nature medicine

Supplementary information

https://doi.org/10.1038/s41591-026-04679-x

Trastuzumab plus nivolumab with gemcitabine and cisplatin as first-line therapy for HER2-positive unresectable biliary tract cancer: a phase 1b/2 trial

In the format provided by the authors and unedited

Protocol

First-line trastuzumab, gemcitabine, cisplatin and nivolumab in advanced HER2-positive biliary tract cancer: a multicenter, open-label, single-arm phase Ib/II trial (HERBOT)

Principal Investigator
Name: Choong-kun Lee
Affiliate: Yonsei Cancer Center, Yonsei University College of Medicine, Korea

Co-Investigators

[redacted]

Contents

Table: Schedule of Activity

Trial Period		Screening	Treatment Cycles[a]				Post-Treatment	
Treatment Cycle/Title		Screening	Cycle 1		Every cycle (from Cycle 2 and beyond_		Follow up visits	Survival follow-up
Remark			Day 1	Day 8	Day 1	Day 8	Within 30day from last dose	Every 3 months from date of last dose administration
Scheduling Window (day)[b]		-28 to 0	±3	±3	±3	±3	±7	±7
Informed consent		X						
Inclusion/Exclusion Criteria		X	X					
Demographics and Medical History		X						
Disease Details and treatments		X						
Pathological findings		X						
Prior and Concomitant medication review		X	X		X		X	X
Review adverse effects[c]		x	X		X		X	X
Trial Treatment administration			X		X			
Post-study anticancer therapy status[d]							X	X
Survival status							X	X
Full Physical examination[e]		X						
Directed Physical examination[e]			X	X	X	X	X	
Vital signs and weight		X	X	X	X	X	X	
ECOG Performance status		X	X	X	X	X	X	
Laboratory tests[f]	Hematology	X	X	X	X	X	X	
	Coagulation	X	If needed*					
	Blood chemistry	X	X	X	X	X	X	
	Urinalysis	X	If needed*					
	Pregnancy test[g]	X	If needed*					
	Thyroid function test	X	Each 3 cycles (C4, 7, …)					
	Hepatitis test (HBsAg, anti-HCV)	X	If needed*					
	Tumor marker (CEA, CA19-9)	X	Each 3 cycles (C4, 7, …)				X	
Quality of Life: Self-reported survey (PRO)		X	Each 3 cycles (C4, 7, …)				X	
ECG		X	Each 3 cycles (C4, 7, …)					
ECHO or MUGA scan[h]		X	Every 12 weeks from first dose of administration ±7 days					
Chest X-ray		X	If needed*					
Tumor imagint Test[i]		X	Each 3 cycles (C4, 7, …)				X[j]	
Tumor tissue sampling[k]		X	C2D1 (optional)				X (optional)	
Blood sampling[l]		X	C2D1, C4D1				X	

Administration of the study drug							
Nivolumab		X		X			
Trastuzumab		X		X			
Gemcitabine		X	X	X	X		
Cisplatin		x	X	X	X		

a. Unless otherwise specified, assessments/procedures are to be performed on Day 1 and prior to the first dose of treatment for each cycle.

b. Unless otherwise specified, the window for each visit is ± 3 days.

c. The investigator will record all previous medications and combination therapy used from the time the subject signs the agreement to 30 days after the last dose of the study drug. If a subject initiates a novel anti-cancer therapy within 30 days from the last dose of the study drug, a history of previous medication/combination therapy and adverse events will no longer be recorded. All medications and combination therapy associated with the SAE must be tracked and recorded until SAE is resolved.

d. If the subjects plan to initiate a new treatment <30 days after their last dose of the study drug, they should schedule a follow-up visit before starting their new treatment. Survival follow-up will be performed following the initial of their new therapy.

e. Full physical examination will include assessments of the following body parts: kidney (only screening/baseline), head and neck, lymph nodes, breast, respiratory system, cardiovascular system, abdomen, genitourinary system, pelvis, rectum, musculoskeletal system, nervous system, mental health, skin, etc. Assessments for directed physical examination will be selected by the investigators based on the subject's clinical performance and symptoms.

f. Clinical laboratory tests should be performed, reviewed, and recorded prior to first dose administration. Refer to protocol 7.10.1 Laboratory assessments for specific details. Additional examinations can be performed under the investigator's judgement.

g. Women of reproductive potential must have a negative pregnancy test result (urine/ serum β-hCG), confirmed within 72 hours prior to first dose of treatment

h. Echo or MUGA will be performed during screening visit prior to treatment administration, and further assessed every 12 weeks from the date of first drug administration (despite treatment delays). The window is + 7 days.

i. A baseline Tumor Imaging Test should be performed within 28 days from the date of first dose administration. A baseline tumor imaging test should be performed within 28 days before the date of the first administration of the study drug. A scan performed as part of a routine clinical management procedure can be used as a baseline scan if it corresponds to the quality level of the diagnostic test and has been performed within the specified screening period. A CT of the thorax, abdomen, and pelvis area is required to assess the tumor response; an MRI or PET-CT can be used in a complementary manner to study the abdomen area. A WBBS can be added if a subject shows bone metastasis. Tumor response imaging tests will be performed every 3 cycles (C4, C7, · · ·), and the window is + 7 days. The imaging technique should remain the same per subject.

j. Subjects, who are discontinuing the trial for reasons other than the progression of the disease, must undertake a tumor imaging test every 2 months- since their last tumor imaging test- until disease progression is confirmed.

k. 1--20 undyed slides or bulk tumor samples will be collected, before, C2D1 and after treatment, among subjects who have agreed to undertake the test. (The number of slides can be negotiated with the main (principal) institution if there are insufficient slides).

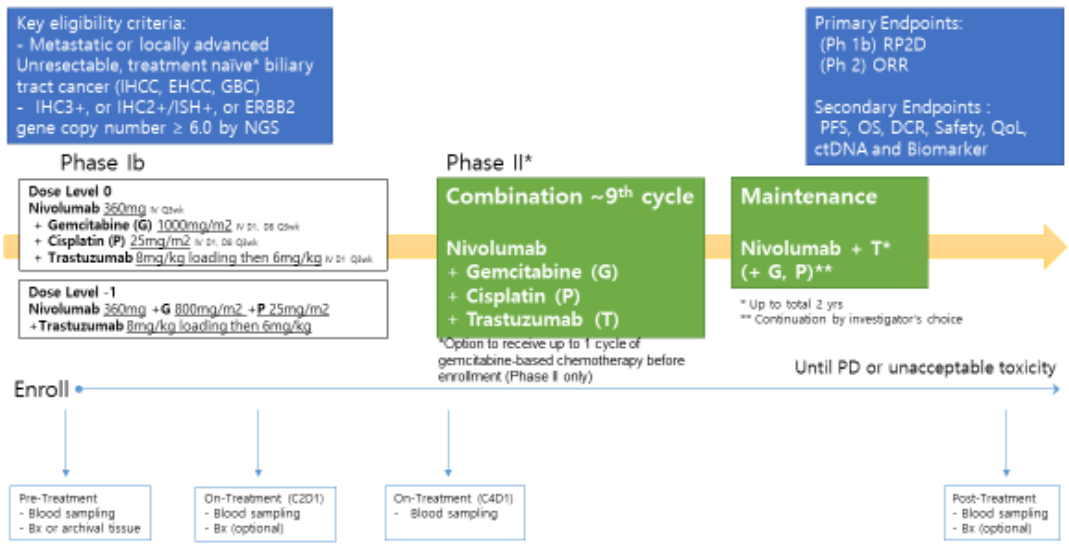
l. Blood specimen will be collected for biomarker research purposes (before, C2D1, C4D1 and after treatment; 10cc *2 (a total of 20cc whole blood)

m. For central confirmation, either two unstained slides for HER2 IHC and SISH or one HER2 IHC-stained slide plus one unstained slide will be collected at screening for confirmation by the central lab. For additional NGS analysis, 10–20 unstained slides or a tumor block will be collected at pre-treatment, C2D1 (optional), and end of study (optional). If the required number of slides is unavailable, consultation with the coordinating center is allowed.

