original reports

# HER2 Expression, Test Deviations, and Their Impact on Survival in Metastatic Gastric Cancer: Results From the Prospective Multicenter VARIANZ Study

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abstract

**PURPOSE** Trastuzumab is the only approved targeted drug for first-line treatment of human epidermal growth factor receptor 2–positive (HER2+) metastatic gastric cancer (mGC). However, not all patients respond and most eventually progress. The multicenter VARIANZ study aimed to investigate the background of response and resistance to trastuzumab in mGC.

**METHODS** Patients receiving medical treatment for mGC were prospectively recruited in 35 German sites and followed for up to 48 months. HER2 status was assessed centrally by immunohistochemistry and chromogenic in situ hybridization. In addition, *HER2* gene expression was assessed using qPCR.

**RESULTS** Five hundred forty-eight patients were enrolled, and 77 had HER2+ mGC by central assessment (14.1%). A high deviation rate of 22.7% between central and local test results was seen. Patients who received trastuzumab for centrally confirmed HER2+ mGC (central HER2+/local HER2+) lived significantly longer as compared with patients who received trastuzumab for local HER2+ but central HER2− mGC (20.5 months, n = 60 *v* 10.9 months, n = 65; hazard ratio, 0.42; 95% CI, 8.2 to 14.4; $P < .001$). In the centrally confirmed cohort, significantly more tumor cells stained HER2+ than in the unconfirmed cohort, and the *HER2* amplification ratio was significantly higher. A minimum of 40% HER2+ tumor cells and a *HER2* amplification ratio of ≥ 3.0 were calculated as optimized thresholds for predicting benefit from trastuzumab.

**CONCLUSION** Significant discrepancies in HER2 assessment of mGC were found in tumor specimens with intermediate HER2 expression. Borderline HER2 positivity and heterogeneity of HER2 expression should be considered as resistance factors for HER2-targeting treatment of mGC. HER2 thresholds should be reconsidered. Detailed reports with quantification of *HER2* expression and amplification levels may improve selection of patients for HER2-directed treatment.

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**ASSOCIATED CONTENT**

**Appendix**

Author affiliations and support information (if applicable) appear at the end of this article.

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## INTRODUCTION

With more than 1 million estimated new cases annually, gastric cancer (GC) is the fifth most commonly diagnosed malignancy worldwide. Mortality from GC is high. With 784,000 deaths in 2018, GC was the third most common cause of cancer-related death globally.[1] Human epidermal growth factor receptor 2 (HER2) overexpression is seen in 12%-30% of GCs. Esophagogastric junction cancers (EGJC) are more likely to be HER2-positive (HER2+) than distal GCs.[2-6]

Trastuzumab, a humanized HER2-targeting immunoglobulin G1 monoclonal antibody in combination with chemotherapy, was approved for first-line treatment of HER2+ metastatic (m)GC based on the randomized controlled Trastuzumab for Gastric Cancer (ToGA) study.[7] However, the improvement in median overall survival (OS) in the subgroup, which was used to license treatment with trastuzumab, of 4.2 months compared with chemotherapy alone was moderate and duration of response is limited in the majority of patients.[7]

Other HER2-targeting drugs such as the tyrosine kinase inhibitor lapatinib and the antibody-drug conjugate trastuzumab-emtansine failed to improve OS in phase III studies in HER2+ metastatic gastric cancer (mGC).[8-10] Also, adding pertuzumab to trastuzumab and chemotherapy did not improve OS.[11] Finally, trastuzumab treatment beyond progression

## CONTEXT

**Key Objective**

We conducted a prospective multicenter study to identify mechanisms of resistance to anti-human epidermal growth factor receptor 2 (HER2)–targeted treatment in patients with metastatic gastric cancer.

**Knowledge Generated**

In more than one of five patients, local and central HER2 results were found to be divergent between local and central pathology assessment. Benefit from trastuzumab in metastatic gastric cancer was found to be limited to patients with centrally confirmed HER2 overexpression. Optimized HER2 expression thresholds by immunohistochemistry and *HER2* gene amplification by in situ hybridization were calculated for predicting benefit from trastuzumab.

**Relevance**

Detailed and quality controlled reports of HER2 test results should be provided in routine use to better select patients who benefit from trastuzumab and limit the risk of overtreatment in patients with gastric cancer where HER2 is not a strong enough oncogenic driver.

in combination with second-line paclitaxel failed to prolong survival.[12]

Patients and the GC medical community are eagerly waiting for better treatment outcomes for HER2+ mGC with novel HER2-targeting drugs and combinations such as Margetuximab, a next-generation anti-HER2 monoclonal antibody with an optimized Fc domain and enhanced antibody-dependent cell-mediated cytotoxicity[13,14]; the antibody-drug conjugate Trastuzumab-Deruxtecan[15]; Poziotinib (HM781-36B), an irreversible pan-HER tyrosine kinase inhibitor[16]; and the combination of trastuzumab and pembrolizumab[17]; among others. Recently presented data demonstrated that Trastuzumab-Deruxtecan is also active in patients with HER2 low tumors.[18]

