receive 5 years of tamoxifen (NCCTG 89-30-52). *CYP2D6* metabolism (extensive or decreased) was based on *CYP2D6*\*4 genotype and presence/absence of a *CYP2D6* inhibitor. RT-PCR profiles for *HOXB13* and *IL-17BR* and the cut-point separating patients into high and low risk categories according to disease-free survival (DFS) were utilized. A risk factor (*CYP2D6:HOXB13/IL17BR*) representing the four categories of combining *CYP2D6* metabolism (extensive or decreased) and *HOXB13/IL17BR* (low or high) was created. The association between

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CYP2D6:HOXB13/IL17BR and DFS and overall survival (OS) was assessed using the log-rank test and proportional hazards modeling.

**Results**—CYP2D6 metabolism and *HOXB13/IL17BR* gene ratio was available in 110/160 (69%) patients. The combined CYP2D6:HOXB13/IL17BR risk factor was significantly associated with DFS (log rank  $p=0.004$ ) and OS ( $p=0.009$ ). Relative to women with extensive CYP2D6 metabolism and low *HOXB13/IL17BR*, those with either decreased metabolism or a high *HOXB13/IL17BR* ratio had significantly worse OS [adjusted hazard ratio (HR) =2.41, 95% confidence interval:1.08-5.37;  $p=0.031$ ] whereas women with both decreased metabolism and high *HOXB13/IL17BR* had the shortest survival (adjusted HR=3.15, 95% CI:1.17-8.52,  $p=0.024$ ).

**Conclusions**—An index comprised of inherited (CYP2D6) and tumor (HOXB13/IL17BR) gene variation identifies patients with varying degrees of resistance to tamoxifen.

## Keywords

Tamoxifen; Cytochrome P450 2D6; HOXB13/IL17BR; Breast cancer

## Introduction

Genetic markers are increasingly utilized to identify patients at increased risk for relapse (prognosis) and to predict the likelihood of a therapeutic response (predictive markers). Given that both tamoxifen and aromatase inhibitors (AIs) are effective therapies for the treatment of estrogen receptor (ER) positive breast cancer,<sup>1, 2</sup> there is great interest in identifying biomarkers that may allow the individualization of therapy.

Cytochrome P450 (CYP) 2D6 is a polymorphic enzyme responsible for the metabolic activation of tamoxifen to metabolites (endoxifen and 4-OH tamoxifen) with a significantly greater affinity for the ER and greater ability to inhibit tumor cell proliferation compared with the parent drug (tamoxifen) or its primary metabolite, N-desmethyltamoxifen.<sup>3, 4</sup> Both genetic (inherited traits) and environmental (drug-induced) factors which alter CYP2D6 enzyme activity affect the concentrations of the active tamoxifen metabolites.<sup>5, 6</sup> We and others have demonstrated the importance of CYP2D6 as a predictor of tamoxifen benefit in the prevention,<sup>7</sup> metastatic,<sup>8</sup> and adjuvant settings.<sup>9-11</sup> Based on these data, an FDA Clinical Pharmacology Subcommittee recently recommended that the tamoxifen label should be updated to reflect the increased risk of breast cancer recurrence for women who are CYP2D6 poor metabolizers, resulting from genetic variation or drug-induced inhibition of CYP2D6.

In a genome-wide microarray analysis of tumors from women with ER positive breast cancer treated with adjuvant tamoxifen, Ma et al discovered the *HOXB13/IL-17BR* gene ratio as an independent predictor of treatment outcome and demonstrated that ectopic expression of *HOXB13* in a nontransformed human mammary epithelial cell confers increased cell migration and invasion.<sup>12</sup> Follow-up studies have demonstrated that both *HOXB13* and *IL17BR* are regulated by estradiol in an ER-dependent manner and this regulation is abrogated by tamoxifen.<sup>13</sup> In hormone sensitive cell lines, *HOXB13* expression rendered cells less sensitive to tamoxifen-induced apoptosis.<sup>14</sup> Further clinical analysis of nearly 2700 patients from four independent clinical databases<sup>15-18</sup> has repeatedly validated the H/I ratio as a prognostic marker<sup>18</sup> and a biomarker predictive of tamoxifen benefit,<sup>15-17</sup> with the effect most pronounced in node-negative, ER positive breast cancer.<sup>15, 16, 18</sup>

