Intra-tumoral FGFR2 Expression Predicts Prognosis and Chemotherapy Response in Advanced HER2-positive Gastric Cancer Patients

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Abstract. *Background/Aim: This study aimed to evaluate the relationship between clinical outcomes and intra-tumoral fibroblast growth factor receptor 2 (FGFR2) expression in human epidermal growth factor receptor 2 (HER2)-positive gastric cancer (GC) patients who had undergone HER2-targeted chemotherapy. Patients and Methods: A retrospective analysis was performed in 22 patients with HER2-positive GC, who had undergone systemic chemotherapy. We performed immunohistochemistry staining of FGFR2 expression using surgically resected specimens or biopsied samples and evaluated clinicopathological characteristic and overall survival (OS) in the FGFR2-negative and -positive GC groups. Results: A total of 8 and 14 patients were placed in the FGFR2-negative and -positive group, respectively. The median OS rates were 56.2 and 16.0 months in the FGFR2-negative and -positive groups, respectively. The FGFR2-negative group had a significantly better prognosis after HER2-targeted chemotherapy [p=0.027 (log-rank test)]. The univariate analysis revealed that performing gastrectomy, response to combination chemotherapy with trastuzumab, and FGFR2 positivity were significantly correlated with OS. In a multivariate analysis, the response to combination chemotherapy with trastuzumab (p=0.008) was significantly correlated with OS. In addition, the proportions of patients who showed CR or PR in response to chemotherapy were 87.5 and 42.9% in the FGFR2-negative and -positive groups, respectively (p=0.031). Conclusion: HER2-positive GC patients, without overexpression of FGFR2, exhibited an improved prognosis and response rate to trastuzumab combination chemotherapy. Assessment of intra-tumoral FGFR2 expression could be helpful in predicting the prognosis and response to trastuzumab in HER2-positive GC patients.*

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Gastric cancer (GC) is one of the most common types of malignant tumors, and the third most common cause of cancer mortality worldwide (1). Although early GC is curable, advanced GC is still associated with a poor survival, and the curative treatment consists of a gastrectomy combined with perioperative chemotherapy (2, 3). A randomized, controlled trial of reduction surgery plus chemotherapy, *versus* chemotherapy alone for stage IV GC (REGATTA trial), failed to show any improved prognosis associated with surgery (4). Thus, systemic chemotherapy has remained the essential treatment for metastatic or recurrent GC. The recent progress of systemic chemotherapeutic and targeted agents could provide significant improvement in the survival rates of GC patients with metastasis. However, the prognosis of metastatic GC patients remains poor with a 5-year overall survival (OS) rate of around 5-20% (5).

Human epidermal growth factor receptor 2 (HER2) is a proto-oncogene, which is encoded by ERBB2 on chromosome 17. It is also a transmembrane tyrosine kinase receptor of 185kDa (6, 7). The rate of HER2-overexpression in GC is reported as between 7% to 34% (8). In HER2-overexpressing metastatic GC, trastuzumab, a recombinant humanized monoclonal antibody directed against HER2, has been used for systemic chemotherapy as first-line treatment. The ToGA trial demonstrated that addition of trastuzumab to a double chemotherapeutic regimen with cisplatin and fluoropyrimidine

improved OS in HER2-positive metastatic gastric or gastroesophageal junction adenocarcinoma patients (9). Trastuzumab significantly improved the prognosis of HER2-positive GC patients, although even responding patients inevitably worsened due to a limited response or acquired resistance to trastuzumab (10). Therefore, understanding and predicting the therapeutic resistance to trastuzumab is critical in deciding upon the therapeutic strategy and thus improving the prognosis in metastatic HER2-positive GC patients.

Fibroblast growth factor receptor 2 (FGFR2), a tyrosine kinase receptor, has been shown to be activated in several types of cancers through a variety of mechanisms, including gene amplification, translocations, and point mutations (11). In a meta-analysis of studies on FGFR2 overexpression, GC patients have a wide range of FGFR2 overexpression frequencies from 2.5 to 61.4% (12). In recent years, the relationship between FGFRs activation and HER2-targeted therapy resistance has been reported in breast and esophageal GC (13-15). In addition, GC patients with a combined overexpression of HER2 and FGFR2 showed poor prognosis after gastrectomy (16). It is suggested that the poor prognosis associated with HER2-positive patients might be caused by HER2-targeted therapy resistance due to FGFR2 activation. However, the predictive significance of FGFR2 expression for prognosis or chemotherapy response in HER2-positive GC patients is still inconclusive. This study aimed to evaluate the relationship between clinical outcomes and intra-tumoral FGFR2 expression in HER2-positive GC patients who had undergone HER2-targeted chemotherapy.

