Original Study

Prognostic and Predictive Impact of Beta-2 Adrenergic Receptor Expression in HER2-Positive Breast Cancer

Rafael Caparica,[1] François Richard,[2] Mariana Brandão,[1] Ahmad Awada,[1] Christos Sotiriou,[3] Evandro de Azambuja[1]

Abstract

ADRB2 mediates trastuzumab resistance in preclinical models of HER2+ breast cancer. We evaluated *ADRB2* gene expression as a prognostic and predictive biomarker in HER2+ early breast cancer patients. Opposing our initial hypothesis, a high *ADRB2* expression may exert antiproliferative, antiangiogenic, and immunogenic effects, and thus be associated with a favorable prognosis in patients with HER2+ early breast cancer.

Background: Beta-2 adrenergic receptor (ADRB2) mediates proliferation and treatment resistance in preclinical models of human epidermal growth factor receptor 2 positive (HER2+) breast cancer. We evaluated *ADRB2* gene expression as a prognostic and predictive biomarker in patients with HER2+ early breast cancer. **Methods:** *ADRB2* expression was retrieved from HER2+ patients enrolled in the FinHer study (N = 202), and 2 public datasets containing data from patients with HER2+ early breast cancer: one including patients who did not receive systemic treatment (disease-free survival [DFS] dataset; n = 175) and another including patients who received neoadjuvant treatment (pathologic complete response [pCR] dataset; n = 207). Survival was estimated with Kaplan-Meier method and Cox regression was used for uni-multivariate analyses. *ADRB2* expression was correlated with several gene signatures. **Results:** *ADRB2* high expression was associated with improved DFS rates in HER2+ patients (hazard ratio [HR] 0.52; 95% confidence interval [CI] 0.32-0.84; $P = .0068$). No association between *ADRB2* expression and pCR was observed (odds ratio 1.14; 95% CI, 0.63-2.10; $P = .67$). No association between *ADRB2* and relapse-free survival (RFS) was observed in HER2+ patients enrolled in the FinHer study (HR 0.93; 95% CI, 0.69-1.25; $P = .61$). *ADRB2* was associated with a low expression of angiogenesis-related (vascular endothelial growth factor −0.38, $P < .001$) and proliferation-related (aurora kinase A −0.36, $P < .001$; genomic grade index −0.028, $P < .001$; signal transducers and activators of transcription −0.17, $P < .001$) genes; and a high expression of immune-related genes (Perez +0.45, $P < .001$; STAT1 +0.28, $P < .001$; immune response gene expression module +0.29, $P < .001$). **Conclusions:** Opposing our initial hypothesis, a high *ADRB2* expression may be a favorable prognostic factor in patients with HER2+ early breast cancer. This association appears to be mediated by antiproliferative, antiangiogenic, and immunogenic effects of *ADRB2*.

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Introduction

The beta-2 adrenergic receptor (ADRB2) is a G protein coupled receptor that mediates physiologic processes such as smooth-muscle relaxation, chronotropism, and inotropism.[1] ADRB2 also exerts a potent trophic effect on the cardiac muscle, inducing proliferation and differentiation of cardiac progenitor cells.[2] The expression of ADRB2 has been demonstrated in breast cancer cells using immunohistochemistry, although its function in these cells is

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unknown.[3] Given the trophic stimuli exerted by ADRB2 on cardiac myocytes, this receptor may also stimulate proliferation and thus function as an oncogene in breast cancer cells.[4]

Preclinical studies demonstrate a significant interaction between human epidermal growth factor receptor 2 (HER2) and ADRB2 pathways: in breast cancer cells that harbor an amplification or overexpression of HER2 (HER2+), the activation of HER2 induces epinephrine production and augments ADRB2 expression, whereas ADRB2 activation increases HER2 expression.[5] The physiologic feedback mechanism of receptor desensitization, which consists in ADRB2 degradation after its activation to prevent exaggerated adrenergic stimuli, is compromised in HER2+ breast cancer cells, allowing a permanent activation of the ADRB2 pathway.[5] Moreover, ADRB2 and HER2 share intracellular effectors, such as mitogen-activated protein kinases (MAPK) and phosphoinositide 3-kinases (PI3K), providing ADRB2 the capacity to drive cell proliferation when HER2 is blocked, and thus to potentially drive resistance to anti-HER2 treatments.[6]

In vitro, ADRB2 activation stimulates mitosis and angiogenesis in HER2+ breast cancer cells, whereas beta-blockers interrupt these effects, suggesting that ADRB2 regulates HER2+ breast cancer proliferation.[5,6] Retrospective studies evaluated the correlation between beta-blocker use and outcomes in patients with breast cancer, with most series observing an improved survival in patients treated with beta-blockers when compared with those who did not receive these medications. Notably, in most of these studies, the population was heterogeneous in terms of breast cancer subtypes and stages. Therefore, although definitive conclusions are precluded, the available data suggest that beta-blockers may improve the prognosis of patients with breast cancer, highlighting the role of *ADRB2* as a potential oncogene in this scenario.[7-12]

In xenograft models of trastuzumab-resistant HER2+ breast cancer, beta-blockers restore trastuzumab sensitivity and induce tumor shrinkage, suggesting that ADRB2 may be also a therapeutic target.[6] Supporting this hypothesis, in a retrospective study of 83 patients with HER2+ early breast cancer treated with neoadjuvant chemotherapy and trastuzumab, a high ADRB2 expression (assessed with immunohistochemistry) was associated with lower pathologic complete response (pCR) rates (13% in ADRB2-high expression group vs. 77% in ADRB2-low expression group, $P < .001$).[6]

In view of the preclinical studies showing that ADRB2 stimulates proliferation and drives treatment resistance in HER2+ breast cancer cells, and the clinical data suggesting that beta-blockers may improve the prognosis of patients with breast cancer, the present study aims to evaluate the expression of the *ADRB2* gene as a predictive and prognostic biomarker in patients with HER2+ early breast cancer.

Objectives

The main objectives of this study were to correlate the expression levels of the *ADRB2* gene with survival outcomes (disease-free survival [DFS]/relapse-free survival [RFS]) and pCR rates in patients with HER2+ early breast cancer.

The secondary objectives were to perform subgroup analyses for the outcomes DFS/RFS and pCR according to *ADRB2* expression in estrogen receptor (ER)-negative and ER-positive patients; to evaluate *ADRB2* expression in different molecular subtypes defined by SCMGENE classification in samples from the DFS and pCR datasets; and to assess the correlation between *ADRB2* expression and gene expression signatures related to proliferation, invasiveness, immune activation, and angiogenesis.

Methods

Study Population: Description of the 3 Datasets Used in the Study

DFS Dataset. Public available datasets were retrieved from Haibe-Kains et al.[13] using the provided link (http://compbio.dfci.harvard.edu/pubs/sbtpaper/). To assess the role of ADRB2 expression as a prognostic biomarker, data from patients with HER2+ early breast cancer, with node-negative disease, who were treated with surgery alone and did not receive any systemic neoadjuvant or adjuvant treatment before the collection of the samples, and who had available information about DFS, RFS, or distant metastasis-free survival (DMFS) were retrieved from this dataset. Patients were defined as HER2+ according to the data retrieved from the dataset: in this dataset, HER2+ classification was based either on immunohistochemistry or in situ hybridization (ISH). DFS was defined as the time between sample collection (surgery) and recurrence or death, whichever occurred first. In case DFS data were not available, DMFS or RFS data were used.

Probes were matched to their gene symbols; in case of multiple matches, the one having the highest variance in the dataset under study was used. Kaplan-Meier curves for DFS according to the categorized *ADBR2* expression level were drawn using *survcomp* package version 1.26 in R version 4.4.1. Hazard ratios (HRs) and confidence intervals (CIs) were computed as univariate analysis with adjustment for datasets, using a Cox regression model. A multivariate analysis adjusted for datasets, age ($\leq$50 vs. $>$50), tumor size in centimeters ($\leq$2 vs. $>$2), histologic grade (1 and 2 vs. 3) and ER status (negative vs. positive), was also performed.

pCR Dataset. Public available datasets were retrieved from Ignatiadis et al.[14] To assess the role of ADRB2 expression as a predictive biomarker, data from patients with HER2+ early breast cancer who received neoadjuvant treatment with anthracyclines $\pm$ taxanes, and for whom information regarding pathological response assessment after neoadjuvant treatment was available, were retrieved from this dataset. Patients were defined as HER2+ according to the data retrieved from the dataset: in this dataset, HER2+ classification was based on either immunohistochemistry or ISH. In the studies included in this dataset, the administration of trastuzumab for HER2+ patients was not mandatory, and information regarding trastuzumab use was not available for all patients. pCR was defined as the absence of residual invasive disease in the breast in one study; and no residual invasive disease in the breast and axillary lymph nodes in the other 7 studies of the dataset. Duplicates across studies have been managed as follows: (1) Spearman coefficients were computed for all patients across studies; (2) patients from MDACC and MAQCIII studies having a coefficient $\geq$0.90 of patients from another study were removed.

Probes were matched to their gene symbols; in case of multiple matches the one having the highest variance in the dataset under study was used. Cox regression models to assess the correlation

between ADBR2 expression and pCR rates were performed, using *ADRB2* expression as a continuous variable. Odds ratios (ORs) and CIs were computed as univariate analysis adjusted for datasets using a linear regression model. A multivariate analysis adjusted for datasets, treatment type (A vs. AT), age (≤50 vs. >50), tumor size (≤T2 vs. ≥T3), nodal status (negative vs. positive), histologic grade (1 and 2 vs. 3) and ER status (negative vs. positive) was also performed.