Although some complete durable tumor responses in trastuzumab-treated patients were reported,[19-22] most patients experience initial or acquired resistance. Beyond the potential impact of tumor heterogeneity and evolution,[23,24] activation of the PI3K/Akt/mammalian target of rapamycin signaling pathway and FGFR2 alterations were identified as potential mechanisms of resistance to HER2-targeted therapy.[25-28] Loss of HER2 expression during trastuzumab treatment was observed and may be a reason for acquired trastuzumab resistance.[12,29-31]

HER2 assessment according to published guidelines[4] and the use of trastuzumab in combination with chemotherapy are standard of care for HER2+ mGC.[32,33] The survival benefit seen in ToGA was reproduced in retrospective[34] and noninterventional studies.[21,35] However, assessment of HER2 in GC has repeatedly been described as challenging. Confirmation in multiple tumor specimens has been recommended to ensure accuracy.[36] Intratumoral HER2 heterogeneity was found in 26% of patients and was associated with worse survival.[37]

Since most evidence thus far is based either on small and monocentric reports or on post hoc analyses, we conducted a prospective multicenter study (VARIANZ; NCT02305043) to determine factors predicting trastuzumab response or resistance in HER2+ mGC.

## METHODS

### Study Design and Cohort

Patients receiving medical treatment for histologically confirmed mGC (including EGJC) were recruited at 35 German centers after having given their informed written consent. The objective was to recruit at least 500 patients to enroll a minimum of 50 patients with HER2+ cancers by central assessment. The study conduct followed the REMARK guidelines.[38]

The VARIANZ study was part of a national research project called "Identification of predictive response and resistance factors to targeted therapy in gastric cancer using a systems medicine approach–SYS Stomach," which was supported by the German Federal Ministry for Education and Research. VARIANZ was performed in accordance with Good Clinical Practice and the Declaration of Helsinki. Approvals of the ethics committees of Leipzig University Medical Faculty and all participating centers were obtained before activation of sites.

Patient data were collected for medical history, cancer-specific evaluation, and GC-directed treatment. Patients were followed for a maximum of 80 months. Formalin-fixed and paraffin-embedded tumor tissue was sent to the central pathology institute at Leipzig University Medical Center.

### HER2 Tests

HER2 status was tested according to published standards[4,39] in the central pathology by two GI pathologists (K.S. and C.W.) using immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH).

IHC was done with the antibody HI608C01 (DCS, Hamburg, Germany), known for high specificity.[40] Tumors were

