Analysis of Fcγ Receptor IIIa and IIa Polymorphisms: Lack of Correlation with Outcome in Trastuzumab-Treated Breast Cancer Patients

Sara A. Hurvitz¹, David J. Betting¹, Howard M. Stern², Emmanuel Quinaux³, Jeremy Stinson², Somasekar Seshagiri², Ying Zhao¹, Marc Buyse³, John Mackey⁴, Adrian Driga⁴, Sambasivarao Damaraju⁴, Mark X. Sliwkowski², Nicholas J. Robert⁵, Vicente Valero⁶, John Crown⁷, Carla Falkson⁸, Adam Brufsky⁹, Tadeusz Pienkowski¹⁰, Wolfgang Eiermann¹¹, Miguel Martin¹², Valerie Bee¹³, Omkar Marathe¹, Dennis J. Slamon¹, John M. Timmerman¹

¹University of California, Los Angeles, 10945 Le Conte Ave., PVUB #3360, Los Angeles, CA 90095, USA
²Genentech, 1 DNA Way, South San Francisco, CA 94080, USA
³International Drug Development Institute, 30 Avenue Provinciale, 1340 Louvain-la-Neuve, Belgium
⁴University of Alberta, Cross Cancer Institute, 11560 University Ave, Edmonton, Alberta, Canada T6G1Z2
⁵Virginia Cancer Specialists, US Oncology, 8503 Arlington Blvd., Suite 400, Fairfax, Virginia, 22031 USA
⁶University of Texas MD Anderson Cancer Ctr, 1515 Holcombe Blvd., #1354, Houston, TX 77030, USA
⁷St. Vincent’s Hospital, Elm Park, Dublin 4, Ireland
⁸University of Alabama at Birmingham, 1530 3rd Avenue S, NP 2540E, Birmingham, Alabama 35294 USA
⁹University of Pittsburgh Medical Center, 300 Halket Street, Suite 4628, Pittsburgh, PA 15213 USA
¹⁰Cancer Center, 5 Roentgen Street, Warsaw 02-781 Poland
¹¹Redcross Women’s Hospital, Taxisstr. 3, Muenchen 80637, Germany
¹²Hospital Gregorio Maranon, Maquez 7 Madrid 28007, Spain
¹³Breast Cancer International Research Group (BCIRG), 11, rue Watt, Paris 75013, France

Research Support: ASCO Young Investigator Award 2007; Genentech

Corresponding Author:
Sara A. Hurvitz, MD¹
Telephone: (310) 829-5471; Facsimile: (310) 829-6192
Email: SHurvitz@mednet.ucla.edu

Running head: FcγR Polymorphisms in Trastuzumab-Treated Breast Cancer


Keywords: Fc-gamma receptor polymorphisms, trastuzumab, breast cancer

Word Count: 3369; Figures: 3; Tables: 2
ABSTRACT

**Purpose**: The mechanisms by which trastuzumab imparts clinical benefit remain incompletely understood. Antibody-dependent cellular cytotoxicity via interactions with Fcγ receptors (FcγR) on leukocytes may contribute to its anti-tumor effects. Single nucleotide polymorphisms (SNPs) in FCGR3A and FCGR2A genes lead to amino acid substitutions at positions 158 and 131 respectively and affect binding of antibodies to FcγR such that 158V/V and 131H/H bind with highest affinity. This study aimed to determine whether high affinity SNPs are associated with disease free survival (DFS) among patients with HER2-positive non-metastatic breast cancer.

**Experimental Design**: Genomic DNA was isolated from 1,286 patients enrolled in a trial of adjuvant trastuzumab-based chemotherapy. Genotyping was performed using Sanger sequencing and Sequenom mass spectrometry.

**Results**: 1,189 patient samples were successfully genotyped for FCGR3A and 1,218 for FCGR2A. Compared to the overall results of the BCIRG006 study, in the subset of patients genotyped in this analysis, a less robust improvement in DFS was observed for the trastuzumab arms compared to control arm (HR=0.842, \( P=0.1925 \)). When stratified for prognostic features, the HR in favor of trastuzumab was consistent with that of the overall study (HR=0.74, \( P=0.036 \)). No correlation between DFS and FCGR3A/2A genotypes was seen for trastuzumab-treated patients (158V/V vs V/F vs F/F, \( P=0.98 \); 131H/H vs H/R vs R/R, \( P=0.76 \); 158V/V and/or 131H/H vs others, \( P=0.67 \)).

