original reports abstract

ERBB2 Copy Number as a Quantitative Biomarker for Real-World Outcomes to Anti–Human Epidermal Growth Factor Receptor 2 Therapy in Advanced Gastroesophageal Adenocarcinoma

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PURPOSE Human epidermal growth factor receptor 2 (HER2) overexpression or amplification (*ERBB2*amp) are biomarkers for approved anti-HER2 therapies. *ERBB2*amp may better predict response compared with immunohistochemistry or in situ hybridization, and quantitative copy number (CN) may further stratify patients. We characterized *ERBB2*amp in advanced gastroesophageal adenocarcinomas (GEA) and hypothesized that increased CN was associated with better outcome to trastuzumab.

METHODS Comprehensive genomic profiling, including assessment of *ERBB2*amp, was performed for 12,905 GEA tissue cases. Clinical outcomes were assessed using a clinicogenomic database linking deidentified electronic health record–derived clinical data to genomic data. Multivariable Cox proportional hazard models were used for real-world progression-free survival (rwPFS) comparisons.

RESULTS *ERBB2*amp (CN ≥ 5) was detected in 15% (1,934 of 12,905) of GEA; median CN 22 (interquartile range 9-73). Median *ERBB2* amplicon size was 0.27 megabase (interquartile range 0.13-0.95), and smaller amplicons were associated with higher CN ($P < .001$). In the clinicogenomic database, of 101 evaluable first-line trastuzumab-treated patients, *ERBB2* CN was a significant predictor of rwPFS as a continuous variable (adjusted hazard ratio = 0.73; 95% CI, 0.60 to 0.89; $P = .002$), whereas *ERBB2* CN was not predictive of rwPFS on chemotherapy (adjusted hazard ratio = 0.93; 95% CI, 0.73 to 1.20; $P = .59$). Among trastuzumab-treated patients, no significant associations with *ERBB2* CN were observed for disease site, age, stage at advanced diagnosis, or most selected coalterations.

CONCLUSION *ERBB2*amp was detected in 15% of GEA tissue samples, with significant diversity in *ERBB2* CN and amplicon focality. *ERBB2* CN was predictive of rwPFS as a continuous variable for patients treated with trastuzumab. Further studies exploring the clinical utility of quantitative *ERBB2* CN, particularly in the setting of the evolving anti-HER2 landscape and combination therapies, are warranted.

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ASSOCIATED CONTENT

Data Supplement

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

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INTRODUCTION

The *ERBB2* gene encodes the human epidermal growth factor receptor 2 (HER2) receptor tyrosine kinase (RTK), a member of the epidermal growth factor receptor (EGFR)-related family of RTKs, and ligand-independent activation of HER2 can result from HER2 overexpression or *ERBB2* amplification (*ERBB2*amp).[1-4] HER2-targeted therapy revolutionized cancer care by providing one of the first demonstrations of clinical utility of biomarker-selected approaches and led to the approval of anti-HER2 therapies in breast cancers with HER2 overexpression.[5-7] The anti-HER2 therapy trastuzumab is also approved in advanced gastroesophageal adenocarcinoma (advGEA) in combination with first-line chemotherapy on the basis of results from the Trastuzumab for Gastric Cancer trial,[8] and investigational use of anti-HER2 therapies is being explored in other tumor types including colorectal cancer.[9-11]

High concordance for HER2 expression by immunohistochemistry (IHC) or in situ hybridization (ISH) and other modalities, among each other and with and *ERBB2*amp by next-generation sequencing (NGS), has been demonstrated in GEA[12-15]; however, in one study evaluating discordant cases, those positive for both expression and amplification had better efficacy outcomes to anti-HER2 therapy compared with those positive for HER2 expression but negative for *ERBB2*amp.[15] Stein et al and others have also presented data suggesting that

CONTEXT

Key Objective

Human epidermal growth factor receptor 2 (HER2) overexpression or *ERBB2* amplification are established biomarkers in gastroesophageal adenocarcinoma (GEA), and studies have suggested that degree of expression or amplification may further inform outcomes to anti-HER2 therapies. We sought to determine whether quantitative *ERBB2* copy number (CN) as determined by next-generation sequencing was directly associated with better outcomes to trastuzumab in a real-world data set of patients with GEA.

Knowledge Generated

ERBB2 amplification is present in multiple tumor types including 15% of GEA, but the degree of CN gain and amplicon focality is variable. We show that *ERBB2* CN is a significant predictor of improved real-world overall survival and real-world progression-free survival as a continuous variable in advanced GEA patients treated with first-line trastuzumab.