*The test is performed when irAE, infection, IP-related side effects are suspected, the researcher determines that the test is necessary.

1.0 SYNOPSIS

Title/Phase	First-line trastuzumab, chemotherapy and nivolumab in advanced HER2-positive biliary tract cancer: a multicenter, open-label, single-arm phase Ib/II trial
Study Drug(s)	Trastuzumab, Nivolumab, Gemcitabine, Cisplatin
Study duration	February 2023 – February 2027 Enrollment period: Feb 2023 – Feb 2025 (2 years) Observation period: Feb 2025 – Feb 2026 (1 year) Data analysis period: Feb 2026 – Feb 2027 (1 year)
Study chair/ Institution	Yonsei Cancer Center
Study method	Multicenter, open-label, single-arm study
Objectives	1) Primary Objective: Phase Ib: Recommended Phase II Dose Phase II: Overall Response Rate (ORR) 2) Secondary Objectives: Progression Free survival (PFS) Overall Survival (OS) Disease Control Rate (DCR) Duration of Response (DOR) Safety/QoL 3) Exploratory Objectives: ctDNA Biomarker AI-based HER2 overexpression or immune phenotype and treatment response
Study Design	



Dosing & Treatment Schedule	Nivolumab 360mg IV q 3weeks Gemcitabine 1000mg/m2 IV D1, D8 q 3weeks Cisplatin 25mg/m2 IV D1, D8 q 3weeks Trastuzumab 8mg/kg (C1) then 6mg/kg IV q 3weeks
Target Accrual and Study duration	Phase Ib: 3 or 6 subjects Phase II: 38 subjects Study duration : from Feb.1st of 2023 to Feb. 1st of 2027
Study Procedures	Phase Ib/II, Open label, Multicenter
Statistical Consideration	Total: 41~44 patients In phase Ib part, 3 to 6 patients will be enrolled. The size of the dose de-escalation cohort was determined using a conventional 3+3 design. If DLTs occur even at dose level -1, the study will be temporarily suspended to allow for additional safety review and consideration of potential modifications to the study design. In phase II part, according to Simon's two-stage minimax design, a minimum sample size of 32 patients was required to accept the hypothesis that the true response rate was 45% with 80% power and to reject the hypothesis that the response rate was less than 27% (reference TOPAZ-1 response rate 26.7%), with a type I error of 0.1. The study was to be stopped if fewer than five responses were observed among the initial 16 patients in the first stage. Considering the 10% drop-out rate, total 38 patients will be enrolled to the phase II part.
Subject selection	Major Inclusion criteria 1) Subjects with histologically- or cytologically-confirmed biliary tract cancer (including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gall bladder cancer) (patients with neuroendocrine tumors, sarcomas, or other histologies without evidence of adenocarcinoma will be excluded.) 2) HER2 positive biliary tract cancer (IHC 3+ or 2+ with ISH + (HER2/CEP17≥2.0 or average HER2 gene copy number ≥ 6 signals/cell) or ERBB2 gene copy number ≥ 6.0 by NGS) 3) Age (at the time of informed consent): 20 years and older 4) Previously untreated if unresectable/metastatic at initial diagnosis; or recurrent disease >6 months after curative surgery or adjuvant thearpy (allow up to 1 cycle of gemcitabine-based chemotherapy for advanced/unresectable or metastatic cholangiocarcinoma prior to enrollment. See appendix) 5) Explicit and voluntary consent to participate in the study obtained using a signed and dated informed consent form clearly and fully describing the purpose, potential risks, and any other critical issues regarding the study 6) Subject with measurable lesions according to RECIST v. 1.1 7) ECOG Performance Status Score 0 or 1 8) Patients with a life expectancy of at least 3 months 9) Patients whose latest laboratory data meet the below criteria within 14 days before enrollment. White blood cells ≥2,000/mm3 and neutrophils ≥1,500/mm3 Platelets ≥100,000/mm3 Hemoglobin ≥9.0 g/dL AST (GOT) and ALT (GPT) ≤3.0-fold the upper limit of normal (ULN) of the study site (or ≤5.0-fold the ULN of the study site in patients with liver metastases) Total bilirubin ≤1.5-fold the ULN of the study site Creatinine ≤1.5-fold the ULN of the study site or creatinine clearance (either the measured or estimated value using the Cockcroft-Gault equation) >45 mL/min

INR ≤1.5-fold or prothrombin time ≤1.5-fold the ULN of the study site
aPTT ≤1.5-fold the ULN of the study site

10) Women of childbearing potential (including women with chemical menopause or no menstruation for other medical reasons) #1 must agree to use contraception#2 from the time of informed consent until 5 months or more after the last dose of the investigational product. Also, women must agree not to breastfeed from the time of informed consent until 5 months or more after the last dose of the investigational product.

11) Men must agree to use contraception#2 from the start of study treatment until 7 months or more after the last dose of the investigational product.

#1. Women of childbearing potential are defined as all women after the onset of menstruation who are not postmenopausal and have not been surgically sterilized (e.g., hysterectomy, bilateral tubal ligation, bilateral oophorectomy). Postmenopause is defined as amenorrhea for ≥12 consecutive months without specific reasons. Women using oral contraceptives, intrauterine devices, or mechanical contraception such as contraceptive barriers are regarded as having childbearing potential.

#2. The subject must consent to use any of the following methods of contraception: vasectomy or condom for patients who are male or female subject's partner and tubal ligation, contraceptive diaphragm, intrauterine device, spermicide, or oral contraceptive for patients who are female or male subject's partner.

12) EF ≥ 50% via Transthoracic echocardiography or MUGA scan

13) Subjects willing to provide tumor biopsy tissue or excisional biopsy tissue.

