Association of Polymorphisms in FCGR2A and FCGR3A With Degree of Trastuzumab Benefit in the Adjuvant Treatment of ERBB2/HER2-Positive Breast Cancer
Analysis of the NSABP B-31 Trial

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IMPORTANCE: Preclinical models and studies in the metastatic and neoadjuvant settings suggest that single nucleotide polymorphisms in FCGR3A and FCGR2A may be associated with differential response to trastuzumab in the treatment of ERBB2/HER2-positive breast cancer, by modulating antibody-dependent cell-mediated cytotoxic effects.

OBJECTIVE: To evaluate the effect of FCGR2A and FCGR3A polymorphisms on trastuzumab efficacy in the adjuvant treatment of ERBB2/HER2-positive breast cancer.

DESIGN, SETTING, AND PARTICIPANTS: This is a retrospective analysis of patients enrolled in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 trial, a phase 3 cooperative group study conducted between 2000 and 2005. The NSABP B-31 trial randomized 2119 women with surgically resected node-positive, ERBB2/HER2-positive breast cancer to treatment with doxorubicin and cyclophosphamide followed by paclitaxel or the same regimen with the addition of 1 year of weekly trastuzumab. Patients were accrued at cooperative group sites across the United States and Canada. This analysis was performed between 2013 and 2016.

INTERVENTIONS: Doxorubicin and cyclophosphamide followed by paclitaxel or the same regimen with the addition of 1 year of weekly trastuzumab.

MAIN OUTCOMES AND MEASURES: Disease-free survival.

RESULTS: The genotyped cohort (N = 1251) resembled the entire B-31 cohort based on clinical variables and the degree of benefit from trastuzumab. Median follow-up time was 8.2 years in the genotyped samples. Disease-free survival probability at 3, 5, and 8 years was 74% (95% CI, 71%-79%), 66% (95% CI, 62%-71%), and 58% (95% CI, 54%-63%) in patients who received ACT and 86% (95% CI, 83%-89%), 82% (95% CI, 79%-85%), and 78% (95% CI, 74%-81%) in patients who received ACTH. Addition of trastuzumab significantly improved patient outcome (hazard ratio [HR], 0.46; 95% CI, 0.37-0.57; P < .001). The expected trend for interaction between polymorphisms and trastuzumab was observed for both genes, but only FCGR3A-158 polymorphism reached statistical significance for interaction (P < .001). As hypothesized, patients with genotypes FCB3A-158V/V or FCB3A-158V/F received greater benefit from trastuzumab (HR, 0.31; 95% CI, 0.22-0.43; P < .001) than patients who were homozygous for the low-affinity allele (HR, 0.71; 95% CI, 0.51-1.01; P = .05).

CONCLUSIONS AND RELEVANCE: The FCGR3A-158 polymorphism is predictive of trastuzumab efficacy in this cohort of patients with early ERBB2/HER2-positive breast cancer. Patients who are homozygous for phenylalanine at this position represent a considerable proportion of the population and, in contrast to previously reported analyses from similarly designed trials, our results indicate that trastuzumab may be less efficacious in these patients.

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Clinical trial National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31, analyzed jointly with the North Central Cancer Treatment Group (NCCTG) N9831, established the benefit of adding trastuzumab, a monoclonal antibody targeting the ERBB2/HER2 protein, to standard chemotherapy in patients with early stage ERBB2/HER2-positive breast cancer. However, only about one-third to one-half of patients benefit from this therapy in advanced or adjuvant settings, respectively.1,2 Although treatment is generally well tolerated, its high cost, associated cardiotoxic effects, and the availability of promising alternatives warrant an attempt to identify patients who will not benefit.

Although many mechanisms have been attributed to the success of trastuzumab, the original patent described 2 primary potential mechanisms. First, by binding to ERBB2/HER2 on the cancer cell membrane, it prevents ERBB2/HER2 dimerization, blocks downstream signaling, and subsequently blocks proliferation. Second, it triggers the host immune system to attack and kill trastuzumab-bound tumor cells. This immune response, known as antibody-dependent cell-mediated cytotoxic effect (ADCC), is initiated when the FCγ receptor on natural killer cells (NK) binds to the Fc portion of trastuzumab. As a result, NK cells release factors including IFN-γ, perforins, and granzymes, which cause tumor cell death via apoptosis. A similar process, known as antibody-dependent cellular phagocytosis (ADCP), is initiated when the FCγ receptor on a macrophage binds to trastuzumab and results in tumor cell death by phagocytosis.

Early studies in ERBB2/HER2-positive breast cancer cell lines demonstrated potent ADCD caused by trastuzumab in vitro.3 Experiments in mice have demonstrated reduced trastuzumab efficacy if the Fc fragment is removed from the antibody or if the mouse is deficient in FCγ receptors.4,5 In addition, an increase in the infiltration of lymphoid cells into tumor samples was observed after trastuzumab treatment compared with paired pretreatment samples.6 Collectively, these findings support a strong role of ADCC or ADCP in the mechanism of trastuzumab efficacy.