Relevance

Genomic profiling to quantitatively assess *ERBB2* amplification should be performed to inform treatment selection and trial enrollment in GEA, particularly as new anti-HER2 therapies and combinations continue to be developed.

higher *ERBB2* copy number (CN) gain as quantified by NGS is associated with better efficacy outcomes to anti-HER2 therapy compared with lower levels of *ERBB2*amp in GEA.[15,16] Earlier studies have also presented data showing that higher *ERBB2* CN by ISH is predictive of better response to anti-HER2 therapy.[17] The NCCN guidelines for esophageal and esophagogastric junction cancer and for gastric cancer (version 2.2021) recommend IHC or ISH for adenocarcinomas as the gold standard for selection of anti-HER2 therapy and NGS as an alternative subsequent option; however, these studies suggest a role for *ERBB2*amp detection by NGS, including CN quantification, as a further predictive marker of HER2 therapy benefit. Thus, further assessment of the utility of *ERBB2*amp as assessed by NGS, as well as the potential additive benefit of quantitative *ERBB2* CN as a biomarker for anti-HER2 therapy, is needed.

We evaluated the frequency and distribution of CN gain and amplicon size in primary disease site subtypes of *ERBB2*amp GEA using a large genomic database of > 14,000 GEA tissue samples. We further analyzed treatment patterns for patients with *ERBB2*amp GEA captured in the Foundation Medicine-Flatiron Health clinicogenomic database (CGDB) and assessed the predictive impact of quantitative *ERBB2* CN on real-world progression-free survival (rwPFS) to anti-HER2 therapy.

METHODS

Foundation Medicine Comprehensive Genomic Profiling

Hybrid capture–based comprehensive genomic profiling (CGP) was performed on formalin-fixed paraffin-embedded tumor tissue samples collected from 12,905 patients with primarily advanced GEA, as well as a comparison cohort of 34,629 primarily advanced breast cancer patients, during routine clinical care (January 2011-September 2020). Testing was performed in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists–accredited, New York State–regulated reference laboratory (Foundation Medicine, Inc, Cambridge, MA). Approval for this study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the WIRB-Copernicus Group Institutional Review Board (IRB; protocol no. 20152817).

As previously described, DNA (> 50 ng) was extracted from formalin-fixed paraffin-embedded specimens, and NGS was performed by hybridization-captured, adaptor ligation–based libraries to high, uniform coverage (> 500×) for all coding exons of up to 324 cancer-related genes (FoundationOne or FoundationOne CDx) plus selected introns.[18] The results were analyzed for base substitutions (subs), short insertions and deletions (indels), CN gains or losses, and rearrangements. *ERBB2*amp was defined as gene CN ≥ +3 of the tumor base ploidy (ie, CN = 5 in diploid, CN = 6 in triploid, CN = 7 in tetraploid, etc) and gene CN of ≥ 5 with 80% of baited targets amplified.[19] For additional methods for tumor mutational burden (TMB) and genomic ancestry determination, see the Data Supplement.

Flatiron Health-Foundation Medicine CGDB

This study used the nationwide deidentified Flatiron Health-Foundation Medicine CGDB (FH-FMI CGDB). Retrospective longitudinal clinical data were derived from electronic health records (EHRs), comprising patient-level structured and unstructured data, curated via technology-enabled abstraction, and were linked to genomic data derived from FMI CGP tests by deidentified, deterministic matching.[20,21] During the study period, the deidentified data originated from approximately 280 US cancer clinics (approximately 800 sites of care). Genomic alterations were identified via CGP as described above. To date, more than 400,000 samples have been sequenced from patients across the United States. The study included 2,270 patients who had a diagnosis of advGEA, received care within the FH network between January 2011 and

September 2020, and underwent tissue CGP (FoundationOne or FoundationOne CDx). IRB approval with waiver of informed consent was obtained before study conduct from WIRB-Copernicus Group IRB.

Patients who were diagnosed with advanced GEA > 90 days before their first structured activity within the FH network or who received their FMI report > 60 days after their last FH structured activity date were excluded to ensure all therapies received before CGP were captured and to exclude patients who left the FH network before CGP. This left 270 unique patients eligible for this study. Clinical characteristics and treatment information were obtained via technology-enabled abstraction of clinical notes and radiology and pathology reports and linked to CGP data. Real-world progression (rwP) events were captured as episodes in which the treating clinician documented in the EHR that there had been growth or worsening of disease.[22,23]

The Fisher exact test or chi-squared test was used to assess significance of categorical relationships, and the Kruskal-Wallis test was used to assess significance of continuous variables. False discovery rate correction was performed by the Benjamin-Hochberg procedure to correct *P* values for multiple tests.

Outcome Assessment

The primary study outcome measurement was rwPFS, which was defined as the time from therapy of interest initiation to the first rwP date > 14 days after therapy initiation or to death, and patients were censored at their last clinic note date if no progression or death was observed. Real-world overall survival (rwOS), which was defined as the time from therapy of interest initiation to death, was also measured as secondary outcome. rwPFS and rwOS were compared across different *ERBB2* CN subgroups using the Kaplan-Meier method and the log-rank test. Median rwPFS and rwOS values were estimated in months with 95% CIs. Multivariable Cox proportional hazard models were fitted on rwPFS or rwOS to estimate the adjusted hazard ratio and its significance level of *ERBB2* CN. Log-transformed CN was used in Cox models. Other items included in the Cox model were age at advanced diagnosis, practice type (academic or community), sex, origin of disease site (esophageal, gastroesophageal junction [GEJ], or gastric), Eastern Cooperative Oncology Group (ECOG) performance status, and therapy line number for rwPFS analysis of chemotherapy only.

RESULTS

Foundation Medicine Genomic Database

Analysis of the Foundation Medicine genomic database of 12,905 GEA tumor samples (Data Supplement) identified *ERBB2*amp in 15.0% (n = 1,934) cases including 19.8% (1,384 of 6,975) of esophageal or GEJ adenocarcinomas and 9.3% (550 of 5,930) of gastric adenocarcinomas (Fig 1A). The clinocogenomic characteristics of 1,934 cases of *ERBB2*amp GEA compared with GEA without *ERBB2* alterations (*ERBB2*wt) are shown in the Data Supplement. For *ERBB2*-amplified cases, median patient age was 63 years, 82.5% of patients were male, and for 71.6% of cases the primary tumor location was esophageal or GEJ. Both male sex and esophageal tumor location were further enriched relative to *ERBB2*wt GEA patients. The most frequent coaltered genes with *ERBB2*amp were *TP53* (89.2%), *CDKN2A*% (27.3%), and *CCNE1* (19.2%; Data Supplement). Coalteration frequencies were similar for *ERBB2*amp versus *ERBB2*wt GEA, although *TP53* alterations were significantly more common in *ERBB2*amp cases (89.2% *v* 71.2%; $P < .001$), whereas *KRAS* alterations were significantly less common in *ERBB2*amp cases (9.2% *v* 21.9%; $P < .001$) and overall gene amplifications were more frequent in the *ERBB2*amp subset (Data Supplement). High microsatellite instability was rare in *ERBB2*amp GEA samples (0.3%) compared with *ERBB2*wt GEA (0.3% *v* 3.2%; $P < .001$), whereas TMB distribution was similar regardless of *ERBB2* amplification status (median 4.3 *v* 3.8 mutations per megabase (Mb) Data Supplement).

In *ERBB2*amp GEA samples, median *ERBB2* CN was 22 (interquartile range [IQR] 9-73) and was similar across esophageal or GEJ (median CN = 22, IQR = 9-72) and gastric (median CN = 20.5, IQR = 9-73) subtypes (Fig 1B). We further assessed the size of the *ERBB2* amplicon in *ERBB2*amp GEA cases. The median *ERBB2* amplicon size was 0.27 Mb (IQR 0.13-0.95) for all GEA cases and was also similar for esophageal or GEJ (median 0.28 Mb, IQR 0.13-1.26) and gastric (median 0.26 Mb, IQR 0.13-0.85) subtypes. We also examined the correlation between *ERBB2* CN and amplicon size in GEA samples and found that more focal amplification significantly correlated with higher CN ($P < .001$) for amplicons larger than 0.16 Mb (Fig 1C). In an orthogonal comparison, *ERBB2* was amplified in 9.2% (3,193 of 34,629) of breast carcinoma cases analyzed, and CN (median 19, IQR 9-40), amplicon size (median 0.32 Mb, IQR 0.13-1.37), and correlation between CN and amplicon focality were similar (Fig 1). In both GEA and breast cases, for amplicons ≤ 0.08 Mb, partial *ERBB2* gene amplification was more common and cases with < 100% of baited *ERBB2* targets amplified were associated with lower *ERBB2* CN gains (Data Supplement).

CGDB Outcomes Analysis

Of 2,270 patients with advGEA in the CGDB, *ERBB2*amp was detected in 15% (342 of 2,164) of cases with CGP performed on tissue biopsies (Data Supplement). Of 39 evaluable patients with *ERBB2*amp whose CGP report was obtained before first-line, 66.7% of patients received a first-line treatment regimen containing anti-HER2 therapy (most commonly chemotherapy plus trastuzumab [61.5%]; Fig 2A). No anti-HER2 therapy was included in the first-line treatment regimen in 25.6% of cases including 20.5% of patients who received chemotherapy alone. The remaining 7.7% of

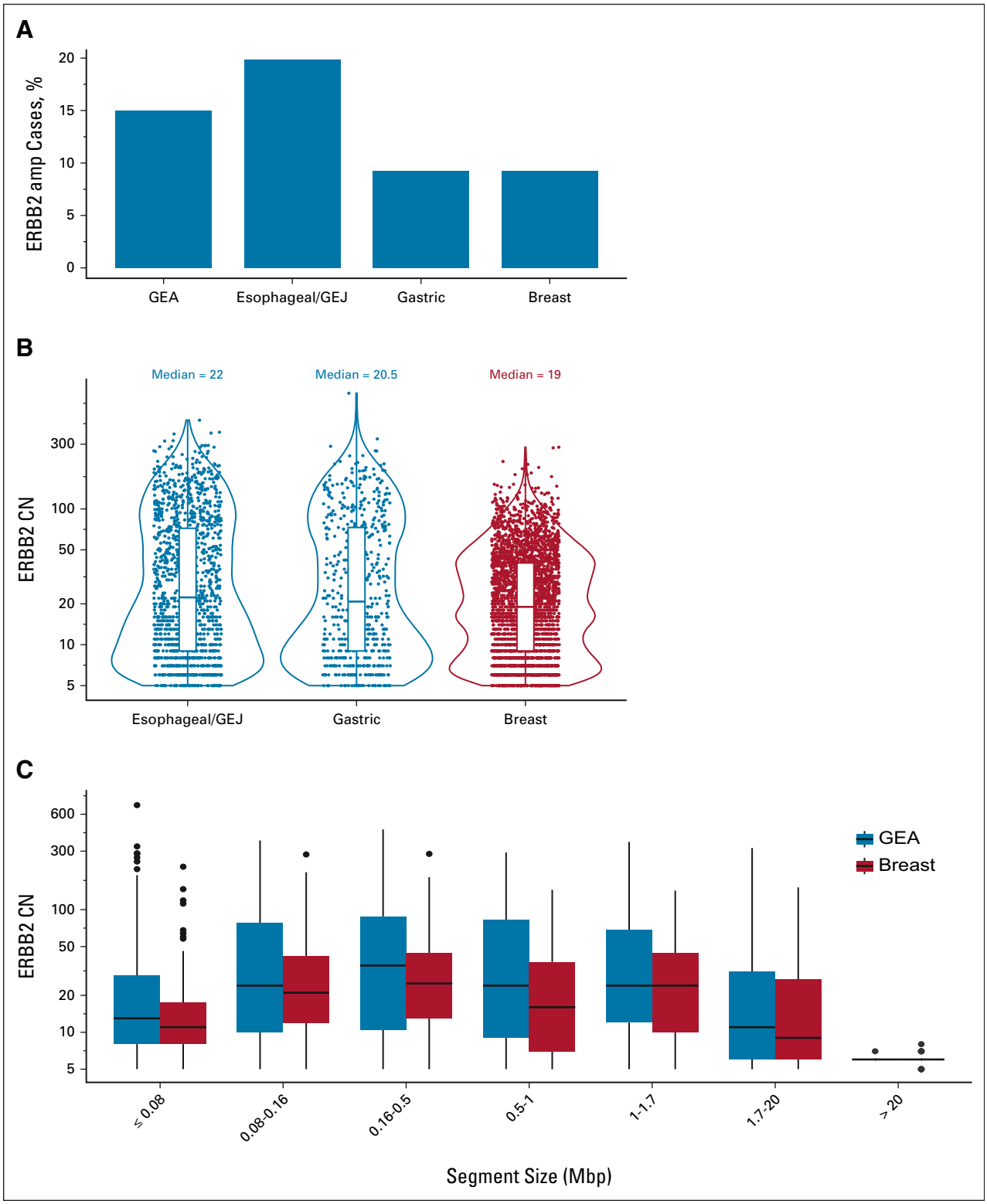

FIG 1. *ERBB2* amplification frequencies, CN distribution, and amplicon size across GEA samples. (A) Frequency of *ERBB2* amplification detected in all GEA tissue samples as well as in esophageal/GEJ and gastric subsets and breast cancer samples in the Foundation Medicine Genomic Database. (B) Similar *ERBB2* CN distribution was observed in *ERBB2*-amplified esophageal/GEJ, gastric, and breast subsets. (C) *ERBB2* CN distribution in *ERBB2*-amplified GEA and breast cancer samples bucketed by *ERBB2* amplicon size. For amplicons > 0.16 Mbp, increased focality significantly correlated with higher *ERBB2* CN ($P < .001$). CN, copy number; GEA, gastroesophageal adenocarcinoma; GEJ, gastroesophageal junction; Mbp, megabase pairs.

cases received an unspecified clinical study drug. In the subset of patients who did not receive anti-HER2 therapy in the first-line post-CGP report, the median *ERBB2* CN was 7 (IQR 6-7.5) and a prior negative HER2 IHC or ISH test was recorded in 20% (2 of 10) of cases; of the remaining patients, four had a prior positive HER2 IHC and/or ISH test and four had no documentation of prior HER2 IHC or ISH testing. Furthermore, of the 10 patients with *ERBB2*amp who did not receive anti-HER2 therapy in the first-line post-CGP, only two had documentation of second-line therapy at the time of data cutoff and in both cases that second-line regimen included an anti-HER2 treatment. By contrast, for *ERBB2*wt patients, the most common first-line therapies post-CGP report were chemotherapy alone (63.6%) or other therapies (20.1%) including immunotherapy, non–HER2-targeted therapies, or unspecified clinical study drugs (Fig 2B). Of 10 patients with *ERBB2*wt tumors on CGP who received an anti-HER2 containing regimen in the first-line post-CGP, seven had prior HER2 and/or IHC test results and 7 of 7 results were positive.

