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ORIGINAL RESEARCH

Phase I/II study of nivolumab plus lenvatinib for advanced biliary tract cancer (JCOG1808/NCCH1817, SNIPE)

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Background: Although cisplatin plus gemcitabine and other combinations have improved the survival of advanced biliary tract cancer (BTC), high unmet medical needs remain. This study aimed to assess the efficacy and safety of nivolumab plus lenvatinib in the second-line treatment for advanced BTC.

Patients and methods: Nivolumab (240 mg) was administered biweekly. Phase I determined the recommended phase II dose of lenvatinib (20 mg or 14 mg). In phase II, the primary endpoint was the objective response rate (ORR). Secondary endpoints were disease control rate (DCR), progression-free survival (PFS), overall survival (OS), and safety. The planned sample size was 32 patients with a power of 80%, a one-sided alpha error of 5%, threshold ORR of 10%, and expected ORR of 30%.

Results: In phase I, the recommended dose of lenvatinib was determined to be 20 mg in six patients, with one dose-limiting toxicity (myocarditis). In phase II, we enrolled 26 patients. ORR, DCR, and median OS and PFS were 9.4% [90% confidence interval (CI) 2.6% to 22.5%], 53.1% (95% CI 34.7% to 70.9%), and 6.4 months (95% CI 4.9-9.7 months) and 2.5 months (95% CI 1.5-4.1 months), respectively. No response was observed *in patients with* the usage of antibiotics. The grade 3 or 4 adverse events were hypertension (59.4%) and biliary tract infection (37.5%). Rash (28.1%) and hypothyroidism (21.9%) were observed as immune-mediated adverse events of any grade.

Conclusions: Nivolumab plus lenvatinib had a manageable safety in advanced BTC, but its efficacy in the second-line treatment was limited.

Key words: biliary tract cancer, nivolumab, lenvatinib, biliary tract infection, immune checkpoint inhibitor

INTRODUCTION

Biliary tract cancer (BTC) includes cancers in the intrahepatic bile duct (IHBD), extrahepatic bile duct (EHBD), gall-bladder (GB), and ampulla of Vater (AV). It causes 2.3 deaths per 100 000 population globally and is most common in Asia.[1] BTC is a lethal disease and is generally diagnosed at an advanced stage.[2] Since 2010, gemcitabine plus cisplatin (GC) has been the standard chemotherapy treatment for advanced/recurrent BTC.[3] In second-line chemotherapy, ABC-06 showed the superiority of FOLFOX compared with active symptom control, and NIFTY showed the superiority of Nal-IRI plus fluorouracil and leucovorin.[4,5] As targeted agents based on next-generation sequencing, inhibitors of fibroblast growth factor receptor (FGFR) aberrations, such as pemigatinib and futibatinib, showed promising activities in the IHBD.[6,7] In addition, dabrafenib plus trametinib for BRAF V600E was reported, and drug developments are continued to be made in the research on human epidermal growth factor receptor 2 (*HER2*) gene abnormalities.[8,9] Moreover, immune checkpoint inhibitors (ICIs) are being actively developed. However, in second-line treatment, the efficacy of a single ICI was limited,[10-12] and attention was focused on combining it with GC therapy in first-line treatment or with a molecular targeted therapy as a combined immunotherapy in second-line treatment. Chen et al. reported that the cancer immunity cycle, including the release of cancer antigens, presentation of cancer antigens to T cells, priming of T cells, and transport of T cells to the tumor, plays an important role in enhancing the effects of ICIs.[13]

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In previous studies, angiogenesis inhibitors were expected to improve the tumor microenvironment through the maturation of dendritic cells, priming of T cells, normalization of tumor vasculature for T-cell trafficking, and reduction of myeloid-derived suppressor cells and regulatory T cells (Tregs).[14-16] The combination of ramucirumab and pembrolizumab, an antiangiogenic antibody drug that had already been reported at that time, showed a response rate of 4%.[17] Lenvatinib is an oral multikinase inhibitor that targets vascular endothelial growth factor receptors 1-3, FGFRs 1-4, platelet-derived growth factor receptorα, rearranged during transfection, and KIT.[18-20] It was very promising as a potentiator of ICIs in BTC, not only because of its potentiation of ICIs via the inhibition of angiogenesis and fibroblast growth factor (FGF)[21] but also because, as a single agent, it showed a response rate of 11.5% in the second-line treatment of BTC.[22] In addition, high response rates were reported for the combination of lenvatinib and an ICI in renal and gynecologic cancers.[23,24]

Therefore, we decided to investigate the efficacy and safety of the combination of nivolumab, an ICI, and lenvatinib, an angiogenesis inhibitor, in the second-line treatment of BTC.

PATIENTS AND METHODS

Study design and patients

This multicenter, single-arm, phase I/II study was conducted at five centers and in compliance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol was approved by the ethics committee or institutional review board of each participating center. All patients provided written informed consent before study entry. This study is registered in the Japan Registry for Clinical Trials as jRCT2091220436. The main eligibility criteria for inclusion were as follows: clinical diagnosis of BTC; unresectable or recurrent disease with a measurable lesion per RECIST version 1.1; age above 20 years; histologically or cytologically confirmed diagnosis of adenocarcinoma; disease progression or treatment failure following one prior gemcitabine-based chemotherapy regimen (in combination with cisplatin or other platinum agent/fluoropyrimidine agent); ability to maintain sufficient food intake; an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, in addition to adequate organ function; and no receipt of any anticancer treatment within 21 days before the first dose of the study drug. Patients with interstitial pneumonia, lung fibrosis, or watery diarrhea were excluded.

Procedures

Patients received lenvatinib orally once daily in 14-day cycles. The nivolumab dose was fixed at 240 mg once every 2 weeks. Treatment continued until the development of an unacceptable toxicity, disease progression, or withdrawal of consent. In phase I, the initial lenvatinib dose was set at 20 mg daily. Safety and tolerability were observed for 28 days (two cycles). In phase II, patients started at 20 mg or 14 mg of lenvatinib, which were the doses determined in phase I. The criteria for intolerable toxicities were defined as any of the following events occurring during the first cycle of treatment: hematological toxicities, which included febrile neutropenia, grade 4 neutropenia for 7 days, grade 4 decrease in platelet count, grade 3 decrease in platelet count for 7 days or with bleeding, and grade 4 anemia; and non-hematological toxicities, which included grade 4 adverse events (AEs), grade 3 gastrointestinal perforation, thromboembolic event, uveitis, pneumonitis, bronchospasm, allergic reaction, infusion-related reaction, wound dehiscence needing treatment, grade 3 AEs for 3 days after proper treatment, grade 2 uveitis, eye pain, blurred vision that was treated locally and not improved to grade 1 during the re-administration period or needed systematic treatment, and adverse reactions requiring lenvatinib discontinuation for >8 days every 2 weeks.

Outcomes

In phase I, the recommended dose of lenvatinib was determined. With six patients, if the occurrence of AEs meeting the criteria for intolerable toxicities ranged from 0 to 2 patients, we proceeded to the phase II part with a recommended dose of 20 mg/day. If not, the dosage of lenvatinib was reduced by one level to 14 mg/day, and an additional six cases were enrolled for safety assessment. In phase II, the efficacy and safety of lenvatinib and nivolumab were evaluated. If efficacy was expected in phase II, we planned to initiate a first-line expansion cohort ($n = 15$). In phase II, the primary endpoint was objective response rate (ORR). Secondary endpoints included overall survival (OS), progression-free survival (PFS), disease control rate (DCR), and safety. Tumor assessments were carried out by a blinded independent central review every 6 weeks until week 24 and then every 12 weeks thereafter using RECIST version 1.1. Complete response (CR) and partial response (PR) required subsequent confirmation of the responses $\geq$4 weeks later. ORR was defined as the proportion of patients with a CR plus those with a PR. DCR was defined as the overall proportion of patients with CR, PR, or stable disease for 4 weeks or longer. The safety profile was assessed by monitoring and recording all AEs, including all the Common Terminology Criteria for Adverse Events, version 5.0, grades. Toxicity was managed with supportive medications, treatment interruption, dose reduction, and/or treatment discontinuation in accordance with the protocol's prespecified dose-modification guidelines.

