

# Molecular Grade Index (MGI) and H/I Ratio are Complementary Prognostic Factors in Breast Cancer

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## BACKGROUND

**HOXB13.1L17BR** Previously, we conducted a genome-wide survey correlating the expression levels of 22,000 genes with the clinical outcome of 60 patients with hormone receptor-positive breast cancer treated with 5-year tamoxifen monotherapy<sup>1</sup>. To facilitate discovery of novel biomarkers of clinical outcome beyond standard prognostic factors, patients who developed recurrences were matched to those who did not with respect to tumor stage and grade. We identified three outcome-associated genes, HOXB13, IL17BR and CHD1, none of which had been previously implicated in breast cancer. We proposed that a simple HOXB13:IL17BR two-gene ratio (hereafter referred to as HOXB13:IL17BR) could serve as a novel biomarker for predicting recurrence in patients with estrogen receptor-positive breast cancer receiving adjuvant tamoxifen therapy. Subsequent studies by us<sup>2</sup> and others<sup>3,4</sup> have further shown that HOXB13:IL17BR is both prognostic (i.e., tumor aggressiveness) and predictive of tamoxifen benefit (i.e., tamoxifen response/resistance) within both retrospective and randomized clinical trials.

**MOLECULAR GRADE INDEX (MGI)** The prognostic importance of tumor grade has long been established<sup>5</sup>; thus, a genomic grade index (GGI) based on 97 tumor grade-associated genes has been shown to be strongly prognostic<sup>6</sup>. Comparative studies of GGI with other prognostic gene signatures including the 70-gene signature<sup>6</sup> and the Oncotype DX 21-gene recurrence score algorithm<sup>7</sup> suggest that tumor grade-related genes are the common denominators of these signatures<sup>6,10,11</sup>. Since HOXB13:IL17BR was developed to be independent of tumor grade<sup>1</sup>, we wish to determine whether it can be used together with a tumor grade signature to provide more accurate prognostic information. In this study, we first developed a simple 5-gene tumor grade signature (MGI for molecular grade index), which could recapitulate tumor grade and predict clinical outcome with comparable performance to the 97-gene GGI in two independent cohorts. We then measured both MGI and HOXB13:IL17BR using real-time reverse transcription-polymerase chain reaction (RT-PCR) in an additional cohort and demonstrate that combining MGI and HOXB13:IL17BR provides superior stratification of risk of recurrence to that by either alone.

## PATIENTS AND TUMOR SAMPLES

**PUBLIC MICROARRAY DATASETS** Two previously published microarray datasets (accessions GSE3494, GSE1456) were downloaded from Gene Expression Omnibus (GEO, <http://ncbi.nlm.nih.gov/geo/>). GSE3494 (Uppsala cohort) consists of 251 patients derived from a population-based cohort treated at Uppsala County, Sweden, from 1987 to 1989, and they were heterogeneous in terms of adjuvant systemic therapy received (untreated or endocrine and/or chemotherapy-treated)<sup>12</sup>. Clinical outcome data (breast cancer-free survival) were available for 236 patients with a median follow-up of 10 years. GSE1456 (Stockholm cohort) consists of a similar series of 150 breast cancer patients treated at the Karolinska Hospital, Stockholm, Sweden from 1994 to 1996/14. Both GSE3494 and GSE1456 contain gene expression data from frozen tumor samples analyzed on the Affymetrix U133A and U133B arrays (Affymetrix, Santa Clara, CA).

**MGH COHORT** The 239 pt. MGH cohort used a retrospective case-cohort design<sup>14</sup> and was derived from 683 stage I-III patients with estrogen receptor-positive breast cancer treated at the Massachusetts General Hospital from 1991 to 1999. Clinical follow-up data were retrieved from clinical and hospital records. Cases were all patients who developed distant metastasis during follow-up; controls were randomly selected from patients who remained disease-free at last follow-up to achieve a 2:1 ratio of controls to cases. In addition, controls were frequency-matched to cases with respect to adjuvant therapy and time of diagnosis. The final cohort consisted of 79 cases and 160 controls, and its patient and tumor characteristics were summarized in Table 1. This study was approved by local Institutional Review Boards.

## REAL-TIME RT-PCR ASSAYS for H/I and MGI

We used primer/probe sequences for HOXB13, IL17BR, ESR1, PGR, CHD1, ACTB, HMBS, SDHA and UBC described previously<sup>2</sup>, and matched primer/probe sequences for the five molecular grade genes (BUB1B, CENPA, NEK2, RACGAP1 and RRM2) and ERBB2 (HER2), using Primer Express (ABI).

For each FFPE sample, two 7-µm tissue sections were used for RNA extraction; gross macro-dissection were used to enrich for tumor content. RNA was reverse transcribed and TaqMan RT-PCR using the ABI 7700iD instrument (Applied Biosystems, Inc) were performed as described before<sup>2</sup>. The cycling thresholds numbers (CTs) were normalized to the mean CT of 4 reference genes (ACTB, HMBS, SDHA and UBC); normalized CTs were taken to represent relative gene expression levels.

## CALCULATION OF H/I, MGI and GGI

Normalized expression levels for the five molecular grade genes from microarrays or RT-PCR were standardized to mean of 0 and standard deviation of 1 across samples within each dataset and then combined into a single index per sample via principle component analysis (PCA) using the first principle component. HOXB13:IL17BR was calculated as the difference in standardized expression levels between HOXB13 and IL17BR as described previously<sup>2</sup>. Genomic Grade Index (GGI) was calculated from microarray data using the 126 Affymetrix probe sets representing 97 genes and scaled within each dataset to have a mean of -1 for grade 1 tumors and +1 for grade 3 tumors as described before<sup>6</sup>.