In a cohort of postmenopausal women with resected ER positive breast treated with adjuvant tamoxifen only, we reported that women with decreased CYP2D6 metabolism (defined as the presence of one or two *CYP2D6* \*4 alleles or the documented co-prescription of a CYP2D6 inhibitor) had significantly shorter disease free survival compared to women with extensive

CYP2D6 metabolism (defined as women who did not carry a *CYP2D6* \*4 allele and were not co-prescribed a CYP2D6 inhibitor).<sup>9</sup> In this same cohort, we demonstrated that a high *HOXB13/IL17BR* ratio was associated with significantly worse DFS and OS, but only in lymph node negative patients.<sup>15</sup>

We hypothesized that because *CYP2D6* and *HOXB13/IL17BR* represent biologically independent markers of tamoxifen resistance, a combined index may provide a better indicator of tamoxifen treatment benefit. Therefore, using the same study population from which we derived our original findings regarding *CYP2D6*<sup>9, 10</sup> and *HOXB13/IL17BR*,<sup>15</sup> we evaluated the combined effect of *CYP2D6* and *HOXB13/IL17BR* on the outcomes of DFS and OS in women with lymph node negative breast cancer treated with adjuvant tamoxifen monotherapy.

## Methods

### Patients

The North Central Cancer Treatment Group (NCCTG) conducted a randomized phase III clinical trial in postmenopausal women with resected ER positive breast cancer to assess the value of administering the androgen flouxymesterone for one year during the standard 5 years of tamoxifen adjuvant therapy (NCCTG 89-30-52). A description of the clinical trial and its outcome has been published.<sup>19</sup> The protocol was amended to assess genetic and environmental factors which may be associated with increase risk of breast cancer relapse. The trial and its amendments were approved by the institutional review board of Mayo Clinic Rochester and the individual NCCTG sites that enrolled patients onto the clinical trial.

The means by which *CYP2D6* genotyping<sup>10</sup> and *HOXB13/IL17BR*<sup>15</sup> were measured and quantified has been previously published. Additionally, we published a comprehensive analysis of both *CYP2D6* genotype and *CYP2D6* enzyme inhibition on tamoxifen treatment outcome.<sup>9</sup> Briefly, a woman who carried either one or two *CYP2D6* \*4 alleles or had any *CYP2D6* genotype but co-prescribed a CYP2D6 inhibitor was considered to have decreased *CYP2D6* metabolism. A woman without a *CYP2D6* \*4 allele and who was not co-prescribed a CYP2D6 inhibitor was considered to have extensive metabolism. A woman was said to have a low *HOXB13/IL17BR* gene ratio if her tumor H/I gene ratio fell below  $<-1.339$ .<sup>15</sup>

**Study Design and End Points**—The primary objective of this study was to assess the association of effect of *CYP2D6* metabolism and the *HOXB13/IL17BR* gene ratio on disease-free survival (DFS) and overall survival (OS) in women with lymph node negative breast cancer receiving adjuvant tamoxifen only. DFS was defined as the time from randomization to documentation of the first of the following events: any recurrence (local, regional or distant) of breast cancer, a contralateral breast cancer, a second primary cancer, or death due to any cause. Patients who were alive without any of these events were censored at the date of their last disease evaluation. OS was estimated as the time from randomization to death due to any cause.

