Trial Protocol

The Efficacy and Safety of **G**emcitabine-Cisplatin Plus **Envafolimab** as **Ne**oadjuvant Therapy in Resectable Biliary Tract Malignancies at High Risk of Recurrence (GENE Trial)

Investigational Product:	Gemcitabine-Cisplatin plus Envafolimab
Study Phase:	II
Leading Study Site:	Sir Run Run Shaw Hospital, Zhejiang University
Principal Investigator:	Prof. Yuelong Liang
Protocol No.:	GENE Trial
Protocol Version No.:	V1.0
Protocol Version Date:	March. 1, 2026

Investigator Signature Page

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Protocol No.: GENE Trial
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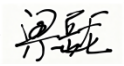
Investigator's Signature:
This protocol has been rigorously reviewed and complies with the Good Clinical Practice (GCP) guideline and the ethical principles of the Declaration of Helsinki. The investigator agrees to conduct the clinical trial in accordance with the protocol design and requirements, and ensures the authenticity and integrity of study data, as well as the protection of participants' rights, safety, and privacy.

Study Site: Sir Run Run Shaw Hospital, Zhejiang University
Signature of Investigator: Yuelong Liang 
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List of Abbreviations

Abbreviation	Definition	Abbreviation	Definition
AE	Adverse event	INR	International Normalized Ratio
ALB	Serum Albumin	MDT	Multidisciplinary Team
ALT	Alanine Aminotransferase	MPR	Major Pathologic Response
AST	Aspartate Aminotransferase	ORR	Objective Response Rate
BTC	Biliary Tract Cancer	OS	Overall Survival
CA19-9	Carbohydrate antigen 19-9	PCR	Pathologic Response Rate
CA125	Carbohydrate Antigen 125	PD-L1	Programmed Death Ligand 1
CEA	Carcinoembryonic antigen	PLT	Platelet
Child-Pugh	Child-Pugh Classification	PPS	Per-protocol Set
Cr	Creatinine	PR	Partial Response
DFS	Disease-free Survival	PS	Performance Status
ECOG	Eastern Cooperative Oncology Group	SAE	Serious Adverse Event
FAS	Full Analysis Set	SD	Stable Disease
HCC	Hepatocellular Carcinoma	SS	Safety Set
HB	Haemoglobin	TBIL	Total Bilirubin
ICC	Intrahepatic Cholangiocarcinoma	WBC	White Blood Cell

1. Protocol Summary

Protocol Title	The Efficacy and Safety of Gemcitabine-Cisplatin Plus Envafolimab as Neoadjuvant Therapy in Resectable Biliary Tract Malignancies at High Risk of Recurrence (GENE Trial)
Type of Clinical Study	Investigator-Initiated Trial
Protocol No.	GENE Trial
Protocol Version No./Date	V1.0/March. 1, 2026
Principal Investigator	Prof. Yuelong Liang
Leading Study Site	Sir Run Run Shaw Hospital, Zhejiang University
Investigation	Envafolimab: 400 mg on Day 1, repeated every 3 weeks (Q3W). Chemotherapy: Gemcitabine: 1000 mg/m^2 in 100 mL of 0.9% sodium chloride injection, administered intravenously over 30 minutes on Days 1 and 8, repeated Q3W. Cisplatin: 25 mg/m^2 in 500 mL of 5% glucose injection, administered intravenously over 2 hours on Days 1 and 8, repeated Q3W.
Study Population	Participants withr resectable BTCs at high risk of recurrence
Study Endpoints	Primary endpoint: Major Pathologic Response (MPR). Secondary endpoints: Overall Survival (OS), Disease-free survival (DFS), Objective Response Rate (ORR) per the Response Evaluation Criteria In Solid Tumors (RECIST v1.1), Pathologic Response Rate (PCR), and R0 resection rate. The incidence and severity of adverse events (AEs), serious adverse events (SAEs), and laboratory test abnormalities judged as per the CTCAE v5.0 criteria. Exploratory endpoint: Relationship between carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), carbohydrate antigen 125 (CA125), Pathological biomarker expression, spatial transcriptome (if samples are sufficient), gut microbiome metagenome sequencing, and treatment response.
Study Design	This trial is a single-arm interventional clinical study to evaluate the efficacy and safety of neoadjuvant gemcitabine-cisplatin plus Envafolimab in resectable biliary tract malignancies patients with high-risk of recurrence. The study includes the following processes: screening visit, neoadjuvant therapy-period visit (every 3 weeks ± 3 days), follow-up visit (every 3 months ± 15 days up to 2 years). 1) Screening visit (within 7 days prior to the first dose of treatment). Within 7 days before the first dose of neoadjuvant therapy, all participants must complete baseline screening examinations, including tumor core needle biopsy for pathological confirmation of BTC, and gut microbiome metagenomic sequencing. Relevant examinations completed by participants prior to providing

	informed consent may be omitted if they were obtained within the screening window (i.e., within 7 days before the first dose) and deemed acceptable by the investigator per protocol requirements. 2) Neoadjuvant therapy-period visit (every 3 weeks ± 3 days) Participants will be administered neoadjuvant therapy combined with perioperative management. Participants who have disease progression during neoadjuvant therapy-period and are assessed as ineligible for radical surgery, develop intolerable toxicity, inoperable, or initiate a new anti-tumor therapy will discontinue treatment and proceed to follow-up visits until withdrawal of consent by the participant, loss to follow-up or death, or end of study (completing the 2-year follow-up period), whichever occurs first. 3) Follow-up visit Postoperative adjuvant therapy will be determined based on pathological findings and the patient's general condition. Safety follow-up and imaging assessments will be performed every 3 months (± 15 days), including contrast-enhanced abdominal CT/MRI and chest CT. Additional imaging may be performed at the investigator's discretion if clinically indicated.
Sample Size	With the primary endpoint set as the major pathological response (MPR) rate, this study adopts a Simon's two-stage design. An MPR rate of ≤5% is considered clinically insignificant, and neoadjuvant therapy is expected to elevate the MPR rate to ≥20%. The type I error rate is set at 5% with a statistical power of 80%, which is sufficient to evaluate the efficacy of the investigational regimen. According to statistical calculation, 16 patients will be enrolled in the first stage. If at least 2 out of these 16 patients achieve MPR, an additional 14 patients will be recruited, resulting in a total sample size of 30 cases. The null hypothesis will be rejected if a cumulative number of at least 4 MPR events are observed among the 30 patients. Considering an approximate 10% loss to follow-up and dropout rate during the study period, the planned total sample size is determined as 34 patients.
Study Period	Study Period: 48 months (May, 2026 to March, 2030).
Inclusion Criteria	1) Participants who have signed a written Informed Consent Form (ICF); 2) Male or female participants aged 18-80 years; 3) Eastern Cooperative Oncology Group performance status (ECOG) score of 0/1; 4) BTC diagnosed by puncture pathology before enrollment; 5) Participants must meet the following requirements for major vital organ function: a. Absolute neutrophil count (ANC) ≥ 1.5×10^9/L; platelet count ≥ 90×10^9/L; hemoglobin ≥ 9 g/dL; b. Coagulation function: international normalized ratio (INR) ≤ 1.2; c. Alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 3 times the upper limit of normal (ULN); d. Serum

