Biomarker Data from the Phase III KATHERINE Study of Adjuvant T-DM1 versus Trastuzumab for Residual Invasive Disease after Neoadjuvant Therapy for HER2-Positive Breast Cancer

Carsten Denkert[1], Chiara Lambertini[2], Peter A. Fasching[3], Katherine L. Pogue-Geile[4], Max S. Mano[5], Michael Untch[6], Norman Wolmark[7], Chiun-Sheng Huang[8], Sibylle Loibl[9], Eleftherios P. Mamounas[10], Charles E. Geyer Jr[7], Peter C. Lucas[7], Thomas Boulet[2], Chunyan Song[11], Gail D. Lewis[11], Malgorzata Nowicka[2], Sanne de Haas[2], and Mark Basik[12]

ABSTRACT

Purpose: In KATHERINE, adjuvant T-DM1 reduced risk of disease recurrence or death by 50% compared with trastuzumab in patients with residual invasive breast cancer after neoadjuvant therapy (NAT) comprised of HER2-targeted therapy and chemotherapy. This analysis aimed to identify biomarkers of response and differences in biomarker expression before and after NAT.

Experimental Design: Exploratory analyses investigated the relationship between invasive disease-free survival (IDFS) and HER2 protein expression/gene amplification, *PIK3CA* hotspot mutations, and gene expression of HER2, PD-L1, CD8, predefined immune signatures, and Prediction Analysis of Microarray 50 intrinsic molecular subtypes, classified by Absolute Intrinsic Molecular Subtyping. HER2 expression on paired pre- and post-NAT samples was examined.

Results: T-DM1 appeared to improve IDFS versus trastuzumab across most biomarker subgroups, except the HER2 focal expression subgroup. High versus low HER2 gene expression in residual disease was associated with worse outcomes with trastuzumab [HR, 2.02; 95% confidence interval (CI), 1.32–3.11], but IDFS with T-DM1 was independent of HER2 expression level (HR, 1.01; 95% CI, 0.56–1.83). Low PD-L1 gene expression in residual disease was associated with worse outcomes with trastuzumab (HR, 0.66; 95% CI, 0.44–1.00), but not T-DM1 (HR, 1.05; 95% CI, 0.59–1.87). *PIK3CA* mutations were not prognostic. Increased variability in HER2 expression was observed in post-NAT versus paired pre-NAT samples.

Conclusions: T-DM1 appears to overcome HER2 resistance. T-DM1 benefit does not appear dependent on immune activation, but these results do not rule out an influence of the tumor immune microenvironment on the degree of response.

Introduction

Patients with HER2-positive early breast cancer (EBC) and residual invasive disease after neoadjuvant therapy (NAT) incorporating chemotherapy and HER2-targeted therapy have poor outcomes—including reduced disease-free survival—compared with patients attaining a pathologic complete response (pCR; refs. 1–4). In the phase III KATHERINE study, adjuvant trastuzumab emtansine (T-DM1) reduced the risk of invasive disease recurrence or death by 50% compared with trastuzumab in patients with residual invasive disease after neoadjuvant HER2-targeted therapy and chemotherapy followed by surgery (5). T-DM1 is now the standard-of-care in these patients.

In EBC, limited data exist on the relationship between tumor biomarkers and outcomes with T-DM1, and on associations between tumor biomarkers in residual disease after NAT and response to adjuvant therapy. The KRISTINE trial investigated neoadjuvant T-DM1/pertuzumab versus trastuzumab/pertuzumab/docetaxel/carboplatin. It found that higher baseline pre-NAT HER2 mRNA and protein expression were associated with increased, albeit numerically, efficacy in both treatments (6). Additionally, studies of HER2-targeted therapies in EBC showed that higher pre-NAT HER2 mRNA and/or protein expression was associated with higher pCR rates in the neoadjuvant setting (7–10) and lower risk of an invasive disease-free survival (IDFS) event in the adjuvant setting (11). Tumor biomarker expression—including HER2—may change as a result of

[1]Institute of Pathology, Philipps University Marburg and University Hospital Marburg (UKGM), Marburg, Germany. [2]F. Hoffmann-La Roche Ltd, Basel, Switzerland. [3]Comprehensive Cancer Center Erlangen-EMN, University Hospital Erlangen, Department of Gynecology and Obstetrics, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany. [4]NSABP Foundation/NRG Oncology, Pittsburgh, Pennsylvania. [5]Instituto do Câncer do Estado de São Paulo, São Paulo, Brazil. [6]AGO-B and HELIOS Klinikum Berlin Buch, Berlin, Germany. [7]NSABP Foundation and University of Pittsburgh/UPMC Hillman Cancer Center, Pittsburgh, Pennsylvania. [8]National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan. [9]German Breast Group, Neu-Isenburg, Germany; Centre for Haematology and Oncology Bethanien, Frankfurt, Germany. [10]NSABP Foundation and Orlando Health Cancer Institute, Orlando, Florida. [11]Genentech, Inc., South San Francisco, California. [12]NSABP Foundation and Jewish General Hospital, McGill University, Quebec, Canada.

Corresponding Author: Carsten Denkert, Institutsdirektor, Institut für Pathologie, UKGM–Universitätsklinikum Marburg, Philipps–Universität Marburg, Baldingerstraße 1, D-35043 Marburg, Germany. Phone: 4906-421-586-2270; E-mail: carsten.denkert@uni-marburg.de

Clin Cancer Res 2023;29:1569–81

doi: 10.1158/1078-0432.CCR-22-1989

Translational Relevance

These exploratory analyses provide the first comprehensive data on the relationship between invasive disease-free survival (IDFS) and biomarkers in patients with residual invasive breast cancer after neoadjuvant therapy (NAT) comprised of chemotherapy plus HER2-targeted therapy for early breast cancer. T-DM1 treatment resulted in a consistent IDFS benefit compared with trastuzumab in all major biomarker subgroups defined by HER2 signaling and tumor immune microenvironment activation. High versus low HER2 gene expression assessed in residual disease after NAT (i.e., in post-NAT tumors) was associated with increased risk of recurrence or death in the trastuzumab, but not in the T-DM1, treatment arm, consistent with T-DM1 overcoming HER2 resistance. Benefit with T-DM1 did not appear to be dependent on immune gene expression. These data further support the use of T-DM1 for the treatment of residual invasive breast cancer after NAT.

NAT (12, 13). Further data are needed on the relationship between HER2 expression after NAT and response to adjuvant treatment.

Tumor-intrinsic molecular subtypes are categories of breast cancer defined by gene expression, including standard markers such as hormone receptors and HER2. Prediction Analysis of Microarray 50 (PAM50) classifies tumors as basal-like, HER2-enriched, luminal A, luminal B, and normal-like intrinsic subtypes using a 50-gene set analysis (14). The HER2-enriched PAM50 intrinsic subtype includes a high proportion of HER2-positive tumors but some are not classified as such based on the totality of gene expression (14). Patients with the HER2-enriched subtype have higher pCR rates in response to neoadjuvant chemotherapy plus HER2-targeted therapy than patients with other intrinsic subtypes (6, 15). However, in the adjuvant setting, patients with luminal intrinsic subtypes generally have similar or better outcomes than those with HER2-enriched tumors (11, 16). In the CALGB40601 study, the HER2-enriched subtype was associated with higher pCR rates, but was a negative predictor of relapse-free survival for patients with residual disease after HER2-targeted NAT who were treated with trastuzumab plus chemotherapy in the adjuvant setting (17). A shift from HER2-enriched subtypes in pre-NAT samples to luminal A subtypes in residual disease after HER2-targeted NAT has been observed (18–20). However, only a limited number of paired samples was available (18–20), the subset of paired samples did not always reflect the overall cohort (18), and the duration of HER2-targeted NAT was sometimes limited (20). Thus, the prognostic or predictive significance of PAM50 intrinsic subtypes and changes in those subtypes in residual disease after HER2-targeted NAT are unclear.

PIK3CA mutations have been implicated in resistance to HER2-targeted therapies (21, 22), with studies finding an association between *PIK3CA* mutations and poorer response to HER2-targeted therapy (6, 8, 9, 11, 23–26). However, some studies have not demonstrated this association (16, 27–30). The prognostic or predictive value of *PIK3CA* mutations in residual disease after NAT is unknown.

Immune cells in the tumor microenvironment may be prognostic and may predict treatment response, especially given the key role of antibody-dependent cell-mediated cytotoxicity in the activity of therapeutic antibodies (31). Immune-system activation, indicated by number of tumor-infiltrating lymphocytes, PD-L1 expression, and/or expression of immune-related genes, has been associated with higher pCR rates and longer disease-free survival in HER2-positive breast cancer (11, 17, 32–35), although not all data are consistent (36, 37).

Here, we present exploratory analyses of biomarker data from KATHERINE, which evaluated the relationship between efficacy and HER2 expression levels, tumor-intrinsic subtypes, immune gene expression, and *PIK3CA* mutation status assessed at eligibility or surgery. We also report on differences in biomarker expression in pre-NAT tumors and residual disease after HER2-targeted NAT.

Materials and Methods

Study design and patients

The KATHERINE study (NCT01772472) evaluated adjuvant T-DM1 versus trastuzumab in patients with histologically confirmed, centrally confirmed, HER2-positive, nonmetastatic, invasive primary breast cancer [T1–4, N0–3, M0 (excluding T1aN0 and T1bN0)] at presentation, and residual invasive disease detected pathologically in the surgical specimen of the breast or axillary lymph nodes after ≥9 weeks of taxane/trastuzumab-based NAT. The study design was published previously (5).

Patients were randomized 1:1 to trastuzumab 6 mg/kg intravenously every 3 weeks for 14 cycles (with 8-mg/kg loading dose if >6 weeks since last dose of trastuzumab) or T-DM1 3.6 mg/kg intravenously every 3 weeks for 14 cycles. The primary endpoint was IDFS, defined as time from randomization until one of the following: recurrence of ipsilateral invasive breast tumor, recurrence of ipsilateral locoregional invasive breast cancer, contralateral invasive breast cancer, distant disease recurrence, or death from any cause. Exploratory objectives included the evaluation of biomarker expression and its relationship with efficacy.

