JJCO Japanese Journal of Clinical Oncology

*Japanese Journal of Clinical Oncology*, 2021, 51(10)1523–1533
https://doi.org/10.1093/jjco/hyab104
Advance Access Publication Date: 13 July 2021
Original Article

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# Cancer-related FGFR2 overexpression and gene amplification in Japanese patients with gastric cancer

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Received 1 February 2021; Editorial Decision 10 June 2021; Accepted 18 June 2021

## Abstract

**Objective:** Fibroblast growth factor receptor 2 (FGFR2) has been proposed as a novel druggable target in unresectable gastric cancer. FGFR2 alteration has been reported as associated with poor prognosis even in patients with gastric cancer who received systemic chemotherapy. This study aimed to evaluate the frequency of FGFR2 overexpression and gene amplification in clinical specimens from Japanese patients with recurrent or unresectable gastric cancer.

**Methods:** This observational study enrolled patients who were histologically or cytologically confirmed with unresectable HER2-negative or unknown gastric or gastroesophageal junctional adenocarcinoma treated with at least one previous chemotherapy. FGFR2 overexpression and gene amplification in the specimens were evaluated by immunohistochemical staining and fluorescence *in situ* hybridization methods, respectively.

**Results:** In a total of 173 eligible cases, FGFR2 immunohistochemistry score was evaluated as 0, 1, 2, 3 and 4 for 20, 80, 35, 28 and 10 cases, respectively. In 151 evaluable cases with FGFR2 immunohistochemistry scores of 1–4, *FGFR2* copy number expressed as fluorescence *in situ* hybridization signals were detected as $<4$, $\geq 4 < 10$ and $\geq 10$ copies for 123, 16 and 12 cases, respectively. *FGFR2* copy number showed an increasing tendency along with higher FGFR2 immunohistochemistry scores in the corresponding specimen. The response rate and time to treatment failure for first line chemotherapy did not have any obvious relationship to FGFR2 immunohistochemistry score and *FGFR2* copy number.

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**Conclusions:** Although FGFR2 overexpression and gene amplification were shown in Japanese patients with unresectable gastric cancer, these alterations did not impact the effects of cytotoxic agents as first line chemotherapy.

**Key words:** FGFR2 overexpression, FGFR2 gene amplification, gastric cancer, immunohistochemical staining, fluorescent *in situ* hybridization

## Introduction

Gastric cancer (GC) is the fifth most prevalent cancer and the third leading cause of cancer-related death worldwide (1). Although surgery is the treatment of choice for GC, prognosis with advanced GC is still poor (2). It has been reported that 22–51% of GC patients who received radical surgery with curative intent develop recurrent disease (3,4). In patients with unresectable advanced or recurrent lesions, systemic chemotherapy can prolong median survival time to 13–14 months (5,6). Trastuzumab in patients with HER2-positive advanced GC, and an antiangiogenic agent (ramucirumab) and immune checkpoint inhibitors (nivolumab and pembrolizumab) introduced as later-line therapy in non-selective patients with metastatic GC have demonstrated modest survival benefits (7–12). Despite improved outcomes with these targeted molecular therapies, however, prognosis with advanced GC still remains wanting, and there is a critical need to develop more efficacious therapeutic agents.

The fibroblast growth factor (FGF)/FGF receptor (FGFR) signaling axis plays an important role in normal organ, vascular and skeletal development. On the other hand, activating *FGFR* gene abnormalities are reported in various tumor types, in which many of these *FGFR* abnormalities are considered a driving event (13–15). Genetic modifications or overexpression of FGFRs have been associated with tumorigenesis and disease progression in breast, lung, gastric, hematologic and other malignancies. The cancer types known to be connected to genetic abnormalities in FGFR include breast cancer [*FGFR1* and *FGFR2* gene amplifications at an incidence of 10 and ~1%, respectively; (16)], squamous cell lung cancer [*FGFR1* gene amplifications at an incidence of 20%; (17)], endometrial cancer [*FGFR2* activating mutation at an incidence of 12%; (16)], intrahepatic cholangiocarcinoma [*FGFR2* gene fusions at an incidence of 14%; (18)], bladder cancer [*FGFR3* activating mutation at an incidence of 50–60% for non-muscle invasive type; (16)], myeloma [*FGFR3* translocation at an incidence of 15%; (16)] and glioma [*FGFR3* gene fusions at an incidence of 8%; (19)]. It has also been reported that *FGFR2* gene amplification and FGFR2 overexpression is found in 1.8–15% (20) and 2.5–61.4% (21) of GC, respectively, and is associated with poor prognosis (22,23). In cases with diffuse type GC, up to a 10% incidence of *FGFR2* gene amplification in those with relatively poor prognosis has been reported (15). It has also been reported that *FGFR2* and *HER2* gene amplifications are mutually exclusive (24). Therefore, *FGFR2* amplification has attracted significant interest as a therapeutic target for *FGFR2*-amplified GC, and several development projects are ongoing (25). In this context, clarifying the frequency of *FGFR2* gene amplification and FGFR2 overexpression in GC may greatly contribute to the development of *FGFR2* inhibitors as a novel therapeutic option. To illuminate the significance of developing *FGFR2* inhibitors for GC, we aimed in this study to find the frequency of *FGFR2* gene amplification and FGFR2 overexpression in clinical specimens from HER2 negative/unknown Japanese patients with recurrent or unresectable GC.

## Patients and methods

### Study design

This study was a multicenter observational study.

### Study population

This study included patients who were diagnosed with unresectable gastric or gastroesophageal junctional adenocarcinoma confirmed by histological or cytological methods. Patients who were diagnosed either to be seen as refractory for at least one systemic chemotherapy or as recurrent during or within 6 months after postoperative adjuvant chemotherapy/chemoradiation therapy were eligible. The other criteria for eligibility were as follows: (i) negative or unknown for HER2/neu status, (ii) age $\geq$ 20 years at written informed consent before enrollment in this study and (iii) clinical GC specimens at diagnosis or surgical resection were available. Patients whom the investigator judged to be ineligible for this study were excluded. It has been reported that *FGFR2* and *HER2* gene amplifications are almost always mutually exclusive (24), so we excluded HER2 positive patients to focus on *FGFR2* amplification in this study.