	albumin ≥ 3.5 g/dL; total bilirubin ≤ 1.5 times the ULN. Patients with obstructive jaundice who meet the eligibility criteria after percutaneous transhepatic cholangial drainage or endoscopic retrograde cholangiopancreatography treatment are also eligible for enrollment; e. Child-Pugh class A or B; f. Serum creatinine ≤ 1.5 times the ULN. 6) Patients with high-risk factors for recurrence. The criteria were defined as meeting at least one of the following: a. Preoperative CA19-9 ≥ 200 U/mL; b. Tumor diameter ≥5 cm or multiple tumor nodules on imaging; c. Regional lymph node metastasis with a short-axis diameter ≥ 1.0 cm on imaging; d. Vascular invasion (portal vein or hepatic artery) on imaging; e. Low or undifferentiated histologic grade. 7) Participants who have at least 1 measurable lesion (RECIST v1.1). 8) Be able to provide fresh stool samples and liver samples if undergoing surgery.
Exclusion Criteria	1) Pathological diagnosis of hepatocellular carcinoma, mixed hepatocellular carcinoma, and other non-cholangiocarcinoma malignant tumor components; 2) Prior systemic therapy for BTC, including immunotherapy, targeted therapy, or chemotherapy; 3) History of other prior or concurrent malignancies, except those with complete treatment and disease-free survival for more than 5 years; 4) Presence of an active, known or suspected autoimmune disease, or requirement for long-term systemic corticosteroid therapy (equivalent to ≥ 10 mg prednisone daily) or other immunosuppressive agents. Participants using inhaled or topical corticosteroids will not be excluded; 5) Presence of ascites, hepatic encephalopathy, sclerosing cholangitis, or other concurrent organ dysfunctions that would preclude tolerance of general anesthesia or hepatectomy; 6) Women who are breastfeeding or pregnant; 7) Any other factors that, in the investigator's judgment, may compromise participant safety or trial compliance, including serious comorbidities requiring ongoing treatment, clinically significant laboratory abnormalities, or relevant social or family-related issues.
Criteria for Discontinuation of Treatment	Discontinuation of trial intervention does not imply termination of the study and subsequent study procedures should be completed under the protocol. Participants must discontinue the trial intervention if they meet any of the following criteria: 1) Development of treatment-related adverse reactions meeting the discontinuation criteria; 2) Disease progression confirmed by imaging assessment; 3) Occurrence of comorbidities, complications, worsening conditions, AEs or

	SAEs that, in the investigator's judgment, render the participant unsuitable for continued treatment; 4) Administration of protocol-prohibited concomitant medications that substantially impair the evaluation of safety and efficacy; 5) Voluntary withdrawal by the participant or premature discontinuation by the investigator; 6) Inadequate compliance that significantly compromises assessment of the primary endpoints; 7) Pregnancy in female participants; 8) Other circumstances in which the investigator deems treatment discontinuation necessary.
Criteria for Withdrawal	1) Participants may voluntarily withdraw from the study at any time, with or without reason. Withdrawn participants will face no discrimination or retaliation, with their medical and legal rights fully preserved. All withdrawal reasons shall be documented in medical records and the eCRF. Withdrawal criteria include but are not limited to: a. Revocation of informed consent or refusal of further follow-up; b. Loss to follow-up: participants missing scheduled visits who cannot be contacted after three documented attempts within one month; c. All-cause death. 2) The investigator shall record reasons for treatment discontinuation in the eCRF and attempt to complete end-of-treatment assessments. Participants discontinuing neoadjuvant therapy will receive continuous survival follow-up for up to 2 years, or until consent withdrawal, loss to follow-up, or death. Ongoing AEs/SAEs related to the study drug will be monitored until resolution, stabilization, loss to follow-up, or initiation of alternative antitumor therapy.
End of Study	End of study is defined as the completion of the last protocol-specified visit by the last participant.
Method of Administration and Surgical Treatment	The detailed schedule for each cycle is as follows: 1) Envafolimab: 400 mg on Day 1, repeated every 3 weeks (Q3W). 2) GC chemotherapy: Gemcitabine: 1000 mg/m^2 in 100 mL of 0.9% sodium chloride injection, administered intravenously over 30 minutes on Days 1 and 8, repeated Q3W. Cisplatin: 25 mg/m^2 in 500 mL of 5% glucose injection, administered intravenously over 2 hours on Day 1 and 8, repeated Q3W.
Permitted and Prohibited Concomitant Medications	1) Concomitant Medications/Therapies: All trial interventions must be documented in the eCRF. Participants shall report any off-site use of investigational products with accurate dosage records. All non-trial medications and therapies administered during the study should also be recorded in the eCRF. 2) Permitted: Essential supportive care and necessary safety treatments are allowed. All adverse events will be closely monitored and promptly managed, with relevant medications documented in the eCRF.

	3) Prohibited: Antitumor therapies other than the study drug, participation in other interventional trials, or additional investigational agents are prohibited. Systemic corticosteroids and immunosuppressants are forbidden prior to Envafolimab initiation, except for immune-related AEs arising after treatment.
Efficacy Evaluation	Primary efficacy evaluation variable: MPR rate will be pathologically evaluated using surgical specimens. MPR is defined as ≤10% residual viable tumor cells in the primary lesion and regional lymph nodes following neoadjuvant therapy, with Pathologic Response Rate (PCR, 0% viable tumor cells) included in the MPR definition. Specimen sampling and sectioning will follow standardized procedures, and assessments will be conducted by specialized pathologists; blinded independent pathological review will be performed as needed. Secondary efficacy evaluation variables: 1) Postoperative resected specimens are analyzed via pathological section to determine the residual viable tumor cell ratio. 2) Overall Survival (OS): OS is defined as the time from participant receiving the first treatment until death from any cause. 3) Disease-free survival (DFS): DFS is defined as the time from the date of surgery to neoplasm recurrence or death for participants without residual lesions after surgery, whichever occurs first. 4) Objective Response Rate (ORR): ORR is defined as the sum of Complete Response (CR) and Partial Response (PR), representing the proportion of participants with sustained tumor regression meeting predefined criteria. 5) Pathologic Response Rate (PCR): No viable tumor cells are found in the review of pathological sections. 6) R0 resection: R0 resection rate refers to the proportion of patients achieving complete surgical resection with negative margins.
Safety Evaluation	All consenting participants will undergo safety assessments, including physical examination, vital signs, hematology, biochemistry, coagulation, urine and stool tests, 12-lead ECG, ECOG status and thyroid function, as well as monitoring of AEs, SAEs and procedure-related adverse events. Assessments will continue from consent signature through study completion. All investigational product-related AEs/SAEs will be followed until resolution, stabilization, participant withdrawal, loss to follow-up or death. AE severity will be graded per CTCAE v5.0.
Biomarker Assessments	Correlations of biomarkers (CEA, CA19-9, CA125, spatial transcriptomics and gut microbiome metagenomics, etc) with treatment efficacy, and relevant basic research, are allowed.
Statistical Analyses	General principles: Detailed statistical methods will be described in the

