Study Protocol Title

A Randomized phase II clinical trial to evaluate the efficacy alternating mFOLFOX/mFOLFIRI chemotherapy regimen as a second-line treatment for advanced biliary tract cancer

Version No: ver. 1.3_2025.01.17

Principal Investigator's Affiliation
: Department of Hematology and Oncology,
Seoul National University Bundang Hospital

Principal Investigator's Name
: Jin Won Kim

Research Protocol

1. Study Title

A Randomized phase II clinical trial to evaluate the efficacy of alternating mFOLFOX/mFOLFIRI chemotherapy regimen as a second-line treatment for advanced biliary tract cancer

2. Research Institution information

1) Principal Investigator:

- Jin Won Kim, Division of Hematology/Medical Oncology, Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine

Co-Investigators:

- Minsu Kang, Division of Hematology/Medical Oncology, Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine
- Jihong Bae, Department of Oncology, Gachon University Gil Medical Center

*Additional site-specific investigators will be recruited

2) Research Coordinator:

- Suna Han, Division of Hematology/Medical Oncology, Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine

3. Funding and support information

Drug support

- Boryung Pharmaceutical (Oxaliplatin, Irinotecan)
  : 136 Changgyeonggung-ro, Jongno-gu, Seoul, Republic of Korea

Research Funding

- None

4. Expected study duration

※ The study will run for 3 years from the date of IRB approval
: 2 years for enrollment, 1 year for follow-up and data analysis

5. Target disease

1) Patients with recurrent or advanced biliary tract cancer who have failed first-line therapy.

- Biliary tract cancer includes intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma gallbladder cancer, and ampulla of Vater cancer.
- Patients must have received at least one cycle of gemcitabine/cisplatin-based chemotherapy and shown confirmed disease progression.

6. Background and objectives

1) Background

- Biliary Tract Cancer (BTC) consists of intrahepatic and extrahepatic cholangiocarcinoma, gallbladder cancer, and ampulla of Vater cancer. BTC is a relatively rare cancer worldwide, with an age-standardized incidence rate (ASIR) of approximately 2.7 cases per 100,000 population, lower than major cancers such as lung, breast, and colorectal cancer. However, the global incidence of BTC has been steadily rising, with higher rates observed in Asia, particularly in Korea, Japan, and China. In the United States, approximately 8,000 BTC cases are reported annually. According to data from the National Cancer Center of Korea, BTC and gallbladder cancer accounted for 2.9% of all cancer cases in Korea in 2020 (7,179 cases, crude incidence rate: 14.0 per 100,000 population), ranking 9th among all cancers. By age group, the highest incidence (35.5%) was observed in individuals in their 70s, indicating that the aging population is expected to contribute to further increases in BTC incidence.
- Surgery remains the primary treatment for BTC; however, only 10% of patients are eligible for surgery at the time of diagnosis, as most are diagnosed at advanced stages. Consequently, systemic chemotherapy is the mainstay of treatment for recurrent or advanced BTC.

  In 2010, the ABC-02 trial demonstrated the superiority of gemcitabine plus cisplatin (Gem/Cis) over gemcitabine monotherapy as first-line treatment for BTC (median overall survival: 11.7 vs. 8.1 months; hazard ratio: 0.64, 95% confidence interval [CI]: 0.52–0.80; $p < 0.001$). Gem/Cis has since been established as the standard first-line treatment for BTC. Recently, immune checkpoint inhibitors, such as Durvalumab and Pembrolizumab, in

combination with Gem/Cis have shown significant efficacy, emerging as new standard therapies. However, median overall survival remains modest (Durvalumab + Gem/Cis: 12.8 months; Pembrolizumab + Gem/Cis: 12.7 months), and resistance ultimately leads to disease progression.

- The ABC-06 trial established FOLFOX as the standard second-line therapy for BTC; however, its efficacy is limited, with a median overall survival of 6.2 months, progression-free survival of 4.0 months, and a tumor response rate of only 5%. Furthermore, there is no established standard therapy for third-line treatment. Targeted therapies, such as those targeting FGFR2 fusion, IDH1 mutation, and BRAF mutation, have shown efficacy but are limited in applicability and effectiveness compared to other cancers.
- Irinotecan is widely used in cancer treatment and has shown efficacy in advanced BTC.
- A 2021 study by Seoul National University Bundang Hospital and Seoul Boramae Medical Center researchers comparing FOLFIRI (irinotecan-based regimen) with FOLFOX as second-line therapy found that FOLFIRI had comparable efficacy to FOLFOX, with a different toxicity profile. Additionally, a study incorporating liposomal irinotecan with 5-fluorouracil demonstrated better efficacy than 5-fluorouracil monotherapy.
- Recent studies have demonstrated the efficacy of alternating chemotherapy regimens in other cancers. For example, in pancreatic cancer, alternating Gem/nab-paclitaxel and FOLFOX resulted in a median progression-free survival of 7.9 months compared to 5.2 months with Gem/nab-paclitaxel alone. The median overall survival was also extended to 16.9 months (mean: 13.2) compared to 12.3 months (mean: 9.7).
- BTC patients often have poor performance status, making third-line treatment uncommon. Exposing patients to novel agents through alternating regimens during second-line treatment may extend survival. Additionally, in Korea, FOLFOX is not reimbursed for BTC beyond the second line, posing practical challenges in clinical settings.
- BTC patients often have poor performance status, making third-line treatment uncommon. Exposing patients to novel agents through alternating regimens during second-line treatment may extend survival. Additionally, in Korea, FOLFOX is not reimbursed for BTC beyond the second line, posing practical challenges in clinical settings.
- This study aims to evaluate whether the alternating mFOLFOX/mFOLFIRI regimen is superior to mFOLFOX monotherapy in patients with advanced BTC who have progressed after first-line treatment.

