Poster 478

Concordance between human epidermal growth factor receptor 2 status by immunohistochemistry and next-generation sequencing in tissue and blood samples from gastric and gastroesophageal junction cancers

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Objectives

- Evaluate the concordance of detecting human epidermal growth factor receptor 2 (*HER2* [*ERBB2*]) gene amplification by tissue or plasma next-generation sequencing (NGS) with HER2 immunohistochemistry (IHC) / in situ hybridization (ISH) status in patients with gastric or gastroesophageal junction cancers (GC/GEJC) using the Tempus® 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
- As NGS was not sufficient to detect all patients with HER2+ GC/GEJC, these data support IHC/ISH as the standard testing 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 of this analysis

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.[1] The levels of HER2 can be measured, which can help identify people who may benefit from treatments that specifically target HER2.[2] 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 capture 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 cancers [GC]) or where the stomach meets the esophagus (gastroesophageal junction cancers [GEJC]).

How did we perform this research?

Clinical records of people with GC/GEJC were sourced from the Tempus® database. 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. These findings support IHC/ISH testing as the gold standard for determining HER2+ cancers. Further study is need 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. Maron at marons@mskcc.org, or the corresponding author, Dr Klempner at sklempner@mgb.org.

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

QR Code

Poster

Supplementary material

Please scan this quick response (QR) code with your smartphone camera or app to obtain a copy of these materials. Alternatively, please click on the following link: [hyperlink to the QR code landing page]

This study was sponsored by AstraZeneca. In March 2019, AstraZeneca entered into a global development and commercialization collaboration agreement with Daiichi Sankyo for trastuzumab deruxtecan (T-DXd; DS-8201).
Poster presented at ASCO GI 2025, January 23–25, San Francisco, CA, US and online, by Dr Steven B Maron. Corresponding author: Dr Samuel J Klempner (sklempner@mgb.org).

Introduction

- HER2 expression is seen in a wide range of solid tumor types and is often associated with poor prognosis in various cancers[1–5]
  - An estimated 18% of patients with GC/GEJC have HER2+ (IHC 3+ or IHC 2+ / ISH+) tumors[6]
- HER2-directed therapy has demonstrated clinically meaningful benefit in patients with HER2+ advanced GC/GEJC,[7,8] with current guidelines recommending HER2 testing to identify those with metastatic disease who may benefit from HER2-directed therapy[9]
- 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* (*ERBB2*) 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

Methods

Patient populations

- De-identified clinical and molecular data from patients with cancer in the US were retrospectively identified in the Tempus® 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; variable allele frequency >5%) were included
  - As this analysis exclusively assessed NGS results, patients were not excluded based on IHC sample collection date

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, 1+, or 2+/ISH−) tumors
- The thresholds to determine tissue and plasma *HER2* amplification were defined as *HER2* copy number ≥8 or log2(copy number) ≥0.5, respectively
  - Additional amplifications were captured by manual inspection
- 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

Results

- A total of 1578 records of patients with GC/GEJC were identified from the Tempus® 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 and blood 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
- According to IHC/ISH status, 229 (15.1%) 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

n (%)	Patients with a tissue NGS result (n=1507)*	Patients with a plasma NGS result (n=305)†
Age, years†		
<50	261 (17.3)	57 (18.7)
50–60	304 (20.2)	74 (24.3)
60–70	450 (29.9)	76 (24.9)
70–80	359 (23.8)	74 (24.3)
≥80	132 (8.8)	24 (7.9)
Not available	1 (0.1)	0 (0)
Sex, male	1005 (66.7)	201 (65.9)
Primary disease site		
Cardia	605 (40.1)	104 (34.1)
Stomach	902 (59.9)	201 (65.9)
Disease stage†		
1–3	191 (12.7)	31 (10.2)
4	973 (64.6)	204 (66.9)
Not available	343 (22.8)	70 (23.0)
HER2 IHC/ISH status		
IHC 0/1+	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	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. HER2, human epidermal growth factor receptor 2; ISH, in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing

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% and *HER2* amplification by plasma NGS with HER2+ tumors was 57.5% (**Table 2**)
- NPA of *HER2* amplification by tissue NGS with HER2− tumors was 99.3% and *HER2* amplification by plasma NGS with HER2− tumors was 99.1% (**Table 3**)
- Correlation between IHC/ISH status with tissue *HER2* major copy number and plasma *HER2* log2(copy number) is shown in **Figures 1** and **2**, respectively

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

	HER2 IHC/ISH status, n		
Tissue *HER2* amplification status, n	**HER2+** (IHC 3+ or IHC 2+ / ISH+)	**HER2−** (IHC 0, IHC 1+, or IHC 2+ / ISH−)	**Total**
Detected	**160**	**8**	168
Not detected	**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; ISH, in situ hybridization; IHC, immunohistochemistry; 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*

	HER2 IHC/ISH status, n		
Plasma *HER2* amplification status, n	**HER2+** (IHC 3+ or IHC 2+ / ISH+)	**HER2−** (IHC 0, IHC 1+, or IHC 2+ / ISH−)	**Total**
Detected	**23**	**2**	25
Not detected	**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; ISH, in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; NPA, negative percent agreement; PPA, positive percent agreement

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

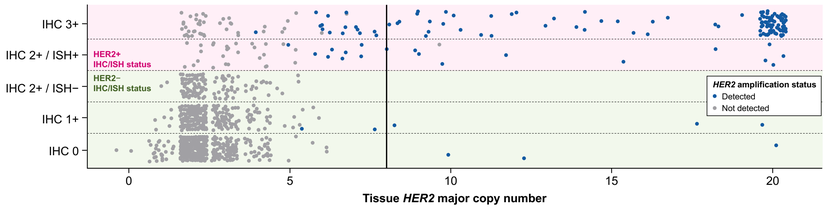


Figure 2. Correlation of plasma NGS with HER2 IHC/ISH status

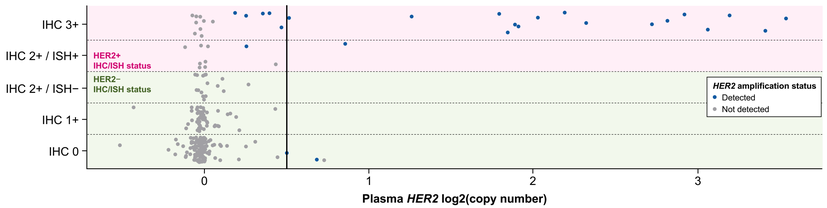
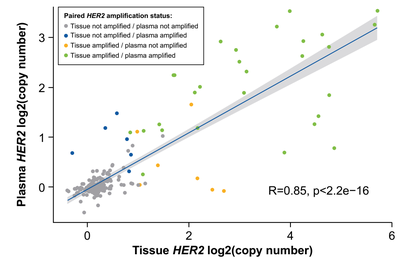


Figure 3. Correlation of paired tissue and plasma *HER2* log2(copy number)



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

- A positive correlation in *HER2* log2(CN) was observed between paired tissue and plasma NGS results (R=0.85; P<2.2e−16; n=307; **Figure 3**)
- Comparison of max allele frequencies in HER2+ GC/GEJC by *HER2* amplification status, and the percentage of patients with HER2 IHC 2+ tumors with *HER2* amplification by ISH status (positive, negative, unknown) are shown in the **Supplementary material**

Study limitations

- The population of patients included in the primary and secondary analyses were limited by size and biases among patient characteristics
- IHC/ISH testing were not performed centrally, with limited insight on testing methods in the database
- As no hypothesis testing was performed, further study is needed to determine the benefit of detecting *HER2* amplification by tissue or plasma NGS compared with standard IHC/ISH testing for HER2+ GC/GEJC in clinical practice

Acknowledgments

The authors acknowledge Arthur Lambert (employee of AstraZeneca) and Angelica Nunes (employee of Daiichi Sankyo) for interpretation and scientific review of data, and feedback, and Yoshinobu Shiose (employee of Daiichi Sankyo) for scientific review of data and feedback. Under the guidance of the authors and in accordance with Good Publications Practice (GPP), medical writing and editorial support was provided by Samuel Shields, PhD, of Helios Medical Communications, part of the Helios Global Group, and was funded by AstraZeneca