Additional preclinical studies have demonstrated an association of single-nucleotide polymorphisms in the genes encoding FCγ receptors with the strength of the immune response. Many studies have focused on position 158 of FCGR3A, which encodes a valine or phenylalanine, and position 131 of FCGR2A, which encodes a histidine or arginine (reviewed in Mello et al).7 In vitro ADCC assays demonstrated greater trastuzumab-mediated ADCD with the FCGR3A-158 valine (V)/V genotype and a trend for association with the FCGR2A-131 histidine (H)/H genotype.8 Analyses of paired pretreatment and posttreatment peripheral blood mononuclear cells (PBMCs) demonstrated more differential changes in gene expression from patients with the V/V or H/H genotypes than did patients with phenylalanine (F)/F or arginine (R)/R genotypes, indicating a difference in the molecular response to trastuzumab according to genotype.9 These observations are consistent with the observation that the FCγRIIA-151H and FCγRIIA-158V have a higher affinity to immunoglobulin (IgG) than the proteins encoded by alternate alleles.10 Cells bearing the high-affinity alleles mediate ADCD more effectively.7

Clinical studies using therapeutic monoclonal antibodies such as rituximab for the treatment of lymphoma and cetuximab for the treatment of colon cancer have shown association of the FCGR3A and FCGR2A genotypes with patient outcomes.11-15 However, clinical studies examining the association of these single-nucleotide polymorphisms with trastuzumab benefit for patients with breast cancer are not clear. Tamura and colleagues16 demonstrated improved response rates to trastuzumab in patients with a FCGR2A-131 H/H genotype in the neoadjuvant setting and improved objective response rate in the metastatic setting. Musolino and colleagues6 observed improved objective response rate and progression-free survival for patients with the FCGR3A-158 V/V genotype.8 However, while conducting our study, 2 large studies of patients enrolled in the NCCTG-N98317 and BCIRG-00618 clinical trials found no association of these loci with trastuzumab efficacy in the adjuvant treatment of ERBB2/HER2-positive breast cancer.

To explore this discrepancy, we retrospectively examined the FCGR3A-158 and FCGR2A-131 genotypes in all available pretreatment blood specimens from NSABP B-31. Our pre-specified primary objective was to determine whether patients with breast cancer with FCGR3A-158 V/V or FCGR3A 158 V/F received greater benefit from trastuzumab than patients with the FCGR3A F/F genotype.

Methods

Patient Cohort

detailed patient characteristics, eligibility criteria, adverse events, and clinical trial results have previously been reported.2,19,20 The NSABP B-31 trial randomly assigned women with surgically resected node-positive ERBB2/HER2-positive breast cancer to treatment with doxorubicin and cyclophosphamide followed by paclitaxel (ACT) or the same regimen with the addition of 1 year of weekly trastuzumab (ACTH). Patients were accrued between February 2000 and April 2005 at cooperative group sites across the United States and Canada. Treatment assignments were balanced according to nodal status, planned hormonal therapy, type of surgery, intended radiontherapy, and institution, with the use of a biased-coin minimization algorithm. Additional inclusion requirements for the
current study include availability of clinical follow-up, appropriate informed consent, and availability of pretreatment blood specimens. Informed consent forms were approved by a local human investigations committee in accordance with an assurance filed with and approved by the US Department of Health and Human Services to permit use of banked tissue and blood samples. These trials were approved by local human investigations committees or institutional review boards in accordance with assurances filed with and approved by the Department of Health and Human Services. Written informed consent was obtained from participants in each trial.

Genotyping
Whole blood was collected before treatment and stored at −80°C. Participant DNA was prepared from 100 μL of whole blood using the Mag-Bind Blood DNA HDQ kit (Omega Biotek) and quantified with picogreen (ThermoFisher). Genotyping of rs1801274 (FCGR2A-131 R/H) and rs396991 (FCGR3A-158 V/F) was performed using iPLEX Pro chemistry and mass spectrometry (Agena) according to the manufacturer’s instructions. Nested polymerase chain reaction was used for FCGR3A-158 to achieve specificity against its homologue FCGR3B. Genotypes were determined with Typer software using default settings after autoclustering (Agena). Two-thirds of the rs396991 assays were performed in duplicate. Bacterial artificial chromosome clones RP11-5K23 (FCGR3A), RP11-100D4 (FCGR3B), and an equimolar mixture of the 2 were used as controls to ensure the rs396991 primers specifically amplified the 3A homologue. Detailed reaction conditions and primer sequences are provided in eMethods in the Supplement. All assays were performed blinded to clinical outcome using deidentified specimens.

