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Original Article

Trastuzumab plus docetaxel and capecitabine as a first-line treatment for HER2-positive advanced gastric or gastroesophageal junction cancer: a phase II, multicenter, open-label, single-arm study

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Abstract: Gastric cancer (GC) is the second most common cancer in China. The ToGA study showed that trastuzumab in combination with fluoropyrimidine plus cisplatin prolonged overall survival (OS) in patients with human epidermal growth factor receptor 2 (HER2)-positive advanced GC (AGC). However, some patients may not be able to receive this regimen. We conducted a clinical study to evaluate the efficacy and safety of trastuzumab in combination with docetaxel+capecitabine (DX) in patients with HER2-positive AGC. This phase II, multi-center, open-label, single arm study enrolled patients with HER2-positive AGC who had not received prior treatment for metastatic disease. Patients were treated with a regimen of trastuzumab (8 mg/kg loading dose followed by 6 mg/kg, day 1), capecitabine (1000 mg/m^2 twice daily, days 1-14) and docetaxel (60 mg/m^2, day 1 for 6 cycles) every 3 weeks. The primary endpoint was progression-free survival (PFS) and the secondary endpoints were objective response rate (ORR), OS and safety profiles. Sixty-seven patients with AGC were enrolled from 14 centers. 64 were included in the full analysis set (FAS). The median PFS was 8.1 months (95% confidence interval [CI]: 5.6-12.8) and the median OS was 20.9 months (95% CI: 15.1-33.0). Response was evaluated in 59 patients. The ORR was 67.8%. The most common adverse events of Grade ≥3 were neutropenia, leukopenia, hand-foot syndrome, febrile neutropenia and anemia. We concluded that combination treatment with trastuzumab and DX was well-tolerated and highly effective in patients with HER2-positive AGC, and may offer an alternative to current treatments.

Keywords: Gastric cancer, trastuzumab, HER2, docetaxel, capecitabine

Introduction

Gastric or gastroesophageal junction (GEJ) cancer is both the second most common and second most lethal cancer in China [1]. China has one of the highest age standardized-incidence rates per 100,000 individuals in the world [2]. Eighty percent of patients diagnosed with gastric cancer (GC) in China are in an advanced stage [3]. The 5-year survival rate for Chinese patients with GC was 35.9% between 2010 and 2014, much lower than that observed in Korea (68.9%) and Japan (60.3%) [4]. Although studies suggest a modest survival advantage can be gained from chemotherapy [5-9], more effective systemic regimens need to be identified for treatment of patients with advanced gastric cancer (AGC).

Some GC tumors are human epidermal growth factor receptor 2 (HER2)-positive and the rate of HER2 overexpression on GC tumors in Chinese patients is 7-12% [10, 11]. Trastuzumab (Herceptin®, Roche, Shanghai Roche Pharmaceuticals Ltd., China) is a humanized antibody targeting HER2 that binds and inhibits tumor proliferation and survival [12]. The ToGA trial demonstrated that trastuzumab in combination with capecitabine or 5-fluorouracil (5-FU) plus cisplatin improved progression-free survival (PFS) and overall survival (OS) in patients with HER2-positive AGC [13]. Trastuzumab is the only targeted therapy currently approved for a first-line treatment of patients with HER2+ AGC [14]. First-line trastuzumab used in combination with cisplatin and capecitabine or 5-FU is the current standard treatment for HER2-positive metastatic GC (mGC) [9, 15]. While the ToGA trial showed the benefits of trastuzumab in combination with capecitabine or 5-FU plus cisplatin, data demonstrating the benefit of other chemotherapy regimens combined with trastuzumab for the treatment of AGC is limited [16].

Despite the survival benefit achieved with trastuzumab, some patients develop resistance to the treatment [12]. While the potential molecular mechanisms behind this resistance are under investigation [17-19], further work is needed to reach a definitive conclusion [20].

Other chemotherapy regimens that may show benefit in combination with trastuzumab include docetaxel plus capecitabine (DX), which has shown a synergistic effect in AGC animal models [21] and activity in patients with AGC or advanced esophageal cancer (AEC) [22, 23]. However, the combination of trastuzumab plus DX for AGC has not yet been studied. We evaluated the efficacy and safety of trastuzumab in combination with DX as a first-line therapy in Chinese patients with HER2-positive AGC.