Disclosures – Dr Steven B Maron

Received honoraria from American Physician CME, Clarion Healthcare, Clinical Care Options,, Medpage, MJH Healthcare Holdings LLC, OncoViews, Physicians' Education Resource, Practicing Clinician Exchange, and Vindico Medical Education; had a consulting or advisory role with Amgen, Calcium Company, Daiichi Sankyo, Elevation Oncology, Novartis, Pinetree Therapeutics, and Purple Biotech; received research funding from Epic Sciences and Guardant Health; and received travel, accommodation, or expenses from AstraZeneca

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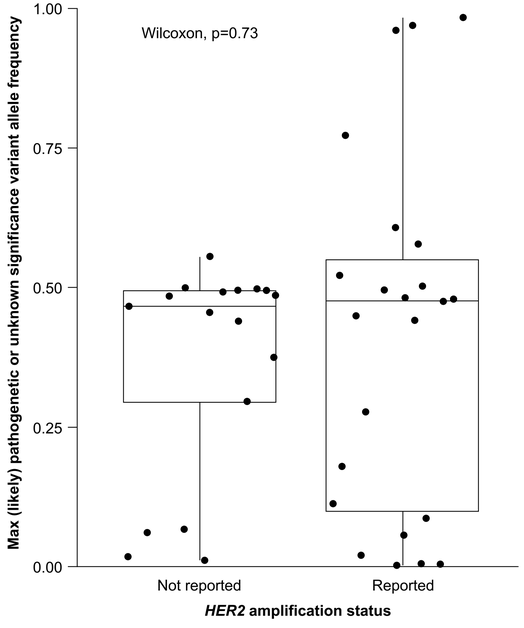
Please note that these data are placed on this slide to allow for easy review. Should these data be included as a supplement, they will be uploaded as images to the QR code.

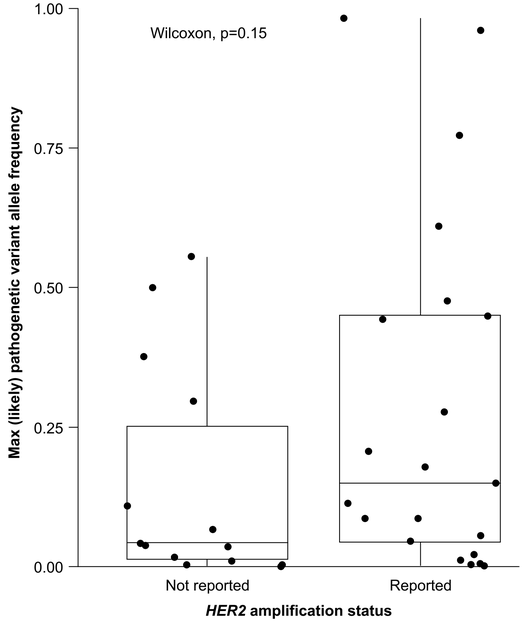
Supplemental results

Figure S1. Comparison of max allele frequencies in HER2+* GC/GEJC by *HER2* amplification status

Includes mutations that are pathogenic, likely pathogenic, and of unknown significance



Includes mutations that are pathogenic and likely pathogenic



Data are shown as supplementary material to the poster (abstract number: 478), titled 'Concordance between human epidermal growth factor receptor 2 status by immunohistochemistry and next-generation sequencing in tissue and blood samples from gastric and gastroesophageal junction cancers', presented at ASCO GI 2025, January 23–25, San Francisco, CA, US and online. *HER2+ was defined as IHC 3+ or IHC 2+ / ISH+ tumors. GC, gastric cancer; GEJC, gastroesophageal junction cancer; HER2, human epidermal growth-factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization

Table S1. *HER2* amplification status in patients with HER2 IHC 2+ GC/GEJC by ISH status

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

Data are shown as supplementary material to the poster (abstract number: 478), titled 'Concordance between human epidermal growth factor receptor 2 status by immunohistochemistry and next-generation sequencing in tissue and blood samples from gastric and gastroesophageal junction cancers', presented at ASCO GI 2025, January 23–25, San Francisco, CA, US and online. *HER2+ was defined as IHC 3+ or IHC 2+ / ISH+ tumors. GC, gastric cancer; GEJC, gastroesophageal junction cancer; HER2, human epidermal growth-factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization