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Beta-2 adrenergic receptor gene (*ADRB2*) expression in HER2-positive early-stage breast cancer patients: a post-hoc analysis of the NCCTG-N9831 (Alliance) trial

Rafael Caparica, MD[1,*], **Yaohua Ma, PhD**[2], **Claudia De Angelis, MD**[3], **François Richard, PhD**[4], **Christine Desmedt, PhD**[4], **Ahmad Awada, MD, PhD**[1], **Martine Piccart, MD, PhD**[1], **Edith A. Perez, MD**[5], **Alvaro Moreno-Aspitia, MD**[5], **Sunil Badve, MD**[6], **E. Aubrey Thompson, PhD**[7], **Evandro de Azambuja, MD, PhD**[1]

[1]Department of Medical Oncology, Institut Jules Bordet, Université Libre de Bruxelles (U.L.B.), Brussels, Belgium

[2]Division of Biomedical Statistics and Informatics, Mayo Clinic, Jacksonville, Florida, USA

[3]Unit of Medical Oncology 2, Azienda Ospedaliera-Universitaria Pisana, Pisa, Italy

[4]Laboratory for Translational Breast Cancer Research, Department of Oncology, KU Leuven, Leuven, Belgium

[5]Division of Hematology and Oncology, Mayo Clinic, Jacksonville, Florida, USA

[6]Department of Pathology, Indiana University School of Medicine, Indiana, USA

[7]Department of Cancer Biology, Mayo Clinic, Jacksonville, Florida, USA

Abstract

Background: Beta-2 adrenergic receptor (ß2AR) modulates immune activation and may enhance trastuzumab activity. We assessed the impact of ß2AR gene *(ADRB2)* expression on the outcomes of patients with HER2-positive early-stage breast cancer enrolled on the NCCTG-N9831 trial.

Patients and Methods: This is a post-hoc analysis of the NCCTG-N9831 trial, which compared chemotherapy (arm A) versus chemotherapy plus trastuzumab (arms B&C) as adjuvant treatment of patients with HER2-positive early-stage breast cancer, with disease-free survival (DFS) as primary endpoint. Gene expression levels retrieved by DASL assay were used to

[*]Corresponding author: Dr. Rafael Caparica, Department of Medical Oncology, Institut Jules Bordet – Boulevard de Waterloo 121, 1000 - Bruxelles, rcaparica@hotmail.com.

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Ethics approval and consent to participate: The Mayo Clinic institutional review board and the Correlative Science Committee of the North American Breast Cancer Group approved this study.

classify patients as *ADRB2*-high or *ADRB2*-low. Hazard ratios (HRs) were calculated by a Cox proportional model adjusted for prognostic variables and *ADRB2* expression. Correlations between *ADRB2* expression and stromal tumor-infiltrating lymphocyte (TIL) levels were assessed with Pearson coefficient. A multivariable Cox regression model with interaction term was performed to assess the interaction between *ADRB2* expression and treatment arm; and *ADRB2* expression and a 8-gene signature previously shown to predict trastuzumab benefit.

Results: Overall, 1,282 patients were included (*ADRB2*-high [*N*=944] / *ADRB2*-low [*N*=338]). A high expression of *ADRB2* was associated with a longer DFS (p=0.01) in the overall population. The addition of trastuzumab to chemotherapy improved DFS only in patients with *ADRB2*-high tumors (p<0.01). *ADRB2* expression was correlated with TIL levels (r=0.24, p<0.001). No association between *ADRB2* expression and the 8-gene trastuzumab benefit signature was observed (p=0.32).

Conclusion: Our findings suggest that a high *ADRB2* expression is a favorable prognostic factor and may identify patients with HER2-positive early-stage breast cancer who benefit from adjuvant trastuzumab.

Trial Registration: clinicaltrials.gov NCT00005970

Micro Abstract:

Beta-2 adrenergic receptor (ß2AR) modulates T-cell activation and previous studies have associated a high expression of the ß2AR gene *(ADRB2)* with a favorable prognosis in HER2-positive breast cancer patients. In this post-hoc analysis of the NCCTG-N9831 trial, a high *ADRB2* expression was associated with longer disease-free survival and also identified patients who benefited from adjuvant trastuzumab.

Keywords

breast cancer; HER2; beta-2 adrenergic receptor; *ADRB2* ; trastuzumab

Introduction

The development of trastuzumab - a monoclonal antibody that targets the human epidermal growth factor receptor 2 (HER2) - has dramatically improved the outcomes of patients with HER2-positive breast cancer, both in the metastatic and the early-disease settings.[1,2] Although more than 90% of patients with HER2-positive early-stage breast cancer remain alive and recurrence-free at 5 years with the standard combination of (neo)adjuvant chemotherapy and HER2 blockade, some of these patients still present with recurrences, and thus the development of prognostic and predictive biomarkers is crucial to support physicians to personalize and refine treatment decisions for this population.[3–5]

The beta-2 adrenergic receptor (ß2AR) is a G protein-coupled receptor that mediates physiologic processes such as smooth muscle relaxation, chronotropism and inotropism when activated by catecholamines.[6] ß2AR is also involved at different stages of inflammatory and immune responses, although its role in these processes is not fully elucidated: whereas some studies show that ß2AR activation leads to the recruitment and

differentiation of macrophages, production of pro-inflammatory cytokines, differentiation of immature T cells into T helper lymphocytes, and T cytotoxic lymphocyte activation, other preclinical data suggest that ß2AR stimulation may impair T-cell function whereas the blockade of the ß2AR pathway with ß - blockers promotes immune activation in murine cancer models.[7–10] As the induction of an immune response against cells that express HER2 is an important mechanism by which trastuzumab exerts its effects, ß2AR may participate in the immune activation and T cell recruitment observed in the tumoral stroma of some patients after trastuzumab administration, and hence ß2AR may influence trastuzumab activity.[11–13]

In line with preclinical studies that show a significant cross-talk between G protein-coupled receptors, such as ß2AR, and epidermal growth factor receptors, such as HER2, tumor samples from patients with HER2-positive breast cancer present high expression levels of the ß2AR gene (*ADRB2*).[14–16] Furthermore, in preclinical models of HER2-positive breast cancer, HER2 activation induces the synthesis of catecholamines (ß2AR agonists), whereas ß2AR activation increases HER2 expression on the cell membrane.[17] As a consequence, ß2AR activation and *ADRB2* expression may render HER2-positive cells more dependent on HER2 signalling.[17] In this context, the potential interaction between ß2AR and HER2 in patients with breast cancer warrants further investigation.

