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# *HER2* Splice Site Mutation c.1899-1G>A as the Potential Acquired Resistance to Trastuzumab in a Patient with HER2-Positive Gastric Adenocarcinoma

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*Disclosures of potential conflicts of interest may be found at the end of this article.*

## Abstract

The addition of trastuzumab to chemotherapy regimen is the standard of care for human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer; however, most patients eventually acquire trastuzumab resistance. Although some resistance mechanisms to trastuzumab-based regimens have been proposed, further understanding is required for developing therapeutic strategies to overcome the resistance. In the present work, we attempted to determine the possible resistance mechanism to trastuzumab in a patient with HER2-positive stage IV gastric adenocarcinoma. In this study, we first report the nucleotide change c.1899-1G>A at the intron 15 acceptor splice site promoting exon 16 deletion of *HER2* as the potential mechanism of trastuzumab resistance in HER2-positive gastric adenocarcinoma. ***The Oncologist*** 2021;26:717–721

## Key Points

- The combination of trastuzumab with chemotherapy is considered to be the standard therapy for HER2-positive advanced gastric cancer (GC), but most of the patients eventually acquire trastuzumab resistance. The mechanisms of resistance to trastuzumab in GC are poorly characterized.
- To the best of the authors' knowledge, this study is the first to implicate *HER2* c.1899-1G>A, which results in exon 16 skpping, as the acquired resistance mechanism to trastuzumab in HER2-positive gastric adenocarcinoma.
- This work provides insights into the potential molecular mechanism of trastuzumab resistance, which is crucial in developing effective therapeutic strategies for HER2-positive GC patients refractory to trastuzumab.

## Patient Story

In September 2018, a 30-year-old female was diagnosed with metastatic gastric cancer (stage IV). The histopathological diagnosis was poorly differentiated adenocarcinoma with HER2 overexpression (3+) (Fig. 1A, B). Her tumor marker levels in the plasma, including carcinoembryonic antigen (CEA; 64.22 μg/L) and cancer antigen (CA)19-9 (5,789 U/mL), were elevated. The primary gastric biopsy and plasma samples underwent capture-based next-generation sequencing (NGS) using a panel consisting of 520 cancer-related genes (OncoScreen Plus; Burning Rock Biotech, Guangzhou, China) for molecular analysis in September 2018. NGS consistently revealed *HER2* amplification from both samples. NGS also showed microsatellite stability (MSS) and low tumor mutation burden (TMB; 2.4 mutations per Megabase [Mb]) in the tissue sample. Table 1 lists the mutations detected from the samples. Figure 1C illustrates the entire treatment history.

The patient was initially treated on October 10, 2018, with trastuzumab combined with oxaliplatin S-1. Chest and abdominal computed tomography (CT) scans, performed

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after two courses, showed partial response (PR; Fig. 1D). Her CEA level decreased to 9.31 μg/L, and CA19-9 decreased to 674.9 U/mL. A review of her CT scans in January 2019 showed her disease remained as PR (Fig. 1D). After another month of treatment, she presented with right shoulder pain. Magnetic resonance imaging scans revealed the emergence of a new lesion in the proximal right humerus, evaluated as progressive disease (PD) with a progression-free survival (PFS) of 4 months (Fig. 1E). At PD, her plasma CEA (171 μg/L) and CA19-9 (3,192 U/mL) levels were markedly elevated.

Subsequently, her second-line treatment was switched to albumin-bound paclitaxel in combination with trastuzumab. Her best response was stable disease (SD); however, after five courses of treatment, she presented with pain on the pelvis and lower limbs. The abdominal CT examinations in May 2019 showed the newly developed pelvic metastasis with a PFS of 3.3 months (Fig. 1F). Her plasma CA19-9 level increased to 8,850 U/mL. Plasma sample obtained was submitted for NGS. Compared with the NGS results prior to first-line treatment, NGS revealed a decreased gene copy number of *HER2* (21.4), the new mutation of c.1899-1G>A in *HER2*, and higher TMB (6.3 mutations/Mb; Table 1). Cell-based splicing reporter minigene assay [1] and sanger sequencing analysis confirmed that the splice site mutation c.1899-1G>A in *HER2* gene resulted in the loss of the entire exon 16 (Fig. 2). As third-line treatment, she was administered a regimen of oxaliplatin, S-1, trastuzumab plus intravenous pembrolizumab, based on the the detection of programmed cell death ligand-1 (PD-L1) expression level by combined positive score (CPS) of ≥10 in gastric biopsy prior to third-line treatment. Treatment-related grade 1 adverse events were reported by the patient. The patient achieved SD lasting for 3.7 months. She succumbed to her disease on October 9, 2019, with an overall survival of 12 months (Fig. 1C).