**Conclusion**: This analysis evaluating the association between FCGR3A/2A genotypes and trastuzumab efficacy in HER2-positive breast cancer did not demonstrate a correlation between FCGR3A-V/F and FCGR2A-H/R SNPs and DFS in patients treated with trastuzumab.
STATEMENT OF TRANSLATIONAL RELEVANCE:
One proposed but unproven mechanism of action (MoA) of the monoclonal antibody (mAb) trastuzumab is induction of antibody dependent cellular cytotoxicity, in which the Fc portion of trastuzumab engages with the Fc-gamma receptor (FcγR) on immune effector cells leading to tumor cell destruction. We sought to determine whether differences in FcγR affinity resulting from two single nucleotide polymorphisms in FcγRIIIa and FcγRIIa impact the clinical outcome of trastuzumab-treated patients. An association between FcγR genotype and outcome was reported with other mAb’s for lymphoma and colon cancer. Such an association would provide evidence that the immune system plays a role in the MoA of trastuzumab. These results also impact the future practice of cancer medicine because a positive association would support the development of engineered antibodies with an increased affinity for FcγR and would support the use of genotyping to preselect patients most likely to respond to trastuzumab.
INTRODUCTION:
Amplification of the HER2 gene is a key driver in the pathogenesis and biological aggressiveness of approximately 25% of breast cancer.\(^1\) Trastuzumab, a humanized anti-HER2 monoclonal IgG1 antibody is known to significantly improve clinical outcome for both early and advanced HER2-positive breast cancer.\(^2\)-\(^4\) Although the mechanisms of action of trastuzumab are not completely understood,\(^5\) preclinical models suggest that growth factor receptor blockade results in critical changes in growth signaling pathways including downregulation of PI3K-AKT signaling leading to decreased cell proliferation and cycle arrest.\(^6\) Other mechanisms suggested from preclinical studies also include inhibition of extracellular domain shedding, decreased angiogenesis, and inhibition of DNA repair.\(^7\),\(^8\)

Therapeutic antibodies of the IgG1 subtype can also mediate antibody dependent cell mediated cytotoxicity (ADCC). This potential mechanism involves antibody binding to HER2 on the surface of tumor cells, followed by the Fragment C (Fc) portion of the antibody engaging Fc-gamma receptors (Fc\(\gamma\)R) expressed on immune effector cells, ultimately resulting in target cell lysis. Preclinical evidence for this mechanism in trastuzumab efficacy was demonstrated in immunodeficient mice bearing human breast cancer xenografts.\(^9\) Furthermore, afucosylated trastuzumab with enhanced affinity to Fc\(\gamma\)R exhibits greater anti-tumor activity in xenograft models than native trastuzumab.\(^10\)

Three classes of Fc\(\gamma\)R [Fc\(\gamma\)RI (CD64), Fc\(\gamma\)RII (CD32), and Fc\(\gamma\)RIII (CD16a)] and their subclasses have been described. Some Fc\(\gamma\)R display allelic polymorphisms that confer differing functional properties.\(^11\) One such polymorphism in the gene encoding Fc\(\gamma\)RIIIa is a single nucleotide substitution at position 559\(^12\) (A559C, rs396991) that leads to the substitution of phenylalanine (F) by valine (V) at amino acid position 158 in the IgG binding domain.\(^13\),\(^14\) IgG1 and IgG3 bind more tightly to Fc\(\gamma\)RIIIa 158 V/V compared to 158 F/F, increasing effector cell
activity in individuals who are homozygous for FcγRIIIa 158 V. A polymorphism in the gene encoding FcγRIIa (A519G, rs1801274) places either histidine (H) or arginine (R) at position 131. IgG1 binds more strongly to cells that are homozygous for FcγRIIa 131 H.

Clinical evidence supporting an association between FCGR3A/2A genotypes and outcomes in patients treated with monoclonal antibody therapy was first reported for rituximab in the treatment of lymphoma. Subsequently, studies evaluating the monoclonal antibody, cetuximab for colon cancer showed an association between FCGR3A/2A genotypes and outcome. However, definitive clinical evidence for the role of Fc-FcγR interactions in breast cancer is lacking. Three small trials, each with fewer than 65 patients, evaluated the association between FCGR3A/2A genotypes and outcome after treatment with trastuzumab-based therapy. Two studies reported an association between at least one FcγR polymorphism and clinical outcome. The other study revealed no such association.

The aim of this study was to further clarify whether FCGR3A and FCGR2A genotypes are correlated with clinical outcome in trastuzumab-treated patients. Such an association would substantiate a role for FcγR-bearing immune effector cells in the anti-tumor activity of trastuzumab.