The World Medical Association Declaration of Helsinki on medical research protocols and ethics was followed throughout the study. Authorization for the use of the clinical specimens for research purposes was obtained from the institutional review board at each study location.

### Study data collection on chemotherapy

In this study, we collected (regimen, duration, efficacy, etc.) data on only one regimen of chemotherapy received first after a diagnosis of unresectable or recurrent GC. Response rates and time to treatment failure (TTF) for chemotherapy prior to enrollment were calculated from case report data extracted from background medical records for each case with first line chemotherapy. Cases with first line chemotherapy were defined as those who had: non-curative resection, received first line chemotherapy and had first line chemotherapy data (on regimens, duration, efficacy, etc.); those who received first line chemotherapy and had first line chemotherapy data (on regimens, duration, efficacy, etc.); those who had curative resection but did not receive adjuvant chemotherapy, had recurrence, received first line chemotherapy and had first line chemotherapy data (on regimens, duration, efficacy, etc.) and; those who had curative resection and recurrence 6 months after adjuvant chemotherapy, received first line chemotherapy and had first line chemotherapy data (on regimens, duration, efficacy, etc.).

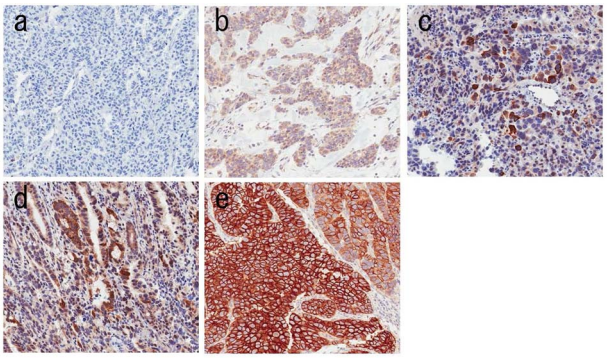


**Figure 1.** Representative immunohistochemical (IHC) images for the expression of fibroblast growth factor receptor 2 (FGFR2) protein in the gastric cancer clinical specimens in this study. Images a, b, c, d and e show IHC score expressions of 0, 1, 2, 3 and 4, respectively. See text for score definitions. Magnification: ×20 objective.

### FGFR2 immunohistochemistry

To evaluate FGFR2 protein expression, immunohistochemistry (IHC) staining was performed using rabbit anti-FGFR2 polyclonal antibody (FGFR2 IHC kit, Nichirei Biosciences Inc., Tokyo, Japan) 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 FGFR2 overexpression in each section was scored by two independent observers as follows: Score 0, <10% of tumor cells expressed weakly with FGFR2 but none expressed highly; Score 1, ≥10% of tumor cells expressed weakly with FGFR2 but none expressed highly; Score 2, <10% of tumor cells expressed highly with FGFR2; Score 3, ≥10%—<50% of tumor cells expressed highly with FGFR2 and Score 4, ≥50% of tumor cells expressed highly with FGFR2 (Fig. 1). The percentage of positive FGFR2 cells was calculated based on the positive area of the tumor cell region. The strong expression ant weak expression was evaluated based on the stainability of the core with strong expression and weak expression of CBA (cell block array) determined in the validation test.

### *FGFR2* fluorescence *in situ* hybridization

To evaluate *FGFR2* gene amplification, we used the fluorescence *in situ* hybridization (FISH) method with the 4 μm serial sections from the tumor specimens used for IHC examination. For this analysis, we used the tumor specimens with FGFR2 IHC scores of 1–4 because it is known that a tumor specimen with a IHC score of 0 rarely shows *FGFR2* gene amplification (26). More specifically, a human *FGFR2* gene probe prepared from genomic sequences of bacterial artificial chromosome clones RP11-7P17 and RP11-62L18 using *FGFR2* reverse and forward primer genes (Hokkaido System Science Co., Ltd., Sapporo, Japan) was fluorescently labeled in orange by nick translation. A human centromere 10 (CEP 10) gene probe (Vysis CEP 10 SpectrumGreen Probe, Abbott Molecular Inc., Des Plaines, USA) as reference, since the *FGFR2* gene is localized on human chromosome 10, was fluorescently labeled in green. After hybridization, single sets of 20 tumor cells in each section were evaluated for their average number of *FGFR2* signals and CEP 10 signals per tumor cell by two independent observers. A ratio of *FGFR2* signals to CEP 10 signals (FGFR2/CEP10) was calculated for each section. A representative FISH image of the *FGFR2* signals is shown in Fig. 2.

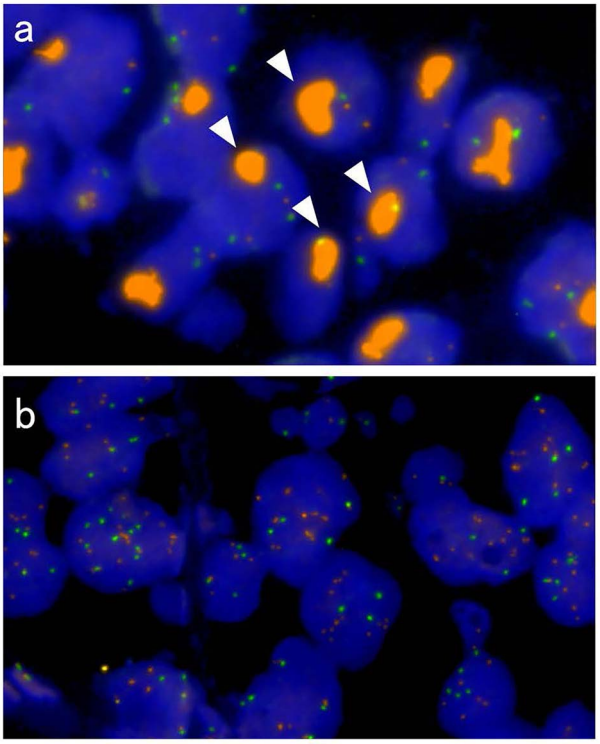


**Figure 2.** Representative FGFR2 fluorescence *in situ* hybridization (FISH) image in the clinical specimen of gastric cancer in this study. Each orange fluorescence image represented FGFR2 gene. (a) This figure showed 40 FGFR2 signals per tumor cell as well as clusters of FGFR2 signals (triangle arrows show representative examples). (b) This figure showed 13 FGFR2 signals per tumor cell.

### Statistical analysis

Statistical significance in the distribution of baseline characteristics according to the FGFR2 IHC score or *FGFR2* copy number expressed by FISH signals per tumor cell was analyzed by $\chi^2$-test or Fisher's exact test with $P < 0.05$ for the two-side significance level. In cases having data on TTF and best response with first line chemotherapy prior to enrollment, Kaplan–Meier plots for the TTF were drawn according to the FGFR2 IHC score or *FGFR2* copy number, and significance between the plots was analyzed using Logrank tests.

## Results

### Disposition and characteristics of cases

Among a total of 176 cases were enrolled maximally during the enrollment period from June 2018 to March 2020 (defined as the full analysis set, FAS); 3 cases did not meet inclusion criteria and were excluded, with the remaining 173 cases being defined as the per protocol set (PPS). Within the PPS, 140 cases having data with which to calculate TTF for a first line chemotherapy regimen just prior to enrollment were defined as the first line chemotherapy set (FLCS) (Table 1 and Fig. 3).

The primary analysis set was the PPS, consisting of 132 (76.3%) males and 41 (23.7%) females. Mean ± standard deviation for age was 67.4 ± 10.1 years (range 34–83 years). In the PPS, 92 cases (53.2%) had a primary tumor lesion at the enrollment. Primary tumors were located in the upper stomach (41 cases, 23.7%), middle

**Table 1.** Structured analysis population proportions: FGFR2 IHC score

| Analysis set | FGFR2 by IHC | | | | | Total |
|---|---|---|---|---|---|---|
| | Score 0 | Score 1 | Score 2 | Score 3 | Score 4 | |
| All enrolled patients | 21 | 82 | 35 | 28 | 10 | 176 |
| PPS | 20 (95.2%) | 80 (97.6%) | 35 (100.0%) | 28 (100.0%) | 10 (100.0%) | 173 (98.3%) |
| Patients excluded from PPS | 1 (4.8%) | 2 (2.4%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 3 (1.7%) |
| FLCS | 13 (61.9%) | 65 (79.3%) | 29 (82.9%) | 24 (85.7%) | 9 (90.0%) | 140 (79.5%) |

*Abbreviations:* PPS, per protocol set; FLCS, first line chemotherapy set.

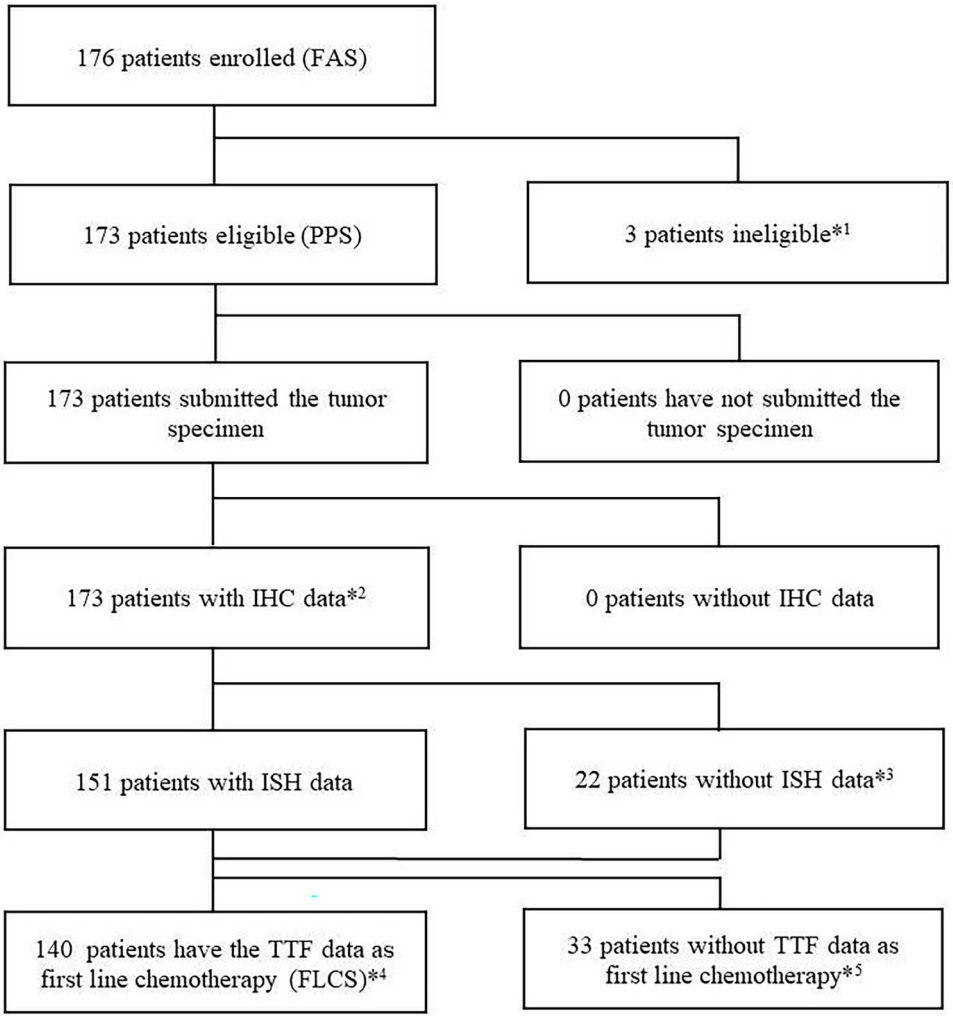


**Figure 3.** Patients flow diagram. FAS, full analysis set; PPS, per protocol set; FLCS, first line chemotherapy set; IHC, immunohistochemistry; ISH, *in situ* hybridization; TTF, time to treatment failure. *1 One patient was excluded from PPS due to deviation of inclusion criteria, 'after primary chemotherapy'. Two patients were excluded from PPS due to deviation of inclusion criteria, 'the patient obtained written informed consent form'. *2 Patients with IHC score 0, 1, 2, 3 or 4. *3 Twenty patients with IHC score 0 and 2 patients with IHC score 1, 2, 3 or 4 who have no ISH data due to specimen failure. *4 FLCS was composed with patients who had: non-curative resection, received first line chemotherapy and had first line chemotherapy data (on regimens, duration, efficacy, etc.); those who received first line chemotherapy and had first line chemotherapy data (on regimens, duration, efficacy, etc.); those who had curative resection but did not receive adjuvant chemotherapy, had recurrence, received first line chemotherapy and had first line chemotherapy data (on regimens, duration, efficacy, etc.) and; those who had curative resection and recurrence 6 months after adjuvant chemotherapy, received first line chemotherapy and had first line chemotherapy data (on regimens, duration, efficacy, etc.). *5 Thirty-three patients were excluded from FLCS for the following reasons. Two patients had no data for the duration of first line chemotherapy. Thirty-one patients had curative resection and recurrence during adjuvant chemotherapy or within 6 months after adjuvant chemotherapy, received second line chemotherapy.