	Statistical Analysis Plan (SAP), finalized before database lock. The SAP will specify all analyses and result presentations and be retained by the investigator. Minor adjustments to protocol-related items are permitted; however, major revisions to primary endpoint definitions and analyses require a formal protocol amendment. Analyzed population: Analyses will be performed in the Full Analysis Set (FAS), defined as all enrolled participants who received at least one dose of study treatment. The MPR rate, as the primary endpoint, will be calculated with the 95% confidence interval estimated via the Clopper-Pearson method. The Per-Protocol Set (PPS) includes participants who met key eligibility criteria, had no major protocol deviations, completed the minimum required treatment dose, and underwent at least one efficacy assessment. MPR rate and corresponding 95% confidence intervals (CIs) will also be analyzed in the PPS, and consistency between FAS and PPS results will be compared to verify the stability of primary findings. Patients who fail to undergo surgical resection after neoadjuvant treatment, without available postoperative pathological specimens for MPR assessment, will still be included in the Full Analysis Set (FAS). Missing primary endpoint data will be handled by non-responder imputation, and such patients will be regarded as not achieving MPR. Time-to-event outcomes, including disease-free survival and overall survival, will be estimated using the Kaplan–Meier method, with median survival and 95% CIs presented. Safety analyses will be conducted in the Safety Set, covering all participants exposed to at least one study treatment. The number and proportion of participants with treatment-emergent adverse events, study drug-related adverse events, and serious adverse events will be summarized. Statistical Analyses: 1) MRP: The primary analysis of the MPR will be performed based on the FAS and PPS. The number and percentage of participants achieving MPR will be summarized and the 95% CI will be calculated via the Clopper-Pearson method. ORR: Analysis will include CR and PR per RECIST v1.1 criteria, and the analysis of the ORR is the same as the analysis of MPR. R0 resection: R0 resection rate is defined as the percentage of participants who achieve complete resection with negative surgical margins. Participants without surgery or pathological assessment are considered to have failed to achieve R0 resection and included in the denominator for calculation. Its analytical method is consistent with that of MPR. DFS: DFS is defined as the time from the date of surgery to neoplasm recurrence or death for participants without residual lesions after surgery, whichever occurs first. OS: OS is defined as the time from the date of receiving the first treatment to death from any cause. 2) Subgroup analysis: No subgroup analysis is designed. 3) Interim analysis:

	The complete result analysis of the first phase is defined as the interim analysis. 4) Safety analysis: Safety assessments include adverse events, vital signs, physical examination, 12-lead ECG, and clinical laboratory tests. All treatment-emergent adverse events (TEAEs) will be summarized by system organ class and preferred term, with their incidence, severity, and causality tabulated. Laboratory parameters and vital signs will be described using descriptive statistics. Clinically significant abnormalities will be summarized in tables. 5) Exploratory analysis: The analysis of exploratory endpoints (biomarkers) is not included in this SAP.

Table 1 Schedule of Activities (SoA) of Clinical Study

Study Period/Activities	Screening Visit[1]	Neoadjuvant therapy-period visit	Follow-up visit
Signing the ICF[2]	●		
Inclusion/exclusion criteria	●		
Enrollment	●		
Pathological confirmation of BTC[3]		●	
Demographics[4]	●		
Past medical history[5]	●		
Physical examination[6]	●	●	●
Laboratory tests[7]	●	●	●
Imaging examination[8]	●	●	●
Concomitant medications[9]	●	●	●
Record of adverse events[10]		●	●
Survival[11]		●	●

1. Screening period: Examination results before informed consent and within the time window specified for this study are permitted for use in screening.

2. Informed consent: should be obtained prior to performing any study-specific procedures.

3. Pathological confirmation of BTC: Participants enrolled will undergo a tumor puncture biopsy prior to the neoadjuvant therapy.

4. Demographics: including age, gender, date of birth, and ethnicity.

5. Past medical history: During screening, participants' medical histories were collected, including tumor history, other past medical conditions, and treatment history.

6. Physical examination: These assessments will be performed by the investigator to evaluate major organ systems at the following visits: screening (within 7 days before first dose), neoadjuvant therapy-period visit (every 3 weeks ± 3 days), follow-up visit (every 3 months ± 15 days).

7. Laboratory tests: Hematology: including red blood cell (RBC) count, haemoglobin (Hb), haematocrit (HCT), white blood cell (WBC) count, differential white blood cell count (neutrophils, eosinophils, lymphocytes, monocytes, and basophils), and platelet (PLT) count. Liver and kidney function and electrolytes: Liver and kidney function indicators include blood urea nitrogen/blood urea (UREA), creatinine (Cr), total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (GGT), total cholesterol (TC), triglycerides (TG), and uric acid (UA). Electrolytes include sodium (Na^+), potassium (K^+), chlorine (Cl^-), calcium (Ca^{2+}), and magnesium (Mg^{2+}). Coagulation: including thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT), and international normalized ratio (INR).

8. Imaging examination: contrast-enhanced abdomen CT/MRI, and chest CT.

9.Concomitant medications: All concomitant medications, including supportive treatment medications (e.g., antiemetic and prophylactic), medications for AEs or concomitant medical histories, and non-drug supportive interventions, should be recorded from the time the subject starts the treatment to the end of study. If participants meet the withdrawal criteria during the study, concomitant medications/therapies will no longer be collected from the time of withdrawal.

10. Adverse events: AEs collection spans from treatment initiation to study end. Baseline abnormalities (ICF signing to first dose) are documented as such. Collection stops for subjects meeting withdrawal criteria, initiating new anti-tumor therapy, or entering long-term follow-up (only product-related AEs then tracked). Product-related AEs are followed until resolution, chronic/stable confirmation, consent withdrawal, LTFU, death, or new anti-tumor therapy—whichever is earliest. SAEs (if product-related) follow the same timeline except for new therapy initiation.

11. Survival: Survival visits will be conducted every 3 months ± 15 days during follow-up period to collect survival information on participants. Follow-up visits will be conducted by telephone until the participant's death, withdrawal of informed consent, or loss to follow-up.

2. Rationality

Biliary tract cancer (BTC) is a group of highly malignant tumors originating from the biliary epithelial system, including intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA), distal cholangiocarcinoma (dCCA), and gallbladder cancer (GBC)[1]. The incidence of BTC is increasing worldwide, and is significantly higher in Asian countries than in Europe and the United States, with an overall poor prognosis[2]. Previous studies reported that preoperative CA19-9>200 U/mL, multiple tumor nodules, metastases to regional lymph nodes, vascular invasion, and low differentiation are the main factors affecting the postoperative survival of BTC patients[3]. Although radical resection can be achieved via direct surgery in such high-risk patients, the high postoperative recurrence rate and low long-term survival rate suggest that surgery alone is insufficient to achieve oncological cure.

Neoadjuvant therapy refers to preoperative treatment for treatment-naive patients with non-metastatic tumors, including chemotherapy, radiotherapy, targeted therapy, and immunotherapy. It aims to shrink tumors, downstage disease, improve radical resection rates, reduce postoperative recurrence, prolong survival. Multiple clinical studies have confirmed that patients with various tumors can benefit from neoadjuvant therapy[4,5]. Previously, in patients with advanced BTC, the TOPAZ-1 study confirmed that immunotherapy (durvalumab) combined with the GC chemotherapy regimen (gemcitabine plus cisplatin) resulted in significantly superior overall survival (OS) compared with GC chemotherapy alone, establishing the first-line role of immunotherapy plus chemotherapy in advanced BTC[6]. However, in contrast to unresectable BTC, studies focusing on neoadjuvant immunotherapy plus chemotherapy for resectable disease, particularly for high-risk patients, remain limited and underexplored[7].

Theoretically, preclinical studies have demonstrated that gemcitabine can promote the transformation of tumor-associated macrophages toward an immunostimulatory phenotype and enhance tumor immunogenicity[8]. Platinum-based drugs can enhance host immune activity and improve T-cell recognition of tumor cells[9]. In BTC, the positive rate of PD-L1 expression is approximately 30%-50%. PD-L1 inhibitors can enhance chemosensitivity by activating $CD8^+$ T cells, strengthening tumor antigen presentation, and reversing the immunosuppressive tumor microenvironment, thereby exerting synergistic effects with chemotherapy[8-10]. Envafolimab, is a PD-L1 single-domain antibody independently developed in China. It offers the advantages of subcutaneous administration, high stability, and low immunogenicity. In 2021, it was approved in China for the treatment of MSI-H/dMMR advanced solid tumors, becoming the first domestically produced PD-L1 inhibitor to receive approval[11]. Collectively, these findings provide a theoretical rationale for the use of GC chemotherapy plus Envafolimab in the high-risk patients.

In clinical, the ZSAB-neoGOLP trial indicated that in patients with intrahepatic cholangiocarcinoma at high risk of recurrence, neoadjuvant therapy with GC chemotherapy regimen combined with immunotherapy and targeted therapy led to a significantly longer median event-free survival (EFS) than the control group (surgery alone): 18.0 months (95% confidence interval [CI]: 13.8-27.6) vs. 8.7 months (95% CI: 7.2-12.4), with a statistically significant difference ($p<0.001$). However, in the neoadjuvant group (four-drug combination), the incidence of adverse events (AEs) during the entire treatment period was 97%, and the rate of grade 3 or higher AEs during the neoadjuvant phase was 28%[12]. Therefore, we propose the following hypothesis: substituting immunotherapy alone for the combination of immunotherapy and targeted therapy, when combined with GC chemotherapy, may achieve comparable oncological efficacy while reducing adverse reactions associated with multidrug regimens.

3. Study Objectives and Endpoints

3.1 Primary objective

To evaluate the efficacy of GC chemotherapy plus Envafolimab neoadjuvant therapy in the patients at high risk of recurrence. Primary endpoint: Major Pathologic Response (MPR).

3.2 Secondary objectives

3.2.1 To evaluate the efficacy of GC chemotherapy plus Envafolimab neoadjuvant therapy in the patients at high risk of recurrence. Secondary endpoints: Postoperative resected specimens are analyzed via pathological section to determine the residual viable tumor cell ratio, Overall Survival (OS), Disease-free survival (DFS), Objective Response Rate (ORR) per the Response Evaluation Criteria In Solid Tumors (RECIST v1.1), Pathologic Response Rate (PCR), and R0 resection rate.
3.2.2 To evaluate the safety of GC chemotherapy plus Envafolimab neoadjuvant therapy in the patients at high-risk of recurrence. Safety endpoints: The incidence and severity of adverse events (AEs), serious adverse events (SAEs), and laboratory test abnormalities judged as per the CTCAE v5.0 criteria.

3.3 Exploratory objective

To evaluate the relationship between biomarkers, radiomics, and treatment efficacy. Exploratory endpoint: Relationship between carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), carbohydrate antigen 125 (CA125), other biomarkers expression, spatial transcriptome (if samples are sufficient), gut microbiome metagenome sequencing, and treatment response.

4. Study Design

4.1 Overall Design

This trial is a single-arm interventional clinical study to evaluate the efficacy and safety of neoadjuvant GC regimen plus Envafolimab in BTC patients at high-risk of recurrence.

With the primary endpoint set as the major pathological response (MPR) rate, this study adopts a Simon's two-stage design. An MPR rate of ≤5% is considered clinically insignificant, and neoadjuvant therapy is expected to elevate the MPR rate to ≥20%. The type I error rate is set at 5% with a statistical power of 80%, which is sufficient to evaluate the efficacy of the investigational regimen. According to statistical calculation, 16 patients will be enrolled in the first stage. If at least 2 out of these 16 patients achieve MPR, an additional 14 patients will be recruited, resulting in a total sample size of 30 cases. The null hypothesis will be rejected if a cumulative number of at least 4 MPR events are observed among the 30 patients. Considering an approximate 10% loss to follow-up and dropout rate during the study period, the planned total sample size is determined as 34 patients.

Study Period: 48 months (from May 2026 to May 2030), among which the first 2 years are the recruitment period and the remaining 2 years are the follow-up period.

4.2.Study procedure

The study includes the following processes: screening visit, neoadjuvant therapy-period visit (every 3 weeks ± 3 days), follow-up visit (every 3 months ± 15 days up to 2 years).

4.2.1 Screening visit (within 7 days prior to the first dose of treatment)

Within 7 days before the first neoadjuvant therapy dose, all participants must complete baseline screening, including tumor core needle biopsy for BTC pathological confirmation and gut microbiome metagenomic sequencing. Relevant examinations completed before informed consent may be omitted if obtained within the 7-day screening window and deemed acceptable by the investigator per protocol.

4.2.2 Neoadjuvant therapy-period visit (every 3 weeks ± 3 days)

Participants in the trial will first receive neoadjuvant therapy before surgery. The neoadjuvant regimen is GC chemotherapy plus Envafolimab, for 3 cycles. During neoadjuvant therapy, safety visits will be conducted on D1 and D8 every 3 weeks ± 3 days, and efficacy visits (imaging examination at the investigator's discretion) will be performed. Participants will discontinue treatment if they have disease progression (ineligible for radical surgery), intolerable toxicity, inoperability, or initiate new anti-tumor therapy, and proceed to follow-up until consent withdrawal, loss to follow-up, death, or the end of the 2-year follow-up period (whichever comes first).

A safety follow-up and imaging examination will be performed 2-4 weeks after neoadjuvant therapy. After 3 cycles of neoadjuvant therapy, pre-operative evaluation will be re-conducted. Eligible participants will undergo radical resection by an experienced hepatic surgeon. If disease progresses or intolerable toxicity occurs during treatment, the investigator will decide whether to perform urgent surgery or recommend alternative therapies. Participants ineligible for resection after neoadjuvant therapy will have subsequent treatment determined by the investigator. Radical resection eligibility will be assessed by the investigator; those with indeterminate resectability require additional MDT discussion confirmation. The criteria for radical resection:

a. No invasion of the main trunk of the hepatic vein, portal vein, or inferior vena cava;

b. No distant metastasis except for hilar lymph node metastases;

c. Minimum distance between surgical margin and tumor border ≥ 0.5 cm;

d. Residual liver volume ≥ 30% (≥ 40% for participants with cirrhosis).

4.2.3 Follow-up visit

Postoperative adjuvant therapy is determined based on pathological findings and the patient's general condition. The referenceable plan is as follows: 6-8 cycles of the original GC regimen, or 8 cycles of capecitabine (1250 mg/m² twice daily). PD-L1 inhibitor (Envafolimab) use is decided on an individual basis, and investigators may individualize postoperative adjuvant regimens. Safety follow-up and imaging assessments (abdominal contrast-enhanced CT/MRI, chest CT) are performed every 3 months (±15 days); additional imaging is conducted as clinically needed. Feces samples are also collected.

Participants who discontinue treatment (excluding postoperative recurrence, new anti-tumor therapy initiation, consent withdrawal, loss to follow-up, or death) will undergo imaging follow-up every 3 months ± 15 days until the first occurrence of these events or study end. For participants with postoperative recurrence who started new anti-tumor therapy, survival information will be collected every 3 months ± 15 days (via phone or other means) until consent withdrawal, loss to follow-up, death, or study end, whichever comes first. The study period is 2 years after initial treatment; mandatory routine follow-up will stop after this period.

4.3 Data and Safety Monitoring Board

An Data and Safety Monitoring Board (DSMB) will be established for this study to regularly review the clinical trial data accumulated in this study, to protect the safety of participants and safeguard the reliability of the trial and the validity of the trial results. The DSMB will review the efficacy analysis data and provided recommendations to the investigator.