PATIENTS & METHODS

FcγR polymorphism genotyping

DNA was purified from serum and whole blood samples using a QIAamp DNA Blood Mini Kit (QIAGEN, CA), and used for nested PCR amplification of regions containing the FCGR3A 158 V/F and FCGR2A 131 H/R SNPs using primers listed in Supplemental Table 1. PCR was performed using Phusion® Hot Start High-Fidelity DNA Polymerase (New England Biolabs, MA).
and manufacturer recommended protocols. The PCR products were purified using a QIAGEN PCR clean kit (QIAGEN, CA), then sequenced on an ABI3730XL (Applied Biosystems, CA) using BigDye® Terminator v3.1 chemistry. PCR products were also analyzed on a MassARRAY Analyzer (Sequenom, CA) using Sequenom’s iPLEX Gold assay. For FCGR3A, rs396991 primers were used to identify the A559C polymorphism. For FCGR2A, rs1801274 primers were used to identify the A519G polymorphism. Each sample underwent a total of four independent rounds of analyses (two Sanger and two Sequenom). The genotype was included for further analysis if there were four concordant results for a given sample. For samples where there were three concordant results and a fourth data point had failed for technical reasons, the genotype was called and included further in data analysis.

**Patient Population**

**Adjuvant Breast Cancer Cohort (BCIRG-006)**

Genomic DNA from serum and whole blood samples was obtained from patients treated in the Breast Cancer International Research Group (BCIRG)-006 study. This adjuvant study compared two trastuzumab-containing arms to a non-trastuzumab containing control arm for treatment of HER2-positive, early breast cancer. In total, 3,222 patients were randomly assigned to one of three treatment arms: (1) AC-T: four cycles of q3 weekly doxorubicin (A, 60 mg/m² IV) plus cyclophosphamide (C, 600 mg/m² IV) followed by four cycles of q3 weekly docetaxel (T, 75 mg/m² IV), (2) AC-TH: AC-T plus trastuzumab (H, 8 mg/kg IV loading dose with first dose of docetaxel followed by 6 mg/kg q 3 weeks for 1 year) or (3) TCH: six cycles of q3 weekly docetaxel, carboplatin (C, AUC 6), trastuzumab (as above, for 1 year). Of these 3,222 patients, 1,286 signed an optional consent upon enrollment to have blood/serum samples sent to our central laboratory for exploratory analyses. A total of 1,189 patient samples (37%) were successfully genotyped for FCGR3A and 1,218 samples (38%) genotyped for FCGR2A. Genotyping failed in 97 samples (7.5%) for FCGR3A and in 68 samples (5.3%) for
FCGR2A. Approximately 860 samples sequenced were from whole blood, and the success rate was over 99% for both polymorphisms from these specimens. The remainder of patients (over 400) only had serum provided. The concentration of DNA is lower in serum compared with whole blood, thus making it technically more challenging to extract an adequate amount of DNA for reliable sequencing from serum. The vast majority of sequencing failures were from serum samples. That said, the fail rate in serum for FCGR3A was higher than that for FCGR2A so there may be a contributing factor that depends on the primers. Due to high homology with FCGR3B, there are unfortunately very limited options for designing primers specific for FCGR3A. The proportion of patients who were genotyped for FCGR3A/2A was well-balanced between the treatment arms (Figure 1).

Advanced Disease Breast Cancer Cohort

Blood samples from 177 participants in the PolyomX and Canadian Breast Cancer Foundation (CBCF—Edmonton, Alberta) tumor banks were collected from 2001 to 2007. All participants had HER2-positive breast cancer and had received at least one course of trastuzumab. A total of 53 participants had unresectable, local/regional recurrence (N=12) or distant metastases (N=41) and had successful determination of at least one FcγR allele. The FCGR3A 158 V/F genotype was successfully determined in 52 participants (29%) and FCGR2A 131 H/R in 53 participants (30%).

Both the early and advanced disease cohort studies were conducted according to institutional review board/ethics committee-approved protocols. Informed consent was obtained from all participating patients. REMARK guidelines were followed in the reporting of these results.
**Statistical Methods and Association Testing**

For the adjuvant cohort, DFS was calculated from the date of randomization to the date of disease recurrence as declared by the treating physician, or death from any cause. This retrospective data analysis was based on the third planned analysis of the BCIRG-006 study. For the advanced disease cohort, PFS was calculated from start of first exposure to trastuzumab (in the metastatic or locally recurrent setting) to the time of disease progression or death from any cause. DFS and PFS curves were estimated using the method of Kaplan-Meier. The effect of trastuzumab and the prognostic impact of genotype were assessed using the log-rank test. The predictive impact of genotype on the effect of trastuzumab was assessed through interaction tests in Cox regression models.

SNPStats software ([http://bioinfo.iconcologia.net/SNPstats](http://bioinfo.iconcologia.net/SNPstats)) was used for determining allele frequencies and Hardy-Weinberg equilibrium (HWE) and the Haploview program ([http://www.broadinstitute.org](http://www.broadinstitute.org)) for pair-wise LD (measured as D’S) patterns between markers. A sample size of N=1133 was used for which we have complete genotype data to determine LD between FCGR2A and FCGR3A gene polymorphisms. Fisher’s exact test was used to assess deviations from HWE, with \( P<0.05 \) suggesting significant deviation from HWE.

