

# Concordance between HER2 status by IHC and NGS in tissue and blood samples from gastric and gastroesophageal junction cancers

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## Objectives

- Evaluate the concordance of detecting *HER2* (*ERBB2*) gene amplification by tissue or plasma next-generation sequencing (NGS) with human epidermal growth factor receptor 2 (HER2) immunohistochemistry (IHC) / in situ hybridization (ISH) status in patients with gastric or gastroesophageal junction cancer (GC/GEJC) using the Tempus<sup>®</sup> database

- Assess the correlation in *HER2* copy number between paired tissue and plasma samples in patients with GC/GEJC

## Conclusions

- This analysis showed moderate concordance of *HER2* amplification detection by NGS in tissue (positive percent agreement [PPA], 69.9%) or plasma (PPA, 57.5%) with HER2-positive (HER2+; IHC 3+ or IHC 2+ / ISH-positive [ISH+]) GC/GEJC determined by IHC/ISH assessment

- High concordance (>90%) per negative percent agreement (NPA) was observed for *HER2* amplification determined by tissue/plasma NGS with HER2-negative (HER2-; IHC 0, IHC 1+, or IHC 2+ / ISH-negative [ISH-]) GC/GEJC determined by IHC/ISH

- NGS testing was not sufficient to detect all patients with HER2+ GC/GEJC; IHC/ISH testing is the standard method for identifying patients with HER2+ GC/GEJC

- Further study is needed to determine if NGS testing could complement IHC/ISH in identifying patients who may benefit from HER2-directed therapy

- Limitations of the population characteristics and statistical analyses should be considered when interpreting the results presented here

## Plain language summary

**Why did we perform this research?** Human epidermal growth factor receptor 2 (HER2) is a protein found at higher-than-normal levels on the cell surface of some tumors.<sup>1</sup> The levels of HER2 can be measured, which can help identify people who may benefit from treatments that specifically target HER2.<sup>2</sup> The standard method to identify people with tumors that have high levels of HER2 (known as HER2-positive [HER2+] tumors) is by testing tissue samples using two techniques called immunohistochemistry (IHC) and in situ hybridization (ISH). Next-generation sequencing (NGS) is a technique already used in clinical practice to identify a range of genetic variations linked to disease, including whether there are higher-than-normal levels of the *HER2* gene (known as *HER2* amplification). NGS may offer an alternative method to identify HER2+ tumors; however, it is currently unclear how well NGS detection of *HER2* amplification can identify people with HER2+ cancers. This study assessed whether *HER2* amplification (determined by NGS testing) could identify people with HER2+ tumors (identified by IHC/ISH) of the stomach (gastric cancer [GC]) or where the stomach meets the esophagus (gastroesophageal junction cancer [GEJC]).

**How did we perform this research?** Clinical records of people with GC/GEJC were sourced from the Tempus<sup>®</sup> database (an electronic library of clinical and molecular data from people with cancer). The primary analysis assessed whether *HER2* gene amplification determined by tissue or blood NGS testing could identify people with HER2+ tumors confirmed by IHC/ISH. Only people who had tissue or blood samples taken for NGS testing within 30 days of their samples taken for IHC/ISH assessment were included in the primary analysis. The secondary analysis evaluated samples from people with GC/GEJC who had both tissue and blood samples (taken within 30 days of each other) that underwent NGS testing and assessed whether the NGS results from the tissue sample were similar to those from the blood sample in the same person.

**What were the findings of this research?** Approximately 70% of people with HER2+ GC/GEJC determined by IHC/ISH had *HER2* amplification detected by NGS testing of cancer tissue and approximately 58% of people with HER2+ GC/GEJC determined by IHC/ISH had *HER2* amplification detected by NGS testing of their blood. Of the people without HER2+ GC/GEJC (known as HER2-negative [HER2-] GC/GEJC), NGS testing of tissue or blood showed that the majority (>90%) of them did not have *HER2* amplification.