stomach (49 cases, 28.3%), lower stomach (62 cases, 35.8%), esophagogastric junction (16 cases, 9.2%) or other (5 cases, 2.9%). Tumor specimens for 168 cases (97.1%) were from a primary lesion and the remaining 5 (2.9%) from a metastatic lesion. The most frequent histological types were: poorly differentiated adenocarcinoma (75 cases, 89.3%); signet ring cell carcinoma (7 cases, 8.3%) and mucinous

**Table 2.** Baseline characteristics of cases according to FGFR2 IHC score

| Category | | FGFR2 by IHC | | | | | Total | *P* value* | 99% CI** |
|---|---|---|---|---|---|---|---|---|---|
| | | Score 0 | Score 1 | Score 2 | Score 3 | Score 4 | | | |
| *N* | | | | | | | | | |
| | | 20 | 80 | 35 | 28 | 10 | 173 | | |
| Age (years) | | | | | | | | | |
| | Mean | 65.2 | 67.6 | 66.0 | 69.3 | 69.4 | 67.4 | | |
| | Std | 11.7 | 9.6 | 12.9 | 6.2 | 9.1 | 10.1 | | |
| | Min | 34 | 42 | 35 | 49 | 47 | 34 | | |
| | Median | 66.5 | 69 | 70 | 69.5 | 71.5 | 69 | | |
| | Max | 80 | 83 | 83 | 79 | 80 | 83 | | |
| Age category (years) | | | | | | | | | |
| | <65 | 7 (35.0%) | 26 (32.5%) | 12 (34.3%) | 5 (17.9%) | 2 (20.0%) | 52 (30.1%) | 0.5323 | |
| | ≧65 | 13 (65.0%) | 54 (67.5%) | 23 (65.7%) | 23 (82.1%) | 8 (80.0%) | 121 (69.9%) | | |
| Gender | | | | | | | | | |
| | Male | 7 (35.0%) | 64 (80.0%) | 27 (77.1%) | 25 (89.3%) | 9 (90.0%) | 132 (76.3%) | 0.0004 | |
| | Female | 13 (65.0%) | 16 (20.0%) | 8 (22.9%) | 3 (10.7%) | 1 (10.0%) | 41 (23.7%) | | |
| Primary tumor (at registration) | | | | | | | | | |
| | Yes | 8 (40.0%) | 42 (52.5%) | 18 (51.4%) | 16 (57.1%) | 8 (80.0%) | 92 (53.2%) | 0.3528 | |
| | No | 12 (60.0%) | 38 (47.5%) | 17 (48.6%) | 12 (42.9%) | 2 (20.0%) | 81 (46.8%) | | |
| Primary site | | | | | | | | | |
| | Upper stomach | 5 (25.0%) | 17 (21.3%) | 12 (34.3%) | 6 (21.4%) | 1 (10.0%) | 41 (23.7%) | 0.2382 | [0.2272, 0.2492] |
| | Middle stomach | 8 (40.0%) | 26 (32.5%) | 7 (20.0%) | 7 (25.0%) | 1 (10.0%) | 49 (28.3%) | | |
| | Lower stomach | 6 (30.0%) | 26 (32.5%) | 13 (37.1%) | 13 (46.4%) | 4 (40.0%) | 62 (35.8%) | | |
| | Esophagogastric junction | 0 (0.0%) | 9 (11.3%) | 3 (8.6%) | 2 (7.1%) | 2 (20.0%) | 16 (9.2%) | | |
| | Others | 1 (5.0%) | 2 (2.5%) | 0 (0.0%) | 0 (0.0%) | 2 (20.0%) | 5 (2.9%) | | |
| Main tissue type | | | | | | | | | |
| | Diffuse type | 13 (65.0%) | 39 (48.8%) | 16 (45.7%) | 11 (39.3%) | 5 (50.0%) | 84 (48.6%) | 0.0567 | |
| | Intestinal type | 3 (15.0%) | 38 (47.5%) | 18 (51.4%) | 15 (53.6%) | 4 (40.0%) | 78 (45.1%) | | |
| | Unspecified adenocarcinoma | 4 (20.0%) | 2 (2.5%) | 1 (2.9%) | 2 (7.1%) | 1 (10.0%) | 10 (5.8%) | | |
| | Others | 0 (0.0%) | 1 (1.3%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 1 (0.6%) | | |
| Diffuse type | | | | | | | | | |
| | Poorly differentiated adenocarcinoma | 12 (92.3%) | 35 (89.7%) | 15 (93.8%) | 9 (81.8%) | 4 (80.0%) | 75 (89.3%) | 0.1893 | |
| | Signet-ring cell carcinoma | 1 (7.1%) | 4 (10.3%) | 0 (0.0%) | 2 (18.2%) | 0 (0.0%) | 7 (8.3%) | | |
| | Mucinous adenocarcinoma | 0 (0.0%) | 0 (0.0%) | 1 (6.7%) | 0 (0.0%) | 1 (20.0%) | 2 (2.4%) | | |
| Intestinal type | | | | | | | | | |
| | Well differentiated | 0 (0.0%) | 12 (31.6%) | 3 (16.7%) | 4 (26.7%) | 1 (25.0%) | 20 (25.6%) | 0.9331 | |
| | Moderately differentiated | 3 (100.0%) | 24 (63.2%) | 14 (77.8%) | 10 (66.7%) | 3 (75.0%) | 54 (69.2%) | | |
| | Papillary adenocarcinoma | 0 (0.0%) | 2 (5.3%) | 1 (5.6%) | 1 (6.7%) | 0 (0.0%) | 4 (5.1%) | | |

Analysis set: per protocol set.

*$P$ value of Fisher's exact test.