**RESULTS**

**Patient Characteristics**

**Adjuvant Breast Cancer Cohort**

The prognostic clinical and pathological features of patients according to treatment arm are shown in Table 1. At the third planned analysis of BCIRG-006 (N=3,222), DFS was significantly improved for patients who received trastuzumab-based therapy compared to control arm therapy (AC-TH vs AC-T: hazard ratio (HR) =0.64, (95% CI 0.53 – 0.78) \( P<0.001 \); TCH vs AC-T: \( \ldots \))
HR=0.75 (95% CI 0.63 – 0.90), \( P=0.002 \) (Supplemental Figure 1) indicating that trastuzumab-based therapy significantly extends DFS compared with chemotherapy alone.\(^{23}\) The clinical and tumor characteristics of the patients genotyped in our study compared to the patients who were not genotyped are shown in Supplemental Table 2. In the subset of patients genotyped in our study (N=1,286), a less robust improvement in DFS was observed for patients treated with trastuzumab compared to control arm therapy (combined trastuzumab-arms vs AC-T HR=0.842, \( P=0.1925 \) (Supplemental Figure 2). Stratified analysis demonstrated that this may be due to genotyped patients in the trastuzumab arms numerically having worse prognostic features than patients in the AC-T arm (Table 1). When stratified for age, node status, hormone receptor status, size and surgery type, the hazard ratio in favor of trastuzumab was consistent with that of the overall patient population and statistically significant (HR=0.74, \( P=0.036 \) (Supplemental Figure 3). Baseline patient characteristics by genotype for this adjuvant cohort are shown in Table 2.

Advanced Disease Breast Cancer Cohort

Patient characteristics by genotype in this 53-patient cohort are shown in Supplemental Table 3. HER2-overexpression/amplification was verified in 50 and was unknown in three participants. Tumors were positive for one or both hormone receptors in 70% (N=37) of patients, negative for both in 23 % (N=12) and unknown in 8% (N=4). A total of 42% of patients were postmenopausal. Tumor grade was grade 1 in 2%, grade 2 in 30%, grade 3 in 53% and unknown in 15% of patients. Visceral metastases were present in 66% of participants. Of the 53 patients, 43 had not received prior chemotherapy and ten had received one to four previous chemotherapy regimens. In terms of specific trastuzumab-based regimens received by patients, 18 (34%) received trastuzumab alone, 28 (53%) received single-agent chemotherapy plus trastuzumab and seven (13%) received doublet (taxane-platinum) chemotherapy plus trastuzumab.
**Genotype and Allele Frequencies**

*Adjuvant Breast Cancer Cohort*

The frequency of FCGR3A/2A genotypes did not differ significantly among treatment arms (Table 2). We observed a minor allele frequency of 0.34 and 0.48 for FCGR3A and FCGR2A, respectively. The frequencies of FCGR3A genotypes deviated from HWE whereas the genotype distributions for FCGR2A were in conformity with the HWE assumptions (Supplemental Table 4). The influence of genotyping errors on the observed deviations from HWE for FCGR3A were ruled out or minimized since the genotyping data from two independent technology platforms (see methods) were concordant. We do not have genotype data from apparently healthy control subjects to assess conformity with HWE assumptions in a case-control setting to suggest putative association of this locus with breast cancer risk or the associated phenotypes, thus limiting the interpretability of our findings. We nonetheless included this allele for further analysis to permit comparisons with the previously reported, smaller studies.19, 20 The LD (D’=0.32) we observed between FCGR2A and FCGR3A were entirely concordant with those previously reported in the literature.27

**FcγR Polymorphisms and Outcome**

*Adjuvant Breast Cancer Cohort*

Baseline patient and tumor characteristics did not differ significantly between the FCGR3A V/V, V/F or F/F polymorphism groups, nor between FCGR2A H/H, H/R or R/R groups (Table 2). In the population of patients genotyped who were in the non-trastuzumab containing control arm (AC-T), there was no statistically significant difference in DFS based on FCGR3A/2A genotypes (FCGR3A V/V vs V/F vs F/F, logrank test P=0.33, and FCGR3A H/H vs H/R vs R/R, logrank test P=0.81). Among those who received trastuzumab (TCH and AC-TH arms combined), there was no statistically significant difference in DFS by FCGR3A genotype (P=0.98) (Figure 2A) or FCGR2A genotype (P= 0.76) (Figure 2B). When cases having the ‘favorable’ FCGR3A V/V
and/or FCGR2A H/H genotypes were compared to others, there was also no statistically significant difference in DFS ($P=0.67$) (Figure 2C). When the trastuzumab-containing treatment arms were analyzed separately, again there was no difference in DFS by the FCGR3A (TCH: $P=0.96$, AC-TH: $P=0.94$), FCGR2A (TCH: $P=0.98$, AC-TH: $P=0.47$) or by combined FCGR3A V/V and/or FCGR2A H/H genotypes (TCH V/V and/or H/H vs TCH others vs. AC-TH V/V and/or H/H vs AC-TH others: logrank $P=0.97$) (Supplemental Figure 4). To evaluate whether genotype is prognostic independently of trastuzumab, the non-trastuzumab containing AC-T arm was analyzed separately. There was no difference in DFS by the FCGR3A ($P=0.33$), FCGR2A ($P=0.81$) or by combined FCGR3A V/V and/or FCGR2A H/H genotypes ($P=0.39$) (Supplemental Figure 5). There was also no difference in overall survival when comparing FCGR3A/2A genotypes (Supplemental Figure 6). Finally, we compared the trastuzumab-containing treatment arms with AC-T in all of the FCGR3A and FCGR2A genotypes. In spite of an apparent trend towards a larger effect of trastuzumab in the FCGR3A V/V and/or FCGR2A H/H genotypes (Figure 3), the difference did not reach significance in any genotype, and the tests for interaction between trastuzumab and genotype were all non-significant.

**Advanced Disease Breast Cancer Cohort**

In the 53-patient advanced disease cohort, baseline prognostic features including age, tumor grade and disease free interval did not differ significantly between the three FCGR3A genotypes or the three FCGR2A genotypes (Supplemental Table 3). Menopausal status, hormone receptor status, and presence of visceral metastases differed significantly between genotypes for one or both SNPs. Menopausal status was statistically different between genotypes for both SNPs ($\chi^2 P=0.0448$ for FCGR3A and $P=0.0287$ for FCGR2A). Hormone receptor status was statistically different between genotypes with FCGR3A (V/V more frequently estrogen receptor (ER) and/or progesterone receptor (PR) positive than other genotypes, $\chi^2 P=0.0488$). Presence of visceral metastases differed significantly between genotypes for FCGR2A (visceral...
metastases less frequent in H/R genotype than others, $\chi^2 P=0.013$). For convenience, we have reported all $P$-values unadjusted for multiplicity, which is standard practice in retrospective analyses, when the number of comparisons is not pre-specified. In actual fact, since many comparisons were performed, $P$-values much less extreme than $P<0.05$ are to be considered statistically significant. As a rough guidance, the Bonferroni correction can be used in the interpretation: if $M$ comparisons are performed, the level of significance that applies to each comparison is equal to 0.05 divided by $M$. Nonetheless, no statistically significant difference in PFS was detected by $FCGR3A$ genotype ($FCGR3A$ V/V vs V/F vs F/F, logrank test $P=0.88$, Supplemental Figure 7A) and by $FCGR2A$ genotypes ($FCGR2A$ H/H vs H/R vs R/R, logrank test $P=0.52$, Supplemental Figure 7B).

DISCUSSION

In addition to perturbation of HER2 signaling, trastuzumab-mediated FcγR engagement by immune effector cells could represent a potential mechanism of action for the antibody in HER2-positive breast cancer. The present study was conducted to determine whether differences in FcγR affinity resulting from SNPs in $FCGR3A$ and $FCGR2A$ had any impact on the outcome of patients treated with trastuzumab-based therapy.

To date, there has been no prospective evaluation of $FCGR3A/2A$ genotypes as determinants of trastuzumab outcome. Three previous retrospective studies investigating the correlation of $FCGR3A/2A$ genotypes with clinical outcome to trastuzumab-based therapy yielded discordant results.$^{19,20,21}$ Each of these studies was limited by a small sample size. Moreover, in contrast to the large adjuvant cohort in the current study, the majority of patients (91%) evaluated in the three published studies had metastatic breast cancer. The first report was a retrospective analysis of a subset of patients enrolled in the pivotal trial of trastuzumab. No difference in the
distribution of the FCGR3A 158V/F genotype was detected among 63 patients who achieved an objective response and those that had progressive disease. Conversely, a subsequent study by Musolino and colleagues reported improved response rates and PFS for those patients with FCGR3A V/V and, to a lesser extent, FCGR2A H/H genotypes among 54 patients with HER2-positive metastatic breast cancer who received trastuzumab and taxane. Tamura and colleagues evaluated whether FCGR3A/2A genotypes are associated with pathological complete response (pCR) or objective response (OR) in patients treated with chemotherapy plus trastuzumab in the neoadjuvant setting (N=15) and whether the genotypes are associated with PFS in patients with metastatic breast cancer (N=35) who received single agent trastuzumab. They also showed a correlation with clinical outcome. Specifically, they found that FCGR2A-H/H genotype was significantly associated with pCR (P=0.015) and OR (P=0.043) in the neoadjuvant setting. They also found a correlation with PFS (P=0.034) in the metastatic setting, however, FCGR3A genotype was not significantly associated with clinical outcome in that study.

The current study involves the largest retrospective analysis to date evaluating an association between FCGR3A/2A genotypes and clinical outcome in trastuzumab-treated HER2-positive breast cancer in the adjuvant setting. No statistically significant correlation between FCGR3A and FCGR2A genotypes and DFS was detected in a cohort of 1,286 patients treated with trastuzumab-based therapy in early breast cancer. Moreover, to expand this study to advanced disease, the retrospective analysis of a cohort of 53 women treated with trastuzumab-based therapy for metastatic breast cancer was performed and also revealed no significant correlation between FCGR3A and FCGR2A genotypes and PFS. While these data do not completely rule out the possibility that trastuzumab acts in part via ADCC, it does suggest that any differences in Fc-FcγR affinity attributed to the SNP’s tested does not result in detectable differences in clinical outcome. We acknowledge that these data are limited by the fact that only 38% of the
patients enrolled in the BCIRG-006 study were genotyped. Thus it is not possible to generalize conclusions originating from the genotyped subset to the entire BCIRG-006.

The trastuzumab benefit in this study appeared less robust in the adjuvant cohort compared to the benefit seen in the overall BCIRG-006 study population, most likely due to the fact that random sampling of study patients for genotyping could not be performed. This was because only those patients who provided informed consent and had separate blood/serum samples sent into the centralized laboratory for biomarker testing were evaluated. As a result, the sample in which FCGR3A/2A genotyping was performed was not representative of the entire patient population. In fact, in this sample, the lower benefit of trastuzumab may have been due to the imbalance in poorer than average prognostic features of trastuzumab-treated patients consenting to provide samples in this sub-study. However, stratified analyses adjusting for known prognostic factors support a statistically significant trastuzumab benefit of the same magnitude in the current cohort as in the overall population. That said, we cannot completely exclude a contribution of FCGR polymorphisms to the trastuzumab-treated patient outcomes.

Our results differ from those of Musolino et al,19 and Tamura et al20 and these discrepancies may reflect differences in intrinsic population factors or chemotherapy regimens. For instance, while the study used by Musolino et al administered only paclitaxel or docetaxel in combination with trastuzumab, patients in our adjuvant cohort received either doxorubicin, cyclophosphamide, and docetaxel, or carboplatin and docetaxel in combination with trastuzumab. In lymphoma patients treated with rituximab, the strong influence of FCGR3A/2A genotypes on outcomes appears to be diminished with concurrent chemotherapy.28 On the other hand, the majority (87%) of breast cancer patients in our advanced disease breast cancer cohort received either trastuzumab monotherapy or single-agent chemotherapy plus trastuzumab. Despite this, PFS still did not correlate with FCGR3A/2A genotypes.
The two receptor polymorphisms considered in this study did not show a significant association with treatment outcomes. The minor allele frequencies and LD between these markers are consistent with previous findings.\textsuperscript{19, 22}

This is the largest analysis evaluating the association between FCGR3A/2A genotypes and clinical outcome in trastuzumab-treated HER2-positive breast cancer. In contrast to previously published smaller studies, we found no statistically significant correlation between FCGR3A/2A genotypes and DFS in the adjuvant setting or PFS in the metastatic setting for patients with HER2-positive breast cancer treated with trastuzumab. These data do not support the hypothesis that naturally-occurring polymorphism-related differences in Fc\textsubscript{\gamma}R affinity result in differential outcome to trastuzumab therapy. However, the data require confirmation in a prospective study and at present time do not completely preclude a potential role for ADCC/Fc\textsubscript{\gamma}R engagement in the treatment of breast cancer with anti-HER2 antibodies. The Fc-engineering of antibodies with much higher affinity Fc-Fc\textsubscript{\gamma}R interactions than those observed with naturally-occurring polymorphisms may provide future opportunities to fully engage this mechanism of action against breast cancer.

**Grant Support**

Funding for this project came from the ASCO Young Investigator Award in 2007 and from Genentech.
References
Legend for Figures and Tables

Figure 1: Consort Diagram for Adjuvant Cohort

Figure 2. Kaplan Meier estimates of disease free survival (DFS) in trastuzumab treated patients within the adjuvant cohort according to FcγR polymorphisms. (A) FCGR3A 158 V/F genotype, \( P=0.98 \) (V/V vs V/F vs F/F) (B) FCGR2A 131 H/R genotype, \( P=0.76 \) (H/H vs H/R vs R/R). (C) FcγRIIIa and FcγRIIa polymorphisms. Others represents patients with neither FCGR3A 158 V/V nor FCGR2A 131 H/H genotype, \( P=0.67 \) (H/H and/or V/V vs others).