The study protocol conformed to Good Clinical Practice guidelines, the Declaration of Helsinki, and applicable local laws and was approved by the institutional review board/ethics committee at each center, and in accordance with assurances approved by the U.S. Department of Health and Human Services, where appropriate. Patients provided written informed consent, including for biomarker analyses on their tumor tissue samples. HER2 protein, gene amplification and gene expression, *PIK3CA* mutation status, and expression of the PD-L1 gene, CD8 gene, and immune gene signatures, including three-gene (PD-L1/IFNγ/CXCL9), five-gene (PD-L1/granzymeB/CD8/IFNγ/CXCL9), T-effector (CD8/granzymeA/granzymeB/perforin/IFNγ), Th1 cytokine (CXCL9/CXCL10/CXCL11), and checkpoint inhibitor (PD-L1/PD-L2/IDO) signatures, were prespecified as biomarkers for exploratory analysis. The effect of treatment and biomarkers on IDFS was assessed.

Tumor samples in the form of a formalin-fixed paraffin-embedded tumor block or partial block obtained from the pretreatment primary tumor biopsy material (or residual tumor tissue from definitive surgery post-NAT) were required for enrollment and submitted for central pathology laboratory assessment of HER2 status by immunohistochemistry (IHC; PATHWAY anti–HER-2/neu 4B5 assay, Ventana Medical Systems, Inc.) and *in situ* hybridization (ISH; INFORM HER2 Dual ISH assay, Ventana Medical Systems, Inc.). HER2-positive status was defined by an IHC3$^+$ score and/or gene amplification by ISH (ratio of ≥2.0 for number of HER2 gene copies to number of chromosome 17 copies). HER2 staining percentage was used for analysis (not prespecified); HER2 staining was categorized as focal (<30%), heterogeneous (30–79%), or homogeneous (≥80%) on the total percentage of cells stained with 2+ and 3+ intensity. Two samples were retested using IQFISH Dako PharmDx (Agilent) after initial invalid results; the

patients were enrolled based on the retesting. Two patients in the trastuzumab arm were enrolled with unknown central HER2 status: one was incorrectly randomized twice, with missing HER2 status at the time of the first randomization and positive HER2 status during rerandomization; the other was randomized based on a local HER2-positive result, but HER2-positive status could not be centrally confirmed, constituting a protocol violation.

Mandatory tumor tissue samples (pre-NAT and post-NAT surgical) underwent additional biomarker analysis. When HER2-positive status used for eligibility was based on pre-NAT samples and a post-NAT surgical sample was available, HER2 status was also performed on the surgical sample for exploratory analyses of changes in HER2 expression after NAT. Surgical specimens were used for *PIK3CA* mutation analysis and for gene expression analyses derived from RNA sequencing, if available. Otherwise, pre-NAT samples were used.

DNA was derived to identify *PIK3CA* hotspot mutations in exons 1, 4, 7, 9, and 20 using the cobas® PIK3CA Mutation Test (Roche Molecular Diagnostics) and cobas® z 480 analyzer (Roche Molecular Systems, Inc.). Whole-transcriptome RNA expression was measured using RNA sequencing (TruSeq RNA Access; Illumina, Inc.) at Expression Analysis on macrodissected tumor samples. The percentage of the tumor area in the macrodissected specimen was captured. Differential gene expression analysis between surgical and pre-NAT samples was performed using limma and voom (38, 39) and was adjusted for tumor content. Gene signatures representing multiple biological pathways and cell types, including the hallmark gene sets, were evaluated using gene set enrichment analysis (40). *P* values were corrected for multiple comparisons (Benjamini and Hochberg methodology), yielding adjusted values. The cutoff of 0.05 was used to identify significantly enriched pathways. PAM50 tumor intrinsic molecular subtypes were determined using Absolute Intrinsic Molecular Subtyping (AIMS; ref. 41).

Statistical analyses

mRNA expression levels were dichotomized at the median into low (≤) and high (>) groups at each sampling time-point (i.e., pre-NAT or post-NAT surgery). Additional validation analyses were performed using mRNA expression stratified into quartiles. We evaluated the potential predictive value of HER2 IHC staining intensity (0/1+ vs. 2+ vs. 3+), HER2 gene copy numbers (<4 vs. 4 to ≤6 vs. >6), and ratios (2 to <4 vs. ≥4), *PIK3CA* status (mutated vs. nonmutated) and mRNA expression of HER2, PD-L1, *CD8A*, T-effector signature, two other signatures relating to T-cell activity (three-gene, five-gene), Th1 cytokine signature, checkpoint inhibitor signature, and PAM50 intrinsic molecular subtypes for IDFS. The predictive value of each biomarker was assessed by comparing IDFS outcomes with T-DM1 versus trastuzumab within biomarker subgroups. Post-hoc analyses were performed to assess the interaction between treatment effect and biomarker subgroups, when suggested by the data, with the limitation that the study was not powered to detect interactions among subgroups. Prognostic value was assessed by comparing IDFS outcomes between biomarker subgroups in pooled-treatment arms (i.e., *PI3KCA* mutation analysis) and within each treatment arm. Composite values of gene signatures were defined as the mean of z-scores from individual genes. Survival curves were estimated with the Kaplan–Meier method. HRs and 95% confidence intervals (CI) were estimated using Cox proportional hazards regression models. Analyses of biomarkers based on mRNA expression were adjusted for tumor content and adjusted in multivariate analyses for clinical stage at presentation, hormone receptor status, preoperative HER2-directed therapy, and pathologic nodal status.

Data availability

For up-to-date details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see https://www.roche.com/innovation/process/clinical-trials/data-sharing/. Individual patient-level biomarker data are available to qualified researchers at The European Genome-Phenome Archive (https://ega-archive.org/access/data-access) under accession number EGAS00001006229. Anonymized records for individual patients across more than one data source external to Roche cannot, and should not, be linked due to a potential increase in risk of patient reidentification.

Results

KATHERINE enrolled 1,486 patients. Demographic characteristics were balanced between the two treatment arms (5). At screening, 1,484 patients had centrally confirmed HER2-positive tumors. HER2 status was assessed on pre-NAT samples in 1,195 (80.4%) patients and on surgical post-NAT samples in the remaining 289 (as pre-NAT samples were not available; **Fig. 1**). HER2 expression for eligibility by IHC was available for 1,483 (99.8%) patients. HER2 amplification by ISH was available for 1,440 (96.9%) patients. For patients enrolled based on pre-NAT samples, HER2 status was also assessed whenever possible on surgical samples for exploratory analyses, yielding 1,002 paired samples. Whole-transcriptome RNA-sequencing data were available for 1,059 (74.1%) of 1,429 patients with tissue available for mRNA extraction. Most data [77.0% (815/1,059 patients)] were derived from samples collected at surgery, to better understand the biology of the tumor post-NAT before randomization. Because samples were missing at the post-NAT time-point or inadequate resection material was available for some patients, pre-NAT samples were also analyzed in another 244 (23.0%) patients. *PIK3CA* mutation status data were available for 1,363 (91.7%) patients. Most [75.3% (1,027/1,363 patients)] were derived from post-NAT surgical samples; the remainder came from pre-NAT samples.

HER2 expression levels and hormone receptor status were balanced between treatment arms (**Table 1**). Tumors were characterized as HER2-positive using ISH (positive rate 96.2%) and/or IHC (76.2% IHC3$^+$). The majority had HER2 gene ratio ≥4 (66.1%), HER2 gene copy number ≥6 (81.5%), and homogeneous HER2 expression (66.8%).

HER2 expression and amplification: study eligibility data

Consistent IDFS benefit with T-DM1 versus trastuzumab was observed across most subgroups, defined by HER2 status assessed for eligibility by IHC and/or ISH, with a less pronounced treatment benefit in IHC2$^+$ versus IHC3$^+$ tumors (**Fig. 2**). In the T-DM1 arm, the 3-year IDFS rate was 89% in the IHC3$^+$ subgroup and 85% in the IHC2$^+$ subgroup; while in the trastuzumab arm, the 3-year IDFS rate was 76% in the IHC3$^+$ subgroup and 81% in the IHC2$^+$ subgroup. Consistent IDFS benefit with T-DM1 was also observed across tumors with homogeneous (≥80%) or heterogeneous (30%–79%) HER2 protein expression. Treatment benefit with T-DM1 was not observed in patients whose tumors had focal (<30%) HER2 expression (HR, 1.21; 95% CI, 0.58–2.51; **Fig. 2**). The interaction test was significant between the HER2 expression pattern of focal versus nonfocal and the treatment effect ($P_{int} < 0.05$). Of 166 samples with focal HER2 expression, most (81.9%) were IHC2$^+$ and derived from pre-NAT samples (75.3%). The corresponding pre-NAT HER2 gene expression levels were lower in focal versus heterogeneous and homogeneous samples (median of 8.88 vs. 10.27 vs. 12.82, respectively), suggesting