**In case of estimation by Monte Carlo Method, 99% confidence interval (CI) is also described together with the $P$ value.

carcinoma (2 cases, 2.4%); well differentiated adenocarcinoma (20 cases, 25.6%); moderately differentiated adenocarcinoma (54 cases, 69.2%) and papillary adenocarcinoma (4 cases, 5.1%; Table 2). None had been reported as positive for HER2/neu.

## FGFR2 IHC score

Of the 173 PPS cases, FGFR2 IHC score was evaluated as 0, 1, 2, 3 and 4 for 20 (11.6%), 80 (46.2%), 35 (20.2%), 28 (16.2%) and 10 (5.8%) cases, respectively (Table 1). Looking at the distribution of baseline characteristics in the PPS according to FGFR2 IHC score, there were no significant differences in age, presence of primary tumor at registration or primary site of tumor and main tissue type, except for gender composition by which the proportion of females was higher than males at Score 0 and that of males was higher than females at Scores 1–4 ($P$ = 0.0004; Table 2). The distribution of gender composition in the FLCS similarly showed a significant

**Table 3.** Structured analysis population proportions: *FGFR2* copy number

| Analysis set | *FGFR2* copy number (copies/cell) | | | Total |
|---|---|---|---|---|
| | <4 | ≥4, <10 | ≥10 | |
| All enrolled patients | 124 | 17 | 12 | 153 |
| PPS | 123 (99.2%) | 16 (94.1%) | 12 (100.0%) | 151 (98.7%) |
| Patients excluded from PPS | 1 (0.8%) | 1 (5.9%) | 0 (0.0%) | 2 (1.3%) |
| FLCS | 101 (81.5%) | 15 (88.2%) | 10 (83.3%) | 126 (82.4%) |

**Table 4.** Relationship between FGFR2 IHC score and *FGFR2* signals

| | FGFR2 by IHC | | | | | Total |
|---|---|---|---|---|---|---|
| | Score 0 | Score 1 | Score 2 | Score 3 | Score 4 | |
| *FGFR2* copy number (copies/cell) | | | | | | |
| <4 | – | 73 (92.4%) | 28 (82.4%) | 21 (75.0%) | 1 (10.0%) | 123 (81.5%) |
| ≥4, <10 | – | 6 (7.6%) | 4 (11.8%) | 5 (17.9%) | 1 (10.0%) | 16 (10.6%) |
| ≥10 | – | 0 (0.0%) | 2 (5.9%) | 2 (7.1%) | 8 (80.0%) | 12 (7.9%) |

Analysis set: per protocol set.

difference ($P$ = 0.0036), whereas no significance was observed in other baseline FLCS characteristics according to FGFR2 IHC score (data not shown).

### *FGFR2* copy number

In the 151 cases of the PPS with FGFR2 IHC scores of 1–4, except for 2 cases who had no FISH result due to specimen failure, *FGFR2* copy numbers per tumor cell were detected as <4, ≥4 < 10 and ≥ 10 for 123 cases, 16 cases and 12 cases, respectively (Table 3). *FGFR2* copy number was moderately correlated with FGFR2/CEP10 ratio ($r$ = 0.41 and $P$ < 0.0001). In these 151 cases, the proportions that showed a ≥4 *FGFR2* copy number per tumor cell according to FGFR2 IHC scores of 1, 2, 3 and 4 were 6/79 (7.6%), 6/34 (17.7%), 7/28 (25.0%) and 9/10 (90.0%), respectively, and that showed a ≥10 *FGFR2* copy number per tumor cell were 0/79 (0.0%), 2/34 (5.9%), 2/28 (7.1%) and 8/10 (80.0%), demonstrating an increased tendency for the proportion of cases with amplified *FGFR2* copy number per tumor cell along with FGFR2 IHC score (Table 4). In addition, the mean ± standard deviation for *FGFR2* copy number per tumor cell according to FGFR2 IHC scores of 1, 2, 3 and 4 were 2.4 ± 0.6 (79 cases), 4.2 ± 6.1 (34 cases), 5.8 ± 11.9 (28 cases) and 25.5 ± 15.6 (10 cases), respectively, demonstrating that the average number of *FGFR2* copies increased along with FGFR2 IHC score and the average number of *FGFR2* copies at IHC score 2 exceeded 4. Looking at the distribution of baseline characteristics in the PPS according to *FGFR2* copy number, there were no significant differences in age, gender, presence of primary tumor at registration or main tissue type except with primary site of tumor ($P$ = 0.0387) in which the proportion of upper or middle stomach primary sites with *FGFR2* copy number category of ≥10 seemed lower than those of <10 categories. Although not significant ($P$ = 0.0956), the proportion of diffuse type primary tumors with a *FGFR2* copy number category of ≥10 seemed higher than those of <10 categories (Table 5).

### Response to chemotherapy prior to enrollment according to FGFR2 IHC score

In the FLCS, the proportion of cases with pyrimidine fluoride plus a platinum anticancer agent as the first line chemotherapy regimen prior to enrollment was 116 cases (82.9%) and other agents accounted for 24 cases (17.1%). Response to chemotherapy regimen prior to enrollment according to FGFR2 IHC score is summarized in Table 6. Response rates for first line chemotherapy according to FGFR2 IHC scores of 0, 1, 2, 3 and 4 were 15.4, 33.8, 34.5, 37.5 and 55.6%, respectively ($P$ = 0.4142). In addition, median values for TTF and Kaplan–Meier plots for TTF with first line chemotherapy (Table 6 and Fig. 4) revealed no statistical differences by FGFR2 IHC score ($P$ = 0.3456, Logrank test).

### Response to chemotherapy prior to enrollment according to *FGFR2* copy number

Response to the chemotherapy regimens according to *FGFR2* copy number is summarized in Table 7. The response rate for first line chemotherapy according to *FGFR2* copy number categories of <4, ≥4 < 10 and ≥10 were 33.7, 60.0 and 30.0%, respectively ($P$ = 0.1464). In addition, the TTF with first line chemotherapy revealed no statistical difference by *FGFR2* copy number ($P$ = 0.4607, Logrank test; Table 7 and Fig. 4).