The overall distributions of DFS and OS were estimated using the Kaplan-Meier method. Log rank tests and univariate proportional hazard models were used to assess whether the endpoint differed with respect to any one of the following factors: age greater than 65 (yes vs. no), ER status at time of entry into the trial (10-49 fmols vs.  $\geq 50$  fmols vs. positive by immunohistochemistry), tumor size 3 cm or greater (yes vs. no), Nottingham grade (3 vs. 1 or 2), HER2 expression (3+ vs. 0, 1+, or 2+). For each clinical outcome, multivariate proportional hazard modeling was performed to obtain a subset of the potential prognostic factors which provided an adequate fit to the data. Residual plots were examined. A risk factor (*CYP2D6:HOXB13/IL17BR*) representing the 4 categories of combining *CYP2D6* metabolism (extensive vs. decreased) and *HOXB13/IL17BR* (low vs. high) was created.

Representing CYP2D6:HOXB13/IL17BR as 3 indicator variables, multivariate proportional hazard modeling was performed to assess whether any one of these factors made a significant contribution to the previous established model for that clinical endpoint.

## Results

Our study population is derived from women with node-negative breast cancer (NNBC) (n=160) who were randomized from December 31, 1990 to April 6, 1995 to the tamoxifen only arm of NCCTG 89-30-52. In 110/160 patients, both *HOXB13/IL17BR* gene expression data and a comprehensive assessment of CYP2D6 metabolism (consisting of *CYP2D6* \*4 genotype and medication history) were known. The characteristics of these 110 patients are listed in Table 1 and the combined CYP2D6 and *HOXB13/IL17BR* phenotype is listed in Table 2.

As of October 25 2006, 12 women were alive with disease progression, a second primary, or contralateral breast disease and 37 women had died. The median length of follow-up for those women still alive is 12.5 years (range: 5.7-15.5 years).

**Univariate analyses of Clinical Outcomes**—Four risk groups were formed by combining CYP2D6 metabolism and *HOXB13/IL17BR* (Table 2). DFS and OS were found to differ significantly with respect to these four patient categories (p=0.004 and p=0.009, respectively). Table 2 presents the Kaplan Meier estimates of 5 year DFS rate and its corresponding confidence interval for each risk group. In order to evaluate the cumulative effect of carrying 1 or more risk factors, we analyzed DFS and OS based on the absence of a risk factor (extensive CYP2D6 and *HOXB13/IL17BR* low), only 1 high risk feature (either CYP2D6 metabolism decreased or *HOXB13/IL17BR* high) and finally 2 high risk features (both CYP2D6 decreased and *HOXB13/IL17BR* high). In this analysis, both DFS (p=0.001) and OS (p=0.005) differed significantly according to risk groups (Figures 1 and 2)

**Multivariate analyses of clinical outcomes**—For each endpoint, proportional hazard modeling was performed utilizing age, extent of surgery, ER status, tumor size, tumor grade and Her2 expression. Once tumor size was accounted for, none of the other traditional factors made a significant contribution to explaining the variability in these clinical outcomes. Further multivariate proportional hazard modeling demonstrated that after adjusting for tumor size, DFS and OS were significantly different among patients with 0, 1 or 2 risk factors as determined by CYP2D6 and *HOXB13/IL17BR*. gene ratio. Relative to women with no risk factors (extensive CYP2D6 and *HOXB13/IL17BR* low), women with at least one risk factor had significantly worse DFS (adjusted HR=2.03, 95% confidence interval:1.04-3.94; p=0.037) and OS (adjusted HR=2.41, 95% confidence interval:1.08-5.37, p=0.031). An even greater effect was observed in women with 2 risk factors (decreased CYP2D6 metabolism and *HOXB13/IL17BR* high). These women had the shortest DFS (adjusted HR=3.10, 95% CI:1.34-7.17; p=0.008), and OS (adjusted HR=3.15, 95% CI:1.17-8.52, p=0.024) (Table 3)