14) Subjects with adequate organ function

Major Exclusion Criteria

1. Patients treated with systemic chemotherapy, biologic therapy, immunotherapy, hormone therapy, or clinical trials for unresectable, locally advanced or metastatic biliary tract cancer. However, the following are excluded.
   a. If disease recurrence occurs 6 months after the last dose of adjuvant chemotherapy, previous adjuvant chemotherapy is permitted.
   b. 1 cycle of Gemcitabine-based anticancer therapy for locally advanced or metastatic cholangiocarcinoma prior to enrollment in this trial is permitted.
2. Patients with multiple primary cancers (with the exception of completely resected basal cell carcinoma, stage I squamous cell carcinoma, carcinoma in situ, intramucosal carcinoma, or superficial bladder cancer, or any other cancer that has not recurred for at least 5 years)
3. Patients with residual adverse effects of prior therapy or effects of surgery that would affect the safety evaluation of the investigational product in the opinion of the investigator or sub-investigator.
4. Patients with current or past history of severe hypersensitivity to any other antibody products
5. Patients with concurrent autoimmune disease or history of chronic or recurrent autoimmune disease
6. Patients with a current or past history of interstitial lung disease or pulmonary fibrosis diagnosed based on imaging or clinical findings. Patients with radiation pneumonitis may be enrolled if the radiation pneumonitis has been confirmed as stable (beyond acute phase) without any concerns about recurrence.
7. Patients with concurrent diverticulitis or symptomatic gastrointestinal ulcerative disease
8. Patients with any metastasis in the brain or meninx that is symptomatic or requires

	treatment. Patients may be enrolled if the metastasis is asymptomatic and requires no treatment. 9. Patients with pericardial fluid, pleural effusion, or ascites requiring treatment (Eligible if adequately controlled with medication or intervention). 10. Patients with uncontrollable, tumor-related pain 11. Patients who have experienced a transient ischemic attack or cerebrovascular accident within 180 days before enrollment 12. Patients with a history of uncontrollable or significant cardiovascular disease meeting any of the following criteria: • Myocardial infarction within 180 days before enrollment • Uncontrollable angina pectoris within 180 days before enrollment • New York Heart Association (NYHA) Class III or IV congestive heart failure • Uncontrollable hypertension despite appropriate treatment (e.g., systolic blood pressure ≥150 mmHg or diastolic blood pressure ≥ 90 mmHg lasting 24 hours or more) • Arrhythmia requiring treatment 13. Patients with uncontrollable diabetes mellitus 14. Patients with systemic infections requiring treatment (infection controlled by oral antibiotics is permitted) 15. Patients who have received systemic corticosteroids (prednisolone or equivalent > 10mg/day) (except for temporary use, e.g., for examination or prophylaxis of allergic reactions) or immunosuppressants within 28 days before enrollment 16. Patients who have received antineoplastic drugs (e.g., chemotherapy agents, molecular-targeted therapy agents, or immunotherapy agents) within 28 days before enrollment 17. Patients who have undergone surgical adhesion of the pleura or pericardium within 28 days before enrollment 18. Patients who have undergone surgery under general anesthesia within 28 days before enrollment 19. Patients who have undergone surgery involving local or topical anesthesia within 14 days before enrollment 20. Patients who have received radiotherapy within 28 days before enrollment (Except for surgical procedures performed solely for tissue biopsy), or radiotherapy to bone metastases within 14 days before enrollment 21. Patients who have received any radiopharmaceuticals (except for examination or diagnostic use of radiopharmaceuticals) within 56 days before enrollment 22. Patients with a positive test result for any of the following: HIV-1 antibody, HIV-2 antibody, HTLV-1 antibody, HBs antigen, or HCV antibody 23. Patients with active hepatitis B or C virus (Patients with hepatitis B are eligible for enrollment if they are HBsAg-positive but have HBV DNA <500 IU/mL and are receiving stable antiviral therapy. Patients with hepatitis C are eligible if they are anti-HCV positive but have no detectable HCV RNA, or if HCV RNA is detectable, they are receiving treatment prior to providing informed consent for the clinical trial.) 24. Women who are pregnant or breastfeeding, or possibly pregnant 25. Patients who have received any other unapproved drug (e.g., investigational use of drugs, unapproved combined formulations, or unapproved dosage forms) within 28 days before enrollment 26. Patients who have previously received Nivolumab, anti-PD-1 antibody, anti-PD-L1 antibody, anti-PD-L2 antibody, anti-CD137 antibody, anti-CTLA-4 antibody or other

	therapeutic antibodies or pharmacotherapies for regulation of T-cells 27. Patients judged to be incapable of providing consent for reasons such as concurrent dementia 28. Other patients judged by the investigator or sub-investigator to be inappropriate as subjects of this study 29. Patient with current or past history of hypersensitivity to Nivolumab.

2.0 TITLE OF THE STUDY

First-line trastuzumab, chemotherapy and nivolumab in advanced HER2-positive biliary tract cancer: a multicenter, open-label, single-arm phase Ib/II trial (HERBOT)

3.0 PARTICIPATING CENTER

Total 12 centers: Yonsei Cancer Center, Gyeongsang National University, Asan Medical Center, Haeundae Paik Hospital, Korea University Anam Hospital, Chungnam National University Hospital, Seoul St. Mary's Hospital, Chonnam National University Hwasun Hospital, CHA Bundang Medical Center, Seoul National University Bundang Hospital, Kangbuk Samsung Hospital, Samsung Medical Center

4.0 PRINCIPAL INVESTIGATOR

Choong-kun Lee, Yonsei Cancer Center

5.0 BACKGROUND & RATIONALE

5.1 Study Rationale

Biliary tract cancer is a rare malignant neoplasm including intrahepatic cholangiocarcinoma (IhCCA), extrahepatic cholangiocarcinoma (EhCCA) and Gallbladder cancer (GBC). Radical surgical excision provide only curative therapy to patients with BTC, but almost all BTCs are diagnosed at unresectable stage. Therefore, survival outcome of advanced BTCs are still poor and heterogeneity of tissue and molecular differences between BTCs limit the clinical studies in BTCs.

Combination therapy of Gemcitabine and Cisplatin has become the standard of care after the ABC-02 trial(1). This trial demonstrated that the addition of cisplatin to gemcitabine improved survival outcomes compared to that with gemcitabine alone. However, the median overall survival (OS) of Gem/Cis chemotherapy is only about one year. Recently reported phase II trial testing first-line Gem/Cis plus nab-paclitaxel(2) showed promising efficacy, but toxicity was also increased, suggesting less toxic agents such as targeted agents or immune checkpoint inhibitors (ICIs) as affordable combination drug to improve poor outcome of first-line regimen for advanced BTC.

Anti-Program cell death-1 (anti-PD-1) inhibitor monotherapy including Nivolumab (OPDIVO) had shown efficacy in refractory, advanced BTC(3–5). In 2nd or 3rd line therapy, however, anti-PD-1 monotherapy showed objective response rate (ORR) of 11.3% in Korean BTC patients(6). This implies that administration of ICIs combined with cytotoxic or target agent chemotherapy in 1st line therapy would be a better therapeutic strategy in advanced BTCs.

Various ICIs combined with Gem/Cis as the 1st line treatment in BTCs are under the trials. Combination of

Nivolumab and Gem/Cis showed improved overall survival (15.4 months) in a small sized study (n=30) with tolerable side effects in advanced BTC patients(4). Recently reported interim analysis of phase III TOPAZ-1 trial (NCT03875235) showed Durvalumab, anti-PD-L1 agent, combined with Gem/Cis showed improvement of overall survival(7). Considering other studies currently ongoing, ICIs combined with Gem/Cis are thought to be the future standard of care in 1st line treatment of advanced stage BTCs.

Recent advances in genome profiling of BTCs showed available molecular targets such as FGFR2 fusion, IDH1 mutation and HER2 amplification/overexpression(8). HER2 amplification/overexpression is presented as many as 15% of total BTC patients. Basket trial of administration of pertuzumab and trastuzumab combination in previously treated HER2 positive advanced BTC patients showed promising overall response rate of 23%. Also, multicenter phase II study conducted by Korean investigators (KCSG-HB19-14) showed promising effect of Trastuzumab combined with modified FOLFOX in Gem/Cis refractory HER2 positive BTC patients with ORR of 29.4%(9). Moreover, preclinical data showed synergistic anti-cancer effect of trastuzumab combined with ICIs in HER2 positive cancers(10,11). Similar data are reported in HER2 positive gastric cancer that phase II and phase III clinical data showed 1st-line ICIs combined with trastuzumab and cytotoxic chemotherapy showed promising overall survival outcomes(12–14).