Figure 3. Kaplan-Meier estimates of disease free survival in trastuzumab (AC-TH/TCH combined) versus no trastuzumab (AC-T) treated patients, by FcγR genotype. (A) V/V (\( P=0.13 \)) (B) V/F (\( P=0.422 \)) (C) F/F (\( P=0.81 \)) (D) H/H (\( P=0.35 \)) (E) H/R (\( P=0.985 \)) (F) R/R (\( P=0.58 \))

Table 1. Patient Characteristics by Treatment Arm (Adjuvant Cohort)

Table 2. Patients Characteristics by Genotype (Adjuvant Cohort)
Figure 1: REMARK24 Diagram for Adjuvant Cohort
### Table 1. Patient Characteristics by Treatment Arm (Adjuvant Cohort)

<table>
<thead>
<tr>
<th></th>
<th>AC-TH (%)</th>
<th>TCH (%)</th>
<th>AC-TH &amp; TCH (Combined) (%)</th>
<th>AC-T (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Positive Lymph Nodes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>N=406</td>
<td>N=402</td>
<td>N=808 (Combined)</td>
<td>N=381</td>
</tr>
<tr>
<td>1-3</td>
<td>112 (28)</td>
<td>133 (33)</td>
<td>245 (30)</td>
<td>120 (31)</td>
</tr>
<tr>
<td>≥ 4</td>
<td>157 (39)</td>
<td>163 (41)</td>
<td>320 (40)</td>
<td>153 (40)</td>
</tr>
<tr>
<td><strong>Hormone Receptor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER/PR Negative</td>
<td>222 (55)</td>
<td>224 (56)</td>
<td>446 (55)</td>
<td>194 (51)</td>
</tr>
<tr>
<td>ER &amp;/or PR Positive</td>
<td>184 (45)</td>
<td>178 (44)</td>
<td>362 (45)</td>
<td>187 (49)</td>
</tr>
<tr>
<td><strong>Tumor Size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 2 cm</td>
<td>248 (61)</td>
<td>240 (60)</td>
<td>488 (60)</td>
<td>210 (55)</td>
</tr>
<tr>
<td>≤ 2 cm</td>
<td>158 (39)</td>
<td>162 (40)</td>
<td>320 (40)</td>
<td>171 (45)</td>
</tr>
<tr>
<td><strong>Menopause Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopause</td>
<td>177 (44)</td>
<td>168 (42)</td>
<td>345 (43)</td>
<td>170 (45)</td>
</tr>
<tr>
<td>Postmenopause</td>
<td>229 (56)</td>
<td>234 (58)</td>
<td>463 (57)</td>
<td>211 (55)</td>
</tr>
<tr>
<td><strong>Age (Years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>49</td>
<td>48</td>
<td>48</td>
<td>49</td>
</tr>
<tr>
<td>Range</td>
<td>24-74</td>
<td>23-73</td>
<td>23-74</td>
<td>26-74</td>
</tr>
<tr>
<td><strong>Tumor Grade</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>3 (0.7)</td>
<td>6 (1.5)</td>
<td>9 (1.1)</td>
<td>6 (1.6)</td>
</tr>
<tr>
<td>G2</td>
<td>102 (25)</td>
<td>113 (28)</td>
<td>215 (27)</td>
<td>83 (22)</td>
</tr>
<tr>
<td>G3</td>
<td>293 (72)</td>
<td>274 (68)</td>
<td>567 (70)</td>
<td>282 (74)</td>
</tr>
<tr>
<td>G4</td>
<td>1 (0.2)</td>
<td>2 (0.5)</td>
<td>3 (0.4)</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td>GX</td>
<td>7 (1.7)</td>
<td>7 (1.7)</td>
<td>14 (1.7)</td>
<td>7 (1.8)</td>
</tr>
</tbody>
</table>