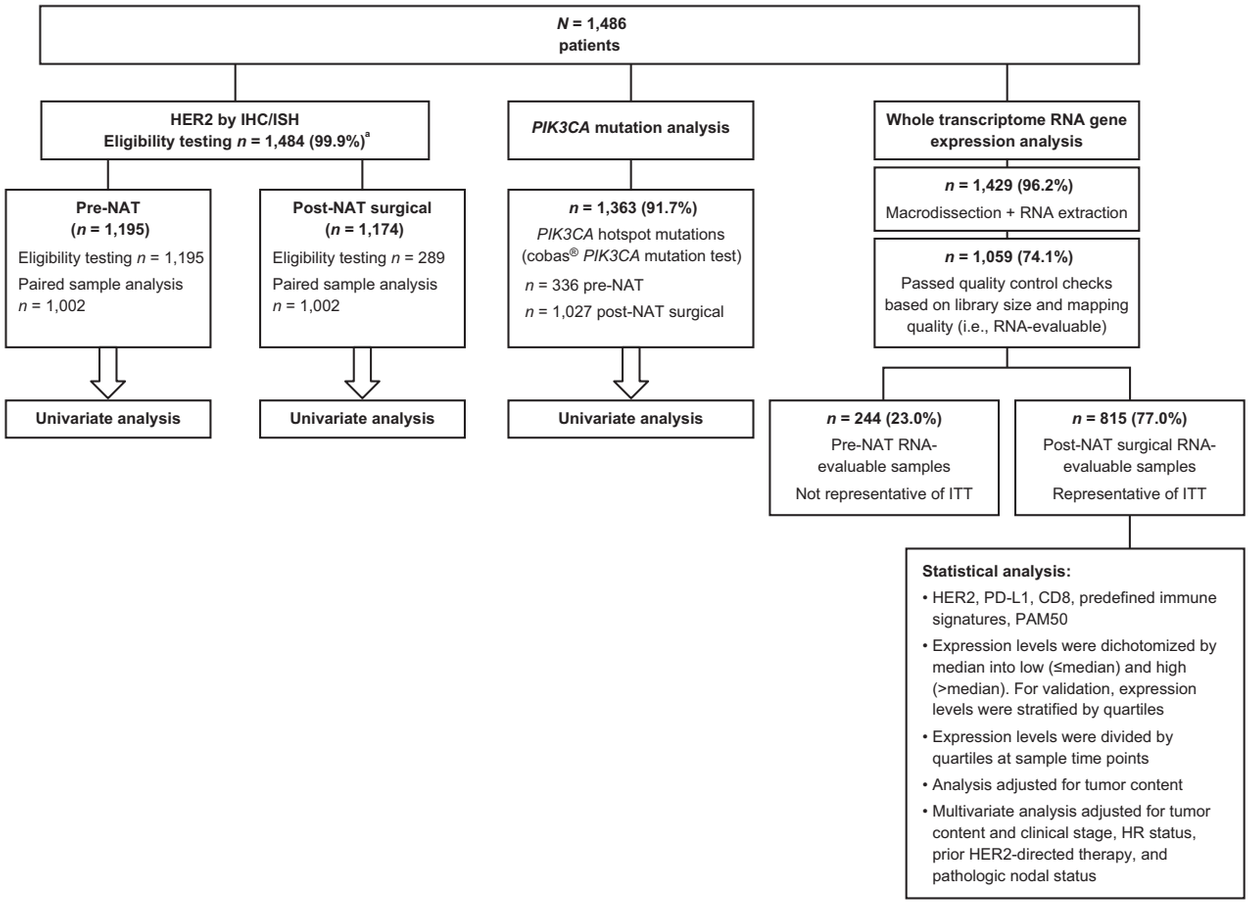

Figure 1.

Study populations for biomarker analyses. [a]Two patients (both in the trastuzumab arm) are not included: one had HER2-positive status that was determined locally but that was not centrally confirmed; the other was randomized twice in error, with a missing HER2 status at the time of the first randomization and a positive HER2 status during rerandomization. HR, hormone receptor.

that focal expression reflects lower HER2 expression and biological heterogeneity pre-NAT and is not a response to NAT. T-DM1 benefit versus trastuzumab did not appear to be affected by HER2 gene ratio or copy number.

HER2 expression by IHC: paired pre- and post-NAT samples

Changes in HER2 expression by IHC were analyzed on paired samples from patients with pre-NAT tumor samples used to assess eligibility and with available post-NAT surgical samples (n = 1,002; **Fig. 1**). Comparison of pre- and post-NAT samples showed increased variability in HER2 expression after NAT, as reflected by the increased prevalence of tumors with IHC2$^+$ and IHC0/1$^+$ scores (Supplementary Fig. S1) and higher incidence of focal expression (Supplementary Fig. S2). Decreases in HER2 staining intensity post-NAT were similar between treatments (IHC3$^+$ to IHC2$^+$, 15.6% for trastuzumab and 14.8% for T-DM1; IHC3$^+$ to IHC0/1$^+$, 5.4% for trastuzumab and 3.1% for T-DM1), as were increases in focal expression (homogeneous/heterogeneous to focal: 15.9% for trastuzumab; 11.9% for T-DM1).

The relationship between IDFS and HER2 IHC score assessed in post-NAT residual disease at surgery (n = 1,174; Supplementary Fig. S3) was similar to that with the HER2 eligibility data (mostly from pre-NAT samples), showing a smaller treatment effect in patients with IHC2$^+$ scores (**Fig. 2**). In the T-DM1 arm, 3-year IDFS rate was similar for patients with an IHC3$^+$ score in residual disease (90%) and those with an IHC2$^+$ score (88%). In the trastuzumab arm, 3-year IDFS was 72% among patients with an IHC3$^+$ score in residual disease and 81% among those with an IHC2$^+$ score, suggesting that the smaller treatment effect in IHC2$^+$ tumors is driven by a differential response in the trastuzumab arm and that T-DM1 is similarly effective in tumors with IHC2$^+$ and IHC3$^+$.

PIK3CA mutation analysis

PIK3CA mutations were identified in 350 (25.7%) of 1,363 patients with available data and were balanced between treatment arms. T-DM1 conferred an IDFS benefit compared with trastuzumab in patients with *PIK3CA*-mutated tumors (HR, 0.54; 95% CI, 0.32–0.90) and nonmutated tumors (HR, 0.48; 95% CI, 0.35–0.65; **Fig. 3A**). *PIK3CA* mutation status was not prognostic overall (HR, 1.04; 95% CI, 0.78–1.38; **Fig. 3B**).

RNA sequencing analysis

RNA sequencing data were derived using pre- and post-NAT tumor samples from 1,059 patients. Clinical and baseline biomarker

Table 1. Tumor HER2 expression/amplification and hormone receptor expression assessed at eligibility.

n (%)	Trastuzumab (*n* = 743)	T-DM1 (*n* = 743)	Total (*N* = 1,486)
Sample used for study eligibility[a]			
Preneoadjuvant therapy	603 (81.2)	592 (79.7)	**1,195 (80.4)**
Surgical	138 (18.6)	151 (20.3)	**289 (19.4)**
HER2 status by ISH			
Negative	4 (0.5)	7 (0.9)	**11 (0.7)**
Positive	722 (97.2)	707 (95.2)	**1,429 (96.2)**
HER2 status by IHC			
IHC0/1$^+$	13 (1.7)	12 (1.6)	**25 (1.7)**
IHC2$^+$	168 (22.6)	158 (21.3)	**326 (21.9)**
IHC3$^+$	559 (75.2)	573 (77.1)	**1,132 (76.2)**
HER2 gene ratio			
<2	4 (0.5)	7 (0.9)	**11 (0.7)**
2 to <4	212 (28.5)	210 (28.3)	**422 (28.4)**
≥4	497 (66.9)	485 (65.3)	**982 (66.1)**
HER2 gene copy number			
<4	13 (1.7)	8 (1.1)	**21 (1.4)**
4 to <6	92 (12.4)	91 (12.2)	**183 (12.3)**
≥6	608 (81.8)	603 (81.2)	**1,211 (81.5)**
HER2 heterogeneity			
Focal (<30%)	81 (10.9)	85 (11.4)	**166 (11.2)**
Heterogeneous (30%–79%)	170 (22.9)	155 (20.9)	**325 (21.9)**
Homogeneous (≥80%)	489 (65.8)	503 (67.7)	**992 (66.8)**
Hormone receptor status			
ER-negative and PgR-negative/unknown	203 (27.3)	209 (28.1)	**412 (27.7)**
ER- and/or PgR-positive	540 (72.7)	534 (71.9)	**1,074 (72.3)**

Note: For all parameters, except hormone receptor expression, there were missing data (not shown).

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.

[a]Two patients were enrolled erroneously without centrally confirmed HER2 status. HER2-positive status was later confirmed for one of these patients.

characteristics were evaluated in the pre- and post-NAT RNA-evaluable populations and compared with the intent-to-treat (ITT) population. Clinical characteristics were similarly distributed in the pre- and post-NAT RNA-evaluable population and were comparable with the ITT population. In the post-NAT RNA-evaluable population ($n = 815$), HER2 expression/amplification assessed at eligibility by IHC and/or ISH in both treatment arms was similar to the ITT population (Supplementary Table S1). However, HER2 IHC and ISH levels in the pre-NAT RNA-evaluable population ($n = 244$) in the trastuzumab arm were higher than in the ITT population. In addition, although IDFS outcomes in the RNA-evaluable population including both pre- and post-NAT samples and in the post-NAT RNA-evaluable population alone closely reflected those of the ITT population, IDFS outcomes in the pre-NAT RNA-evaluable population were less consistent with the ITT population (Supplementary Fig. S4A and S4B). These data suggested that the pre-NAT RNA-evaluable population may not be reflective of the ITT population. Further, multidimensional scaling analysis and differential gene-expression analysis showed potentially different gene expression in pre- and post-NAT samples suggesting different biology triggered by the NAT (Supplementary Fig. S5A–S5G). Therefore, in-depth analyses of biomarker gene expression and IDFS outcomes were conducted exclusively on the post-NAT RNA-evaluable population (**Fig. 1**).