Statistical Methods
The use of patient blood specimens and a detailed statistical analysis plan were approved by the Cancer Therapy Evaluation Program (CTEP) of the National Cancer Institute (NCI) before starting the current investigation. The primary end point for survival analyses was disease-free survival. Disease-free survival events included local, regional, and distant recurrence; contralateral breast cancer; a second primary cancer; or death from any cause. Follow-up included events recorded before June 30, 2012. In 2005, during a planned joint analysis with NCCTG-N9831, the early-stopping boundary was reached and the early-stopping criterion was met. Follow-up was required at 3, 5, and 8 years after randomization. Of 883 assays performed in duplicate, 3 discordances were observed (0.3%) for FCGR3A-158 (rs396991). These 3 observations were excluded from further analysis. Approximately 2% of FCGR2A-131 and 7% of FCGR3A-158 assays failed default genotyping quality controls in the Typer software, yielding 21 and 87 missing values, respectively. Some of the FCGR3A reactions excluded by the software may actually represent heterozygous patients with 3 germline copies of the gene.21 However, these wells are indistinguishable from wells with contamination or poor reaction conditions and their expected frequency of 2.5% limits our power to detect meaningful clinical significance. For these reasons, any sample that failed quality control was eliminated. Thirteen samples failed both assays, suggesting poor-quality DNA. The patients genotyped showed 25% H/H, 49% H/R, and 26% R/R alleles for FCGR3A-158 (rs396991). These 3 observations were excluded from further analysis. Approximately 2% of FCGR2A-131 and 7% of FCGR3A-158 assays failed default genotyping quality controls in the Typer software, yielding 21 and 87 missing values, respectively. Some of the FCGR3A reactions excluded by the software may actually represent heterozygous patients with 3 germline copies of the gene.21 However, these wells are indistinguishable from wells with contamination or poor reaction conditions and their expected frequency of 2.5% limits our power to detect meaningful clinical significance. For these reasons, any sample that failed quality control was eliminated. Thirteen samples failed both assays, suggesting poor-quality DNA. The patients genotyped showed 25% H/H, 49% H/R, and 26% R/R alleles for FCGR2A-131 and 46% F/F, 42% F/V, and 12% V/V for FCGR3A-158. Both loci conformed to the Hardy-Weinberg Equilibrium in our data. In 1156 specimens with both assays successful, linkage disequilibrium indicated the FCGR3A and FCGR2A genes are strongly linked (D’ = 0.30; P < .001) as expected from their close genomic proximity and previous studies.18

FCGR Polymorphisms and Clinical Characteristics
FCGR3A-158 single-nucleotide polymorphisms were not associated with nodal status, ER, PR, tumor size, or race (eTable 3 in the Supplement). However, patients with the homozygous V/V genotype tended to be older (P < .001). The mean age
The box size is proportional to the precision. ACT, doxorubicin and cyclophosphamide followed by paclitaxel with the addition of 1 year of weekly trastuzumab; DFS, disease-free survival; HR, hazard ratio; M, multivariable; U, univariable.

was 52 years for V/V patients and 49 to 50 years for the other genotypes. FCGR2A-131 was not associated with nodal status, ER, PR, tumor size, or age. However, it was weakly associated with race (Table 4 in the Supplement).

**FCGR3A Polymorphism and Trastuzumab Efficacy**

Patients with genotypes that included the higher-affinity alleles FCGR3A-158 V/V or V/F received greater benefit from trastuzumab (HR, 0.31; 95% CI, 0.23-0.48; P < .001) than patients who were homozygous for the low-affinity allele (HR, 0.71; 95% CI, 0.51-1.01; P = .05) (Figure 1A and B). Genotype by treatment-interaction test indicates an association between FCGR3A-158 and benefit from trastuzumab (P < .001). In an exploratory analysis, the FCGR3A-158, high-affinity V/V homozygous patients received the most benefit (HR, 0.12; 95% CI, 0.05-0.28; P < .001), heterozygous V/F patients received intermediate benefit (HR, 0.34; 95% CI, 0.23-0.48; P < .001), and homozygous low-affinity F/F patients received the least benefit (HR, 0.71; 95% CI, 0.51-1.01; P = .05) (Figure 2). Similar results were observed using univariable models.
**Polymorphisms in FCGR3A and Degree of Trastuzumab Benefit**