Among 270 patients with *ERBB2*amp advGEA meeting criteria for assessment, 101 received anti-HER2 therapy in the first-line setting regardless of CGP report timing (cohort A). A control set (cohort B) of 87 *ERBB2*amp patients treated with chemotherapy alone was also assessed; these patients received chemotherapy in any line because of prohibitively small numbers available for assessment when limited to the first-line setting. Clinical characteristics, genomic characteristics, and therapeutic regimens comparing cohorts A and B are shown in Table 1. Patients with *ERBB2* amplified GEA by CGP who received first-line anti-HER2 therapy (cohort A) were significantly more likely to have had a documented positive HER2 IHC or FISH result than those who received chemotherapy alone in the first-line (82.2% *v* 57.9%; $P < .001$) or in any line (cohort B, 82.2% *v* 57.5%; $P < .001$).

In patients with *ERBB2*amp advGEA treated with first-line trastuzumab (cohort A), higher *ERBB2* CN was a significant predictor as a continuous variable of longer rwPFS (hazard ratio [HR], 0.73; 95% CI, 0.60 to 0.89; $P < .01$). In contrast to the anti-HER2 cohort, higher *ERBB2* CN was not significantly associated with longer rwPFS in patients receiving chemotherapy (cohort B, HR, 0.93; 95% CI, 0.73 to 1.20; $P = .59$). Age at advanced diagnosis, practice type, sex, and disease site did not show a statistically significant association with rwPFS in cohort A. Other patient characteristics assessed were also not significantly associated with rwPFS in the chemotherapy cohort (Data Supplement). ECOG performance status at the start of therapy was marginally lower for patients receiving HER2-targeted therapy versus chemotherapy (Table 1), and higher ECOG significantly correlated with worse outcome in trastuzumab-treated patients (Data Supplement). Also, as expected, increasing line number was significantly associated with an increased risk of progression or death in patients receiving chemotherapy.

No single *ERBB2* CN cutoff was identified as an optimal predictor of rwPFS on trastuzumab-based therapy (Data Supplement). To visualize the relationship between *ERBB2* CN and rwPFS, patients in cohort A were divided into five subgroups by quintiles of quantitative *ERBB2* CN. For each increasing CN quartile, median rwPFS increased from 4.5 months for patients with CN 5-9 to 11.0 months for patients with CN > 107 (Fig 3). To assess whether longer rwPFS might have been the result of *ERBB2* CN association with other factor(s), we analyzed the relationship between *ERBB2* CN and multiple covariates in cohort A and saw no significant differences in *ERBB2* CN for disease site, age and stage at advanced diagnosis, sex, ancestry, practice type, co-*KRAS* mutation, co-*EGFR* amplification, or co-*FGFR1* or *FGFR2* amplification (Data Supplement). We did see a suggestive association between *PIK3CA* mutation and lower *ERBB2* CN (unadjusted $P = .03$). In patients with *ERBB2*amp advGEA treated with first-line trastuzumab, higher *ERBB2* CN was a significant predictor as a continuous variable of longer rwOS (HR, 0.79; 95% CI, 0.64 to 0.97; $P = .02$), although the increasing trend for rwOS by each *ERBB2* CN quintile was not as consistent as that for rwPFS (Data Supplement). Patients with CN 34 approximately 107 had longest median rwOS (20.4 months), whereas patients with CN > 107 had similar median rwOS to other subgroups. We did observe longer rwOS for patients with advGEA positive for HER2 by IHC or FISH treated with first-line trastuzumab with *ERBB2* amplification detected by NGS versus those negative for amplification by NGS (Data Supplement), consistent with existing literature.[15]

DISCUSSION

In a large genomic data set of 12,905 GEA cases, *ERBB2*amp was detected in 15% of tissue samples tested using NGS-based CGP, with enrichment in esophageal or GEJ samples relative to gastric samples consistent with published studies.[24] The *ERBB2*amp CN distribution and amplicon size were similar across esophageal or GEJ and gastric subsets, and more focal amplification generally correlated with higher copy gains. We compared a cohort of *ERBB2*amp breast carcinoma samples and observed a similar distribution of *ERBB2* CN gain and amplicon size, as well as correlation between focality and CN. However, more comprehensive pan-tumor analysis is needed to further elucidate the genomics of *ERBB2* amplification across disease subtypes.