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### Genotyping Results and Interpretation of the Molecular Results

Capture-based targeted sequencing results showed that the patient with HER2-positive metastatic gastric adenocarcinoma harbored *HER2* amplification with MSS and low TMB prior to first-line treatment.

Although several HER2-targeted therapies such as pertuzumab, lapatinib, trastuzumab emtansine, and trastuzumab deruxtecan (T-DXd) have been approved for the treatment of patients with HER2-positive breast cancer in either adjuvant or metastatic setting, these agents except for T-DXd have failed to demonstrate significant survival benefits in patients with HER2-positive advanced gastric or gastroesophageal junction (GEJ) cancer [2]. In recent years, an array of promising and novel anti-HER2 therapeutic agents and their combinations for HER2-positive gastric cancer (GC) have entered various stages of clinical development, such as tucatinib, margetuximab, and ZW45 [2].

Targeted therapeutic agents, such as trastuzumab and pembrolizumab, have been approved by the U.S. Food and Drug Administration (FDA) for use in gastric cancer. Trastuzamb is based on testing for HER2 positivity. Pembrolizumab is based on testing for microsatellite instability by polymerase chain reaction (PCR), mismatch repair deficiency by immunohistochemistry, PD-L1 expression by CPS, or high TMB by NGS. TMB has emerged as a potential biomarker for predicting the tumor response to immune checkpoint inhibitors (ICIs). Elevated TMB increases the odds of generating immunogenic neoantigens, thus inducing a response to ICIs [3].

Trastuzumab, a humanized monoclonal antibody, specifically binds to HER2 and thus inhibits its homodimerization and phosphorylation, resulting in inhibition of the proliferation of HER2-overexpressing tumor cells [4]. The combination of trastuzumab with chemotherapy is considered to be

**Table 1.** Results from NGS-based mutation analysis prior to trastuzumab treatment and after resistance to treatment with trastuzumab

| | At baseline prior to trastuzumab treatment | | | | | |
|---|---|---|---|---|---|---|
| | Primary tumor tissue | | Plasma sample | | After trastuzumab resistance: plasma sample | |
| Gene/biomarker | Variation | Abundance | Variation | Abundance | Variation | Abundance |
| *ARID1A* | p.Glu1958fs | 5.63% | p.Glu1958fs | 17% | p.Glu1958fs | 28.09% |
| *FGFR1* | ND | | amplification | 3.86 CN | amplification | 4.4 CN |
| *TP53* | p.Arg267Trp | 9.58% | p.Arg267Trp | 22.26% | p.Arg267Trp | 31.95% |
| *HER2* | amplification | 31.58 CN | amplification | 27.31 CN | amplification | 21.4 CN |
| *HER2* | ND | | ND | | c.1899-1G>A | 6.67% |
| TMB | | 2.4 mutations/Mb | | 2.4 mutations/Mb | | 6.3 mutations/Mb |
| MSI status | MSS | | NA | NA | NA | NA |

Abbreviations: CN, copy number; fs, frameshift; Glu, glutamic acid; Mb, megabase; MSI, microsatellite instability; MSS, microsatellite stability; NA, not applicable/not tested; ND, not detected; NGS, next-generation sequencing; Pro, proline; Ser, serine; TMB, tumor mutation burden; Trp, tryptophan.

