

Original Article

Fibroblast growth factor receptor 2 (*FGFR2*) fusions in Japanese patients with intrahepatic cholangiocarcinoma

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Abstract

Objective: Fibroblast growth factor receptor gene alterations have emerged as promising drug targets for intrahepatic cholangiocarcinoma, a rare cancer that has a poor prognosis. This study evaluated the frequency of fibroblast growth factor receptor 2 fusions in clinical specimens from Japanese patients with iCCA.

Methods: This study enrolled 116 patients who had histologically or cytologically confirmed adenocarcinoma and been diagnosed as relapsing after resection or with unresectable intrahepatic cholangiocarcinoma. We evaluated the frequency of *fibroblast growth factor receptor 2* fusions-positive cells in their specimens using break-apart fluorescent *in situ* hybridization 'for 114 patients who met the study protocol'.

Results: Of a total of 114 cases, six (5.3%) were identified as fibroblast growth factor receptor 2 fusions-positive with a high frequency (87% or more) of fibroblast growth factor receptor 2 fusions-positive tumour cells whereas the remainder, with the exception of three cases with indeterminate

results, were identified as fibroblast growth factor receptor 2 fusions-negative. The patients' baseline characteristics as well as their objective response rates, disease control rates, times to progression, and times to treatment failure with previous or ongoing first-line chemotherapy did not have any obvious relationship to the proportion of fibroblast growth factor receptor 2 fusions-positive case.

Conclusions: Further detailed elucidation of fibroblast growth factor receptor 2 fusion status is expected to contribute to the development of promising therapeutic options for patients suffering from recurrent or unresectable intrahepatic cholangiocarcinoma.

Key words: *FGFR2* fusions, intrahepatic cholangiocarcinoma, break-apart fluorescent *in situ* hybridization

Introduction

Fibroblast growth factor (FGF) signals through transmembrane tyrosine kinase receptors (*FGFR1* to *4*) to regulate angiogenesis, wound repair and many developmental pathways in the early embryo. FGF and FGFRs express in various types of human cells and play crucial roles in their proliferation, migration, differentiation and survival; thus aberrant FGFR signalling can promote tumour development (1,2). In many cases of developmental disorder and cancer types, an association to the FGF/FGFR signalling pathway dysregulation has been reported (3). Examples of cancer types where genetic *FGFR* abnormalities are known are: gastric cancer (*FGFR2* gene amplification incidence 10%), breast cancer (*FGFR1* and *FGFR2* gene amplifications incidence 10% and ~1%, respectively), bladder cancer (*FGFR3* activating mutation incidence 50–60% for the non-muscle invasive type), endometrial cancer (*FGFR2* activating mutation incidence 12%), and myeloma (*FGFR3* translocation incidence 15%) (1). It has also been reported that *FGFR2* fusions by chromosomal translocation are found in 10–14% of intrahepatic cholangiocarcinoma (iCCA) (4,5).

iCCA accounts for 10–20% of newly diagnosed liver cancers (6). Incidence and mortality rates for iCCA have risen over the last 15 years (7), and the prognosis for such patients is poor. In patients with unresectable disease or who relapse after surgery, standard first-line treatment with gemcitabine-cisplatin results in a median survival of ~1.5 years (8–11), highlighting the need for more effective systemic therapies (6,12). Recently, it has been reported that *FGFR* aberrations may be prognostic and may provide improved outcomes for patients treated with FGFR-targeted therapy (13). Several small-molecule FGFR inhibitors are being investigated across multiple tumour types with *FGFR* alterations, including iCCA (14). Recently, pemigatinib, a selective inhibitor of *FGFR1*, 2 and 3, achieved a significant response in a phase 2 study (15) and was approved for iCCA in the USA. In addition, futibatinib, a selective inhibitor of *FGFR1–4*, resulted in a 34.3% objective response rate with interim data in iCCA patients harbouring *FGFR2* gene fusions in a phase 2 study (16). Given this context, clarifying the frequency of *FGFR2* gene abnormalities in iCCA is expected to contribute greatly to the development of novel *FGFR2* kinase inhibitors as a therapeutic option. Thus, the aim of our study was to find the frequency of *FGFR2* fusions in clinical specimens from Japanese patients with recurrent or unresectable iCCA.

Patients and methods

Study design

This study was a multicenter observational study. The aim of this study was to determine the frequency of *FGFR2* fusions in clinical specimens from Japanese patients with recurrent or unresectable iCCA.