Methods

Patients

This study was a phase II, multicenter, open label, single-arm clinical study designed to evaluate the efficacy and safety of trastuzumab in combination with DX as a first-line treatment in Chinese patients with inoperable, locally advanced or recurrent and/or HER2-positive metastatic GC or GEJ adenocarcinoma.

Additional eligibility criteria included >18 years of age, Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-1, and an expected survival of ≥3 months. Major exclusion criteria included previous chemotherapy for advanced/metastatic disease, previous or current use of any epidermal growth factor receptor-targeted drugs, loss of upper gastrointestinal tract physical integrity or malabsorption syndrome, active (significant or uncontrolled) gastrointestinal bleeding, history or evidence of brain metastases, residual relevant toxicity from previous therapy (except alopecia), other malignancy within the last 5 years (exceptions: carcinoma *in situ* of the cervix or basal cell carcinoma), congestive heart failure, baseline left ventricular ejection fraction (LVEF) less than 50%, angina pectoris requiring medication, evidence of transmural myocardial infarction on electrocardiogram (ECG), poorly controlled hypertension (systolic blood pressure [BP] >180 mmHg or diastolic BP >100 mmHg), clinically significant valvular heart disease or high risk uncontrollable arrhythmias.

This study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The protocol was approved by the institutional review board at each participating center. All patients provided written informed consent before enrollment and were capable of protocol compliance. This study was registered at ClinicalTrials.gov (NCT02004769).

Tumor specimens and HER2 status confirmation

All tumor specimens collected from patients who received tumor resection during surgery or biopsy at our hospital were processed as formalin-fixed paraffin-embedded (FFPE) tissues. The HER2 status of tumors was evaluated using immunohistochemistry (IHC) with anti-HER2/neu (4B5) monoclonal antibody (Ventana Medical Systems, AZ, USA) [24]. If the IHC score was 2+, fluorescence *in situ* hybridization (FISH) was performed using the PathVysion HER-2 DNA Probe Kit (Abbott Molecular, IL, USA). A tumor was considered HER2-positive if it scored IHC2+ and had a confirmed FISH+ result (HER2: chromosome enumeration probe 17 ratio ≥2), or if it scored IHC3+. FISH analyses for HER2 status were carried out according to the manufacturer's protocol (Vysis, Inc., IL, USA). HER2 testing was performed at either Sun Yat-Sen

University Cancer Center or a local hospital laboratory.

Treatment plan and tumor assessment

Patients received one cycle of treatment with trastuzumab in combination with DX every 3 weeks. All patients were treated until either disease progression, occurrence of unacceptable toxicity or withdrawal from the study for other reasons. Each cycle of treatment included trastuzumab plus DX administered as follows: trastuzumab was administered by intravenous (IV) infusion at a loading dose of 8 mg/kg on treatment Cycle 1 Day 1, followed by a 6 mg/kg dose every 3 weeks (i.e., per cycle); capecitabine was administered for the first 14 days of each cycle (evening of Day 1 to morning of Day 15) and given orally at a dose of 1000 mg/m^2 twice daily (total daily dosage, 2000 mg/m^2); and docetaxel was administered at a dose of 60 mg/m^2 as an IV infusion (infusion time: 1 h) on Day 1 of each cycle (maximally 6 cycles), starting after completion of the trastuzumab infusion.

The primary end point was progression-free survival (PFS). The secondary end points were objective response rate (ORR), overall survival (OS) and safety. Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, using only measurable target lesions [25]. To be considered measurable by computerized tomography (CT) or magnetic resonance imaging (MRI), tumor lesions had to be at ≥10 m in at least one dimension. For a lymph node to be considered pathologically enlarged and measurable, it must be ≥15 mm in the shorter axis when assessed by CT scan.

Complete tumor assessment was performed ≤21 days before the first dose of study treatment; follow-up tumor assessments were performed at 6-week intervals from the start of study treatment. The duration of follow-up was either when 80% of patients died or 18 months after the last patient was enrolled, whichever occurred first.