2) Study hypothesis and objectives

Hypothesis: The mFOLFOX/mFOLFIRI regimen will improve the 6-month overall survival (OS) rate by 25% compared to the mFOLFOX regimen (assumed 6-month OS rate of 50%).

A. Primary objective – 6-month overall survival (OS) rate

B. Secondary objectives

① Objective Response Rate (ORR)
② Disease Control Rate (DCR)
③ Overall Survival (OS)
④ Progression-Free Survival (PFS)
⑤ Toxicity and Adverse Events (AEs)
⑥ Quality of Life (QoL)

7. Investigational Medicinal Products and Devices (Code Name or Generic Name), Dosage Strength, Formulation, etc.(including Control Drugs)

<The following agents to be used in this clinical trial are commonly utilized in gastrointestinal cancers such as gastric and colorectal cancer, with established dosing schedules and regimens.>

1) 5-fluorouracil

- ✓ Generic Name: Fluorouracil
- ✓ Composition, Strength, and Formulation: Available in 1000 mg (20 ml), 500 mg (10 ml), or 250 mg (5 ml), containing 50 mg of 5-fluorouracil per ml.
- ✓ Storage Conditions: Store in a sealed, light-resistant container at room temperature, diluted solutions are stable for 24 hours at room temperature.
- ✓ Indications:
  - ① Carcinomas - Colorectal cancer, rectal cancer, gastric cancer, esophageal cancer, liver cancer, pancreatic cancer, gallbladder cancer, lung cancer, oral cancer, bladder cancer, kidney cancer, ovarian cancer, uterine cancer, and skin cancer.
  - ② Sarcomas - Reticulosarcoma, Hodgkin's disease, lymphosarcoma, and fibrosarcoma.
- ✓ Mechanism of Action

① Inhibits DNA synthesis and induces RNA dysfunction.

② Fluorouracil itself does not directly inhibit pyrimidine nucleotide synthesis but becomes activated through intracellular metabolism.

③ Deactivated by dihydropyrimidine dehydrogenase (DPD) in the liver and kidneys.

④ Excreted as unchanged drug in urine (7-20%), most of the drug is exhaled as CO_2, with minimal biliary excretion.

✓ Characteristics

① The anticancer effects and toxicity of fluorouracil can be enhanced by leucovorin calcium.

② Highly effective in combination regimens with other chemotherapeutic agents.

③ Used as a standard treatment for colorectal and rectal cancers.

2) Irinotecan

✓ Generic Name: Irinotecan

✓ Trade Name: Campto Injection (Manufactured by Boryung Pharmaceutical Co., Ltd.)

✓ Formulation: 40 mg/2 ml, 100 mg/5 ml per vial.

✓ Indications:

① Recurrent or progressive metastatic colorectal cancer after 5-fluorouracil treatment.

② Advanced colorectal cancer (chemotherapy-naïve) in combination with 5-fluorouracil and leucovorin.

③ Unresectable or recurrent gastric cancer.

④ Small-cell lung cancer.

⑤ Advanced non-small cell lung cancer.

⑥ Pancreatic cancer.

⑦ Advanced epithelial ovarian cancer (chemotherapy-naïve) in combination with cisplatin.

✓ Mechanism of Action: Irinotecan is a camptothecin derivative that specifically inhibits topoisomerase-I, preventing DNA replication.

✓ Adverse Effects: Diarrhea, mucositis, and bone marrow suppression, dose-limiting

toxicities include diarrhea and bone marrow suppression.

3) Oxaliplatin

- ✓ Generic Name: Oxaliplatin
- ✓ Trade Name: OXALItin Injection (Manufactured by Boryung Pharmaceutical Co., Ltd.)
- ✓ Formulation: 150mg(5mg/ml) per vial.
- ✓ Indications
  - ① First-line treatment for metastatic colorectal cancer in combination with 5-fluorouracil and folinic acid (leucovorin).
  - ② Adjuvant therapy for Stage III (Duke's C) colorectal cancer after complete resection of the primary tumor in combination with 5-fluorouracil and folinic acid.
  - ③ Unresectable advanced or metastatic gastric cancer.
- ✓ Adverse Effects: Bone marrow suppression, mucositis, and neurological symptoms (paresthesia, dysesthesia).

8. Inclusion Criteria, Exclusion Criteria, Target Sample Size, and Sample Size Justification

1) Inclusion criteria (All eligibility criteria must be fulfilled)

A. Age ≥ 19 years.

B. Diagnosed with biliary tract cancer, including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer, or ampulla of Vater cancer.

C. Either unresectable advanced disease or recurrence after curative surgery.

D. ECOG (Eastern Cooperative Oncology Group) performance status of 0–2

E. First-Line Treatment Failure: Disease progression after at least one cycle of gemcitabine/cisplatin-based therapy or discontinuation of therapy due to adverse effects.

F. Presence of evaluable or measurable lesions according to RECIST v1.1 criteria.

G. Laboratory Criteria: optima bone marrow, liver, and kidney function within one week prior to enrollment:

  A. Hemoglobin > 9.0 g/dL

  B. Absolute neutrophil count (ANC) > 1,000/uL

  C. Platelet count > 75,000/uL

D. Serum creatinine < 1.5× upper limit of normal (ULN)

E. AST/ALT < 3× ULN

F. Total bilirubin < 1.5× ULN (*biliary drainage is allowed*).

H. Patients who understand the study protocol, can provide written informed consent, and are aware of their right to withdraw at any time without penalty.

I. Effective Contraception (for patients of childbearing potential receiving oxaliplatin):

A. Male patients:

Must use effective contraception during the study and for 12 months after treatment completion.

B. Female patients:

Must use effective contraception during the study and for 15 months after treatment completion.

< Permitted effective contraceptive methods>

<table>
<tr><th>Highly effective contraceptive methods</th><th>Effective contraceptive methods</th></tr>
<tr><td rowspan="3">● Complete Abstinence (Refraining from any sexual activity from the time of consent until the end of the study)
● Oral, vaginal, or transdermal contraceptives containing estrogen and progestin, which suppress ovulation.
● Progestin-only contraceptives, including oral, implantable, or injectable forms, which suppress ovulation.
● Hormone-releasing intrauterine system
● Intrauterine devices (IUDs) with a failure rate of less than 1%.
● Bilateral tubal occlusion or ligation.
● Vasectomy(with documented azoospermia confirmed by semen analysis, conducted at least 90 days after the procedure).</td><td>● Progestin-only oral hormonal contraceptives where ovulation suppression is not the primary mechanism of action.
● Male or female condoms, with or without spermicide.
● Caps, diaphragms, or sponges containing spermicide.
● Combination of a cap, diaphragm, or sponge containing spermicide with a male condom (dual barrier method).</td></tr>
<tr><td>Contraceptive Methods Not Permitted</td></tr>
<tr><td>● Periodic Abstinence (e.g., calendar method, symptothermal method, post-ovulation method).
● Withdrawal Method (Coitus Interruptus)
● Spermicide Alone
● Lactational Amenorrhea Method (LAM)</td></tr>
</table>

2) Exclusion Criteria (Patients meeting any of the following criteria will be excluded from the study.)

A. Patients with prior exposure to oxaliplatin or irinotecan in previous cancer treatments.

B. Patients with metastatic or unresectable biliary tract cancer who have received second-line or higher chemotherapy.

C. Pregnant or breastfeeding women.

D. Patients with a history of other malignancies within the past 3 years, except for papillary or follicular thyroid cancer.

E. Patients with uncontrolled infections or other systemic diseases.

F. Patients with a history of myocardial infarction, unstable angina, or heart failure (NYHA Class III-IV) within the last 6 months.

G. Patients with Grade 3 or higher peripheral neuropathy caused by prior chemotherapy.

H. Patients with known allergic reactions to the investigational drugs.

I. Known patients with Gilbert's syndrome, DPD (dihydro-pyrimidine dehydrogenase) deficiency, or Homozygous UGT1A1*28 alleles.

J. Patients currently taking potent CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, ritonavir, clarithromycin), or potent CYP3A4 inducers (e.g., rifampin, carbamazepine, St. John's Wort).

L. Patients who are eligible for targeted therapy, including FGFR inhibitors or IDH1 inhibitors. (eligible for patient who unable to use these targeted agents due to drug cost.)

M. Patients with active CNS metastases and/or carcinomatous meningitis.

N. Patients who meet contraindications for the investigational drugs as per domestic regulatory guidelines, including patients with infections, Interstitial pneumonitis or pulmonary fibrosis, Severe diarrhea, Chronic inflammatory bowel disease, Intestinal paralysis or obstruction, Functional impairment due to peripheral sensory neuropathy, Severe renal dysfunction.

K. Patients deemed ineligible by the investigator for any other reason.

3) Target sample size and calculation basis

Assuming a 6-month survival rate of 50% for the mFOLFOX group and 75% for the

mFOLFOX/mFOLFIRI group, a total of 58 evaluable patients per group is required to achieve 80% power at a significance level of p=0.05.

Considering a 10% drop-out rate, 65 patients per group need to be randomized, resulting in a total of 130 patients for the study. The enrollment period is planned for 2 years, with 1 year of follow-up.

4) Plan for enrollment of participants

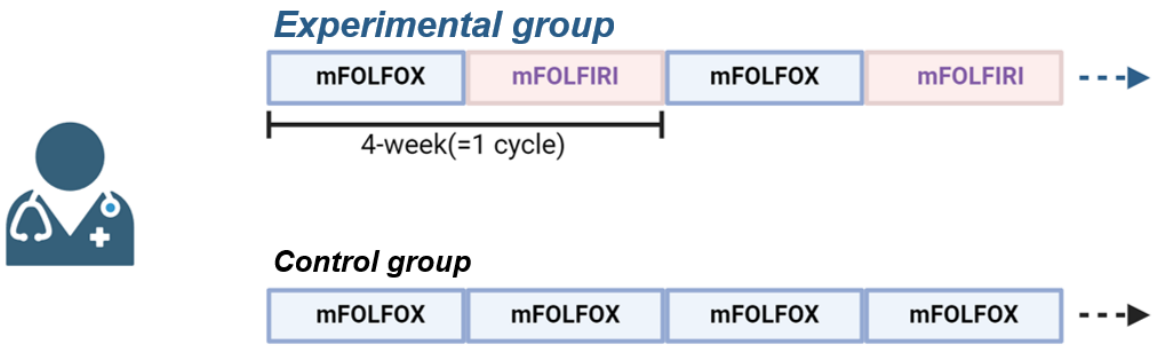
The study will recruit patients who are already receiving treatment, without utilizing a separate [Participant Recruitment Advertisement]. The principal investigator and co-investigators will ensure that potential participants are not excluded from this study based solely on race or socioeconomic status. Efforts will be made to include all eligible patients who meet the inclusion criteria, and potential participants will be fully informed about the purpose of the study. Recruitment will aim to ensure that the study population represents the overall population of biliary tract cancer patients treated at the study institution.

9. Methods

1) Specific research methods

A. Clinical trial design

- 2nd-line treatment, Phase II
- *Alternating mFOLFOX / FOLFIRI*



< Study design >

- Screening will be conducted between Day -14 and Day 0 (the day of randomization). Radiological assessments for tumor evaluation are valid up to 2 weeks prior to the start of the study (Day 1).
- The initiation criteria for each cycle of chemotherapy administration are ANC > 1.0 × 10^9/L and platelets > 75 × 10^9/L.
- The window period for chemotherapy administration visits is set at ±3 days.
- Response evaluations will be conducted every 8 weeks (after every 2 cycles). The window period is set at ±7 days.
- The administered dose may be rounded to one decimal place.
- The treatment will continue until the occurrence of any of the following events.

• Disease progression • Occurrence of unacceptable adverse effects • Withdrawal of consent

- The end-of-treatment visit will be conducted on the day treatment is discontinued or within 30 days of the last administration.
- The survival status will be assessed every 8 weeks after the end-of-study visit, with a window period of ±2 weeks (assessment can be conducted via phone).

B. General principles for chemotherapy dose adjustment

① Dose reduction or treatment delay is planned for severe hematologic or non-hematologic adverse events occurring during clinical trial treatment. Dose adjustment should be performed based on the severity of the adverse event in the most severely affected organ system. Adverse events will be graded using NCI CTCAE version 5.0.

② If the subject experiences multiple adverse events with conflicting recommendations, the most conservative dose adjustment recommendation will be followed.

③ It should be noted that doses reduced due to adverse events cannot be re-escalated.

④ All adverse events must be resolved to Grade 1 or lower before resuming treatment. Dose adjustments should be based on the severity of the adverse event in the most severely affected organ system. Missed doses during the treatment cycle should not be made up.

⑤ If the investigator determines that an adverse event is unlikely to progress to a severe or life-threatening level (e.g., alopecia, taste alterations), treatment will continue at the same dose without dose reduction or interruption. Additionally, (non-hemolytic) anemia can be managed satisfactorily with transfusion, and therefore dose reduction or treatment interruption is not required in such cases.

⑥ Dose adjustments for a single abnormal hematologic laboratory value will be based

on hematologic parameters at the start of the treatment cycle. Since specimen collection is not scheduled during the treatment cycle, nadir level measurements will not be conducted.

⑦ If recovery does not occur within a maximum of 3 weeks after the scheduled resumption of treatment, resulting in a delay in the treatment cycle, the protocol treatment will be discontinued unless both the investigator and the subject determine that continuing chemotherapy is the only viable option.

⑧ The dosing amount is based on baseline body weight, and recalculation of BSA and dose adjustment is required if there is a weight change of 10% or more. (If the institution has a chemotherapy prescription system, it may be appropriately adjusted in consultation with the principal investigator's institution.)

C. Chemotherapy dose adjustment

- Dose reductions for adverse events can be adjusted based on the recommendations below, in accordance with the institution's standard treatment guidelines and the investigator's discretion.

<Dose Levels>

	Dose levels	Dose	
Oxaliplatin	**Full Dose**	**85 mg/m^2**	**100%**
	Dose level -1	68 mg/m^2	80 %
	Dose level -2	51 mg/m^2	60%
Irinotecan	**Full Dose**	**150 mg/m^2**	**100%**
	Dose level -1	120 mg/m^2	80 %
	Dose level -2	90 mg/m^2	60%
5-fluorouracil	**Full Dose**	**2400 mg/m^2**	**100 %**
	Dose level -1	1920 mg/m^2	80 %
	Dose level -2	1440 mg/m^2	60%
5-fluorouracil	**Full Dose**	**400 mg/m^2**	**100 %**
	Dose level -1	320 mg/m2	80 %
	Dose level -2	240mg/m2	60%
Leucovorin	**No dose reduction**		

< Dose adjustment for hematologic abnormalities>

The hematologic criteria (absolute neutrophil count and platelet count) can be adjusted based on the domestic approval conditions of the investigational drug, referring to the criteria below.

	1.0≤ANC<1.5 x10^9/L 75K≤ PLT <100K	**0.5≤ANC<1.0 x10^9/L 50K≤ PLT < 75K**	**ANC< 0.5 x10^9/L PLT < 50K**
First event	No dose adjustment	Dose reduction by one level for all drugs (oxaliplatin, irinotecan, and 5-FU).	Dose reduction by one level for all drugs.
Second event	No dose adjustment	Dose reduction by two levels for oxaliplatin and irinotecan. Dose reduction by one level for 5-FU.	Dose reduction by two levels for all drugs.
Third event	Dose adjustment will not be applied if continuing treatment is considered beneficial for the participant.	If continuing treatment is considered beneficial for the participant, further dose reduction or omission of certain drugs may be implemented. Otherwise, treatment or study participation will be discontinued.	If the continuation of treatment is determined to be beneficial for the participant, further dose reduction or omission of certain drugs may be applied. Otherwise, treatment or study participation will be discontinued.

★ For mFOLFOX and Neutropenia, It is recommended to first reduce the 5-FU bolus dose by 25% or discontinue it before following the above recommendations in cases of neutropenia. Treatment cannot be initiated unless hematologic adverse events are resolved to ANC > 1.5 × 10^9/L and platelets > 75 × 10^9/L. However, in subsequent cycles, participants who had their dose reduced in the previous cycle due to persistent cytopenia may begin treatment at the reduced dose level if ANC ≥ 1.0 × 10^9/L. For febrile neutropenia, additional dose reductions are permitted at the investigator's discretion, taking into account the patient's safety.

<**Dose adjustment for non-hematologic adverse events >**

	Grade 2	**Grade 3**	**Grade 4**
First event	Wait until recovery to	Wait until recovery to	If the investigator

	grade 0-1, then restart with the full dose from the previous cycle.	grade 0-1, then reduce the dose of Oxaliplatin, Irinotecan, and 5-FU by one level.	determines that discontinuation of the protocol or continuation of treatment is necessary for a specific subject, wait until recovery to grade 0-1, then administer a reduced dose of Oxaliplatin, Irinotecan, and 5-FU by two levels.
The second event of the same non-hematologic adverse reaction	Wait until recovery to grade 0-1, then reduce the dose of Oxaliplatin, Irinotecan, and 5-FU by one level.	Wait until recovery to grade 0-1, then reduce the dose of Oxaliplatin, Irinotecan, and 5-FU by two levels.	Not applicable.
The third event of the same non-hematologic adverse reaction	Wait until recovery to grade 0-1, then reduce the dose of Oxaliplatin, Irinotecan, and 5-FU by two level.	Treatment discontinuation /Study termination	Not applicable.
The fourth event of the same non-hematologic adverse reaction	Treatment discontinuation /Study termination	Not applicable.	Not applicable.

<Expected Adverse Reactions and Management Strategies>

In cases where neurological toxicity, reversible posterior leukoencephalopathy syndrome, septic shock, disseminated intravascular coagulation, hemolytic uremic syndrome, QT interval prolongation, or rhabdomyolysis are suspected or occur, appropriate treatment should be

administered, and the patient should be referred to a relevant specialist. Based on the investigator's judgment, treatment may be resumed with dose adjustment according to the toxicity grade once recovery is achieved.

2) Control group setting and randomization method

The investigational product will be administered only to participants enrolled in this clinical trial in accordance with the procedures specified in the clinical trial protocol. Participants who withdraw from the trial will retain their participant number. A new participant number must always be assigned to new participants. All eligible participants must be registered prior to the initiation of treatment.

3) Administration/Dosage of investigational products, method of administration, combination therapy, and use of control drugs

The chemotherapy regimens to be used in this clinical trial are mFOLFOX and mFOLFIRI. The methods of administration and schedules for the drugs are as follows:

A. mFOLFOX (Administered as a single regimen every 2weeks)

1) mFOLFOX
D1 Oxaliplatin 85mg/m2 over 2hr Leucovorin 400mg/m2 over 2hr Fluorouracil 400mg/m2 FU 2400mg/m2 over 46hr

B. mFOLFOX/mFOLFIRI (Administered alternately every 2 weeks.)

1) mFOLFOX	2) mFOLFIRI
D1 Oxaliplatin 85mg/m2 over 2hr Leucovorin 400mg/m2 over 2hr Fluorouracil 400mg/m2	D1 Irinotecan 150mg/m2 over 2hr Leucovorin 100mg/m2 over 2hr 5FU 2400mg/m2 over 46hr

FU 2400mg/m2 over 46hr	

- The treatment will continue to be repeated until disease progression occurs, treatment is no longer feasible due to toxicity, or the patient declines further therapy.

4) Observation parameters, clinical laboratory tests, and methods of assessment

<Clinical study tests and observation items, schedule >

A. The screening procedures must be conducted within 14 days prior to the initiation of treatment. (Screening tests may be used as Day 1 Cycle 1 tests.) Evaluation timeline: Day -14 to Day -1 (before drug administration)

① Informed Consent: A signed consent form in the designated format.

② Vital Signs and Physical Measurements: Body temperature, blood pressure, heart rate, height, and weight.

③ ECOG Performance Status

④ History of Biliary Tract Cancer: Initial histopathological diagnosis and prior treatment history for biliary tract cancer.

⑤ Medical History: Comorbid conditions and ongoing treatment history.

⑥ Physical Examination: Clinical examination.

⑦ Tumor Evaluation (only for areas with lesions): CT (abdomen and pelvis CT, chest CT), chest X-ray, bone scan (if needed), PET-CT (if needed).

⑧ Tumor evaluation must be conducted within 14 days prior to the initiation of treatment, and the same evaluation method must be used.

⑨ Laboratory Tests

a. Hematologic Tests: Hemoglobin, hematocrit, WBC (including differential count), platelet count.

b. Biochemical Tests: Total bilirubin, γ-GTP (if needed), AST/ALT, alkaline phosphatase, total protein, albumin, serum creatinine, BUN, tumor markers (CA19-9, CEA), creatinine clearance (Ccr based on the MDRD calculation formula).

⑩ Quality of Life Assessment: FACT-G7 questionnaire.

B. Evaluation parameters during drug administration period

① Vital signs and physical measurements

② Clinical laboratory tests

③ Hematologic tests (evaluated every cycle): Hemoglobin, hematocrit, WBC (including differential count), platelet count.

④ Blood biochemical tests (assessed per cycle): Total bilirubin, γ-GTP (if needed), AST/ALT, alkaline phosphatase, total protein, albumin, serum creatinine, BUN, tumor markers (CA19-9, CEA) (tumor markers are assessed at each response evaluation), creatinine clearance.

⑤ Radiological measurement of lesions (Tumor Evaluation: CT (abdomen, pelvis CT, chest CT) (and/or MRI), chest X-ray, bone scan (if needed), PET-CT (if needed)): Evaluations should be conducted using the same methods. Additional tests may be used if symptoms are present or if deemed necessary. Tumor evaluations are performed every 8 weeks, with a window period of +/- 1 week.

⑥ Clinical findings regarding adverse events

⑦ Quality of life assessment (FACT-G7) – performed at each response evaluation (to be done before the consultation, with a window period of +/- 2 weeks).

C. Conditions for discontinuation of the investigational drug administration

In the cases described below, the administration of the drug should be discontinued, and the reason must be documented in the case report form.

① Patients with obvious disease progression or those for whom treatment cannot be administered due to the condition.

② Patients who experience clinically severe or persistent side effects that make continued administration difficult (if the next dose is delayed by more than 3 weeks).

③ Cases where clinically significant abnormal test results are observed (if the next dose is delayed by more than 3 weeks).

④ If the patient wishes to discontinue the treatment.

⑤ If the patient refuses to receive the drug administration.

⑥ If the patient passes away.

⑦ If follow-up observation is not possible.

⑧ If the clinical trial investigator deems it necessary for any other reason.

D. End-of-treatment assessment

① Vital signs, physical measurements: Height, weight

② Clinical laboratory tests

a. Hematologic tests: Hemoglobin, hematocrit, WBC (including differential count), platelet count.

b. Blood biochemical tests: Total bilirubin, γ-GTP, AST/ALT, alkaline phosphatase (ALP), total protein, albumin, serum creatinine, BUN, tumor markers (CA19-9, CEA), creatinine.

③ Radiological measurement of lesions (if needed)

④ Clinical findings regarding adverse reactions

E. Follow-up investigation

① Adverse events related to the investigational drug and the tumor will be followed up until recovery. The reasons for trial discontinuation and the date of discontinuation must be documented in the case report form for all patients. The investigator is recommended to ensure that all necessary tests and procedures are completed when a participant is excluded from the clinical trial.

② The follow-up period begins after the end-of-trial visit and is conducted every 8 weeks. During the follow-up period, the following evaluation variables will be recorded. (Medical record review and survival confirmation can be done by phone):

a. Duration until disease progression for patients who did not experience disease progression up to the end of investigational drug administration.

b. Third-line treatment (including start date): Additional cancer treatments if known (e.g., chemotherapy, immunotherapy, targeted therapy, radiotherapy, and surgery).

c. Confirmation of survival status.

5) Criteria for efficacy evaluation and evaluation methods

A. Death - Confirm the patient's date of death.

B. 6-month survival rate - The proportion of patients who are alive at 6 months from the initiation of second-line chemotherapy (analyzed using the survival function).

C. Overall Survival - Defined as the time from the initiation of second-line chemotherapy to the occurrence of death from any cause, analyzed as the event.

D. Treatment Response - Analyzed based on RECIST 1.1 criteria, including complete response (CR), partial response (PR), and disease progression (PD).

E. Disease Progression - Confirm the date of the CT scan showing disease progression based on RECIST 1.1 criteria.

F. Progression-Free Survival - Defined as the time from the initiation of second-line chemotherapy to the occurrence of either disease progression or death without prior progression, analyzed as the event.

G. Toxicity - Toxicity will be assessed at every treatment cycle through medical history, physical examination, and blood tests, and will be recorded based on CTCAE version 5.0.

6) Uniqueness compared to existing treatments and studies

There are currently no proven effective treatments for advanced or recurrent biliary tract cancer that is unresponsive to first-line therapy. However, recent systemic reviews suggest that second-line chemotherapy may provide some benefit. This study aims to identify the most effective combination of chemotherapy regimens. While most previous studies on second-line therapy for biliary tract cancer have been phase ii trials with a single-arm design, this clinical trial is a randomized phase ii study. It is designed to evaluate the most promising chemotherapy regimens based on their effectiveness in other gastrointestinal cancers, particularly pancreatic cancer. The study design, including both experimental and control groups, adds significant value to the research.

7) Benefits and risks to study participants

A. The drugs used in this study are already effectively utilized in other cancer types, and their side effects and risks are well-documented. Appropriate management of these adverse effects is feasible within clinical practice.

B. Second-line chemotherapy is considered potentially effective for biliary tract cancer,

and it is currently administered in routine clinical practice when deemed appropriate for the patient. This study aims to determine which of the two most promising drug combinations is more effective. Patients enrolled in this study will not receive under-treatment or excessive treatment.

8) Criteria for discontinuation or withdrawal

Cases meeting the following criteria will result in discontinuation of drug administration, and the reason will be documented in the case report form (CRF).

A. Definite disease progression or patients unable to continue treatment due to their condition.
B. Patients experiencing severe or persistent adverse events that make continued administration clinically challenging (e.g., when the next treatment cycle is delayed by more than 3 weeks).
C. Clinically significant abnormal laboratory findings (e.g., when the next treatment cycle is delayed by more than 3 weeks).
D. Patients who wish to discontinue treatment.
E. Patients who refuse further drug administration.
F. Patients who have died.
G. Patients who are unable to undergo follow-up.
H. Any other case deemed necessary by the investigator.

9) Safety evaluation criteria, methods, and reporting for adverse events

Severe adverse events (SAEs) will be reported according to the regulations of each institution's IRB. Reports to the IRB will also be submitted to the primary investigator (PI) institution.

10) Data and Safety Monitoring Plan (DSMP)

A. Monitoring Responsibility - The principal investigator at each institution.
B. Reporting of Adverse Events and Non-Compliance - Reports will follow the internal regulations and schedule established by the institution's IRB. The PI will also be informed during IRB reporting.
C. Monitoring is scheduled at the following milestones: upon the registration of the first participant at the institution, when 20% of participants are enrolled, and at

the conclusion of the study.

D. Safety Meetings - In the event of a significant safety issue, any investigator can request a meeting. Meetings must be held within one week of the request to address the issue.

11) Data analysis and statistical methods

A. Analysis population

1. Overall efficacy analysis will be performed on the ITT (intention-to-treat) population, defined as all randomized patients.
2. Safety analysis will include all patients who received treatment.

B. Primary analysis

The primary analysis will measure the 6-month overall survival rate (OS rate) using the Kaplan-Meier method.

C. Secondary analysis

Efficacy analysis will be based on the evaluation of patient responses according to RECIST v1.1 response criteria. The effectiveness of the investigational drug will be measured in terms of overall response rate (ORR) with 95% confidence intervals. The best overall response (BOR) during the period from drug administration to either disease progression or study termination will be reported. Target and non-target lesion evaluations will follow RECIST v1.1 criteria and will be conducted every 6 weeks. If clinical disease progression is suspected, the evaluation will be performed immediately prior to the termination of the clinical trial. All lesion evaluations for each patient must maintain consistent imaging methods throughout the treatment period, including the use of CT scans and X-rays to ensure uniformity. Lesions smaller than 10 mm at baseline must be documented using spiral CT in the medical record and consistently evaluated throughout the clinical trial. Where clinically feasible, oral and intravenous contrast agents should be used consistently. Tumor measurements during the clinical trial should be performed by the same investigator or radiologist. Additionally, tumor evaluation personnel at each institution must be trained in RECIST standards through participation in prior multi-center

clinical studies. Overall survival (OS) and progression-free survival (PFS) from the start of investigational drug administration will be analyzed using the Kaplan-Meier method. Analyses of the time to disease progression or death will include all available follow-up information.