Statistical analysis

In the phase II part including patients who received the dose recommended in the phase I part, the threshold for ORR (under the null hypothesis) was set as 10%, and the expected ORR (under the alternative hypothesis) was set at 30% based on results of previous studies,[25] which provided an 80% power for the primary endpoint with a one-sided alpha error of 5%. A total sample size of at least 32 patients was estimated to be required. In the phase II part, if there are 7 or more patients showing efficacy out of 32 patients, it is possible to reject the null hypothesis.

Table 1. Patient characteristics ($N = 32$)

Median age, years (range)	**63 (44-78)**
Sex, *n* (%)	
Male	23 (71.9)
Female	9 (28.1)
ECOG PS, *n* (%)	
0	23 (71.9)
1	9 (28.1)
Primary site, *n* (%)	
Gall-bladder	7 (21.9)
Extrahepatic bile duct	5 (15.6)
Intrahepatic bile duct	15 (46.9)
Ampulla of Vater	5 (15.6)
Extent of disease, *n* (%)	
Unresectable	25 (78.1)
Recurrent	7 (21.9)
Primary chemotherapy (first line), *n* (%)	
GEM + CDDP	23 (71.9)
GEM + CDDP + S-1	9 (28.1)
Microsatellite instability status, *n* (%)	
Stable	27 (84.4)
High	0 (0.0)
Unknown	5 (15.6)
Biliary drainage, *n* (%)	
Presence	14 (43.8)
Absence	18 (56.3)
Use of antibiotics, *n* (%)[a]	
Yes	9 (28.1)
No	23 (71.9)

CDDP, cisplatin; ECOG PS, Eastern Cooperative Oncology Group performance status; GEM, gemcitabine.
[a]Within 1 month before the start of treatment.

The PFS and OS were estimated by the Kaplan—Meier method. The confidence interval (CI) for ORR and DCR was estimated by the Clopper—Pearson method. All statistical analyses were carried out with SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Patient characteristics

At the five centers, six patients were enrolled in phase I between August and October 2019, and a total of 32 patients were enrolled in phases I and II, which ended in November 2020, as shown in Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2024.103919. The recommended dose of lenvatinib was determined to be 20 mg in the six patients in phase I, with one AE corresponding to the toxicity evaluation criteria of myocarditis. The trial was completed without a first-line expansion cohort because the efficacy in phase II was limited. Primary tumor locations included the GB ($n = 7$), EHBD ($n = 5$), IHBD ($n = 15$), and AV ($n = 5$). Most patients were male (71.9%), had an ECOG PS score of 0 (71.9%), and had an unresectable disease (78.1%). Additionally, data regarding prior chemotherapy, microsatellite instability status, biliary drainage, and usage of antibiotics within 1 month before the start of treatment in this trial are shown in Table 1. For 32 patients, the median number of treatment courses was 4 (range 1-25). The breakdown of reasons for protocol treatment discontinuation included 28 patients (87.5%) due to disease progression, 2 patients (6.3%) due to toxicity, 1 patient due to patient refusal, and 1 case due to other reasons.

Table 2. Best overall response ($N = 32$)

	n (%)
Complete response	0 (0.0)
Partial response	3 (9.4)
Stable disease	14 (43.8)
Progressive disease	14 (43.8)
Not evaluated	1 (3.1)
ORR (90% CI)	3 (9.4; 2.6-22.5)
DCR (95% CI)	17 (53.1; 34.7-70.9)

CI, confidence interval; DCR, disease control rate; ORR, objective response rate.

Efficacy

The ORR following nivolumab plus lenvatinib was 9.4% (90% CI 2.6% to 22.5%); 3 patients (9.4%) experienced a PR, and the disease became stable in 14 patients (43.8%; Table 2). With median follow-up of 6.4 months for 32 patients, the median PFS was 2.5 months (95% CI 1.5-4.1 months; Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2024.103919). The median OS was 6.4 months (95% CI 4.9-9.7 months; Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2024.103919). In the overall response rate subgroup analysis, tumor response was observed only in the GB (28.6%) and AV (20.0%) and without the usage of antibiotics within 1 month before the start of treatment (13.0%). No tumor response was observed with the usage of antibiotics (Table 3).

Safety

AEs are shown in Table 4. The most common AEs were hypertension (78.1%), proteinuria (62.5%), anorexia (53.1%), platelet count decreased (50.0%), hoarseness (43.8%), biliary tract infection (40.6%), and malaise (40.6%). The most common AEs of $\geq$grade 3 were hypertension (59.4%) and biliary tract infection (37.5%). No treatment-related deaths occurred. AEs led to nivolumab discontinuation in 9.4% of patients and to lenvatinib discontinuation in 21.9% of patients. However, most AEs were manageable: 62.5% of patients required a lenvatinib dose reduction, 93.8% of patients required a lenvatinib dose interruption, and 81.3% of patients required a nivolumab dose interruption. Immune-mediated adverse events (IMAEs; considered causally related to nivolumab) are shown in Table 5. The most common IMAEs were rash (28.1%), hypothyroidism (21.9%), malaise (18.8%), fever (12.5%), and anorexia (12.5%). AEs and IMAEs of the phase I part, respectively, are shown in Supplementary Tables S1 and S2, available at https://doi.org/10.1016/j.esmoop.2024.103919.

DISCUSSION

In recent years, ICIs and combined immunotherapies have demonstrated activity in multiple cancers. However, nivolumab plus lenvatinib did not show sufficient efficacy for BTC in this study.

Table 3. Subgroup analysis of objective response rate

Factor	ORR (%) (95% CI)
MSI status	
Stable	7.4 (0.9-24.3)
Unknown	20.0 (0.5-71.6)
Primary site	
Gall-bladder	28.6 (3.7-71.0)
Extrahepatic bile duct	0.0 (0.0-52.2)
Intrahepatic bile duct	0.0 (0.0-21.8)
Ampullary	20.0 (0.5-71.6)
Biliary drainage	
Presence	7.1 (0.2-33.9)
Absence	11.1 (1.4-34.7)
Use of antibiotics[a]	
Yes	0.0 (0.0-33.6)
No	13.0 (2.8-33.6)

CI, confidence interval; ORR, objective response rate; MSI, microsatellite instability.
[a]Within 1 month before the start of treatment.

Table 4. Adverse events ($N = 32$)

CTCAE ver. 5.0	Any grade, *n* (%)	Grade 3/4, *n* (%)
Hypertension	25 (78.1)	19 (59.4)
Proteinuria	20 (62.5)	2 (6.3)
Anorexia	17 (53.1)	2 (6.3)
Platelet count decreased	16 (50.0)	3 (9.4)
Hoarseness	14 (43.8)	0
Biliary tract infection	13 (40.6)	12 (37.5)
Malaise	13 (40.6)	1 (3.1)
Rash	12 (37.5)	2 (6.3)
Fever	11 (34.4)	1 (3.1)
Hypothyroidism	11 (34.4)	0
AST increased	9 (28.1)	0
Lymphocyte count decreased	9 (28.1)	3 (9.4)
Diarrhea	9 (28.1)	1 (3.1)
Nausea	9 (28.1)	0
Hypoalbuminemia	9 (28.1)	2 (6.3)
Weight loss	8 (25.0)	0
Palmar-plantar erythrodysesthesia syndrome	8 (25.0)	0
Vomiting	7 (21.9)	0
ALT increased	6 (18.8)	1 (3.1)
Fatigue	6 (18.8)	0

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTCAE, Common Terminology Criteria for Adverse Events.

Table 5. Immune-mediated adverse events ($N = 32$)

CTCAE ver. 5.0	Any grade, *n* (%)	Grade 3/4, *n* (%)
Rash	9 (28.1)	1 (3.1)
Hypothyroidism	7 (21.9)	0
Malaise	6 (18.8)	1 (3.1)
Fever	4 (12.5)	0
Anorexia	4 (12.5)	0
Diarrhea	3 (9.4)	1 (3.1)
Blood corticotrophin increased	3 (9.4)	0
Hyperthyroidism	2 (6.3)	0
ALT increased	2 (6.3)	0
Mucositis oral	2 (6.3)	1 (3.1)
Pneumonitis	2 (6.3)	0
Pruritus	2 (6.3)	0
Increase in blood thyroid-stimulating hormone	2 (6.3)	0
Atrial fibrillation	2 (6.3)	0
Infusion reaction	2 (6.3)	0

ALT, alanine aminotransferase; CTCAE, Common Terminology Criteria for Adverse Events.