**Discussion**—Our analysis of this cohort of estrogen receptor positive, lymph node negative patients treated with adjuvant tamoxifen strongly suggests that a combination of inherited (*CYP2D6*) and tumor (*HOXB13/IL17BR*) genetic variation influences the risk of breast cancer recurrence and death, independent of standard prognostic factors. Furthermore, we demonstrated a stepwise increased risk of recurrence and death, with the greatest risk in those patients who carry both high risk features (e.g. both decreased CYP2D6 metabolism and a high *HOXB13/IL17BR* gene ratio). In contrast to prior studies in lymph node negative breast cancer that have simply focused on tumor genetic variation,<sup>20</sup> the paradigm of accounting for host genetic variation affecting drug metabolism, as well as tumor somatic variation, offers the potential for a more accurate estimate of drug benefit and the identification of groups of patients for whom alternative or additional therapies could be considered.

The potential synergy between *HOXB13/IL17BR* and *CYP2D6* as biomarkers for tamoxifen resistance may be related to the differential effects of tamoxifen and its metabolites in the setting of tumor cells with dysfunctional estrogen signaling. Endoxifen exhibits a significantly greater affinity for the ER than tamoxifen<sup>3</sup> and multiple studies suggest that only two tamoxifen metabolites, endoxifen and 4-OH tamoxifen, have significant anti-proliferative activity.<sup>3, 22</sup> Tamoxifen and other metabolites exhibit only weak antagonist/agonist properties.<sup>23</sup> The importance of these pharmacologic differences may be magnified in women with a high *HOXB13/IL17BR* gene ratio, as *in vitro*, *HOXB13* and *IL17BR* are regulated by estradiol, which suppresses *HOXB13* and augments *IL17BR* expression.<sup>13</sup> In tumors with a high *HOXB13/IL17BR* ratio, the loss of both suppression of *HOXB13* expression and induction of *IL17BR* expression may not only be a marker for impaired ER signaling, but also for increased growth factor signaling. Based on studies demonstrating that ER-positive tumors with a high *HOXB13/IL17BR* ratio are more likely to overexpress HER2,<sup>13</sup> *in vivo* ER binding of tamoxifen and other non-potent metabolites (as opposed to endoxifen) may activate growth factor signaling and thus tumor growth<sup>18</sup>. This hypothesis is supported by preliminary data wherein nontransformed human mammary epithelial cells expressing *HOXB13* demonstrated a synergistic increase in cell motility and invasion in the presence of epidermal growth factor.<sup>12</sup>

Most of the published breast cancer gene signatures appear to provide similar information in the setting of ER positive breast cancer<sup>24</sup> and proliferation-related genes appear to be an essential component accounting for the concordance between these expression signatures.<sup>12, 20, 25-28</sup> While “proliferation” signatures appear to identify those patients who derive the greatest benefit from adjuvant chemotherapy,<sup>29</sup> the role of *HOXB13* and *IL17BR* as a tool for clinical decision making has been unclear. However, our data suggest that postmenopausal patients with decreased *CYP2D6* metabolism (especially *CYP2D6* poor metabolizers) may preferentially benefit from alternative hormonal therapy (e.g. aromatase inhibitor) in the setting of tumors with a high *HOXB13/IL17BR* ratio. Further retrospective studies of large adjuvant trials comparing tamoxifen to aromatase inhibitors are necessary to validate this hypothesis.

In summary, our data suggest that an index comprised of *CYP2D6* and *HOXB13/IL17BR* may provide a robust indicator of tamoxifen resistance. A prospective approach incorporating both factors may lead to the improved individualization of endocrine therapy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Dr. Matthew Goetz had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