**What are the implications of this research?** Results from NGS testing for *HER2* amplification could identify almost all people with HER2- GC/GEJC and some people with HER2+ GC/GEJC. NGS was not sufficient to detect all people with HER2+ GC/GEJC (identified by IHC/ISH); IHC/ISH testing is the standard method for identifying people with HER2+ GC/GEJC. Further study is needed to determine if NGS testing could complement standard IHC/ISH testing in identifying people with GC/GEJC who may benefit from HER2-directed therapy.

**Where can I access more information?** For more information about this study, please reach out to the presenter, Dr. Steven B Maron, at [marons@mskcc.org](mailto:marons@mskcc.org), or the corresponding author, Dr. Samuel J Klempner, at [sklempner@mgb.org](mailto:sklempner@mgb.org)

1. Uzunpamak B, et al. *Ann Oncol*. 2023;34:1035–1046; 2. Zhang H, et al. *Histopathology*. 2024;85:3–19

## Introduction

- HER2 expression is seen in a wide range of solid tumor types and is often associated with poor prognosis in various cancers<sup>1–5</sup>
  - An estimated 18% of patients with GC/GEJC have HER2+ (IHC 3+ or IHC 2+/ISH+) tumors<sup>6</sup>
- HER2-directed therapy has demonstrated clinically meaningful benefit in patients with HER2+ advanced GC/GEJC,<sup>7,8</sup> with current guidelines recommending HER2 testing to identify those with metastatic disease who may benefit from HER2-directed therapy<sup>9</sup>
- IHC/ISH are the standard testing methods for determining HER2 positivity in clinical practice
- Although NGS is a testing method commonly used by clinical oncology specialists to identify a panel of disease biomarkers, including *HER2* amplification, there are limited data on whether NGS can identify patients with HER2+ GC/GEJC
- This study assessed the concordance of detecting *HER2* amplification by tissue or plasma NGS with HER2 status determined by IHC/ISH in advanced GC/GEJC

## Results

- A total of 1578 records of patients with GC/GEJC were identified from the Tempus<sup>®</sup> database for the primary analysis
  - Tissue NGS testing was performed in 1507/1578 patients and plasma NGS testing in 305/1578 patients; demographics and clinical characteristics of these patients are shown in **Table 1**
  - In patients with tissue (n=1507) and blood (n=305) NGS results, 973 (64.6%) and 204 (66.9%) had Stage 4 disease and 189 (12.5%) and 21 (6.9%) had received prior chemotherapy, respectively
- 229 (15.2%) and 40 (13.1%) patients were HER2+ among those with tissue and plasma NGS testing, respectively

**Table 1. Demographics and clinical characteristics of patients identified for the primary analysis**

| Demographic/characteristic         | Patients with a tissue NGS result (n=1507)* | Patients with a plasma NGS result (n=305)* |
|------------------------------------|---------------------------------------------|--------------------------------------------|
| Median age, years                  | 64.3                                        | 63.3                                       |
| Age, years, n (%)                  |                                             |                                            |
| <50                                | 261 (17.3)                                  | 57 (18.7)                                  |
| 50 to <60                          | 304 (20.2)                                  | 74 (24.3)                                  |
| 60 to <70                          | 450 (29.9)                                  | 76 (24.9)                                  |
| 70 to <80                          | 359 (23.8)                                  | 74 (24.3)                                  |
| ≥80                                | 132 (8.8)                                   | 24 (7.9)                                   |
| Not available                      | 1 (0.1)                                     | 0                                          |
| Sex, male, n (%)                   | 1005 (66.7)                                 | 201 (65.9)                                 |
| Primary site, n (%)                |                                             |                                            |
| GEJ/cardia                         | 605 (40.1)                                  | 104 (34.1)                                 |
| Non-cardia stomach                 | 902 (59.9)                                  | 201 (65.9)                                 |
| Stage, n (%)                       |                                             |                                            |
| 1–3                                | 191 (12.7)                                  | 31 (10.2)                                  |
| 4                                  | 973 (64.6)                                  | 204 (66.9)                                 |
| Not available                      | 343 (22.8)                                  | 70 (23.0)                                  |
| HER2 status, n (%)                 |                                             |                                            |
| IHC 0 or IHC 1+                    | 987 (65.5)                                  | 217 (71.1)                                 |
| IHC 2+/ISH unknown‡                | 158 (10.5)                                  | 32 (10.5)                                  |
| IHC 2+/ISH–                        | 133 (8.8)                                   | 16 (5.2)                                   |
| IHC 2+/ISH+                        | 47 (3.1)                                    | 10 (3.3)                                   |
| IHC 3+                             | 182 (12.1)                                  | 30 (9.8)                                   |
| Received prior chemotherapy, n (%) | 189 (12.5)                                  | 21 (6.9)                                   |