Previous studies suggest that a high *ADRB2* expression may be associated with a favorable prognosis in patients with early-stage breast cancer.[14,15] The present study assessed the influence of *ADRB2* expression on the outcomes of patients with HER2-positive early-stage breast cancer enrolled on the North Central Cancer Treatment Group (NCCTG) - N9831 trial (NCCTG is now part of the Alliance for Clinical Trials in Oncology [Alliance]).

Methods

Patients

The NCCTG-N9831 trial enrolled 3,505 patients with HER2-positive early-stage breast cancer previously submitted to surgery to receive 4 cycles of doxorubicin and cyclophosphamide q21 days (all patients), followed by either weekly paclitaxel alone (arm A); paclitaxel followed by trastuzumab (arm B); or paclitaxel administered concomitantly with trastuzumab (arm C) (Supplementary Figure 1 and protocol available as Supplementary material). The trial's main objective was to evaluate the benefit of adding trastuzumab to adjuvant chemotherapy and disease-free survival (DFS) was the primary endpoint.[18,19] A statistically and clinically significant DFS improvement yielded by the addition of trastuzumab to adjuvant chemotherapy was demonstrated in the NCCTG-N9831 trial.[18–20]

The present study is an exploratory post-hoc analysis of the NCCTG-N9831 trial. For the purpose of this study, arms B and C were pooled (arms B&C) in order to represent patients who received adjuvant trastuzumab plus chemotherapy, whereas arm A represents patients who received chemotherapy alone. The Mayo Clinic institutional review board and the Correlative Science Committee of the North American Breast Cancer Group approved this study. Signed informed consent for use of data and samples was obtained from all patients prior to study enrolment.

Gene expression analyses

From the overall 3,505 patients enrolled on the NCCTG-N9831 trial, 1,282 samples (arm A, 433; arm B, 477; arm C, 372) were evaluable for gene expression profiling, and gene expression data were retrieved by DASL assay as previously described (Supplementary Figure 2).[21,22] All gene expression analyses were performed centrally at Mayo Clinic Comprehensive Cancer Center, Rochester, USA. The ideal cut-point for *ADRB2* expression assessed as a continuous variable for the outcome survival was determined in the overall population per SAS software (=11.903), with levels above this cut-point defined as high (*ADRB2*-high) and levels below this cut-point as low (*ADRB2*-low). The cut-point adopted in this study was tested in a previous dataset from patients with HER2-positive early-stage breast cancer as a validation cohort.[23]

Tumor-infiltrating lymphocytes (TILs)

Histopathologic analysis of the percentage of stromal TILs was performed in a single hematoxylin-eosin–stained section from each tumor using criteria proposed by Denkert et al, Loi et al, Adams et al, and Salgado et al.[24–27] TIL levels were defined as the percentage of tumor stroma containing infiltrating lymphocytes that were not in direct contact with tumor cells from an assessment of the entire tumor-containing area of the section. Areas of non-invasive cancer or artefacts were excluded. Histopathologic analyses were conducted centrally by 2 pathologists who were blinded to patient's treatment, tumor staging and outcome. Patients were classified into 3 categories according to TIL levels (<5%; 5–20%; and >20%) and the correlation between *ADRB2* expression and TIL levels was assessed. DFS according to TIL levels was assessed in the overall population and according to *ADRB2* expression.

8-Gene Trastuzumab-benefit Signature

A gene expression signature consisting of eight genes associated with the HER2 (ERBB2, c17orf37, GRB7) and the estrogen receptor (ESR1, NAT1, GATA3, CA12, IGF1R) pathways has been shown to predict the magnitude of adjuvant trastuzumab benefit in patients with HER2-positive early breast cancer from NSABP B-31 and NCCTG N9831 trials by categorizing patients into 3 subgroups according to trastuzumab benefit (large, moderate and no-benefit), as previously described.[28,29] We investigated the association between *ADBR2* expression and the trastuzumab-benefit subgroups according to the 8-gene signature in our population. The interaction between *ADBR2* expression and the subgroups that benefit from trastuzumab (large and moderate) per the 8-gene signature was also assessed.

Objectives

The main objectives of this study were:

1. To evaluate DFS according to *ADRB2* expression.
2. To evaluate *ADRB2* expression as a predictor of trastuzumab benefit.

Subgroup analyses to evaluate patient outcomes according to *ADRB2* expression per treatment arm were performed.

Secondary objectives were to assess the correlation between *ADRB2* expression and TIL levels, to analyse patient outcomes according to *ADRB2* expression per TIL levels and to evaluate the association between ADRB2 expression and the 8-gene trastuzumab-benefit signature subgroups.

Endpoint

As in the NCCTG-N9831 trial, the primary endpoint of this study is DFS, defined as the time between randomization and occurrence of loco-regional or distant recurrence of breast cancer; development of contralateral breast cancer or second primary cancer other than squamous or basal cell carcinoma of the skin, carcinoma in situ of the cervix, or lobular carcinoma in situ of the breast; or death from any cause.[18,19]

Statistical Analysis

DFS curves were estimated with Kaplan Meier method and *p* values for comparisons were calculated with log-rank test; Hazard ratios were calculated by a Cox proportional hazard model of multivariable analysis adjusted for age (≤50 vs >50 years); lymph node status (positive vs negative); tumor size (≤2 vs >2cm); histologic grade (3 vs <3); hormone-receptor status (negative vs positive); and *ADRB2* expression (high vs. low), with 95% confidence intervals (CI) estimated by chi-square test and *p* values calculated by Wald test. A multivariable Cox regression model with interaction term was performed to assess the interaction between *ADRB2* expression and treatment arm and the interaction between *ADRB2* expression and the 8-gene trastuzumab-benefit signature. The correlations between *ADRB2* expression and TIL levels were assessed by the Pearson-correlation coefficient. Associations between categorical variables were estimated by chi-square test. The *p*-values were considered significant if ≤0.05. Statistical analyses were performed with SAS software (version 9.4).