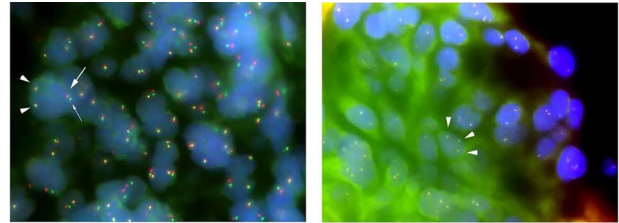


Figure 1. Representative fluorescence images for clinical cholangiocarcinoma specimens stained with *FGFR2* FISH probes in this study. In *FGFR2* fusions-positive cells (left panel), green signal fluorescence (thin dotted arrow; indicates the *FGFR2* gene centromere region) was observed to stand apart from red signals (thick solid line arrow; indicates the *FGFR2* gene telomere region). In the *FGFR2* fusions-negative cells (right panel), only yellow signals (arrow heads; indicates a mixed fluorescence of red and green signals) were observed.

Study population

This study included 116 patients who were diagnosed with adenocarcinoma confirmed by histological or cytological findings and who were diagnosed by imaging to have either iCCA that was recurrent after curative resection for a primary tumour, or unresectable. Other criteria for eligibility were as follows: (i) first-line chemotherapy for the patient was planned, ongoing or complete, (ii) age \geq 20 years at written consent, (iii) Eastern Cooperative Oncology Group Performance Status score of 0 or 1 and (iv) clinical cholangiocarcinoma specimens 'from a primary lesion or metastatic lesions' at diagnosis or surgical resection were available. Any patients whom the investigator determined ineligible for this study were excluded.

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 of each study location.

Break-apart fluorescent *in situ* hybridization

To identify the presence of *FGFR2* fusions, we used break-apart fluorescent *in situ* hybridization (FISH) methods, and 4 μ m sections from formalin-fixed, paraffin-embedded tumour specimens (4). More specifically, two kinds of human *FGFR2* break-apart gene probes, *FGFR2* telo (RP11-78A18 recognizing the *FGFR2* gene telomere region) and *FGFR2* cen (RP11-7P17 recognizing the *FGFR2* gene centromere region), fluorescently labelled by nick translations in green and red, respectively, were purchased from Chromosome Science Labo (Sapporo, Japan). After hybridization, single sets of 100 tumour cells, each such tumour specimen being evaluated by two independent observers, were classified as *FGFR2* fusions-positive or -negative under fluorescent microscopy (Fig. 1). In the event 7% or more *FGFR2* fusions-positive cells were observed

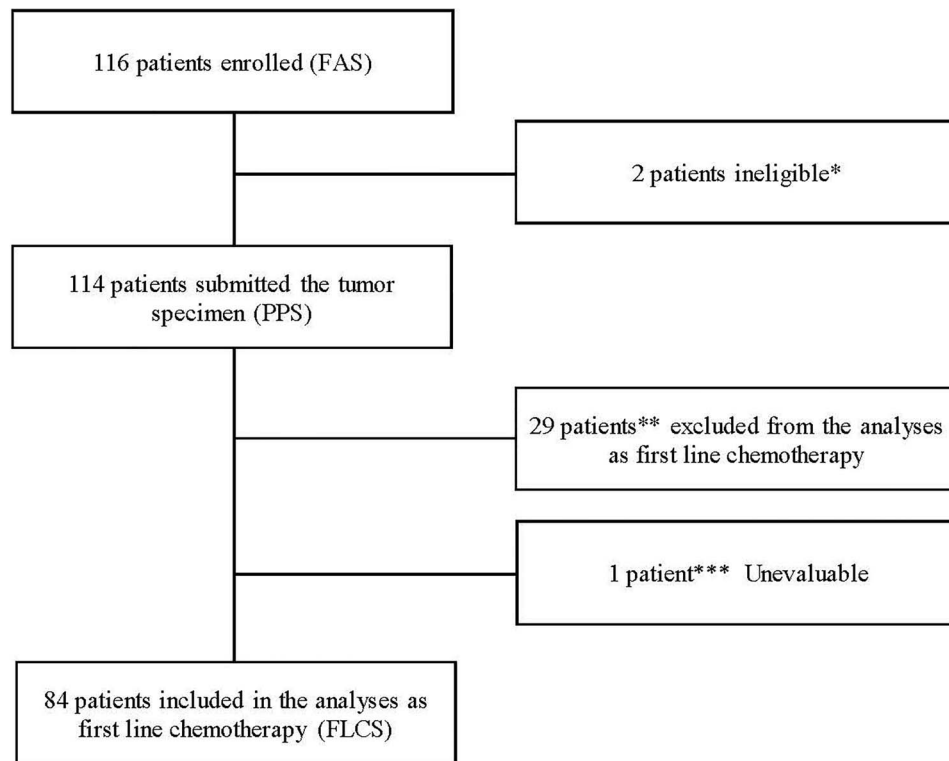


Figure 2. Disposition of cases FAS, full analysis set; PPS, per protocol set; FLCS, first-line chemotherapy set. *Deviation from inclusion criteria no. 1: Patient has histologically or cytologically confirmed adenocarcinoma and been diagnosed as relapsing or with unresectable intrahepatic cholangiocarcinoma by imaging. Patients histologically confirmed as bile duct cell carcinoma were enrolled in this trial. These patients were excluded from the PPS. **Six patients received first-line chemotherapy after giving informed consent from. Twenty-three patients did not receive first-line chemotherapy. These patients were excluded from FLCS. ***One patient could not be analysed for time to progression and time to treatment failure. Because the start and end date of first-line chemotherapy were unknown, this patient was excluded from FLCS.

in a tumour specimen, it was classed as *FGFR2* fusions-positive (a positive case), and otherwise it was classed as *FGFR2* fusions-negative (a negative case). This cut-off value for the FISH analysis, was derived from our previous work on *FGFR2* fusion-positive and -negative cases from surgically resected specimens. Fusion-negative cases validated by RNA sequencing showed background FISH signals at 6.4% (mean + 2 standard deviations) (17). From the results, we initially defined the cut-off value of $\geq 7\%$ for the positive cells in the FISH analysis.

Statistical analysis

Statistical significances in baseline characteristic distributions according to *FGFR2* fusions status were analysed by Fisher's exact test with a $P < 0.05$ two-side significance level. Age, gender, smoking history, alcohol consumption history, hepatitis B virus infection history and hepatitis C virus infection history were tested in these analyses. Where a first-line chemotherapy had been established, 95% confidence intervals for response rates and chemotherapy disease control rates were also examined according to *FGFR2* fusions status. In addition, Kaplan–Meier Plots for time to progression and time to treatment failure with first-line chemotherapy were generated according to *FGFR2* fusions status, and significance between the plots was analysed using Log-rank tests.

Results

Disposition of cases

A total of 116 cases were recruited maximally during the enrollment period from 2 October 2018 to 30 September 2019 (defined as the

full analysis set: FAS). Two cases were excluded by reason of inclusion criteria violation, and the remaining 114 cases (defined as the per protocol set: PPS) comprised the 72 (63.2%) males and 42 (36.8%) females who were examined. The mean \pm standard deviation for age was 68.0 ± 10.0 years (range 37–84 years). At diagnosis, the primary tumour location for all the cases was determined the intrahepatic bile duct. Tumour specimens from 98 cases (86.0%) were from a primary lesion and the remaining 16 (14.0%) were from liver or other metastatic sites. The most frequent histological classifications were moderately differentiated adenocarcinoma (49 cases, 43.0%) followed by poorly differentiated adenocarcinoma (29 cases, 25.4%) and well differentiated adenocarcinoma (15 cases, 13.2%). These specimens were carried forward to histologically examine the frequency of *FGFR2* fusions. Within the PPS, 84 cases for whom first-line chemotherapy was ongoing or complete prior to study inclusion, and who had definitive data on the start and end date of their chemotherapy were defined as the first-line chemotherapy set (FLCS) (Fig. 2).

Frequency of *FGFR2* fusions

In this study, we focused on results from the 114 cases in the PPS (Fig. 2). FISH evaluation of *FGFR2* fusion status identified six cases (5.3%) with an *FGFR2* fusions-positive tumour specimen, with high frequencies of 87%, 92%, 93%, 94%, 96% and 97% FISH-positive cells, respectively (Fig. 3). With exception of the three unevaluable (indeterminate) cases, all others were identified as *FGFR2* fusions-negative. Results on *FGFR2* fusions status in the FLCS were similar to those in the PPS (Table 1).