Performance status and safety assessments

ECOG PS was assessed at baseline and every 21 days during the scheduled visits. The National Cancer Institute Common Toxicity Criteria for Adverse Events (version 4.0) was used for safety evaluations, with the exception of cardiac failure which was graded according to the New York Heart Association classification system. Complete medical histories, including demographics, were recorded during the screening visits. Physical examinations were performed at baseline and at each study visit, or as clinically indicated. 12-lead ECG was performed on each patient at baseline, before initiating each treatment cycle and at any time during the study if clinically indicated. LVEF was measured using a multiple-uptake gated acquisition scan, cardiac MRI or ECG. Baseline LVEF assessments were performed ≤21 days prior to the start of study treatment and then every 12 weeks (more often if clinically indicated) during the study and in the 6 months following the end of treatment/withdrawal from the study.

Statistical analysis

The sample size was determined to be 65 patients assuming an improvement in PFS from 5.5 months to 6.7 months with the addition of trastuzumab [13] and allowing for a 5% drop out rate. The full analysis set (FAS) consisted of all intention-to-treat patients who received at least one dose of any drug as part of the study treatment and was the main population for efficacy evaluation. The safety analysis set (SAF) consisted of all patients who had received at least one dose of any drug as part of the study treatment and completed at least one safety evaluation.

The primary efficacy parameter, duration of PFS, was defined as the time from the initiation of treatment to the date of progressive disease (PD) or death. OS was measured from the initiation of treatment to the date of the last follow-up or death by any cause. Overall tumor response was defined as the occurrence rate of a confirmed complete response (CR) or a partial response (PR) determined by confirmed radiographic evaluations of target and non-target lesions. PFS and OS were analyzed using the Kaplan-Meier method and 95% confidence intervals (CI) were calculated using the Clopper-Pearson Exact method [26]. The change in tumor size from baseline was analyzed using a waterfall plot. The ORR and PFS analyses and the safety evaluation were conducted after 80% of PFS events were observed. OS and safety analyses were conducted when 80% of deaths were observed, or 18 months after the

Table 1. Baseline patient characteristics (FAS)

Parameter	FAS (*N*=64)
Median age (range), years	58 (19-76)
Sex, *n* (%)	
Male	47 (73.4)
Female	17 (26.6)
ECOG PS, *n* (%)	
0	14 (21.9)
1	50 (78.1)
History of gastric cancer surgery, *n* (%)	
No	44 (68.8)
Yes	20 (31.3)
HER2 status, *n* (%)	
HER2++ plus FISH+	13 (20.3)
HER2+++	51 (79.7)
Location of primary lesion, *n* (%)	
Gastric	53 (82.8)
Gastroesophageal junction	11 (17.2)
Lauren classification, *n* (%)	
Intestinal	15 (23.4)
Diffuse	7 (10.9)
Mixed	5 (7.8)
Unknown	37 (57.8)
Differentiation, *n* (%)	
G1	3 (4.7)
G2	21 (32.8)
G3	22 (34.4)
Gx	5 (7.8)
Unknown	13 (20.3)
Metastasis, *n* (%)	
Liver	27 (42.2)
Lung	16 (25.0)
Lymph node	46 (71.9)
Ovary	2 (3.1)
Peritoneum	8 (12.5)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; FAS, full analysis set; HER2, human epidermal growth factor 2.

last patient was enrolled, whichever occurred first. Descriptive statistics were calculated for safety analyses.

All statistical analyses were performed using SPSS software (SPSS 11.0, Chicago, Ill., USA). All *p* values were two sided and a *p* value ≤0.05 was considered statistically significant.

Whole exome sequencing

All FFPE tumor specimens of twenty seven patients were sent to HaploX (Shenzhen, Guangdong, China) for next generation high throughput sequencing. The resulting tumor DNA libraries were sequenced using the Illumina HiSeq Genome Analyzer (Illumina, San Diego, CA, USA), which yielded approximately 150 bp of paired end sequence. FASTQ files were then generated.