## Discussion

FGFR2 overexpression and *FGFR2* gene amplification have been identified as a novel oncogenic (15) and druggable target (27) in cancers including GC. In addition, FGFR2 overexpression and *FGFR2* gene amplification have been reported as associated with poor prognosis and lower response to chemotherapy in GC (22,23). Furthermore, bemarituzumab, a novel FGFR2b inhibitor, plus chemotherapy demonstrated significant progression-free and overall survival benefit compared with placebo plus chemotherapy in patients with

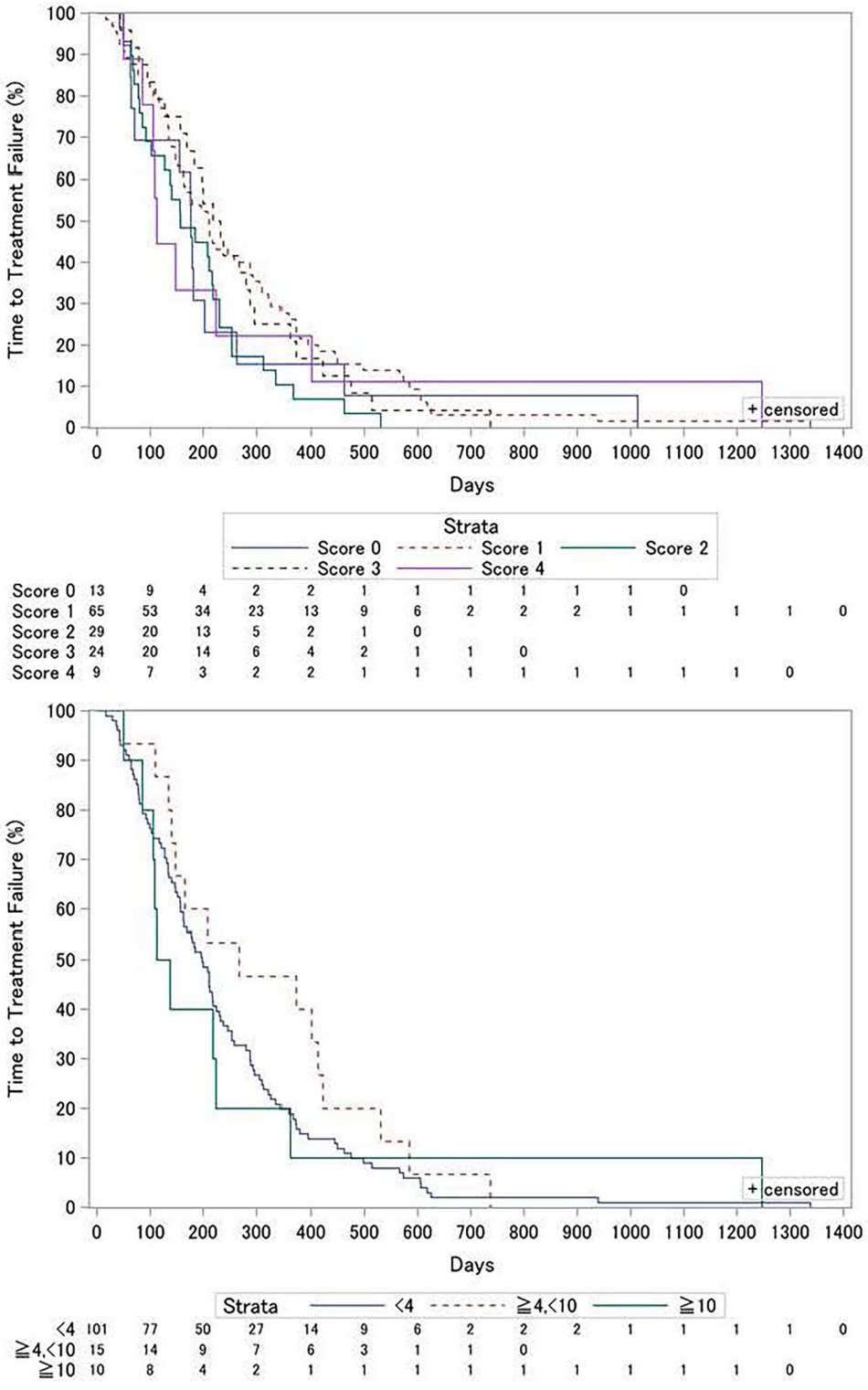


**Figure 4.** Kaplan–Meier plots of the TTF for first line chemotherapy. The upper and lower panel represented Kaplan–Meier plots according to FGFR2 IHC score 0–4 ($P$ = 0.3456, Logrank test) and FGFR2 copy number category of $<4$, $\geq 4 < 10$ and $\geq 10$ copies/cell ($P$ = 0.4607, Logrank test), respectively.