In a real-world CGDB, we observed that most patients with *ERBB2*amp detected by CGP received trastuzumab in combination with chemotherapy postreceipt of their genomic test results, whereas those without *ERBB2*amp detected received chemotherapy alone, consistent with current guidelines. However, a smaller subset of *ERBB2*amp

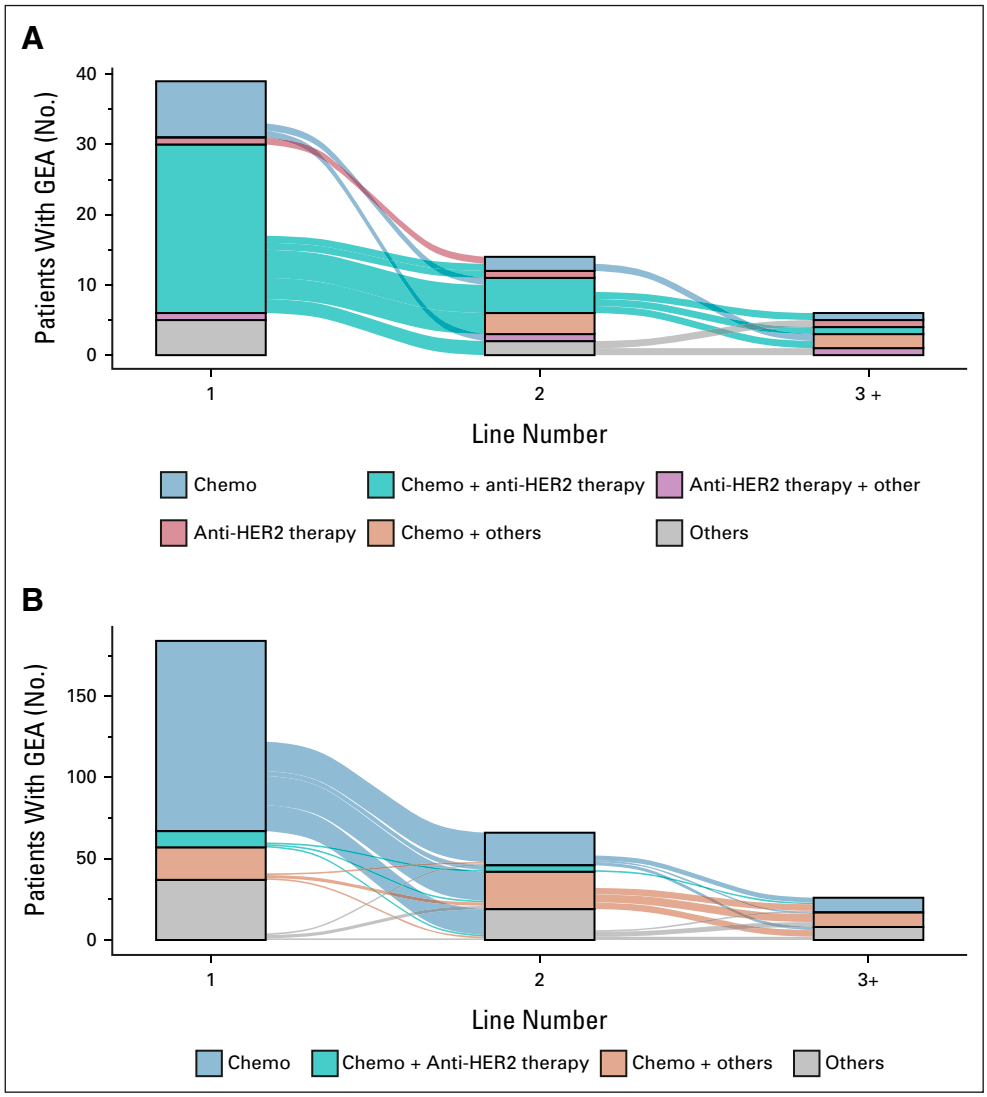

FIG 2. Treatment patterns for patients with GEA with *ERBB2* amplification or *ERBB2* wild-type tumors. Sankey diagrams showing treatment patterns for patients with GEA receiving first-line therapy post-CGP report. (A) Patients with GEA with *ERBB2* amplification detected by CGP commonly received anti–HER2-containing regimens. (B) Patients with GEA without *ERBB2* amplification or other *ERBB2* alterations detected by CGP rarely received anti-HER2 therapy. Therapies grouped as others include immunotherapy, non–HER2-targeted therapies, and unknown clinical study drugs. CGP, comprehensive genomic profiling; chemo, chemotherapy; GEA, gastroesophageal adenocarcinoma; HER2, human epidermal growth factor receptor 2.

cases received first-line treatment regimens absent of anti-HER2 therapy, which was likely because of relatively low-level *ERBB2*amp (median CN 7), and in two cases, a prior negative HER2 IHC and/or ISH result. Furthermore, for two of these patients with documented second-line therapy at the time of data cutoff, both received anti-HER2 therapy at that time. We also observed that a small subset of patients without *ERBB2*amp detected on CGP received chemotherapy in combination with anti-HER2 therapy following CGP report, and in the majority of these cases, the patient had a prior positive HER2 IHC and/or ISH result, despite the generally high reported concordance between IHC and ISH testing for HER2 expression and the CGP methodology used for *ERBB2* amplification detection in this study (88%-98%).[14,15]