Gene variant calling and filtering

Variant calling was carried out using a series of published software in accordance with official instructions as follows: we used Burrows-Wheeler Aligner (version 0.7.10-r789) [27] and Sambamba (version 0.5.9) [28] to process paired-end FASTQ sequences, then generated duplicate-marked, sorted, base-recalibrated, indexed Binary Alignment/Map (BAM) files; SpeedSeq (version 0.1.2) [29] and GATK4 HaplotypeCaller (version 4.0.12.0) were used to call germline single nucleotide polymorphisms and insertions/deletions simultaneously. Variants identified by both methods were considered as high confidence mutations. Bcftools software (version 1.9-94-g9589876) was used to create intersections of variant call format files. Next, we used ANNOVAR with online databases (version hg38) to annotate the variant calls. The online databases used included COSMIC v76, refGene, avsnp147, ALL.sites.2015_08_edit, EAS.sites.2015_08_edit, esp6500siv2_all, exac03 and clinvar_20170905. Mutations with single nucleotide variants (SNVs) in the ALL.sites.2015_08_edit database that were detected at a frequency of ≤0.1% were analyzed. SNVs annotated with "intergenic" or "intronic" in the Func.refGene database and "synonymous SNV" in the ExonicFunc.refGene database were removed. The R package maftools was used to process and visualize the filtered SNVs [30].

Results

Patient characteristics

Between November 2013 and June 2016, a total of 67 patients were enrolled from 14 hospitals in China. Fifty-eight patients completed follow-up and nine discontinued or withdrew from the study (patient withdrew consent, *n*=5; death or lost to follow-up, *n*=3; other, *n*=1). The FAS comprised 64 patients; three patients were not included in the FAS because they did not receive any drugs related to the study treatment. Patient demographics and baseline disease characteristics are shown in **Table 1**. The

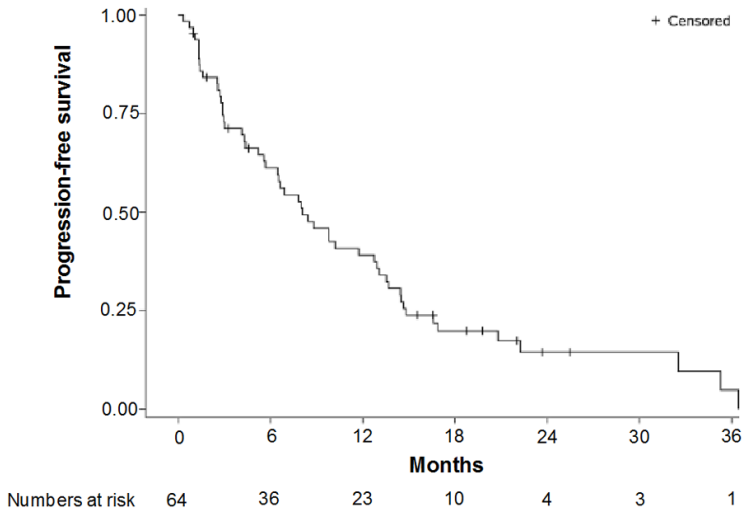

Figure 1. Progression-free survival, Kaplan-Meier.

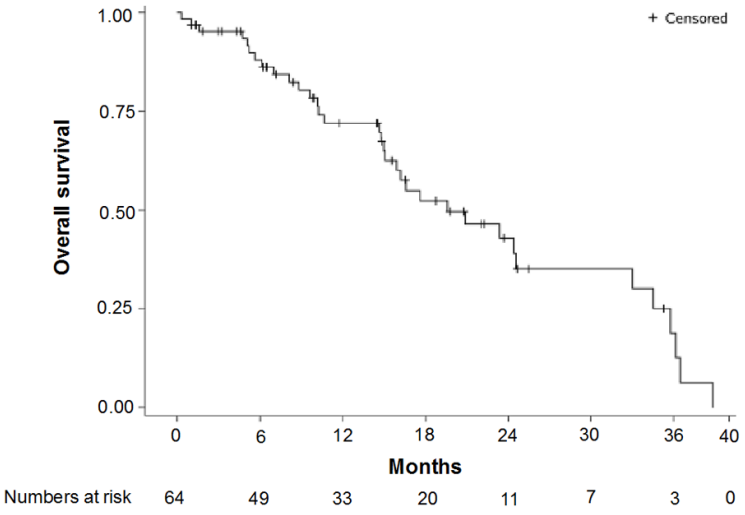

Figure 2. Overall survival, Kaplan-Meier.