Table 1. Disposition of cases according to *FGFR2* fusions status

Analysis Sets	<i>FGFR2</i> fusions			Total
	Positive (<i>n</i> = 6)	Negative (<i>n</i> = 107)	Indeterminate (<i>n</i> = 3)	
All enrolled patients	6	107	3	116
FAS	6 (100.0%)	107 (100.0%)	3 (100.0%)	116 (100.0%)
Patients Excluded from FAS	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
PPS	6 (100.0%)	105 (98.1%)	3 (100.0%)	114 (98.3%)
Patients excluded from PPS	0 (0.0%)	2 (1.9%)	0 (0.0%)	2 (1.7%)
FLCS	4 (66.7%)	77 (73.3%)	3 (100.0%)	84 (73.7%)
Patients excluded from FLCS	2 (33.3%)	28 (26.7%)	0 (0.0%)	30 (26.3%)

Analysis set: All enrolled patients.

FAS, full analysis set; PPS, per protocol set; FLCS, first-line chemotherapy set

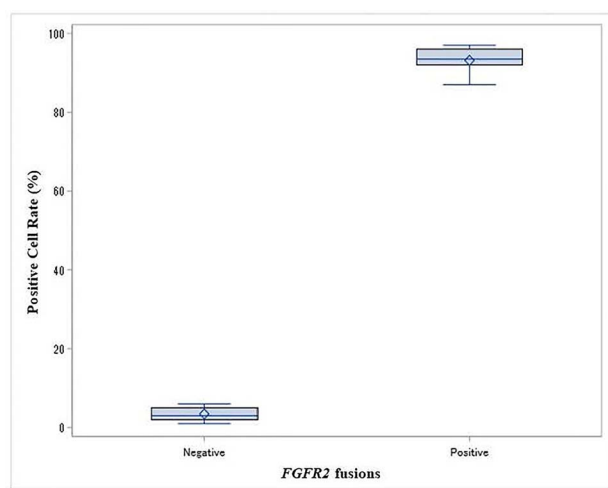


Figure 3. *FGFR2* fusions-positive cell rates in tum or specimens from patients with intrahepatic cholangiocarcinoma. Each column represents the box plot of *FGFR2* fusions-negative (*n*=107) and positive (*n*=6) cases composed of the minimum value, the lower quartile, the median, the mean value (white diamond), the upper quartile and the maximum value. The individual frequency of the *FGFR2* fusions-positive cases were 87%, 92%, 93%, 94%, 96% and 97%, respectively.

The total success rate of the FISH assay was 97.4% (113/116), including 94.0% (109/116) succeeded in the first evaluations. A median turnaround time (TAT) was 18 days (range 13–27 days), which was results from 116 cases of FAS. TAT was calculated as the difference in calendar days between the date the specimen was submitted by the site to the date of the report for the specimens.

Baseline characteristics according to the *FGFR2* fusions status

Baseline characteristics for the PPS: mean age \pm standard deviation was 64.7 ± 7.4 years (range 52–73 years) for *FGFR2* fusions-positive cases (positive cases, *n* = 6) and 68.2 ± 10.1 years (range 37–84 years) for *FGFR2* fusions-negative cases (negative cases, *n* = 105), respectively. Gender for all positive cases was male whereas the negative group was composed of 63 males (60.0%) and 42 females (40.0%). As for smoking, all positive cases had a smoking history, whereas there were 60 cases (57.1%) with smoking history among the negative cases. Alcohol consumption history was noted in five (83.3%) of the positive cases and 56 (53.3%) of the negative. History of hepatitis B virus infection was reported in three (50.0%) of the

positive cases and 28 (26.7%) of the negative. Hepatitis C virus infection history was not reported in any positive cases whereas six (5.7%) of the negative cases did report this history (Table 2). Thus maleness, smoking history, alcohol consumption history and hepatitis B virus infection history proportions were higher in the positive cases than in the negative. In addition, the proportion of hepatitis C virus infection histories in the positive cases was lower than that in the negative. Nevertheless, we found no statistically significant differences, by way of age, gender, smoking history, alcohol consumption history, hepatitis B virus infection history or hepatitis C virus infection history ($P > 0.05$, Fisher's exact test).