**Table 5.** Baseline characteristics of cases according to *FGFR2* copy number

| Category | *FGFR2* copy number (copies/cell) | | | *P* value* |
|---|---|---|---|---|
| | <4 | ≥4, <10 | ≥10 | |
| *N* | 123 | 16 | 12 | |
| Age (years) | | | | |
| Mean | 67.2 | 70.3 | 67.4 | |
| Std | 10.2 | 8.2 | 9.4 | |
| Min | 35 | 46 | 47 | |
| Median | 69 | 72.5 | 70 | |
| Max | 83 | 77 | 80 | |
| Age category (years) | | | | |
| <65 | 39 (31.7%) | 2 (12.5%) | 4 (33.3%) | 0.3194 |
| ≧65 | 84 (68.3%) | 14 (87.5%) | 8 (66.7%) | |
| Gender | | | | |
| Male | 99 (80.5%) | 14 (87.5%) | 10 (83.3%) | 0.9201 |
| Female | 24 (19.5%) | 2 (12.5%) | 2 (16.7%) | |
| Primary tumor (at registration) | | | | |
| Yes | 67 (54.5%) | 9 (56.3%) | 8 (66.7%) | 0.7404 |
| No | 56 (45.5%) | 7 (43.8%) | 4 (33.3%) | |
| Primary site | | | | |
| Upper stomach | 27 (22.0%) | 6 (37.5%) | 1 (8.3%) | 0.0387 |
| Middle stomach | 34 (27.6%) | 6 (37.5%) | 1 (8.3%) | |
| Lower stomach | 48 (39.0%) | 3 (18.8%) | 5 (41.7%) | |
| Esophagogastric junction | 12 (9.8%) | 1 (6.3%) | 3 (25.0%) | |
| Others | 2 (1.6%) | 0 (0.0%) | 2 (16.7%) | |
| Main tissue type | | | | |
| Diffuse type | 56 (45.5%) | 7 (43.8%) | 8 (66.7%) | 0.0956 |
| Intestinal type | 63 (51.2%) | 7 (43.8%) | 3 (25.0%) | |
| Unspecified adenocarcinoma | 4 (3.3%) | 1 (6.3%) | 1 (8.3%) | |
| Others | 0 (0.0%) | 1 (6.3%) | 0 (0.0%) | |
| Diffuse type | | | | |
| Poorly differentiated adenocarcinoma | 49 (87.5%) | 7 (100.0%) | 7 (87.5%) | 0.4839 |
| Signet-ring cell carcinoma | 6 (10.7%) | 0 (0.0%) | 0 (0.0%) | |
| Mucinous adenocarcinoma | 1 (1.8%) | 0 (0.0%) | 1 (12.5%) | |
| Intestinal type | | | | |
| Well differentiated | 13 (20.6%) | 4 (57.1%) | 1 (33.3%) | 0.0736 |
| Moderately differentiated | 47 (74.6%) | 2 (28.6%) | 2 (66.7%) | |
| Papillary adenocarcinoma | 3 (4.8%) | 1 (14.3%) | 0 (0.0%) | |
| Specimen collection sites | | | | |
| Primary tumor | 119 (96.7%) | 16 (100.0%) | 12 (100.0%) | 1 |
| Liver | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | |
| Lung | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | |
| Abdominal lymph nodes | 1 (0.8%) | 0 (0.0%) | 0 (0.0%) | |
| Peritoneal dissemination | 2 (1.6%) | 0 (0.0%) | 0 (0.0%) | |
| Others | 1 (0.8%) | 0 (0.0%) | 0 (0.0%) | |

Analysis set: per protocol set.
**P* value of Fisher's exact test.

advanced GC (28). Thus, we aimed in this multicenter observational study to clarify the frequency of FGFR2 overexpression and *FGFR2* gene amplification using IHC and FISH methods as well as reliable baseline factors in Japanese patients with recurrent or unresectable GC.

In the present study, the proportion of the cases with FGFR2 overexpression as expressed by IHC scores of ≥1, ≥2, ≥3 or 4 was revealed to be 88.4, 42.2, 22.0 or 5.8%, respectively. It has been reported in a meta-analysis of studies on FGFR2 overexpression that GC patients have a wide range of FGFR2 overexpression frequencies from 2.5 to 61.4% (21). The frequency of FGFR2 overexpression found in the present study conducted in Japanese GC patients was demonstrated to be no less than in those studies.

It has been recognized that FGFR2 overexpression is often led by *FGFR* gene amplification (15). There have been multiple reports to-date that *FGFR2* gene amplification is associated with FGFR2 overexpression in gastric cancer (21,29), and FGFR2 protein overexpression has been noted to strongly correlate with *FGFR2* gene amplification, according to a report by Ahn et al. (26). On the other hand, Tuner et al. reported that FGFR2 overexpression was result

**Table 6.** Response to chemotherapy prior to enrollment according to FGFR2 IHC score

| Category | FGFR2 by IHC | | | | | *P* value* |
|---|---|---|---|---|---|---|
| | Score 0 | Score 1 | Score 2 | Score 3 | Score 4 | |
| *N* | 13 | 65 | 29 | 24 | 9 | |
| Best overall response: first line chemotherapy | | | | | | |
| Complete response (CR) | 0 (0.0%) | 1 (1.5%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0.5074 |
| Partial response (PR) | 2 (15.4%) | 21 (32.3%) | 10 (34.5%) | 9 (37.5%) | 5 (55.6%) | |
| Stable disease (SD) | 8 (61.5%) | 17 (26.2%) | 8 (27.6%) | 6 (25.0%) | 2 (22.2%) | |
| Non-CR/Non-PD | 1 (7.7%) | 13 (20.0%) | 2 (6.9%) | 5 (20.8%) | 1 (11.1%) | |
| Progressive disease (PD) | 1 (7.7%) | 11 (16.9%) | 9 (31.0%) | 4 (16.7%) | 1 (11.1%) | |
| Not evaluable (NE) | 1 (7.7%) | 2 (3.1%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | |
| Response rate (CR + PR) | 2 (15.4%) | 22 (33.8%) | 10 (34.5%) | 9 (37.5%) | 5 (55.6%) | 0.4142 |
| 95% Confidence interval (%) | [4.3, 42.2] | [23.5, 46.0] | [19.9, 52.7] | [21.2, 57.3] | [26.7, 81.1] | |
| Disease control rate (CR + PR + SD + Non-CR/Non-PD) | 11 (84.6%) | 52 (80.0%) | 20 (69.0%) | 20 (83.3%) | 8 (88.9%) | 0.663 |
| 95% Confidence interval (%) | [57.8, 95.7] | [68.7, 87.9] | [50.8, 82.7] | [64.1, 93.3] | [56.5, 98.0] | |
| Time to treatment failure (TTF): first line chemotherapy | | | | | | |
| *N* | 13 | 65 | 29 | 24 | 9 | |
| Median TTF | 176 | 211 | 157 | 225 | 112 | |
| 95% Confidence interval (%) | [64.0, 202.0] | [162.0, 289.0] | [92.0, 218.0] | [157.0, 288.0] | [50.0, 401.0] | |

Analysis set: FLCS ($n$ = 140).

*$P$ value of Fisher's exact test.

of abnormal transcriptional upregulation of the *FGFR2* gene (16). We also evaluated *FGFR2* gene amplification in this study. Because the *FGFR2* gene is known to localize on human chromosome 10, we evaluated the number of *FGFR2* copies per tumor cell on a basis of 4 copies/cell, or the equivalent of 2 times 2 copies/cell in normal cells, and set 3 categories for *FGFR2* copy number per tumor cell, i.e. $<4$, $\geq 4 < 10$ and $\geq 10$ copies/cell. As a result, *FGFR2* copy numbers of $<4$, $\geq 4 < 10$ and $\geq 10$ copies/cell were observed in 123, 16 and 12 cases out of 151 cases with an FGFR2 IHC score of $\geq 1$, respectively. In addition, although no statistically significant difference was noted, the fact that an increasing tendency was observed in the proportion of cases who showed amplified *FGFR2* copy number per tumor cell along with their FGFR2 IHC score suggests a relationship between IHC score (FGFR2 overexpression) and *FGFR2* copy number expressed by FISH signals (*FGFR2* gene amplification). Taking these results into account, we consider it possible to estimate the *FGFR2* gene amplification with high reliability in clinically available GC specimen screening samples using the IHC method, which is more convenient than the FISH method.