Table 2. Overall objective response rate based on RECIST

Response	*n* (%)
Total	59 (100)
CR	5 (8.5)
PR	35 (59.3)
SD	11 (18.6)
PD	8 (13.6)
ORR	40 (67.8)

Abbreviations: CR, complete response; ORR, objective response rate; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria In Solid Tumors; SD, stable disease.

median age at the time of enrollment was 58 years (range: 19-76). 31.3% of patients had a history of GC surgery. The number of HER2-positive tumors based on IHC3+ and IHC2+ plus FISH+ were 51 (79.7%) and 13 (20.3%), respectively. All patients had differentiated adenocarcinoma; 11 (17.2%) patients had GC and 53 (82.8%) had GEJ cancer. The most frequent site of metastasis was the lymph node (71.9%), followed by the liver (42.2%), lung (25.0%) and ovary (3.1%).

Efficacy

The median duration of follow-up at the time of analysis (FAS; *n*=64) was 14.5 months. The median PFS was 8.1 months (95% CI: 5.6-12.8 and the median OS was 20.9 months (95% CI: 15.1-33.0) (**Figures 1** and **2**). The ORR was evaluated in 59 patients (5 patients were missing) using the RECIST (version 1.1) criteria. Five patients achieved CR and 35 achieved PR; the ORR was 67.8% (95% CI 0.54-0.79) (**Table 2**). The median time to response was 1.77 months (95% CI: 1.44-4.3) and among the 40 patients who achieved CR or PR, the median duration of response was 11.5 months (95% CI: 6.8-13.2). **Figure 3** shows a waterfall plot of tumor size reduction over time, demonstrating that most patients experienced a reduction of tumor size compared to baseline.

Safety

Adverse events (AEs) are listed in **Table 3**. The median number of trastuzumab treatment cycles was 8.0 (1.0-51.0). Adverse events were observed in 63 of the 67 patients who received at least one dose of the study treatment. The most common Grade ≥3 AEs were neutropenia (22.4%), followed by leukopenia (19.4%), hand-foot syndrome (9.0%), febrile neutropenia (4.5%) and anemia (3.0%) (**Table 3**). We observed acceptable cardiac safety in this study. An LVEF decrease by ≥15% was reported for one patient. One AE (febrile neutropenia) leading to death was reported in this study.

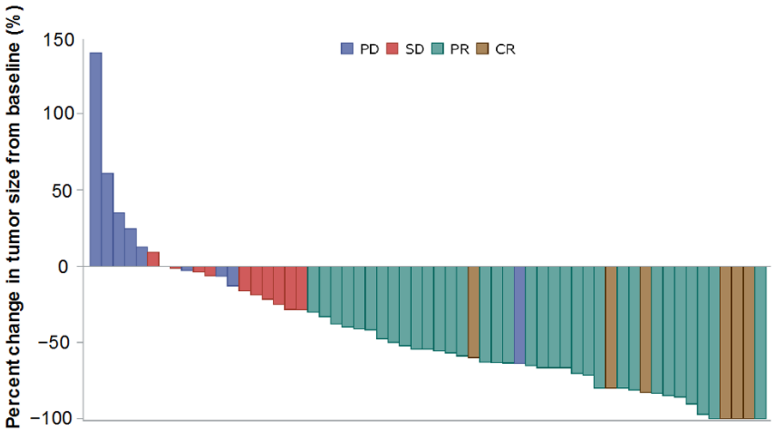

Figure 3. Waterfall plot of percent change in tumor size from baseline. Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Table 3. Adverse events (SAF)

	SAF (*N*=67), n %	
Any AEs	63 (94.0)	
Any SAEs	16 (23.9)	
Treatment-related SAEs	14 (20.9)	
AEs leading to treatment withdrawal	7 (10.5)	
AEs leading to death	1 (1.5)	
All cause AEs (≥10% incidence rate)	All grade, n (%)	Grade ≥3, n (%)
Metabolic and nutritional diseases		
Hypoalbuminemia	11 (16.4)	0
Hypocalcemia	7 (10.45)	0
Laboratory test		
ALT increase	13 (19.4)	1 (1.5)
AST increase	17 (25.4)	1 (1.5)
TBIL increase	11 (16.4)	1 (1.5)
Blood pressure increase	8 (11.9)	0
Neutropenia	29 (43.3)	15 (22.4)
Platelet count decrease	13 (19.4)	0
Leukopenia	37 (55.2)	13 (19.4)
Skin and subcutaneous tissue diseases		
Hand-foot syndrome	17 (25.4)	6 (9.0)
Gastrointestinal diseases		
Diarrhea	8 (11.9)	0
Nausea	7 (10.45)	0
Vomiting	7 (10.45)	1 (1.49)
Blood and lymphatic diseases		
Anemia	26 (38.8)	2 (3.0)

Abbreviations: AE, adverse event; ALT, alanine transaminase; AST, aspartate aminotransferase; SAE, serious adverse event; SAF, safety analysis set; TBIL, total bilirubin.

Genomic analysis

To explore gene candidates suitable for the prediction of patients with GC most likely to respond to trastuzumab/DX treatment, we applied a batch of tests for each gene mutation status and the study treatment response in seventeen patients' tumor samples. We found seven genes that were statistically significantly related to treatment response (Figure S1). These seven genes were *CTAGE15*, *RIMBP3*, *BRSK2*, *AP3B1*, *IQCE*, *MGA* and *NPBWR2*, in which 76%, 76%, 71%, 53%, 53%, 24% and 6% of patients had mutations, respectively. Among the seven mutated genes, two were previously reported to be related to GC: *BRSK2* and *AP3B1*. The response rates for mutant and wild-type *AP3B1* in this study were 33.3% and 87.5%, respectively (*P*= 0.0498); response rates for mutant and wild-type *BRSK2* were 41.7% and 100%, respectively (*P*=0.0441). Patients with wild-type *AP3B1* or *BRSK2* genes had a better response to the study treatment.

Discussion

This phase II, multi-center, open-label, single arm study examined trastuzumab with DX as a treatment regimen for first-line therapy in patients with HER2-positive advanced gastric or GEJ cancer. The results of this study showed a favorable ORR of 67.8% (95% CI: 0.54-0.79), median PFS of 8.1 months (95% CI: 5.6-12.8) and median OS of 20.9 months (95% CI: 15.1-33.0). Treatment outcomes were comparable to previously investigated treatment regimens for this patient population, including the ToGA trial, which reported an ORR of 47%, median PFS of 6.7 months (95% CI: 6-8) and median OS of 13.8 months (95% CI: 12-16) for trastuzumab plus

cisplatin and capecitabine/5-FU treated patients [13]. This treatment regimen demonstrated an acceptable safety profile with only 23.9% of patients experiencing an SAE. This drug combination appears to be an effective and safe alternative to the standard cisplatin and 5-FU treatment regimens currently recommended for AGC; however, further clinical studies using trastuzumab plus DX in AGC patients are warranted.

Our results are in line with previous reports from phase II studies evaluating similar regimens of DX in patients with AGC or AEC [22, 23]. These studies reported an ORR of 39-46%, median PFS of 4.2-6.1 months and median OS of 9.4-15.8 months [22, 23]. Our study used this chemotherapy regimen with the addition of trastuzumab and demonstrated for the first time the clinical efficacy of trastuzumab in combination with DX in HER2-positive AGC. While there are promising options available for a first-line treatment of metastatic AGC that are reported to have good response rates and survival benefits, there is still a lack of effective treatment options for patients who have relapsed after treatment with a cisplatin-containing regimen especially those who recur in a short term. HER2-positive AGC patients have a higher rate of disease recurrence after resection. There is currently no standard second-line therapy for patients with recurrent HER2-positive AGC [9, 31] and while several clinical studies have investigated potential second-line treatments in these patients, none have been successful to date [32]. The combination of DX might be an option for these patients if they have maintained a suitable ECOG PS. Patients unable to tolerate platinum-based therapies may also be candidates for this treatment regimen. Thirty-three (51.6%) patients received second line chemotherapy after disease progression. Two patients received surgery after enrolling into the trial and two patients received radiotherapy.

The overall incidence of Grade ≥3 AEs in our study was 49.3% (95% CI: 37.3-61.2). The relative absence of Grade ≥3 AEs other than neutropenia (*n*=15, 22.4%), leukopenia (*n*=13, 19.4%) and hand-foot syndrome (*n*=6, 9.0%) observed in this study was remarkable and is lower than that reported in the two phase II studies evaluating the DX treatment regimen in patients with AGC [22, 23]. The ToGA study reported an overall incidence rate of Grade ≥3 AEs of 68%, which is higher than that reported in the present study; incidence rates of 30% and 2% were reported for neutropenia and hand-foot syndrome Grade ≥3, respectively. The incidences of all grades of diarrhea, nausea and vomiting were lower in the present study than that reported for the ToGA trial; the incidence of all grades anemia was higher in this trial (38.8%) than in the ToGA trial (28%) but the occurrence of Grade ≥3 anemia was lower (3.0% and 12%, respectively) [13]. One treatment-related death was reported in this study. This rate is similar to that observed in the ToGA trial (3%) [13] and two phase II trials evaluating DX treatment, which reported 1 (2.2%) and 0 deaths [22, 23].

In addition, we found that a lack of response to this treatment regimen was significantly related to missense mutations for two genes, *BRSK2* and *AP3B1*. *BRSK2* encodes a serine/threonine-protein kinase of the AMPK family that acts as a checkpoint kinase [33] and was previously found to be amplified in cases of GC [34]. The clinical implications of BRSK2 expression in pancreatic ductal adenocarcinoma have been published; BRSK2 was found to be induced by nutrient deprivation in pancreatic ductal adenocarcinoma cells and to suppress mTORC1 activity [35]. The *AP3B1* gene encodes the large B1 subunit of the adaptor-related protein complex-3 and is involved in protein trafficking to lysosomes or specialized endosomal-lysosomal organelles [36]. A previously published expression profile analysis of premalignant lesions of GC revealed that the *AP3B1* gene was upregulated [37]. Though some reports demonstrate an association between *BRSK2* and *AP3B1*, the potential roles of these two genes in GC have not been well studied. Additional studies are needed to determine the underlying functions of these two genes in AGC and to confirm the clinical implications of the present findings.

This study was limited in that it was a relatively small, single-arm study so it could not be directly compared to other treatment regimens, and tumor samples were not available from all patients for testing correlations between gene mutations and treatment response.

Conclusions

The combination of trastuzumab plus DX was an effective and safe first-line treatment regi-

men for AGC. Further studies with larger numbers of patients are warranted to directly compare trastuzumab in combination with DX versus in combination with other platinum-based agents plus fluorouridine regimens and to confirm the results of the present study.

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All patients provided written informed consent before enrollment and were capable of complying with the protocol.

Disclosure of conflict of interest

None.

Abbreviations

5-FU, 5-fluorouracil; AE, adverse event; AEC, advanced esophageal cancer; AGC, advanced gastric cancer; BP, blood pressure; CI, confidence interval; CR, complete response; CT, computerized tomography; DX, docetaxel plus capecitabine; ECG, electrocardiogram; ECOG, Eastern Cooperative Oncology Group; FAS, full analysis set; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence *in situ* hybridization; GC, gastric cancer; GEJ, gastroesophageal junction; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; IV, intravenous; LVEF, left ventricular ejection fraction; mAb: monoclonal antibody; mGC, metastatic gastric cancer; MRI, magnetic resonance imaging; ORR, objective response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; PS, performance status; RECIST, Response Evaluation Criteria in Solid Tumors; SNV, single nucleotide variant.