\*Patients with results from a tissue NGS xT panel (xT.v1, xT.v2, xT.v3, xT.v4); †patients with results from a plasma NGS xF.v2 panel; ‡patients with HER2 IHC 2+ tumors without an ISH result were excluded from concordance analysis  
GEJ, gastroesophageal junction; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; NGS, next-generation sequencing

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## Methods

### Patient populations

- De-identified clinical and molecular data from patients with cancer in the US were retrospectively identified in the Tempus<sup>®</sup> database
- For the primary analysis, patients with GC/GEJC, a HER2 IHC score (IHC 0–3+) curated by clinicians, and tissue or plasma samples that underwent NGS testing were included
  - Only patients who had samples for NGS collected within 30 days of the reported IHC sample collection date were evaluated
- For the secondary analysis, patients with GC/GEJC and a curated HER2 IHC score who underwent tissue and plasma NGS testing (NGS samples collected within 30 days of each other; blood variable allele frequency >5%) were included
  - As this analysis exclusively assessed NGS results, patients were not excluded based on IHC sample collection date

### Concordance of *HER2* amplification status, determined by NGS, with HER2 IHC/ISH status

- Of the patients identified as *HER2* amplified by tissue and plasma NGS, 95.2% and 92.0% were HER2+ by IHC/ISH, respectively
- PPA of *HER2* amplification by tissue NGS with HER2+ tumors was 69.9% (**Table 2**) and *HER2* amplification by plasma NGS with HER2+ tumors was 57.5% (**Table 3**)
- NPA of *HER2* amplification by tissue NGS with HER2– tumors was 99.3% (**Table 2**) and *HER2* amplification by plasma NGS with HER2– tumors was 99.1% (**Table 3**)
- Scatter plots of IHC/ISH status with tissue *HER2* major copy number and plasma *HER2* log2(copy number) are shown in **Figures 1** and **2**, respectively

**Table 2. Comparison of tissue *HER2* amplification status, determined by NGS, with HER2 IHC/ISH status\***

| Tissue <i>HER2</i> amplification status, n | HER2 IHC/ISH status, n        |                                       |       |
|--------------------------------------------|-------------------------------|---------------------------------------|-------|
|                                            | HER2+ (IHC 3+ or IHC 2+/ISH+) | HER2– (IHC 0, IHC 1+, or IHC 2+/ISH–) | Total |
| Amplified                                  | 160                           | 8                                     | 168   |
| Not amplified                              | 69                            | 1112                                  | 1181  |
| Total                                      | 229                           | 1120                                  |       |
|                                            | PPA 69.9%                     | NPA 99.3%                             |       |

\*Patients with HER2 IHC 2+ tumors with an unknown ISH result were excluded  
HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; NGS, next-generation sequencing; NPA, negative percent agreement; PPA, positive percent agreement