IDFS benefit with T-DM1 compared with trastuzumab was observed irrespective of mRNA expression levels [i.e., high (>median) and low (≤median)] of the biomarkers, including HER2, PD-L1, and CD8, and immune gene signatures assessed post-NAT (**Fig. 4**). Further evaluation using quartile analysis of HER2 gene expression showed treatment benefit of T-DM1 versus trastuzumab in all quartiles, with the lowest HER2 quartile showing a similar HR as the highest, and the second quartile showing the least benefit (Supplementary Fig. S6A–S6D). Although sample size and number of events are small in these subgroups, limiting interpretation, overall benefit of T-DM1 seems independent of HER2 gene expression. Analysis of the effect of biomarker gene expression levels within treatment arms revealed that high versus low HER2 gene expression was associated with worse outcome in the trastuzumab arm (HR, 2.02; 95% CI, 1.32–3.11), but not the T-DM1 arm (HR, 1.01; 95% CI, 0.56–1.83; **Fig. 5A**; Supplementary Table S2). The effect was sustained for trastuzumab (HR, 1.67; 95% CI; 1.06–2.62) after adjusting for tumor content, clinical stage, hormone receptor status, prior HER2-directed therapy, and pathologic nodal status in a multivariate analysis. Similarly, as discussed above, lower 3-year IDFS rates were observed in the trastuzumab arm in patients with IHC3$^+$ versus IHC2$^+$ measured in post-NAT residual tumors ($n = 1,174$; Supplementary Fig. S3). Additionally, within the trastuzumab arm, worse outcomes were observed in patients with the AIMS-HER2–enriched subtype versus other AIMS subtypes (except the AIMS-basal-like subtype; **Fig. 5B**). This was not evident with T-DM1 (**Fig. 5C**). However, the majority of samples were the AIMS-HER2–enriched subtype, with the prevalence of other AIMS subtypes too low for robust analysis. Low PD-L1 gene expression was associated with worse outcomes in the trastuzumab, but not the T-DM1, arm [high vs. low PD-L1 expression: HR, 0.66; 95% CI, 0.44–1.00 (trastuzumab); HR, 1.05; 95% CI, 0.59–1.87 (T-DM1); **Fig. 5D**; Supplementary Table S2]. The effect was sustained with trastuzumab (HR, 0.62; 95% CI, 0.41–0.94) after adjusting for tumor content, clinical stage, hormone receptor status, prior HER2-directed therapy, and pathologic nodal status in a multivariate analysis. A similar trend was observed with trastuzumab when quartile analysis compared the lowest to the higher quartiles, but the 95% CI crossed 1 for all quartiles (Supplementary Fig. S6H; see Supplementary Fig. S6E–S6H for analysis of IDFS by PD-L1 gene-expression quartile). The checkpoint inhibitor signature expression data were similar to the PD-L1 expression data [HR, 0.69; 95% CI, 0.46–1.04 (trastuzumab); HR, 1.31; 95% CI, 0.74–2.34 (T-DM1); Supplementary Table S2]; this was sustained in the multivariate analysis [HR, 0.63; 95% CI, 0.42–0.95 (trastuzumab); HR, 1.30; 95% CI, 0.72–2.34 (T-DM1)]. Above-median expression of the five-gene immune signature was associated with worse outcomes in the T-DM1 arm (HR, 1.89; 95% CI, 1.04–3.42) but not in the trastuzumab arm (HR, 1.01; 95% CI, 0.68–1.50; Supplementary Table S2). The effect was sustained in the T-DM1 arm (HR, 2.12; 95% CI, 1.14–3.91) after adjusting for tumor content, clinical stage, hormone receptor status, prior HER2-directed therapy, and pathologic nodal status in a multivariate analysis. However, further analysis by quartiles for the five-gene immune signature did not confirm this finding (Supplementary Fig. S6I–S6L).

Although mRNA data from pre-NAT samples were not used for outcome analyses, they were used to compare gene-expression levels between pre-NAT and post-NAT surgical samples regardless of treatment arm. Profiles at these time points differed. Residual tumors following HER2-targeted NAT appeared to have lower HER2 gene expression (Supplementary Fig. S5A) than pre-NAT samples, consistent with the paired pre- and post-NAT sample analysis by IHC. However, pretreatment mRNA data were available for fewer patients and, unlike HER2 IHC results, data were not produced on paired samples. In addition, based on mRNA levels, there was a greater

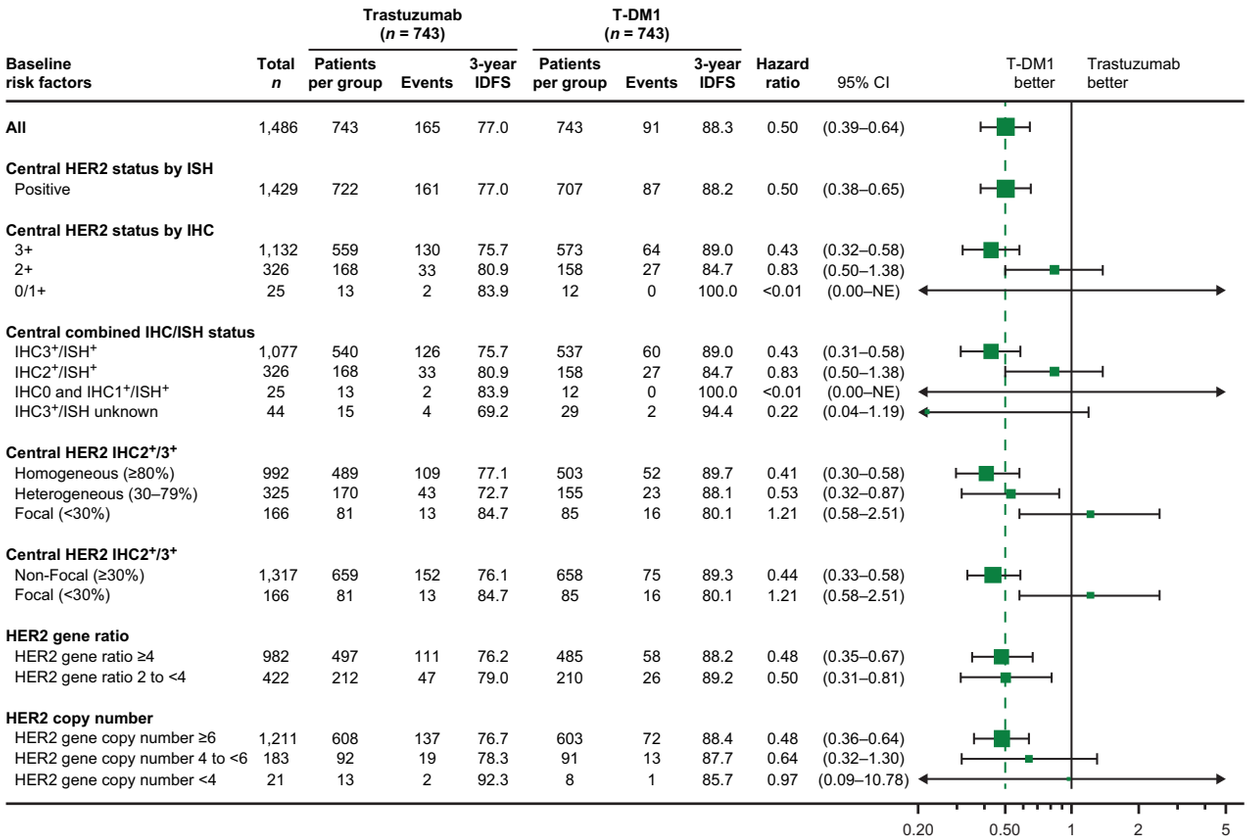

Figure 2.

Forest plot of IDFS by HER2 expression subgroups assessed at eligibility. Results for HER2 expression by IHC and ISH are from pre-NAT biopsies in 80.4% (1,195/1,486) of patients and from surgical tissue in the remaining 19.4% (289/1,486) of patients. Data for groups with fewer than 15 patients are not shown. Two patients did not have confirmed HER2-positive disease. NE, not estimable.

proportion of AIMS-HER2–enriched intrinsic subtype tumors in the pre-NAT (72.8%) versus post-NAT (65.2%) samples (Supplementary Fig. S5B). The same was observed in hormone receptor–negative (88.6% AIMS-HER2–enriched in pre-NAT; 65.2% AIMS-HER2–enriched in post-NAT) and hormone receptor–positive (74.9% AIMS-HER2–enriched in pre-NAT; 61.7% AIMS-HER2–enriched in post-NAT) tumors. Irrespective of pre-NAT or post-NAT status, AIMS-luminal subtypes were more common among hormone receptor–positive than hormone receptor–negative tumors. AIMS-basal subtypes predominated in hormone receptor–negative tumors. More AIMS-HER2–enriched tumors were present in those with HER2 IHC 3^+ and those with HER2 gene expression above median (Supplementary Fig. S5C and S5D). There was a lower proportion of tumors of AIMS-luminal B subtype in post-NAT versus pre-NAT samples. Furthermore, HER2 protein and gene expression levels were higher in the AIMS-HER2–enriched PAM50 subtypes compared with the other subgroups irrespective of whether they were collected pre- or post-NAT (Supplementary Fig. S5C and S5D). No consistent differences were observed in gene expression of PD-L1 and other immune markers (Supplementary Fig. S5A). Differences between post-NAT surgical and pre-NAT samples were noted in gene set enrichment analysis of the hallmark gene sets (Supplementary Fig. S5E and S5F). Some pathways (e.g., epithelial–mesenchymal transition, inflammatory response, hypoxia, coagulation, apoptosis, oxidative phosphorylation, fatty acid metabolism, angiogenesis) were upregulated in post-NAT versus pre-NAT samples, whereas genes related to the cell cycle and interferon-α response were downregulated in post-NAT versus pre-NAT samples.

Discussion

Exploratory biomarker analyses of KATHERINE data were undertaken to identify biomarkers beyond clinical parameters typically used to define patients at higher risk of breast cancer recurrence and to determine if specific subgroups have a differential response to T-DM1. We evaluated the relationship between efficacy and expression of key biomarkers assessed in pre-NAT and post-NAT samples of residual disease at surgery. IDFS benefit with T-DM1 versus trastuzumab was observed in all major subgroups, regardless of whether the analysis was primarily on pre-NAT (e.g., IHC and ISH analysis) or post-NAT surgical (e.g., *PIK3CA*, mRNA gene expression analysis) samples. Notably, there were more tumors with HER2 IHC2^+ score (22%), HER2 gene ratio ≥2 to <4 (28%), and hormone receptor–positive status (72%) than in other studies of HER2-positive EBC (6, 11). This may be due to selection of patients without a pCR following NAT for this trial, which likely resulted in an enrichment of characteristics associated with a lower likelihood of achieving a pCR (e.g., lower HER2 expression, estrogen receptor–positive status). We did not observe a higher