Results

Patient characteristics

A total of 1,282 patients (arm A, *N*=433; arms B&C, *N*=849) were included in the present study. Median age was 50 years (22–80); most patients had positive lymph nodes (86.1%), tumors ≤2cm (89.9%), with histologic grade 3 (72.8%) and approximately half had hormone receptor-positive disease (51.6%). In comparison to the 2,223 patients enrolled in the NCCTG-N9831 trial who were not included, high-grade and hormone receptor-negative tumors were more frequent amongst patients included in the present analysis. Nevertheless, the differences in terms of patient outcomes between the three arms for the patients included in this study were similar to those observed in the trial as a whole, as previously reported.[21]

According to *ADRB2* expression levels, 944 patients were classified as *ADRB2*-high and 338 were classified as *ADRB2*-low. No significant differences in terms of clinicopathological characteristics were observed between patients with *ADRB2*-high and those with *ADRB2*-low tumors (Table 1).

DFS according to *ADRB2* expression – overall population

In the overall population (*N*=1,282), DFS was significantly longer in patients with *ADRB2*-high tumors when compared to those with *ADRB2*-low tumors (HR 0.77; 95% CI 0.63–0.96; *p*=0.01) (Figure 1).

In multivariable analysis adjusted for clinicopathological factors, age >50 years, lymph-node positive status and tumor size >2cm were associated with a worse prognosis, whereas hormone-receptor positive status was a favorable prognostic factor (Table 2).

DFS according to ADRB2 expression – per treatment arm—In patients from arm A (*N*=433), no significant difference in terms of DFS was observed between patients with *ADRB2*-high tumors versus those with *ADRB2*-low tumors (HR 0.93; 95% CI, 0.65–1.32; *p*=0.68). In patients from arms B&C (*N*=849), DFS was significantly longer in those with *ADRB2*-high tumors when compared to those with *ADRB2*-low tumors (HR 0.69; 95% CI, 0.53–0.91; *p*<0.01) (Supplementary Table 1).

ADRB2 expression cut-point validation

Using the same cut-point for ADRB2 expression from this study in a publicly available dataset from patients with HER2-positive early-stage breast cancer (N=175), we observed a trend for a longer DFS in the ADRB2-high expression group (HR 0.55; 95% CI, 0.30–1.01; Supplementary Figure 3).[23]

Trastuzumab benefit according to *ADRB2* expression

In patients with *ADRB2*-high tumors (*N*=944), the benefit of adding trastuzumab to chemotherapy was observed: DFS was significantly longer in arms B&C when compared to arm A (HR 0.65; 95% CI, 0.51–0.82; *p*<0.01) (Figure 2 A). In patients with *ADRB2*-low tumors (*N*=338), no significant DFS difference was observed between arms B&C versus arm A (HR 0.87; 95% CI, 0.60–1.26; *p*=0.47) (Figure 2 B). Patients from arms B&C with *ADRB2*-high tumors had a significantly longer DFS in comparison to the other subgroups (*p*<0.01) (Figure 2 C), however no significant interaction between *ADRB2* expression and treatment arm was observed for the outcome DFS (*p*=0.19).

ADRB2 expression and TILs

Out of the 1,282 patients included in this study, 566 had tumor samples evaluable for stromal TIL level assessment, out of whom 222 had TILs <5%; 222 had TILs between 5% and 20%; and 122 had TILs >20% (Supplementary Figure 2). *ADRB2* expression was significantly correlated with TIL levels (r=0.24; *p*<0.01) (Figure 3).

In patients with *ADRB2*-high tumors (*N*=409), DFS was significantly longer in those whose tumors had TILs >20% when compared to those with TILs <5% (*p*=0.03), whereas no significant DFS difference was observed between patients whose tumors had TIL levels between 5–20% versus those with TILs <5% (*p*=0.60).

In patients with *ADRB2*-low tumors (*N*=157), no significant difference in terms of DFS was observed according to TIL levels (*p*=0.12) (Figures 4 A–B). Multivariable analyses adjusted

for clinicopathological factors including TILs were performed in the 566 patients who had evaluable samples for TIL levels assessment (Supplementary Table 2).

ADRB2 expression and 8-gene trastuzumab-benefit signature subgroups

When applying the 8-gene signature to our population, a total of 140 patients were categorized as no-benefit, 522 as moderate-benefit and 570 as large-benefit from trastuzumab. No significant association between *ADRB2* expression and the 8-gene trastuzumab-benefit signature subgroups was observed in our study (p=0.32). No significant interaction occurred between *ADRB2-high* expression and moderate-benefit (p=0.59) or large-benefit (p=0.68) trastuzumab subgroups for the outcome DFS.

Discussion

In the present post-hoc analysis of the NCCTG-N9831 trial, a high expression of *ADRB2* was associated with a favorable prognosis in patients with HER2-positive early-stage breast cancer, with this effect being mainly observed in those who received trastuzumab plus chemotherapy (arms B&C). Interestingly, the *ADRB2-high* expression profile also identified a subgroup of patients who appeared to benefit from adjuvant trastuzumab in the trial. The observed correlation between *ADRB2* expression and TIL levels suggests that *ADRB2* may exert its effects by participating in lymphocyte recruitment and immune activation to potentialize trastuzumab anti-tumor effect.

Previous studies assessed the impact of *ADBR2* expression on the outcomes of patients with breast cancer. When evaluating the expression of adrenergic receptor genes in 1,924 breast cancer patients (out of whom only 50 were HER2-positive), Rivero et al. found that a high expression of *ADRB2* was associated with a longer distant-metastasis-free survival.[14] Our group has previously assessed *ADRB2* expression in 175 patients with HER2-positive early-stage breast cancer and observed a longer DFS in those whose tumors presented high levels of *ADRB2* expression. In this study, *ADRB2* expression was correlated with the expression of genes involved in immune activation, whereas it was negatively correlated with genes involved in angiogenesis and cell proliferation, suggesting potential biological mechanisms through which *ADRB2* may exert its effects.[15] By assessing *ADRB2* expression in patients enrolled on a phase III trial, the present study reinforces the role of *ADRB2* high expression as a potential prognostic factor in patients with HER2-positive early-stage breast cancer.