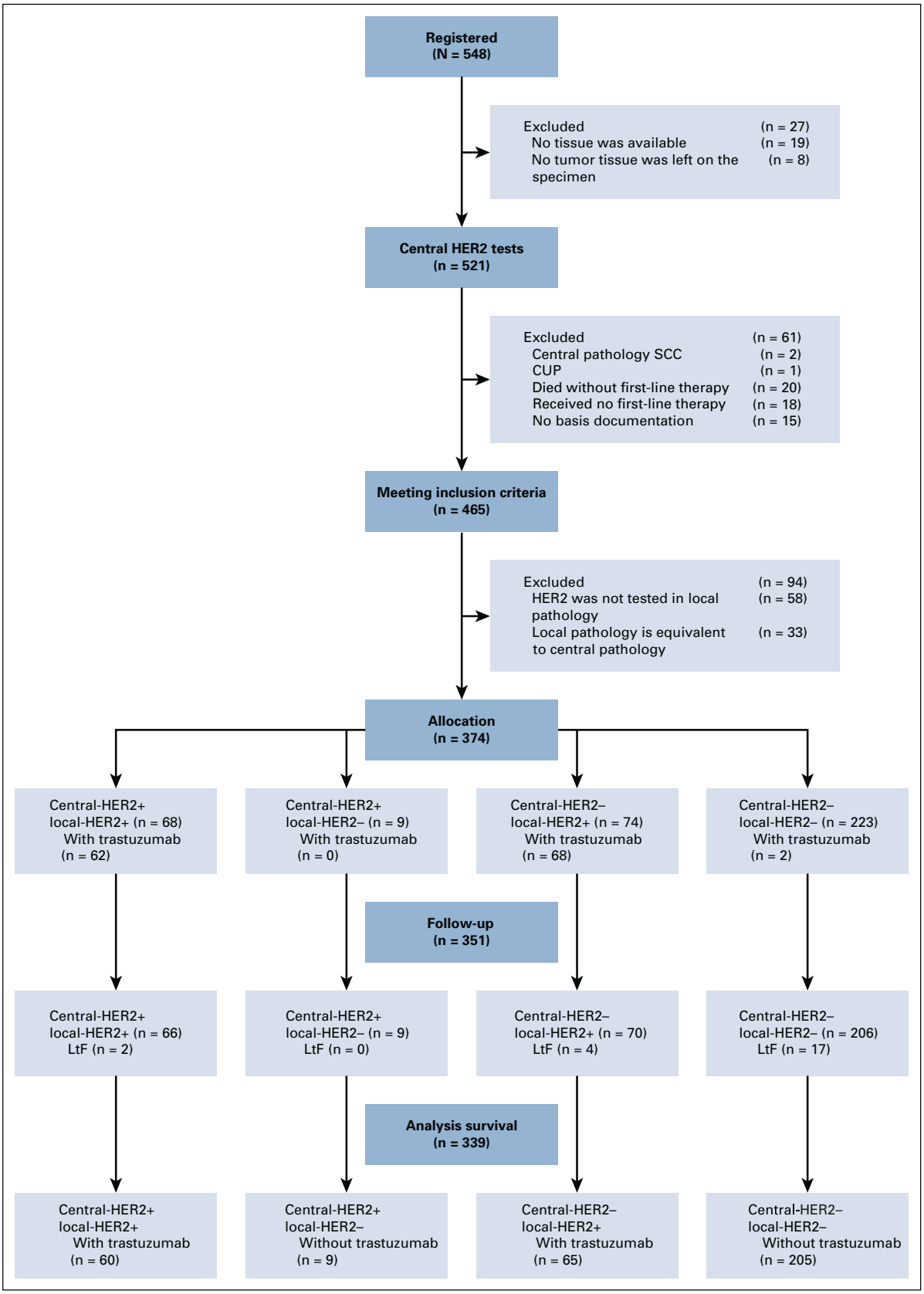


**FIG 1.** CONSORT diagram of the VARIANZ study. Patients were assigned to groups according to central HER2 test results, confirmation of local HER2 status, and treatment with trastuzumab. CUP, carcinoma of unknown primary; HER2, human epidermal growth factor receptor 2; Ltf, lost to follow-up; SCC, squamous cell carcinoma.

**TABLE 1.** Patient Characteristics and Patient Treatment Groups According to Central HER2 Status and Confirmation of Local HER2 Status

| Patient Characteristic | Central-HER2+ Local-HER2+ (HER2+/HER2+) Concordant HER2 | Central-HER2+ Local-HER2− (HER2+/HER2−) Deviating HER2 | Central-HER2− Local-HER2+ (HER2−/HER2+) Deviating HER2 | Central-HER2− Local-HER2− (HER2−/HER2−) Concordant HER2 | Overall Population |
|---|---|---|---|---|---|
| No. of patients | n = 60 | n = 9 | n = 65 | n = 205 | N = 339 |
| Age (mean) | 69.0 ± 12.2 | 64.1 ± 8.6 | 68.2 ± 11.0 | 63.9 ± 11.1 | 65.6 ± 11.4 |
| Sex (male/female) | 46/14 | 9/0 | 55/10 | 129/76 | 239/100 |
| BMI (mean) | 26.1 ± 3.2 | 25.8 ± 5.1 | 24.2 ± 3.7 | 24.5 ± 4.9 | 24.8 ± 4.5 |
| ECOG | | | | | |
| 0-1 | 52 | 5 | 60 | 184 | 301 |
| > 1 | 7 | 3 | 4 | 17 | 31 |
| Primary tumor location | | | | | |
| Cardia cancer (EGJ I-III) | 41 | 7 | 28 | 96 | 172 |
| Noncardia GC | 19 | 2 | 34 | 107 | 162 |
| First-line treatment | | | | | |
| Chemotherapy-mono | 6 | 0 | 5 | 8 | 19 |
| Chemotherapy-doublet | 40 | 3 | 41 | 89 | 173 |
| Chemotherapy-triplet | 14 | 6 | 17 | 96 | 133 |
| Study treatment | 0 | 0 | 2 | 12 | 14 |
| Trastuzumab | 60 | 0 | 65 | 0 | 125 |
| Primary tumor resection | | | | | |
| Yes | 12 | 2 | 25 | 84 | 123 |
| No | 48 | 7 | 40 | 121 | 216 |
| R0 | 11 | 2 | 17 | 57 | 87 |
| Grading | | | | | |
| Grade 1-2 | 41 | 2 | 22 | 56 | 121 |
| Grade 3-4 | 19 | 7 | 42 | 148 | 216 |
| No. of metastatic sites | | | | | |
| 1 | 33 | 4 | 31 | 130 | 198 |
| > 1 | 27 | 5 | 34 | 74 | 140 |
| Best response | | | | | |
| Complete response | 3 | 0 | 4 | 8 | 15 |
| Partial response | 19 | 1 | 14 | 35 | 69 |
| Stable disease | 19 | 1 | 18 | 55 | 93 |
| Progressive disease | 11 | 4 | 18 | 79 | 112 |
| Central HER2 IHC score | | | | | |
| 0-1 | 0 | 0 | 44 | 179 | 223 |
| 2 | 11 | 4 | 21 | 26 | 62 |
| 3 | 49 | 5 | 0 | 0 | 54 |
| Local HER2 IHC score | | | | | |
| 0-1 | 0 | 6 | 0 | 149 | 155 |
| 2 | 2 | 1 | 8 | 22 | 33 |
| 3 | 51 | 0 | 51 | 0 | 102 |

Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; EGJ, esophagogastric junction; GC, gastric cancer; HER2, human epidermal growth factor receptor 2; HER2−, HER2 negative; HER2+, HER2 positive; IHC, immunohistochemistry; R0, complete resection.