Pre-existing immune responses to tumor antigens are associated with the clinical activity of ICI. Enabling these responses through the use of tumor-targeted antibodies for the promotion of chemotherapeutic agents or cross-priming to induce immunogenic cell death or reduce immunosuppressive populations is the logical next step in the development of combination anticancer therapies. In preclinical models and patient-derived xenograft models, trastuzumab stimulates HER2-specific T cell responses by increasing HER2 internalization and cross-presentation by dendritic cells(15–17). Trastuzumab has also been shown to upregulate the expression of PD-1 and PD-L1 ligands, induce the expression of tumor-infiltrating lymphocytes, and regulate the expression of major histocompatibility complex class II(10,18–21). After chemotherapy, dying cancer cells stimulate dendritic cells to enhance antigen processing and presentation and promote priming of CD8+ tumor-specific T cells(22,23). Combination of ICI with trastuzumab has been shown to enhance HER2-specific T cell responses, promote immune cell trafficking, and induce expansion of peripheral memory T cells(24). A possible mechanistic interaction of this modality was demonstrated in a HER2-positive transgenic mouse model in which dual inhibition of PD-1 and HER2 resulted in tumor eradication.

In treating HER2-positive advanced BTC, the triple combination of nivolumab, trastuzumab, and cytotoxic chemotherapy (Gem/Cis) may overcome innate resistance and activate an immune response to cancer along with inhibiting oncogenic signal from HER2 pathway, resulting in a synergistic effect with a longer response. This study plan to test the efficacy and safety of first-line nivolumab plus trastuzumab and Gem/Cis for the treatment of HER2-positive advanced BTC.

5.2 Background Information

5.2.1 Background of Nivolumab

Immunotherapy represents a promising breakthrough treatment in advanced biliary tract cancer (BTC). Phase I study of combination therapy of Nivolumab, Gemcitabine and Cisplatin in previously untreated BTC showed progression free survival of 4.2 months and overall survival of 15.4 months. Phase II study of the identical regimen showed PFS of 6.6 months and OS of 10.6 months. The results seem quite disappointing, but interestingly 2 year survival was 35.4%. This improvement in the tail of the survival curve has been previously described in literature across multiple cancers highlighting a potential role of immunotherapy in biliary cancer in this subset. Nivolumab was not prospectively tested with targeted agent and cytotoxic chemotherapy for BTC patients.

5.2.2 Background of Trastuzumab

Recent studies of Next Generation Sequencing (NGS) revealed that targetable genetic abnormalities are found in about 50% of BTC patients. Multiple studies in solid tumors including BTC have demonstrated durable responses

to immunotherapy in MSI-high patients leading to the tumor agnostic approval of immunotherapy in this setting (with ORR of BTC 17%). Human epidermal growth factor receptor 2 (HER2; erythroblastic oncogene B2, ERBB2) is one of the most common oncogenic receptor tyrosine kinase, of which overexpression/amplification is reported in breast cancer (20-30%), gastric cancer (10-20%), and colorectal cancer. Monoclonal antibodies such as Trastuzumab, Pertuzumab and small-molecular inhibitors such as Lapatinib are known to show effective in HER2 amplified cancers. Also, HER2 is overexpressed or amplified in approximately 14.8–24.0% of gallbladder cancers (GBC), 7.8–12.9% of extrahepatic cholangiocarcinomas (EhCCA), and 3.1–4.5% of intrahepatic cholangiocarcinomas (IhCCA).

Few trials have shown the efficacy of HER2-targeting agents against HER2-positive BTC. BTC cohort results of a single-arm, open-label, phase IIa, multiple basket study (MyPathway) were recently reported. Among 39 HER2-positive patients, metastatic BTC patients with a median of two previous treatment lines were treated with pertuzumab and trastuzumab, a chemotherapy-free regimen. Pertuzumab plus trastuzumab resulted in an ORR of 23%, a median PFS of 4.0 months, and a median OS of 10.9 months, with relatively low grade 3 or higher incidences of treatment-related adverse events (46%)(25). The recently presented phase II HERB trial tested the efficacy of the HER2 antibody-drug conjugate trastuzumab deruxtecan for previously treated patients with BTC, resulting in an ORR of 36.4% for 22 HER2-positive patients with a median PFS and OS of 4.4 months and 7.1 months, respectively. Treatment-related adverse events leading to drug discontinuation occurred in eight patients, and two patients had grade 5 interstitial lung disease(26). In the recently presented phase II KCSG-HB19-14 trial, mFOLFOX in combination with trastumab was tested for Gem/Cis-refractory HER2-BTC patients as 2nd- or 3rd – line treatment(9). Thirty-four patients were enrolled and the regimen showed favorable efficacy with 29.4% (95%CI 15.1–47.5) of ORR and 79.4% disease control rate. Median progression-free survival was 5.1 months (95%CI 3.6–6.7); median overall survival was 10.7 (95%CI 7.9–not reached). The most common treatment-related grade 3 or 4 adverse events were neutropenia, grade 3 anemia, and grade 3 peripheral sensory neuropathy. There were no treatment-related cardiac toxicities or deaths. Therefore, HER2 amplification is thought to be an important treatment target in HER2 positive BTC, and unmet need exist to develop effective 1st line therapies for HER2 positive BTC.

5.2.3 Background of Chemotherapy

The randomized phase III ABC-02 trial have established gemcitabine/cisplatin as the standard-of-care (SoC) regimen as the palliative first-line chemotherapy for advanced BTC (Valle et al 2010)(1). Gemcitabine/cisplatin combination therapy is the most commonly used standard treatment regimen or backbone regimen to add novel drugs as first-line treatment. The recently reported randomized phase III TOPAZ-1 study is a first study show positive result on adding immune checkpoint inhibitor to gemcitabine/cisplatin(7). The study showed durvalumab with gemcitabine and cisplatin (SoC) of 8 cycles followed by durvalumab maintenance showed both improved OS and PFS compared to placebo with SoC regimen (Gemcitabine and Cisplatin) (12.8 month vs. 11.5 month, p=0.021). ICI plus gemcitabine and cisplatin is now thought as the standard regimen for the first-line treatment of advanced BTC.

5.3 Summary of Investigational Agents

Immune checkpoint blockade is a rapidly advancing therapeutic approach in the field of immuno-oncology and treatment with investigational agents targeting this mechanism has induced regressions in several types of cancer. Nivolumab (BMS-936558; anti-PD-1 monoclonal antibody) is a fully human monoclonal immunoglobulin (Ig) G4 antibody that binds to the PD-1 cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Inhibition of the interaction between PD-1 and its ligands promote immune responses and antigen-specific T cell responses to both foreign and self-antigens. Results from a Phase I/II study (CA209003/MDX1106-03) indicate that nivolumab is active in multiple tumor types.

Refer to the Investigator's Brochure for more preclinical and clinical data.