5. Study Population

5.1 Inclusion Criteria

1) Participants who have signed a written Informed Consent Form (ICF);
2) Male or female participants aged 18-80 years;
3) Eastern Cooperative Oncology Group performance status (ECOG PS) score of 0/1;
4) BTC diagnosed by puncture pathology before enrollment;
5) Participants must meet the following requirements for major vital organ function: a. Absolute neutrophil count (ANC) ≥ 1.5×10^9/L; platelet count ≥ 90×10^9/L; hemoglobin ≥ 9 g/dL; b. Coagulation function: international normalized ratio (INR) ≤ 1.2; c. Alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 3 times the upper limit of normal (ULN); d. Serum albumin ≥ 3.5 g/dL; total bilirubin ≤ 1.5 times the ULN. Patients with obstructive jaundice who meet the eligibility criteria after percutaneous transhepatic cholangial drainage or endoscopic retrograde cholangiopancreatography treatment are also eligible for enrollment; e. Child-Pugh class A or B; f. Serum creatinine ≤ 1.5 times the ULN.
6) Patients with high-risk factors for recurrence. The criteria were defined as meeting at least one of the following: a. Preoperative CA19-9 ≥ 200 U/mL; b. Tumor diameter ≥5 cm or multiple tumor nodules on imaging; c. Regional lymph node metastasis with a short-axis diameter ≥ 1.0 cm on imaging; d. Vascular invasion (portal vein or hepatic artery) on imaging; e. Low or undifferentiated histologic grade.
7) Participants who have at least 1 measurable lesion (RECIST v1.1).
8) Be able to provide fresh stool samples and liver samples if undergoing surgery.

5.2 Exclusion Criteria

1) Pathological diagnosis of hepatocellular carcinoma, mixed hepatocellular carcinoma, and other non-cholangiocarcinoma malignant tumor components;
2) Prior systemic therapy for BTC, including immunotherapy, targeted therapy, or chemotherapy;
3) History of other prior or concurrent malignancies, except those with complete treatment and disease-free survival for more than 5 years;
4) Presence of an active, known or suspected autoimmune disease, or requirement for long-term systemic corticosteroid therapy (equivalent to >10 mg prednisone daily) or other immunosuppressive agents. Participants using inhaled or topical corticosteroids will not be excluded;
5) Presence of ascites, hepatic encephalopathy, sclerosing cholangitis, or other concurrent organ dysfunctions that would preclude tolerance of general anesthesia or hepatectomy;
6) Women who are breastfeeding or pregnant;
7) Any other factors that, in the investigator’s judgment, may compromise participant safety or trial compliance, including serious comorbidities requiring ongoing treatment, clinically significant

laboratory abnormalities, or relevant social or family-related issues.

5.3 Criteria for Discontinuation of Treatment

Discontinuation of trial intervention does not imply termination of the study and subsequent study procedures should be completed under the protocol. Participants must discontinue the trial intervention if they meet any of the following criteria:
1) Development of treatment-related adverse reactions meeting the discontinuation criteria;
2) Disease progression confirmed by imaging assessment;
3) Occurrence of comorbidities, complications, worsening conditions, AEs or SAEs that, in the investigator's judgment, render the participant unsuitable for continued treatment;
4) Administration of protocol-prohibited concomitant medications that substantially impair the evaluation of safety and efficacy (as determined by the investigator);
5) Voluntary withdrawal by the participant or premature discontinuation by the investigator;
6) Inadequate compliance that significantly compromises assessment of the primary endpoints;
7) Pregnancy in female participants;
8) Other circumstances in which the investigator deems treatment discontinuation necessary.

5.4 Criteria and Management after Treatment Withdrawal

1) Participants may voluntarily withdraw from the study at any time, with or without reason. Withdrawn participants will face no discrimination or retaliation, with their medical and legal rights fully preserved. All withdrawal reasons shall be documented in medical records and the eCRF. Withdrawal criteria include but are not limited to: a. Revocation of informed consent or refusal of further follow-up; b. Loss to follow-up: participants missing scheduled visits who cannot be contacted after three documented attempts within one month; c. All-cause death.
2) The investigator shall record reasons for treatment discontinuation in the eCRF and attempt to complete end-of-treatment assessments. Participants discontinuing neoadjuvant therapy will receive continuous survival follow-up for up to 2 years, or until consent withdrawal, loss to follow-up, or death. Ongoing AEs/SAEs related to the study drug will be monitored until resolution, stabilization, loss to follow-up, or initiation of alternative antitumor therapy.

6. Trial Intervention

6.1 Investigation

All investigational products will be labeled per GCP requirements and shipped as study supplies. Labels will be standardized, including protocol number, participant ID, product name, dosage, strength, storage conditions, batch number, expiry date, and collaborator information, with complete records maintained in the Trial Master File. Provided by the collaborator and distributed to sites as scheduled, the products will be overseen by the investigator to ensure administration only to trial participants per protocol, with no transfer to non-trial parties and unused products returned to the collaborator. The investigator will assign a responsible individual for product storage, dispensing, return, and documentation (quantity, shipping, receipt, dispensing, return). All products must be stored in a locked facility with temperature monitoring and regular recording by the drug manager. The monitor will oversee product supply, use,

storage, and disposal. Post-trial, products will be promptly reconciled (monitored and investigator-signed); used products may be destroyed by the institution, and unused ones returned to the collaborator for disposal.

The detailed schedule for each cycle is as follows: 1) Envafolimab: 400 mg on Day 1, repeated every 3 weeks (Q3W). 2) GC chemotherapy: Gemcitabine: 1000 mg/m^2 in 100 mL of 0.9% sodium chloride injection, administered intravenously over 30 minutes on Days 1 and 8, repeated Q3W. Cisplatin: 25 mg/m^2 in 500 mL of 5% glucose injection, administered intravenously over 2 hours on Days 1 and 8, repeated Q3W. A total of 3 cycles will be administered.

Drug interruption or dose reduction is allowed for drug-related adverse reactions, with dose adjustments as needed; dose modification rules for such reactions are as follows. Overdose is defined as accidental or intentional administration of the investigational product exceeding the protocol-specified dose. In case of overdose, the investigator must immediately notify the collaborator, closely monitor the participant for adverse events (AEs), and document the overdose, related AEs, and treatments in the participant’s medical records. Overdose itself is not an AE but must be recorded in the eCRF; AEs or SAEs resulting from overdose shall be managed and reported per standard AE/SAE principles.

6.2 Blinding and Unblinding

This is a single-arm trial and the blind method is not applicable.

6.3 Concomitant Medication/Therapy

1) Concomitant Medications/Therapies: All trial interventions must be documented in the eCRF. Participants shall report any off-site use of investigational products with accurate dosage records. All non-trial medications and therapies administered during the study should also be recorded in the eCRF.
2) Permitted: Essential supportive care and necessary safety treatments are allowed. All adverse events will be closely monitored and promptly managed, with relevant medications documented in the eCRF.
3) Prohibited: Antitumor therapies other than the study drug, participation in other interventional trials, or additional investigational agents are prohibited. Systemic corticosteroids and immunosuppressants are forbidden prior to Envafolimab initiation, except for immune-related AEs arising after treatment.