Chronic stress and adrenergic activation induce angiogenesis, stimulate cell motility and facilitate metastases development in preclinical models of breast cancer.[30,31] In response to high catecholamine levels observed in states of chronic stress, a negative feedback mechanism occurs, consisting in the downregulation of adrenergic receptors mediated by an increase in their degradation and suppression of their expression.[32] In this context, a high *ADRB2* expression might be an indirect marker of low stress levels, which would potentially justify the prognostic effect of *ADRB2* expression observed in our study. Moreover, as *ADRB2* expression can be modulated by factors such as physical exercise (increases expression) and chronic stress (reduces its expression), interventions to minimize chronic stress or stimulate exercise are worthy of evaluation in future studies.[33–35] Notably, the

practice of physical exercise has been shown to reduce the risk of recurrence in patients with early-stage breast cancer.[36]

In patients with HER2-positive early-stage breast cancer, high TIL levels are usually associated with a favorable prognosis as well as predictors of pathologic complete response in several studies.[37,38] Preclinical data suggest that ß2AR participates in several stages of immune activation by inducing the differentiation of T cells, increasing interferon gamma secretion and enhancing the activity of T cytotoxic lymphocytes.[8] On this basis, the correlation between TIL levels and *ADRB2* expression observed in our study highlights the role of the ß2AR as a potential mediator of immune activation in breast cancer patients. Furthermore, high TIL levels were prognostic mainly in patients whose tumors presented a high *ADRB2* expression, suggesting that ß2AR may not only participate in TIL recruitment but also stimulate their anti-cancer activity.

The amplification and/or hyperexpression of HER2 is the standard method adopted in clinical practice to select patients who will receive HER2 blockade.[1] Nonetheless, the fact that a significant proportion of patients with HER2-positive early-stage breast cancer present recurrences despite receiving the standard treatment whereas some patients enrolled on the NCCTG-N9831 trial are alive and recurrence-free after being treated with chemotherapy alone highlights the importance of identifying those who may not benefit from HER2 blockade, to whom alternatives to trastuzumab are needed.[4] Moreover, trastuzumab administration is associated with substantial financial costs; with a 1-year length; and with a risk (albeit modest) of clinically relevant toxicities such as cardiac events.[2,39–42] The identification of patients who do not benefit from trastuzumab would thus spare these individuals from unnecessary toxicities, offer them an opportunity to receive alternative treatments or to be enrolled on clinical trials, as well as to optimize financial resource application.[4] In our study, only patients whose tumors had a high *ADRB2* expression appeared to benefit from trastuzumab. If these findings are further validated, *ADRB2* expression has the potential to be explored as a predictive biomarker to help clinicians in identifying patients who need alternatives to trastuzumab treatment. Furthermore, by providing prognostic information, *ADRB2* expression may also identify a subset of patients with a favorable prognosis who may be candidates to de-escalation strategies.[43]

To further understand the mechanisms by which *ADRB2* exerts its effects in HER2-positive early breast cancer, in light of the preclinical data that shows a significant cross-talk between HER2 and ß2AR pathways, we investigated the association between *ADRB2* expression and a 8-gene trastuzumab-benefit signature that relies on the expression of genes related to the estrogen receptor and HER2 pathways.[17] In the studies that described this signature, patients with HER2-positive early breast cancer who did not benefit from trastuzumab presented high levels of estrogen receptor and intermediate/low levels of HER2 expression, being most frequently categorized as luminal A and B per PAM50 subtypes, whereas those who benefitted from trastuzumab presented more frequently high levels of HER2 expression.[44] *ADRB2* expression was not associated with the 8-gene signature profiles that predict trastuzumab benefit in the present study, suggesting that *ADRB2* effects may occur independently of its interaction with HER2 signalling. Notably, in previous studies with the 8-gene signature, patients who benefited from trastuzumab had higher TIL levels than those

who identified as having no trastuzumab benefit.[28,45] As in our study, in which *ADRB2* expression was associated with TIL levels, these results suggest that the 8-gene signature might also be a marker of T-cell recruitment and immune activation in these patients. Noteworthy, these findings have to be interpreted cautiously as TIL levels alone were not predictive of trastuzumab benefit in the NCCTG-N9831 population.[45]

A high expression of ß2AR in immunohistochemistry has been previously associated with a worse prognosis in patients with HER2-positive breast cancer, whereas our study assessing the same pathway - but at the gene expression level - shows different results.[46] Previous studies have demonstrated a very poor correlation between gene expression and protein synthesis.[47,48] The lack of immunohistochemistry for ß2AR is a limitation of the present study. Whether *ADRB2* expression correlates with ß2AR or if ß2AR expression in immunohistochemistry is prognostic/predictive in patients with HER2-positive breast cancer is a question that remains open and needs to be addressed in future studies. Hypothetically, *ADRB2* may exert its effects through intracellular signalling at the RNA level rather than at the protein level (ß2AR).

Potential limitations have to be considered when interpreting our findings. Not all patients included in the NCCTG-N9831 trial had evaluable samples for gene expression analyses and TIL assessment, although patients enrolled in this study were representative of the trial's population.[21] An interaction between *ADRB2* expression and treatment arm for the outcome DFS – which would strengthen our findings - could not be demonstrated. As this is to our knowledge the largest cohort of breast cancer patients in which *ADRB2* expression has been evaluated as a biomarker, we defined the ideal cut-point to categorize *ADRB2*-high versus low expression groups based on our own population, increasing the risk of "overfitting". However, the validation of the cut-point in a different cohort of patients with HER2-positive early-stage breast cancer yielded similar results, suggesting this cut-point may be applicable for future studies exploring the role of *ADRB2* expression in patients with breast cancer.[23]