classified as HER2+ if at least 10% of tumor cells stained positive in resection specimens and if at least five adjacent tumor cells stained positive in biopsies (IHC-score 3+ and 2+ if confirmed by CISH).[39] In cases with more than one specimen sent to central pathology, one HER2 test per patient was taken for evaluation in the following order: first HER2 test of endoscopic biopsies, second resection specimen, and only if no other specimen was sent, HER2 tests of metastatic sites. For the detailed IHC report, the percentage of HER2+ tumor cells in relation to all tumor cells was calculated. Local HER2 testing was performed according to local standards. The use of specific antibodies, certification of the laboratory, or participation in round robin tests was not requested, in line with the real-world approach of HER2 testing in Germany. *HER2* gene amplification was determined by CISH using the Zytomed System (C-3022-40, Bremerhaven, Germany). For gene expression analysis, RNA was extracted from formalin-fixed and paraffin-embedded tissue (n = 102) as described previously by STRATIFYER Molecular Pathology GmbH, Cologne, Germany.[41] RT-qPCR was applied for relative quantification of *HER2* mRNA, and *CALM2* (calmodulin 2; housekeeping gene) expression was determined by using gene-specific TaqMan-based assays as published previously.[42]

### Data Analyses or Statistical Methods

VARIANZ was designed as an observational study. For this reason, we did not establish a detailed statistical analysis plan and included rough estimates for the expected number of observed cases. Data management used the BioXM Knowledge Management Environment version 6.0 provided by Biomax, which integrated clinical and molecular data.

Survival data were analyzed using the Kaplan-Meier estimation and Cox regression analysis available in Matlab R2016b. For the Kaplan-Meier estimation, significant differences between patient groups were assessed using the log-rank test. For the Cox regression analysis, the 95% CI was calculated for the estimated hazard ratios (HRs) to determine significance. The analysis set comprised all patients belonging to one of the following groups: HER2-positive concordant with local report (HER2+/HER2+), HER2-positive deviating from local report (HER2+/HER2–), HER2− deviating from local report (HER2−/HER2+), and HER2− concordant with local report (HER2−/HER2−; Fig 1).

The optimal cutoff value of HER2 IHC, *HER2/CEP17* amplification ratio, and *HER2* gene expression was obtained based on the Cox regression *P* value. The threshold was used to divide the patients into high- and low-risk groups. We defined values higher than or equal to the optimal cutoff value as positive marker or high expression, whereas lower values were defined as negative marker or low expression.

For gene expression analyses, a selection of the HER2− patients (HER2−/HER2−) was analyzed to compare with trastuzumab-treated patients (HER2+/HER2+ and HER2−/HER2+). This HER2− subgroup was selected to match age, sex, and tumor type distributions of the other groups. This was performed by iteratively selecting random patients minimizing the Kolmogórov-Smirnov statistic.

## RESULTS

### Study Cohort

VARIANZ recruited 548 patients with mGC in 35 German study centers between May 2014 and January 2018. Five hundred twenty-one patients were centrally tested for HER2. In 27 patients, no sufficient tumor material for central HER2 testing was available. As outlined in Figure 1, 465 patients met all inclusion criteria, and all necessary information about medical history, treatment, and follow-up was supplied by investigators. For 351 patients, sufficient follow-up data were collected. Patient characteristics are listed in Table 1.

No significant differences were detected between HER2+ and HER2− patients regarding age, sex, ECOG performance status, body mass index, tumor localization, and grading and number of metastatic sites.

For 374 patients, the HER2 status was determined in both central and local pathology institutes. In 77 cases (20.6%), central testing revealed HER2+ mGC. However, in 22.7% of patients, the locally assessed HER2 status was not confirmed by central testing (n = 83, Fig 1). In the majority of these cases, a local HER2+ status was not confirmed centrally (HER2−/HER2+; n = 74), whereas in nine patients, HER2 was tested positive centrally in contrast to a negative local assessment (HER2+/HER2−).

### HER2 Test Deviations

To examine reasons for HER2 deviations between central and local testing, we quantified the number and percentage of tumor cells stained HER2+. Examples of HER2 staining in VARIANZ are given in Figure A1 (online only). We found expression to be higher and more homogenous in the HER2+/HER2+ population as compared with the HER2−/HER2+ population (58.6% ± 31.4% [SD], n = 60) *v* 12.8% ± 19.6% [SD], n = 65); $P < .001$; Fig 2A). Similarly, a higher *HER2/CEP17* ratio was found in the HER2+/HER2+ cohort (n = 56) (7.2 ± 5.8 [SD] *v* the HER2−/HER2+ cohort of patients (n = 59); 1.5 ± 0.9 [SD]; $P < .001$; Fig 2B). Furthermore, *HER2* gene expression (ΔCt) was higher in confirmed HER2+/HER2+ tumors (n = 27) (41.8 ± 1.6 [SD] *v* HER2−/HER2+ tumors (n = 28); 39.1 ± 1.7 [SD]; $P < .001$) although the number of tumor specimens left for analysis was limited (Fig 2C).

In 75% of the patients, tumor specimens originating from the same location (endoscopic biopsy of the primary tumor, or resection specimen, or biopsy from metastases) were tested for HER2 in both the central and local pathologies.

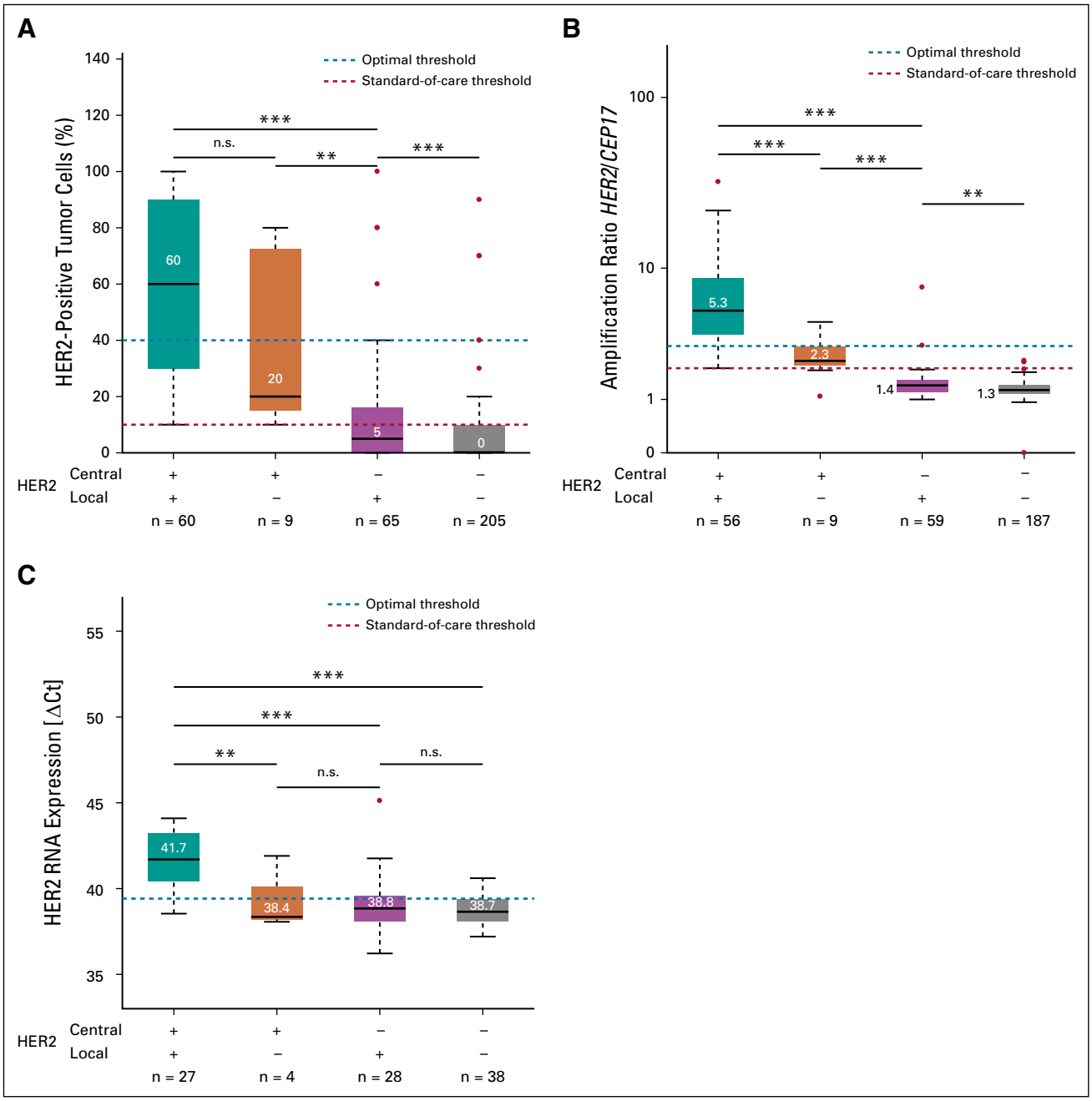


**FIG 2.** Detailed central HER2 test results: number of tumor cells staining HER2+ by immunohistochemistry (A), amplification ratio for *HER2/CEP17* by chromogenic in situ hybridization (B), and *HER2* gene expression (ΔCt) (C) according to central HER2 status, central confirmation of HER2 status, and treatment with trastuzumab. Significant differences between the patient groups are calculated using the one-way analysis of variance test. Thresholds displayed are used in routine HER2 assessment or are calculated as optimized thresholds, best separating overall survival (Fig 4) of patients treated with trastuzumab. Significance is shown for *$.01 < P \leq .05$, **$.001 < P \leq .01$, and ***$P \leq .001$. HER2, human epidermal growth factor receptor 2; HER2−, HER2-negative; HER2+, HER2-positive.

For the remainder, tumor specimens originated from separate locations. More than 60% were endoscopic biopsies from the primary tumor. Frequency in deviating and concordant HER2 test results did not differ between the two groups.

In 45 patients, specimens from more than one location were tested in the central pathology. In nine patients, samples were from the same tumor location, and in 36 patients, they originated from different locations. Of all 45 patients with more than one available specimen tested in the central pathology, only in four patients (8.9%), HER2 tests deviated between endoscopic biopsies, resection specimen, or biopsies from metastases. In two cases, biopsies were tested positive for HER2, whereas resection

specimen or metastases were HER2− and vice versa in the other two discordant cases.