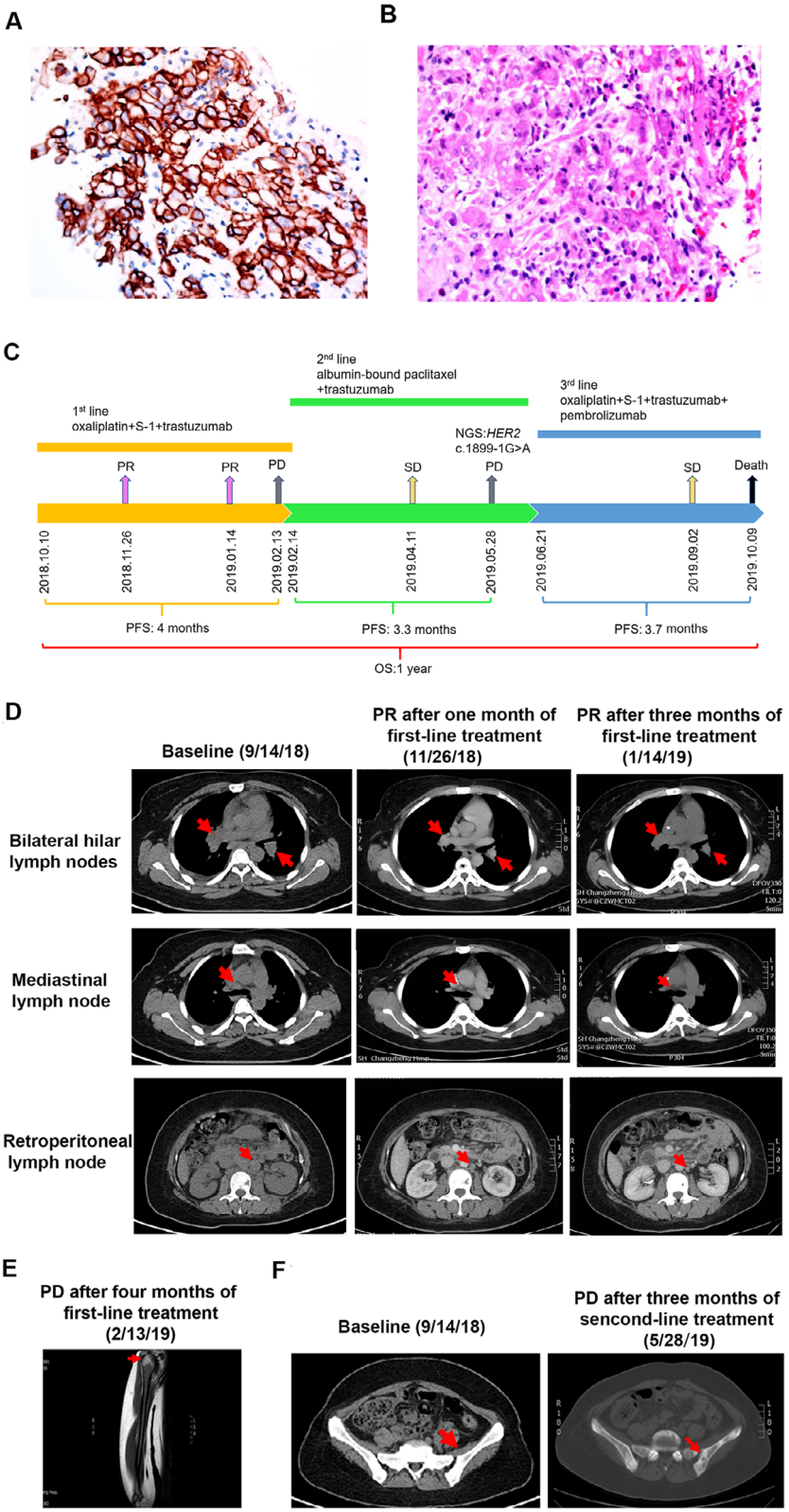


**Figure 1.** A summary of patient's treatment history. **(A):** Immunohistochemistry staining analyses showed the tumor cells were positive for HER2 expression (3+). **(B):** H&E staining showed a poorly differentiated adenocarcinoma. **(C):** The entire treatment procedure. **(D):** Chest and abdominal computed tomography (CT) scans at baseline and in November 2018 and January 2019 demonstrating PR in bilateral hilar lymph nodes, mediastinal lymph node, and retroperitoneal lymph node. **(E):** Magnetic resonance imaging scans in February 2019 showed the patient developed metastasis to right humerus after failure of first-line treatment. **(F):** CT scans in May 2019 showed the patient developed metastasis to pelvis after failure of second-line treatment.
Abbreviations: *HER2*: human epidermal growth factor receptor 2; NGS, next-generation sequencing; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