Patients and Methods

Patients. A retrospective analysis was performed using the medical records of 22 patients with HER2-positive GC, who had undergone systemic chemotherapy for metastases or postoperative recurrences at the Kanazawa Medical University Hospital, between January 2009 and December 2020. Clinicopathological data before the treatment, such as patient sex, age, tumor type, tumor location, tumor differentiation, and HER2 status by means of immunohistochemistry (IHC), were collected from the records of our Hospital. We also extracted the results of blood examinations before chemotherapy, that included information on the serum levels of carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA 19-9). Based on the results of the clinicopathological examination, patients were staged using the 8th edition of the UICC (17) according to the extent of lymph node metastasis (N) and distant metastasis (M). Gastrectomy and lymph node dissection were performed according to the 2014 Japanese GC treatment guidelines (version 4) (18). This study was approved by the Medicine Ethics Committee of Kanazawa Medical University. The research reported in this paper was in compliance with the Helsinki Declaration. We obtained written informed consent from all patients.

Evaluations. We performed IHC staining of FGFR2 expression using surgical resected specimens or biopsied samples of HER2-positive GC patients. We used the ant-FGFR2 antibody (ab58201,

Abcam, Cambridge, UK; 1:200 dilution) with 4 μm sections from formalin-fixed and paraffin-embedded tumor specimens. The staining intensity of each tumor cell, and proportion of tumor cells with an FGFR2 overexpression in each section, was assessed according to a previous report (19). In IHC, ≥50% of the tumor cells expressed high FGFR2 was defined as FGFR2-positive. Representative IHC images are shown in Figure 1. We evaluated overall survival (OS) in the FGFR2-negative and FGFR2–positive GC patients. OS was measured from the date that chemotherapy was initiated for metastases or postoperative recurrences, until the patient's death by GC or other causes. In the case of patients who survived during our analysis, the date of the last follow-up was December 31, 2020. The association between clinicopathological factors and OS was assessed by means of univariate and multivariate analyses.

Treatment. Postoperative adjuvant chemotherapy for advanced GC consisted of S-1 monotherapy or S-1 combination with another drug. Patients with residual tumors, metastases, or postoperative recurrences underwent systemic chemotherapy according to the Japanese GC treatment guidelines (18). As first-line chemotherapy, trastuzumab combined with capecitabine plus cisplatin, capecitabine plus oxaliplatin, or S-1 plus oxaliplatin were administered to patients. Responses of the target lesions to chemotherapy were classified according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (20), as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). When the patients showed PD during chemotherapy, we changed the regimen or performed palliative care based on the guidelines and the patients' general conditions.

Statistical analysis. Data were expressed as n (%) or mean (±standard deviation). Continuous variables and categorical variables were compared using the Student's *t*-test and the χ^2 test, respectively. All *p*-values were two-sided, and differences with *p*<0.05 were considered to be statistically significant. An OS analysis was performed using the Kaplan-Meier method, and results were examined using the log-rank test. Cox's proportional hazards regression model was used to identify clinicopathological factors that were independently associated with survival. Variables that were associated with an OS rate of *p*≤0.10 in the univariate analysis, were included in the multivariate analysis. The JMP software version 8.0 (SAS Institute, Cary, NC, USA) was used for all statistical analyses.

Results

Patient characteristics in the FGFR2-negative and -positive groups. Of 22 patients, 8 were placed in the FGFR2-negative group, and 14 were placed in the FGFR2 positive group. The clinicopathological characteristics of both groups are shown in Table I. The mean age for the negative group was 71.8 and the mean age for the positive group was 73.8 years. There was no significant difference between groups in terms of HER2 status and tumor characteristics including tumor type, location, and differentiation. The proportion of patients with stage IV GC before treatment was more than 60% in both groups. Although the proportion of patients who underwent gastrectomy tended to be higher in the FGFR2-negative
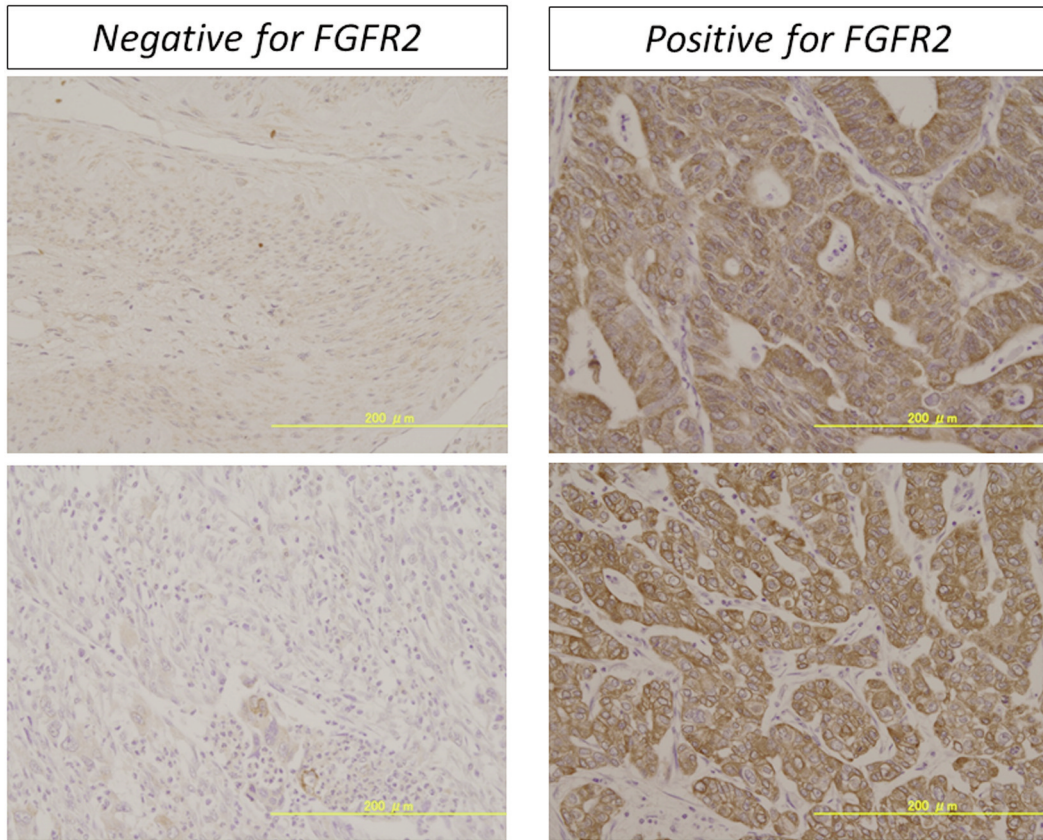
Figure 1. *Representative immunohistochemistry (IHC) images for the expression of Fibroblast growth factor receptor 2 (FGFR2) protein in the FGFR2-negative and -positive groups.*

Table I. *Patient characteristics.*

	FGFR2-negative (n=8)	FGFR2-positive (n=14)	*p*-Value
Sex (male)	7 (87.5%)	11 (78.6%)	0.59
Age	71.8 (±3.0)	73.8 (±2.3)	0.59
HER2 score=3	5 (62.5%)	11 (78.6%)	0.42
Tumor type 3 or 4	6 (75.0%)	6 (42.9%)	0.14
Tumor location (U/M/L)	2/5/1 (25.0/62.5/12.5%)	4/4/6 (28.6/28.6/42.9%)	0.21
Tumor differentiation (por-sig)	2 (25.0%)	1 (7.1%)	0.25
Stage IV[a] (before the treatment)	5 (62.5%)	9 (64.3%)	0.93
Postoperative recurrence (+)	3 (37.5%)	5 (35.7%)	0.93
Gastrectomy (+)	6 (75.0%)	6 (42.9%)	0.14
CEA (ng/ml)	14.6 (±72.8)	98.9 (±55.0)	0.37
CA19-9 (U/ml)	261.3 (±109.2)	177.4 (±82.5)	0.55

Values are in n (%) or mean (±standard deviation). [a]The 8th edition of the International Union Against Cancer (UICC) tumor-node-metastasis (TNM) classification (17). CEA, Carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.

group, there was no significant difference between the groups (*p*=0.14). Regarding the pretreatment levels of tumor markers (CEA and CA 19-9), no significant difference was observed between the groups.

Overall survival in the FGFR2-negative and -positive groups. In all the patients with a HER2-positive GC, the median OS rate was 24.6 months. The median OS rates were 56.2 and 16.0 months in the FGFR2-negative and -positive
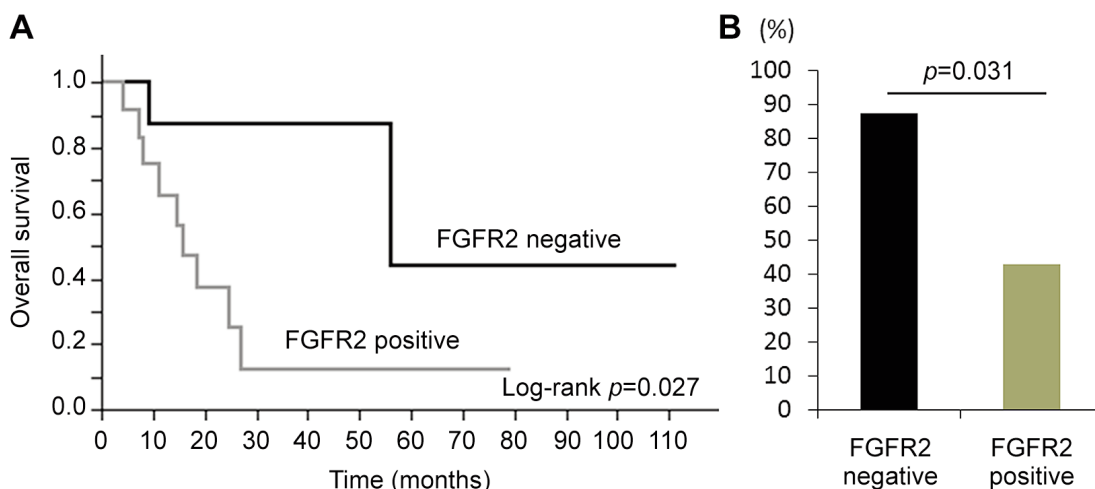
Figure 2. *Overall survival rates and response rates to trastuzumab in the FGFR2-negative and -positive groups. (A) The median overall survival rates were 56.2 and 16.0 months in the FGFR2-negative and -positive groups, respectively [p=0.027 (log-rank)]. (B) The proportion of patients who showed complete response (CR) or partial response (PR) to the combination chemotherapy with trastuzumab.*

groups, respectively (Figure 2A). The FGFR2-negative group had a significantly better prognosis after HER2-targeted chemotherapy [*p*=0.027 (log-rank)].

Correlation between clinicopathological factors and overall survival. The univariate analysis revealed that performing gastrectomy (*p*=0.06), response to the combination chemotherapy with trastuzumab (CR or PR; *p*=0.0007), and FGFR2-positive (*p*=0.03) were significantly correlated with OS (Table II). In a multivariate analysis, the response to combination chemotherapy with trastuzumab (*p*=0.008) was significantly correlated with OS. However, FGFR2-positivity was not an independent prognostic factor in HER2-positive GC patients who had undergone chemotherapy.

Response to trastuzumab in the FGFR2-negative and -positive groups. Combination chemotherapy with trastuzumab, capecitabine plus cisplatin, capecitabine plus oxaliplatin, and S-1 plus oxaliplatin regimens were combined in nine, four, and eight patients, respectively. One patient was administered a combined trastuzumab and paclitaxel regimen. The proportions of patients who showed CR or PR in response to chemotherapy were 87.5 and 42.9% in the FGFR2-negative and -positive groups, respectively (*p*=0.031) (Figure 2B). In the FGFR2-positive and FGFR2-negative groups, the proportion of patients who underwent a second-line regimen and more than a third-line regimen were 62.5 *vs.* 78.6% (*p*=0.42) and 25.0 *vs.* 28.6% (*p*=0.86), respectively. There were no significant differences in the response rates to second- and third-line chemotherapy between the groups. These data indicated that the response to a trastuzumab combination

regimen could contribute towards a good prognosis in FGFR2-negative patients with HER2-positive GC.

Discussion

In this study, we assessed the relationship between clinical outcomes and intra-tumoral FGFR2 expression in HER2-positive GC patients with metastases or postoperative recurrences. We found that HER2-positive GC patients, without an overexpression of FGFR2, showed an improved prognosis due to a better response to chemotherapy combined with trastuzumab. Generally, HER2- as well as FGFR2-positive GC is more frequently associated with venous invasion and an advanced tumor stage, compared to -negative GCs (21). In a recent study of a Japanese cohort of HER2-negative GC patients, no obvious relationships between the response rate for first line chemotherapy and FGFR2 expression in IHC were found (19). However, the role of FGFR2 expression as a prognostic factor remains unclear in HER2-positive GC patients who have undergone systemic chemotherapy using trastuzumab for metastases or postoperative recurrences.

The FGFR2 receptor, tyrosine kinase, regulates cell proliferation, differentiation and motility, and its frequent dysregulation, like gene amplification, is linked to tumor formation in GC (22, 23). It has been reported that FGFR2 gene amplification and FGFR2 expression is found in 1.8-15% and 2.5-61.4% of GC, respectively (15, 24). In addition, both amplification and expression have been associated with poor prognosis in GC patients (25, 26). Shoji et al. also reported that advanced GC patients with FGFR2 amplification had

Table II. *Univariate and multivariate analyses to identify prognostic factors in HER2-positive GC patients who underwent chemotherapy.*

	Univariate analysis		Multivariate analysis	
	Hazard ratio	*p*-Value	Hazard ratio	*p*-Value
Sex (male)	0.35	0.26		
Age (>75)	1.58	0.48		
HER2 score 3	0.89	0.86		
Tumor type 3 or 4	1.58	0.46		
Tumor location (U)	1.89	0.34		
Tumor differentiation (por-sig)	1.62	0.56		
Stage IV[a] (before the treatment)	1.47	0.55		
Postoperative recurrence (+)	0.60	0.42		
Gastrectomy (+)	0.28	0.06	0.52	0.36
CEA (>5 ng/ml)	0.66	0.49		
CA19-9 (>37 U/ml)	0.94	0.92		
CR or PR to chemotherapy with trastuzumab	0.08	0.0007	0.12	0.008
FGFR2 positive in IHC	4.55	0.03	1.76	0.55

[a]The 8th edition of the International Union Against Cancer (UICC) tumor-node-metastasis (TNM) classification (17). CEA, Carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.

significantly shorter OS than those without FGFR2 amplification (9.1 *vs*. 16.5 months) (27). While we did not assess FGFR2 amplification, overexpression of the FGFR2 protein in the tumor legion was associated with poor prognosis, even in our analysis. Regarding FGFR2 amplification, a previous report has shown that there is a non-overlap with HER2 expression in GC patients (28). On the other hand, 5.6% of GC patients were reported to have a co-expression of FGFR2 and HER2 proteins in their tumor lesions (16). In our cohort, more than 60% of patients had a co-expression of FGFR2 and HER2. We defined that ≥50% tumor cells expressed high FGFR2 as positive for FGFR2 in IHC, so the degree of the FGFR2 expression might affect the FGFR2 positivity rate. The median OS in the FGFR2-positive group was 16.0 months, significantly shorter than that of the FGFR2-negative group. A median survival rate of 21 months was confirmed in patients manifesting co-expressions of FGFR2 and HER2 (16). It was considered that as we had analyzed only metastatic HER2-positive GC patients, we may have obtained a lower OS rate in the FGFR2-positive group, in comparison with previous research. In contrast, the FGFR2-negative group exhibited an improved prognostic rate (median OS=56.2 months). A multivariate analysis determined that responses to the combination chemotherapy with trastuzumab were independently correlated with OS, in spite of FGFR2 expression status. This indicates that the good response to trastuzumab combination chemotherapy could be essential in improving the prognosis of HER2-positive patients. Although there was no significant difference in the clinicopathological characteristics between the groups, the proportion of patients who underwent gastrectomy, regardless of whether they had

received a curative or non-curative resection, tended to be higher in the FGFR2-negative group, compared with the FGFR2-positive group. Therefore, a primary tumor resection by gastrectomy might contribute to improved prognosis in the FGFR2-negative group.

We also evaluated that more than 80% patients in the FGFR2-negative group revealed CR or PR in response to combination chemotherapy with trastuzumab. Although the role of FGFR2 expression in the chemotherapy response to HER2-positive GC has yet to be identified, FGFR activation was reported to affect resistance to HER2-targeted therapies in breast cancer, as well as esophageal GC (12-14). For instance, FiGhTeR trial is a phase II trial that aims to assess the safety and activity of the FGFR (1, 2, and 3) inhibitor in HER2-trastuzumab resistant GC patients (29). As mentioned before, in the ToGA study, the median OS was 13.8 months, and the response rate was 47.3% in patients who had been treated with trastuzumab plus chemotherapy (9). Compared to the results of the ToGA study, our results showed greater median OS and response rates in the FGFR2-negative group. The FGFR2 expression itself was not a significant independent factor contributing to OS in multivariate analysis, but the response to trastuzumab plus chemotherapy obviously promoted the improvement in prognosis of HER2-positive GC patients. Our results suggest that intra-tumoral FGFR2 expression may predict the response to trastuzumab plus chemotherapy and be a useful factor to take into account when deciding upon a chemotherapy strategy that may improve the prognosis of HER2-positive GC patients. However, we were not able to unravel the mechanism of FGFR2 signaling as a key mediator of resistance to anti-

HER2 therapy. A previous study showed that tumor-associated fibroblasts promoted HER2-targeted therapy resistance through FGFR2 activation. Therefore, the tumor microenvironment including the immune cells, might be essential players in HER2-targeted therapy resistance that is induced by FGFR2 activation or signaling.

While we believe that our study brings a new perspective to the relationship between clinical outcomes and intra-tumoral FGFR2 expression in HER2-positive GC patients, it also has certain limitations. It was a retrospective study performed at a single Institution, and the sample size was small. To confirm the significance of FGFR2 expression in HER2-positive GC patients who undergo anti-HER2 chemotherapy, further work with a prospective cohort study in multiple institutions is, therefore, warranted.

Conclusion

We showed that HER2-positive GC patients, without FGFR2 overexpression, exhibited an improved prognosis and response rate to trastuzumab combination chemotherapy for metastases or postoperative recurrences. Assessment of intra-tumoral FGFR2 expression in IHC could be helpful in predicting the prognosis and response to trastuzumab in HER2-positive GC patients.

Conflicts of Interest

The Authors declare no competing interest in connection with this study.

Authors' Contributions

NN designed the study. NN, DK, YT, TM, and TM performed data acquisition, data analysis, and interpretation. NN prepared the manuscript. SK, HF, NU, and HT revised paper critically. All Authors read and approved the final manuscript.

References

1 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136(5): E359-E386, 2015. PMID: 25220842. DOI: 10.1002/ijc.29210

2 Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ and MAGIC Trial Participants: Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 355(1): 11-20, 2006. PMID: 16822992. DOI: 10.1056/NEJMoa055531

3 Brenkman HJ, Haverkamp L, Ruurda JP and van Hillegersberg R: Worldwide practice in gastric cancer surgery. World J Gastroenterol 22(15): 4041-4048, 2016. PMID: 27099448. DOI: 10.3748/wjg.v22.i15.4041

4 Fujitani K, Yang HK, Mizusawa J, Kim YW, Terashima M, Han SU, Iwasaki Y, Hyung WJ, Takagane A, Park DJ, Yoshikawa T, Hahn S, Nakamura K, Park CH, Kurokawa Y, Bang YJ, Park BJ, Sasako M, Tsujinaka T and REGATTA study investigators: Gastrectomy plus chemotherapy versus chemotherapy alone for advanced gastric cancer with a single non-curable factor (REGATTA): a phase 3, randomised controlled trial. Lancet Oncol 17(3): 309-318, 2016. PMID: 26822397. DOI: 10.1016/S1470-2045(15)00553-7

5 Wagner AD, Syn NL, Moehler M, Grothe W, Yong WP, Tai BC, Ho J and Unverzagt S: Chemotherapy for advanced gastric cancer. Cochrane Database Syst Rev 8: CD004064, 2017. PMID: 28850174. DOI: 10.1002/14651858.CD004064.pub4

6 Akiyama T, Sudo C, Ogawara H, Toyoshima K and Yamamoto T: The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. Science 232(4758): 1644-1646, 1986. PMID: 3012781. DOI: 10.1126/science.3012781

7 Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin JA, Greene MI and Weinberg RA: The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature 312(5994): 513-516, 1984. PMID: 6095109. DOI: 10.1038/312513a0

8 Valtorta E, Martino C, Sartore-Bianchi A, Penaullt-Llorca F, Viale G, Risio M, Rugge M, Grigioni W, Bencardino K, Lonardi S, Zagonel V, Leone F, Noe J, Ciardiello F, Pinto C, Labianca R, Mosconi S, Graiff C, Aprile G, Frau B, Garufi C, Loupakis F, Racca P, Tonini G, Lauricella C, Veronese S, Truini M, Siena S, Marsoni S and Gambacorta M: Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. Mod Pathol 28(11): 1481-1491, 2015. PMID: 26449765. DOI: 10.1038/modpathol.2015.98

9 Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK and ToGA Trial Investigators: Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 376(9742): 687-697, 2010. PMID: 20728210. DOI: 10.1016/S0140-6736(10)61121-X

10 Meric-Bernstam F, Johnson AM, Dumbrava EEI, Raghav K, Balaji K, Bhatt M, Murthy RK, Rodon J and Piha-Paul SA: Advances in HER2-targeted therapy: novel agents and opportunities beyond breast and gastric cancer. Clin Cancer Res 25(7): 2033-2041, 2019. PMID: 30442682. DOI: 10.1158/1078-0432.CCR-18-2275

11 Hierro C, Rodon J and Tabernero J: Fibroblast growth factor (FGF) receptor/FGF inhibitors: novel targets and strategies for optimization of response of solid tumors. Semin Oncol 42(6): 801-819, 2015. PMID: 26615127. DOI: 10.1053/j.seminoncol.2015.09.027

12 Kim HS, Kim JH, Jang HJ, Han B and Zang DY: Pathological and prognostic impacts of FGFR2 overexpression in gastric cancer: a meta-analysis. J Cancer 10(1): 20-27, 2019. PMID: 30662521. DOI: 10.7150/jca.28204

13 Wei W, Liu W, Serra S, Asa SL and Ezzat S: The breast cancer susceptibility FGFR2 provides an alternate mode of HER2 activation. Oncogene, 2015. PMID: 25639874. DOI: 10.1038/onc.2014.440

14 Piro G, Carbone C, Cataldo I, Di Nicolantonio F, Giacopuzzi S, Aprile G, Simionato F, Boschi F, Zanotto M, Mina MM, Santoro R, Merz V, Sbarbati A, de Manzoni G, Scarpa A, Tortora G and Melisi D: An FGFR3 autocrine loop sustains acquired resistance to trastuzumab in gastric cancer patients. Clin Cancer Res *22(24)*: 6164-6175, 2016. PMID: 27267856. DOI: 10.1158/1078-0432.CCR-16-0178

15 Hanker AB, Garrett JT, Estrada MV, Moore PD, Ericsson PG, Koch JP, Langley E, Singh S, Kim PS, Frampton GM, Sanford E, Owens P, Becker J, Groseclose MR, Castellino S, Joensuu H, Huober J, Brase JC, Majjaj S, Brohée S, Venet D, Brown D, Baselga J, Piccart M, Sotiriou C and Arteaga CL: HER2-overexpressing breast cancers amplify FGFR signaling upon acquisition of resistance to dual therapeutic blockade of HER2. Clin Cancer Res *23(15)*: 4323-4334, 2017. PMID: 28381415. DOI: 10.1158/1078-0432.CCR-16-2287

16 Jia YX, Li TF, Zhang DD, Fan ZM, Fan HJ, Yan J, Chen LJ, Tang H, Qin YR and Li XY: The coexpression and prognostic significance of c-MET, fibroblast growth factor receptor 2, and human epidermal growth factor receptor 2 in resected gastric cancer: a retrospective study. Onco Targets Ther *9*: 5919-5929, 2016. PMID: 27729801. DOI: 10.2147/OTT.S111778

17 In H, Solsky I, Palis B, Langdon-Embry M, Ajani J and Sano T: Validation of the 8th edition of the AJCC TNM staging system for gastric cancer using the National Cancer Database. Ann Surg Oncol *24(12)*: 3683-3691, 2017. PMID: 28895113. DOI: 10.1245/s10434-017-6078-x

18 Japanese Gastric Cancer Association: Japanese gastric cancer treatment guidelines 2014 (ver. 4). Gastric Cancer *20(1)*: 1-19, 2017. PMID: 27342689. DOI: 10.1007/s10120-016-0622-4

19 Minashi K, Yamada T, Hosaka H, Amagai K, Shimizu Y, Kiyozaki H, Sato M, Soeda A, Endo S, Ishida H, Kamoshida T, Sakai Y and Shitara K: Cancer-related FGFR2 overexpression and gene amplification in Japanese patients with gastric cancer. Jpn J Clin Oncol *51(10)*: 1523-1533, 2021. PMID: 34258618. DOI: 10.1093/jjco/hyab104

20 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D and Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer *45(2)*: 228-247, 2009. PMID: 19097774. DOI: 10.1016/j.ejca.2008.10.026

21 Nagatsuma AK, Aizawa M, Kuwata T, Doi T, Ohtsu A, Fujii H and Ochiai A: Expression profiles of HER2, EGFR, MET and FGFR2 in a large cohort of patients with gastric adenocarcinoma. Gastric Cancer *18(2)*: 227-238, 2015. PMID: 24626858. DOI: 10.1007/s10120-014-0360-4

22 Turner N and Grose R: Fibroblast growth factor signalling: from development to cancer. Nat Rev Cancer *10(2)*: 116-129, 2010. PMID: 20094046. DOI: 10.1038/nrc2780

23 Xie L, Su X, Zhang L, Yin X, Tang L, Zhang X, Xu Y, Gao Z, Liu K, Zhou M, Gao B, Shen D, Zhang L, Ji J, Gavine PR, Zhang J, Kilgour E, Zhang X and Ji Q: FGFR2 gene amplification in gastric cancer predicts sensitivity to the selective FGFR inhibitor AZD4547. Clin Cancer Res *19(9)*: 2572-2583, 2013. PMID: 23493349. DOI: 10.1158/1078-0432.CCR-12-3898

24 Kim HS, Kim JH and Jang HJ: Pathologic and prognostic impacts of *FGFR2* amplification in gastric cancer: a meta-analysis and systemic review. J Cancer *10(11)*: 2560-2567, 2019. PMID: 31258762. DOI: 10.7150/jca.29184

25 Hosoda K, Yamashita K, Ushiku H, Ema A, Moriya H, Mieno H, Washio M and Watanabe M: Prognostic relevance of FGFR2 expression in stage II/III gastric cancer with curative resection and S-1 chemotherapy. Oncol Lett *15(2)*: 1853-1860, 2018. PMID: 29434882. DOI: 10.3892/ol.2017.7515

26 Seo S, Park SJ, Ryu MH, Park SR, Ryoo BY, Park YS, Na YS, Lee CW, Lee JK and Kang YK: Prognostic impact of fibroblast growth factor receptor 2 gene amplification in patients receiving fluoropyrimidine and platinum chemotherapy for metastatic and locally advanced unresectable gastric cancers. Oncotarget *8(20)*: 33844-33854, 2017. PMID: 27802183. DOI: 10.18632/oncotarget.12953

27 Shoji H, Yamada Y, Okita N, Takashima A, Honma Y, Iwasa S, Kato K, Hamaguchi T and Shimada Y: Amplification of FGFR2 gene in patients with advanced gastric cancer receiving chemotherapy: prevalence and prognostic significance. Anticancer Res *35(9)*: 5055-5061, 2015. PMID: 26254407.

28 Liu YJ, Shen D, Yin X, Gavine P, Zhang T, Su X, Zhan P, Xu Y, Lv J, Qian J, Liu C, Sun Y, Qian Z, Zhang J, Gu Y and Ni X: HER2, MET and FGFR2 oncogenic driver alterations define distinct molecular segments for targeted therapies in gastric carcinoma. Br J Cancer *110(5)*: 1169-1178, 2014. PMID: 24518603. DOI: 10.1038/bjc.2014.61

29 Merz V, Zecchetto C, Simionato F, Cavaliere A, Casalino S, Pavarana M, Giacopuzzi S, Bencivenga M, Tomezzoli A, Santoro R, Fedele V, Contarelli S, Rossi I, Giacomazzi S, Pasquato M, Piazzola C, Milleri S, de Manzoni G and Melisi D: A phase II trial of the FGFR inhibitor pemigatinib in patients with metastatic esophageal-gastric junction/gastric cancer trastuzumab resistant: the FiGhTeR trial. Ther Adv Med Oncol *12*: 1758835920937889, 2020. PMID: 32684989. DOI: 10.1177/1758835920937889