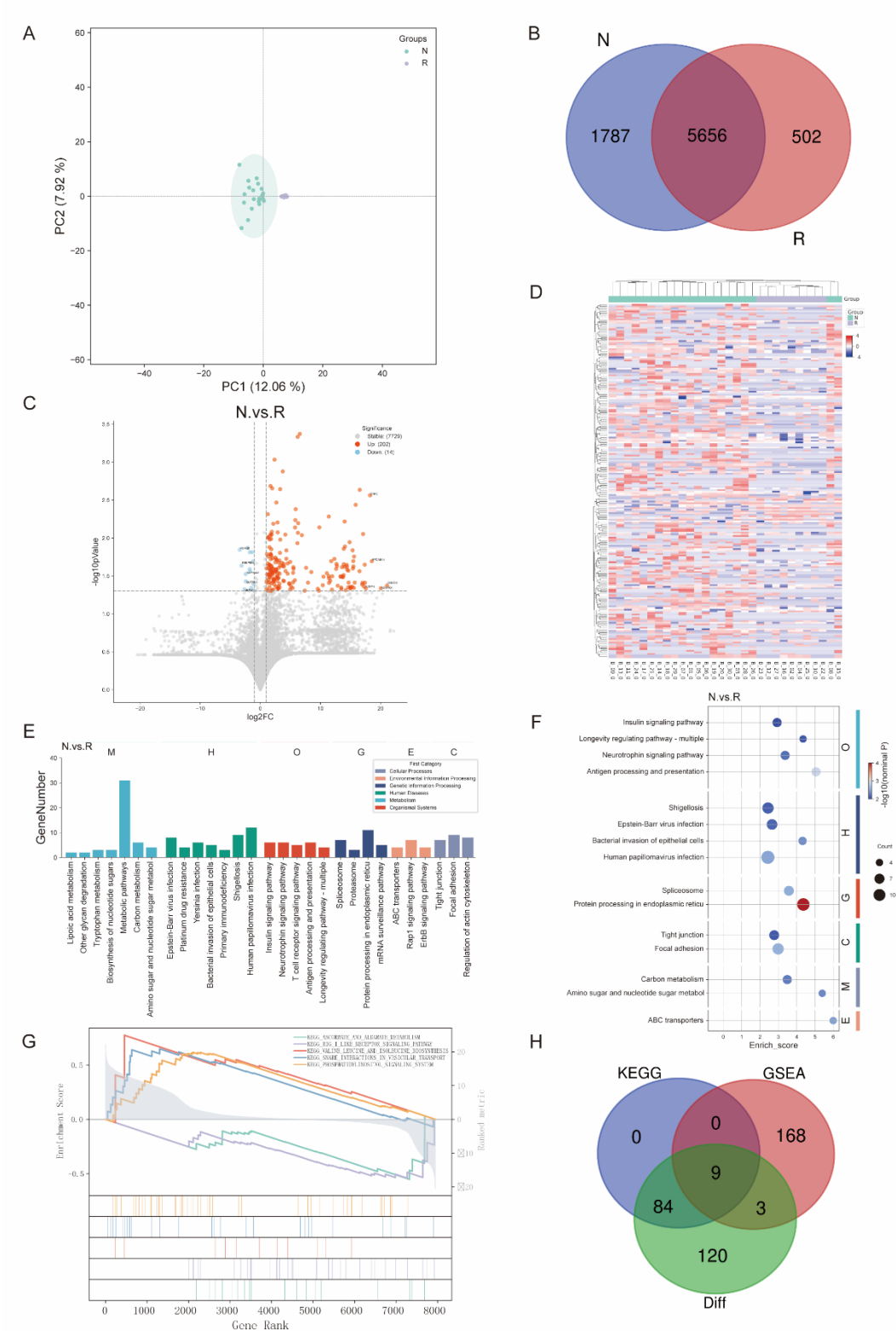
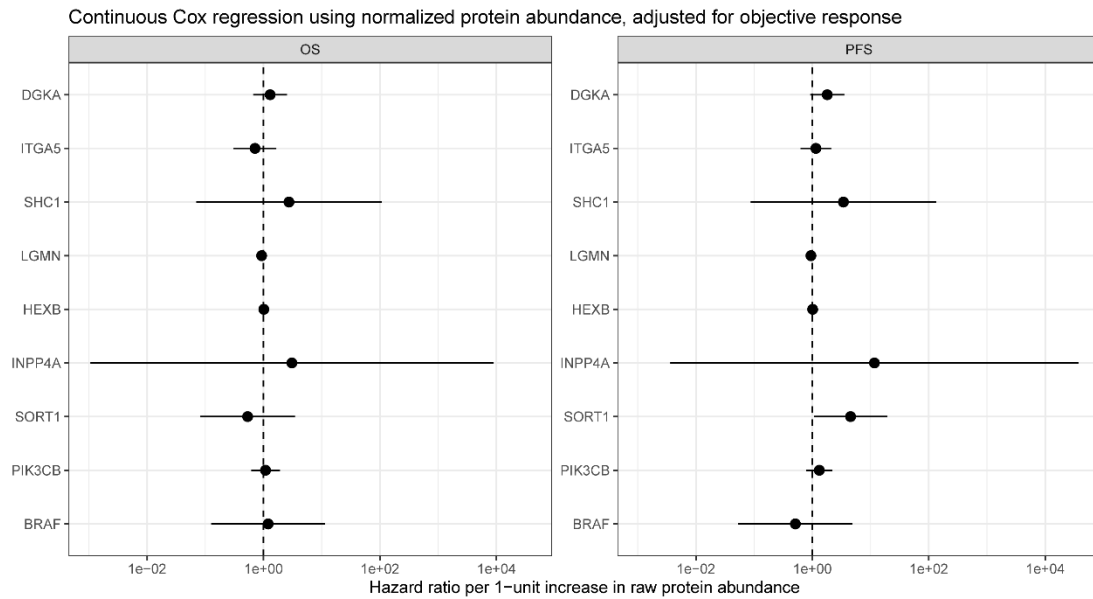




Supplementary Figure 1: Proteomic landscape and identification of exploratory candidate markers associated with treatment response. All panels are based on the same 30 pretreatment plasma samples from responders (R, n = 8) and non-responders (N, n = 22), unless otherwise indicated. (A) Principal component analysis (PCA) of proteomic profiles from responders (R, n = 8) and

non-responders (N, n = 22) in the BicareX trial. (B) Venn diagram showing overlapping and unique quantified proteins in responders and non-responders. (C) Volcano plot showing nominally differentially abundant proteins, defined using an unadjusted $P < 0.05$ threshold for exploratory visualization. (D) Hierarchical clustering heatmap of nominally differentially abundant proteins after Z-score normalization. (E, F) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of nominally differentially abundant proteins, shown by protein count (E) and enrichment score (F). (G) Gene set enrichment analysis (GSEA) of activated or suppressed KEGG pathways in responders. Only protein processing in the endoplasmic reticulum remained significant after false discovery rate (FDR) correction ($q = 0.01049$); all other KEGG and GSEA signals shown were nominal and exploratory, and no GSEA pathway passed FDR correction. (H) Integrated Venn diagram showing the intersection of nominally differentially abundant proteins and nominal pathway-level signals, identifying nine exploratory candidate proteins.



Supplementary Figure 2. Continuous Cox regression analysis of candidate proteins adjusted for objective response. Forest plots showing hazard ratios for overall survival (OS) and progression-free survival (PFS) from Cox proportional hazards regression models using continuous normalized protein abundance. Models were adjusted for objective response, defined as complete response (CR)/partial response (PR) (n = 8) versus stable disease (SD)/progressive disease (PD) (n = 22). HRs are shown per 1-unit increase in normalized protein abundance. Horizontal lines indicate 95% confidence intervals, and the vertical dashed line indicates HR = 1. No candidate protein remained significantly associated with OS or PFS after false discovery rate (FDR) correction.

Supplementary Table 1. HBV monitoring, antiviral prophylaxis, and causality adjudication strategy

Domain	Protocol / Criteria
Baseline HBV assessment	HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc, HBV DNA, and liver biochemistry were assessed before treatment initiation.
Definition of HBV exposure	HBsAg positivity and/or anti-HBc positivity.
Definition of chronic HBV infection	HBsAg positivity.
Definition of resolved HBV infection	HBsAg negativity with anti-HBc positivity.
HBV-DNA monitoring	HBV DNA was assessed at baseline in all enrolled patients. During treatment, HBV DNA was monitored every 3 months in patients with chronic HBV infection (HBsAg-positive) and in patients with resolved HBV infection (HBsAg-negative and anti-HBc-positive). Routine repeat HBV-DNA testing was not scheduled for patients without serological evidence of prior HBV exposure. Additional HBV-DNA testing was performed at any time in patients with unexplained ALT or AST elevation, bilirubin increase, hepatic decompensation, suspected hepatitis, or liver failure.
Liver function monitoring	Liver function tests were performed before each treatment cycle.
Antiviral prophylaxis policy	Patients with HBsAg positivity or detectable HBV DNA were recommended to receive prophylactic antiviral therapy with a high genetic barrier nucleos(t)ide analogue, including entecavir, tenofovir disoproxil fumarate, or tenofovir alafenamide, before or at the initiation of systemic anticancer therapy.
Management of resolved HBV infection	Patients with resolved HBV infection were managed with serial HBV-DNA monitoring. Pre-emptive antiviral therapy was initiated if HBV DNA became detectable or HBsAg seroreversion occurred.
HBV reactivation definition	HBV reactivation was defined as a ≥ 2 log ₁₀ IU/mL increase in HBV DNA from baseline or nadir, newly detectable HBV DNA in patients with previously undetectable HBV DNA, or HBsAg seroreversion in patients with resolved HBV infection.
HBV-associated hepatitis definition	HBV reactivation accompanied by ALT elevation or hepatic dysfunction after exclusion of other causes.

Domain	Protocol / Criteria
Differential diagnosis of hepatic injury	Immune-mediated hepatitis, HBV flare, biliary obstruction, cholangitis, intrahepatic tumour progression, portal vein tumour thrombosis, chemotherapy-associated hepatotoxicity, sepsis, ischaemic liver injury, and other drug-induced liver injury were considered.
Causality adjudication process	Clinically significant hepatic adverse events and liver-related deaths were reviewed by a multidisciplinary team including medical oncologists, hepatobiliary surgeons, and hepatologists.
Data used for adjudication	HBV serology, baseline and serial HBV-DNA results, antiviral treatment status, liver biochemistry pattern, imaging findings, biliary obstruction or cholangitis, tumour progression, vascular invasion, infection, concomitant hepatotoxic medications, temporal relationship to treatment, and response to treatment interruption, corticosteroids, antibiotics, biliary drainage, or antiviral therapy.
HBV reactivation outcome	No definite HBV reactivation was documented among HBsAg-positive patients receiving antiviral prophylaxis. No HBsAg-positive patient developed HBV DNA conversion from undetectable to detectable status during treatment.

Supplementary Table 2. Baseline HBV characteristics and reactivation outcomes

HBV-related variable	No. of patients / total	Percentage
Baseline HBV status		
Current or prior HBV infection	41/61	67.2
HBsAg-positive chronic HBV infection	10/61	16.4
Resolved HBV infection	31/61	50.8
No HBV exposure	20/61	32.8
Baseline HBV-DNA assessment		
Baseline HBV-DNA testing, overall	61/61	100
Baseline detectable HBV DNA	7/61	11.5
Baseline undetectable HBV DNA	54/61	88.5
Baseline HBV-DNA testing among HBsAg-positive patients	10/10	100
Baseline HBV-DNA testing among patients with resolved HBV infection	31/31	100
Baseline HBV-DNA testing among patients without prior HBV exposure	20/20	100
Antiviral treatment and on-treatment monitoring		
HBsAg-positive patients receiving antiviral therapy	10/10	100
HBsAg-positive patients with baseline HBV DNA <500 IU/mL	10/10	100
HBV reactivation outcomes		
HBV DNA conversion during treatment among HBsAg-positive patients	0/3	0
Definite HBV reactivation among HBsAg-positive patients	0/10	0
Newly detectable HBV DNA among patients with resolved HBV infection	0/31	0
HBsAg seroreversion among patients with resolved HBV infection	0/31	0
Definite HBV reactivation among patients with resolved HBV infection	0/31	0

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBs, hepatitis B surface antibody; HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody; anti-HBc, hepatitis B core antibody; HBV DNA, hepatitis B virus DNA.

Note: Percentages were calculated using the total population as the denominator unless otherwise specified. Current or prior HBV infection was defined as HBsAg positivity and/or anti-HBc positivity. Chronic HBV infection was defined as HBsAg positivity. Resolved HBV infection was defined as HBsAg negativity with anti-HBc positivity. All HBsAg-positive patients received antiviral therapy before or at the initiation of systemic anticancer treatment, and baseline HBV DNA levels were below 500 IU/mL in all HBsAg-positive patients. No HBsAg-positive patient developed HBV DNA conversion from undetectable to detectable status during treatment, and no definite HBV

reactivation event was documented among HBsAg-positive patients receiving antiviral prophylaxis

Supplementary Table 3. Treatment exposure, dose modification and supportive care interventions

Variable / intervention	Definition or notes	Value
Section: Treatment exposure		
Safety population	All enrolled patients who received at least one dose of cadonilimab, gemcitabine, or cisplatin	61
Median cadonilimab cycles, median (range)	Number of administered cadonilimab cycles	8 (1–34)
Median gemcitabine cycles, median (range)	Number of administered gemcitabine cycles	8 (1–8)
Median cisplatin cycles, median (range)	Number of administered cisplatin cycles	8 (1–8)
Completed 8 planned GC cycles, n (%)	Patients completing all planned gemcitabine/cisplatin combination cycles	34 (55.7%)
Entered cadonilimab maintenance, n (%)	Patients who continued cadonilimab monotherapy after GC cycles	30 (49.2%)
Median duration of maintenance, months (range)	Duration of cadonilimab maintenance therapy	4.2 (0.7–19.7)
Still on maintenance at data cutoff, n (%)	Patients still receiving cadonilimab maintenance on Nov 30, 2025	3 (4.9%)
Completed maintenance therapy	Completion of cadonilimab therapy for up to 24 months	2 (3.3%)
Section: Dose modification and discontinuation		
Any dose delay, n (%)	Delay of any study drug because of AE, scheduling issues, or clinical decision	10 (16.4%)
Cadonilimab interruption, n (%)	Temporary interruption of cadonilimab	6 (9.8%)
Gemcitabine dose reduction, n (%)	Dose reduction from protocol dose	12 (19.7%)
Cisplatin dose reduction, n (%)	Dose reduction from protocol dose	10 (16.4%)
Any chemotherapy dose reduction, n (%)	Dose reduction of gemcitabine and/or cisplatin	12 (19.7%)
Relative dose intensity of gemcitabine, median (IQR)	Administered dose intensity / planned dose intensity	0.95 (0.52–1.00)
Relative dose intensity of cisplatin, median (IQR)	Administered dose intensity / planned dose intensity	0.94 (0.55–1.00)
Permanent discontinuation due to AE, n (%)	Permanent discontinuation of any study treatment due to AE	4 (6.6%)
Discontinuation due to disease progression, n (%)	Study treatment stopped because of progression	45 (73.8%)
Discontinuation due to personal reasons, n (%)	Patient decision / non-medical reason	4 (6.6%)

Variable / intervention	Definition or notes	Value
Discontinuation due to death, n (%)	Death as the reason for study treatment discontinuation	3(4.9%)
Section: Supportive care interventions		
G-CSF, n (%)	For neutropaenia or febrile neutropaenia	27 (44.3%)
Red blood cell transfusion, n (%)	For anaemia	6 (9.8%)
Platelet transfusion, n (%)	For thrombocytopaenia	4 (6.6%)
Thrombopoietic agents, n (%)	TPO receptor agonist or other platelet-supportive agent	20 (32.8%)
Anti-infective therapy, n (%)	For infection, fever, cholangitis, etc.	7 (11.5%)
Biliary drainage or stent placement, n (%)	For biliary obstruction/cholangitis	3 (4.9%)
Hepatoprotective treatment, n (%)	For liver enzyme elevation or hepatic dysfunction	27 (44.3%)
Corticosteroids for irAE, n (%)	Systemic corticosteroids used to manage immune-related toxicity	11 (18.0%)

Data are presented as median (range) for treatment exposure variables, median (IQR) for relative dose intensity, or n (%) for categorical variables, as indicated. Percentages are based on the safety population (N = 61). Categories of dose modification and supportive care interventions were not mutually exclusive; patients could receive more than one intervention. Data cutoff was November 30, 2025. AE, adverse event; GC, gemcitabine plus cisplatin; G-CSF, granulocyte colony-stimulating factor; IQR, interquartile range; TPO, thrombopoietin.

Supplementary Table 4. Subsequent therapies after study discontinuation

Variable	No. of patients / total (Percentage)
Discontinued study treatment	56/61 (91.8%)
Received any subsequent anticancer therapy	33/56 (58.9%)
No subsequent antitumor therapy or no subsequent therapy recorded	23/56 (41.1%)
Targeted therapy for driver gene mutations (HER2, FGFR2)	4/56 (7.1%)
TKI-based therapy	20/56 (35.7%)
Immunotherapy-containing subsequent therapy	14/56 (25.0%)
Chemotherapy-containing subsequent therapy	6/56 (10.7%)
TACE/interventional therapy-containing subsequent therapy	6/56 (10.7%)
Radiotherapy/SBRT-containing subsequent therapy	7/56 (12.5%)
Traditional Chinese medicine alone	3/56 (5.4%)

TKI, tyrosine kinase inhibitor; TACE, transarterial chemoembolization; PD-1, programmed death 1; SBRT, stereotactic body radiotherapy.

Treatment categories were not mutually exclusive because some patients received multimodal or sequential therapies.

Supplementary Table 5. Subgroup analysis of ORR, DCR, PFS and OS

Subgroup	Level	N	ORR, n/N (%; 95% CI)	P	DCR, n/N (%; 95% CI)	P	Median PFS, months (95% CI)	P	Median OS, months (95% CI)	P
Overall	Overall	61	34/61 (55.7; 42.4-68.5)		52/61 (85.2; 73.8-93.0)		8.0 (6.8-9.8)		15.6 (12.2-NR)	
CPS score	CPS <1	22	11/22 (50.0; 28.2-71.8)	0.53	20/22 (90.9; 70.8-98.9)	1.00	8.8 (7.8-NR)	0.72	20.0 (12.8-NR)	0.55
	CPS ≥1	39	12/19 (63.2; 38.4-83.7)		17/19 (89.5; 66.9-98.7)		7.5 (6.4-NR)		13.1 (11.0-NR)	
Anatomical site	iCCA	36	17/36 (47.2; 30.4-64.5)	0.20	31/36 (86.1; 70.5-95.3)	0.29	8.8 (7.1-12.7)	0.27	19.0 (12.9-NR)	0.08
	eCCA	9	7/9 (77.8; 40.0-97.2)		9/9 (100.0; 66.4-100.0)		8.7 (6.3-NR)		12.1 (7.5-NR)	
	GBC	16	10/16 (62.5; 35.4-84.8)		12/16 (75.0; 47.6-92.7)		6.8 (5.7-NR)		16.6 (9.6-NR)	
Sex	Male	31	21/31 (67.7; 48.6-83.3)	0.07	28/31 (90.3; 74.2-98.0)	0.30	7.8 (6.4-11.0)	0.67	19.0 (12.9-NR)	0.22
	Female	30	13/30 (43.3; 25.5-62.6)		24/30 (80.0; 61.4-92.3)		8.0 (6.8-10.0)		12.9 (10.3-NR)	
Age	<60	33	21/33 (63.6; 45.1-79.6)	0.20	28/33 (84.8; 68.1-94.9)	1.00	7.8 (6.8-10.8)	0.80	16.2 (12.8-NR)	0.89
	≥60	28	13/28 (46.4; 27.5-66.1)		24/28 (85.7; 67.3-96.0)		8.0 (6.3-11.0)		14.4 (10.3-NR)	
ECOG PS	ECOG 0	32	19/32 (59.4; 40.6-76.3)	0.61	29/32 (90.6; 75.0-98.0)	0.28	8.8 (7.8-11.1)	0.16	16.5 (13.2-NR)	0.55
	ECOG 1	29	15/29 (51.7; 32.5-70.6)		23/29 (79.3; 60.3-92.0)		6.4 (5.3-10.8)		12.2 (9.8-NR)	
HBV status	No infection	20	10/20 (50.0; 27.2-72.8)	0.59	17/20 (85.0; 62.1-96.8)	1.00	7.6 (6.8-NR)	0.71	19.5 (12.2-NR)	0.46
	Past or present infection	41	24/41 (58.5; 42.1-73.7)		35/41 (85.4; 70.8-94.4)		8.3 (6.4-10.1)		14.6 (12.0-NR)	
Disease classification	Locally advanced	17	8/17 (47.1; 23.0-72.2)	0.56	14/17 (82.4; 56.6-96.2)	0.69	8.8 (8.3-NR)	0.47	19.0 (12.8-NR)	0.80
	Metastatic	44	26/44 (59.1; 43.2-73.7)		38/44 (86.4; 72.6-94.8)		7.5 (6.4-8.8)		13.7 (12.0-NR)	
Disease status	Initially unresectable	51	28/51 (54.9; 40.3-68.9)	1.00	43/51 (84.3; 71.4-93.0)	1.00	8.0 (6.8-9.8)	0.64	14.2 (12.0-NR)	0.17
	Recurrent	10	6/10 (60.0; 26.2-87.8)		9/10 (90.0; 55.5-99.7)		7.6 (6.3-NR)		NR (13.2-NR)	

ORR, objective response rate; DCR, disease control rate; PFS, progression-free survival; OS, overall survival; CI, confidence interval; iCCA, intrahepatic cholangiocarcinoma; eCCA, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; NR, not reached.

Exact binomial 95% CIs are shown for ORR and DCR. Response-rate P values were calculated by Fisher's exact test. PFS and OS are Kaplan-Meier medians with 95% CIs; P values were calculated by log-rank tests. Patients with missing CPS were excluded from CPS subgroup comparisons.

All subgroup P values are exploratory, nominal, and unadjusted for multiplicity.

Supplementary Table 6: Comparison of baseline characteristics between patients included and not included in plasma proteomic analysis

Characteristic	Overall, N = 61	Not included in analysis, N = 31	Included in analysis, N = 30	P-value
Sex				0.127
Male	31 (50.8%)	19 (61.3%)	12 (40.0%)	
Female	30 (49.2%)	12 (38.7%)	18 (60.0%)	
Age, years	58 (31-70)	56 (38-70)	59 (31-70)	0.750
Disease status				0.301
Initially unresectable	51 (83.6%)	24 (77.4%)	27 (90.0%)	
Recurrent	10 (16.4%)	7 (22.6%)	3 (10.0%)	
Disease classification				0.402
Locally advanced	17 (27.9%)	7 (22.6%)	10 (33.3%)	
Metastatic	44 (72.1%)	24 (77.4%)	20 (66.7%)	
Site of origin				0.821
Intrahepatic	36 (59.0%)	17 (54.8%)	19 (63.3%)	
Extrahepatic	9 (14.8%)	5 (16.1%)	4 (13.3%)	
Gallbladder	16 (26.2%)	9 (29.0%)	7 (23.3%)	
HBV status				0.106
No infection	20 (32.8%)	7 (22.6%)	13 (43.3%)	
Past or present infection	41 (67.2%)	24 (77.4%)	17 (56.7%)	
Site of metastases				
Liver	35 (57.4%)	20 (64.5%)	15 (50.0%)	0.297
Lung	4 (6.6%)	1 (3.2%)	3 (10.0%)	0.354
Peritoneum	9 (14.8%)	6 (19.4%)	3 (10.0%)	0.473
Bone	1 (1.6%)	0 (0.0%)	1 (3.3%)	0.492
Others	6 (9.8%)	1 (3.2%)	5 (16.7%)	0.104
TMB				0.495
<10 Muts/Mb	42 (68.9%)	23 (74.2%)	19 (63.3%)	
≥10 Muts/Mb	7 (11.5%)	2 (6.5%)	5 (16.7%)	
Unknown	12 (19.7%)	6 (19.4%)	6 (20.0%)	
MSI status				0.184
MSS	39 (63.9%)	17 (54.8%)	22 (73.3%)	
Unknown	22 (36.1%)	14 (45.2%)	8 (26.7%)	
PD-L1 combined positive score				0.729
<1	22 (36.1%)	11 (35.5%)	11 (36.7%)	
≥1	19 (31.1%)	11 (35.5%)	8 (26.7%)	
Unknown	20 (32.8%)	9 (29.0%)	11 (36.7%)	

HBV, Hepatitis B Virus; Mb, Megabase; MSS, Microsatellite Stable; MSI, Microsatellite Instability; Mut, Mutation; SD, Standard Deviation; TMB, Tumor Mutational Burden.

Sample median (range), for age characteristic; Sample size n (percent %), for categorical characteristics. P values for categorical variables were calculated using two-sided Fisher's exact tests. P values for age were calculated using the two-sided Wilcoxon rank-sum test. All P values are descriptive and nominal.

**INTERNAL DOCUMENT
CONFIDENTIAL**

BIOMEDICAL ETHICS RESEARCH PROTOCOL

(Interventional Clinical Study)

Study of the Efficacy and Safety of Cadonilimab Combined with Gemcitabine and Cisplatin as First-Line Treatment for Unresectable Locally Advanced or Metastatic Biliary Tract Malignancies

Research Institution	West China Hospital, Sichuan University
Principal Investigator (Signature)	Wentao Wang
Responsible Department	Department of Liver Surgery
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Lead Institution	West China Hospital, Sichuan University
Participating Institution	West China Hospital, Sichuan University
Study Period	July 2023 - July 2025
Version	V4.0
Version Date	20 June 2023

PROTOCOL SYNOPSIS

Study Design	☐ Case-control study ☐ Cohort study ☐ Cross-sectional study ☐ Randomized controlled study ☐ Blinding applied ☒ Other: single-arm clinical study
Study Type (select according to project type)	Category A: High Risk ☐ Class III new clinical technology (established safety and efficacy, high technical difficulty and high risk) ☐ Research involving special populations (children, pregnant women, persons with intellectual disability, subjects with mental disorders, etc.) ☒ Off-label drug study: off-label indication ☐ Off-label medical-device study ☐ Other (investigator determination; specify)
	Category B: Moderate Risk ☐ Post-marketing biologic study (preventive or therapeutic) ☐ Post-marketing therapeutic vaccine study ☐ Post-marketing orphan-drug study ☐ Class II new clinical technology (established safety and efficacy, some technical difficulty, and certain medical and ethical risks) ☐ Other (investigator determination; specify)
	Category C: Low Risk ☐ Study of a drug marketed for at least 5 years, including chemical drugs and generics ☐ Study of a marketed medical device, including AI and imaging software ☐ Class I new clinical technology (established safety and efficacy, low technical difficulty, and almost no ethical risk) ☐ Other (investigator determination; specify)
Total Sample Size	65 subjects
Risk/Benefit Analysis	The study regimen may provide clinical benefit, but adverse reactions may also occur, as with any treatment. If an adverse reaction occurs, the study physician will assess the subject and provide timely and appropriate medical management. No antitumour treatment is completely effective; disease may continue to progress during participation, and tumour-related complications may occur. Conversely, a regimen containing the study drug may control or relieve the disease. This study may also help provide a new treatment strategy for patients with advanced biliary tract malignancies.
Risk Assessment	☐ No greater than minimal risk ☒ Greater than minimal risk Minimal risk means that the probability and magnitude of anticipated harm or discomfort are not greater than those ordinarily encountered in daily life or during routine physical or psychological examinations.

I. BACKGROUND

1. Epidemiology of biliary tract cancer

Biliary tract cancers (BTCs) are aggressive and heterogeneous malignancies arising from the epithelial cells of the bile ducts and gallbladder. They mainly include intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder cancer.¹ BTCs account for approximately 10%-15% of primary liver tumours and 3% of gastrointestinal malignancies.² Over recent decades, the incidence of BTC has increased rapidly worldwide, particularly in Asia.³ Surgical resection is the only potentially curative treatment for BTC.⁴⁻⁶ However, because of the insidious onset of BTC and the limited specificity and sensitivity of available diagnostic methods, most patients present with advanced disease and are not candidates for surgery. Even after radical resection, many patients eventually develop metastasis or tumour recurrence.⁷ The 5-year overall survival (OS) rate after resection is only 10%-40%,⁸ and the overall 5-year OS rate for BTC is less than 5%.⁹ Therefore, optimizing clinical treatment strategies and improving outcomes in BTC remain urgent clinical priorities.

For unresectable or metastatic advanced BTC, gemcitabine plus cisplatin (GC) was established as standard first-line treatment based on the ABC-02 trial. ABC-02 was the first large phase III randomized clinical trial in patients with locally advanced or metastatic BTC. The study showed that GC improved chemotherapy efficacy and confirmed a synergistic effect between gemcitabine and cisplatin. Median OS was 11.7 months in the combination group and 8.1 months in the gemcitabine-alone group (HR=0.64; 95% CI, 0.52-0.80; $P<0.001$). Median progression-free survival (PFS) was 8.0 and 5.0 months, respectively (HR=0.63; 95% CI, 0.51-0.77; $P<0.001$).¹⁰ Based on the phase III JCOG1113 and FUGA-BT studies, gemcitabine plus S-1 and capecitabine plus oxaliplatin may also be used as alternative regimens for advanced BTC.¹¹ Nevertheless, median survival remains less than 1 year, underscoring the need for new therapeutic agents.

2. Progress in first-line immune checkpoint inhibitor therapy for advanced BTC

In recent years, increasing understanding of the tumour immune microenvironment has opened new avenues for immunotherapy in advanced BTC. Immune checkpoint inhibitors (ICIs) are a rapidly developing class of immunotherapeutic agents that enhance immune responses by relieving immune suppression, thereby promoting tumour-cell killing.

Multiple clinical studies have investigated ICIs as first-line treatment for advanced BTC and have reported promising early findings. Pembrolizumab, an anti-PD-1 antibody, has been approved by the US Food and Drug Administration for patients with MSI-H/dMMR BTC.¹² Small studies of immunotherapy combined with chemotherapy have also reported encouraging activity. A Korean phase II study evaluated durvalumab (D) with or without tremelimumab (T) plus GC as first-line treatment for unresectable or recurrent BTC. Patients were enrolled into three cohorts: a biomarker cohort (BMC; $n=30$), a GC+D cohort ($n=45$), and a GC+D+T cohort ($n=46$). In the BMC cohort, one cycle of GC was followed by GC+D+T every 3 weeks until disease progression. The GC+D and GC+D+T cohorts received their respective regimens until progression. Mean follow-up was 28.5, 11.3, and 11.9 months, respectively. ORRs were 50.0%, 73.4%, and 73.3%; median PFS was 13.0, 11.0, and 11.9 months; and median OS was 15.0, 18.1, and 20.7 months. The most common adverse events (AEs) were nausea (59.5%), neutropenia (54.5%), and pruritus (55.4%). The most common grade 3/4 AEs were neutropenia (50.4%), anaemia (35.5%), and thrombocytopenia (16.5%). The chemoimmunotherapy regimens were tolerated and showed promising efficacy.¹³

A phase II, single-arm, multicentre study from China evaluated camrelizumab plus GEMOX as first-line treatment for advanced BTC. Thirty-eight subjects were enrolled. At a median follow-up of 11.8 months, median OS and PFS were 11.8 and 6.1 months, respectively; the 6-month PFS rate was 50%, and the ORR was 54%. Camrelizumab plus GEMOX therefore also showed antitumour activity in first-line advanced BTC.¹¹

TOPAZ-1 was a randomized, double-blind, placebo-controlled, global phase III trial that enrolled 685 patients with locally advanced or metastatic BTC to evaluate durvalumab plus standard chemotherapy versus chemotherapy alone as first-line treatment. At the prespecified interim analysis presented at ASCO GI 2022, durvalumab plus

chemotherapy reduced the risk of death by 20% compared with chemotherapy alone (HR=0.80; 95% CI, 0.66-0.97; P=0.021). Median OS was 12.8 months versus 11.5 months. Approximately 25% of patients in the combination group were alive at 2 years compared with 10% in the chemotherapy-alone group. Durvalumab plus chemotherapy reduced the risk of disease progression or death by 25% (HR=0.75; 95% CI, 0.64-0.89; P=0.001). Median PFS was 7.2 months versus 5.7 months. The ORR increased from 18.7% to 26.7%, and 26.1% of responders in the combination group had an ongoing response at 12 months. On 3 September 2022, the US FDA approved durvalumab plus GC as first-line treatment for locally advanced or metastatic biliary tract malignancies. The 2022 Chinese Society of Clinical Oncology (CSCO) guideline recommended gemcitabine, cisplatin, and durvalumab as first-line treatment for patients able to tolerate intensive chemotherapy (category 1A). The 2022 National Comprehensive Cancer Network (NCCN) guideline initially listed durvalumab plus gemcitabine and cisplatin as category 2B evidence in version 1 and subsequently upgraded it to a preferred category 1 regimen in version 2. Nevertheless, median OS with chemoimmunotherapy in TOPAZ-1 remained approximately 12 months and ORR remained around 20%, indicating an ongoing unmet clinical need.

Tumour pathogenesis is complex, and simultaneous blockade of multiple targets may provide greater activity. Bispecific antibodies can recognize and bind two different antigens and may offer greater sensitivity and specificity than monospecific antibodies, potentially reducing off-target effects. Interest in combined PD-1/PD-L1 and CTLA-4 blockade in biliary tract malignancies has increased. Combinations of different ICIs may overcome resistance to single-agent immunotherapy by targeting multiple mechanisms of immune escape. These combinations are intended to provide coordinated, rather than merely additive, antitumour effects.

IMMUCHEC was a prospective, randomized, multicentre phase II trial evaluating five combinations of gemcitabine, cisplatin, durvalumab, and/or tremelimumab in advanced BTC.¹⁵ A total of 138 patients were allocated according to regimen and dose to gemcitabine+durvalumab+tremelimumab (4 x 75 mg), gemcitabine+cisplatin+durvalumab+tremelimumab (4 x 75 mg), gemcitabine+cisplatin, gemcitabine+cisplatin+durvalumab+tremelimumab (1 x 300 mg), or gemcitabine+cisplatin+durvalumab. The primary endpoint was ORR, and secondary endpoints included PFS, OS, and duration of response (DoR). ORRs were 4.6%, 18.2%, 28.6%, 26.7%, and 20.7%, respectively. The addition of immunotherapy did not improve PFS, although the gemcitabine+cisplatin+durvalumab+tremelimumab (1 x 300 mg) group had a median OS of 22.73 months (95% CI, 9.53-25.1), suggesting a possible OS trend. These preliminary findings suggested that dual-checkpoint inhibition combined with chemotherapy might warrant further study in advanced BTC.

3. Mechanism of action and clinical rationale for cadonilimab

Cadonilimab is a humanized immunoglobulin G1 (IgG1) bispecific antibody (BsAb) with crystallizable fragment (Fc) mutations designed to eliminate Fc receptor- and complement-mediated cytotoxicity. It simultaneously binds programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), thereby blocking PD-1/PD-L1, PD-1/PD-L2, CTLA-4/B7.1, and CTLA-4/B7.2 interactions. PD-1 is mainly expressed on activated T lymphocytes. Binding to PD-L1 or PD-L2 inhibits T-cell proliferation and cytokine production, and upregulation of PD-1 ligands on some tumour cells can suppress immune surveillance by activated T cells. CTLA-4 is expressed on activated CD4⁺ and CD8⁺ T cells and acts as a negative regulator of T-cell activity through binding to B7.1 and B7.2.

Cadonilimab uses an IgG-single-chain variable fragment (IgG-scFv) structure that supports differential antigen binding and high retention in tumour tissue while rapidly mediating independent internalization of PD-1 or CTLA-4. Cadonilimab shows higher affinity for lymphocytes in the tumour microenvironment than at peripheral sites and reduces CTLA-4 binding in peripheral blood and normal tissues, potentially mitigating CTLA-4-related toxicity. The proposed advantages include: (1) simultaneous blockade of PD-1 and CTLA-4 with binding capacities comparable to the corresponding monospecific antibodies; (2) Fc optimization to eliminate antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), reducing Fc-mediated immune activation, and to reduce antibody-dependent cytokine release (ADCR), potentially lowering IL-6-mediated immune toxicity while reducing IL-8 release; (3) stability more than 100-fold greater than native IgG4, no binding to other IgG molecules, and low host-cell protein residue; and (4) strong antitumour activity.

A phase Ia/Ib dose-escalation study of cadonilimab in Australia enrolled subjects with advanced solid tumours that had failed standard treatment, including non-small-cell lung cancer, gastric cancer, pancreatic cancer, mesothelioma, triple-negative breast cancer, neuroendocrine carcinoma, and endometrial cancer. Dose-escalation decisions followed a 3+3+3 design. This was the first-in-human study of cadonilimab. By 30 April 2020, 92 subjects had been enrolled in the phase Ia study. Doses ranged from 0.2 mg/kg Q2W through 0.5, 1, 2, 4, 6, and 10 mg/kg Q2W, a fixed dose of 450 mg Q2W, and 15 mg/kg Q3W. Eighteen subjects received 10 mg/kg Q2W and three received 15 mg/kg Q3W.

No maximum tolerated dose was reached at any dose level by 30 April 2020. One dose-limiting toxicity, grade 3 AST elevation, occurred in the 1.0 mg/kg group. Among the 18 subjects who received 10 mg/kg Q2W, the incidence of grade 3/4 treatment-related adverse events (TRAEs) was 22.2%; fever, hyperthyroidism, polyneuropathy, and nephritis each occurred in one subject. A 10 mg/kg Q2W dose-escalation study in China was ongoing without a reported DLT. Among three subjects treated with 15 mg/kg Q3W, no grade ≥ 3 TRAE occurred; TRAEs included grade 2 anaemia, grade 1 facial rash, and grade 1 thyroid dysfunction. Across early studies conducted in China and abroad, 277 patients had received cadonilimab by 10 July 2020, with dose escalation from 0.2 mg/kg Q2W to 25 mg/kg Q3W. Only one DLT (grade 3 AST elevation at 1.0 mg/kg) was reported; no DLT occurred at 15 or 25 mg/kg Q3W. TRAEs occurred in 73.6% of patients and generally resolved or improved after treatment interruption and supportive care.

Across four early monotherapy studies (AK104-101 [N=119], AK104-201 [N=240], AK104-202 [N=68], and AK104-101 [N=119], as listed in the source protocol), 458 patients were included. The overall incidence of adverse reactions was 83.6%. Adverse reactions occurring in at least 10% of patients included rash (23.1%), anaemia (16.4%), hypothyroidism (16.2%), increased AST (14.6%), increased ALT (13.8%), pruritus (10.9%), fatigue (10.3%), and proteinuria (10.0%). Grade ≥ 3 adverse reactions occurred in 20.3%, including infusion-related reactions/hypersensitivity (2.2%) and anaemia (2.0%). These preliminary data suggested an acceptable safety and tolerability profile.

Multiple clinical studies of cadonilimab have been conducted in China and internationally. In a phase II study of cadonilimab plus lenvatinib as first-line treatment for unresectable hepatocellular carcinoma (HCC), eligible patients had unresectable BCLC stage B/C, Child-Pugh A HCC and no prior systemic therapy. Cadonilimab 6 mg/kg intravenously Q2W was combined with oral lenvatinib 8 mg for body weight < 60 kg or 12 mg for body weight ≥ 60 kg once daily. Among 18 efficacy-evaluable patients, ORR was 44.4% (8/18) and DCR was 77.8% (8 PR and 6 SD; two patients had tumour reductions of 28.4% and 29.2%). The 12-month OS rate was 83.6%. TRAEs occurred in 83.3% of patients; grade 3 AEs occurred in 26.7%, and no grade 4 TRAEs were reported. The most common TRAEs ($\geq 15\%$) were elevated AST (36.7%), elevated ALT (36.7%), decreased platelet count (33.3%), decreased neutrophil count (30.0%), and elevated bilirubin (26.7%), most of which were grade 1 or 2. Cadonilimab plus lenvatinib showed antitumour activity with acceptable safety in first-line unresectable HCC.

Several registration-oriented studies were ongoing, including cadonilimab plus lenvatinib and transarterial chemoembolization for intermediate-stage HCC, cadonilimab as adjuvant treatment after resection of high-risk HCC, and cadonilimab in advanced HCC progressing after anti-PD-1/PD-L1 therapy combined with antiangiogenic targeted therapy. Cadonilimab has also shown activity in other solid tumours. In a phase II study presented at AACR 2020 (NCT03852251), cadonilimab plus capecitabine and oxaliplatin (XELOX) in gastric cancer achieved a DCR of 94.7%, including one complete response; some patients received treatment for more than 42 weeks, and 13 remained on treatment. As of 2 April 2020, ORR was 57.9% and DCR was 94.7%. Grade ≥ 3 AEs occurred in 9.5% of patients.

4. Risk/benefit assessment

In BTC, increased expression of PD-1 and CTLA-4 and the resulting inhibition of T-cell immune responses are important contributors to tumour growth and spread. Cadonilimab targets these immune checkpoints and may activate the immune system against tumour cells. As noted above, immunotherapy combined with chemotherapy has generally been tolerable. For D +/- T plus GC in first-line advanced BTC, the most common AEs of any grade were nausea (59.5%), neutropenia (54.5%), and pruritus (55.4%), and the most common grade 3/4 AEs were neutropenia

(50.4%), anaemia (35.5%), and thrombocytopenia (16.5%). No new safety signals were identified beyond the known safety profiles of the individual agents. Cadonilimab plus chemotherapy is expected to have synergistic antitumour activity and may benefit patients with advanced BTC without introducing unacceptable additional risk.

In summary, PD-1 blockade combined with chemotherapy has shown preliminary safety and encouraging activity in advanced BTC and warrants further investigation. This study is designed to evaluate the safety and efficacy of cadonilimab combined with GC in patients with unresectable locally advanced or metastatic BTC.

II. STUDY OBJECTIVES

1. Primary objective: To evaluate the objective response rate (ORR) according to RECIST version 1.1.
2. Secondary objectives:
 - Disease control rate (DCR) according to RECIST version 1.1
 - Duration of response (DoR) according to RECIST version 1.1
 - Progression-free survival (PFS) according to RECIST version 1.1
 - Overall survival (OS)
 - Safety
3. Exploratory objective: To explore potential tumour biomarkers predictive of efficacy, including but not limited to PD-L1 expression and tumour mutational burden (TMB), and their associations with clinical efficacy.

III. STUDY DESIGN, METHODS, AND PROCEDURES

1. Study design

This is a single-arm phase II clinical study evaluating the safety and efficacy of cadonilimab combined with GC chemotherapy in patients with unresectable locally advanced or metastatic BTC.

After signing informed consent, subjects will receive cadonilimab plus GC. GC chemotherapy will be administered for a maximum of 8 cycles. After completion of chemotherapy, cadonilimab will be continued until disease progression, death, unacceptable toxicity, withdrawal of informed consent, initiation of new antitumour therapy, another protocol-specified reason for discontinuation, or a maximum total treatment duration of 24 months. Tumour imaging will be assessed according to RECIST version 1.1 every 9 weeks (+/-7 days) from the first dose; after week 48, imaging will be performed every 12 weeks (+/-7 days). Safety will be assessed from the first dose through 90 days after the last dose using NCI CTCAE version 5.0. The primary endpoint is ORR. Secondary endpoints include DCR, DoR, PFS, OS, and safety. Exploratory analyses will assess potential predictive biomarkers, including but not limited to PD-L1 expression and TMB.

The first administration of cadonilimab will be recorded as Cycle 1 Day 1. For all subsequent treatment cycles, study treatment will begin on Day 1 after completion of predose assessments. For administrative reasons, the investigator may allow dosing within 3 days before or after the scheduled Day 1 of each cycle.

Table 1. Study Treatments and Dosing Schedule

Drug	Dose	Frequency	Route	Course/Treatment Cycle
Cadonilimab [†]	10 mg/kg	Q3W	Intravenous infusion	Each cycle is 21 days; administered on Day 1 of each cycle and continued thereafter.
Gemcitabine	1,000 mg/m ²	Days 1 and 8	Intravenous infusion	Each cycle is 21 days; administered on Days 1 and 8 for a total of 8 cycles.
Cisplatin	25 mg/m ²	Days 1 and 8	Intravenous infusion	Each cycle is 21 days; administered on Days 1 and 8 for a total of 8 cycles.

¹ Cadonilimab should be infused before administration of the chemotherapy drugs.

2. Study procedures

Eligible patients with previously untreated, unresectable locally advanced or metastatic BTC; no prior systemic treatment, with curative surgery or neoadjuvant/adjuvant therapy allowed if completed more than 6 months earlier; at least one measurable lesion according to RECIST version 1.1; expected survival >3 months; ECOG performance status 0-1. Subjects will receive cadonilimab 10 mg/kg Q3W plus gemcitabine 1,000 mg/m² and cisplatin 25 mg/m² on Days 1 and 8 of each 21-day cycle for a maximum of 8 cycles, followed by continued cadonilimab until progression or another discontinuation criterion is met.

IV. SUBJECT SELECTION

1. Inclusion criteria

- (1) Written informed consent obtained before any study-related procedure.
- (2) Age >18 years and ≤70 years, regardless of sex.
- (3) Histologically or cytologically confirmed unresectable locally advanced or metastatic BTC, including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gallbladder cancer.
- (4) No prior systemic treatment; curative surgery or neoadjuvant/adjuvant therapy completed more than 6 months previously is permitted.
- (5) Expected survival >3 months.
- (6) At least one measurable lesion according to RECIST version 1.1.
- (7) ECOG performance status of 0-1.
- (8) Adequate organ function, meeting all of the following laboratory criteria:
 1. Absolute neutrophil count (ANC) $\geq 1.5 \times 10^9/L$ without granulocyte colony-stimulating factor use within the preceding 14 days.
 2. Platelet count $\geq 90 \times 10^9/L$ without transfusion within the preceding 14 days.
 3. Haemoglobin >9 g/dL without transfusion or erythropoietin within the preceding 14 days.
 4. Total bilirubin $\leq 1.5 \times$ upper limit of normal (ULN).
 5. AST and ALT $\leq 2.5 \times$ ULN; AST or ALT $\leq 5 \times$ ULN is permitted in patients with liver metastases.
 6. Serum creatinine $\leq 1.5 \times$ ULN and creatinine clearance ≥ 60 mL/min calculated using the Cockcroft-Gault formula.
 7. Adequate coagulation, defined as international normalized ratio (INR) or prothrombin time (PT) $\leq 1.5 \times$ ULN.
 8. Normal thyroid function, defined as TSH within the normal range. Subjects with baseline TSH outside the normal range may be enrolled if total T3 (or FT3) and FT4 are within the normal range.
 9. Cardiac enzyme panel within the normal range; isolated laboratory abnormalities considered clinically insignificant by the investigator are permitted.
- (9) Women of childbearing potential must have a negative urine or serum pregnancy test within 3 days before the first dose of study treatment (Cycle 1 Day 1). If a urine pregnancy test cannot be confirmed as negative, a serum pregnancy test is required. Women not of childbearing potential are defined as postmenopausal for at least 1 year, surgically sterilized, or status post hysterectomy.
- (10) If there is a risk of conception, all subjects, male or female, must use a contraceptive method with an annual failure rate below 1% throughout treatment and for 120 days after the last dose of study treatment.

2. Exclusion criteria

- (1) Diagnosis of a malignancy other than BTC within 5 years before the first dose, except cured basal-cell carcinoma of the skin, squamous-cell carcinoma of the skin, and/or carcinoma in situ treated with curative resection.
- (2) Ampullary tumour.
- (3) Current participation in an interventional clinical study, or receipt of another investigational drug or treatment with an investigational device within 4 weeks before the first dose.

- (4) Prior treatment with anti-PD-1, anti-PD-L1, or anti-PD-L2 therapy, or an agent directed against another stimulatory or coinhibitory T-cell receptor, such as CTLA-4, OX-40, or CD137.
- (5) Prior palliative radiotherapy for BTC, excluding postoperative adjuvant radiotherapy.
- (6) Systemic treatment within 2 weeks before the first dose with a traditional Chinese patent medicine indicated for antitumour treatment or an immunomodulatory agent, including thymosin, interferon, or interleukin, except local use for pleural-effusion control.
- (7) Active autoimmune disease requiring systemic treatment within 2 years before the first dose, such as disease-modifying drugs, corticosteroids, or immunosuppressants. Replacement therapy such as thyroxine, insulin, or physiological corticosteroid replacement for adrenal or pituitary insufficiency is not considered systemic treatment. Known primary immunodeficiency. Subjects with positive autoimmune antibodies alone require investigator assessment to determine whether autoimmune disease is present.
- (8) Systemic corticosteroid therapy within 4 weeks before the first dose, excluding intranasal, inhaled, or other local corticosteroids, or any other form of immunosuppressive therapy. Physiological corticosteroid doses of prednisone ≤ 10 mg/day or equivalent are permitted.
- (9) Clinically uncontrolled pleural effusion or ascites. Subjects who do not require drainage or whose effusion has not increased significantly for 3 days after drainage is discontinued may be enrolled.
- (10) Known allogeneic organ transplantation, except corneal transplantation, or allogeneic haematopoietic stem-cell transplantation.
- (11) Known hypersensitivity to the active ingredient or excipients of cadonilimab.
- (12) Failure to recover adequately from toxicity and/or complications caused by any prior intervention before treatment initiation, defined as grade ≤ 1 or return to baseline, excluding fatigue or alopecia.
- (13) Known history of human immunodeficiency virus infection, defined as positive HIV-1/2 antibody.
- (14) Untreated active hepatitis B, defined as HBsAg positivity with HBV DNA above the upper limit of normal of the study-centre laboratory. Subjects with HBV may be enrolled if the viral load before the first dose is $< 2.5 \times 10^3$ copies/mL (500 IU/mL) and anti-HBV treatment is administered throughout study treatment. Subjects who are anti-HBc positive, HBsAg negative, anti-HBs negative, and HBV-DNA negative do not require prophylactic anti-HBV therapy but require close monitoring for viral reactivation.
- (15) Active HCV infection, defined as positive HCV antibody with HCV RNA above the lower limit of detection.
- (16) Receipt of a live attenuated vaccine within 4 weeks before the first dose.
- (17) Pregnant or breastfeeding women.
- (18) Any severe or uncontrolled systemic disease, including the following:
 1. Major, symptomatic, or difficult-to-control abnormalities in rhythm, conduction, or morphology on resting ECG, such as complete left bundle-branch block, second-degree or higher atrioventricular block, ventricular arrhythmia, or atrial fibrillation.
 2. Unstable angina, congestive heart failure, or chronic heart failure of New York Heart Association class ≥ 2 .
 3. Any arterial thrombosis, embolism, or ischaemic event within 6 months before enrolment, including myocardial infarction, unstable angina, cerebrovascular accident, or transient ischaemic attack.
 4. Major surgery, including craniotomy, thoracotomy, or laparotomy, within 4 weeks before the first dose; an unhealed wound, ulcer, or fracture; tissue needle biopsy or other minor surgery within 7 days before the first dose, excluding venous catheterization for infusion.
 5. Poorly controlled blood pressure, defined as systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg.
 6. Active pulmonary tuberculosis.
 7. Active or uncontrolled infection requiring systemic treatment.
 8. Clinically active diverticulitis, abdominal abscess, or gastrointestinal obstruction.
 9. Liver disease such as cirrhosis, decompensated liver disease, or acute or chronic active hepatitis.
 10. Poorly controlled diabetes mellitus, defined as fasting blood glucose > 10 mmol/L.
 11. Urine protein $\geq ++$ with confirmed 24-hour urinary protein > 1.0 g.
 12. A psychiatric disorder that prevents cooperation with treatment.

- (19) Any medical history, evidence of disease, treatment, or abnormal laboratory value that may interfere with the study results or prevent full participation, or any other condition or potential risk that, in the investigator's opinion, makes the subject unsuitable for the study.

3. Criteria for discontinuation

(1) Discontinuation of study treatment

A subject may discontinue treatment at any time for any reason, or the investigator may discontinue treatment in the event of an adverse event. The investigator may also discontinue treatment if the subject is unsuitable for continued treatment, violates the protocol, or for administrative and/or other safety reasons.

Treatment may be discontinued if the subject withdraws informed consent and requests withdrawal from the clinical study, or if the investigator determines that continued study treatment is unfavourable to the subject or that the subject cannot tolerate GC chemotherapy or cadonilimab-related adverse reactions.