FinHer Dataset. In the FinHer study, 232 women with HER2+ early breast cancer were randomized to receive adjuvant chemotherapy alone or combined with 9 weeks of trastuzumab (loading dose 4 mg/kg followed by 8 weekly doses of 2 mg/kg).[15] In the primary analysis of the study, after a median follow-up of 36 months, 3-year RFS was significantly longer in patients who received trastuzumab versus those who did not receive trastuzumab (89% vs. 78%, respectively; HR 0.42; 95% CI, 0.21-0.83; $P = .01$).[15] After 62 months of follow-up, a sustained trend in favor of trastuzumab was observed in terms of distant DFS (HR 0.65; 95% CI, 0.38-1.12; $P = .12$).[16]

Publicly available gene expression data from the patients enrolled in the FinHer study were retrieved from the Gene Expression Omnibus portal under the accession GSE65095. HER2+ was defined according to the study protocol as a HER2 expression ≥2 on a scale from 0 to 3 in immunohistochemistry confirmed by a copy number gain in chromogenic in situ hybridization.[15,17] All HER2+ patients with available data regarding the expression of the *ADRB2* gene were included. The definition of RFS was specified in the study protocol as the time from randomization to the detection of local or distant recurrence, contralateral invasive breast cancer, or death, whichever occurred first.[15] Kaplan-Meier method was used to estimate RFS according to the categorized *ADBR2* expression level. HRs and CIs were computed as univariate analysis using a Cox regression model.

Definition of Low and High Expression of ADRB2

In all survival analyses, *ADRB2* expression was evaluated as a categorical variable (high vs. low). The median expression of *ADRB2* was initially estimated in the overall samples from each dataset evaluated. Patients were classified as either "*ADRB2*-high" group, when *ADRB2* expression level in their samples was above the median value of their dataset, or as "*ADRB2*-low" group, when *ADRB2* expression level in their samples was below the median value of their dataset.

Additional analyses were performed to explore different cutoff points of *ADRB2* expression (25% and 75% of the samples) to define "*ADRB2*-high" and "*ADRB2*-low" groups.

ER and HER2 Status

When available, ER and HER2 status of the patients was retrieved from the GeneSys Export files. When missing, they were inferred using the function bimod from the genefu package version 2.6.0.[18]

Molecular Classification of Samples According to Gene Expression: SCMGENE

The SCMGENE, a breast cancer classification model based on the expression levels of 3 genes (*ER*, *HER2,* and aurora kinase A [*AURKA*]) was used to categorize samples from the datasets as previously described.[13] According to SCMGENE classification, 5 subgroups were identified: LumA, LumB, HER2+/ER+, HER2+/ER−, and HER2−/ER− (TNBC).[19] LumA and LumB differ by their proliferative rate, either low or high, respectively. To define LumA and LumB subgroups according to proliferation rates, the median expression levels of *AURKA* in the overall ER+/HER2− patients were used as a cutoff: patients whose tumors had *AURKA* expression levels lower than the median were defined as LumA, whereas those whose tumors had levels higher than the median were defined as LumB. In some of the analyses performed, the HER2+/ER− and HER2+/ER+ groups were pooled to form the HER2+ subgroup, representing the overall population of HER2+ patients.

Correlation of ADRB2 Expression With Gene Expression Signatures

In the pooled samples from the DFS and the pCR datasets, the correlation of *ADRB2* expression with 9 gene signatures associated with the following characteristics was performed: AURKA (proliferation, 219 genes); signal transducers and activators of transcription (STAT3) (proliferation, 123 genes); plasminogen activator, urokinase (invasion, 68 genes); vascular endothelial growth factor (VEGF) (angiogenesis, 14 genes); genomic grade index (GGI) (grading, 129 genes); immune response gene expression module (IRM) (immune, 7 genes); STAT1 (immune, 95 genes); Perez (immune, 14 genes); MAPK (activation of the MAPK pathway, 315 genes).[20-25] The correlations were assessed by computing the Spearman coefficient between the 2 variables and the associated *P* value was considered significant if ≤ .05.

Statistics

All analyses were performed using the R software version 4.4.1 (https://www.r-project.org/) and *P* values were corrected for multiple testing by false discovery rate.

Results

Patient Characteristics

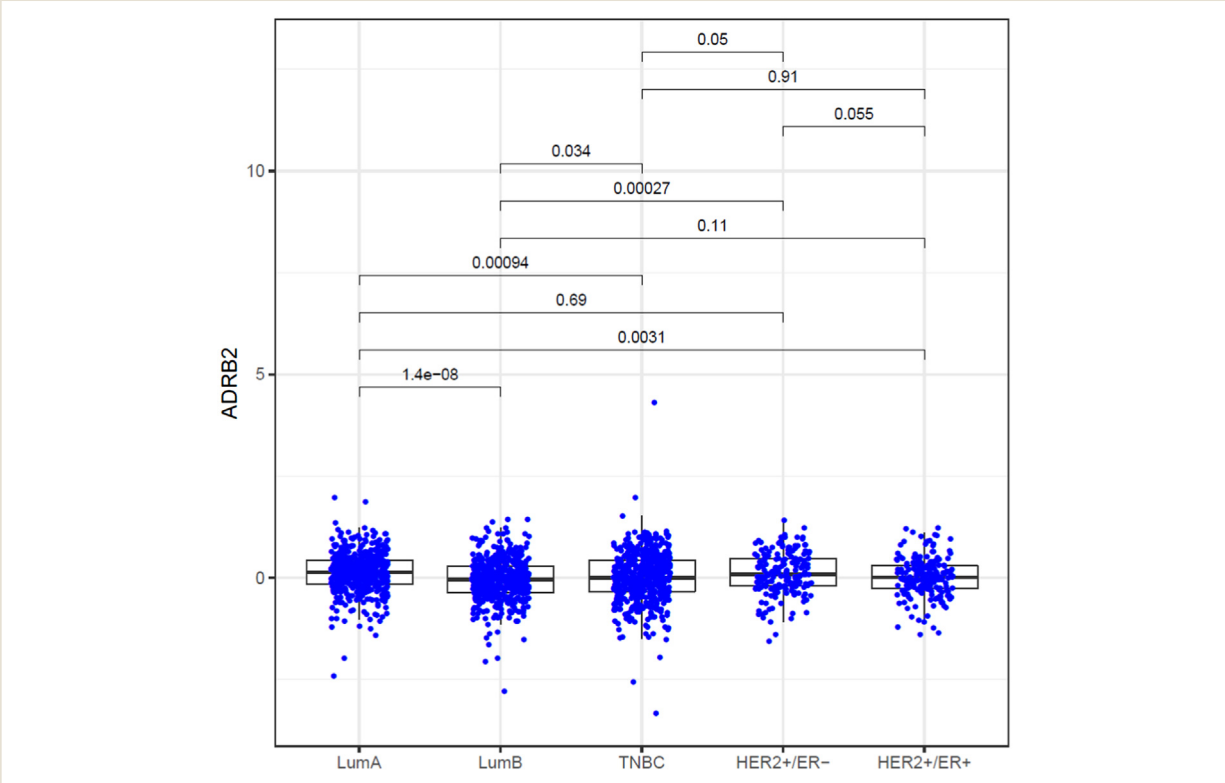
Two datasets have been designed aggregating publicly available gene expression data reproducing Haibe-Kains et al.[13] (n = 1258) and Ignatiadis et al.[14] (n = 1013) to study DFS and pCR, respectively. These datasets comprise a total of 237 and 207 patients with HER2+ early breast cancer respectively, of whom 175 (73.8%) and 207 (100%) had available data regarding *ADRB2* expression. The characteristics of the patients and the studies comprised in each dataset are presented in Supplemental Tables 1 and 2 in the online version.

From the FinHer study, data from 202 patients with HER2+ early breast cancer was publically available (102 patients randomized to chemotherapy and trastuzumab and 100 patients randomized to chemotherapy alone). The characteristics of the patients included from FinHer study's dataset are displayed in Supplemental Table 3 in the online version.

ADRB2 Expression According to Breast Cancer Subtypes

Samples from 2 datasets (DFS and pCR, n = 382) were pooled and categorized into 5 subtypes (LumA, LumB, HER2+/ER+, HER2+/

Figure 1 ADRB2 Expression According to Breast Cancer Subtypes Defined per SCMGENE in the Pooled Samples From the Disease-Free Survival and Pathologic Complete Response Datasets (n = 382)



Abbreviations: ADRB2 = beta-2 adrenergic receptor; ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; Lum = luminal; TNBC = triple-negative breast cancer.

ER−, and TNBC) using the SCMGENE signature, as previously described.[13] The expression of *ADRB2* was then quantified in the 5 subtypes, being significantly higher in the LumA and the HER2+/ER− subtypes (Figure 1).

Correlation of ADRB2 Expression With DFS

In HER2+ patients from the DFS dataset (n = 175), DFS was significantly longer in the *ADRB2*-high group in comparison with the *ADRB2*-low group (median 119 months vs. 68 months; HR 0.52; 95% CI, 0.32-0.84; P = .0068) (Figure 2). When using different cutoff values of *ADRB2* expression to define *ADRB2*-high and -low (25% and 75%), a trend for improved DFS in patients with *ADRB2*-high was observed in both scenarios, apparently with a more robust association observed with the 75% cutoff. (Supplemental Figure 1 in the online version)

The prognostic impact of *ADRB2*-high was significant in the subgroup of HER2+/ER+ patients (n = 117) (median DFS 131 months vs. 102 months; HR 0.45; 95% CI, 0.24-0.84; P = .0097), whereas in HER2+/ER− patients (n = 58), the difference was not statistically significant (median DFS 120 months vs. 69 months; HR 0.68; 95% CI, 0.29-1.58; P = .37) (Figure 3).