**Table 3. Comparison of plasma *HER2* amplification status, determined by NGS, with HER2 IHC/ISH status\***

| Plasma <i>HER2</i> amplification status, n | HER2 IHC/ISH status, n        |                                       |       |
|--------------------------------------------|-------------------------------|---------------------------------------|-------|
|                                            | HER2+ (IHC 3+ or IHC 2+/ISH+) | HER2– (IHC 0, IHC 1+, or IHC 2+/ISH–) | Total |
| Amplified                                  | 23                            | 2                                     | 25    |
| Not amplified                              | 17                            | 231                                   | 248   |
| Total                                      | 40                            | 233                                   |       |
|                                            | PPA 57.5%                     | NPA 99.1%                             |       |

\*Patients with HER2 IHC 2+ tumors with an unknown ISH result were excluded  
HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; NGS, next-generation sequencing; NPA, negative percent agreement; PPA, positive percent agreement

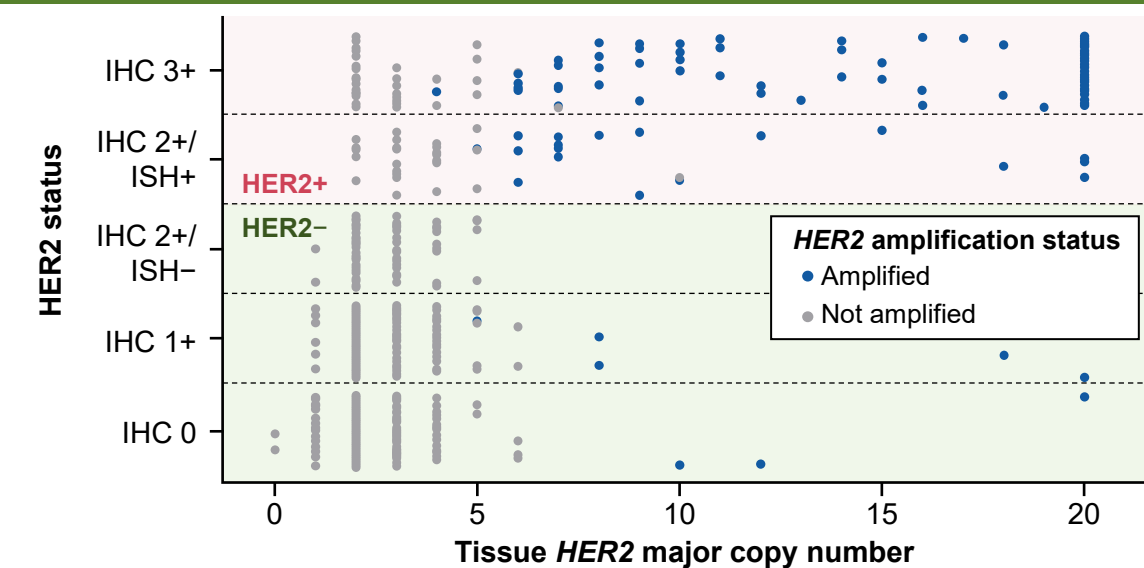