A subject must discontinue treatment for any of the following reasons but may continue to be monitored in the study:

- The subject or the subject's legally authorized representative requests discontinuation of treatment.
- A protocol-specified adverse event requiring discontinuation occurs.
- Another malignancy requiring active treatment develops.
- An intercurrent disease prevents further treatment.
- The investigator decides to withdraw the subject from treatment.
- The subject has a positive serum pregnancy test.
- The subject has poor compliance.
- The investigator determines that continued study treatment would expose the subject to unnecessary risk because of the subject's disease status or individual circumstances.
- The subject completes protocol-specified treatment.

Subjects who discontinue treatment but remain in the study for follow-up should complete all visits and procedures listed in the schedule of assessments.

(2) Withdrawal from the study

A subject must withdraw from the study if the subject or the subject's legally authorized representative withdraws informed consent for participation. After withdrawal, the subject will no longer receive treatment or attend scheduled study visits. With the subject's consent, survival follow-up may continue after withdrawal. A subject who is lost to follow-up must be considered withdrawn from the study.

V. ALTERNATIVE DIAGNOSTIC AND TREATMENT OPTIONS

This clinical study enrolls patients with unresectable BTC. According to current guidelines, chemotherapy and/or immunotherapy are preferred treatment options. Other available treatments include targeted therapy, immunotherapy, radiotherapy, and traditional Chinese medicine. For subjects who discontinue the study, second-line treatment will be selected according to current clinical guidelines.

VI. ASSESSMENTS AND TIME POINTS

Assessment	Screening Day -28 to -1	C1/D1 Day 1 (+3 d)	C2/D1 Day 22 (+/-3 d)	Cn/D1 Day 21(n-1)+1 (+/-3 d)	Safety visit 30 d after last dose (+/-7 d)	Survival visit Every 90 d (+/-7 d)
General study procedures						
Written informed consent ¹	•					
Inclusion/exclusion criteria	•					
Demographics/medical history/prior cancer treatment history ²	•					
Prior and concomitant medications	•	•	•	•	•	
Vital signs ³	•	•	•	•	•	
Weight/height ⁴	•	•	•	•	•	
Complete physical examination	•	•	•	•	•	
ECOG performance status	•	•	•	•	•	
12-lead ECG ⁵	•		•	•	•	
Histopathological examination	•					
Child-Pugh score	•					
Laboratory assessments						
Complete blood count ⁶	•		•	•	•	
Blood chemistry (glucose, amylase, electrolytes, etc.) ⁶	•		•	•	•	
Urinalysis ⁶	•		•	•	•	
Coagulation function ⁷	•		•	•	•	
Pregnancy test ⁸	•					
Thyroid function ⁹	•		•	•	•	
Cardiac enzyme panel ⁹	•		•	•	•	
Viral serology (HIV, HBV, HCV) ¹⁰	•					
HCV RNA if HCV antibody positive ¹¹	•		•	•	•	
HBV DNA if HBsAg and/or HBcAb positive ¹²	•		•	•	•	
Safety monitoring and survival						
Adverse-event assessment ¹³	•	•	•		•	
Subsequent antitumour treatment						•

Assessment	Screening Day -28 to -1	C1/D1 Day 1 (+3 d)	C2/D1 Day 22 (+/-3 d)	Cn/D1 Day 21(n-1)+1 (+/-3 d)	Safety visit 30 d after last dose (+/-7 d)	Survival visit Every 90 d (+/-7 d)
Survival status						•
Efficacy assessments						
Tumour imaging assessment ¹⁴	•			•		
Tumour markers	•		•	•	•	
PET/CT	As clinically indicated					
Study drug administration ¹⁵		•	•	•		
Cadonilimab		•	•	•		
Gemcitabine		•	•	•		
Cisplatin		•	•	•		
Exploratory biomarkers						
Archived/fresh tumour tissue ¹⁶	•					
Whole blood ¹⁷	•			•	•	

In addition to the scheduled visits and recording of the above assessments, concomitant medications must be followed and documented in detail, including anti-HBV drugs, interferon, thymosin, other immune-enhancing agents, and traditional Chinese medicines. Start and stop dates, dose, and dosing frequency must be recorded.

VII. EFFICACY ASSESSMENT CRITERIA

1. Efficacy endpoints

Objective response rate (ORR): the proportion of all subjects who achieve complete response (CR) or partial response (PR).

Progression-free survival (PFS): the time from enrolment to the first radiographic disease progression or death, whichever occurs first.

Disease control rate (DCR): the proportion of all subjects who achieve CR, PR, or stable disease (SD).

Duration of response (DoR): the interval from the first documented response to disease progression or death, whichever occurs first.

Overall survival (OS): the time from enrolment to death from any cause.

ORR, PFS, DCR, and DoR will be assessed by the investigator according to RECIST version 1.1.

2. Safety assessment

During the study and follow-up, the severity of adverse events will be graded according to NCI CTCAE version 5.0. Safety assessments include:

- The incidence, severity, and relationship to study treatment of all AEs, treatment-emergent adverse events (TEAEs), treatment-related adverse events (TRAEs), serious adverse events (SAEs), and immune-related adverse events (irAEs).
- The number and proportion of subjects who discontinue treatment because of the above adverse events.
- Changes in vital signs, physical examination findings, and laboratory results before, during, and after study treatment.

VIII. OBSERVATION, DOCUMENTATION, AND MANAGEMENT OF ADVERSE EVENTS

An adverse event (AE) is any untoward medical occurrence associated with any medical intervention in the study, whether or not considered related to the study intervention. All observed AEs or AEs spontaneously reported by subjects, regardless of study group or causality, must be recorded on the AE page of the case report form (CRF). Investigators will assess all AEs according to NCI CTCAE version 5.0. Any change in CTCAE grade will be recorded in the AE CRF/work sheet. All AEs, regardless of CTCAE grade, must be assessed for seriousness. Detailed AE assessment criteria are presented in Table 2.

Table 2. Criteria for Assessment of Adverse Events

Item	Assessment Criteria
NCI CTCAE v5.0 grading	Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. Grade 2: Moderate; minimal, local, or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living. Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living. Grade 4: Life-threatening consequences; urgent intervention indicated. Grade 5: Death related to an AE.

Item	Assessment Criteria
Seriousness	An SAE is any event occurring at any dose or during use of a study drug that results in death; is life-threatening; results in persistent or significant disability/incapacity; requires inpatient hospitalization or prolongation of existing hospitalization; results in a congenital anomaly/birth defect; or is another important medical event that, based on appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Hospitalization for a pre-existing condition that has not worsened during the study, including elective surgery, does not constitute an SAE.
Duration	Record the start and end dates of the AE. If the event lasts less than 1 day, record the appropriate duration and unit.
Action taken	Did the AE lead to discontinuation of study treatment?
Relationship to study drug	A medically qualified investigator must assess whether the study drug caused the AE and sign/date the source document or work sheet supporting the causality assessment. Factors include evidence of exposure, temporal relationship, alternative causes, dechallenge, rechallenge, and consistency with the known pharmacological or toxicological profile of the study drug or drug class.
Exposure	Is there evidence that the subject was actually exposed to the study drug, such as reliable medical history, acceptable compliance assessment, expected pharmacological effects, or measurement of the drug/metabolite in biological specimens?
Temporal relationship	Is there a reasonable temporal sequence between study-drug treatment and the AE? Is the timing consistent with a drug-induced event?
Alternative causes	Can the AE be explained by another aetiology, such as the underlying disease, other drugs/vaccines, or host or environmental factors?
Dechallenge	Was the study drug discontinued or was the dose/exposure/frequency reduced? If so, did the AE resolve or improve? A positive dechallenge indicates improvement; a negative dechallenge indicates no improvement. This criterion may not apply if the AE caused death or permanent disability, resolved despite continued treatment, or the study involved a single administration.
Rechallenge	Was the subject re-exposed to the study drug, and if so, did the AE recur or worsen? Rechallenge is not recommended for severe events that may have been caused by the study drug when re-exposure may pose serious risk, unless potential benefit is considered to outweigh the risk, no alternative treatment is available, and prior approval is obtained from the principal investigator.
Consistency with treatment characteristics	Are the clinical/pathological manifestations of the AE consistent with previously available pharmacological and toxicological information about the study drug or drug class?
Causality category: Related	There is evidence of exposure to the study drug, the temporal relationship is reasonable, and the AE is more likely explained by the study drug than by other causes.
Causality category: Unrelated	The subject did not receive the study drug; the temporal relationship is unreasonable; or another cause is more likely than the study drug to explain the AE. This category also applies to overdose without an associated AE.

1. Recording of adverse events

Investigators should use appropriate medical terminology or concepts to record AEs and SAEs. Colloquial expressions and abbreviations should be avoided. All AEs, including SAEs, must be recorded on the AE form of the CRF.

2. AE collection period

Investigators will identify AEs by asking subjects non-leading questions. All AEs, including SAEs, whether observed by the investigator or spontaneously reported by the subject, will be collected from the time informed consent is signed through 90 days after the last dose. From 30 to 90 days after the last dose, investigators must report all SAEs and all AEs related to study treatment or study procedures. After 90 days following the last dose,

investigators should report SAEs considered related to the study drug or study procedures. If a new antitumour treatment is initiated within 90 days after the last dose, only SAEs related to the study drug need to be recorded thereafter.

3. Follow-up of adverse events

AEs should be followed until recovery to baseline or grade 0-1, or until the investigator determines for a reasonable cause, such as irreversibility or adequate improvement, that further follow-up is unnecessary. If an AE does not resolve, the reason must be documented in the CRF. Recovery status and date must be recorded in the CRF and medical record regardless of relationship to study treatment.

4. Information to be recorded for AEs

For each AE, investigators must record the diagnosis or, if no diagnosis is available, the symptoms and signs including laboratory abnormalities; start and end dates and times if applicable; CTCAE severity grade and changes in grade for grade 3 or higher events; seriousness; action taken with study treatment; treatment administered for the AE; outcome; and relationship to study treatment. For an SAE, investigators must additionally provide the date on which SAE criteria were met, the date the investigator became aware of the SAE, the basis for seriousness, dates of admission and discharge, possible cause and date of death, whether an autopsy was performed, causality in relation to study procedures and other drugs, other possible causes, and the rationale for the causality assessment. The SAE narrative should include subject number, age, sex, height, weight, study-drug indication, disease stage and general condition; clinical course, development, resolution, and outcome; relevant laboratory findings with dates, units, and normal ranges; relevant medical history and concomitant conditions; relevant medication history and concomitant drugs; and details of study-drug dosing and treatment duration.

Diagnosis, symptoms, and signs

If a diagnosis is available, the diagnosis rather than individual symptoms and signs should be recorded in the CRF, for example, liver failure rather than jaundice, elevated transaminases, and asterixis. If symptoms and signs cannot be attributed to the diagnosis at the time of reporting, they should be recorded as separate AEs/SAEs. If they are later confirmed to be caused by the diagnosis, only the diagnosis should remain separately reported; symptoms and signs are then considered part of the diagnosis. AE entries for symptoms and signs should be removed, and an SAE follow-up report should be submitted.