After the publication of the NCCTG-N9831 trial, pertuzumab, neratinib and trastuzumab-emtansine (T-DM1) have been incorporated into the treatment of HER2-positive early-stage breast cancer.[41,42,49–51] It is unknown if the results of this study can be extrapolated to patients who received these contemporary therapies. Patients whose tumors were classified as *ADRB2*-high may represent those who exercise regularly, and thus physical exercise habits may have impacted their prognosis.[35] Data regarding patient exercise habits and concomitant medications such as beta-blockers/beta-agonists that could have influenced our results were not evaluable, precluding analyses adjusted for these factors. Gene expression analyses were performed in samples obtained before enrolment, therefore any potential effect of trastuzumab in *ADRB2* expression could not be evaluated dynamically. Although preclinical data supports a cross-talk between HER2 and ß2AR pathways, we did not observe an association between *ADRB2* expression and the 8-gene signature containing genes related to HER2 and estrogen receptor pathways, reinforcing that further studies are needed to understand the potential mechanism through which ß2AR exerts its effects in HER2-positive breast cancer. Although the correlation between *ADRB2* expression and TIL levels suggests that *ADRB2* may act as an immune-modulator, TIL levels alone were not prognostic in patients who received trastuzumab in the NCCTG-N9831 trial.[52]

Despite the above-mentioned limitations, having the analyses performed in a subset of patients enrolled in a randomized controlled trial strengthens our findings. Moreover, the fact that a single gene (*ADRB2*) was used instead of complex multigene signatures renders the study's methods more feasible to be reproduced in future studies.

Conclusion

This post-hoc analysis of the NCCTG-N9831 trial suggests that a high *ADRB2* expression is a favorable prognostic factor and may identify a subset of patients with HER2-positive early-stage breast cancer who benefit from adjuvant trastuzumab. The ß2AR may exert its effects on HER2-positive breast cancer by rendering the tumoral stroma more immunogenic and stimulating TIL infiltration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Competing interests

RC is currently employed by Novartis Pharma – Switzerland (but was not at the time this study was conceived, developed and finalized); received speaker honoraria from Boehringer Ingelheim, AstraZeneca and Janssen, and travel grants from AstraZeneca and Pfizer, outside of the submitted work. EP and AMA received research grants from Genentech and NIH (to the institution) for the NCCTG-N9831 study. AA has acted as advisor, received fees for lectures and research grants from Roche, Lilly, Amgen, ESAI, BMS, Pfizer, Novartis and MSD. MP is a board member of Radius; has received consultant honoraria from: AstraZeneca, Lilly, MSD, Novartis, Odonate, Pfizer, Roche-Genentech, Crescendo Biologics, Periphagen, Huya, Debiopharm, PharmaMar, G1 Therapeutics, Menarini, Seattle Genetics and Immunomedics. Institut Jules Bordet has received research grants from Roche/GNE, Radius, AstraZeneca, Lilly, MSD, GSJ/Novartis, Synthon, Servier, and Pfizer. SB received speaker honoraria from Targos, MSD and Natera, and research funds for his institution from Dako/Agilent and Roche/Ventana. EdA received honoraria from Roche-Genentech, research grant from Roche-Genentech (to the institution), and travel grants from Roche-Genentech and GlaxoSmithKline, outside the submitted work. FR, CD, EAT, YM and CDA declare no disclosures.

Glossary

ß2AR	Beta-2 adrenergic receptor
ADRB2	Beta-2 adrenergic receptor gene
CI	confidence interval
Cm	centimetres
DFS	disease-free survival
HER2	human epidermal growth factor receptor 2

HR	hazard ratio
NCCTG	North Central Cancer Treatment Group
REMARK	Reporting Recommendations for Tumor Marker Prognostic Studies
TILs	tumor infiltrating lymphocytes
T-DM1	trastuzumab-emtansine

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Highlights

- Beta-2 adrenergic receptor (ß2AR) mediates inflammation and T cell activation.
- Previous data suggest ß2AR gene (*ADRB2*) expression is prognostic in HER2+ breast cancer.
- We assessed if *ADRB2* expression impacted outcomes of patients enrolled in the NCCTG-N9831 trial.
- High *ADRB2* expression was associated with a favorable prognosis and predicted trastuzumab benefit.
- *ADRB2* may exert its effects via immune activation and lymphocyte recruitment.

Clinical practice points

Although the development of HER2 blockade has dramatically improved the outcomes of patients with HER2-positive breast cancer, additional prognostic and predictive biomarkers are needed to identify patients who do not benefit from HER2 blockade and to spare these individuals from unnecessary toxicities, offering them an opportunity to receive alternative treatments or to be enrolled on clinical trials, as well as to optimize financial resource application. Previous studies suggest that a high expression of the beta-2 adrenergic receptor gene (*ADRB2*) – involved in inflammation and lymphocyte activation - is associated with a favorable prognosis in patients with HER2-positive breast cancer. In this post-hoc analysis of the NCCTG-N9831 trial, a high *ADRB2* expression was associated with a longer disease-free survival and also identified a subset of patients who benefited from the addition of trastuzumab to adjuvant chemotherapy. The expression of *ADRB2* correlated with stromal tumor infiltrating lymphocyte (TIL) levels, suggesting that the beta-2 adrenergic receptor may interact with the tumoral immune microenvironment in HER2-positive breast cancer. *ADRB2* expression arises as a promising biomarker to be further explored in patients with HER2-positive breast cancer.