Table 2 Dose Modification Rules Based on Adverse Drug Reactions

Name of AE	Grade 2	Grade 3	Grade 4
Neurotoxicity	No dose modification indicated	Cisplatin reduced to 60mg/m^2 if AE persists >7d; Cisplatin discontinued if AE persists during the chemotherapy cycle; if the symptoms are improved after discontinuation of Cisplatin, Cisplatin 75 mg/m^2 can be continued	Cisplatin discontinued
Neutropenia	No dose modification indicated	Chemotherapy suspended until ANC>1.5×10^9/L, gemcitabine reduced to 80%	Gemcitabine discontinued, chemotherapy suspended until ANC>1.5×10^9/L, and Cisplatin reduced to 60 mg/m^2
Thrombocytopenia	Chemotherapy suspended until PLT>75×10^9/ L	Chemotherapy suspended until PLT>75×10^9/L, gemcitabine reduced to 80%, and Cisplatin to 60 mg/m^2	Gemcitabine and Cisplatin discontinued
Renal insufficiency	If the symptoms are resolved to Grades 0-1 or baseline after discontinuation of Envafolimab, Envafolimab can be continued	Envafolimab suspended first and continued if the symptoms are resolved to Grades 0-1 or baseline; if the symptoms are not resolved to Grades 0-1 or baseline after discontinuation of Envafolimab.	Envafolimab discontinued
Hepatotoxicity	If the symptoms are resolved to Grades 0-1 or baseline after discontinuation of Envafolimab, Envafolimab can be continued	Envafolimab suspended first and continued if the symptoms are resolved to Grades 0-1 or baseline; if the symptoms are not resolved to Grades 0-1 or baseline after discontinuation of Envafolimab	Envafolimab discontinued
Diarrhea	If the symptoms are resolved to Grades 0-1 or baseline after suspension of Envafolimab, Envafolimab can be continued	Envafolimab suspended first and continued if the symptoms are resolved to Grades 0-1 or baseline; if the symptoms are not resolved to Grades 0-1 or baseline after discontinuation of Envafolimab; Cisplatin should be discontinued if the symptoms are not resolved to Grades 0-1 or baseline; if the symptoms are improved, Cisplatin can be continued at 60 mg/m^2	Cisplatin and Envafolimab discontinued
Pneumonitis	Envafolimab suspended until improvement to Grades 0-1	Envafolimab discontinued	Envafolimab discontinued
Endocrine disorders	Envafolimab suspended until improvement to Grades 0-1	Envafolimab suspended first and continued if the symptoms are resolved to Grades 0-1 or baseline; for hypothyroidism, if the symptoms are not resolved to Grades 0-1 after suspension of Envafolimab	Envafolimab discontinued;
Skin adverse reactions	No dose modification indicated	Envafolimab suspended first and continued if the symptoms are resolved to Grades 0-1	Envafolimab discontinued
Infusion reactions	Envafolimab dripped at a slower speed or suspended, and infusion can be resumed with close observation after the symptoms are relieved.	Envafolimab must be permanently stopped immediately, with symptomatic treatment given	Envafolimab must be permanently stopped immediately with symptomatic treatment given
Myocarditis	Envafolimab discontinued	Envafolimab discontinued	Envafolimab discontinued
Pancreatitis	If the symptoms are resolved to Grades 0-1 after discontinuation of Envafolimab, Envafolimab can be continued	If the symptoms are resolved to Grades 0-1 after discontinuation of Envafolimab, Envafolimab can be continued	Envafolimab discontinued
Adverse reactions are graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.			

7. End and Termination of Study

7.1.End of Study

End of study (EOS) is defined as the completion of the last protocol-specified visit by the last participant.

7.2 Criteria for Early Termination of Study

Reasons for early study termination include, but are not limited to:
1) Termination during neoadjuvant therapy if disease progression precludes surgical opportunity, drug intolerance occurs, or the mortality rate exceeds 20%.
2) Identification of major flaws in the trial protocol that compromise the validity of study drug evaluation.
3) Discovery of unexpected or significant safety risks to participants.
4) Termination by the investigator for scientific, medical, or ethical reasons, with priority given to participants' rights, safety, and well-being.
5) Any other circumstances in which the investigator deems continuation of the trial inappropriate.

8. Study Procedures

8.1.Screening Visit

Eligible participants will be screened, with ICF obtained before screening. Each screened participant will be assigned a unique screening number, which will not be reused for withdrawn participants. The screening window is within 7 days before the first treatment, and all screening assessments must be completed before enrollment. Valid tests performed before ICF signing, if within the protocol-specified time window and approved by the investigator, may be used without repetition. Participants meeting all inclusion criteria and no exclusion criteria may be enrolled.
1) Inclusion/exclusion criteria;
2) Demographics: including age, gender, date of birth, and ethnicity.
3) Past medical history: During screening, tumor history (diagnosis date, results, initial and screening clinical stage), other medical/treatment history, pre-ICF pre-existing conditions (as concurrent illnesses), 28-day pre-intervention treatments (prescription/over-the-counter drugs, Chinese herbal medicines, other procedures), allergies and drug dependence will be documented.
4) Physical examination: It is mainly performed to evaluate the major body systems;
5) ECOG scoring;
6) Child-Pugh classification;
7) AJCC staging;
8) 12-lead electrocardiogram (ECG);
9) Pulmonary test;
10) Cardiac ultrasound;
11) Laboratory tests: hematology, urinalysis, stool routine, liver and kidney function, coagulation, myocardial enzyme spectrum, thyroid function, pregnancy test (if clinically indicated);
12) Detection of tumor markers: including AFP, CEA, CA19-9, and CA125;

13) Pathological test: biomarker (e.g., PD-L1) expression;
14) Imaging examination: If imaging was performed before providing informed consent for this study and within 7 days prior to the first treatment dose, repeat imaging is not required. Color Doppler ultrasound/contrast-enhanced ultrasound will be performed at the investigator's discretion based on clinical circumstances.

8.2 Neoadjuvant therapy-period visit (every 3 weeks ± 3 days)

For neoadjuvant therapy-period visits, relevant examinations will be performed on D1 and D8 as follows:
1) Physical examination;
2) ECOG scoring;
3) Laboratory tests: hematology, liver and kidney function, coagulation, myocardial enzyme spectrum (only on D1 for each cycle), thyroid function (only on D1 for each cycle);
4) Detection of tumor markers: including AFP, CEA, CA19-9, and CA125 (only on D1 for each cycle);
5) Imaging examination will be decided by the investigator according to the situation and performed only on D1 for each cycle such Color Doppler ultrasonography; Contrast-enhanced abdomen CT/MRI; PET-CT/low-dose PET-CT.
6) Concomitant medications;
7) Adverse events;
8) Survival.

8.3 Follow-up visit

Postoperative adjuvant therapy will be determined based on pathological findings and the patient's general condition. Safety follow-up and imaging assessments will be performed every 3 months (±15 days), including contrast-enhanced abdominal CT/MRI and chest CT. Additional imaging may be performed at the investigator's discretion if clinically indicated.

8.4 Efficacy Evaluation

Primary efficacy evaluation variable
MPR rate will be pathologically evaluated using surgical specimens. MPR is defined as ≤10% residual viable tumor cells in the primary lesion and regional lymph nodes following neoadjuvant therapy, with Pathologic Response Rate (PCR, 0% viable tumor cells) included in the MPR definition. Specimen sampling and sectioning will follow standardized procedures, and assessments will be conducted by specialized pathologists; blinded independent pathological review will be performed as needed.
Secondary efficacy evaluation variables:
1) Postoperative resected specimens are analyzed via pathological section to determine the residual viable tumor cell ratio.
2) Overall Survival (OS): OS is defined as the time from participant receiving the first treatment until death from any cause.
3) Disease-free survival (DFS): DFS is defined as the time from the date of surgery to neoplasm recurrence or death for participants without residual lesions after surgery, whichever occurs first.
4) Objective Response Rate (ORR): ORR is defined as the sum of Complete Response (CR) and Partial Response (PR), representing the proportion of participants with sustained tumor regression meeting

predefined criteria.
5) Pathologic Response Rate (PCR): No viable tumor cells are found in the review of pathological sections.
6) R0 resection: R0 resection rate refers to the proportion of patients achieving complete surgical resection with negative margins.

8.5 Safety Evaluation

All consenting participants will undergo safety assessments, including physical examination, vital signs, hematology, biochemistry, coagulation, urine and stool tests, 12-lead ECG, ECOG status and thyroid function, as well as monitoring of AEs, SAEs and procedure-related adverse events. Assessments will continue from consent signature through study completion. All investigational product-related AEs/SAEs will be followed until resolution, stabilization, participant withdrawal, loss to follow-up or death. AE severity will be graded per CTCAE v5.0.