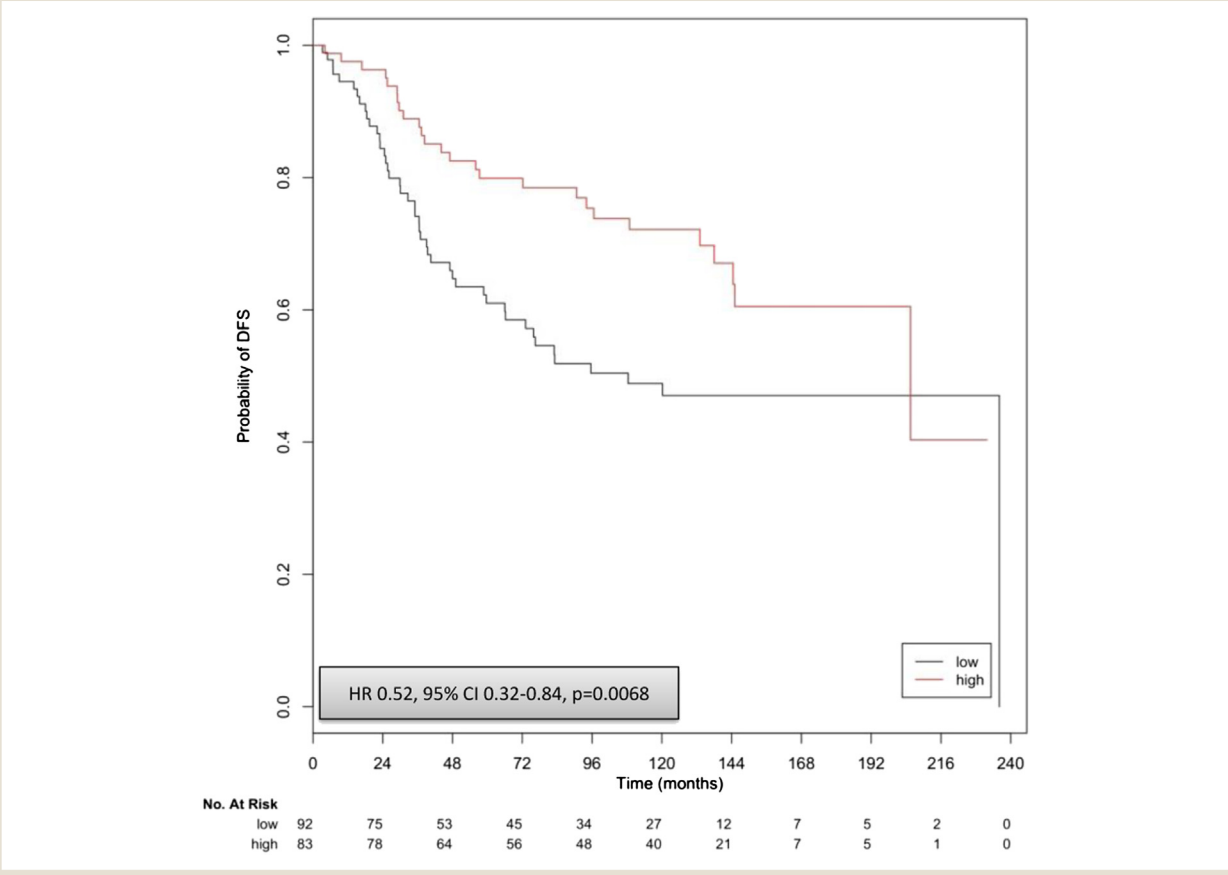
A significant difference in terms of DFS was observed between *ADRB2*-high and *ADRB2*-low groups in univariate analysis (HR 0.54; 95% CI, 0.34-0.87; P = .001), although in multivariate analysis the difference was no longer significant (HR 0.67; 95% CI, 0.36-1.23; P = .19) (Supplemental Table 4 in the online version). In the subgroup of HER2+/ER+ patients, a superior DFS was observed in *ADRB2*-high compared with the *ADRB2*-low group in univariate (HR 0.39; 95% CI, 0.19-0.79; P = .008) and multivariate analyses (HR 0.36; 95% CI, 0.14-0.93; P = .035). No significant interaction was observed between ER status and *ADRB2* expression in an independent Cox model using only these 2 variables ($P_{interaction}$ = .42). In the subgroup of HER2+/ER− patients, no significant difference was observed between *ADRB2*-high and *ADRB2*-low groups in univariate (HR 0.71; 95% CI, 0.36-1.41; P = .33) or multivariate analyses (HR 0.78; 95% CI, 0.32-1.92; P = .59) (Supplemental Table 4 in the online version).

Correlation of ADRB2 Expression With pCR Rates

In the pCR dataset, 70 pCR events were recorded among 207 HER2+ patients (33%). No significant difference was observed in terms of pCR rates between *ADRB2*-high and *ADRB2*-low groups in univariate (OR 1.14; 95% CI, 0.63-2.10; P = .67) and multivariate analyses (OR 1.52; 95% CI, 0.76-3.16; P = .25) (Supplementary Table 5 in the online version).

In the subgroup of HER2+/ER+ patients (n = 80), no significant difference in terms of pCR was observed between the *ADRB2*-high and *ADRB2*-low groups in univariate (OR 0.98; 95% CI,

Figure 2 Kaplan-Meier Curves of Disease-Free Survival (DFS) According to ADRB2 Expression in the Overall HER2+ Population of the DFS Dataset (n = 175)



Abbreviations: ADRB2 = beta-2 adrenergic receptor; CI = confidence interval; HER2 = human epidermal growth factor receptor 2; HR = hazard ratio.

0.30-3.61; $P = .98$) and multivariate analyses (OR 1.75; 95% CI, 0.47-8.05; $P = .43$).

In the subgroup of HER2+/ER− patients (n = 127), no significant difference in terms of pCR was observed between the *ADRB2*-high and *ADRB2*-low groups in univariate (OR 1.06; 95% CI, 0.49-2.28; $P = .88$) and multivariate analyses (OR 1.41; 95% CI, 0.56-3.72; $P = .47$) (Supplemental Table 5 in the online version).

RFS in the FinHer Dataset

In the overall HER2+ patients enrolled in the FinHer trial (n = 202), no significant difference in terms of RFS was observed between *ADRB2*-high and *ADRB2*-low groups (HR 0.93; 95% CI, 0.69-1.25; $P = .61$) (Figure 4).

No significant difference in terms of RFS was observed between *ADRB2*-high and *ADRB2*-low groups both in patients randomized to chemotherapy and trastuzumab (n = 102; HR 0.73; 95% CI, 0.46-1.15; $P = .17$) and in patients randomized to chemotherapy alone (n = 100; HR 1.07; 95% CI, 0.67-1.71; $P = .78$) (Figure 5).

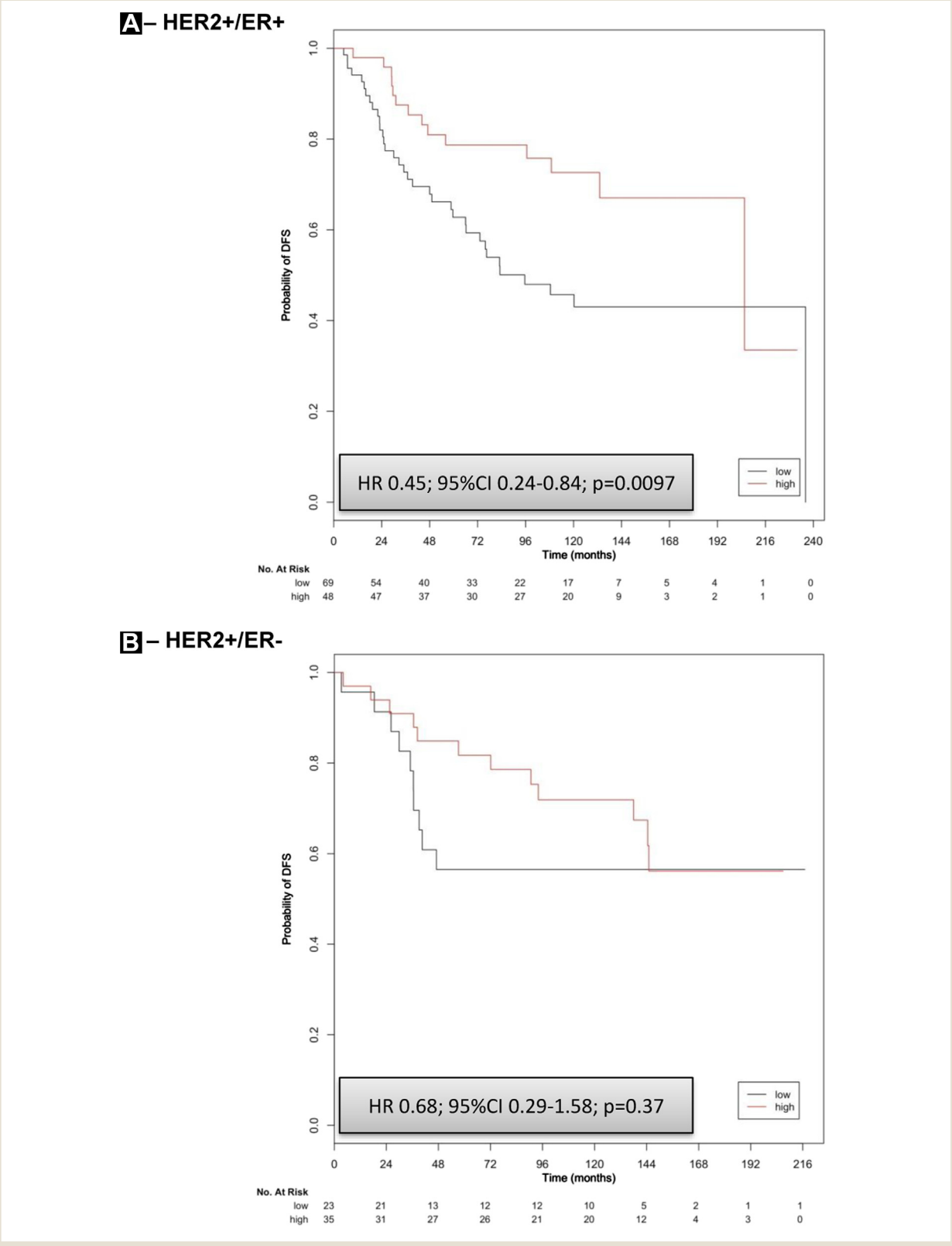
No significant difference in terms of RFS was observed between *ADRB2*-high and *ADRB2*-low groups both in HER2+/ER+ (n = 97; HR 0.82; 95% CI, 0.54-1.26; $P = .37$) and HER2+/ER− patients (n = 105; HR 1.1; 95% CI, 0.72-1.69; $P = .66$) (Figure 6).