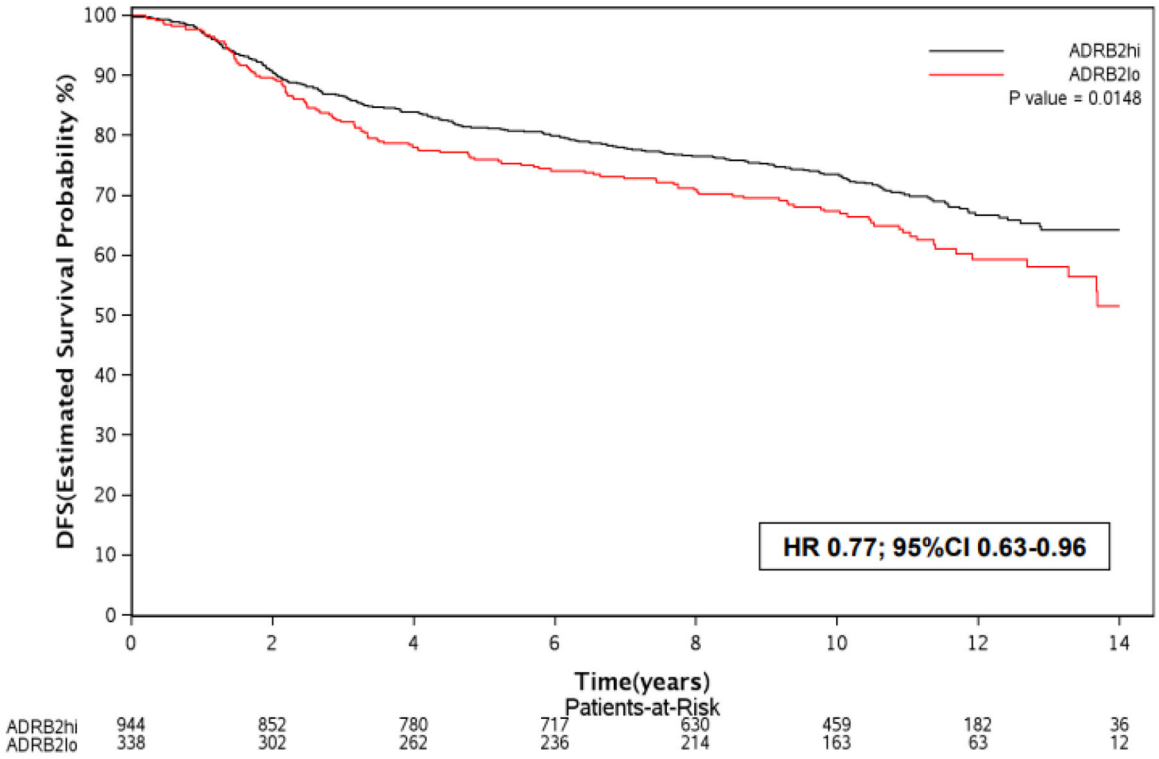
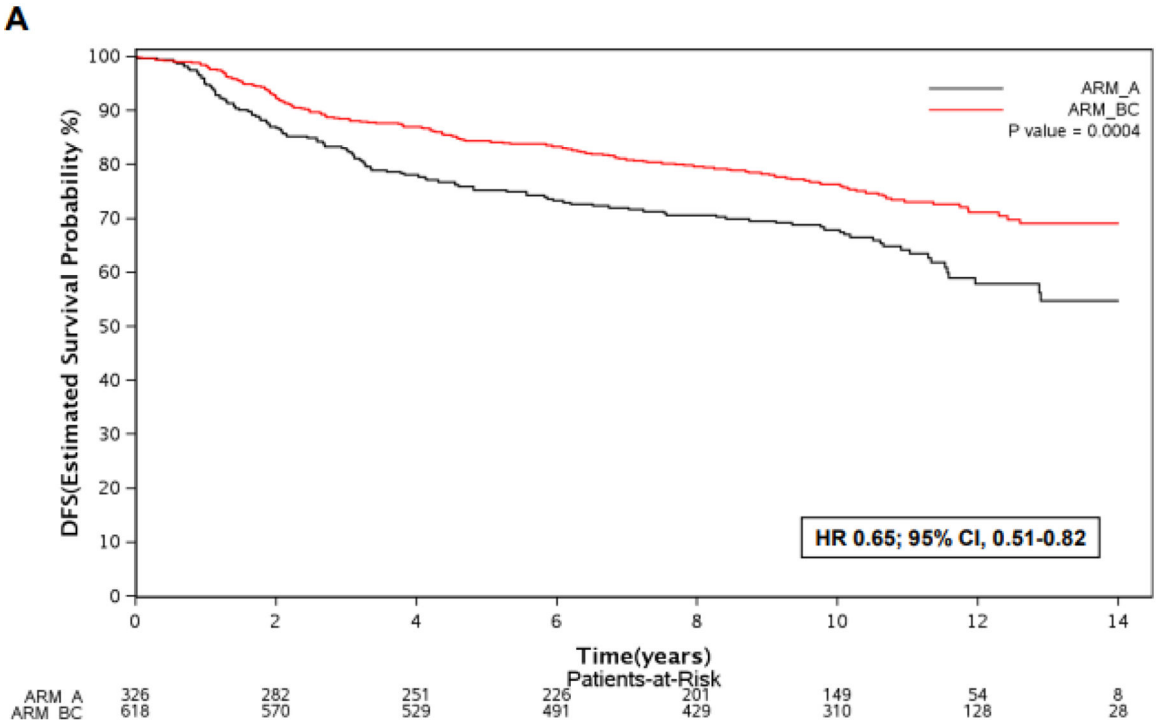


Figure 1 –.

Kaplan Meier curves for DFS according to *ADRB2* expression (high vs. low) in the overall population.

Abbreviations: *ADRB2,* beta-2 adrenergic receptor gene; DFS, disease-free survival; hi, high; lo, low.