### Survival

Patients with centrally confirmed HER2+ mGC (HER2+/HER2+, n = 60) had significantly longer OS when treated with trastuzumab plus chemotherapy as compared with HER2−/HER2+ tested patients who were treated with trastuzumab plus chemotherapy (n = 65) (20.5 months [95% CI, 15.7 to 31.5] *v* 10.9 months [95% CI, 8.2 to 14.4; HR, 0.42]; $P < .001$; Fig 3).

OS of trastuzumab-treated patients with centrally unconfirmed HER2+ mGC (HER2−/HER2+ [n = 65] was not significantly different from that of trastuzumab-untreated HER2-tested patients, Fig 3).

Patients with HER2+/HER2− mGC (n = 9) and patients with centrally confirmed HER2− (HER2−/HER2−) mGC (n = 205) did not receive trastuzumab. In HER2+/HER2− mGC (n = 9), all central HER2 results were obtained after the start of first-line therapy (mean 67 days [95% CI, 9 to 407 days]). Consequently, the initially chosen treatment regimen was not changed in these patients, which was in accordance with the noninterventional character of the VARIANZ study.

### HER2 Expression Thresholds

To suggest alternative criteria for HER2-directed treatment, we determined the thresholds optimizing the statistical significance for survival benefit. We found that a portion of 40% of HER2+ tumor cells in biopsies and resection specimen was an optimized threshold for treatment with trastuzumab. The mean survival in patients with ≥ 40% of tumor cells staining positive for HER2 was 20.5 months (95% CI, 15.6 to 32.6) versus 11.4 months (95% CI, 9.3 to 15.0; HR, 0.46; $P = .001$; Fig 4A). The optimized *HER2/CEP17* ratio for indicating benefit from trastuzumab was 3.0 with a median survival of 22.8 months (95% CI, 16.5 to 81.7) in patients with an *HER2/CEP17* ratio ≥ 3.0 versus 11.7 months (95% CI, 9.3 to 14.8) for patients with an *HER2/CEP17* ratio < 3.0 (HR, 0.36; $P < .001$; Fig 4B). For *HER2* gene expression, the optimized threshold was 39.41 ΔCT. The median survival in patients with ΔCT > 39.41 was 22.8 months (95% CI, 10.0 to 29.5) versus 5.0 months (95% CI, 2.5 to 9.0; HR, 0.28; $P < .001$; Fig 4C).

## DISCUSSION

Treatment of metastatic GC and EGJC remains challenging. Trastuzumab, the only approved anti-HER2–targeted therapy in mGC in the year 2021, improves survival in HER2+ patients,[7,34] but response rates are still moderate and the majority of initial responders eventually experience progression. Here, we report the results from a prospective multicenter noninterventional study including 125 trastuzumab-treated patients, suggesting that trastuzumab-treated patients with deviating HER2 test results, performed by two independent laboratories, do not benefit from trastuzumab. Patients with equivocal HER2 test results show only intermediate HER2 expression, characterized by < 40% of tumor cells staining positive for HER2. These patients did seemingly not benefit from the addition of trastuzumab to chemotherapy in our study as OS equals the survival of HER2− patients who were not treated with

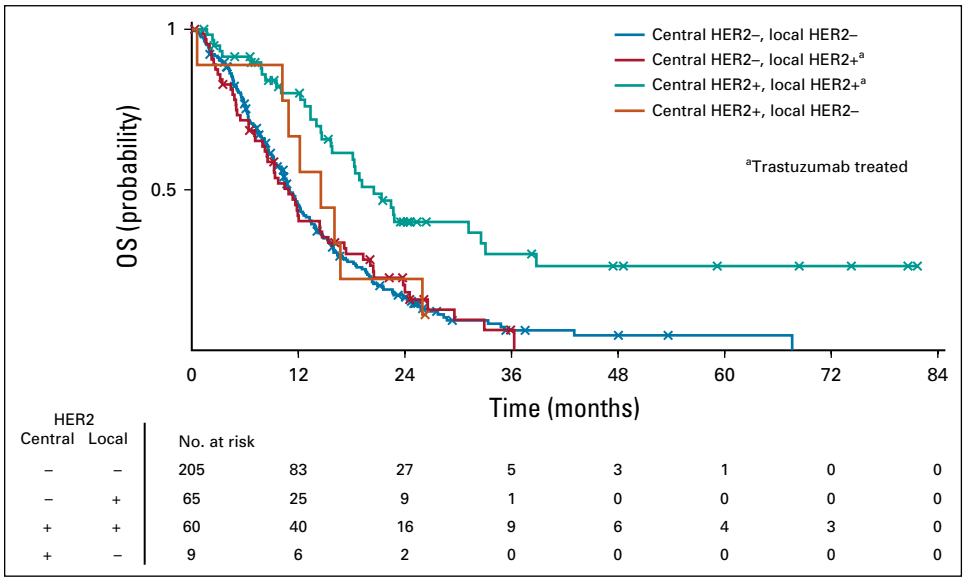


**FIG 3.** OS of patients according to central HER2 status (HER2+ [green], HER2− [blue]) and central confirmation of HER2 status (green and blue represent confirmed HER2 status, and red represents deviating HER2 status). [a]Treatment with trastuzumab. HER2, human epidermal growth factor receptor 2; HER2−, HER2-negative; HER2+, HER2-positive. OS, overall survival.

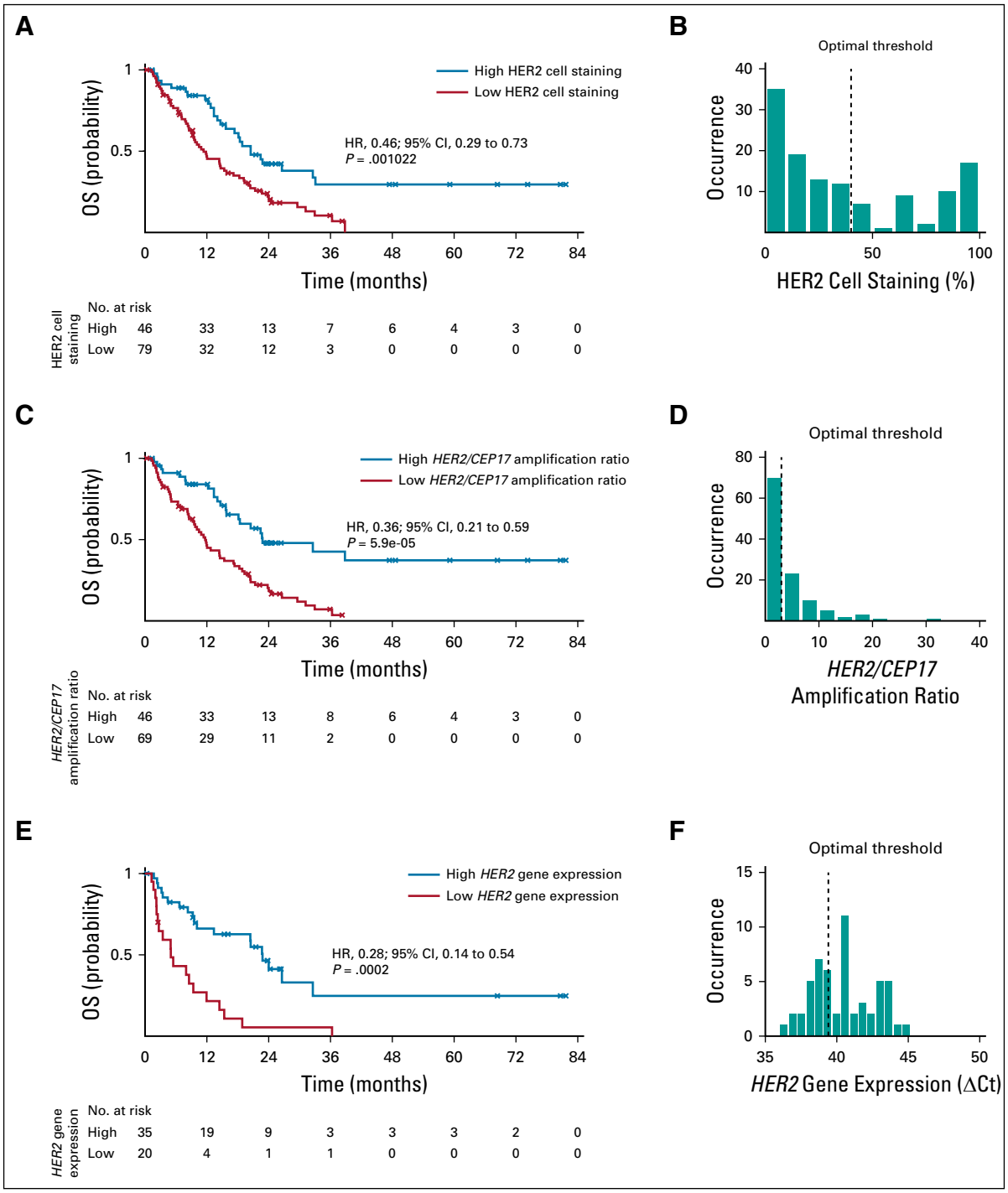


**FIG 4.** Calculation of optimized thresholds best separating OS of patients treated with trastuzumab. (A) Number of tumor cells staining HER2+ by immunohistochemistry, (B) amplification ratio for HER2/CEP17 by chromogenic in situ hybridization, and (C) *HER2* gene expression (ΔCt). HER2, human epidermal growth factor receptor 2; HR, hazard ratio; OS, overall survival.

trastuzumab. Survival of trastuzumab-treated patients with confirmed HER2+ status, in contrast, was longer in our study compared with other studies.[7] Elevated thresholds for diagnosing HER2 positivity could be the reason according to our analyses. Our results suggest optimized thresholds to be used for patient selection in the future: ≥ 40% of HER2+ tumor cells by IHC, and a gene amplification ratio of ≥ 3.0 *HER2/CEP17* selects those patients who benefit from trastuzumab.