8.6 Biomarker Evaluation

Correlations between these biomarkers (including CEA, CA19-9, CA125, spatial transcriptome, and gut microbiome metagenomic sequencing) and overall treatment efficacy, as well as subsequent basic research, are permitted.

9. Adverse Events and Serious Adverse Events

9.1 Definitions

9.1.1 Adverse event
An AE is any untoward medical occurrence in a participant administered an investigational product, without necessarily having a causal relationship to treatment. Operation-related AEs are events caused by surgical complications resulting in hospitalization, prolonged hospitalization, disability, life-threatening conditions, or death.
9.1.2 Events Meeting the AE Definition
1) Clinically significant abnormal laboratory or safety assessments, including worsening from baseline.
2) Exacerbation of pre-existing chronic or intermittent conditions.
3) Newly diagnosed conditions arising after study intervention initiation.
4) Signs or sequelae of suspected drug-drug interactions.
5) Signs or sequelae of suspected overdose; intentional overdose with suicidal/self-harm intent must be reported regardless of sequelae.
6) Progressive disease (PD), as a study endpoint, is not reported as an AE/SAE.
9.1.3 Events Not Meeting the AE Definition
1) Expected progression or baseline manifestations of the studied disease, unless unexpectedly severe.
2) Medical/surgical procedures unrelated to the study disease.
3) Hospitalization for social or elective non-medical reasons.
4) Non-worsening fluctuations of pre-existing conditions.
5) Pre-existing conditions documented as medical history.

9.2 Adverse Drug Reaction (ADR)

An ADR is a noxious and unintended response to a drug at normal therapeutic doses. For investigational products with undefined therapeutic doses, any harmful, unexpected reaction causally related to the product is considered an ADR.

9.3 Serious Adverse Events

A SAE is any untoward medical occurrence regardless of causality that:
1) Results in death (excluding tumor progression-related death).
2) Is life-threatening (imminent risk of death at the time of the event).
3) Requires or prolongs hospitalization (not for elective or non-medical reasons).
4) Causes persistent or significant disability/incapacity.
5) Results in a congenital anomaly or birth defect.
6) Is medically significant (requires intervention to prevent serious outcomes).

9.4 Suspected Unexpected Serious Adverse Reactions (SUSAR)

A SUSAR is a serious adverse reaction whose nature, severity, or frequency is inconsistent with the known safety profile of the investigational product

9.5 AE Severity Grading Criteria

AE and SAE severity will be graded by the investigator in accordance with CTCAE v5.0, which includes general severity criteria followed by event-specific grading. General severity scale:
Grade 1: Mild-asymptomatic or mild symptoms; observation only; no treatment required.
Grade 2: Moderate-requiring minimal or non-invasive intervention; limits instrumental activities of daily living (ADL).
Grade 3: Severe or medically significant but not immediately life-threatening; requires or prolongs hospitalization; disabling; limits self-care ADL.
Grade 4: Life-threatening; requires urgent intervention.
Grade 5: Fatal.
Note: Instrumental ADL: meal preparation, shopping, telephone use, financial management, etc. Self-care ADL: bathing, dressing, feeding, hygiene, medication intake, and ambulation. AE seriousness and severity are distinct. A severe-grade AE is not automatically an SAE. For example, severe headache does not qualify as an SAE unless it meets SAE criteria.

9.6 Assessment of Relationship between Adverse Events and Investigational Products

Causality Assessment of Adverse Events:
Definitely related: Event occurs temporally after dosing, consistent with known reactions, improves upon drug withdrawal and recurs on rechallenge, and cannot be explained by the patient's condition or other therapies.
Possibly related: Event shows temporal association and consistency with known product reactions, but may also be attributed to the patient's condition or concomitant treatments.
Unrelated/Unlikely related: Event lacks temporal association or consistency with known drug reactions,

and is likely explained by the patient's condition or other therapies.
Not assessable: Event has unclear temporal relationship, resembles known reactions, but may be confounded by concomitant medications.
Note: AEs assessed as definitely related, possibly related, or not assessable are classified as adverse drug reactions.

9.7 Disposal of AE and SAE

1) All AEs occurring during the study, regardless of causality, must be documented with onset date, resolution date, description, severity, causality, interventions, and outcome. AE/SAE collection starts at study treatment initiation and continues through study completion. Abnormalities between ICF signing and treatment start are recorded as concurrent conditions. AE collection ceases upon participant withdrawal or initiation of new antitumor therapy. For long-term follow-up, only treatment-related AEs are collected.
2) Treatment-related AEs are followed until resolution, stabilization, withdrawal, loss to follow-up, death, or new antitumor therapy. Treatment-related SAEs are followed until resolution, stabilization, withdrawal, loss to follow-up, or death. Medical events occurring after ICF signing but before treatment are documented in the medical history section of the eeCRF, not as AEs.
3) AEs are monitored until resolution or stabilization. Missing outcome data due to non-compliance is classified as loss to follow-up. Following initial reporting, the investigator must conduct active follow-up. Additional tests or consultations may be performed to clarify event characteristics and causality. New or updated information must be added to the eCRF.
4) To avoid bias, AEs/SAEs should be elicited via open-ended, non-leading inquiries by the investigator. All SAEs, regardless of causality, must be reported to the principal investigator and ethics committee within 24 hours of identification. Treatment-related SAEs also require reporting to the DSMB within 24 hours.

10. Statistical Considerations

10.1 General principles

Detailed statistical methods will be described in the Statistical Analysis Plan (SAP), finalized before database lock. The SAP will specify all analyses and result presentations and be retained by the investigator. Minor adjustments to protocol-related items are permitted; however, major revisions to primary endpoint definitions and analyses require a formal protocol amendment.

10.2 Analyzed Population

Analyses will be performed in the Full Analysis Set (FAS), defined as all enrolled participants who received at least one dose of study treatment. The MPR rate, as the primary endpoint, will be calculated with the 95% confidence interval estimated via the Clopper-Pearson method. The Per-Protocol Set (PPS) includes participants who met key eligibility criteria, had no major protocol deviations, completed the minimum required treatment dose, and underwent at least one efficacy assessment. MPR rate and corresponding 95% confidence intervals (CIs) will also be analyzed in the PPS, and consistency between FAS and PPS results will be compared to verify the stability of primary findings. Patients who fail to

undergo surgical resection after neoadjuvant treatment, without available postoperative pathological specimens for MPR assessment, will still be included in the Full Analysis Set (FAS). Missing primary endpoint data will be handled by non-responder imputation, and such patients will be regarded as not achieving MPR. Time-to-event outcomes, including disease-free survival and overall survival, will be estimated using the Kaplan-Meier method, with median survival and 95% CIs presented. Safety analyses will be conducted in the Safety Set, covering all participants exposed to at least one study treatment. The number and proportion of participants with treatment-emergent adverse events, study drug-related adverse events, and serious adverse events will be summarized.

10.3 Statistical Analyses

1) MRP: The primary analysis of the MPR will be performed based on the FAS and PPS. The number and percentage of participants achieving MPR will be summarized and the 95% CI will be calculated via the Clopper-Pearson method. ORR: Analysis will include CR and PR per RECIST v1.1 criteria, and the analysis of the ORR is the same as the analysis of MPR. R0 resection: R0 resection rate is defined as the percentage of participants who achieve complete resection with negative surgical margins. Participants without surgery or pathological assessment are considered to have failed to achieve R0 resection and included in the denominator for calculation. Its analytical method is consistent with that of MPR. DFS: DFS is defined as the time from the date of surgery to neoplasm recurrence or death for participants without residual lesions after surgery, whichever occurs first. OS: OS is defined as the time from the date of receiving the first treatment to death from any cause.
2) Subgroup analysis:
No subgroup analysis is designed.
3) Interim analysis:
The complete result analysis of the first phase is defined as the interim analysis.