5.3.1 Preclinical Summary of Nivolumab

Nivolumab was shown to inhibit the binding of PD-1 to its ligands, PD-L1 and PD-L2, and to promote proliferation of human T cells in a variety of assays. Nivolumab blockade of the PD-1 pathway was studied using activity in the MLR as assessed by T-cell proliferation and IFN-γ secretion. PD-1 blockade resulted in a reproducible concentration-dependent enhancement of both proliferation and IFN-γ release in the mixed lymphocyte reaction up to 50 μg/mL. No effect was observed with a human IgG4 isotype control or CD4+ T cells and DC control(27). The role of anti-PD-1 in generating an anti-tumor response has been tested in a number of murine transplantable tumor models. A surrogate rat anti-mouse PD-1 antibody (4H2) was derived and expressed as a chimeric IgG1 murine antibody. Antibody 4H2 inhibited the binding of murine PD-1 to its ligands and is expected to have an improved in vivo half-life over the parental rat antibody(28).

5.3.2 Preclinical Summary of Trastuzumab

Refer to the Investigator's Brochure for more preclinical and clinical data.

5.3.3 Summary of Safety

Combination therapy of immune checkpoint inhibitor and Trastuzumab were done, typically in Gastric cancer. AIO INTEGA Randomized Clinical Trial showed tolerable adverse events of combination of Nivolumab, Trastuzumab and mFOLFOX regimen in HER2 positive gastric cancer. Specifically, grade 3 or more AE were reported in 88% of patients (38/44) among patients treated with Nivolumab, Trastuzumab and mFOLFOX. Neutropenia was the most common adverse event among the patients, reported in 10 patients (23%). 7 patients (16%) terminated the trial due to adverse events, which was judged to be expected by the investigators.

In phase II PANTHERA trial, trastuzumab, anti-PD-1 inhibitor pembrolizumab in combination with capecitabine and cisplatin was tested to HER2-positive advanced gastric cancer patients as palliative first-line treatment. Treatment-related adverse events occurred in 42 patients (97.7%). Non-hematologic adverse events were mostly graded 1 or 2, and the commonly reported grade 3 adverse events were mostly hematologic, including neutropenia (39.5%), anemia (16.3%), febrile neutropenia (9.3%), and thrombocytopenia (7%). Grade 4 adverse events occurred in two patients (neutropenia and thromboembolic event). One patient died from grade 5 pneumonia related to disease progression. Thirty-nine (90.7%) patients required one or more reductions in the dose of capecitabine or cisplatin. Nine (20.9%) patients discontinued capecitabine due to adverse events, although treatment was resumed in three after the resolution of toxicity. Seven (16.3%) patients discontinued cisplatin due to adverse events, two of whom resumed treatment after the resolution of toxicity. None of the treatment-related adverse events led to the discontinuation of trastuzumab. Immune-related adverse events occurred in 16 patients (37.2%), but none required pembrolizumab discontinuation. Grade 3 immune-related adverse events included colitis (3/43, 7%) and allergic reaction (1/43, 2.3%).

In PANACEA study, a single-arm, multicenter, phase Ib/II trial, assessed Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced, HER2-positive breast cancer. Adding Pembrolizumab showed promising efficacy with tolerable safety in HER2-positive breast cancer.

Most of the adverse events are thought to be from cytotoxic chemotherapy, and there were no added adverse events due to triple combination.

5.3.4 Summary of Clinical Activity

Preclinical animal studies have shown that trastuzumab treatment leads to an increase in the levels of cytotoxic T

cells, which can subsequently be stimulated by PD-1 blockade, leading to an improved response if antibodies targeting HER2 and antibodies blocking PD-1 are combined.

No clinical trials have assessed combination of Trastuzumab and Immune checkpoint inhibitor in BTCs yet. However, a randomized, double-blind, placebo-controlled phase KEYNOTE-811 study, which was designed to assess the Pembrolizumab combined to standard of care, Trastuzumab, Capecitabine with Oxaliplatin, for unresectable or metastatic, HER2-positive gastric or gastro-oesophageal junction adenocarcinoma showed promising efficacy and safety. In interim analysis of KEYNOTE-811, ORR was 74.4% (95% CI, 66.2–81.6) in the pembrolizumab group and of 51.9% (95% CI, 43.0–60.7) in the placebo group. This resulted in a statistically significant 22.7% improvement in objective response rate in the pembrolizumab group (P = 0.00006). As shown in other HER2-positive tumor type, anti-HER2 treatment in combination with ICI and cytotoxic chemotherapy will show anti-tumor survival benefit with tolerable safety profile.

Nivolumab 360mg every 3 weeks are thought to be equivalent to nivolumab 240mg every 2 weeks. Nivolumab 360mg every 3 weeks or nivolumab 240mg every 2 weeks in combination with fluoropyrimidine and platinum-containing chemotherapy for advanced or metastatic gastric or gastroesophageal junction cancer, and esophageal adenocarcinoma has been approved by Food and Drug Administration according to randomized phase III CHECKMATE-649 trial(29).

6.0 OBJECTIVES

6.1 Primary objectives

Phase Ib: Recommended Phase II Dose (RP2D)

Phase II: Objective Response Rate (ORR)

6.2 Secondary objectives

Progression-free survival (PFS)

Disease control rate (DCR)

Duration of Response (DOR)

Overall survival (OS)

Safety based on CTCAE 5.0.

Quality of life questionnaire (EORTC-QLQ-C30)

6.3 Exploratory objectives

Correlative biomarker exploration including ctDNA

AI-based HER2 overexpression or immune phenotype and treatment response

7.0 METHODOLOGY

Table. Trial Treatment (during phase Ib)

	Level 0	Level -1
Nivolumab	360mg fixed dose	360mg fixed dose
Samfenet® (Trastuzumab biosimilar)	6 mg/kg (8mg/kg loading dose)	6 mg/kg (8mg/kg loading dose)
Gemcitabine	1,000 mg/m^2	800 mg/m^2
Cisplatin	25 mg/m^2	25 mg/m^2

Table: Trial Treatment (during phase II)

Medicine	Dose	Duration	Dosage formulation	Route of administration
Nivolumab	360mg	Once in 3weeks	NS 100mL mix	IV infusion for 30min
Samfenet® (Trastuzumab biosimilar)	8mg/kg (Cycle 1) 6mg/kg (Cycle 2 and beyond)	Once in 3weeks	NS 250mL mix	IV infusion Cycle 1: 90min Cycle 2 and later: 30~90min
Gemcitabine	1000mg/m^2 (Dose level 0) 800mg/m^2 (Dose level -1)	D1 & D8 in 3weeks	NS 100mL mix*	IV infusion for 30min
Cisplatin	25mg/m^2 (Dose level 0)	D1 & D8 in 3weeks	NS 150mL mix*	IV infusion for 60min

*The type and dose of mix fluid can be altered as per guidelines of each institution. Cisplatin infusion should be performed with adequate hydration according to the guidelines of each institution.

Ono Pharmaceutical/Bristol-Myers Squibb will supply Nivolumab. Boryung Pharmaceutical will supply Samfenet® (Trastuzumab biosimilar) and Gemzar® (Gemcitabine). Cisplatin, and other solutions will be supplied locally.

7.1 Study Design

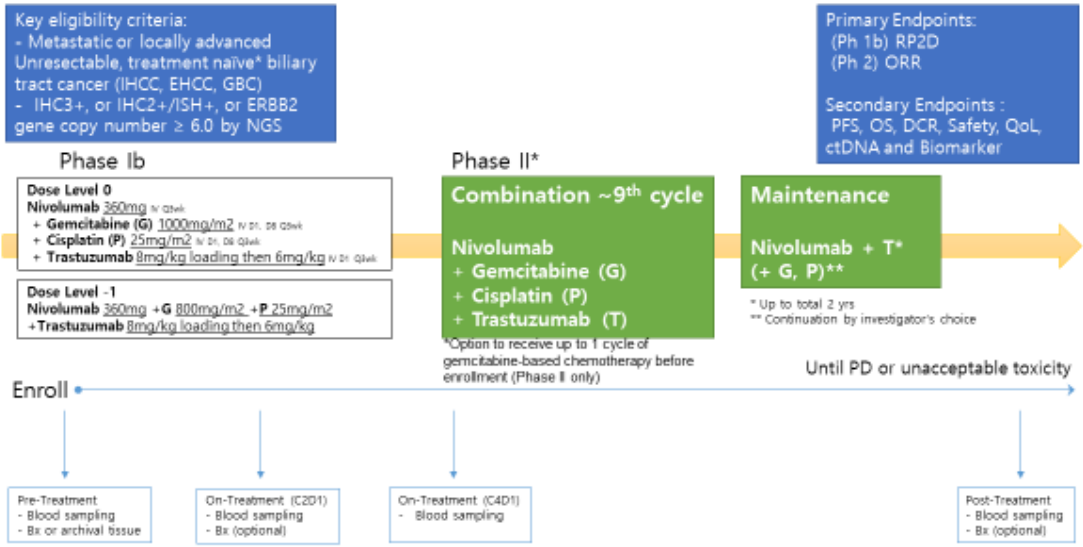


Figure 1: Study Design

This study assesses the combination therapy of Nivolumab, Trastuzumab and Gemcitabine, Cisplatin in previously untreated unresectable HER2 positive BTC. The study is a multicenter, open-label, single-arm phase Ib/II trial. Only patients without previous treatment are to be included, but one cycle of gemcitabine-based chemotherapy for locally advanced or metastatic cholangiocarcinoma is permitted before enrollment.

Nivolumab and trastuzumab will be administered up to 2 years, and continuation of gemcitabine/cisplatin beyond 9 cycle is at the discreation of the investigator.

7.2 Primary Endpoint:

The primary endpoint of the phase I study is determination of Recommended Phase II Dose (RP2D). This study will follow a standard 3+3 design, and dosing within a cohort will not continue if the dose is determined to have exceeded the MTD based on the incidence of DLTs. The study will begin with dose level 0. If dose level 0 is found to exceed the MTD, dose level -1 will be explored.

The primary efficacy endpoint of the phase II study is ORR (defined as the proportion of participants who have achieved confirmed CR or PR). This study will use ORR based on RECIST 1.1 criteria as assessed by investigator as the primary endpoint. Objective response rate is an acceptable measure of clinical benefit for a late-stage study that demonstrates superiority of a new antineoplastic therapy, especially if the magnitude of the effect is large and the therapy has an acceptable risk/benefit profile.

7.3 Secondary Endpoint:

The secondary efficacy endpoints include PFS, OS, DCR and DOR. PFS is defined as the time from enrollment to the first documented PD per RECIST 1.1 assessed by investigator or death from any cause, whichever occurs first. DOR is defined as the time from the first documented evidence of CR or PR until PD per RECIST 1.1 or death due to any cause.

Safety parameters frequently used for evaluating investigational systemic anticancer treatments are included as safety endpoints including, but not limited to, the incidence of causality, and outcome of AEs/SAEs, and changes in vital signs and laboratory values. AEs will be assessed as defined by NCI CTCAE v5.0.

Quality of life questionnaire (EORTC-QLQ-C30) is a psychometrically and clinically validated instrument appropriate for assessing HRQoL in oncology studies. The EORTC QLQ-C30 is the most widely used cancer specific HRQoL instrument, which contains 30 items and measures 5 functional dimensions (physical, role, emotional, cognitive and social), 3 symptom items (fatigue, nausea/vomiting and pain), 6 single items (dyspnea, sleep disturbance, appetite loss, constipation, diarrhea, and financial impact), and a global health and QoL scale.

7.4 Exploratory Endpoints

If available and agreed by the subjects, tissue slides, either biopsied or operation specimen will be obtained for exploratory endpoints before the administration of the study. H&E stained slide and HER2 IHC stained slides will be collected/analyzed to exploratory analyses the relation between treatment response and AI-based HER2 overexpression (Lunit SCOPE HER2) or immune phenotype (Lunit SCOPE IO).

Additional blood samples will be taken at selected time points specified in the applicable assessment schedules for assessment of biomarkers, including but not limited to ctDNA. If a study subject is independently tested for other biomarkers while on-study, the results may be collected.

8.0 STUDY PROCEDURES

8.1 Participant selection

8.1.1 Inclusion Criteria

Having provided written consent before participation in the study, patients must fulfill all of the following criteria to be eligible for enrollment. If a enrolled subject is found not to meet any of the following criteria before the first dose of the investigational product, the subject will not be started on the study treatment and will be withdrawn from the study.

1. Subjects with histologically- or cytologically-confirmed biliary tract cancer (including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and gall bladder cancer) (patients with neuroendocrine tumors, sarcomas, or other histologies without evidence of adenocarcinoma will be excluded.)
2. HER2 positive biliary tract cancer (IHC 3+ or 2+ with ISH + (HER2/CEP17≥2.0 or average HER2 gene copy number ≥ 6 signals/cell) or ERBB2 gene copy number ≥ 6.0 by NGS)
3. Age (at the time of informed consent): 20 years and older
4. Previously untreated if unresectable/metastatic at initial diagnosis; or recurrent disease >6 months after curative surgery or adjuvant therapy (allow up to 1 cycle of gemcitabine-based chemotherapy for advanced/unresectable or metastatic cholangiocarcinoma prior to enrollment. See appendix)
5. Explicit and voluntary consent to participate in the study obtained using a signed and dated informed consent form clearly and fully describing the purpose, potential risks, and any other critical issues regarding the study.
6. Subject with measurable lesions according to RECIST v. 1.1
7. ECOG Performance Status Score 0 or 1
8. Patients with a life expectancy of at least 3 months
9. Patients whose latest laboratory data meet the below criteria within 14 days before enrollment. If the date of the laboratory tests at the time of screening is not within 7 days before the first dose of the investigational product, testing must be repeated within 7 days before the first dose of the investigational product, and these latest laboratory tests must meet the following criteria. Of note, laboratory data will not be valid if the patient has received a granulocyte colony-stimulating factor (G-CSF) or blood transfusion within 14 days before testing.
    - White blood cells ≥2,000/mm3 and neutrophils ≥1,500/mm3
    - Platelets ≥100,000/mm3
    - Hemoglobin ≥9.0 g/dL
    - AST (GOT) and ALT (GPT) ≤3.0-fold the upper limit of normal (ULN) of the study site (or ≤5.0-fold the ULN of the study site in patients with liver metastases)
    - Total bilirubin ≤1.5-fold the ULN of the study site
    - Creatinine ≤1.5-fold the ULN of the study site or creatinine clearance (either the measured or estimated value using the Cockcroft-Gault equation) >45 mL/min
    - INR ≤1.5-fold or prothrombin time ≤1.5-fold the ULN of the study site
    - aPTT ≤1.5-fold the ULN of the study site
10. Women of childbearing potential (including women with chemical menopause or no menstruation for other medical reasons) #1 must agree to use contraception#2 from the time of informed consent until 5 months or more after the last dose of the investigational product. Also, women must agree not to breastfeed from the time of informed consent until 5 months or more after the last dose of the investigational product.

11. Men must agree to use contraception#2 from the start of study treatment until 7 months or more after the last dose of the investigational product.

#1. Women of childbearing potential are defined as all women after the onset of menstruation who are not postmenopausal and have not been surgically sterilized (e.g., hysterectomy, bilateral tubal ligation, bilateral oophorectomy). Postmenopause is defined as amenorrhea for ≥12 consecutive months without specific reasons. Women using oral contraceptives, intrauterine devices, or mechanical contraception such as contraceptive barriers are regarded as having childbearing potential.
#2. The subject must consent to use any of the following methods of contraception: vasectomy or condom for patients who are male or female subject's partner and tubal ligation, contraceptive diaphragm, intrauterine device, spermicide, or oral contraceptive for patients who are female or male subject's partner.

12. EF ≥ 50% via Transthoracic echocardiography or MUGA scan
13. Subjects willing to provide tumor biopsy tissue or excisional biopsy tissue.
14. Subjects with adequate organ function

8.1.2 Exclusion Criteria

If a subject is found to meet any of the following criteria before the first dose of the investigational product, the subject will not be started on the study treatment and will be withdrawn from the study.

1. Patients treated with systemic chemotherapy, biologic therapy, immunotherapy, hormone therapy, or clinical trials for unresectable, locally advanced or metastatic biliary tract cancer. However, the following are excluded.
   a. If disease recurrence occurs 6 months after the last dose of adjuvant chemotherapy, previous adjuvant chemotherapy is permitted.
   b. 1 cycle of Gemcitabine-based anticancer therapy for locally advanced or metastatic cholangiocarcinoma prior to enrollment in this trial is permitted.
2. Patients with multiple primary cancers (with the exception of completely resected basal cell carcinoma, stage I squamous cell carcinoma, carcinoma in situ, intramucosal carcinoma, or superficial bladder cancer, or any other cancer that has not recurred for at least 5 years)
3. Patients with residual adverse effects of prior therapy or effects of surgery that would affect the safety evaluation of the investigational product in the opinion of the investigator or sub-investigator.
4. Patients with current or past history of severe hypersensitivity to any other antibody products
5. Patients with concurrent autoimmune disease or history of chronic or recurrent autoimmune disease
6. Patients with a current or past history of interstitial lung disease or pulmonary fibrosis diagnosed based on imaging or clinical findings. Patients with radiation pneumonitis may be enrolled if the radiation pneumonitis has been confirmed as stable (beyond acute phase) without any concerns about recurrence.
7. Patients with concurrent diverticulitis or symptomatic gastrointestinal ulcerative disease
8. Patients with any metastasis in the brain or meninx that is symptomatic or requires treatment. Patients may be enrolled if the metastasis is asymptomatic and requires no treatment.
9. Patients with pericardial fluid, pleural effusion, or ascites requiring treatment (Eligible if adequately controlled with medication or intervention).
10. Patients with uncontrollable, tumor-related pain
11. Patients who have experienced a transient ischemic attack or cerebrovascular accident within 180 days

before enrollment

12. Patients with a history of uncontrollable or significant cardiovascular disease meeting any of the following criteria:
    - Myocardial infarction within 180 days before enrollment
    - Uncontrollable angina pectoris within 180 days before enrollment
    - New York Heart Association (NYHA) Class III or IV congestive heart failure
    - Uncontrollable hypertension despite appropriate treatment (e.g., systolic blood pressure ≥150 mmHg or diastolic blood pressure ≥ 90 mmHg lasting 24 hours or more)
    - Arrhythmia requiring treatment
13. Patients with uncontrollable diabetes mellitus
14. Patients with systemic infections requiring treatment (infection controlled by oral antibiotics is permitted)
15. Patients who have received systemic corticosteroids (prednisolone or equivalent > 10mg/day) (except for temporary use, e.g., for examination or prophylaxis of allergic reactions) or immunosuppressants within 28 days before enrollment
16. Patients who have received antineoplastic drugs (e.g., chemotherapy agents, molecular-targeted therapy agents, or immunotherapy agents) within 28 days before enrollment
17. Patients who have undergone surgical adhesion of the pleura or pericardium within 28 days before enrollment
18. Patients who have undergone surgery under general anesthesia within 28 days before enrollment
19. Patients who have undergone surgery involving local or topical anesthesia within 14 days before enrollment
20. Patients who have received radiotherapy within 28 days before enrollment, or radiotherapy to bone metastases within 14 days before enrollment
21. Patients who have received any radiopharmaceuticals (except for examination or diagnostic use of radiopharmaceuticals) within 56 days before enrollment
22. Patients with a positive test result for any of the following: HIV-1 antibody, HIV-2 antibody, HTLV-1 antibody, HBs antigen, or HCV antibody
23. Patients with active hepatitis B or C virus (Patients with hepatitis B are eligible for enrollment if they are HBsAg-positive but have HBV DNA <500 IU/mL and are receiving stable antiviral therapy. Patients with hepatitis C are eligible if they are anti-HCV positive but have no detectable HCV RNA, or if HCV RNA is detectable, they are receiving treatment prior to providing informed consent for the clinical trial.)
24. Women who are pregnant or breastfeeding, or possibly pregnant
25. Patients who have received any other unapproved drug (e.g., investigational use of drugs, unapproved combined formulations, or unapproved dosage forms) within 28 days before enrollment
26. Patients who have previously received Nivolumab, anti-PD-1 antibody, anti-PD-L1 antibody, anti-PD-L2 antibody, anti-CD137 antibody, anti-CTLA-4 antibody or other therapeutic antibodies or pharmacotherapies for regulation of T-cells
27. Patients judged to be incapable of providing consent for reasons such as concurrent dementia
28. Other patients judged by the investigator or sub-investigator to be inappropriate as subjects of this study
29. Patient with current or past history of hypersensitivity to Nivolumab.

8.2 Treatment Plan

8.2.1 Dosing and Administration

Dose modification and discontinuation for Nivolumab, Trastuzumab, Gemcitabine and Cisplatin are hematological and non-hematological adverse events

8.2.1.1 Dose modification

The criteria below are for reference only. Matters not described below can be adjusted by referring to the approvals of each test drug.

8.2.1.1.1 Dose modification of Nivolumab

Nivolumab should be administered in 360mg q3weeks, and dose reduction is not allowed.

8.2.1.1.2 Dose modification and delay of Trastuzumab

Caution should be exercised when treating participants with clinically significant cardiovascular disease such as preexisting coronary artery disease or CHF. Participants with symptomatic CHF, unstable angina pectoris, or symptomatic or poorly controlled cardiac arrhythmia should not be enrolled in clinical trials with Trastuzumab. Trastuzumab should be discontinued in the event of any Grade 3 or 4 events consistent with CHF.

There will be no dose modifications of trastuzumab. Trastuzumab dose delays are permitted for Grade 3/4 clinical toxicity or at investigator discretion. Dose delays are not required for laboratory abnormalities unless associated with clinical symptoms. Omitted doses of trastuzumab are not replaced or restored; instead, the participant should resume the planned treatment cycles. Participants who develop signs and symptoms of CHF should have trastuzumab held and should receive treatment for CHF. Participants with an asymptomatic absolute decrease in LVEF of >16 percentage points or an absolute decrease in LVEF of 10 to 15 percentage points to below the lower limit of normal should have trastuzumab held as outlined below.