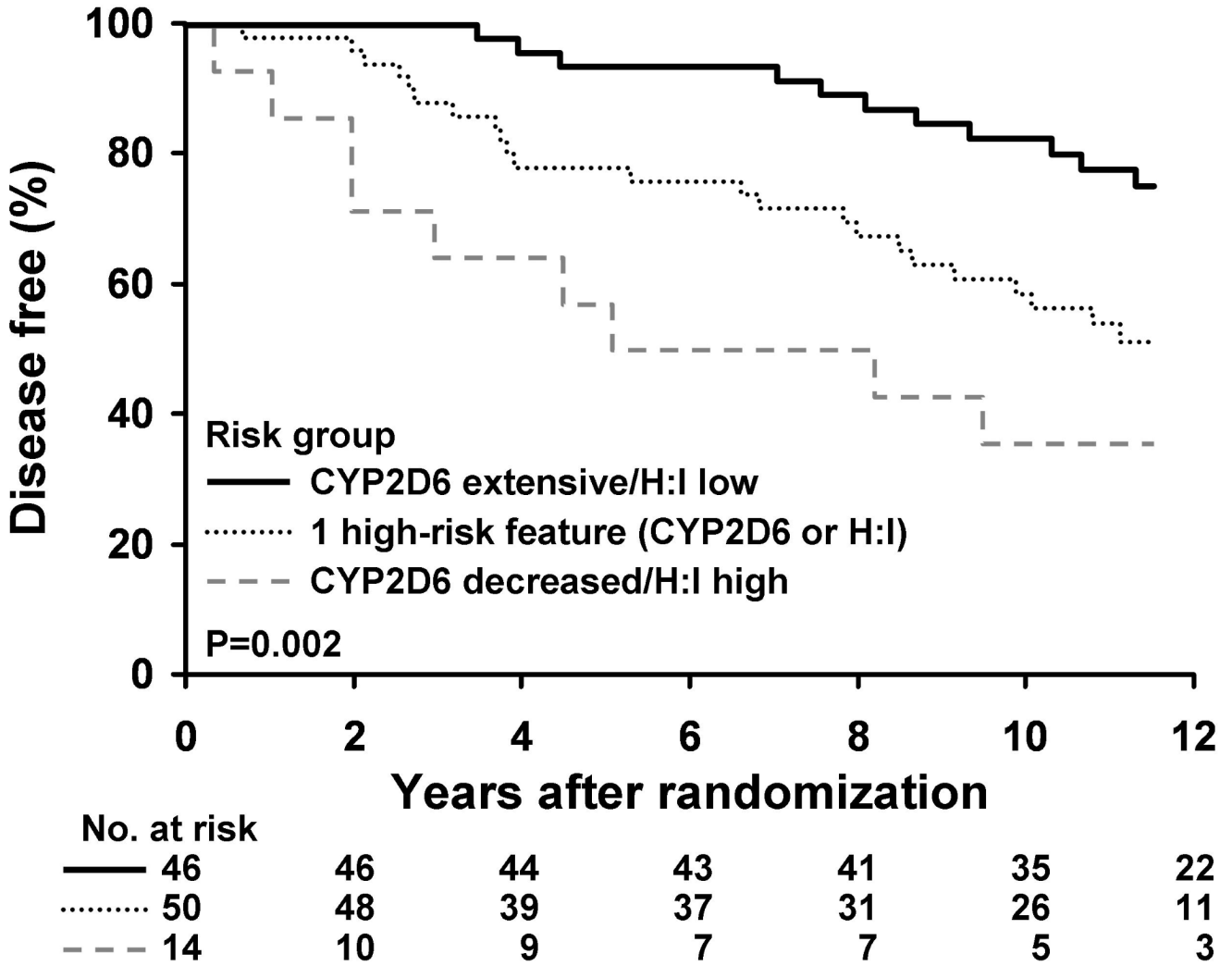
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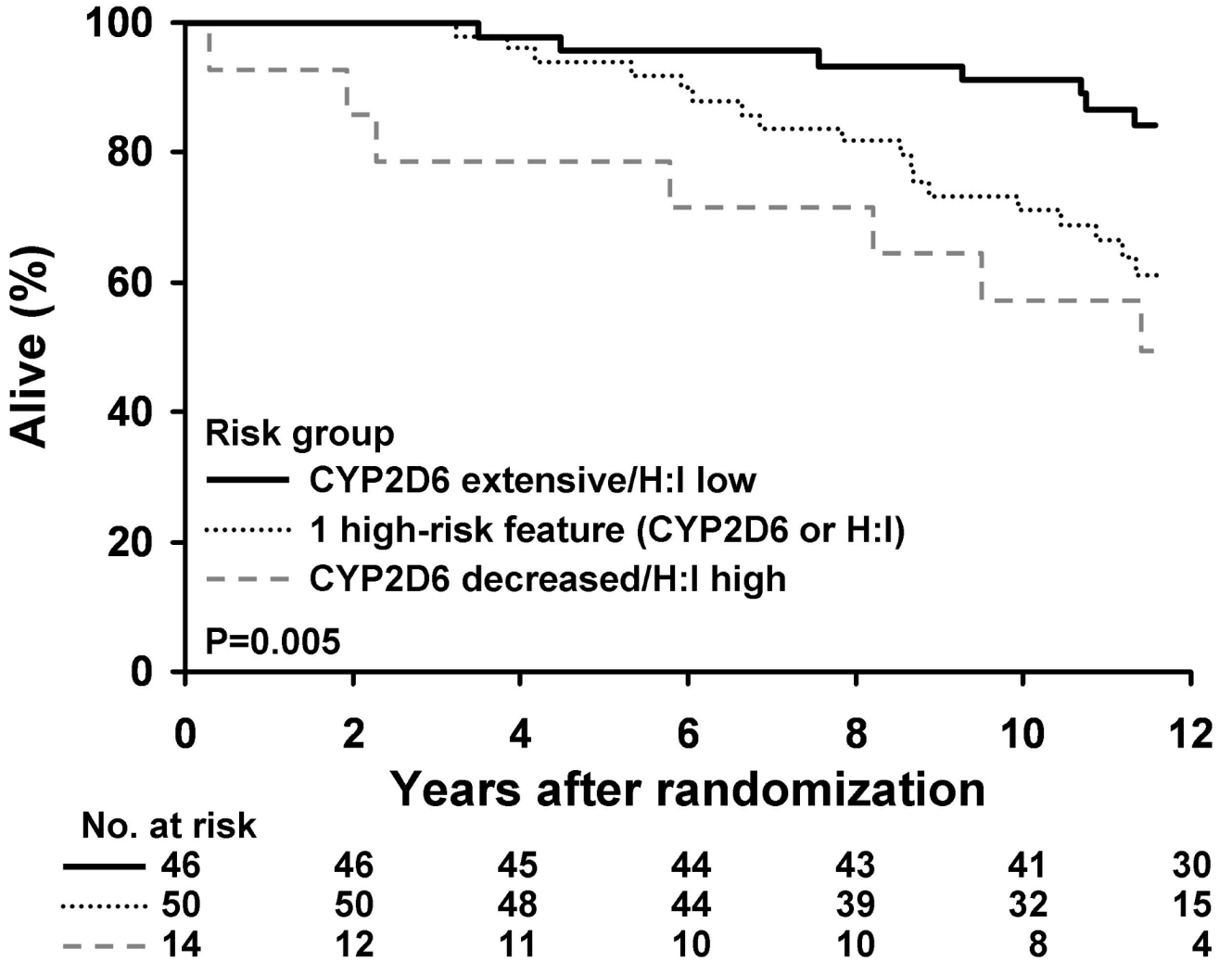
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**Figure 1.** Kaplan-Meier Estimates of Disease-Free Survival in lymph node negative patients according to the presence of no high risk features (extensive CYP2D6 metabolism and HOXB13/IL17BR <math>< -1.339</math>), only 1 high risk feature (either decreased CYP2D6 metabolism or HOXB13/IL17BR  $\geq -1.339</math>), and 2 high risk features (both decreased CYP2D6 metabolism and HOXB13/IL17BR  $\geq -1.339</math>)$$