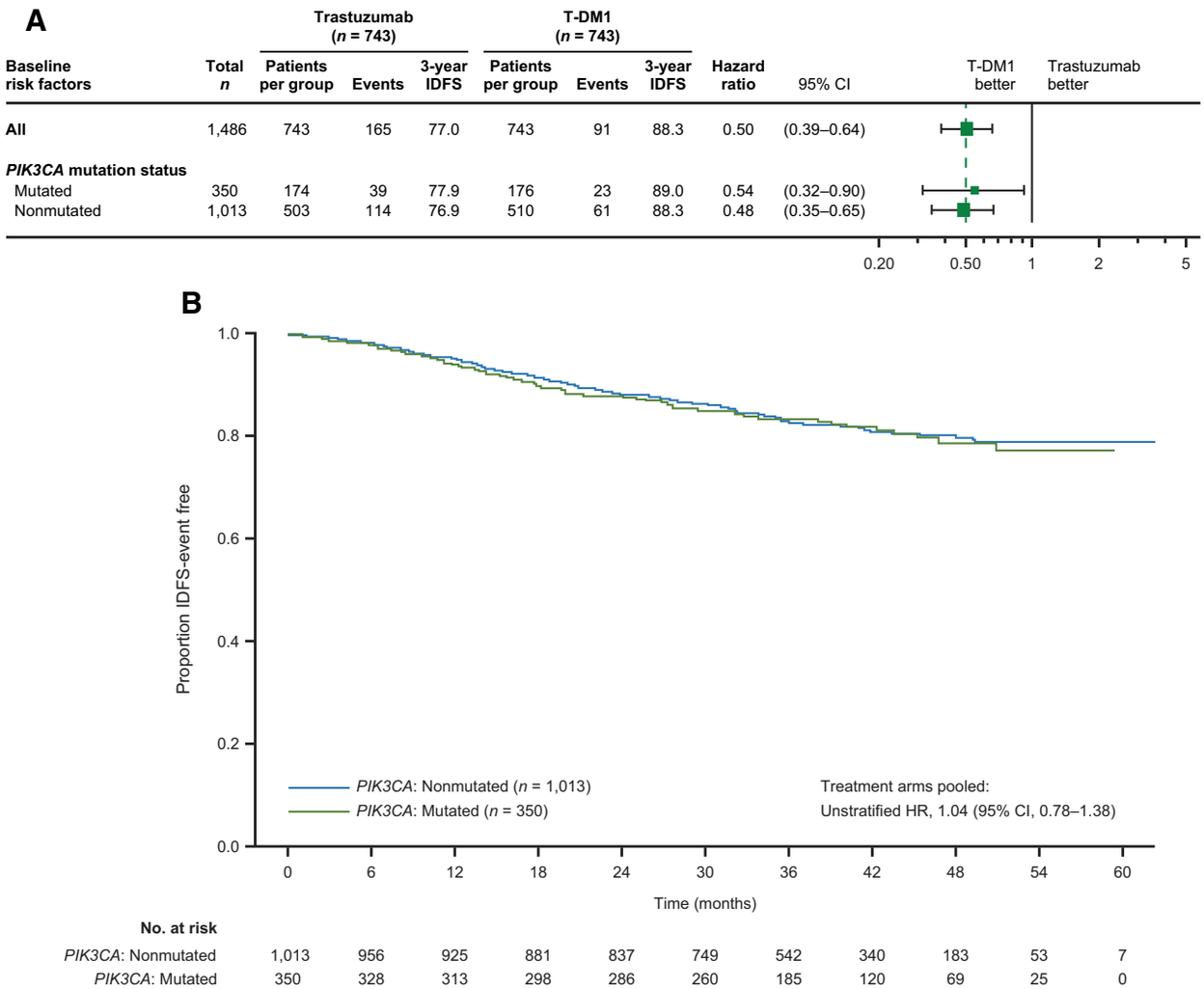

Figure 3.

T-DM1 improved IDFS regardless of *PIK3CA* mutation status, and *PIK3CA* mutations were not prognostic overall. Of the 1,363 samples available, 1,027 (75.3%) were post-NAT surgical samples and 336 (24.7%) were pre-NAT samples. **A,** *PIK3CA* mutation analysis by treatment arm. **B,** *PIK3CA* mutation analysis in pooled-treatment arms.

PIK3CA mutation rate versus other EBC trials (6, 9, 11), even though *PIK3CA* mutations have been previously associated with lower pCR rates (9, 25). However, *PIK3CA* mutation was assessed in post-NAT surgical samples for most patients, whereas previous studies used pre-NAT samples.

HER2-targeted therapies in the metastatic setting have shown more benefit from T-DM1/trastuzumab/pertuzumab in patients with higher HER2 expression (HER2 gene copy number, HER2 mRNA, and HER2 protein; refs. 24, 26, 28, 29). In adjuvant/neoadjuvant trials combining trastuzumab with chemotherapy, higher HER2 expression was associated with higher pCR rates and better outcomes (16, 42–44). Consistent with this, the IDFS benefit with T-DM1 was more pronounced in patients with HER2 IHC3$^+$ versus IHC2$^+$ scores assessed at eligibility (mostly from pre-NAT samples). It was therefore surprising to see the opposite association in KATHERINE for trastuzumab-treated patients, where those with lower HER2 expression (e.g., IHC2$^+$ or focal HER2 expression) had greater IDFS benefit. The smaller IDFS benefit with T-DM1 and better benefit with trastuzumab in the IHC2$^+$ versus IHC3$^+$ subgroups resulted in a smaller difference in treatment benefit between the two arms in that patient subgroup. However, the higher prevalence of luminal biology in lower HER2-expressing tumors may have influenced IDFS, and potentially influenced the relatively small IHC2$^+$ subgroups differently in each arm. Consistent IDFS benefit with T-DM1 was observed in tumors with different HER2 gene ratios or copy numbers, or with heterogeneous/homogeneous HER2 expression. In contrast with the majority of the biomarker subgroups, lack of T-DM1 benefit over trastuzumab was observed in tumors with focal (<30%) HER2 expression—most (80%) of which were pretreatment samples—showing a similar trend as the IHC2$^+$ subgroup. However, it should be noted that the number of patients with focal expression ($n = 166$) is comparably small with few IDFS events ($n = 29$) leading to a wide 95% CI (0.58–2.51) in this subgroup. Therefore, the results of the interaction test between focal status and the treatment effect should be interpreted with caution. Still, these results are interesting from a translational point of view, raising the hypothesis that with a therapeutic approach that targets an antigen

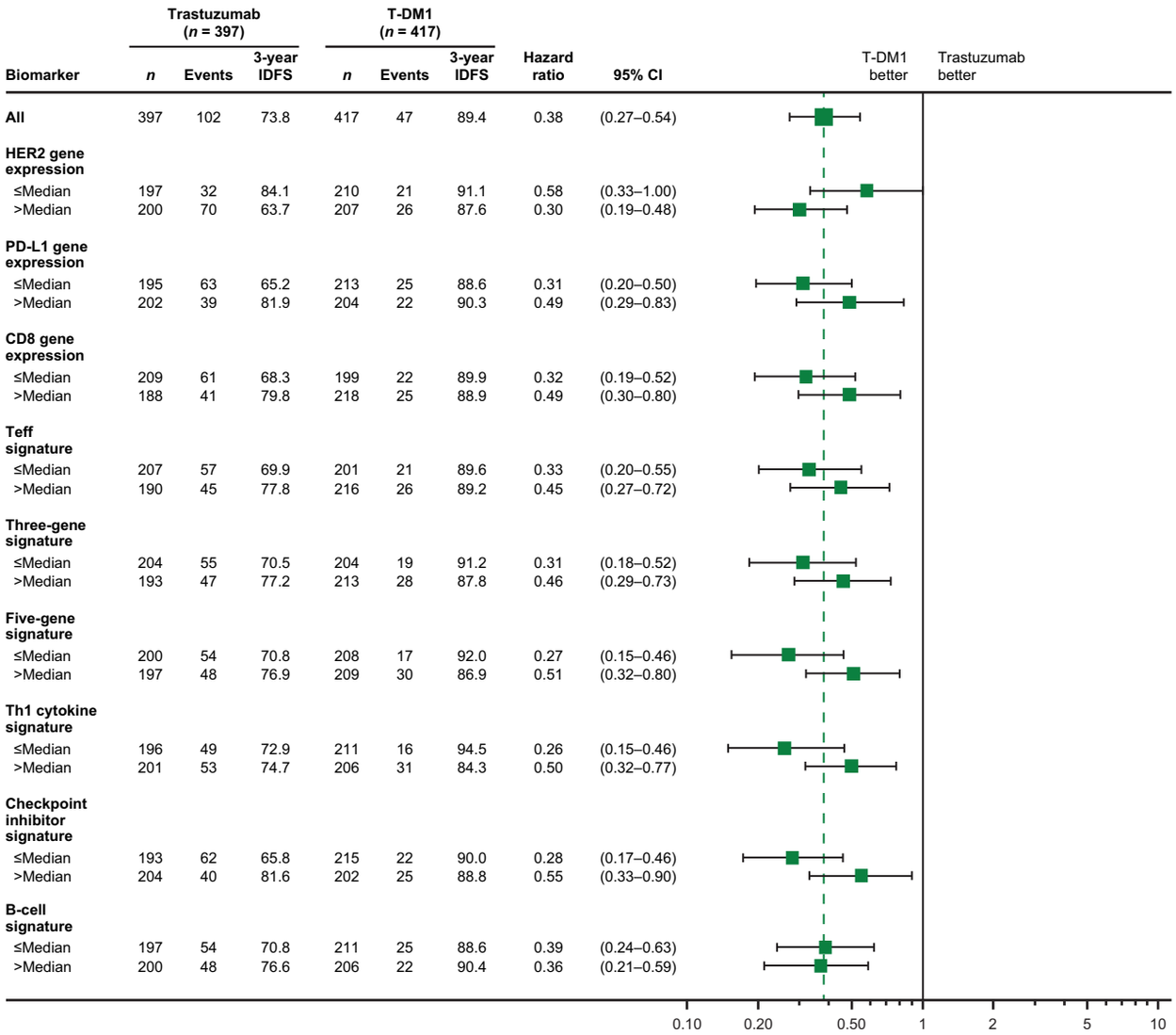

Figure 4.

Predictive value of mRNA expression levels on IDFS. Forest plot of treatment effect on IDFS by mRNA expression level subgroups (>median vs. ≤median) in RNA-evaluable surgical tissue samples. Dashed line indicates the overall treatment effect in the population with biomarker-evaluable surgical samples ($n = 814$). The analysis is adjusted for tumor content (percentage of tumor in the marked area). One sample could not be included because there was insufficient information on tumor content. Teff, T-effector signature (CD8/granzymeA/granzymeB/perforin/IFNγ); three-gene signature (PD-L1/IFNγ/CXCL9); five-gene signature (PD-L1/granzymeB/CD8/IFNγ/CXCL9); Th1 cytokine signature (CXCL9/CXCL10/CXCL11); checkpoint inhibitor signature (PD-L1/PD-L2/IDO); B-cell signature (CD19/CD79A/FCRL5/MS4A1/POU2AF1/STAP1).

with an antibody–drug conjugate (ADC), the additional benefit from the chemotherapy component of the ADC might be decreased if the target antigen is expressed only focally, particularly with ADCs thought to have minimal bystander effects. However, it should be noted that this was not observed in the trastuzumab arm in our study where the 3-year IDFS rate was higher in the focal versus nonfocal subgroups. Previous data have shown reduced benefit for T-DM1 in patients with focal/heterogeneous expression versus nonfocal expression in the (neo-)adjuvant setting (6, 30), but lower pCR rates for focal/heterogeneous HER2 expression have also been observed for trastuzumab/pertuzumab/chemotherapy (6). Although the proportion of patients with lower HER2 levels (IHC0–2+) and focal expression increased in surgical compared with pre-NAT samples, this increase was seen in both the trastuzumab and T-DM1 arms; therefore, it does not explain the reduced/lack of response to T-DM1 in these subgroups.

Analyses of the effect of HER2 gene expression levels also showed that high expression was associated with worse outcomes in the trastuzumab but not the T-DM1 arm; this contrasts with studies showing better outcomes with trastuzumab in tumors with higher HER2 expression (16, 42–44). KATHERINE data are derived from patients with residual disease after HER2-targeted therapy. However, our data are consistent with CALGB40601, which evaluated lapatinib/trastuzumab in the neoadjuvant setting followed by adjuvant

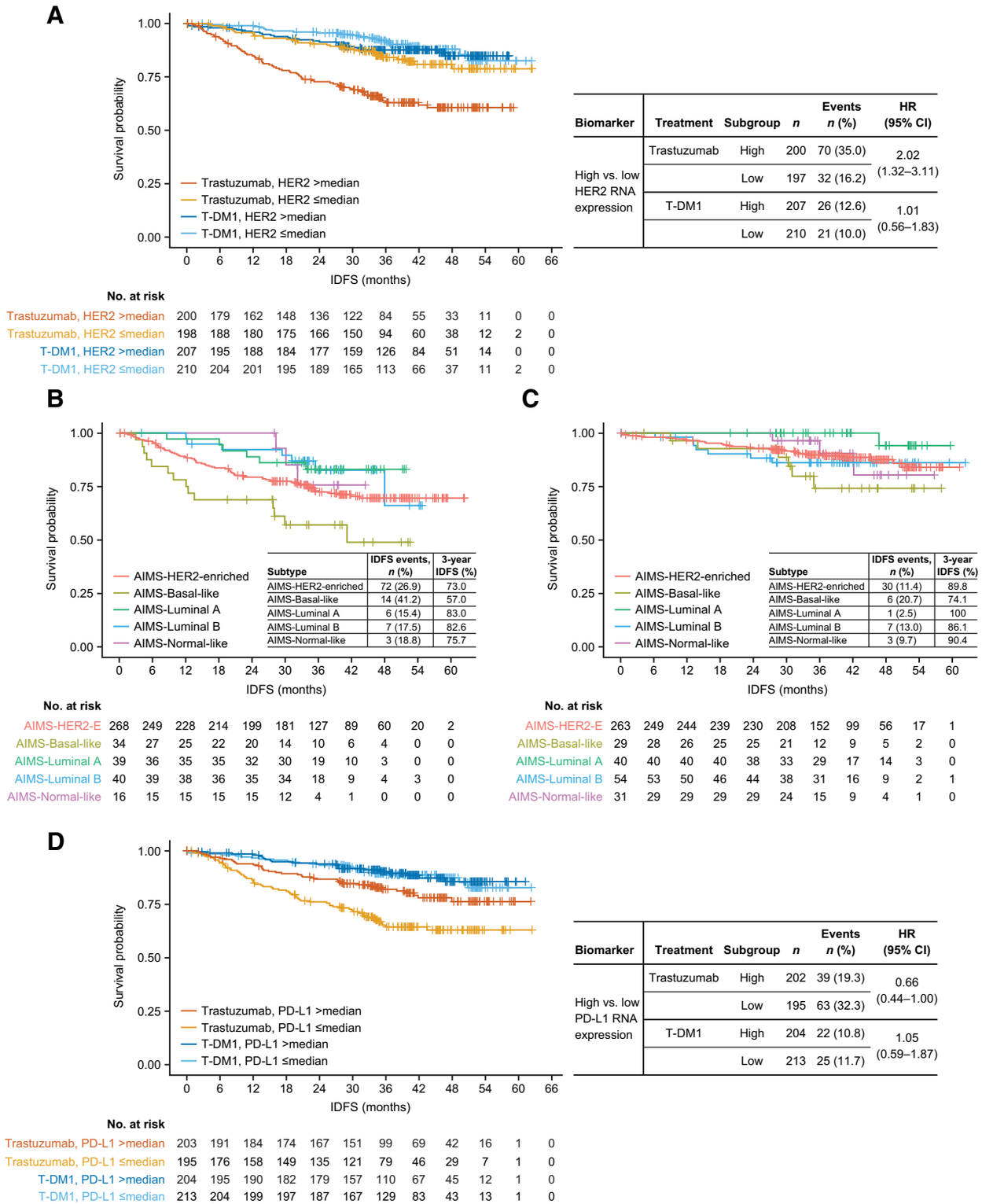

Figure 5.

Biomarker gene expression analysis in surgical tissue samples. Kaplan–Meier plots of IDFS by (**A**) HER2 mRNA expression level (>median vs. ≤median) and treatment group, (**B**) AIMS PAM50 intrinsic subtypes in trastuzumab-treated patients, (**C**) AIMS PAM50 intrinsic subtypes in T-DM1–treated patients, and (**D**) PD-L1 mRNA expression level (>median vs. ≤median) and treatment group. One sample could not be included because there was insufficient information on tumor content. AIMS-HER2-E, AIMS HER2-enriched.

trastuzumab plus chemotherapy (17). In CALGB40601, in pre-NAT tissue, the HER2-enriched subtype was associated with higher pCR rates than other subtypes, but in residual disease the HER2-enriched subtype was associated with worse relapse-free survival. These, and our findings, suggest the presence of resistance mechanisms to trastuzumab in the adjuvant setting in those with high HER2 gene and protein expression, and residual disease after neoadjuvant trastuzumab exposure. T-DM1 may overcome these resistance mechanisms, potentially by targeted delivery of DM1 to HER2-positive cells. It should be noted that different PAM50 classifiers exist and that the prevalence of the PAM50 subtypes may differ among classifiers, due to the training of these classifiers using different methodologies and data sets (45). However, efficacy results using the gold standard (14) were consistent with our data showing an increased benefit of T-DM1 over trastuzumab, and worse outcomes for the AIMS-HER2–enriched subtype in the trastuzumab arm but not in the T-DM1 arm.

Resistance to HER2-targeted therapies can be mediated by dysregulation of the PI3K/AKT pathway downstream from HER2 (21, 22, 46). Data on the effect of *PIK3CA* mutations on response to HER2-targeted therapy in the adjuvant setting have been inconsistent. *PIK3CA* mutations were associated with unfavorable prognosis in a pooled-treatment arm, case-controlled analysis of APHINITY (11). However, in post hoc analyses of the NSABP B-31 trial, *PIK3CA* mutation did not affect benefit of trastuzumab (16). Analysis of combined treatment arms in the KAITLIN study showed no effect of *PIK3CA* mutations on outcomes in the adjuvant setting (30). In a large, exploratory, pooled analysis, although *PIK3CA* mutations were associated with a lower pCR rate in response to systemic therapy containing HER2-targeted agents in the neoadjuvant setting, there was no statistically significant difference in disease-free survival or overall survival (25). However, limited events were available for this long-term outcome analysis. In the current study, *PIK3CA* mutation status did not influence outcomes with trastuzumab or T-DM1, and IDFS benefit was observed with T-DM1 versus trastuzumab, irrespective of mutation status.

Although the data on the effect of *PIK3CA* mutation on IDFS are inconsistent, to our knowledge, this is the first analysis primarily on tissue from post-NAT residual disease. These post-NAT samples may be enriched for acquired (e.g., driven by NAT) or *de novo* alterations/resistance mechanisms, and *PIK3CA* mutations may no longer be a prominent driver of tumorigenesis. In the MARIANNE MBC study, which showed an association between *PIK3CA* mutations and poor outcome, most patients (60%–70%) had no prior exposure to HER2-targeted therapy (47). *PIK3CA* mutation status in micrometastases (e.g., by postsurgery ctDNA) may more accurately reflect the molecular profile of remaining tumor cells if assays with high sensitivity can be applied. Despite promising preclinical results, the relevance of *PIK3CA* mutation in the adjuvant setting for HER2-positive breast cancer remains poorly understood, with variable results in clinical studies.

The immune system is an important component of the tumor microenvironment. Markers of immune activation, such as a higher number of tumor-infiltrating lymphocytes (32, 33, 35) and greater expression of genes indicative of an activated immune microenvironment (11, 17, 33, 36, 48), have generally been associated with better outcomes in HER2-positive EBC. The prognostic value of PD-L1 expression in HER2-positive breast cancer and higher PD-L1 gene expression has been associated with improved survival in multivariate analysis (49). Further, RNA expression of immunosuppressive checkpoint molecules, such as PD-L1, strongly correlates with other immune markers with proimmune activity (33) and an immune signature of genes associated with strong cytotoxic activity (50). In KATHERINE, T-DM1 benefit was consistent across immune function gene expression groups (Supplementary Table S2). High versus low PD-L1 expression was associated with improved outcomes with trastuzumab, but not T-DM1. A similar trend was observed in the checkpoint inhibitor signature subgroup. This suggests that, in contrast to trastuzumab, T-DM1 may be less dependent on immune activation. Notably, patients with low immune activation in their tumor microenvironment, who generally do worse (as observed in the trastuzumab arm), benefit from T-DM1 treatment. No robust associations between the remaining immune signatures and IDFS were observed in either arm. It would be interesting to assess additional immune infiltration signatures and genes. It should be noted that the current analyses are based on bulk RNA sequencing, providing information on gene expression of the entire sample, not by individual cell type. The contributions of tumor, stromal, and immune cells cannot therefore be distinguished.