Correlation of ADRB2 With Gene Expression Signatures

Samples from the DFS and the pCR datasets were pooled into a single dataset (n = 382) that was used to assess the correlation between *ADRB2* expression and gene signatures associated with angiogenesis, proliferation, invasion, and immune activation. *ADRB2* expression was significantly associated with low expression levels of angiogenesis-related (VEGF −0.38, $P < .001$) and proliferation-related genes (AURKA −0.36, $P < .001$; GGI −0.28, $P < .001$; STAT3 −0.17, $P < .001$); whereas the expression of genes related to immune activation was directly associated with *ADRB2* expression (Perez +0.45, $P < .001$; STAT1 +0.28, $P < .001$; IRM +0.29, $P < .001$). A positive, although weak, correlation between *ADRB2* and MAPK (+0.14, $P < .001$) and invasion-related genes (PLAU, +0.12, $P < .001$) was also observed (Figure 7).

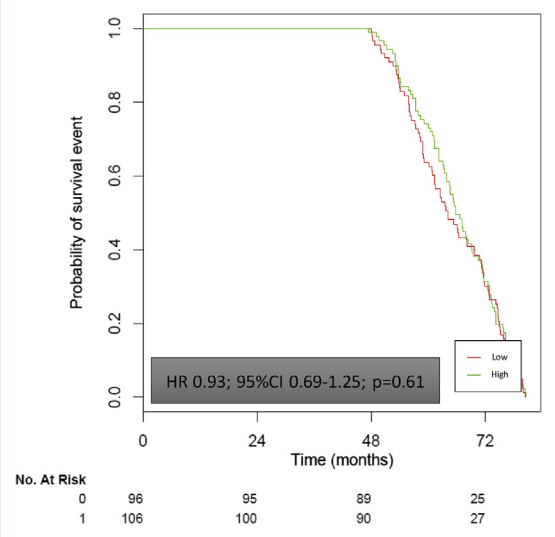
An additional analysis restricted to HER2+/ER+ patients (n = 116), the subgroup in which *ADRB2* high expression appeared to have a more pronounced prognostic effect, was performed (Supplemental Figure 2 in the online version). Similar to what was observed in the overall HER2+ population, *ADRB2* expression was significantly associated with a low expression of angiogenesis-related (VEGF −0.35, $P \leq .001$) and proliferation-related genes

Figure 3 Kaplan-Meier Curves of Disease-Free Survival (DFS) According to ADRB2 Expression in HER2+/ER+ (n = 117, A) and HER2+/Er− (n = 58, B) Subgroups of Patients From the DFS Dataset



Abbreviations: ADRB2 = beta-2 adrenergic receptor; CI = confidence interval; ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; HR = hazard ratio.

Figure 4 Kaplan-Meier Curves of Relapse-Free Survival According to ADRB2 Expression in the Overall HER2+ Patients Enrolled in the FinHer Study (n = 202)



Abbreviations: ADRB2 = beta-2 adrenergic receptor; CI = confidence interval; HR = hazard ratio.

(AURKA −0.21, $P = .004$; GGI −0.18, $P < .01$), whereas the expression of genes related to immune activation (Perez +0.5, $P < .001$; STAT1 +0.35, $P < .001$; IRM +0.31, $P < .001$) was directly associated with *ADRB2* expression.

Discussion

We observed a higher expression of *ADRB2* in the LumA and HER2+/ER− subtypes, suggesting that *ADRB2* is active in HER2+ breast cancer, as observed in preclinical studies.[5,26,27] Opposing our initial hypothesis, *ADRB2* high expression was associated with improved DFS in HER2+ patients from our DFS dataset, although in multivariate analysis this association was not sustained. This positive prognostic impact of *ADBR2*-high expression was more pronounced in HER2+/ER+ patients, with a trend being also observed in the HER2+/ER− subgroup. Interestingly, *ADRB2* was associated with a low expression of genes involved in angiogenesis and proliferation, and a high expression of genes related to immune activation, a finding that may justify the better prognosis observed in the *ADRB2*-high expression group. No association between *ADRB2* expression and pCR was observed. In the FinHer dataset, no association between *ADRB2* expression and RFS was found both in patients who received chemotherapy and trastuzumab and in those who received chemotherapy alone. Therefore, the hypothesis that *ADRB2* mediates treatment resistance was not supported by our findings.

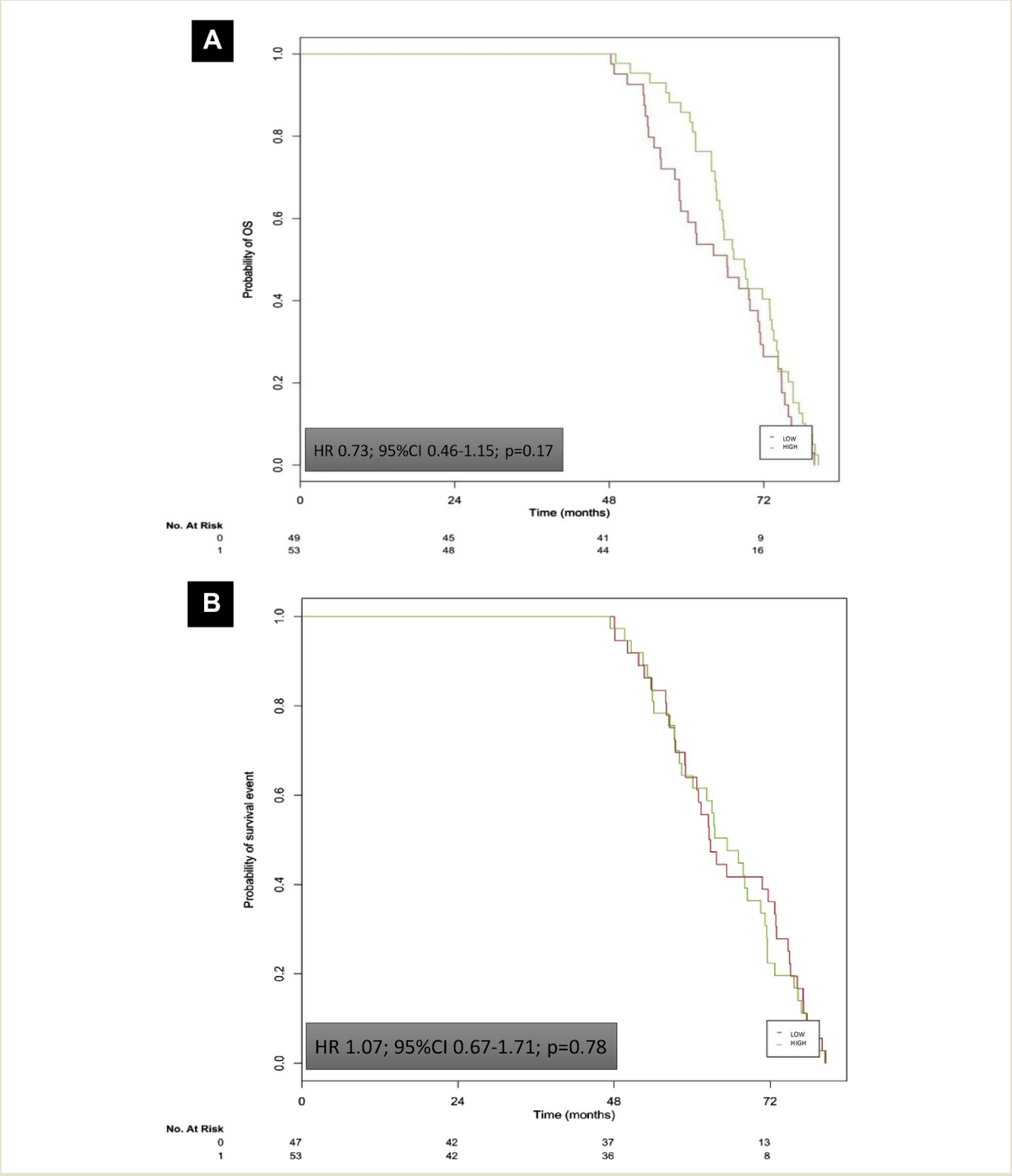
The practice of regular physical activity reduces the risk of recurrence and improves the prognosis of patients with breast cancer.[28] Serum samples obtained both from patients with breast cancer and from healthy women immediately after exercise induce the expression of the Hippo tumor suppressor pathway genes in cell models of HER2−/ER+ breast cancer, compromising the viability and survival of these cells. However, when the same cells are pretreated with beta-blockers, the antiproliferative effect of the post-exercise serum is not observed, suggesting that its anticancer effect is mediated by beta-adrenergic stimulation.[29] Physical exercise induces the expression of *ADRB2* in leukocytes.[30] Hypothetically, if *ADRB2* expression also increases in breast cancer cells as a consequence of exercise, the better prognosis observed in the *ADRB2*-high expression group in our study may be the result of an indirect effect, meaning that physical exercise might be more frequent in the *ADRB2*-high expression group.