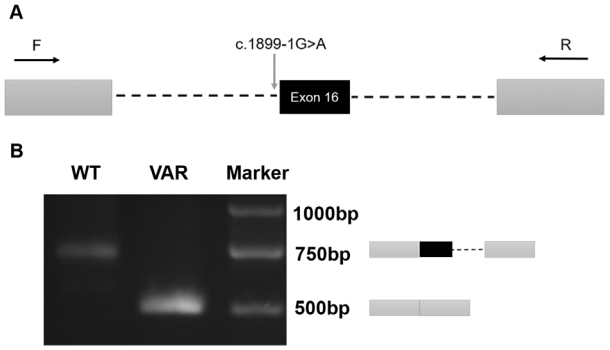


**Figure 2.** The *HER2* c.1899-1G>A variant resulted in aberrant splicing of exon 16. **(A):** Minigene assay was performed using human embryonic kidney 293T cells to investigate the impact of *HER2* c.1899-1G>A on the splicing of exon 16. *HER2* exon 16 coding sequence are indicated by black boxes, 150 base-pairs of 5' and 3' flanking intronic sequences are indicated by dash lines, two exons derived from *SERPING1/CINH* gene are indicated by dark gray boxes, and polymerase chain reaction (PCR) primers are indicated by black arrowhead. **(B):** Reverse transcription PCR (RT-PCR) analysis of the spliced transcripts expressed from the wild-type and mutant (c.1899-1G>A) minigene constructs. RT-PCR products were analyzed by agarose gel separation followed by sequencing of the different bands. The inclusion or the exclusion of *HER2* exon 16 in each transcript is schematically indicated on the right.
Abbreviations: F, forward primer; R, reverse primer; VAR, mutant construct containing the c.1899-1G>A variant; WT, wild-type minigene construct containing reference sequence.

the standard therapy for HER2-positive advanced GC, on the basis of the results from the Trastuzumab for Gastric Cancer trial indicating that HER2-positive patients treated with trastuzumab plus chemotherapy have a longer overall survival (13.8 months vs. 11.1 months) [5]. In this study, the case benefited from trastuzumab combined with oxaliplatin S-1 as first-line treatment with a PFS of 4 months.

Although an objective response rate of 47% to 71% is observed when trastuzumab is used in combination with chemotherapy for first-line treatment of HER2-positive advanced GC, most of the patients eventually acquire trastuzumab resistance [2]. However, The molecular mechanisms of acquired resistance to trastuzumab in HER2-positive GC remain elusive, which is due to the fact that NGS-based molecular testing is still not a common practice for monitoring trastuzumab resistance in HER2-positive GC. Compared with the NGS results prior to first-line treatment, NGS on plasma smaple after progression on second-line treatment revealed a decreased gene copy number of *HER2* (21.4), a new mutation of c.1899-1G>A in *HER2* (Table 1), and a higher TMB. CPS of ≥10 was shown in gastric biopsy prior to third-line treatment for testing PD-L1 expression.

Several potential mechanisms of acquired resistance to trastuzumab-based treatment in advanced HER2-positive gastric or GEJ cancers have been identified, such as tumor heterogeneity in HER2 positivity and loss of HER2 positivity. The previous studies have demonstrated that 32%–69% of patients with HER2-positive gastric cancer undergo HER2 loss in tumor rebiopsies at progression on trastuzumab-based treatment [6–8]. Furthermore, approximately 6% of initially HER2-negative patients with metastatic or recurrent gastric or GEJ cancers can turn out to have HER2-positive metastatic lesions [9]. Tumor rebiopsies from metastatic lesions should be performed to ascertain HER2 status and heterogeneity in patients who progress on trastuzumab-based treatment but is not likely possible in this case. The progression appears to be in bone only in the patient.

## Functional and Clinical Significance of the Specific Mutation in the Particular Cancer

Our work revealed that *HER2* c.1899-1G>A resulted in the deletion of exon 16, which was consistent with a recent study [10]. Exon 16 of *HER2* is 48 bases long, and its skipping causes an in-frame loss of 16 amino acids in the juxtatransmembrane region. The alternative mRNA splice variant of *HER2* lacking exon 16 frequently occurs in breast cancers [10–12]. However, exon 16 deletion of *HER2* (*HER2*D16) as a product of splice site mutation in *HER2* gene is rare, with the frequency of 0.15% in GC [10].