In this study, post-NAT surgical samples had lower HER2 mRNA expression and proportionally fewer AIMS-HER2–enriched or AIMS-luminal B subtypes than pre-NAT samples, reflecting the increased presence of AIMS-luminal A and AIMS-normal-like subtypes in the post-NAT samples. Gene expression was adjusted for tumor content using exact tumor percentage as a covariate in the regression model; thus, these changes in expression were not simply due to smaller tumor samples. Although gene expression analysis was not paired, the data are consistent with studies of pre- and posttreatment paired samples from patients with residual disease after HER2-targeted NAT, showing downregulation of the HER2-enriched and luminal B signatures after NAT (19, 20), and from a subgroup of paired samples in KATHERINE, showing a change from HER2-positive status pre-NAT to HER2-negative or unknown status post-NAT in 8% of patients (51). We also identified other genes that were differentially expressed between pre-NAT and post-NAT samples, suggesting that they differ biologically.

There was greater expression of genes related to epithelial–mesenchymal transition, inflammatory response, hypoxia, coagulation, apoptosis, oxidative phosphorylation, fatty acid metabolism, and angiogenesis in the post-NAT surgical versus pre-NAT samples. This suggests mechanisms of invasiveness and metabolic pathways potentially transmitted via the administration of neoadjuvant treatment including HER2-targeted therapy. Lower expression of cell cycle–related genes and interferon-α response was observed in the post-NAT surgical compared with the pre-NAT samples, potentially due to NAT.

That our gene-expression analysis was performed primarily on post-NAT surgical samples is a study strength, as these samples reflect tumor status closer to the time of treatment administration in the adjuvant setting, possibly providing the most accurate data on the relationship between treatment, IDFS, and tumor biomarker expression in these patients. These data also increase our understanding of tumor biology following HER2-targeted NAT. A limitation is that the number of tumor cells in residual disease is generally lower than that in pre-NAT samples, which could present technical issues in biomarker analysis, resulting in potentially skewed results even though the analyses were adjusted for tumor content.

In summary, this exploratory biomarker analysis of the KATHERINE trial demonstrates that T-DM1 confers clinical benefit in a wide range of subgroups defined by HER2 signaling and immune biomarkers. High HER2 gene expression was associated with increased risk of recurrence or death in the trastuzumab, but not T-DM1, treatment arm, consistent with T-DM1 overcoming HER2 resistance. Benefit with T-DM1 does not appear dependent on immune activation, but

these results do not rule out that the tumor immune microenvironment may influence the degree of response. To our knowledge, this analysis of post-NAT samples provides the first comprehensive data on the relationship between biomarker expression and IDFS in residual disease after HER2-targeted therapy. The poor prognostic impact of *PIK3CA* mutations, as seen in metastatic and neoadjuvant settings, was not observed.

Authors' Disclosures

C. Denkert reports other support from Sividon Diagnostics; personal fees from Teva, Novartis, Pfizer, Roche, Amgen, AstraZeneca, MSD Oncology, and Daiichi Sankyo; and grants from Roche, Myriad, and German Breast Group outside the submitted work. In addition, C. Denkert has a patent for VMscope digital pathology software with royalties paid, a patent for EP18209672 pending, and a patent for EP20150702464 pending. C. Lambertini reports personal fees from F. Hoffmann-La Roche Ltd during the conduct of the study. P.A. Fasching reports personal fees from Novartis, Pfizer, Daiichi Sankyo, AstraZeneca, Eisai, Merck Sharp & Dohme, Lilly, Pierre Fabre, SeaGen, Roche, Agendia, and Sanofi Aventis, as well as grants from BioNTech and Cepheid during the conduct of the study. K.L. Pogue-Geile reports other support from NSABP during the conduct of the study. M.S. Mano reports personal fees from Roche outside the submitted work. M. Untch reports personal fees from AbbVie, Amgen, AstraZeneca, Daiichi Sankyo, Eisai, Lilly, MSD, Mylan, Myriad Genetics, Novartis, Pierre Fabre, Pfizer, Gilead, Roche, Sanofi Aventis, SeaGen, and Celgene outside the submitted work. C.-S. Huang reports grants, personal fees, and nonfinancial support from Roche during the conduct of the study. C.-S. Huang also reports grants and personal fees from Daiichi Sankyo and Eli Lilly; grants, personal fees, and nonfinancial support from AstraZeneca, Pfizer, Roche, and Novartis; and grants from EirGenix, MSD, and OBI Pharma outside the submitted work. S. Loibl reports grants and other support from Roche during the conduct of the study. S. Loibl also reports grants and other support from AZ, AbbVie, Amgen, DSI, Gilead, Celgene/BMS, Novartis, and Pfizer; grants from Molecular Health; and other support from SeaGen, Sanofi, Relay, Olema, EirGenix, Merck kG, Lilly, GSK, Pierre Fabre, and Esai outside the submitted work. In addition, S. Loibl has a patent for VM Scope with royalties paid, a patent for EP14153692.0 pending, a patent for EP21152186.9 pending, and a patent for EP15702464.7 pending. E.P. Mamounas reports personal fees from Genentech/Roche, Exact Sciences, Merck, Hologic, Tersera, and Puma Biotechnology outside the submitted work. C.E. Geyer Jr reports grants and nonfinancial support from Genentech/Roche during the conduct of the study; C.E. Geyer Jr also reports grants and nonfinancial support from AstraZeneca, grants and nonfinancial support from Daiichi Sankyo, and personal fees from Exact Sciences outside the submitted work. P.C. Lucas reports stock ownership from Amgen outside the submitted work; in addition, P.C. Lucas has a patent for small molecules and their use as MALT1 inhibitors issued. T. Boulet reports grants from F. Hoffmann-La Roche Ltd during the conduct of the study. C. Song reports other support from Genentech Inc during the conduct of the study, as well as other support from Genentech Inc outside the submitted work. G.D. Lewis reports other support from Genentech and Roche outside the submitted work. M. Nowicka reports personal fees from F. Hoffmann-La Roche, as well as other support from F. Hoffmann-La Roche outside the submitted work. S. de Haas reports personal fees and other support from F. Hoffmann-La Roche. Ltd. during the conduct of the study. M. Basik reports other support from Roche Canada during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

C. Denkert: Conceptualization, supervision, writing–review and editing. **C. Lambertini:** Conceptualization, supervision, investigation, writing–original draft, project administration, writing–review and editing. **P.A. Fasching:** Conceptualization, supervision, writing–review and editing. **K.L. Pogue-Geile:** Conceptualization, writing–review and editing. **M.S. Mano:** Resources, writing–review and editing. **M. Untch:** Resources, writing–review and editing. **N. Wolmark:** Resources, funding acquisition, project administration, writing–review and editing. **C.-S. Huang:** Writing–review and editing. **S. Loibl:** Conceptualization, resources, writing–review and editing. **E.P. Mamounas:** Resources, writing–review and editing. **C.E. Geyer Jr:** Conceptualization, resources, writing–review and editing. **P.C. Lucas:** Resources, writing–review and editing. **T. Boulet:** Conceptualization, software, formal analysis, supervision, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing. **C. Song:** Conceptualization, supervision, writing–review and editing. **G.D. Lewis:** Conceptualization, supervision, writing–review and editing. **M. Nowicka:** Conceptualization, data curation, software, formal analysis, supervision, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing. **S. de Haas:** Conceptualization, supervision, funding acquisition, investigation, writing–original draft, project administration, writing–review and editing. **M. Basik:** Conceptualization, supervision, writing–review and editing.

Acknowledgments

This study was funded by F. Hoffmann-La Roche, Ltd. We thank the patients who participated in this trial and the clinical site teams. Medical writing support, funded by F. Hoffmann-La Roche, was provided by Holly Strausbaugh, PhD, and Tracy McNally, PhD, on behalf of Twist Medical.

The publication costs of this article were defrayed in part by the payment of publication fees. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Received June 22, 2022; revised October 31, 2022; accepted February 1, 2023; published first February 2, 2023.

References

1. Untch M, Fasching PA, Konecny GE, Hasmüller S, Lebeau A, Kreienberg R, et al. Pathologic complete response after neoadjuvant chemotherapy plus trastuzumab predicts favorable survival in human epidermal growth factor receptor 2–overexpressing breast cancer: results from the TECHNO trial of the AGO and GBG study groups. J Clin Oncol 2011; 29:3351–7.
2. de Azambuja E, Holmes AP, Piccart-Gebhart M, Holmes E, Cosimo SD, Swaby RF, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): survival outcomes of a randomised, open-label, multicentre, phase 3 trial and their association with pathological complete response. Lancet Oncol 2014;15:1137–46.
3. Gianni L, Eiermann W, Semiglazov V, Lluch A, Tjulandin S, Zambetti M, et al. Neoadjuvant and adjuvant trastuzumab in patients with HER2-positive locally advanced breast cancer (NOAH): follow-up of a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet Oncol 2014;15:640–7.
4. Schneeweiss A, Chia S, Hickish T, Harvey V, Eniu A, Waldron-Lynch M, et al. Long-term efficacy analysis of the randomised, phase II TRYPHAENA cardiac safety study: evaluating pertuzumab and trastuzumab plus standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer. Eur J Cancer 2018;89:27–35.
5. von Minckwitz G, Huang C-S, Mano MS, Loibl S, Mamounas EP, Untch M, et al. Trastuzumab emtansine for residual invasive HER2-positive breast cancer. N Engl J Med 2019;380:617–28.
6. de Haas SL, Hurvitz SA, Martin M, Kiermaier A, Lewis Phillips G, Xu J, et al. Biomarker analysis from the neoadjuvant KRISTINE study in HER2-positive early breast cancer. Presented at the San Antonio Breast Cancer Symposium; 2016 Dec 6–10; San Antonio, TX. Poster number P6-07-09.
7. Denkert C, Huober J, Loibl S, Prinzler J, Kronenwett R, Darb-Esfahani S, et al. HER2 and ESR1 mRNA expression levels and response to neoadjuvant trastuzumab plus chemotherapy in patients with primary breast cancer. Breast Cancer Res 2013;15:R11.
8. Schneeweiss A, Chia S, Hegg R, Tausch C, Deb R, Ratnayake J, et al. Evaluating the predictive value of biomarkers for efficacy outcomes in response to pertuzumab-and trastuzumab-based therapy: an exploratory analysis of the TRYPHAENA study. Breast Cancer Res 2014;16:R73.
9. Bianchini G, Kiermaier A, Bianchi GV, Im Y-H, Pienkowski T, Liu M-C, et al. Biomarker analysis of the NeoSphere study: pertuzumab, trastuzumab, and