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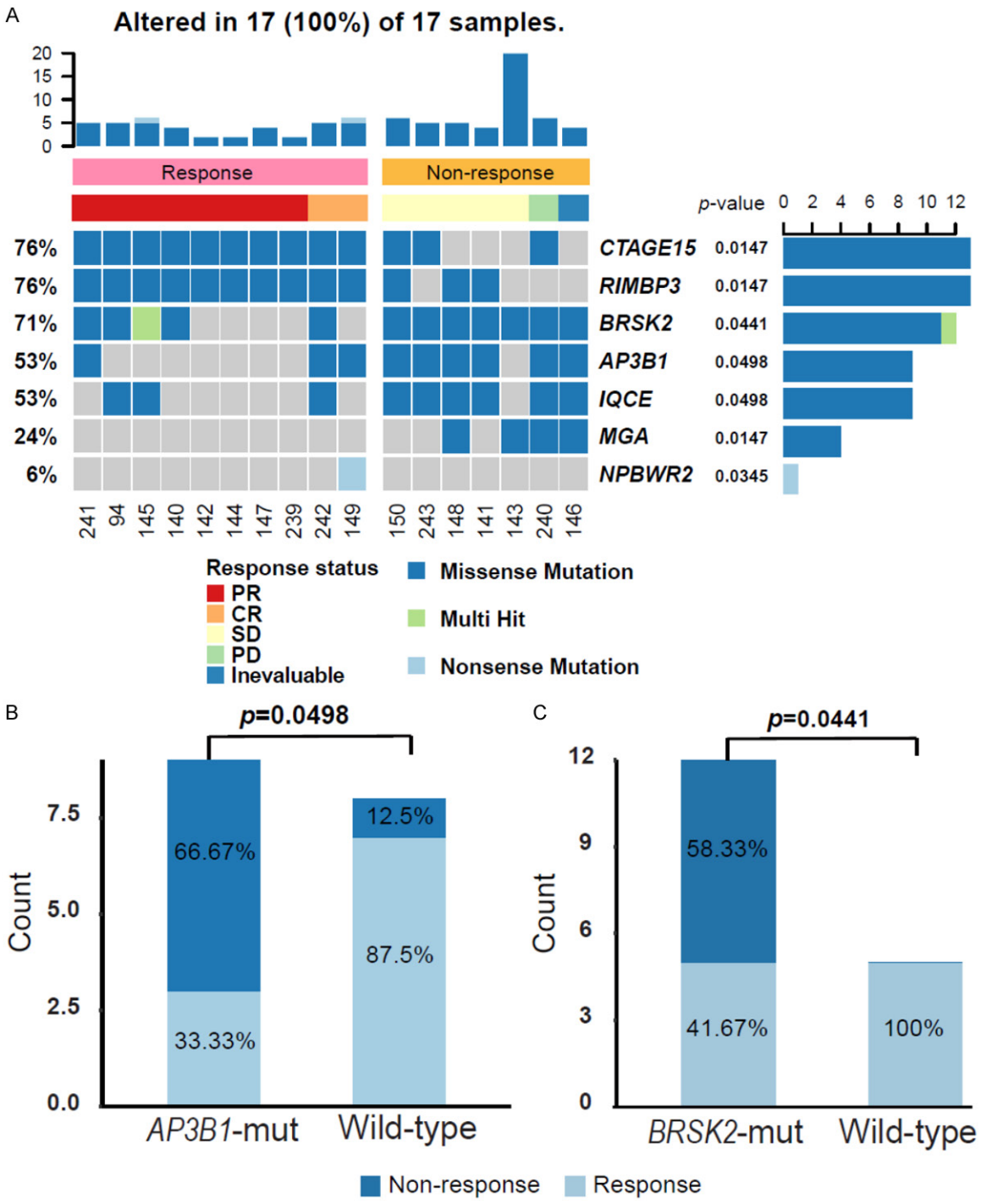

Figure S1. Mutations in specific genes associated with response to trastuzumab and chemotherapy. A. Response-associated mutations in patients with the response status of CR/PR versus SD/PD/Inevaluable (two-tailed Fisher's exact test; all mutation rates of specific genes were significantly different between the two groups). Bottom numbers represent sample names. Mutation types are indicated using different colors. B and C. Bar graphs showing the proportion of patients that responded to trastuzumab treatment according to *AP3B1* and *BRSK2* wild-type or mutant status. Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.