MCF-7 is a lineage of HER2−/ER+ breast cancer cells that are highly dependent on ER signaling, frequently used to reproduce in vitro the features of luminal breast cancer.[31] Gargiulo et al.[26] evaluated the effects of *ADRB2* activation in MCF-7 and in non-tumoral immature breast cells (MCF-10A). After *ADRB2* expression was induced by gene transfection into MCF-7 cells, treatment with epinephrine inhibited their proliferation and migration. Interestingly, isoproterenol (an ADRB2 agonist) increased ER expression and induced the differentiation of MCF-10A cells into mature normal breast epithelial cells. The differentiation mediated by isoproterenol was not observed when either fulvestrant or tamoxifen was administered, demonstrating that the effect exerted by ADRB2 depends on the ER pathway, supporting our findings of a significant prognostic impact of *ADRB2* high expression in HER2+/ER+ patients, and also the higher *ADRB2* expression levels observed in HER2+ and luminal tumors.[30]

The proliferation of vascular smooth-muscle cells and the synthesis of VEGF are key steps in angiogenesis, which is essential for tumor growth and metastases development.[32,33] *ADRB2* expression was associated with a low expression of genes related to angiogenesis in our study. In a lineage of vascular smooth-muscle cells (A7r5) transfected with the ER gene, Walters et al.[27] observed that estradiol stimulated proliferation, while isoproterenol inhibited the proliferative effects of estradiol. The antiproliferative effects of isoproterenol were not observed in the presence of propranolol (beta-2 adrenergic blocker), suggesting that ADRB2 activation may inhibit angiogenesis in ER+ tumors.[33] The effect of ADRB2 to antagonize the proangiogenic effects of estradiol may also explain the positive prognostic impact of *ADRB2*-high expression in HER2+/ER+ patients in our study.

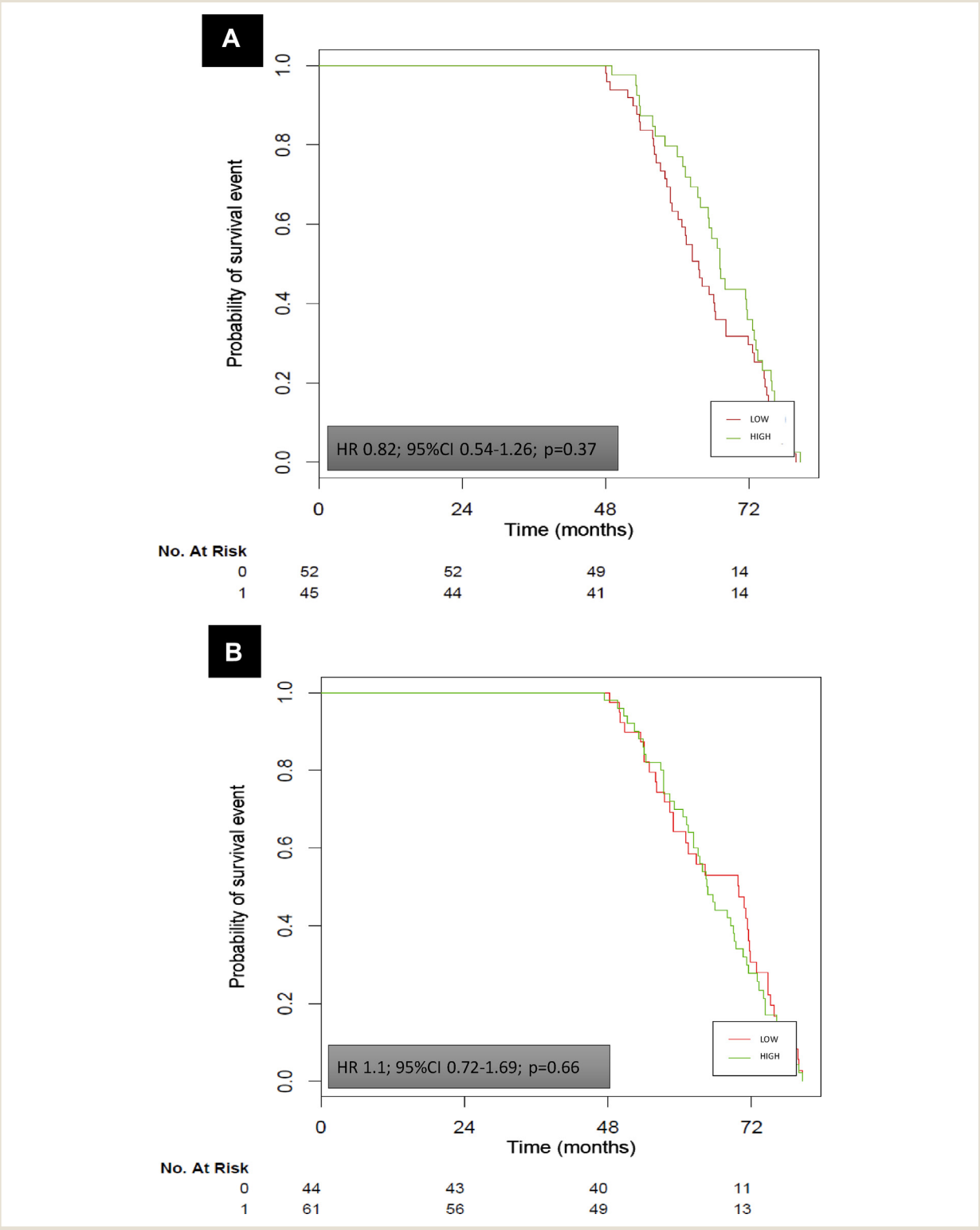
Preclinical studies evaluating the interactions of ADRB2 with the immune system present contradictory results. Wrobel et al.[34] observed that propranolol increased the infiltration of cytotoxic lymphocytes and decreased the concentration of immunosuppressive myeloid cells into the stroma of melanoma models in mice, suggesting that ADRB2 activation may render the tumoral microenvironment less immunogenic. On the other hand, Swanson et al.[35] demonstrated that ADRB2 stimulation induces differentiation of immature T cells into T-helper lymphocytes type 1, increasing the production of interferon-gamma, which enhances the activity of T-cytotoxic lymphocytes. We observed a significant association between the expression of *ADRB2* and genes involved in immune activation. The induction of an antitumor immune response by immune checkpoint inhibitors has proven to be an effective treatment in a variety of tumors, including breast cancer.[36,37] Indeed, high levels of tumor-infiltrating lymphocytes are associated with a good prognosis in HER2+ breast cancer, and

Figure 5 Kaplan-Meier Curves of Relapse-Free Survival According to ADRB2 Expression in Patients Who Received Chemotherapy and Trastuzumab (n = 102, A); and in Those Who Received Chemotherapy Alone (n = 100, B) in the FinHer Study



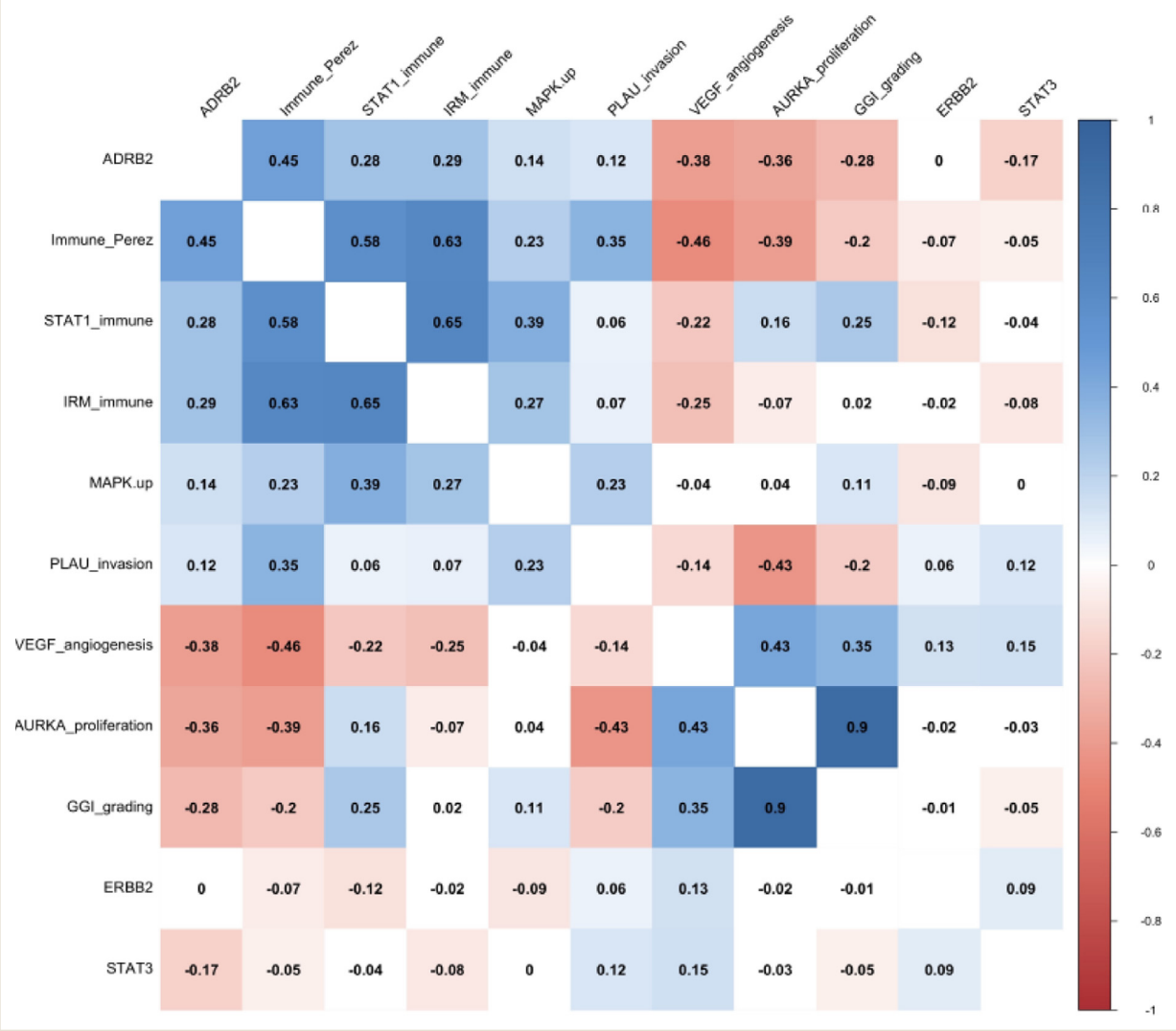
Abbreviations: ADRB2 = beta-2 adrenergic receptor; CI = confidence interval; HR = hazard ratio.