Although many questions on the role of FGFR2 overexpression and *FGFR2* gene amplification in the pathogenesis and progression of GC have yet to be answered, it has been reported that a GC cell line established from GC patient with *FGFR2* gene amplification demonstrates significant inhibition of tumor cell growth and survival by the induction of *FGFR2* downregulation (30). Those results suggest that tumor progression in GC patients with FGFR2 overexpression and *FGFR2* gene amplification may in large part be associated with these FGFR abnormalities, and thus the establishment of optional chemotherapies that target these molecular factors would be highly desirable.

We also examined relationships between baseline characteristics and response to first line chemotherapy prior to enrollment, with FGFR2 IHC score and *FGFR2* copy number, to investigate predictive factors for FGFR2 overexpression and *FGFR2* gene amplification. For gender composition, the proportion of females with an FGFR2 IHC score of 0 was higher, whereas the proportion of males with FGFR2 IHC scores of 1–4 was higher, and an imbalance was thus observed. However, no difference was shown by way of *FGFR2* copy number. In addition, no gender effects on FGFR2 overexpression have been observed in other studies of FGFR2 overexpression in the primary tumors of GC patients by IHC (26,31). Our examination of other baseline characteristics revealed no relationships between FGFR2 IHC score and *FGFR2* copy number, and was consistent with other studies on FGFR2 overexpression (26,31) and *FGFR2* gene amplification (32,33) involving GC patients. Furthermore, we found no relationship to first line chemotherapy response in this study. At this point, it is widely recognized that a high-level *FGFR2* gene amplification and FGFR2 overexpression is associated with decreased overall survival and lower response to chemotherapy (30,34). Because our present study was small-sized, limited to HER2 negative cases, did not control for background chemotherapy regimen and did not evaluate overall survival, there are still issues to be investigated by way of confirming the association of FGFR2 with the response to chemotherapy.

Based on the above considerations, we believe it essential to clarify FGFR2 protein overexpression and/or *FGFR2* gene amplification in GC patients to confirm altered FGFR2 expression, and to develop the potential molecular-targeting therapeutic agents with FGFR2 inhibitors.

## Conclusions

The present multicenter observational study took a detailed look at the frequency of FGFR2 overexpression and *FGFR2* gene amplification in Japanese patients with GC, and the effect of cytotoxic agents were similar regardless of whether patients had FGFR overexpression and gene amplification. These findings may contribute the development of promising therapeutic option for patients with recurrent or unresectable GC.

**Table 7.** Response to chemotherapy prior to enrollment according to *FGFR2* copy number

| Category | *FGFR2* copy number (copies/cell) | | | *P* value* |
|---|---|---|---|---|
| | <4 | ≧4,<10 | ≧10 | |
| *N* | 101 | 15 | 10 | |
| Best overall response: first line chemotherapy | | | | |
| Complete response (CR) | 1 (1.0%) | 0 (0.0%) | 0 (0.0%) | 0.7098 |
| Partial response (PR) | 33 (32.7%) | 9 (60.0%) | 3 (30.0%) | |
| Stable disease (SD) | 28 (27.7%) | 2 (13.3%) | 3 (30.0%) | |
| Non-CR/Non-PD | 16 (15.8%) | 3 (20.0%) | 2 (20.0%) | |
| Progressive disease (PD) | 21 (20.8%) | 1 (6.7%) | 2 (20.0%) | |
| Not evaluable (NE) | 2 (2.0%) | 0 (0.0%) | 0 (0.0%) | |
| Response rate (CR + PR) | 34 (33.7%) | 9 (60.0%) | 3 (30.0%) | 0.1464 |
| 95% Confidence interval (%) | [25.2, 43.3] | [35.7, 80.2] | [10.8, 60.3] | |
| Disease control rate (CR + PR + SD + Non-CR/Non-PD) | 78 (77.2%) | 14 (93.3%) | 8 (80.0%) | 0.417 |
| 95% Confidence interval (%) | [68.1, 84.3] | [70.2, 98.8] | [49.0, 94.3] | |
| Time to treatment failure (TTF): first line chemotherapy | | | | |
| *N* | 101 | 15 | 10 | |
| Median TTF | 198 | 267 | 124.5 | |
| 95% Confidence interval (%) | [157.0, 218.0] | [135.0, 413.0] | [50.0, 224.0] | |

Analysis set: FLCS with IHC score 1–4 ($n$ = 126).

*$P$ value of Fisher's exact test.

## Funding

This work was supported by Taiho Pharmaceutical Co., Ltd., Tokyo, Japan.

## Conflict of interest statement

Keiko Minashi received research grant from Astellas Pharma Inc., Daiichi Sankyo Co., Ltd., Mediscience Planning Inc., Merck Biopharma Co., Ltd., MSD K.K., Ono Pharmaceutical Co., Ltd., Taiho Pharmaceutical Co., Ltd. Takeshi Yamada received lecture fee from Daiichi Sankyo Co., Ltd., Eli Lilly Japan K.K., Merck Biopharma, Novartis, Ono Pharmaceutical Co., Ltd., Taiho Pharmaceutical Co., Ltd., Yakult Honsha. Kohei Shitara reports paid consulting or advisory roles for AbbVie, Astellas Pharma Inc., Bristol-Myers Squibb K.K., Eli Lilly Japan K.K., GSK, MSD K.K., Novartis, Ono Pharmaceutical Co., Ltd., Pfizer, Taiho Pharmaceutical Co., Ltd. and Takeda; honoraria from AbbVie, Novartis and Yakult; and research funding from Astellas Pharma Inc., Chugai Pharmaceutical Co., Ltd., Eli Lilly Japan K.K., Daiichi Sankyo Co. Ltd., MSD K.K., Ono Pharmaceutical Co., Ltd., Sumitomo Dainippon Pharma, Taiho Pharmaceutical Co., Ltd. and Medi Science, outside the submitted work.

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