**Discussion**

Analysis of FCGR3A-158 polymorphism in NSABP B-31 demonstrated differential trastuzumab benefit predicted by preclinical models, studies of other antibodies, and smaller studies of trastuzumab in the metastatic and neoadjuvant settings. Treatment interaction tests indicate polymorphisms at this position are associated with variability in the degree of benefit from adjuvant trastuzumab. Patients with the 158 F/F genotype have a better prognosis when treated with ACT and received less relative benefit (HR, 0.71) from the addition of trastuzumab, whereas patients with 158 F/V or V/V have a worse prognosis with ACT and receive significantly more relative benefit from trastuzumab (HR, 0.31). These results indicate that ADCC may play a substantial component in the efficacy of trastuzumab for the treatment of breast cancer in the adjuvant setting; ADCC also activates tumor-antigen-specific cellular immunity via intercellular crosstalk among NK and dendritic cells, which may also enhance the efficacy of anti-ERBB2/HER2 therapy.22,23

We also observed that the FCGR2A-131 polymorphism showed the expected trend for trastuzumab benefit but treatment interaction tests indicated no evidence of differential trastuzumab treatment effect according to FCGR2A genotypes. Perhaps this may be owing to the lack of expression of FCyRIIa on NK cells because NK cells are thought to be the main effectors of ADCC; although cytotoxic effects mediated through other effector cells via FCyRIIa have been reported.24 Our observation that the significant differential benefit from trastuzumab was limited to the FCGR3A-158 polymorphism and was not seen in the FCGR2A-131 single-nucleotide polymorphisms may also be owing to the fact that FCyRIIa-158V was found to bind to IgG1 immune complexes at low concentrations but not low FCyRIIa-158F. Conversely, no binding differences were detected between FCyRIIa-131H and I31R at low IgG1 concentrations.25

Our results differ from recent reports from the NCTCT-N983127 and BCIRG-006 trials,18 which found no association of FCGR3A-V158 and benefit from trastuzumab. These trials also examined the addition of trastuzumab to ACT in the adjuvant treatment of ERBB2/HER2-positive breast cancer and differed from NSABP B-31 primarily in the timing of the treatments. Both studies had sample sizes remarkably similar to our own and demonstrated substantial benefits from the addition of trastuzumab to chemotherapy. The methodologies used were rigorous: BCIRG-006 measured each single-nucleotide polymorphisms in quadruplicate (duplicate measurements on 2 platforms) and NCTCT-N9831 had 100% concordance in one-third of the population measured in duplicate. However, both of the previous FCGR studies suffered from sampling bias. In the BCIRG-006 study, the 1286 patients who were genotyped did not show significant benefit from trastuzumab (HR, 0.84;
We have shown that patients with breast cancer with 1 or 2 of the FCGR3A-158V alleles receive greater benefit from trastuzumab than patients who are homozygous for the FCGR3A-158F allele. Although multivariable analysis showed that patients with the F/F genotype had a slightly higher residual risk, this difference was not remarkable. Thus, genotyping does not seem to be sufficient for selection of patients for additional treatment.

Currently, many therapies, including new anti-HER2/ HER2 monoclonal antibodies or pan-tyrosine kinase inhibitors are being used in addition to trastuzumab to treat patients with breast cancer in the metastatic and neoadjuvant settings. These agents also engage the immune system and boost trastuzumab-induced ADCC.28-30 Recent investigation of additional immune checkpoint inhibitors to anti-HER2/ HER2 therapies in patients with ERBB2/HER2–positive metastatic breast cancer is based on the hypothesis that a substantial component of trastuzumab benefit is mediated through ADCC and cross-priming of antigen-specific cytotoxic T cells,22,23,31 which could enable the inhibition of immune checkpoints to improve the long-term efficacy of anti-ERBB2/ HER2 therapy. However, carefully designed clinical trials would be needed to determine if additional agents enhance trastuzumab activity in specific genotypes.

Studies on NK cell mediated therapies suggest that the Fc region on monoclonal antibodies such as trastuzumab could be re-engineered to augment the affinity for a variety of inhibitory and activating FCGR allelotypes.10,32,33 Single amino acid changes to the antibody at the binding site can strongly boost trastuzumab-induced ADCC.28-30 Recent investigation of additional immune checkpoint inhibitors to anti-HER2/ HER2 therapies in patients with ERBB2/HER2–positive metastatic breast cancer is based on the hypothesis that a substantial component of trastuzumab benefit is mediated through ADCC and cross-priming of antigen-specific cytotoxic T cells,22,23,31 which could enable the inhibition of immune checkpoints to improve the long-term efficacy of anti-ERBB2/ HER2 therapy. However, carefully designed clinical trials would be needed to determine if additional agents enhance trastuzumab activity in specific genotypes.

Conclusions

This study supports the hypothesis that ADCC activity plays an important role in the efficacy of trastuzumab. However, a great number of different molecules are involved in determining ADCC activity, therefore it may be useful to collect peripheral blood monocytes from patients in future clinical trials so that functional ADCC activity can be assessed and associated with treatment efficacy of trastuzumab and other therapeutic monoclonal antibodies.34
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