First-line chemotherapy prior to inclusion according to the *FGFR2* fusions status

The 84-case of FLCS was composed of 45 recurrent cases (53.6%), being recurrent after curative resection for primary tumours, and 39 who were unresectable (46.4%). The most frequent chemotherapy regimen was gemcitabine plus cisplatin for 57 cases (67.9%) followed by S-1 monotherapy for 15 (17.9%), S-1 plus gemcitabine plus cisplatin for six (7.1%) and gemcitabine monotherapy for six cases (7.1%).

Response to these chemotherapy regimens is summarized in Table 3. The response rates in the *FGFR2* fusions-positive and -negative cases were 0% (95% confidence interval: 0.0–49.0%) and 14.3% (95% confidence interval: 8.2–23.8%), respectively. Because of the overlapping 95% confidence intervals, no difference could be declared between the positive and negative cases. In addition, Kaplan–Meier Plots of both time to progression and time to chemotherapy treatment failure (data not shown) revealed no statistical difference between the positive and negative cases ($P = 0.5357$ and $P = 0.9783$, respectively, Log-rank test).

Discussion

FGFR2 tyrosine kinase fusions have been identified as novel oncogenic (3) and druggable targets (13) in various cancers, especially in iCCA. Thus, the aim of this multicenter observational study was to elucidate *FGFR2* fusion frequency using FISH methods, as well as reliable baseline factors, in Japanese patients with recurrent or unresectable iCCA.

The results of our study revealed that 6 out of the 114 cases examined were identifiable as *FGFR2* fusions-positive. In addition, the individual proportion of *FGFR2* fusions-positive cells counted in the FISH-labelled tumour specimens was as high as 87% or more in these cases. Although the role of *FGFR2* fusions in the pathogenesis

Table 2. Baseline characteristics according to *FGFR2* fusions status

Item	Category	<i>FGFR2</i> fusions			Total
		Positive (<i>n</i> = 6)	Negative (<i>n</i> = 105)	Indeterminate (<i>n</i> = 3)	
Age	<65	3 (50.0%)	32 (30.5%)	1 (33.3%)	36 (31.6%)
	≥65	3 (50.0%)	73 (69.5%)	2 (66.7%)	78 (68.4%)
Gender	Male	6 (100.0%)	63 (60.0%)	3 (100.0%)	72 (63.2%)
	Female	0 (0.0%)	42 (40.0%)	0 (0.0%)	42 (36.8%)
Smoking history	Current or former	6 (100.0%)	60 (57.1%)	2 (66.7%)	68 (59.6%)
	Never	0 (0.0%)	45 (42.9%)	1 (33.3%)	46 (40.4%)
Drinking history	Current or former	5 (83.3%)	56 (53.3%)	3 (100.0%)	64 (56.1%)
	Never	1 (16.7%)	49 (46.7%)	0 (0.0%)	50 (43.9%)
Hepatitis B virus infection history	None	3 (50.0%)	77 (73.3%)	3 (100.0%)	83 (72.8%)
	Yes	3 (50.0%)	28 (26.7%)	0 (0.0%)	31 (27.2%)
Hepatitis C virus infection history	None	6 (100.0%)	99 (94.3%)	3 (100.0%)	108 (94.7%)
	Yes	0 (0.0%)	6 (5.7%)	0 (0.0%)	6 (5.3%)

Analysis set: PPS.

Table 3. Response to first-line chemotherapy prior to inclusion according to *FGFR2* fusions status

Responses	<i>FGFR2</i> fusions			Total
	Positive (<i>n</i> = 4)	Negative (<i>n</i> = 77)	Indeterminate (<i>n</i> = 3)	
Complete response (CR)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Partial response (PR)	0 (0.0%)	11 (14.3%)	1 (33.3%)	12 (14.3%)
Stable disease (SD)	1 (25.0%)	25 (32.5%)	1 (33.3%)	27 (32.1%)
Non-CR/Non-PD	0 (0.0%)	1 (1.3%)	0 (0.0%)	1 (1.2%)
Progressive disease (PD)	1 (25.0%)	14 (18.2%)	0 (0.0%)	15 (17.9%)
Not evaluable (NE)	2 (50.0%)	26 (33.8%)	1 (33.3%)	29 (34.5%)
Response rate (CR + PR)	0 (0.0%)	11 (14.3%)	1 (33.3%)	12 (14.3%)
95% confidence interval (%)	[0.0, 49.0]	[8.2, 23.8]	[6.1, 79.2]	[8.4, 23.3]
Disease control rate (CR + PR + SD + Non-CR/Non-PD)	1 (25.0%)	37 (48.1%)	2 (66.7%)	40 (47.6%)
95% confidence interval (%)	[4.6, 69.9]	[37.3, 59.0]	[20.8, 93.9]	[37.3, 58.2]