AEs secondary to other events

AEs secondary to another event, such as clinical sequelae, should generally be recorded under the primary event unless the secondary event is severe or itself an SAE. A clinically significant secondary event occurring at a different time from the primary event should be recorded separately. If the relationship between events is unclear, each event should be recorded separately in the CRF.

Persistent or recurrent AEs

A persistent AE is an AE that remains present without resolution between two assessment time points and should be recorded only once in the CRF. The initial severity should be recorded and updated if the event worsens so that the maximum severity is captured. A recurrent AE is an event that resolves between two assessment time points and later recurs; each occurrence should be recorded separately.

Laboratory abnormalities

Clinically significant abnormal laboratory results should be reported as AEs. Investigators are responsible for reviewing all laboratory abnormalities and applying medical judgment to determine whether each abnormality should be reported as an AE.

Deaths

All deaths occurring during the study, including during the 90-day follow-up after the last dose, must be recorded on the CRF death-report form and reported promptly to the sponsor, regardless of relationship to study

treatment. If the cause of death is known, the cause should be recorded as an AE with outcome of death and reported as an SAE. Death due to tumour progression is not recorded or reported as an AE/SAE, but must be documented on the death-report form and communicated promptly to the sponsor. If the cause of death is unknown at the time of reporting, “death of unknown cause” should be recorded on the AE form and initially reported as an SAE while the specific cause is investigated.

Pre-existing medical conditions

Symptoms or signs present during screening should be recorded as AEs after study entry only if their severity, frequency, or nature worsens, excluding worsening of the disease under study. The record should reflect the change relative to baseline, for example, “increased frequency of headache.”

Disease progression

Disease progression is defined as worsening of the subject’s condition caused by the primary tumour targeted by study treatment, development of new lesions related to the primary tumour, or progression of existing lesions. Expected disease progression is not reported as an AE. Death, life-threatening consequences, hospitalization or prolongation of hospitalization, persistent or significant disability/incapacity, congenital anomaly/birth defect, or other important medical events caused by symptoms and signs of expected disease progression are not subject to expedited SAE reporting.

New antitumour treatment

If a subject starts a new antitumour treatment within 90 days after the last dose, only SAEs considered related to the study drug will subsequently be recorded and reported.

5. Expected adverse events and prevention/management plans

(1) Infusion reactions

Subjects should be monitored for signs and symptoms of infusion reactions, including fever and/or chills, flushing and/or pruritus, changes in heart rate or blood pressure, dyspnoea or chest discomfort, and rash, as well as systemic hypersensitivity reactions such as generalized urticaria, angioedema, asthma, hypotension, and tachycardia. Primary prophylaxis for infusion reactions is not permitted in subjects who have not experienced a prior infusion event. The investigator may use appropriate secondary prophylaxis after an initial infusion-related reaction at his or her discretion:

- Acetaminophen/paracetamol and/or an antihistamine such as diphenhydramine may be administered approximately 30 minutes before subsequent infusions, and/or a corticosteroid or equivalent may be given according to local practice.
- A non-sedating antihistamine such as cetirizine may be considered for subjects with recurrent infusion reactions.
- If symptoms persist despite the above measures, corticosteroid treatment should be considered.
- According to local practice, intravenous pethidine/meperidine or morphine sulfate plus promethazine, or equivalent agents, may be considered before subsequent infusions.

Premedications may need to be repeated during the infusion; this should be considered when determining the dose. Any major corticosteroid-related reaction or requirement must be discussed with the medical monitor.

For a grade ≤ 2 infusion reaction, the cadonilimab infusion rate may be reduced by 50%, or the infusion may be interrupted until the event resolves, for up to 6 hours. After resolution, the infusion may be restarted at 50% of the original infusion rate and continued to completion. Once the infusion rate has been reduced by 50% or an infusion has been interrupted because of an infusion reaction, all subsequent infusions must be administered at the reduced rate. If a second grade ≥ 2 infusion reaction occurs at the slower rate, the infusion should be stopped. Cadonilimab must be discontinued permanently for any grade 3 or 4 infusion reaction.

If a severe hypersensitivity reaction (CTCAE grade 3 or 4) is observed during infusion, the infusion must be stopped immediately and no further cadonilimab administered. Supportive treatment should be provided according to standard medical practice. Appropriate drugs and medical equipment for management of anaphylaxis or type I

hypersensitivity, including corticosteroids, epinephrine, and antihistamines, must be readily available at all study centres. Subjects should be instructed to report any delayed reaction immediately.

Table 3. Cadonilimab Treatment Modifications for Infusion Reactions

NCI CTCAE Grade	Cadonilimab Treatment Modification
Grade 1, mild Mild transient reaction; no interruption or treatment required.	Reduce the cadonilimab infusion rate by 50% and monitor closely for worsening. Total infusion time should not exceed 120 minutes.
Grade 2, moderate Requires treatment or interruption, but responds promptly to symptomatic treatment; prophylactic medication indicated for <24 hours.	Interrupt the cadonilimab infusion. Once the reaction has resolved or improved to grade ≤ 1 , restart at 50% of the previous infusion rate and monitor closely. If a second grade ≥ 2 reaction occurs at the slower rate, stop the infusion and discontinue cadonilimab.
Grade 3 or 4, severe or life-threatening Grade 3: prolonged response or recurrence after initial improvement; hospitalization indicated. Grade 4: life-threatening; urgent intervention required.	Immediately stop the cadonilimab infusion and disconnect the infusion line. Permanently discontinue cadonilimab; no further cadonilimab may be administered.

(2) Immune-related adverse events

An irAE is an AE associated with drug exposure, consistent with an immune-mediated mechanism, and without a clear alternative aetiology. Because cadonilimab targets PD-1 and CTLA-4 and activates and expands T cells, irAEs may occur during the study. Potential irAEs may resemble those associated with anti-PD-1/PD-L1 therapy and include immune-mediated colitis, dermatitis, pneumonitis, hepatitis, encephalitis, nephritis, and endocrine disorders. In the absence of an alternative cause such as infection or disease progression, an immune-mediated aetiology should be considered for signs or symptoms consistent with these conditions.

Cadonilimab treatment modifications for potential irAEs will be based on toxicity severity according to NCI CTCAE version 5.0. The maximum permitted duration of cadonilimab interruption is 12 weeks. If the subject does not recover to a level permitting reinitiation within 12 weeks, cadonilimab should be permanently discontinued and the subject should enter follow-up. Exceptions include interruption longer than 12 weeks because of corticosteroid tapering for an irAE or because of an AE considered possibly unrelated or unrelated to cadonilimab; continuation in these circumstances requires discussion with the medical monitor.

In general, irAEs should be managed according to the following principles:

- Perform a comprehensive evaluation and exclude alternative causes such as disease progression, concomitant medication, or infection. Serological, immunological, and histological/biopsy data should be used where appropriate to support the diagnosis.
- Consider systemic corticosteroids, such as prednisone or an intravenous equivalent, for persistent low-grade or severe grade ≥ 3 events when appropriate.
- If symptoms recur or worsen during corticosteroid tapering, increase the corticosteroid dose until symptoms stabilize or improve, then restart tapering at a slower rate.
- For events that do not respond to systemic corticosteroids, consider a more potent immunosuppressant, such as infliximab or mycophenolate mofetil, after discussion with the medical monitor.
- Grade 3 or 4 inflammatory reactions caused by local tumour response, such as inflammation at metastatic sites or lymph nodes, do not mandatorily require discontinuation of study treatment.
- Arrange timely consultation with an appropriate specialist, such as a cardiologist or autoimmune-disease specialist.
- If multiple low-grade AEs occur simultaneously, the investigator should determine whether cadonilimab should be discontinued even if each event alone would not require discontinuation.
- After study-drug discontinuation for a grade 3 or 4 event, cadonilimab may be restarted after discussion with the medical monitor if the subject responds promptly and the toxicity resolves rapidly.
- For laboratory abnormalities, interruption or permanent discontinuation should be determined according to associated clinical symptoms/signs and the investigator's clinical judgment.

Recommended dose modifications and toxicity-management principles for immune-related AEs are shown in Table 4. For irAEs not covered by the table or for special circumstances, refer to the American Society of Clinical

Oncology clinical practice guideline on management of immune-related adverse events in patients treated with immune checkpoint inhibitors.¹⁶ Diagnostic evaluation guidance for immune-related adverse events is provided in the appendix referenced in the source protocol.

Table 4. Recommended Cadonilimab Dose Modifications and Toxicity Management for Immune-Related AEs

Immune-related AE	Toxicity grade	Action for cadonilimab	irAE management	Monitoring and follow-up
Pneumonitis	Grade 2	Interrupt	Corticosteroid, initial prednisone 1-2 mg/kg or equivalent, followed by gradual taper.	Monitor signs and symptoms; perform imaging for suspected pneumonitis; initiate corticosteroid treatment; consider prophylactic antibiotics against opportunistic infection.
Pneumonitis	Grade 3-4 or recurrent grade 2	Permanently discontinue	Corticosteroid management as clinically indicated.	As above.
Diarrhoea/colitis	Grade 2-3	Interrupt	Corticosteroid, initial prednisone 1-2 mg/kg or equivalent, followed by gradual taper.	Monitor for gastroenteritis and intestinal perforation. For grade ≥ 2 diarrhoea with suspected colitis, consider gastroenterology consultation and endoscopy.
Diarrhoea/colitis	Grade 4	Permanently discontinue	Corticosteroid management as clinically indicated.	As above.
AST/ALT or bilirubin elevation	Grade 2	Interrupt	Corticosteroid, initial prednisone 0.5-1 mg/kg or equivalent, followed by gradual taper.	Monitor liver function weekly or more frequently until liver enzymes return to baseline or stabilize.
AST/ALT or bilirubin elevation	Grade 3	Interrupt or permanently discontinue [†]	Corticosteroid, initial prednisone 1-2 mg/kg or equivalent, followed by gradual taper.	As above.
AST/ALT or bilirubin elevation	Grade 4	Permanently discontinue	Corticosteroid, initial prednisone 1-2 mg/kg or equivalent, followed by gradual taper.	As above.
Type 1 diabetes mellitus or hyperglycaemia	New-onset T1DM or hyperglycaemia related to beta-cell failure	Interrupt	Initiate insulin replacement for T1DM or glucose-lowering treatment for hyperglycaemia.	Monitor blood glucose and other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Interrupt	Use corticosteroids and initiate hormone replacement if clinically indicated.	Monitor signs and symptoms, including hypopituitarism and adrenal insufficiency.
Hypophysitis	Grade 3-4	Interrupt or permanently discontinue [†]	Use corticosteroids and hormone replacement as clinically indicated.	As above.
Hyperthyroidism	Grade 2	Continue	Use a non-selective beta blocker such as propranolol or a thionamide as appropriate.	Monitor signs and symptoms of thyroid disease.
Hyperthyroidism	Grade 3-4	Interrupt or permanently discontinue [†]	Treat as clinically indicated.	As above.
Hypothyroidism	Grade 2-4	Continue	Initiate thyroid-hormone replacement, such as levothyroxine or liothyronine, according to standard practice.	Monitor signs and symptoms of thyroid disease.
Nephritis and renal dysfunction	Grade 2	Interrupt	Corticosteroid, initial prednisone 1-2 mg/kg or equivalent, followed by gradual taper.	Monitor changes in renal function.
Nephritis and renal dysfunction	Grade 3-4	Permanently discontinue	Corticosteroid management as above.	Monitor renal function.
Myocarditis	Grade 1	Interrupt	Use corticosteroids according to AE type and severity.	Ensure adequate evaluation to confirm aetiology and/or exclude other causes.
Myocarditis	Grade 2	Interrupt or permanently discontinue [†]	Use corticosteroids according to AE type and severity.	As above.
Myocarditis	Grade 3-4	Permanently discontinue	Use corticosteroids according to AE type and severity.	As above.