## Disclosures – Dr. Steven B Maron

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### Testing methods

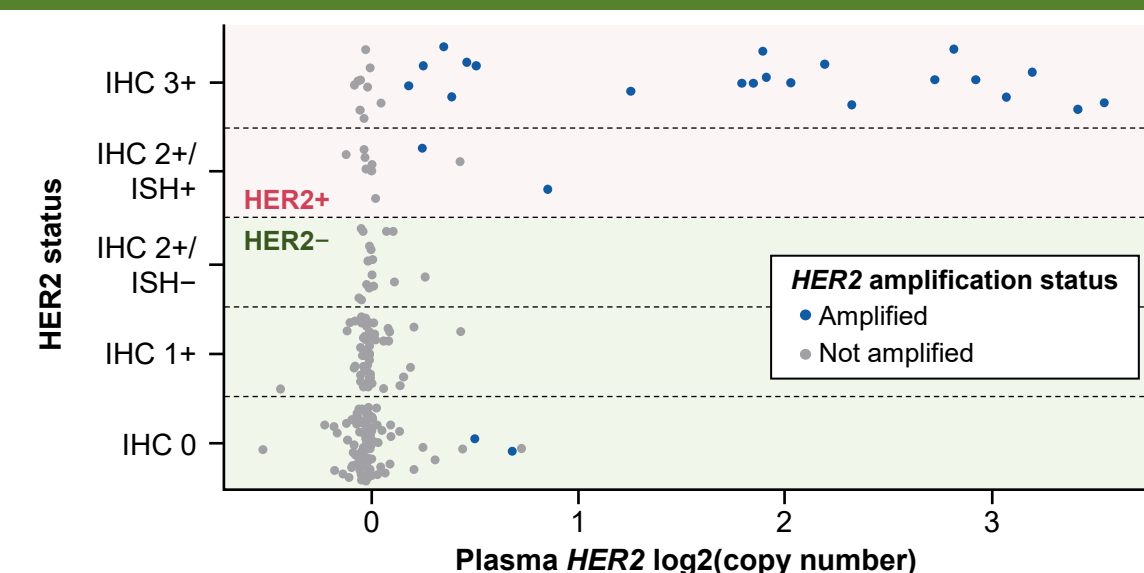
- Concordance was assessed according to PPA and NPA
  - PPA was defined as the percentage of patients with *HER2* amplification detected in the subgroup with HER2+ (IHC 3+ or IHC 2+/ISH+) tumors
  - NPA was defined as the percentage of patients with *HER2* amplification not detected in the subgroup with HER2– (IHC 0, IHC 1+, or IHC 2+/ISH–) tumors
- Maximum allele frequencies in patients with HER2+ GC/GEJC and *HER2* amplification in patients with IHC 2+ were described
- HER2* amplification was determined according to copy number ratio thresholds (tissue: copy number ≥8; plasma: log2[copy number] ≥0.03) and prespecified filters (which included segment sizes, confidence intervals, tumor purity)
  - In tissue NGS analyses, gains of seven copies or copy number variations detected in samples with tumor purities >30% were considered as amplified in circumstances where corroborative or prior data were available
- Additional amplifications were captured by manual inspection

**Figure 1. Scatterplot of tissue NGS with HER2 IHC/ISH status**



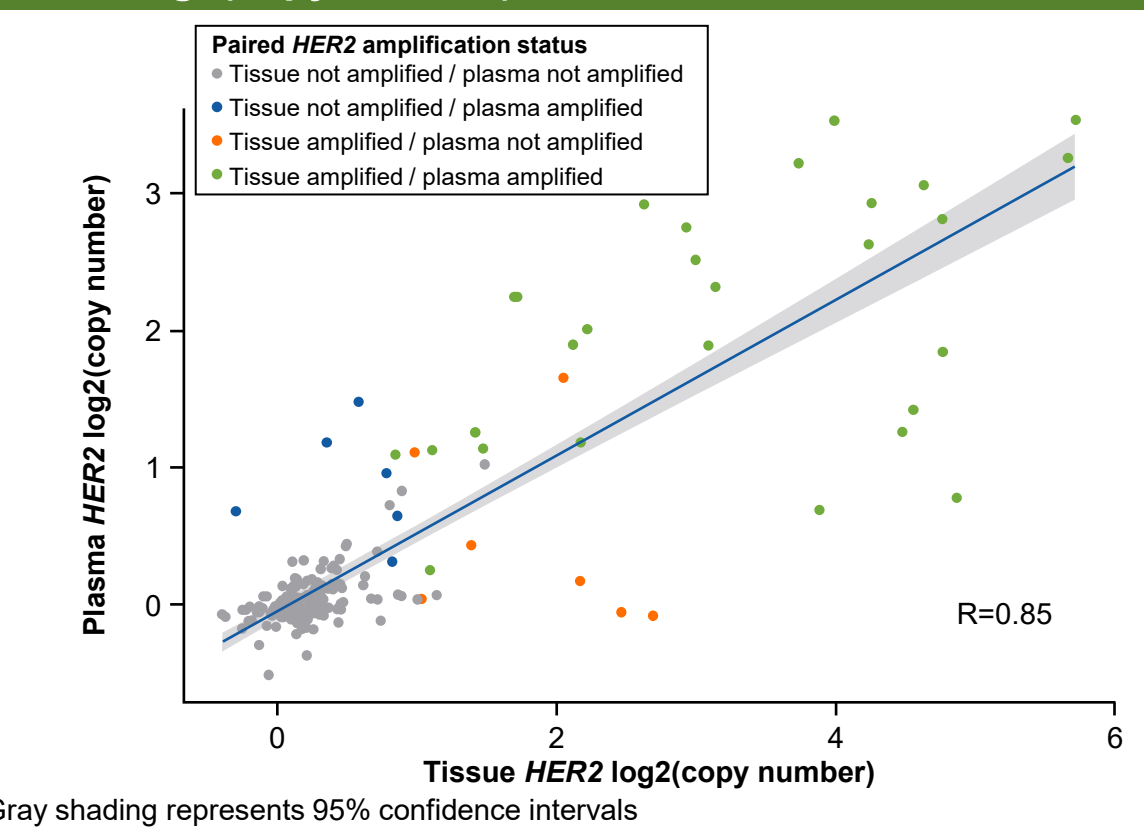
HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; NGS, next-generation sequencing