**Figure 2.** Kaplan-Meier Estimates of Overall Survival in lymph node negative patients according to the presence of no high risk features (extensive CYP2D6 metabolism and HOXB13/IL17BR < -1.339), only 1 high risk feature (either decreased CYP2D6 metabolism or HOXB13/IL17BR ≥ -1.339), and 2 high risk features (both decreased CYP2D6 metabolism and HOXB13/IL17BR ≥ -1.339)

**Table 1**

## Baseline Characteristics

Characteristic	H/I and CYP2D6 phenotype available (N=110)	H/I and CYP2D6 phenotype NOT available (n=50)
Median Age (Range):	65 (42-84)	63 (47-82)
Race		
Caucasian	105 (95%)	42 (84%)
African-American	1 (1%)	1 (2%)
Unknown	4 (4%)	7 (14%)
Extent of Surgery		
Mastectomy	91 (83%)	38 (76%)
Breast Sparing	19 (17%)	12 (24%)
ER status		
10-49 fmols	27 (25%)	7 (14%)
>= 50 fmols	65 (59%)	34 (68%)
Positive	18 (16%)	9 (18%)
Tumor Size ≥ 3cm	22 (20%)	7 (14%)
Tumor Grade		
1-2	86 (78%)	22 (44%)
3	17 (15%)	5 (10%)
Unknown	7 (6%)	23 (46%)
IHC Her 2 expression		
0	13 (12%)	2 (4%)
1+	34 (31%)	10 (20%)
2+	35 (32%)	9 (18%)
3+	21 (19%)	6 (12%)
Unknown	7 (6%)	23 (46%)

**Table 2**

Risk groups as defined by CYP2D6 Metabolism and HOXB13/IL17BR gene ratio. The Kaplan Meier estimates of 5-year disease-free survival (DFS) rates and corresponding confidence intervals (CI) for each group are given. CYP2D6 inhibitors co-administered during tamoxifen in 9 patients were as follows: cimetidine (n=4), fluoxetine (n=2), paroxetine (n=2), sertraline (n=1), and haloperidol (n=1)\*

Risk Group	HOXB13/IL17BR gene ratio	CYP2D6 Metabolism Phenotype (CYP2D6*4 Genotype: CYP2D6 inhibitor use)	N=110		estimated 5 yr DFS rate (95% CI)
1	< -1.339	Extensive (Wt/Wt: no)	46	46	93.5% (86.6-99.9%)
2	< -1.339	Decreased (Unknown: yes Wt/Wt:yes Wt/*4:yes Wt/*4:no *4/*4:no)	18	2 5 1 9 1	83.8% (67.8-99.9%)
3	≥ -1.339	Extensive (Wt/Wt: no)	32	32	75.0% (61.4-91.6%)
4	≥ -1.339	Decreased (Wt/Wt:yes Wt/*4:no *4/*4:no)	14	1 11 2	57.1% (36.3-89.9%)

\* One patient was co-prescribed two CYP2D6 inhibiting drugs

**Table 3****Results of multivariate modeling of DFS and OS**

Multivariate modeling based on tumor size, and the three risk group model comparing presence of 1 high risk feature (defined as the presence of either decreased CYP2D6 metabolism or a *HOXB13/IL17BR* gene ratio  $\geq -1.339$ ) or 2 high risk features (defined as the presence of both decreased CYP2D6 metabolism and a *HOXB13/IL17BR* gene ratio  $\geq -1.339$ ) relative to CYP2D6 extensive metabolizers with a *HOXB13/IL17BR*  $< -1.339$  Hazard Ratios (HR) and corresponding 95% confidence intervals

Factor	Clinical Outcome	
	DFS	OS
Tumor size $\geq 3$ cm	2.64 (1.42-4.92)	2.52 (1.23-5.14)
Either <i>HOXB13:IL17BR</i> high or decreased CYP2D6 metabolism	2.03 (1.04-3.94)	2.41 (1.08-5.37)
Both <i>HOXB13:IL17BR</i> high and decreased CYP2D6 metabolism	3.10 (1.34-7.17)	3.15 (1.17-8.52)