With the exception of the nucleotide change (-1G>A), other point mutations at the intron 15 acceptor splice site, such as -1G>C, have been previously reported as an oncogenic driver in lung cancer [10]. *HER2*D16 can drive lung tumorigenesis with an immunosuppressive tumor microenvironment in the genetically engineered mouse models [10]. *HER2*D16 resulting from c.1899-1G>A has been implicated as a novel mechanism of osimertinib resistance in a patient with epidermal growth factor receptor (*EGFR*) L858R/T790M-positive non-small cell lung cancer (NSCLC). Furthermore, the combination of osimertinib with afatinib can reverse the osimertinib resistance driven by *HER2*D16 in *EGFR* T790M/L858R-positive NSCLC cells [13]. Recently, a real-world retrospective cohort study has demonstrated that *HER2* exon 16 skipping contributes to the resistance mechanism to tyrosine kinase inhibitors in patients with *EGFR*-mutated lung cancer and might be an oncogenic driver in breast, colorectal, gastric, and ovarian cancers [14]. Our and a previous study suggest that *HER2*D16 might potentially contribute to the mechanism of acquired resistance to trastuzumab and might serve as a novel therapeutical target in gastric cancer. *HER2*D16 as the potential mechanisms of resistance to trastuzumab-based regimens is proposed in this work. However, we can not conclude that *HER2* splice site mutation c.1899-1G>A is the mediator of trastuzumab resistance in the context of lacking the validation from firm functional assays. Further *in vitro* and *in vivo* studies are needed to elucidate the oncogenic properties of point mutation c.1899-1G>A in HER2-positive gastric cancer and validate this splice site mutation as the mechanism of acquired resistance to trastuzumab in HER2-positive gastric cancer.

## Potential Strategies to Target the Pathway and Implications for Clinical Practice

Preclinical studies have revealed the effect of trastuzumab on inhibiting *HER2*D16 oncogenic activity, but yielded controversial results. Trastuzumab resistance has been demonstrated *in vitro* in *HER2*D16-expressing breast cancer cell line [15], but several studies from the same group have demonstrated contradicting results, with one reporting that *HER2*D16-transformed cells shows reduced sensitivity to

trastuzumab and the other reporting that the cell proliferation induced by an increased *HER2*D16 expression in HER2-positive GC patient-derived xenografts is effectively inhibited by trastuzumab [11, 16, 17].

Our patient obtained clinical benefit from trastuzumab in combination with pembrolizumab and chemotherapy as the third-line treatment with a PFS of 2 months, but she succumbed to her disease after another month treatment. Because the multidrug treatment with targeted agent, immune checkpoint inhibitor, and chemotherapy were administered simultaneously, we cannot delineate the lack of therapeutic effect of each agent. However, we speculate that the trastuzumab resistance mediated by the emergence of the *HER2* c.1899-1G>A contributed to the lack of response and short disease control with the multidrug regimen. T-DXd is a novel HER2-targeting antibody-drug conjugate. Based on the findings of DESTINY-Gastric01 trails [18], T-DXd has been approved on January 15, 2021, by the FDA for the treatment of patients with locally advanced or metastatic HER2-positive gastric or GEJ adenocarcinoma who have received a prior trastuzumab-based regimen. Further studies are needed to illuminate the efficacy of T-DXd and other anti-HER2 agents in GC patients with acquired *HER2*D16.

## Glossary of Genomic Terms and Nomenclature

**Adenine (A):** A nitrogenous purine base, which bonds with thymine (T) to form the A–T base pair in DNA and the A-U base pair in RNA

**Guanine (G)**: A nitrogenous purine base, which bonds with cytosine (C) to form the G-C base pair

**c.**: a cDNA sequence

**>**: nucleotide substitutions

**c.1899-1G>A**: the G to A substitution at the last nucleotide of intron 15 of the HER2 gene

**Messenger RNA (mRNA)**: RNA that serves as a template for protein synthesis

**Megabase (Mb)**: Unit of length for DNA fragments equal to 1 million nucleotides

**Exon**: DNA sequence portion of a gene that codes for the protein

**Intron**: DNA base sequence between exons

## Acknowledgments

This study was funded by the Shanghai Municipal Science and Technology Committee (17511103400). This study was approved by the Ethics Committee of the Changzheng Hospital. Informed consent was obtained from the participant.

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## Disclosures

**Jianxing Xiang:** Burning Rock (E). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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