Historically, IHC testing for HER2 overexpression has been considered the gold standard and remains so according to published guidelines. However, many testing modalities including NGS for *ERBB2*amp are now clinically available as validated US Food and Drug Administration–approved assays and studies correlating testing results with clinical outcomes to anti-HER2 therapies are needed. Published data in GEA as well as in colorectal cancer show that borderline positivity for HER2 expression and lower associated levels of *ERBB2* amplification are associated with reduced benefit from anti-HER2 therapies[17,25,26] Recent data from our group suggest that patients with GEA with positive IHC and/or ISH results for HER2 expression but negative results for *ERBB2*amp by NGS have worse outcomes on anti-HER2 therapy compared with patients testing positive by both or multiple methodologies.[15] Further exploratory analysis using median *ERBB2* CN as a cutoff suggested that *ERBB2* CN can be used effectively to further stratify best responders to anti-HER2 therapy, suggesting additional utility for quantitative NGS testing.[15,16]

In the current study, we expanded on these analyses and showed that *ERBB2* CN is predictive as a continuous variable for efficacy outcome to anti–HER2-targeted therapy in advGEA. Although no single CN cutoff appeared to be clinically optimal, increased rwPFS was observed for

TABLE 1. Characteristics of Patients With *ERBB2*-Amplified GEA in the Clinicogenomic Database

Characteristics	Cohort A: Anti-HER2 Therapy (first-line only, n = 101)	Cohort B: Chemotherapy Alone (any line, n = 87)
Disease site, No. (%)		
Esophageal/GEJ	82 (81.2)	76 (87.4)
Gastric	19 (18.8)	11 (12.6)
Sex, No. (%)		
Male	88 (87.1)	74 (85.1)
Female	13 (12.9)	13 (14.9)
Stage at diagnosis, No. (%)		
I/II	11 (10.9)	11 (12.6)
III	8 (7.92)	8 (9.20)
IV	77 (76.2)	64 (73.6)
Unknown	5 (4.95)	4 (4.60)
Median age at advanced diagnosis, years (IQR)	62.0 (56.0-68.0)	62.0 (55.0-70.0)
Ancestry, No. (%)		
EUR	86 (85.1)	72 (82.7)
AMR	10 (9.90)	12 (13.8)
Other	4 (3.96)	3 (3.53)
Unknown	1 (0.99)	0 (3.45)
Practice type, No. (%)		
Community	92 (91.1)	80 (91.6)
Academic	9 (8.91)	7 (8.04)
HER2 IHC/FISH status,[a] No. (%)		
Positive	83 (82.2)	50 (57.5)
Equivocal	1 (0.99)	1 (1.15)
Negative	4 (3.96)	16 (18.4)
Unknown	13 (12.9)	20 (23.0)
ECOG PS at therapy initiation,[b] No. (%)		
0-1	61 (60.4)	44 (50.6)
2+	31 (30.7)	35 (40.2)
Missing	9 (8.91)	8 (9.20)

Abbreviations: AMR, ad mixed American; CGP, comprehensive genomic profiling; ECOG, Eastern Cooperative Oncology Group; EUR, European; GEA, gastroesophageal adenocarcinoma; GEJ, gastroesophageal junction; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; IQR, interquartile range.

[a]HER2 IHC/FISH status: from test results within 90 days of CGP specimen collection date.

[b]The closest ECOG performance status from up to 30 days before the therapy initiation, or up to 7 days after the therapy initiation.

each increase in *ERBB2* CN quintile, with a median rwPFS of 5.4 months for patients with *ERBB2* CN of 5-9 compared with 11.0 months for patients with *ERBB2* CN > 107. We further assessed whether covariates associated with higher *ERBB2* CN could actually be driving anti-HER2 therapy outcome; however, none of the covariates assessed were significantly associated with *ERBB2* CN in our cohort of patients treated with first-line trastuzumab with the exception of *PIK3CA* mutations, which were suggestively associated with lower *ERBB2* CN (P = .03) and could contribute to anti-HER2 resistance. In our genomic analysis, we also found that higher *ERBB2* CN was associated with more focal gene amplification; however, *ERBB2* amplicon size itself was not an independent predictor of outcome to anti-HER2 therapy (HR, 1.12; 95% CI, 0.94 to 1.35; P = .21).

Additional RTKs have been shown to be oncogenic drivers, and therapeutic strategies have been implemented targeting overexpression or amplification of *EGFR*, *MET*, and *FGFR2*.[27] Historically, IHC and/or ISH methodologies, similar to those approved for HER2, have been used in trials investigating EGFR and MET expression as biomarkers with limited success.[28] However, more recently, promising results for *MET* amplification as a biomarker in NSCLC have been reported, and within the *METamp* cohort, increased *MET* CN was further predictive of improved outcome to

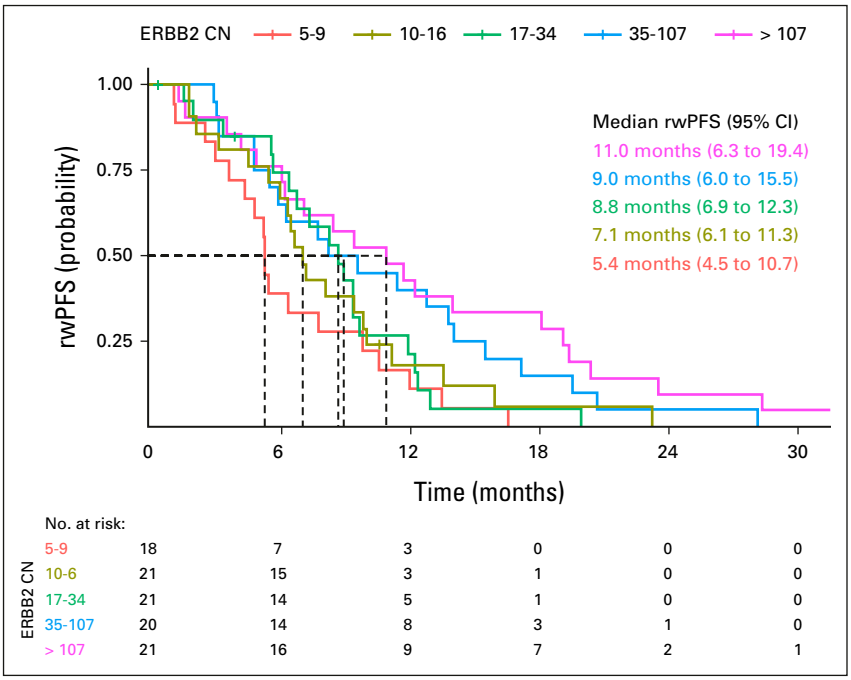

FIG 3. *ERBB2* CN as a predictor of rwPFS in patients with advanced GEA treated with anti-HER2 therapy in the first-line setting. Association of *ERBB2* CN with rwPFS in patients with GEA treated with first-line trastuzumab in the CGDB. *ERBB2* CN was predictive as a continuous variable, and no single CN cutoff was identified as an optimal predictor of rwPFS. To visualize Kaplan-Meier rwPFS curves, the cohort was split into *ERBB2* CN quintiles. Curves are truncated at 30 months as the number at risk was reduced to one in the entire ERBB2amp cohort. Median and IQR rwPFS for each CN quintile is shown. CGDB, clinicogenomic database; CN, copy number; GEA, gastroesophageal adenocarcinoma; HER2, human epidermal growth factor receptor 2; IQR, interquartile range; rwPFS, real-world progression-free survival.

MET-targeted therapy.[29] Promising studies assessing *EGFR* amplification as a predictive biomarker in GEA have also been presented.[30] Finally, innovative trial designs have been implemented in which personalized antibody therapy was selected and prioritized on the basis of presence and degree of gene amplification.[31] These data suggest that the observations made herein for *ERBB2* in GEA may potentially be applicable to other RTKs and disease subtypes.

Limitations of this study include absence of clinical information and treatment history for most patients in the FMI genomic database. For the CGDB cohort, clinical data were derived from EHR and data not documented in the EHR may be incomplete or missing, particularly for events occurring outside of the FH network. rwPFS was defined as time from therapy start to progression or death, where rwP events are abstracted from EHR and are limited by clinician interpretation and documentation. Selection bias is likely also present due to all patients in this study having received CGP. Additional retrospective and prospective analyses to assess quantitative *ERBB2* CN as a more sensitive predictive biomarker in GEA, and potentially in other tumor types, are needed.

In conclusion, in patients with advGEA treated with first-line trastuzumab, quantitative *ERBB2* CN was a significant predictor of efficacy outcome to anti-HER2 therapy, where higher CN was associated with longer rwPFS. These data along with results of prior studies suggest that although traditional IHC and ISH methodologies for detection of HER2 expression are generally concordant, NGS-based methodologies to detect and quantify *ERBB2* copy gains may allow for more robust stratification of HER2-positive patients and better prediction of efficacy benefit from approved and investigational HER2-targeted therapies. Ultimately, in caring for patients, we seek to gather maximal data to inform prognostic and predictive clinical abilities and feel our data suggest that quantitative *ERBB2* CN information may help extend these goals in *ERBB2*amp GEA.

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DATA SHARING STATEMENT

All data relevant to the study are included in the article or uploaded in the Data Supplement.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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