## CUT-POINTS & STATISTICAL ANALYSES

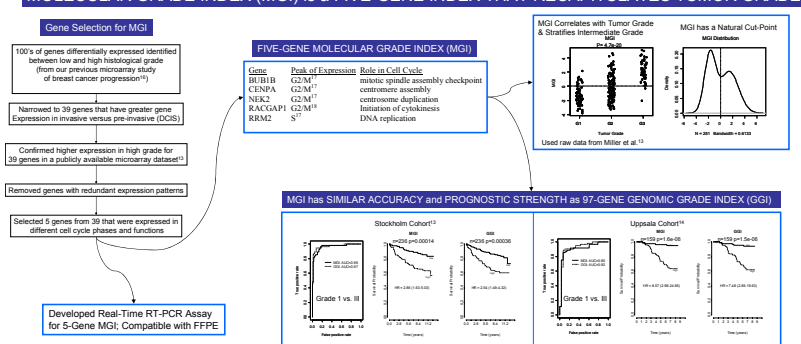
**H/I CUT-POINT** The calculation of 0.06 for HOXB13:IL17BR, previously defined to stratify patients treated with adjuvant tamoxifen into low and high risk of recurrence<sup>1</sup>, was applied directly in this study.

**MGI CUT-POINT** The calculation and the cutoff for MGI was defined without using any clinical outcome data and instead was a natural cutoff. Initial analysis of MGI in the Uppsala cohort indicated good discrimination of grade 1 and grade 3 tumors using the mean (0) as cutoff, and model-based clustering of MGI also indicated a bimodal distribution with a natural cutoff around 0. This cutoff was further supported by receiver operating characteristic (ROC) analysis.

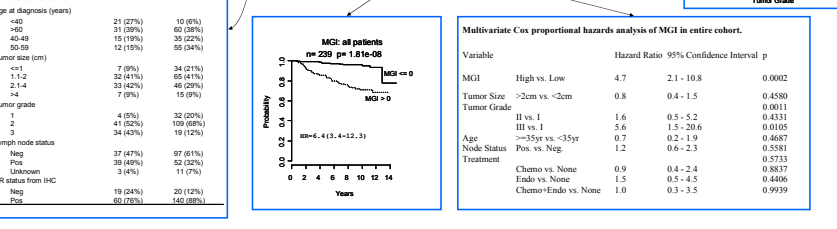
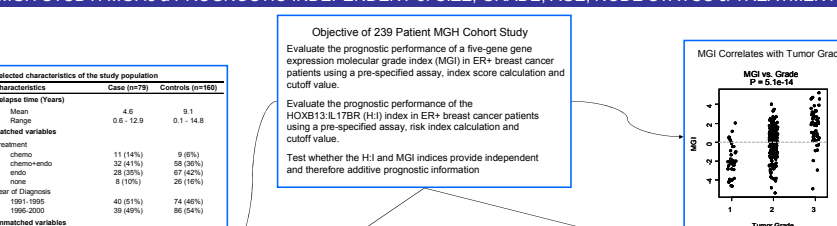
**GENOMIC GRADE INDEX (GGI)** GGI was dichotomized at the cutoff point of 0 as described previously<sup>6</sup>.

**STATISTICAL ANALYSES** Kaplan-Meier analysis with logrank test and Cox proportional hazards regression were performed to assess the association of gene expression indices with clinical outcome. Multivariate Cox regression models were performed to assess the prognostic capacity of gene expression indexes after adjusting for known prognostic factors.

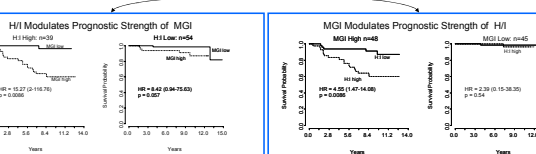
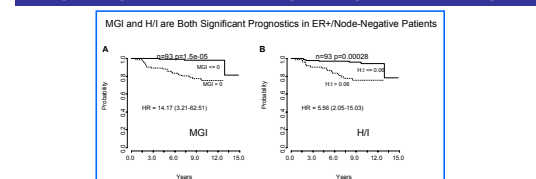
## MOLECULAR GRADE INDEX (MGI) is a FIVE-GENE INDEX THAT RECAPITULATES TUMOR GRADE



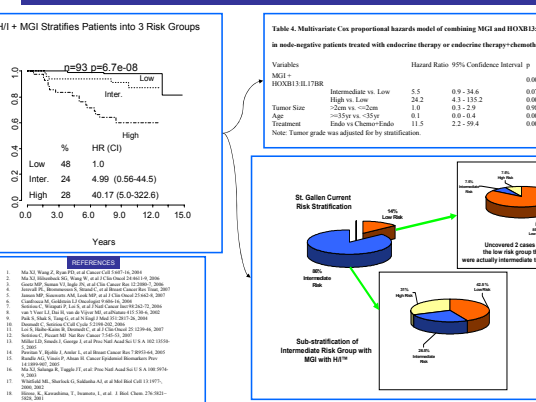
## MGH STUDY: MGI is a PROGNOSTIC INDEPENDENT of SIZE, GRADE, AGE, NODE STATUS & TREATMENT



## MGH STUDY: H/I and MGI MODIFY EACH OTHER'S PROGNOSTIC STRENGTH in ER+ LYMPH-NODE NEGATIVE PATIENTS



## MGH STUDY: H/I and MGI are COMPLEMENTARY PROGNOSTICS and STRATIFY PATIENTS SUPERIOR to St. GALLEN<sup>19</sup>



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