Immune-related AE	Toxicity grade	Action for cadonilimab	irAE management	Monitoring and follow-up
All other irAEs	Intolerable or persistent grade 2	Interrupt	Use corticosteroids according to AE type and severity.	Ensure adequate evaluation to confirm aetiology and/or exclude other causes.
All other irAEs	Grade 3	Interrupt or permanently discontinue according to AE type; permanent discontinuation is required for events including, but not limited to, Guillain-Barre syndrome and encephalitis.	Use corticosteroids according to AE type and severity.	As above.
All other irAEs	Grade 4 or recurrent grade 3	Permanently discontinue	Use corticosteroids according to AE type and severity.	As above.

¹ Whether to interrupt or permanently discontinue treatment should be decided after discussion between the investigator and the medical monitor.

Notes:

- When an AE improves to grade 0-1 or baseline, corticosteroids should be tapered over at least 4 weeks.
- For cadonilimab interruption, treatment may be restarted if the AE improves to grade 0-1 or baseline and corticosteroids have been tapered to ≤ 10 mg/day prednisone or equivalent. If the AE has not resolved within 12 weeks after the last dose, or corticosteroids cannot be tapered to ≤ 10 mg/day within 12 weeks, cadonilimab should be permanently discontinued.
- For severe or life-threatening irAEs, intravenous corticosteroids should be initiated and followed by oral corticosteroids. If response is inadequate, other immunosuppressants should be considered.
- For grade 3-4 immune-related endocrine disorders requiring interruption, cadonilimab may be resumed when the AE improves to grade ≤ 2 and is controlled with hormone replacement, or when metabolic stability is achieved, such as in T1DM.

If these principles differ from other immune-checkpoint-inhibitor toxicity-management guidelines or local standard clinical practice, the investigators should discuss the case and manage toxicity accordingly.

(3) Non-immune-related adverse events

AEs related to cadonilimab are generally expected to arise through an immune-mediated mechanism and therefore to be classified as irAEs. For a grade ≥ 3 non-immune-related AE considered related to cadonilimab by the investigator, cadonilimab may be interrupted and clinical management provided. After the event resolves or improves, continuation may be considered at the investigator's discretion. AEs clearly unrelated to cadonilimab or clinically insignificant laboratory abnormalities do not necessarily require treatment interruption. All toxicities will be graded according to NCI CTCAE version 5.0. Non-immune-related reactions will be managed according to routine clinical practice at each study centre.

(4) Toxicity management and dose modification for gemcitabine and cisplatin

Two dose reductions are permitted for chemotherapy. The first reduction is to 75% of the original dose and the second to 50%. Once a dose reduction is required, the reduced dose will be maintained in subsequent cycles. If a third reduction would be required because of toxicity after two prior reductions, the relevant chemotherapy drug must be discontinued.

Chemotherapy may be withheld for safety reasons for up to 6 weeks from the previous chemotherapy dose. In general, if a chemotherapy-related AE has not recovered to grade ≤ 1 or baseline despite active symptomatic and supportive treatment within 6 weeks, or if a chemotherapy-related safety risk remains, the investigator should consider discontinuation in light of the potential clinical benefit. If treatment is withheld for more than 6 weeks because of an AE considered possibly unrelated or unrelated to chemotherapy, the investigator should consider whether treatment may continue and discuss with the medical monitor if necessary. If toxicity is attributed to only one chemotherapy drug, dose adjustment of the other drug is not required. Toxicities caused by gemcitabine or cisplatin should be managed according to prescribing information and standard clinical practice.

6. Clinical treatment subsidy and compensation

Cadonilimab will be stored by the GCP centre and provided free of charge to subjects enrolled in the clinical study. The sponsor will purchase insurance for study participants in accordance with local regulations and minimum requirements. If a subject experiences any side effect or other bodily injury related to study-drug treatment or

diagnostic examinations required by the protocol, medical expenses will be compensated through the insurer to the extent permitted by Chinese law, excluding injury caused by the natural progression of an underlying disease or by a medical accident. Insurance coverage extends from enrolment of the first subject until completion of the study by the last subject. The deductible is RMB 5,000 per subject per accident, the maximum compensation is RMB 500,000 per subject per accident, and the premium is RMB 300 per subject.

IX. TIMING AND METHOD OF COMMUNICATING NEW STUDY INFORMATION

Investigators should promptly communicate any amendment to the study protocol. All protocol amendments require approval by the institutional review board/independent ethics committee (IRB/IEC). Except where an immediate change is necessary to protect subject safety, amendments may be implemented only after IRB/IEC approval and approval by the national regulatory authority where applicable. If an immediate change is required, the circumstances must be documented and reported to the IRB/IEC within 5 working days. If a protocol amendment requires revision of the site informed consent form (ICF), the revised ICF must be approved by the site IRB/IEC before use.

The study will be conducted in strict accordance with applicable laws and regulations to protect subject safety and rights. New information may arise during the study, including updates to treatment, changes in the study hypothesis, or safety warnings. Such information will be provided promptly to subjects. A plan has been established to communicate supplementary information. Within 24 hours after new information is identified, subjects will be informed by written notice and telephone, and a face-to-face meeting will be arranged to address questions. Subjects will be asked to confirm that they understand the information; a written confirmation is not required. Communication and subject confirmation will be documented and archived. Supplementary information will be updated monthly, and urgent information will be communicated promptly. A hotline and email address will be available for subjects to contact study personnel, and regular face-to-face visits will be arranged during the study to assess health status and answer questions.

X. QUALITY CONTROL AND QUALITY ASSURANCE

The principal investigator will organize relevant training to ensure standardized study conduct. Completion of CRFs or study records and all other reports must comply with Good Clinical Practice (GCP) and the study protocol. GC chemotherapy and cadonilimab administration for all patients will be overseen by Professor Ji Ma of the Department of Oncology, West China Hospital, Sichuan University. Patients will purchase GC chemotherapy, while cadonilimab will be provided free of charge for the study, stored by the GCP centre, and supplied to patients at no cost.

All data and treatments must be verifiable. To ensure reliability, all observations and findings must be capable of source verification. Quality-control measures will be applied at every stage to ensure data reliability and procedural accuracy. The principal investigator must ensure protocol compliance, verify the accuracy of data and completeness of records and reports, and ensure that informed consent is obtained from all subjects before study procedures begin. Any protocol violation or deviation must be reported promptly to the ethics committee. Where necessary, the study team will develop standard operating procedures and implement quality-control processes throughout study conduct and data handling to ensure standardized and reliable operations.

XI. DATA AND SAFETY MONITORING

A data and safety monitoring plan will be developed according to study risk. All AEs will be documented in detail, managed appropriately, and followed until resolution or stabilization. SAEs and unexpected events will be reported promptly to the ethics committee, competent authorities, sponsor, and drug regulatory authority as required. The principal investigator will periodically conduct cumulative reviews of all AEs and convene investigator meetings when necessary to assess study risks and benefits. In blinded studies, emergency unblinding may be performed when necessary to protect subject safety and rights.

XII. STATISTICAL ANALYSIS

1. Sample-size calculation

This study uses a two-stage design. Historical data from the ABC-02 phase III trial of standard gemcitabine and cisplatin treatment are used as the reference.¹⁰ The ORR according to RECIST was approximately 26% (42/161). Assuming an ORR of 40% for cadonilimab plus GC, a one-sided alpha of 0.10, beta of 0.20 (80% power), and a 10% dropout rate, 65 subjects are expected to be required. Under the two-stage design, at least 8 responses among the first 26 subjects are required to proceed to the second stage.

2. Statistical methods

Continuous variables will be summarized using the number of observations, mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized using frequencies and percentages.

Safety measures, including AEs, laboratory tests, and vital signs, will be summarized for patients with unresectable locally advanced or metastatic BTC treated with cadonilimab plus GC.

ORR and DCR and their 95% confidence intervals will be calculated. Median PFS and median OS will be estimated using the Kaplan-Meier method. For subjects with an objective response, DoR will be calculated as of the data-analysis cutoff.

XIII. ETHICAL PRINCIPLES AND REQUIREMENTS

The clinical study will comply with the World Medical Association Declaration of Helsinki and relevant Chinese regulations, including the Measures for Ethical Review of Biomedical Research Involving Humans issued by the National Health and Family Planning Commission of the People's Republic of China. Requirements regarding informed consent, privacy protection, free study-related care and compensation, risk control, protection of vulnerable subjects, and compensation for research-related injury will be implemented.

The study may begin only after the protocol has been approved by the ethics committee. Before enrolment, the investigator is responsible for fully explaining the study objectives, procedures, and potential risks to each subject and/or the subject's legally authorized representative and obtaining written informed consent. Subjects must be informed that participation is entirely voluntary, that they may refuse to participate or withdraw at any time without discrimination or retaliation, and that their medical care and rights will not be affected. The informed consent form must be retained as a clinical-study document, and subject privacy and data confidentiality must be protected.

XIV. STUDY TIMELINE

- July 2023 - December 2024: Complete subject enrolment.
- January 2025 - February 2026: Complete follow-up for all subjects.
- February 2026 - July 2026: Analyse and summarize study results.

XV. STUDY PERSONNEL

Name	Professional Title	Specialty	Task	GCP Training Certificate
Wentao Wang	Director, Department of Liver Surgery			Yes
Ji Ma	Associate Chief Physician			Yes
Tao Wang	Attending Physician			No
Yiwen Qiu	Resident Physician			No
Yi Yang	Resident Physician			No

XVI. KEY REFERENCES

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English translation of the Chinese Protocol Version 4.0 dated 20 June 2023.