docetaxel versus trastuzumab plus docetaxel, pertuzumab plus trastuzumab, or pertuzumab plus docetaxel for the neoadjuvant treatment of HER2-positive breast cancer. Breast Cancer Res 2017;19:16.
10. Prat A, Pascual T, De Angelis C, Gutierrez C, Llombart-Cussac A, Wang T, et al. HER2-enriched subtype and ERBB2 expression in HER2-positive breast cancer treated with dual HER2 blockade. J Nat Cancer Inst 2020;112:46–54.
11. Krop IE, Paulson J, Campbell C, Kiermaier AC, Andre F, Fumagalli D, et al. Genomic correlates of response to adjuvant trastuzumab (H) and pertuzumab (P) in HER2+ breast cancer (BC): Biomarker analysis of the APHINITY trial. J Clin Oncol 2019;37:1012.
12. Zhou X, Zhang J, Yun H, Shi R, Wang Y, Wang W, et al. Alterations of biomarker profiles after neoadjuvant chemotherapy in breast cancer: tumor heterogeneity should be taken into consideration. Oncotarget 2015;6:36894–902.
13. Niikura N, Tomotaki A, Miyata H, Iwamoto T, Kawai M, Anan K, et al. Changes in tumor expression of HER2 and hormone receptors status after neoadjuvant chemotherapy in 21,755 patients from the Japanese breast cancer registry. Ann Oncol 2016;27:480–7.
14. Parker JS, Mullins M, Cheang MCU, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27:1160–7.
15. Schettini F, Pascual T, Conte B, Chic N, Brasó-Maristany F, Galván P, et al. HER2-enriched subtype and pathological complete response in HER2-positive breast cancer: A systematic review and meta-analysis. Cancer Treat Rev 2020;84:101965.
16. Pogue-Geile KL, Song N, Jeong JH, Gavin PG, Kim SR, Blackmon NL, et al. Intrinsic subtypes, PIK3CA mutation, and the degree of benefit from adjuvant trastuzumab in the NSABP B-31 trial. J Clin Oncol 2015;33:1340–7.
17. Fernandez-Martinez A, Krop IE, Hillman DW, Polley M-Y, Parker JS, Huebner L, et al. Survival, pathologic response, and genomics in CALGB 40601 (Alliance), a neoadjuvant phase III trial of paclitaxel-trastuzumab with or without lapatinib in HER2-positive breast cancer. J Clin Oncol 2020;38:4184–93.
18. Carey LA, Berry DA, Cirrincione CT, Barry WT, Pitcher BN, Harris LN, et al. Molecular heterogeneity and response to neoadjuvant human epidermal growth factor receptor 2 targeting in CALGB 40601, a randomized phase III trial of paclitaxel plus trastuzumab with or without lapatinib. J Clin Oncol 2016;34:542–9.
19. Pernas S, Petit A, Climent F, Paré L, Perez-Martin J, Ventura L, et al. PAM50 subtypes in baseline and residual tumors following neoadjuvant trastuzumab-based chemotherapy in HER2-positive breast cancer: a consecutive-series from a single institution. Front Oncol 2019;9:707.
20. Brasó-Maristany F, Griguolo G, Pascual T, Paré L, Nuciforo P, Llombart-Cussac A, et al. Phenotypic changes of HER2-positive breast cancer during and after dual HER2 blockade. Nat Commun 2020;11:385.
21. Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 2004;6:117–27.
22. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 2007;12:395–402.
23. Gianni L, Bianchini G, Kiermaier A, Bianchi G, Im Y-H, Pienkowski T, et al. Neoadjuvant pertuzumab and trastuzumab: biomarker analyses of a 4-arm randomized phase II study (Neosphere) in patients with HER2-positive breast cancer. Cancer Res 2011;71(Suppl.):Abstract S5–1.
24. Baselga J, Cortés J, Im SA, Clark E, Ross G, Kiermaier A, et al. Biomarker analyses in CLEOPATRA: a phase III, placebo-controlled study of pertuzumab in human epidermal growth factor receptor 2–positive, first-line metastatic breast cancer. J Clin Oncol 2014;32:3753–61.
25. Loibl S, Majewski I, Guarneri V, Nekljudova V, Holmes E, Bria E, et al. PIK3CA mutations are associated with reduced pathological complete response rates in primary HER2-positive breast cancer: pooled analysis of 967 patients from five prospective trials investigating lapatinib and trastuzumab. Ann Oncol 2016;27:1519–25.
26. Perez EA, de Haas SL, Eiermann W, Barrios CH, Toi M, Im Y-H, et al. Relationship between tumor biomarkers and efficacy in MARIANNE, a phase III study of trastuzumab emtansine ± pertuzumab versus trastuzumab plus taxane in HER2-positive advanced breast cancer. BMC Cancer 2019;19:517–31.
27. Loi S, Michiels S, Lambrechts D, Fumagalli D, Claes B, Kellokumpu-Lehtinen P-L, et al. Somatic mutation profiling and associations with prognosis and trastuzumab benefit in early breast cancer. J. Natl Cancer Inst 2013;105:960–7.
28. Baselga J, Lewis Phillips GD, Verma S, Ro J, Huober J, Guardino AE, et al. Relationship between tumor biomarkers and efficacy in EMILIA, a phase III study of trastuzumab emtansine in HER2-positive metastatic breast cancer. Clin Cancer Res 2016;22:3755–63.
29. Kim SB, Wildiers H, Krop IE, Smitt M, Yu R, deHaas SL, et al. Relationship between tumor biomarkers and efficacy in TH3RESA, a phase III study of trastuzumab emtansine (T-DM1) vs. treatment of physician's choice in previously treated HER2-positive advanced breast cancer. Int J Cancer 2016;139:2336–42.
30. Metzger O, Lambertini C, Krop IE, Lewis Phillips G, Perou C, Symmans WF, et al. Biomarker analysis from KAITLIN, a randomised phase 3 study of adjuvant trastuzumab emtansine (TDM-1; K) plus pertuzumab (P) versus trastuzumab (H) plus taxane (T) plus P after anthracyclines (AC) for high-risk HER2-positive early breast cancer (EBC). ESMO Breast Cancer Virtual Congress 2021, Abstract 420.
31. Bianchini G, Gianni L. The immune system and response to HER2-targeted treatment in breast cancer. Lancet Oncol 2014;15:e58–68.
32. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol 2014;25:1544–50.
33. Denkert C, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2–positive and triple-negative primary breast cancers. J Clin Oncol 2015;33:983–91.
34. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner B, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol 2018;19:40–50.
35. Kim SR, Song N, Gavin PG, Salgado R, Bandos H, Kos Z, et al. Stromal tumor-infiltrating lymphocytes in NRG Oncology/NSABP B-31 Adjuvant Trial for early-stage HER2-positive breast cancer. J Natl Cancer Inst 2019;111:867–71.
36. Bianchini G, Pusztai L, Piekowski T, Im Y-H, Bianchi GV, Tseng L-M, et al. Immune modulation of pathologic complete response after neoadjuvant HER2-directed therapies in the NeoSphere trial. Ann Oncol 2015;26:2429–36.
37. Perez EA, Ballman KV, Tenner KS, Thompson EA, Badve SS, Bailey H, et al. Association of stromal tumor-infiltrating lymphocytes with recurrence-free survival in the N9831 adjuvant trial in patients with early-stage HER2-positive breast cancer. JAMA Oncol 2016;2:56–64.
38. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. *limma* powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47.
39. Law CW, Chen Y, Shi W, Smyth GK. Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Genome Biol 2014;15:R29.
40. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102:15545–50.
41. Paquet ER, Hallett MT. Absolute assignment of breast cancer intrinsic molecular subtype. J Natl Cancer Inst 2015;107:1–9.
42. Pogue-Geile KL, Kim C, Jeong J-H, Tanaka N, Bandos H, Gavin PG, et al. Predicting degree of benefit from adjuvant trastuzumab in NSABP trial B-31. J Natl Cancer Inst 2013;105:1782–8.
43. Scaltriti M, Nuciforo P, Bradbury I, Sperinde J, Agbor-Tarh D, Campbell C, et al. High HER2 expression correlates with response to the combination of lapatinib and trastuzumab. Clin Cancer Res 2015;21:569–76.
44. Fumagalli D, Venet D, Ignatiadis M, Azim HA Jr Maetens M, Rothé F, et al. RNA sequencing to predict response to neoadjuvant anti-HER2 therapy: a secondary analysis of the NeoALTTO randomized clinical trial. JAMA Oncol 2017;3:227–34.
45. Schettini F, Brasó-Maristany F, Kuderer NM, Pratt A. A perspective on the development and lack of interchangeability of the breast cancer intrinsic subtypes. NPJ Breast Cancer 2022;8:85.
46. Eichhorn PJ, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, et al. Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. Cancer Res 2008;68:9221–30.
47. Perez EA, Barrios C, Eiermann E, Toi M, Im Y-H, Conte P, et al. Trastuzumab emtansine with or without pertuzumab versus trastuzumab plus taxane for human epidermal growth factor receptor 2–positive, advanced breast cancer:

primary results from the phase III MARIANNE Study. J Clin Oncol 2017;35:141–8.
48. Perez EA, Thompson EA, Ballman KV, Anderson SK, Asmann YW, Kalari KR, et al. Genomic analysis reveals that immune function genes are strongly linked to clinical outcome in the North Central Cancer Treatment Group N9831 Adjuvant Trastuzumab Trial. J Clin Oncol 2015;33:701–8.
49. Matikas A, Zerdes I, Lovrot J, Richard F, Sotiriou C, Bergh J, et al. Prognostic implications of PD-L1 expression in breast cancer: systematic review and meta-analysis of immunohistochemistry and pooled analysis of transcriptomic data. Clin Cancer Res 2019;25:5717–26.
50. Sabatier R, Finetti P, Mamessier E, Adelaide J, Chaffanet M, Ali HR, et al. Prognostic and predictive value of PDL1 expression in breast cancer. Oncotarget 2015;6:5449–64.
51. Loibl S, Huang C-S, Mano MS, Mamounas EP, Geyer CE, Untch M, et al. Adjuvant trastuzumab emtansine in breast cancer patients with HER2-negative residual invasive disease in KATHERINE. NPJ Breast Cancer 2022;8:106.