Analysis set: FLCS.

of cholangiocarcinoma is still being actively investigated, *FGFR2* fusions are already thought to drive oncogenesis in cholangiocarcinoma (18). The high frequency of *FGFR2* fusions-positive cells found in a subset of this study suggest that enhanced tumour cell proliferation driven by the presence on *FGFR2* fusions was the main contributor to tumour progression in those. As for the percentage of our *FGFR2* fusions-positive cases, previous genomic analysis studies that analysed the surgically resected cases have reported that *FGFR2* fusions are implicated in 13–14% of patients with iCCA (4,5). In contrast, the present study with recurrent or unresectable iCCA and recent reports (15,19) that targeted advanced/recurrent patients indicated lower frequency (5.3% and 7.4–9%, respectively). Recently, it was reported that *FGFR2* fusions were related to better overall survival (12,20). In addition, it was reported that the activation of the FGF/FGFR pathway associates with better prognosis in patients with iCCA (21). Low frequency of *FGFR2* fusions in the patients with recurrent or unresectable iCCA in the present study may be related to this point. However, the percentage of *FGFR2* fusions-positive cases found in this study was less than in previous reports. Our lower percentage of positive cases may be explained by our study's relatively small sample size, which may have skewed our results due to a large deviation in the proportion of positive cases, although

we did reaffirm that the considerable percentage of *FGFR2* gene abnormalities for patients suffering from recurrent or unresectable iCCA was present.

Previously, Kuwata et al. reported a next-generation sequencing (NGS) success rate of 82.9% (22). In this study, we observed a FISH success rate ~14% higher than their NGS success rate. On the other hand, our median TAT was 18 days longer than that previous reported with the NGS (23) because of limitations whereby we had to do our analyses after pooling samples from multiple centers in this study. If we had had no analysis time restrictions, our TAT would have been shorter. We therefore posit that FISH analyses may have more clinical utility, since physicians and patients deserve the most accurate diagnostic test results, in the shortest possible TAT.

We also analysed baseline characteristics and results for first-line chemotherapy prior to study inclusion to look for factors presumably due to the presence of an *FGFR2* fusions-positive tumour. Here, the proportions of maleness, smoking history, alcohol consumption history and hepatitis B virus infection history were higher in our *FGFR2* fusions-positive cases than in our negative. In contrast, the proportion of hepatitis C virus infection histories in our positive cases was lower than that in our negative. However, no statistical significance was noted for any of these factors. In addition, there were

no significant differences between our *FGFR2* fusions-positive and -negative cases due to first-line chemotherapy prior to study inclusion.

It has been reported that *FGFR2* fusions are associated with female sex (5,24) and young age of onset (5,19,24). Furthermore, Arai et al. (4) reported no significant differences in age, gender, tumour differentiation, clinical stage and prognosis except for a propensity of hepatitis virus infections, between their fusion-positive and -negative cases. The results from our present study were similar to those reported. At the same time, our results imply that, unless one does chromosomal tumour examinations, the development of any screening algorithm comprised of several baseline characteristics, to find possible *FGFR2* fusions-positive cases in an iCCA patient population, in whom to apply FGFR inhibitors in future, will likely be difficult.

Further detailed elucidation of patients' *FGFR2* fusion status is expected to contribute to the development of promising therapeutic options for those suffering from iCCA.

Conclusion

The present multicenter observational study has elucidated *FGFR2* fusions status in a population of Japanese patients with recurrent or unresectable iCCA.

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Conflict of interest statement

D. Sakai received lecture fee from Chugai Pharmaceutical Co. Ltd, Daiichi-Sankyo Co. Ltd and received grant from Chugai Pharmaceutical Co. Ltd, Daiichi-Sankyo Co. Ltd, Eli Lilly Japan K.K., Ono Pharmaceutical Co. Ltd, Yakult Honsha. E. Hatano received lecture fee and donation from Taiho Pharmaceutical Co., Ltd. C. Morizane received honoraria from Eisai, MSD K.K., Novartis, Taiho Pharmaceutical, Teijin Pharma, Yakult Honsha and received grant

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