The combination of GC and ICIs has proven to be superior in the first-line treatment of BTC.[26,27] On the other hand, after second-line treatment, neither ICIs alone nor in combination have shown efficacy. In this study, however, the patients were not necessarily in poor condition, as 70% of them had an ECOG PS of 0, but the prognosis was limited, with a median survival of 6.4 months, which may have limited the efficacy of the ICI.

The use of antibiotics has recently been reported to alter the intestinal microbiota, negatively affecting the effectiveness of ICIs. While there are reports of a negative impact of antibiotic administration on treatment efficacy defined as administration within 1 month before the start of treatment,[28] no such difference was observed when an ICI was used in combination with GC therapy in the first-line treatment of BTC.[29] The impact of antibiotics may be not so large when an ICI is combined with chemotherapy. The combination in this study did not include chemotherapy but instead included a molecular targeted therapy and this may have had a greater impact. As a result, no response was observed in the group that had a history of antibiotic use within 1 month before the start of treatment. This corresponds to 28.1% of the patients in this study, so the impact was not small. Furthermore, during the treatment course, biliary tract infections were observed in 40.6% of cases, which suggests that antibiotics were used. This is also important as one of the potential reasons for the poor outcomes in this study.

Compared with a previous phase II trial of lenvatinib for second-line treatment of BTC,[22] there was no increase in the response rate; rather, it tended to be lower. The dose of lenvatinib was set at 24 mg daily in the previous lenvatinib study, whereas it was set at 20 mg in our study. On the other hand, grade 3/4 hypertension occurred at a higher rate, suggesting that a sufficient dose intensity may not have been maintained due to dose interruption and dose reduction. In phase I of this study, the dose of lenvatinib was considered acceptable in only one case of intolerable toxicities; however, it was also considered important to select appropriate subjects for this combination therapy, such as those with no history of hypertension.

In another study, the combination of lenvatinib and ICI proved to be superior to chemotherapy regimens for endometrial cancer. The response rate in a group with mismatch repair-proficient endometrial cancer was 30.3%.[30] This study was a second-line treatment like ours, but the response rates were very different. Furthermore, a pembrolizumab plus lenvatinib trial of a second-line treatment for BTC also showed a limited response rate of 10%, which was similar to our study.[31] In the development of ICIs, differences in carcinomas seem to be important.

Subsite-specific outcomes are often discussed for BTC. In a phase III trial of durvalumab as a first-line treatment, intrahepatic cholangiocarcinoma showed better outcomes in a subgroup analysis. In our study, however, responses were observed in GB and AV but not in extrahepatic or

intrahepatic cholangiocarcinoma. Furthermore, among the 20 patients with extrahepatic or intrahepatic cholangiocarcinoma, 7 patients had received antibiotics within 1 month before the start of treatment, and biliary tract infections occurred during the treatment course in another 6 patients. In the treatment of BTC with ICIs, treatment efficacy may depend on the presence or absence of a biliary tract infection and history of antibiotic use.

Some data suggest that patients who experience IMAEs more are more likely to respond to ICIs.[32] In this study, the three cases with PR had IMAEs such as hypothyroidism, hyperthyroidism, diarrhea, and increased blood corticotrophin. IMAEs may also be biomarkers for treatment of BTC with ICIs.

A limitation of this study was that it was a single-arm study with a small number of cases. However, the five centers that participated in this study handle high volumes of patients, and this contributes to the quality assurance of this study. On the other hand, a randomized trial would have been necessary to confirm the definitive effects of the ICI. In addition, the effects of antibiotic administration have been previously reported in other cancer types, and perhaps the study should have been set up to exclude such cases.

In conclusion, nivolumab plus lenvatinib was found to be safe but not sufficiently effective in the second-line treatment of BTC.

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DISCLOSURE

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