Figure 6 Kaplan-Meier Curves of Relapse-Free Survival According to ADRB2 Expression in Hormone-Receptor Positive (n = 97, A) and Hormone-Receptor Negative Patients (n = 105, B) in the FinHer Study



Abbreviations: ADRB2 = beta-2 adrenergic receptor; CI = confidence interval; HR = hazard ratio.

Figure 7 Spearman Correlation Between ADRB2 Expression in HER2+ Patients From DFS and pCR Datasets (n = 382) and 9 Gene Signatures associated with the following characteristics: AURKA (proliferation, 219 genes); STAT3 (proliferation, 123 genes); PLAU (invasion, 68 genes); VEGF (angiogenesis, 14 genes); GGI (grading, 129 genes); IRM (immune, 7 genes); STAT1 (immune, 95 genes); Perez (immune, 14 genes); MAPK (activation of the MAPK pathway, 315 genes).[20-25] Only the Associations that Reached Statistical Significance Are Colored, With Blue Meaning a Positive Correlation (High ADRB2 Expression is Associated With a High Expression of that Signature) and Pink Meaning a Negative Correlation (High ADRB2 Expression is Associated With a Low Expression of that Signature)



Abbreviations: ADRB2 = beta-2 adrenergic receptor; AURKA = aurora kinase A; GGI = genomic grade index; HER2 = human epidermal growth factor receptor 2; IRM = immune response gene expression module; PLAU = plasminogen activator, urokinase; STAT3 = signal transducers and activators of transcription; VEGF = vascular endothelial growth factor.

immunotherapy presents promising results in this subgroup of patients.[38,39] A potential action of *ADRB2* to render the tumoral microenvironment more immunogenic may explain its positive prognostic impacts. The role of *ADRB2* expression as a predictive biomarker for immunotherapy response and the use of ADRB2 agonists with immune checkpoint inhibitors are thus potential strategies to be further explored.

In the pCR dataset, no association between *ADBR2* expression and pCR was observed. Notably, the information regarding trastuzumab use in this population could not be retrieved. Given the robust survival benefit yielded by trastuzumab in patients with HER2+ early breast cancer, a potential imbalance between trastuzumab use in both groups (*ADRB2*-high vs. *ADRB2*-low) could have influenced our results.[40] However, the analysis of the FinHer study also found no significant association between *ADRB2* expression and RFS both in patients who received and in those who did not receive trastuzumab, even though the 9-week duration of trastuzumab used in this trial is now considered suboptimal.[41]

Rivero et al.[42] evaluated the correlation between *ADRB2* expression and distant-metastasis-free survival in 1924 patients with early breast cancer, demonstrating that a high expression of *ADBR2* was significantly correlated with a favorable prognosis in the overall study population ($P = .006$). Notably, no significant association between *ADRB2* expression and prognosis was observed in the HER2+ subgroup ($P = .412$); however, the limited number of HER2+ patients included (n = 50) precludes definitive conclusions. Interestingly, *ADRB2* expression was correlated with genes involved in T-cell activation and inflammatory response in this study, corroborating our findings.[42]

Potential limitations have to be considered when interpreting our results. Data were extracted from publicly available datasets and not collected prospectively. *ADRB2* expression levels were not available for all patients in the DFS dataset, and the small sample size of the HER2+/ER− subgroup may have limited the analysis in this subset. Also, no data regarding the use of beta-blockers, beta-agonists, and patients' physical exercise habits were available, which are potential factors that could have influenced our results. However, given the encouraging preclinical evidence suggesting that ADRB2 might be involved in treatment resistance in HER2+ breast cancer, this hypothesis warranted further investigation, and to our knowledge this is the first study evaluating *ADRB2* expression as a biomarker specifically in patients with HER2+ breast cancer. Notably, the population of HER2+ patients who did not receive any systemic treatment in our DFS dataset created an interesting scenario to study *ADRB2* expression as a prognostic biomarker.

In conclusion, our results suggest that *ADRB2*-high expression can be a favorable prognostic factor in patients with HER2+ early breast cancer, especially in the HER2+/ER+ subgroup. This effect might be the result of a tumoral microenvironment that is more immunogenic and less favorable to angiogenesis and proliferation in *ADRB2*-high tumors. *ADRB2* arises as a promising biomarker and potential therapeutic target in patients with HER2+ breast cancer.

Clinical Practice Points

- Preclinical studies suggest that beta-2 adrenergic receptor (ADRB2) stimulates proliferation and mediates treatment resistance in HER2+ breast cancer cells.
- This study evaluated the expression of the *ADRB2* gene as a prognostic and predictive biomarker in patients with HER2+ early breast cancer.
- *ADRB2* expression did not predict the occurrence of pathologic complete response.
- Opposing our initial hypothesis, a high expression of *ADRB2* appeared to be associated with a favorable prognosis in patients with HER2+ early breast cancer.
- *ADRB2* may exert antiproliferative, antiangiogenic, and immunogenic effects that could justify its positive prognostic impact.
- The role of *ADRB2* expression as a biomarker in patients with HER2+ early breast cancer should be further validated in prospective studies.

Acknowledgments

Author contributions: RC, FR, and EdA conceived the project. FR and CS performed the bioinformatics and statistical analysis. RC wrote the manuscript. All authors collaborated with their expertise and revised the final version of this manuscript before submission.

Disclosure

R. Caparica received speaker honoraria from Boehringer-Ingelheim, AstraZeneca, and Janssen; amd travel grants from Pfizer and AstraZeneca, none related to the present work. A. Awada has acted as advisor, and received fees for lectures and research grants from Roche, Lilly, Amgen, ESAI, BMS, Pfizer, Novartis, and MSD. E. de Azambuja has received honoraria and served on the advisory board of Roche/GNE, and received travel grants from Roche/GNE and GSK/Novartis. The Institute he works for has received research grants from AstraZeneca, Lilly, MSD, Novartis, Pfizer, Roche-Genentech, Synthon, Radius, and Servier. All other authors state that they have no conflicts of interest.

Supplemental Data

Supplemental tables and figures accompanying this article can be found in the online version at https://doi.org/10.1016/j.clbc.2020.01.007.

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Supplemental Table 1 Characteristics of Patients and Studies Included in the Dataset of Haibe-Kains et al[13]

	N	CAL	DFHCC	EMC2	MAINZ	NCI	NKI	STNO2	TRANSBIG	UCSF	UNT	UPP	VDX
	1258	8	6	48	200	10	165	7	198	11	133	128	344
Age													
≤50	567	4	0	0	51	3	137	4	142	6	61	30	129
>50	585	4	6	0	149	7	28	3	56	5	72	98	157
Missing	106	0	0	48	0	0	0	0	0	0	0	0	58
Tumor size													
≤2cm	788	5	3	0	112	5	98	7	102	8	83	87	278
>2cm	364	3	3	0	88	5	67	0	96	3	50	41	8
Missing	106	0	0	48	0	0	0	0	0	0	0	0	58
ER													
Negative	367	4	5	4	38	5	42	5	64	4	44	17	135
Positive	891	4	1	44	162	5	123	2	134	7	89	111	209
HER2													
Negative	1014	8	5	28	164	9	136	6	154	2	115	104	283
Positive	237	0	1	20	36	1	29	1	44	2	18	24	61
Missing	7	0	0	0	0	0	0	0	0	7	0	0	0
Grade													
1	186	2	0	0	29	2	36	0	30	1	32	47	7
2	435	1	1	0	136	1	49	3	83	6	51	62	42
3	418	5	5	0	35	7	80	4	83	4	29	18	148
Missing	219	0	0	48	0	0	0	0	2	0	21	1	147
SCMGENE													
LumA	278	2	0	9	59	1	55	0	37	0	59	56	0
LumB	279	2	0	17	78	4	46	2	72	1	24	33	0
TNBC	277	4	5	2	27	4	32	4	45	1	32	15	106
HER2+/ER-	88	0	0	2	11	1	10	1	19	1	12	2	29
HER2+/ ER+	149	0	1	18	25	0	19	0	25	1	6	22	32
Missing	187	0	0	0	0	0	3	0	0	7	0	0	177

Patients with HER2+ early breast cancer included in the disease-free survival analysis were retrieved from the dataset of Haibe-Kains et al.[13]
Datasets were retrieved from the author's or Institution's Web sites. Each dataset was assigned a short acronym and an instance number as follows: CAL = dataset of patients with breast cancer from the University of California, San Francisco, and the California Pacific Medical Center (United States); DFHCC = Dana-Farber Harvard Cancer Center (United States); EMC = Erasmus Medical Center (the Netherlands); MAINZ = Mainz hospital (Germany); NCI = National Cancer Institute (United States); NKI = National Kanker Instituut (the Netherlands); STNO = Stanford/Norway (United States and Norway); TRANSBIG = dataset collected by the TransBIG consortium (Europe); UCSF = University of California, San Francisco (United States); UNT = cohort of patients with untreated breast cancer from the Oxford Radcliffe (United Kingdom) and Karolinska (Sweden) hospitals; UPP = Uppsala Hospital (Sweden); VDX = Veridex (the Netherlands).
Abbreviations: ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2.

Supplemental Table 2 Characteristics of Patients and Studies Included in the Dataset of Ignatiadis et al[14]

		EORTC (A)	EORTC (AT)	ISPY (AT)	LBJ/IN/GEI (AT)	MDACCT (A)	MDACCT (AT)	TOP (A)	MDACC (AT)	MAQCIII (A)	USO (AT)
	1013	102	59	79	57	62	116	114	271	92	61
Age											
≤50	540	38	30	51	30	35	65	69	137	50	35
>50	435	28	29	28	27	27	51	45	133	41	26
Missing	38	36	0	0	0	0	0	0	1	1	0
Tumor size											
T0	7	0	0	0	0	1	1	0	4	1	0
T1	54	2	1	1	1	4	7	16	20	1	1
T2	501	38	38	32	19	31	45	79	149	50	20
T3	266	26	20	38	18	10	27	5	58	24	40
T4	146	0	0	8	19	15	36	14	38	16	0
Missing	39	36	0	0	0	1	0	0	2	0	0
Nodal status											
N0	331	27	22	25	16	23	36	52	88	21	21
N1	422	34	32	46	25	21	50	57	113	12	32
N2	119	5	5	6	15	15	23	3	32	10	5
N3	56	0	0	2	1	2	7	2	37	2	3
Missing	85	36	0	0	0	1	0	0	1	47	0
ER											
Negative	559	65	59	36	21	29	51	114	105	50	29
Positive	454	37	0	43	36	33	65	0	166	42	32
HER2											
Negative	857	69	39	76	57	53	99	81	230	92	61
Positive	156	33	20	3	0	9	17	33	41	0	0
Missing	0	0	0	0	0	0	0	0	0	0	0
Grade											
G1	45	2	0	6	5	3	12	2	11	3	1
G2	307	21	16	24	19	24	37	20	98	29	19
G3	515	32	38	27	23	25	47	87	152	47	37
Missing	131	47	5	22	10	10	20	5	10	13	4
pCR											
No	778	63	32	65	46	58	94	98	216	65	41
Yes	235	39	27	14	11	4	22	16	55	27	20

Supplemental Table 2 Continued

		EORTC (A)	EORTC (AT)	ISPY (AT)	LBJ/IN/GEI (AT)	MDACCT (A)	MDACCT (AT)	TOP (A)	MDACC (AT)	MAQCIII (A)	USO (AT)
SCMGENE											
LumA	205	19	0	14	11	21	39	12	71	16	2
LumB	205	9	0	22	25	10	19	12	79	22	7
TNBC	396	54	43	28	21	22	41	58	80	37	12
HER2+/ER-	127	11	16	8	0	7	10	20	25	13	17
HER2+/ER-	80	9	0	7	0	2	7	12	16	4	23

Patients with HER2+ early breast cancer included in the pCR analysis were retrieved from the dataset of Ignatiadis et al.[14]

Abbreviations: A = anthracycline-based neoadjuvant chemotherapy; AT = anthracycline plus taxane-based neoadjuvant chemotherapy; EORTC = European Organisation for Research and Treatment of Cancer; ER = estrogen receptor; GEICAM = Grupo Español de Investigación en Cáncer de Mama; GEO = gene expression omnibus; HER2 = human epidermal growth factor receptor 2; INEN = Instituto Nacional de Enfermedades Neoplásicas; I-SPY = Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis; LBJ = Lyndon B. Johnson Hospital; MAQCII = MicroArray Quality Control Consortium II; MDACC = MD Anderson Cancer Center; pCR = pathologic complete response; TOP = Trial of Principle; USO = US Oncology.

Supplemental Table 3 Characteristics of the Patients From the FinHer Study

	Chemotherapy	Chemotherapy and Trastuzumab
Overall	100	102
Age		
≤50	57	48
>50	43	54
Tumor size		
≤2 cm	33	41
>2 cm	67	60
Missing	0	1
Nodal status		
Negative	20	9
Positive	80	93
ER		
Negative	54	51
Positive	46	51
HER2		
Negative	0	0
Positive	100	102
Grade		
1	3	2
2	29	35
3	64	63
Missing	4	2

Abbreviations: ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2.

Supplemental Table 4 Cox Regression Models for the Correlation Between *ADRB2* Expression and Disease-Free Survival in HER2+ (n = 175), HER2+/ER+ (n = 117), and HER2+/ER− Patients (n = 58), Univariate and Multivariate

	n	Hazard Ratio and 95% Confidence Interval
Univariate analysis		
HER2+	175	0.54 (0.34-0.87; $P = .01$)
HER2+/ER+	117	0.39 (0.19-0.79; $P = .008$)
HER2+/ER−	58	0.71 (0.36-1.41; $P = .33$)
Multivariate analysis		
HER2+	175	0.67 (0.36-1.23; $P = .19$)
HER2+/ER+	117	0.36 (0.14-0.93; $P = .03$)
HER2+/ER−	58	0.78 (0.32-1.92; $P = .59$)

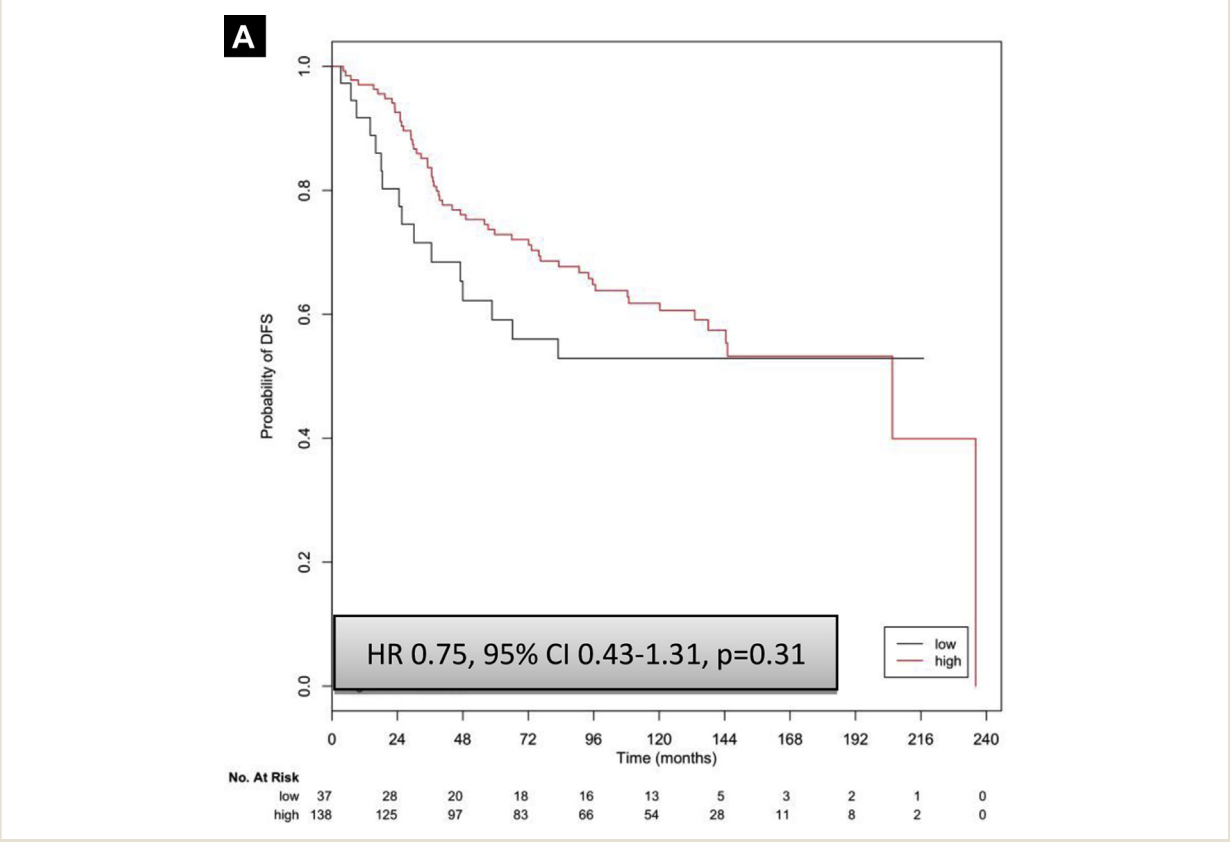
Multivariate analysis was adjusted for datasets, age (≤50 vs. >50), tumor size (≤2 vs. >2 cm), histologic grade (1, 2 vs. 3) and ER status (negative vs. positive).
Abbreviations: ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2.