**Figure 2. Scatterplot of plasma NGS with HER2 IHC/ISH status**



HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; NGS, next-generation sequencing

**Figure 3. Correlation of paired tissue and plasma *HER2* log2(copy number)**



Gray shading represents 95% confidence intervals

- Correlation of paired tissue and blood *HER2* log2(copy number) was evaluated by Pearson's correlation analysis
- Statistical analyses are reported in a descriptive manner; no confirmatory hypothesis testing was planned

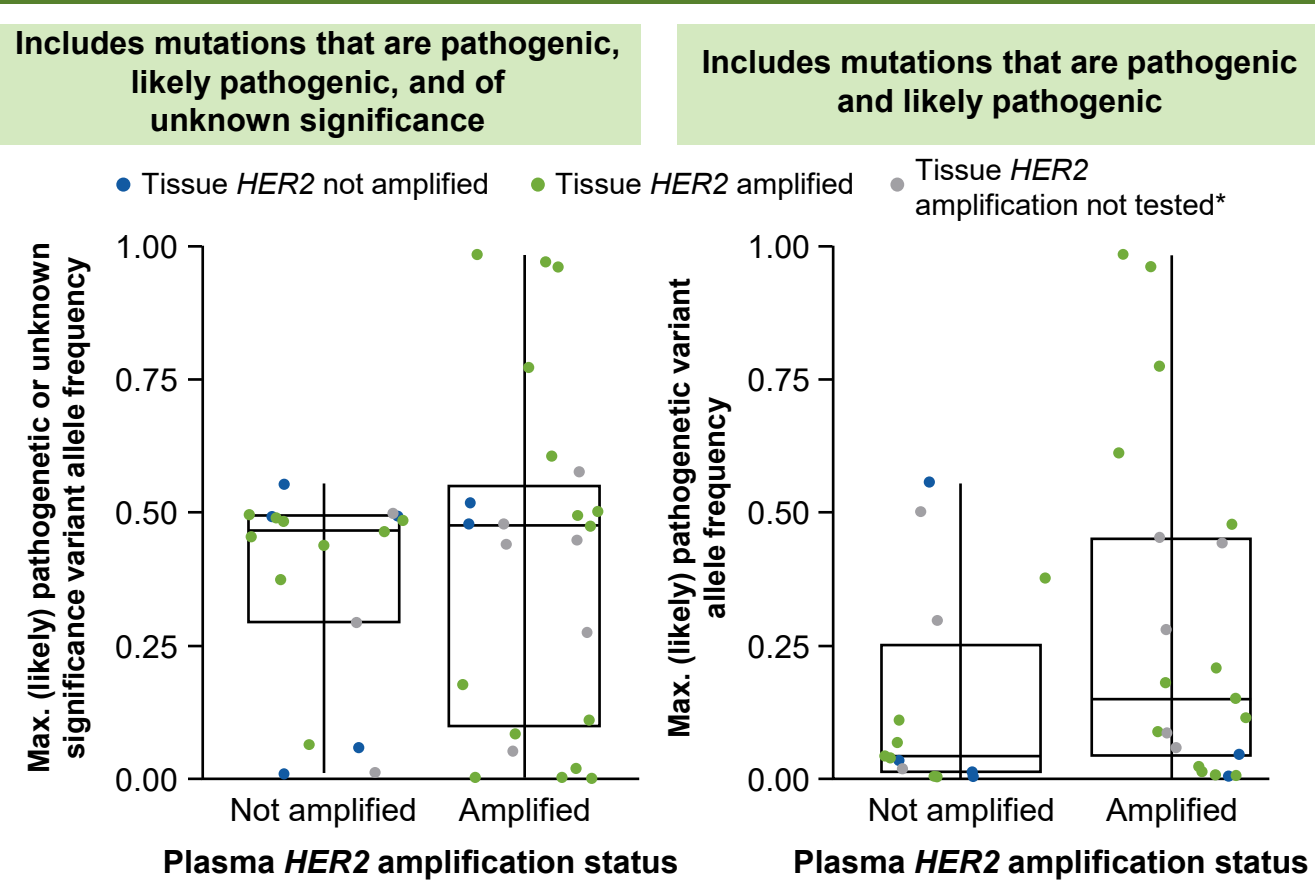
### Primary analysis

- Evaluate the concordance of tissue *HER2* amplification status, determined by NGS, with HER2 IHC/ISH status
- Evaluate the concordance of plasma *HER2* amplification status, determined by NGS, with HER2 IHC/ISH status

### Secondary analysis

- Assess the correlation between *HER2* log2(copy number) calling in paired tissue and plasma NGS

**Figure 4. Comparison of maximum allele frequencies in HER2+ GC/GEJC by plasma *HER2* amplification status**



Each box plot shows the minimum, 25th percentile, median, 75th percentile, and maximum  
\*Within 30 days of plasma NGS test. GC, gastric cancer; GEJC, gastroesophageal junction cancer; HER2, human epidermal growth factor receptor 2; NGS, next-generation sequencing

**Table 4. Plasma *HER2* amplification status in patients with HER2 IHC 2+ GC/GEJC by ISH status**

| ISH status (IHC 2+ patients) | <i>HER2</i> copy number variant amplified | <i>HER2</i> copy number variant not amplified | Proportion amplified, % |
|------------------------------|-------------------------------------------|-----------------------------------------------|-------------------------|
| Positive                     | 9                                         | 21                                            | 30.0                    |
| Negative                     | 0                                         | 16                                            | 0                       |
| Unknown                      | 5                                         | 27                                            | 15.6                    |

GC, gastric cancer; GEJC, gastroesophageal junction cancer; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization

### Correlation of paired tissue and plasma *HER2* log2(copy number)

- A positive correlation in *HER2* log2(copy number) was observed between paired tissue and plasma NGS results (R=0.85, n=307; **Figure 3**)
- Comparisons of max. allele frequencies in HER2+ GC/GEJC by *HER2* amplification status are shown in **Figure 4**
- Proportion of patients with HER2 IHC 2+ tumors with *HER2* amplification by ISH status (positive, negative, unknown) is shown in **Table 4**

### Study limitations

- The population of patients included in the primary and secondary analyses were limited by size and potential biases among patient characteristics
- IHC/ISH testing were not performed centrally, with limited insight on testing methods in the database, and IHC/ISH and NGS testing may not have been performed on the same samples
- As other NGS testing panels are available, which use different copy number cutoffs for determining *HER2* amplification, the generalizability of these results may be limited

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