Abbreviations: ER Estrogen Receptor; PR Progesterone Receptor; A Adriamycin; C Cytoxan; T Taxotere; H Herceptin; GX: Grade unknown
### Table 2. Patients Characteristics by Genotype (Adjuvant Cohort)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>V/V (%)</th>
<th>V/F (%)</th>
<th>F/F (%)</th>
<th>H/H (%)</th>
<th>H/R (%)</th>
<th>R/R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Positive Lymph Nodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>62 (37)</td>
<td>131 (28)</td>
<td>172 (31)</td>
<td>93 (29)</td>
<td>187 (31)</td>
<td>94 (32)</td>
</tr>
<tr>
<td>1-3</td>
<td>55 (32)</td>
<td>207 (44)</td>
<td>211 (38)</td>
<td>129 (40)</td>
<td>242 (41)</td>
<td>113 (38)</td>
</tr>
<tr>
<td>&gt; 4</td>
<td>52 (31)</td>
<td>133 (28)</td>
<td>166 (30)</td>
<td>101 (31)</td>
<td>168 (28)</td>
<td>91 (31)</td>
</tr>
<tr>
<td>Hormone Receptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER/PR negative</td>
<td>92 (54)</td>
<td>261 (55)</td>
<td>287 (52)</td>
<td>183 (57)</td>
<td>328 (55)</td>
<td>149 (50)</td>
</tr>
<tr>
<td>ER &amp;/or PR positive</td>
<td>77 (46)</td>
<td>210 (45)</td>
<td>262 (48)</td>
<td>140 (43)</td>
<td>269 (45)</td>
<td>149 (50)</td>
</tr>
<tr>
<td>Tumor Size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 2 cm</td>
<td>93 (55)</td>
<td>279 (59)</td>
<td>326 (59)</td>
<td>195 (60)</td>
<td>341 (57)</td>
<td>176 (59)</td>
</tr>
<tr>
<td>&lt; 2 cm</td>
<td>76 (45)</td>
<td>192 (41)</td>
<td>223 (41)</td>
<td>128 (40)</td>
<td>256 (43)</td>
<td>122 (41)</td>
</tr>
<tr>
<td>Menopause Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopause</td>
<td>77 (46)</td>
<td>192 (41)</td>
<td>246 (45)</td>
<td>143 (44)</td>
<td>250 (42)</td>
<td>133 (45)</td>
</tr>
<tr>
<td>Postmenopause</td>
<td>92 (54)</td>
<td>279 (59)</td>
<td>303 (55)</td>
<td>180 (56)</td>
<td>347 (58)</td>
<td>165 (55)</td>
</tr>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>49.3</td>
<td>48.5</td>
<td>48.6</td>
<td>49.0</td>
<td>48.0</td>
<td>49.0</td>
</tr>
<tr>
<td>Range</td>
<td>27.0-74.0</td>
<td>23.0-74.0</td>
<td>24.0-74.0</td>
<td>28.0-74.0</td>
<td>22.0-74.0</td>
<td>24.0-73.0</td>
</tr>
<tr>
<td>HER2 Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>154</td>
<td>435</td>
<td>496</td>
<td>290</td>
<td>557</td>
<td>263</td>
</tr>
<tr>
<td>Mean</td>
<td>8.3</td>
<td>8.2</td>
<td>8.0</td>
<td>8.3</td>
<td>8.1</td>
<td>8.1</td>
</tr>
<tr>
<td>Range</td>
<td>2.2-19.3</td>
<td>2.2-19.5</td>
<td>2.3-18.1</td>
<td>2.2-23.5</td>
<td>2.3-19.5</td>
<td>2.2-19.2</td>
</tr>
<tr>
<td>Treatment Arm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC-T</td>
<td>56 (33)</td>
<td>149 (32)</td>
<td>176 (32)</td>
<td>110 (34)</td>
<td>182 (30)</td>
<td>95 (32)</td>
</tr>
<tr>
<td>AC-TH</td>
<td>53 (31)</td>
<td>162 (34)</td>
<td>191 (35)</td>
<td>114 (35)</td>
<td>210 (35)</td>
<td>91 (31)</td>
</tr>
<tr>
<td>TCH</td>
<td>60 (36)</td>
<td>160 (34)</td>
<td>182 (33)</td>
<td>99 (31)</td>
<td>205 (34)</td>
<td>112 (38)</td>
</tr>
</tbody>
</table>

**Abbreviations:** ER Estrogen Receptor; PR Progesterone Receptor; A Adriamycin; C Cytoxan; T Taxotere; H Herceptin
Figure 2. Kaplan Meier estimates of disease free survival (DFS) in trastuzumab treated patients within the adjuvant cohort according to FcγR polymorphisms. (A) FCGR3A 158 V/F genotype, $P=0.98$ (V/V vs V/F vs F/F) (B) FCGR2A 131 H/R genotype, $P=0.76$ (H/H vs H/R vs R/R). (C) FcγRIIIa and FcγRIIa polymorphisms. Others represents patients with neither FCGR3A 158 V/V nor FCGR2A 131 H/H genotype, $P=0.67$ (H/H and/or V/V vs others).
Figure 3. Kaplan-Meier estimates of disease free survival in trastuzumab (AC-TH/TCH combined) versus no trastuzumab (AC-T) treated patients, by FcγR genotype. (A) V/V (P=0.13) (B) V/F (P=0.422) (C) F/F (P=0.81) (D) H/H (P=0.35) (E) H/R (P=0.985) (F) R/R (P=0.58)
Analysis of Fcγ Receptor IIIa and IIa Polymorphisms: Lack of Correlation with Outcome in Trastuzumab-Treated Breast Cancer Patients


Clin Cancer Res  Published OnlineFirst April 13, 2012.

Updated version  Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-11-2294

Supplementary Material  Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2012/06/12/1078-0432.CCR-11-2294.DC1

Author Manuscript  Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.