Heterogeneity of HER2 expression is evident for GC and EGJC. HER2 tests were routinely performed at participating centers according to recommended algorithms.[43] IHC of

HER2 is well-established for the characterization of mGC in Germany, but deviating results between central and local testing were reported previously and were not influenced by the type of pathology (university, private practice, or others), fixation method, commercial kit, antibody manufacturer, number of tumor-carrying documented samples, or regular participation in round robin tests.[2] Longer OS was seen in trastuzumab-treated patients with homogenous mGC HER2 expression (100% of HER2+ stained tumor cells), and response rates were improved compared with heterogenous mGC.[22] Interestingly, even less HER2 homogeneity was found in our cohort compared with Korean and Japanese cohorts.[22,44] Our data also demonstrate that for patients with more than one tumor specimen tested in the central pathology, a minor deviation rate for HER2 results (in only four of 45 patients) was found, despite the fact that most tests were performed in specimens originating from different tumor locations such as primary tumor biopsy, resection specimen, and metastasis (36 of 45 patients). Deviating HER2 results between two probes tested within one pathological institute were recently reported in 25% of patients, when two surgical specimens were analyzed.[40] When multiple endoscopic biopsies were tested, 26% of patients showed deviating HER2 results and these were associated with worse survival.[37] However, these results were obtained in a retrospective case series of limited sample size, which requires validation.

Additionally, intermediate HER2 IHC2+ scores were more often detected in patients with trastuzumab-resistant compared with trastuzumab-sensitive mGC,[27] and patients with IHC2+ scores had worse survival than patients with IHC3+ mGC when treated with trastuzumab.[29] The level of HER2 amplification was also shown to be prognostic in trastuzumab-treated patients.[30,35]

We conclude that benefit from trastuzumab seems to be limited in patients with intermediate and heterogenous HER2-expressing mGC. Furthermore, loss of HER2 positivity was observed in several rebiopsy studies[29-31] and may also be assessed by liquid biopsies during HER2-directed therapy.[45]

Our study has some obvious limitations. It is a noninterventional study, which, for methodological reasons, has no control arm. In the decade after the ToGA trial, conduct of a randomized trial evaluating trastuzumab benefit of HER2+ patients with intermediate HER2 expression would have been impossible for ethical reasons. Second, the percentage of HER2-positive cells was much higher in concordant cases and the question whether the central pathologist was less likely to call a low HER2-positive tumor positive or a low HER2-positive tumor second slide from the block was more likely to be negative could not be addressed. Obvious strengths are the prospective design, the availability of tumor material at one central laboratory allowing for quality-controlled assessment of HER2 status, and the homogenous data set received from the participating centers.

Our results suggest that for routine HER2 assessment, more detailed reporting of HER2 test results should be provided and an elevated threshold for HER2 expression (40% of HER2+ stained tumor cells) and HER2 amplification (ratio 3.0 *HER2/CEP17*) should be considered to better select patients who benefit from trastuzumab. Furthermore, stratifying patients in HER2-targeting interventional trials according to HER2 expression levels could give a proof of this concept and eventually limit the risk of overtreatment and failing trials.


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## PRIOR PRESENTATION

Presented at the Multidisciplinary Gastrointestinal Cancer Meeting (ASCO-GI) 2017 in San Francisco, CA (abstract #12); Annual Meeting of the American Association for Cancer Research (AACR) 2018, Chicago, IL (abstract #2615); 13th International Gastric Cancer Congress (IGCC) 2019, Prague (Czech Republic) Best abstract talk #0783.

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## CLINICAL TRIAL INFORMATION

VARIANZ; NCT02305043

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

**HER2 Expression, Test Deviations, and Their Impact on Survival in Metastatic Gastric Cancer: Results From the Prospective Multicenter VARIANZ Study**

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## APPENDIX

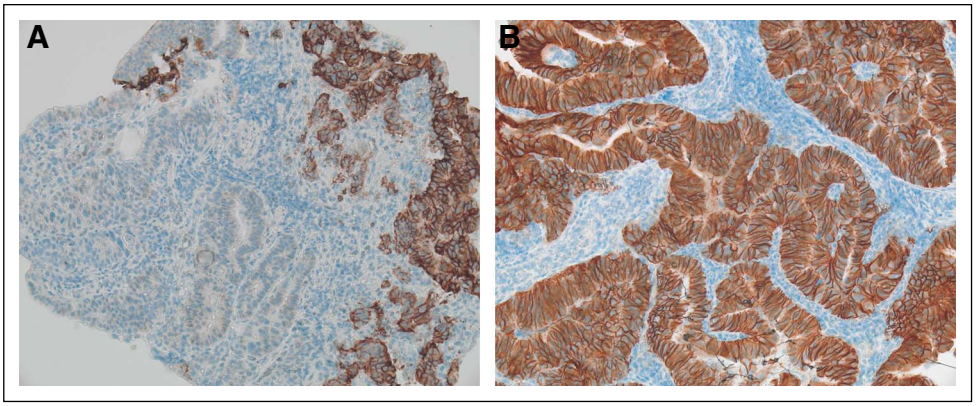


**FIG A1.** HER2 staining examples in the VARIANZ study. (A) Example of intratumoral heterogeneity: unstained HER2 negative tumor cells on the left side, some normal stomach glands in the middle, and strongly staining HER2-positive tumor cells on the right side, (B) example of homogenously HER2-positive staining tumor cells. HER2, human epidermal growth factor receptor 2.