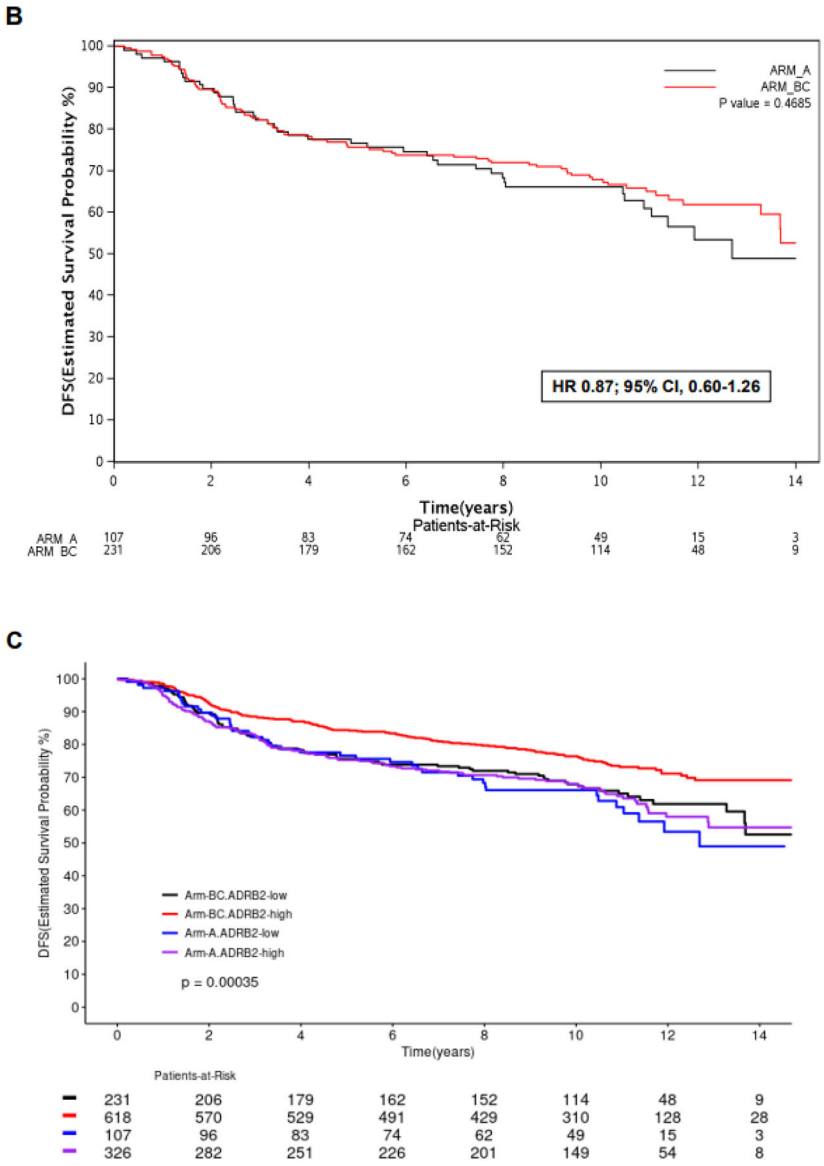


Figure 2 –.

Kaplan Meier curves for DFS according to treatment arm (B&C vs. A) in patients with *ADRB2*-high tumors (A), *ADRB2*-low tumors (B), and DFS according to ADRB2 expression and treatment arm (C).

Abbreviations: *ADRB2*, beta-2 adrenergic receptor gene; DFS, disease-free survival; hi, high; lo, low; HR, hazard ratio.

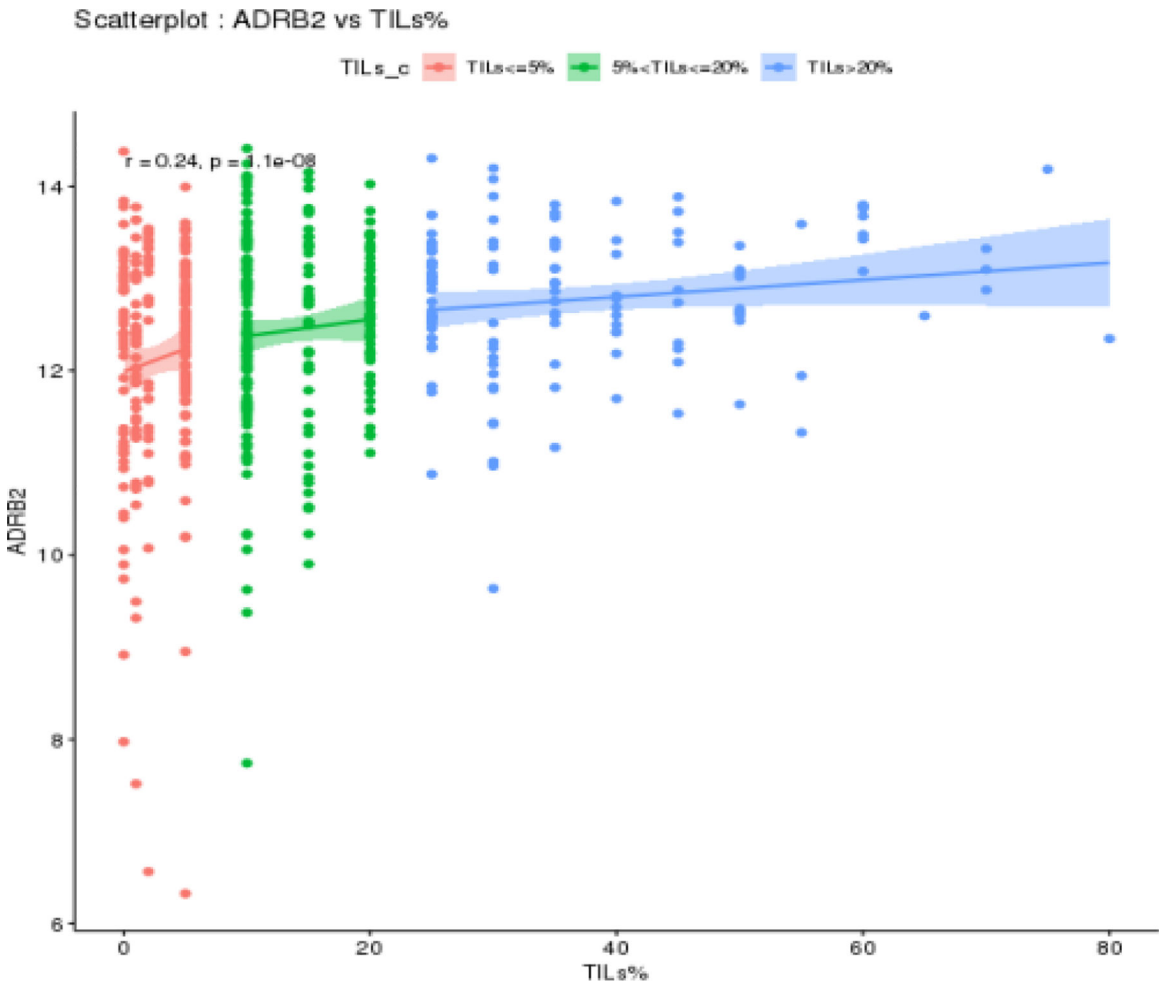


Figure 3 –.

Scatterplot illustrating the correlation between ADRB2 expression and TIL levels (>20%, 5–20% and <5%) in patients who had samples evaluable for gene expression analyses and TIL levels assessment (N=566).

Abbreviations: *ADRB2*, beta-2 adrenergic receptor gene; DFS, disease-free survival; TILs, tumor-infiltrating lymphocytes.