10.4 Safety analysis

Safety assessments include adverse events, vital signs, physical examination, 12-lead ECG, and clinical laboratory tests. All treatment-emergent adverse events (TEAEs) will be summarized by system organ class and preferred term, with their incidence, severity, and causality tabulated. Laboratory parameters and vital signs will be described using descriptive statistics. Clinically significant abnormalities will be summarized in tables.

10.5 Exploratory analysis

The analysis of exploratory endpoints (biomarkers) is not included in this SAP.

11. Data Management

11.1 Database Establishment

This study uses an eCRF for remote data entry. The data manager designs and validates the database for accuracy. Only investigators and authorized site personnel may enter, correct, or modify study data in the eCRF; monitors are not permitted to enter data. All eCRF entries are automatically marked with the

creator’s unique user ID. Investigators must electronically sign to confirm data accuracy, ensuring data are true, complete, and properly filled. Corrections must be crossed out with the revised data, reason, investigator’s signature, and date documented; original records must not be erased or overwritten. A full audit trail is maintained for all entries and changes. The eCRF is not a source document unless otherwise specified. Monitors verify eCRF integrity and consistency against source data. Data are reviewed per the Data Management Plan (DMP); queries are issued for inconsistent, invalid, or missing data. Quality control is applied throughout data processing, and the DMP defines roles and responsibilities for all parties.

11.2 Data Verification

Data verification includes logic checks, manual verification, medical verification, and statistical pre-analysis. Queries will be sent electronically via the Electronic Data Capture (EDC) system for site response. Queries meeting requirements will be closed; unresolved queries or new queries from data updates will require re-response by the investigator or Clinical Research Coordinator (CRC), repeating until all data are confirmed.

11.3 Database Lock

All source data shall be entered into the Epidata database, then reviewed by the PI, statistician, and data manager to confirm no unresolved issues. All parties will sign the database lock request form, and the data manager will lock the database. Post-lock, the data manager will export the analysis database to the statistician; locked data are non-editable. Non-critical data issues identified post-lock will be corrected and documented in the statistical analysis program upon confirmation. Unlocking post-lock requires approval from the investigational product sponsor if justified by definite evidence.

12. General Considerations: Regulatory, Ethical, and Study Oversight

12.1 Regulatory and Ethical Considerations

The investigator ensures this study is conducted in accordance with the Declaration of Helsinki to maximize protection of participants. The study will strictly comply with the ethical guidelines for human medical research under the Declaration of Helsinki and relevant Chinese clinical trial regulations. Prior to study initiation, the protocol and participant ICF must be approved by the site’s Ethics Committee (EC). The EC shall provide written approval comments to the investigator, who shall submit the EC’s approval documents, as well as the EC member list and their qualifications, to collaborators. The investigator shall report all SAEs or deaths occurring during the study to the EC. During the study, the investigator shall submit study progress reports to the EC as required; after study completion, a written final report shall be submitted to the EC.

12.2 Informed Consent

In accordance with applicable local laws and regulations, signed and dated ICFs must be obtained from participants prior to initiation of any study-related procedures. Written informed consent, signed and

dated by each participant in duplicate, shall be acquired before study enrollment. One copy will be retained by the participant, and the original will be filed by the investigator as part of the study records. No study-specific assessments or procedures may be performed by the investigator prior to obtaining written informed consent. The content of consent and the time of consent acquisition must be properly documented, and the ICF must be stored in the investigator site file. Original signed ICFs will be maintained by the investigator as essential study documents, and the date of ICF signature will be recorded in the EDC system. In the event of protocol amendments, the ICF may require corresponding revision to reflect updated content. Any revised ICF must be submitted to the Independent EC for written approval, and signature confirmation must be obtained from both newly enrolled and ongoing participants.

12.3 Protocol Approval and Retention

1) Retention of Study Materials: The investigator shall properly keep all study-related records, including inpatient medical records and original clinical trial documents. The electronic eCRF backup discs shall be preserved as required.
2) Protocol Approval and Amendments: In line with local regulations, the study protocol and relevant documents must be approved by the Institutional Review Board (IRB) and competent authorities before study initiation. The sponsor shall ensure all ethical and regulatory requirements are met prior to enrolling the first participant.

12.4 Confidentiality

1) All study-related findings and documents are strictly confidential and shall not be disclosed by the investigator or study team without the collaborators' written consent.
2) All identifiable information of participants must be pseudonymized. In the EDC system and documents submitted to the sponsor or relevant parties, participants are identified only by patient identification, initials, and/or date of birth, with no full names included.
3) Documents containing participants' personal identification information (e.g., signed ICFs) shall be kept confidential by the investigator.
4) The sponsor may publicize, publish, or report study-related information/data to drug regulators. Any other individual or entity wishing to publicize or publish study results/data must obtain prior consent from the Principal Investigator (PI) and collaborators. If the investigator's name needs to be included in any publicity, publication, or advertisement, the collaborators must first obtain the investigator's consent.

13. References

1. Valle JW, Kelley RK, Nervi B, et al. Biliary tract cancer. Lancet. 2021 Jan 30;397(10272):428-444. .

2. Zhao ZF, Wu HY, Han J, et al. Global trends and disparities in gallbladder and biliary tract cancers: insights from the global burden of disease study 2021. Eur J Gastroenterol Hepatol. 2025 May 1;37(5):573-584.

3. Wang YC, Xun ZY, Yang X, et al. Local-regional therapy combined with toripalimab and lenvatinib in patients with advanced biliary tract cancer. Am J Cancer Res. 2023 Mar 15;13(3):1026-1037.

4. von Minckwitz G, Huang CS, Man MS, et al. Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer. N Engl J Med. 2019 Feb 14;380(7):617-628.

5. Roy BL, Gelli M, Pittau G, et al. Neoadjuvant chemotherapy for initially unresectable intrahepatic cholangiocarcinoma. Br J Surg. 2018 Jun;105(7):839-847.

6. Oh DO, He AR, Qin SK, et al. Durvalumab plus Gemcitabine and Cisplatin in Advanced Biliary Tract Cancer. NEJM Evid. 2022 Aug;1(8):EVIDoa2200015.

7. Pereira RA, Barcellos G, Lenz G, et al. Neoadjuvant Chemotherapy in Resectable Biliary Tract Cancer: A Systematic Review and Metanalysis. J Surg Oncol. 2026 Mar;133(3):313-325.

8. Chen F, Sheng J, Li XP, et al. Tumor-associated macrophages: orchestrators of cholangiocarcinoma progression. Front Immunol. 2024 Sep 3:15:1451474.

9. Chen C, Chen Z, Chen D, Zhang B, Wang Z, Le H. Suppressive effects of gemcitabine plus cisplatin chemotherapy on regulatory T cells in non-small cell lung cancer. J Int Med Res. 2015;43(2):180-187.

10. Xue YY, Gao S, Gou JX, et al. Platinum-based chemotherapy in combination with PD-1/PD-L1 inhibitors: preclinical and clinical studies and mechanism of action. Expert Opin Drug Deliv. 2021 Feb;18(2):187-203.

11. Chen YW, Zhang JL, Hu WD, et al. Envafolimab plus lenvatinib and transcatheter arterial chemoembolization for unresectable hepatocellular carcinoma: a prospective, single-arm, phase II study. Signal Transduct Target Ther. 2024 Oct 9;9(1):280.

12. Shi GM, Huang XY, Liang F, et al. Neoadjuvant GOLP in Resectable High-Risk Intrahepatic Cholangiocarcinoma. N Engl J Med. 2026 Mar 5;394(10):983-995.