D. Safety data analysis

Adverse events will be documented according to NCI CTCAE version 5.0. Additional analyses will be conducted based on the severity of adverse events and their causal relationship to the investigational drug. Standard safety analysis will be performed, including details on dose adjustments, serious adverse events (e.g., hospitalizations), and early study withdrawals.

E. Quality of life assessment (QoL)

Quality of life will be assessed using QoL questionnaire scores (FACT-G7). If the data satisfy normality assumptions, a two-sample t-test will be performed; otherwise, the Mann-Whitney U test will be used.

10. Measures to ensure participant safety

1) Ethical conduct of the study

The study will comply with the Declaration of Helsinki (2013 revision) and the guidelines of Good Clinical Practice (ICH-GCP). The research will only be conducted after receiving approval from the Institutional Review Board (IRB).

2) Informed consent process

The investigator will personally explain the study to participants and obtain consent during routine care (e.g., outpatient clinics or hospital rooms). The consent process will use language understandable to participants and guardians, and participation will be voluntary without any disadvantage for refusal.

3) Compensation for study participants

The investigational drugs (irinotecan, oxaliplatin, and 5-fluorouracil) are approved and marketed in Korea, having been in use for over five years. No specific injuries related to this study are anticipated. In the event of injury caused by the investigational drug or

study procedures, appropriate medical care will be provided. Since the treatment methods in this study are already used for gastric and colorectal cancer, no financial compensation will be provided to participants.

4) Protection of Participant Privacy

Patient medical record numbers and pathology numbers will be stored in separate, coded files under the supervision of the principal investigator. Research data will be password-protected and stored in a locked research facility. In compliance with Article 15 of the Bioethics and Safety Act Enforcement Rules, study-related records will be retained for three years after the study's completion. Documents will be destroyed in accordance with Article 16 of the Personal Information Protection Act Enforcement Decree after the retention period.

5) Additional Protections for Vulnerable Participants

※ This study does not include vulnerable participants.

11. Storage and Disposal of Human-Derived Materials

※ Not applicable

12. Reference

1) Lamarca A, Hubner RA, Ryder WD, Valle JW. Second-line chemotherapy in advanced biliary cancer: a systematic review. Ann Oncol. 2014 Apr 25.

2) AJCC Cancer Staging Manual. 8th ed. Springer; 2018.

3) Su J, Liang Y, He X. Global, regional, and national burden and trends analysis of gallbladder and biliary tract cancer from 1990 to 2019 and predictions to 2030: a systematic analysis for the Global Burden of Disease Study 2019. Front Med (Lausanne). 2024;11:1384314.

4) Eckel F, Schmid RM. Chemotherapy in advanced biliary tract carcinoma: a pooled analysis of clinical trials. Br J Cancer. 2007;96(6):896-902.

5) Laughter MR, Maymone MBC, Mashayekhi S, et al. The global burden of atopic dermatitis:

lessons from the Global Burden of Disease Study 1990-2017. Br J Dermatol. 2021;184(2):304-309.

6) Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med. 2010;362(14):1273-1281.

7) American Cancer Society. Bile Duct Cancer Statistics. Available at: https://www.cancer.org/cancer/types/bile-duct-cancer/about/key-statistics.html.

8) 국가암정보센터. Available at: https://cancer.go.kr/.

9) Oh DY, He AR, Qin S, et al. Durvalumab plus gemcitabine and cisplatin in advanced biliary tract cancer. NEJM Evid. 2022;1(8)

10) Kelley RK, Ueno M, Yoo C, et al. Pembrolizumab in combination with gemcitabine and cisplatin compared with gemcitabine and cisplatin alone for patients with advanced biliary tract cancer (KEYNOTE-966): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2023;402(10406):964.

11) Choi IS, Kim KH, Lee JH, et al. A randomised phase II study of oxaliplatin/5-FU (mFOLFOX) versus irinotecan/5-FU (mFOLFIRI) chemotherapy in locally advanced or metastatic biliary tract cancer refractory to first-line gemcitabine/cisplatin chemotherapy. Eur J Cancer. 2021;154:288-295.

12) Carrato A, Pazo-Cid R, Macarulla T, et al. Nab-paclitaxel plus gemcitabine and FOLFOX in metastatic pancreatic cancer. NEJM Evid. 2024;3(2)

13) Bridgewater J, Palmer D, Cunningham D, Iveson T, Gillmore R, Waters J, et al. Outcome of second-line chemotherapy for biliary tract cancer. Eur J Cancer. 2012.

14) Moretto R, Raimondo L, De Stefano A, Cella CA, Matano E, De Placido S, et al. FOLFIRI in patients with locally advanced or metastatic pancreatic or biliary tract carcinoma: a

monoinstitutional experience. Anticancer Drugs. 2013;24(9):980-985.

15) Lee HY. Second-line treatment with mFOLFOX6 in gemcitabine-pretreated pancreato-biliary cancer. World Gastrointestinal Congress; 2013 (P0165). 2013.