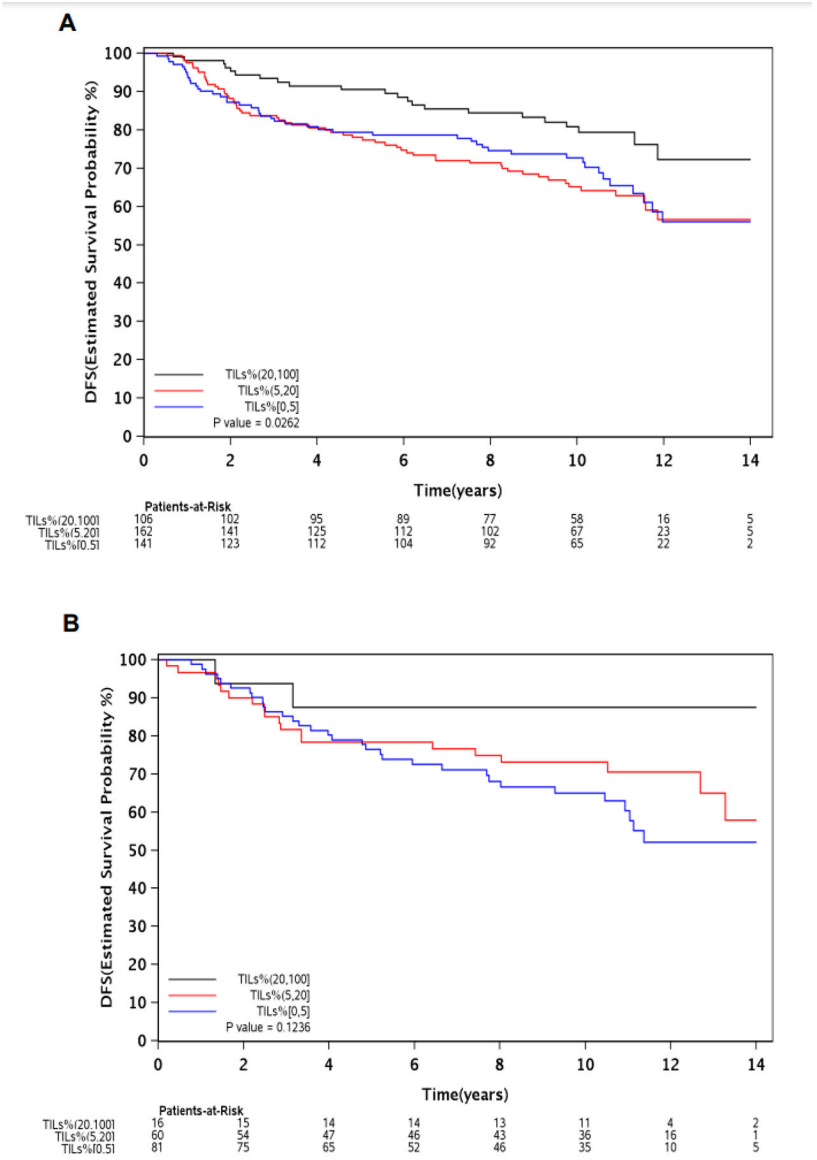


Figure 4 –.

DFS according to TIL levels (>20%, 5–20% and <5%) in patients with *ADRB2*-high (A), and *ADRB2*-low (B) tumors.

Abbreviations: *ADRB2*, beta-2 adrenergic receptor gene; DFS, disease-free survival; HR, hazard ratio; TILs, tumor-infiltrating lymphocytes.

Table 1 –

Characteristics of the patients included in this study.

Variable	*ADRB2*-high (*N*=944)	*ADRB2*-low (*N*=338)	Total (*N*=1,282)	*p* value
Age				0.6224
Median	49.5 (22–79)	50.0 (23–80)	50 (22 – 80)	
≤50 years	488 (51.7%)	180 (53.3%)	668 (52.1%)	
>50 years	456 (48.3%)	158 (46.7%)	614 (47.9%)	
Lymph Node Status				0.7235
Negative	133 (14.1%)	45 (13.3%)	178 (13.9%)	
Positive	811 (85.9%)	293 (86.7%)	1104 (86.1%)	
Tumor size				0.4006
≤2cm	853 (90.4%)	300 (88.8%)	1153 (89.9%)	
>2cm	91 (9.6%)	38 (11.2%)	129 (10.1%)	
Histologic grade				0.4675
Low/intermediate	259 (27.7%)	86 (25.7%)	345 (27.2%)	
High	675 (72.3%)	249 (74.3%)	924 (72.8%)	
Missing	10	3	13	
Hormone receptor status				0.3935
Negative	464 (49.2%)	157 (46.4%)	621 (48.4%)	
Positive	480 (50.8%)	181 (53.6%)	661 (51.6%)	
Menopausal status				0.283
Pre	479 (50.7%)	183 (54.1%)	662 (51.6%)	
Post	465 (49.3%)	155 (45.9%)	620 (48.4%)	
Arm				0.337
Arm A	326 (34.5%)	107 (31.7%)	433 (33.8%)	
Arms B&C	618 (65.5%)	231 (68.3%)	849 (66.2%)	

Abbreviations: *ADRB2*, beta-2 adrenergic receptor gene; cm, centimeters.

Table 2 –

Multivariable Cox regression model assessing the impact of clinicopathological variables in terms of DFS in the overall population.

Variable	Number of patients	DFS events	HR	95% CI lower	95% CI higher	*p* value
ADRB2 expression (high vs low)	1269	393	0.77	0.63	0.96	0.02
Age (>50 years vs ≤50 years)	1269	393	1.22	1.00	1.49	0.05
Lymph node (positive vs negative)	1269	393	1.56	1.11	2.19	0.01
Tumour size (>2cm vs ≤2cm)	1269	393	1.49	1.11	1.99	<0.01
Histologic grade (high vs not high)	1269	393	1.02	0.81	1.28	0.87
Hormone receptor (positive vs negative)	1269	393	0.77	0.63	0.95	0.01

Abbreviations: *ADRB2*, beta-2 adrenergic receptor gene; CI, confidence interval; cm, centimeters; HR, hazard ratio.