Supplemental Table 5 Cox Regression Models for the Correlation Between *ADRB2* Expression and Pathologic Complete Response in HER2+ (n = 207), HER2+/ER+ (n = 80), and HER2+/ER− patients (n = 127), Univariate and Multivariate

	n	Hazard Ratio and 95% Confidence Interval
Univariate analysis		
HER2+	207	1.14 (0.63-2.10; $P = .67$)
HER2+/ER+	80	0.98 (0.30-3.61; $P = .98$)
HER2+/ER-	127	1.06 (0.49-2.28; $P = .88$)
Multivariate analysis		
HER2+	207	1.52 (0.76-3.16; $P = .25$)
HER2+/ER+	80	1.75 (0.47-8.05; $P = .43$)
HER2+/ER−	127	1.41 (0.56-3.72; $P = .47$)

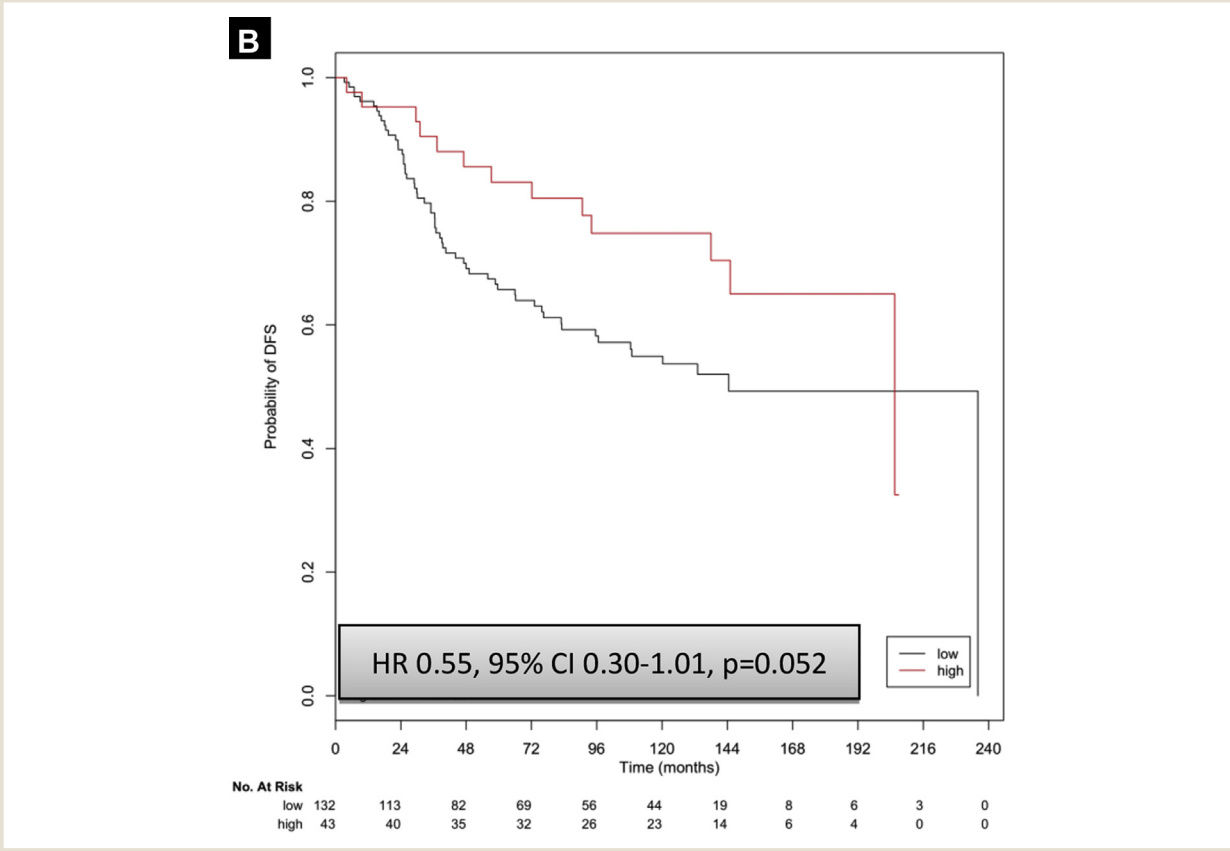
Multivariate analysis was adjusted for datasets, treatment type (A vs. AT), age (≤50 vs. >50), tumor size (≤T2 vs. ≥T3), nodal status (negative vs. positive), histologic grade (1 and 2 vs. 3) and ER status (negative vs. positive).
Abbreviations: ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2.

Supplemental Figure 1 **Kaplan Meier Curves of Disease-free Survival (DFS) According to ADRB2 Expression in the Overall HER2+ Population of the DFS Dataset (N = 175) Using the Alternative Cutoff Values of 25% (A) and 75% of the Samples (B) to Define ADRB2 Expression as High. Patients Were Classified as Either "ADRB2-High" Group, when ADRB2 Expression Level in Their Samples was Above the Cutoff Value of Their Dataset; or as "ADRB2-Low" Group, when ADRB2 Expression Level in Their Samples was Below the Cutoff Value of Their Dataset**

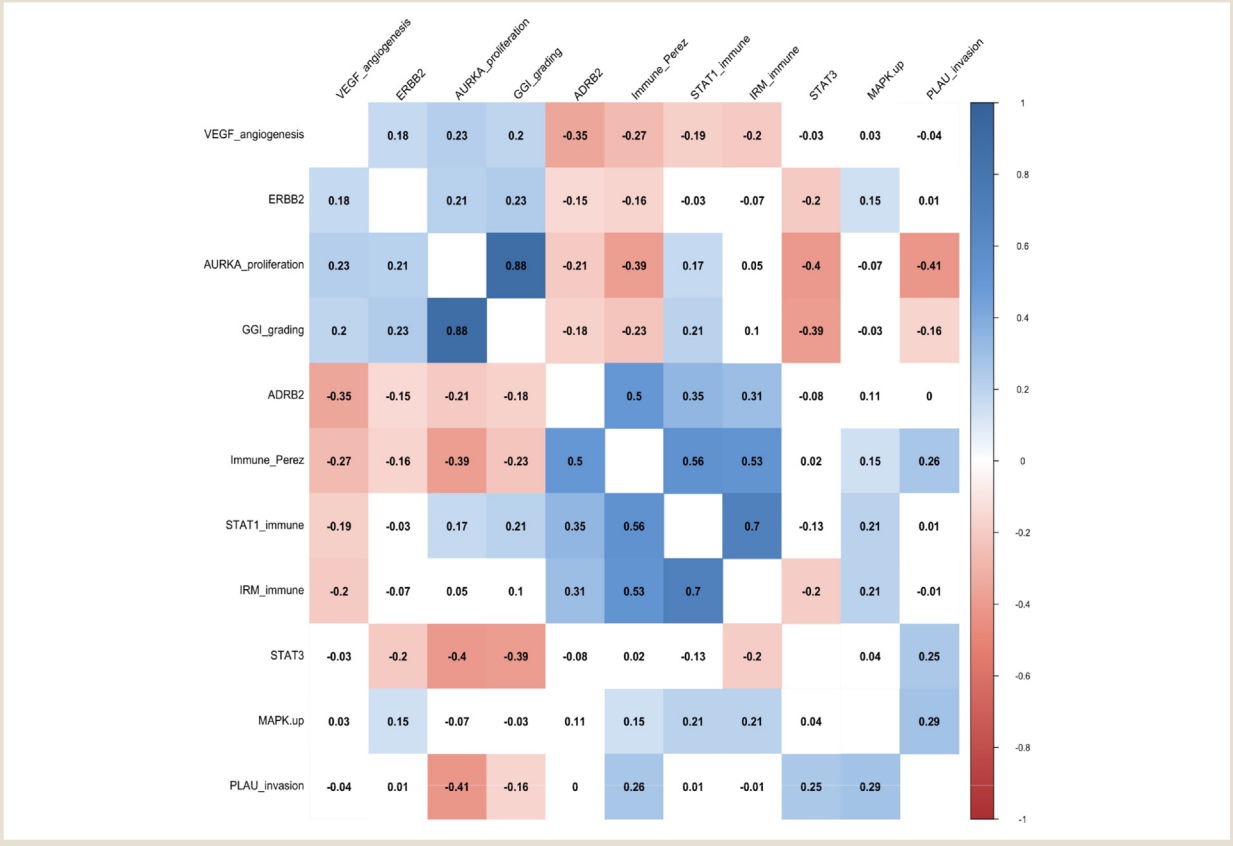


Abbreviations: ADRB2 = beta-2 adrenergic receptor; CI = confidence interval; HER2 = human epidermal growth factor receptor 2; HR = hazard ratio.

Supplemental Figure 1 Continued



Supplemental Figure 2 **Spearman Correlation Between ADRB2 Expression in HER2+/ER+ Patients From Both Datasets (n = 116) and 9 Gene Signatures associated with the following characteristics: AURKA (proliferation, 219 genes); STAT3 (proliferation, 123 genes); PLAU (invasion, 68 genes); VEGF (angiogenesis, 14 genes); GGI (grading, 129 genes); IRM (immune, 7 genes); STAT1 (immune, 95 genes); Perez (immune, 14 genes); MAPK (activation of the MAPK pathway, 315 genes).[20-25] Only the Associations that Reached Statistical Significance Are Colored, With Blue Meaning a Positive Correlation (High ADRB2 Expression is Associated With a High Expression of that Signature) and Pink Meaning a Negative Correlation (High ADRB2 Expression is Associated With a Low Expression of that Signature)**



Abbreviations: ADRB2 = beta-2 adrenergic receptor; AURKA = aurora kinase a; GGI = genomic grade index; HER2 = human epidermal growth factor receptor 2; IRM = immune response gene expression module; PLAU = plasminogen activator, urokinase; STAT3 = signal transducers and activators of transcription; VEGF = vascular endothelial growth factor.