Congestive Heart Failure and other Cardiac Dysfunction

All participants must have a baseline evaluation of cardiac function including a measurement of LVEF by ECHO (with speckle tracking) prior to entry into the study. If an ECHO cannot be performed or is technically limited, a MUGA scan can alternatively be performed. Only participants with normal LVEF (≥ 50%) should be entered into this study. All participants will undergo regular cardiac monitoring throughout the study, including at baseline and repeated at Cycles 3 and 5 and every 4 cycles starting from Cycle 9. During the course of trastuzumab therapy, participants should be monitored for signs and symptoms of CHF (ie, dyspnea, tachycardia, new unexplained cough, neck vein distention, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnea, orthopnea, peripheral edema, and rapid unexplained weight gain). Participants who develop signs or symptoms of CHF will be further evaluated with a repeat LVEF assessment using the same method selected at baseline (either ECHO or MUGA) if possible.

Management of Symptomatic Cardiac Changes

Participants who develop signs and symptoms of CHF should have trastuzumab held and should receive treatment for CHF as recommended by the ACC/AHA (eg, angiotensin converting enzyme inhibitors, angiotensin-II receptor blockers, β-blockers, diuretics, and cardiac glycosides, as needed) with referral to cardiology for consultation. If the symptoms of CHF resolve with treatment, and/or cardiac function improves to baseline, reinitiating of trastuzumab can be considered at the discretion of the investigator, after discussion with the participant concerning the risks and benefits of continued therapy and in consultation with a cardiologist. If the participant is benefiting clinically from trastuzumab, the benefit of continued treatment may outweigh the risk of cardiac dysfunction or heart failure. If trastuzumab is restarted, continued surveillance with noninvasive measures

of LVEF (MUGA or ECHO) will resume as regularly scheduled. Additional LVEF assessments prior to the next regularly scheduled LVEF measurement may be performed at the investigator's discretion.

Management of Asymptomatic Decreases in LVEF

Trastuzumab can be continued in participants experiencing an asymptomatic absolute decrease in LVEF of <16 percentage points from baseline, when the ejection fraction remains within the imaging center's range of normal limits. Repeat measures of LVEF should be obtained using the methodology selected at baseline if possible. Close follow-up of such participants is recommended. Participants with an asymptomatic absolute decrease in LVEF of >16 percentage points or an absolute decrease in LVEF of 10 to 15 percentage points to below the lower limit of normal should have trastuzumab held. Referral to cardiology should be considered for evaluation and management of left ventricular systolic dysfunction with adherence to ACC/AHA guidelines. In light of the variability inherent in the assessment of ejection fraction, consideration should be given to repeating the study within 4-7 days to confirm an observed decline. Repeat measures of LVEF should be obtained using the same methodology selected at baseline if possible, but at the discretion of the investigator or consulting cardiologist. If trastuzumab has been held for an asymptomatic decline in LVEF, a repeat measure of LVEF will be obtained within 1 month to evaluate for recovery of LVEF. If LVEF does not improve after repeat assessment within 1 month, the participant should be monitored with monthly or as clinically indicated ECHOs/MUGAs until LVEF is improved. If cardiac function improves and LVEF no longer meets "hold" criteria as defined above, trastuzumab may be restarted. If trastuzumab is restarted, continued surveillance with noninvasive measures of LVEF (MUGA or ECHO), using the optimal methodology as determined by the investigator or consulting cardiologist, will resume per the standard schedule. Additional LVEF Assessments prior to the next regularly scheduled LVEF measurement may be performed at the investigator's discretion. If the participant is benefiting clinically from trastuzumab, the benefit of continued treatment may outweigh the risk of cardiac dysfunction even in the setting of an asymptomatic LVEF decline, and reinitiating of trastuzumab can be considered at the discretion of the investigator after consultation with a cardiologist and discussion with the participant concerning the risks and benefits of continued therapy.

8.2.1.1.3 Dose modification of Gemcitabine/Cisplatin

The standard of care agents (Gemcitabine/cisplatin) will be administered according to prescribing information or treatment guidance in general use by the investigating site. With regard to gemcitabine/cisplatin, patients may delay and subsequently resume dosing per local standard clinical practice. If dosing must be delayed for reasons other than treatment related toxicity, dosing will occur as soon as feasible. In the event that chemotherapy is discontinued due to treatment-related toxicity, treatment with Nivolumab + Trastuzumab may be administered as per protocol.

Table: Dose Modification and Toxicity Management Guidelines for Gemcitabine/Cisplatin

* Final decision for treatment discontinuation should be made based on a risk-benefit analysis by the investigator.

Criteria	Toxicity	Grade	Gem dose	Cis dose	Restart of drug	Dose of the drug afterwards
Hematologic toxicity	Neutropenia	1-2	Maintain	Maintain	N/A	N/A
		3	75%	Maintain	N/A	After resolution of hematologic toxicity, the dose of Gemcitabine should be escalated to 100%. Consecutive neutropenia events necessitating dose reduction, the 25% of dose of Gemcitabine should be reduced permanently.
		4	Withhold	Withhold	Resolution of ANC >1000/mm3	The dose of Gemcitabine should be reduced 25%
	Febrile	3-4	Withhold	Withhold	Resolution of ANC	The dose of Gemcitabine should

	neutropenia				>1000/mm3	be reduced 25%
	Thrombocy-topenia	3	75%	Maintain	N/A	After resolution of hematologic toxicity, the dose of Gemcitabine should be escalated to 100%. Consecutive neutropenia events necessitating dose reduction, the 25% of dose of Gemcitabine should be reduced permanently.
		4	Withhold	Withhold	Resolution of platelet > 50,000/mm3	25% dose reduction from previous dose of Gemcitabine
Non-hematologic toxicty	Renal functionloss	Creatinine level above ULN	100%	Further administration of Cisplatin depends on CrCl(mL/min), >60: 100% dose maintain 51– 60: 75% of Cisplatin 40 – 50: 50% of Cisplatin		
	Peripheral neuropathy	2	100%	75%	N/A	25% dose reduction from previous dose of Cisplatin
		3	100%	Withhold	Resolution of toxicity to Grade≤2	25% dose reduction from previous dose of Cisplatin. Additional episode leads to continuous 25% dose reduction
		4	100%	N/A	N/A	Discontinuation of the drug. Can be restarted by investigator's judgement.
	Nausea and vomiting	3-4	Withhold	Withhold	Resolution of toxicity to Grade≤2	Restart the dose with full dose
	Other non-hematologic toxicities	3-4	Withhold	Withhold	Resolution of toxicity to Grade≤2	Follow standard care guidelines

8.2.2 Dose Delay Criteria for Nivolumab

Nivolumab should be delayed for the following:

- Grade 2 non-skin, drug-related adverse event, with the exceptions of fatigue
- Grade 2 drug-related serum creatinine more than 1.5 and up to 6 times the ULN
- First occurrence of any grade 3 adverse reaction
- Grade 3 drug-related laboratory abnormality, with the following exceptions:
- Grade 3 lymphopenia or asymptomatic amylase or lipase does not require dose delay
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.
- Dose delay for AST, ALT, or Total Bilirubin abnormalities should be managed as follows:
- If a participant has a baseline AST or ALT, or Bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity (2 grade shift)
- If a participant has baseline AST, ALT or Bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity (2 grade shift)
- If a participant has baseline AST, ALT, or Bilirubin within the Grade 2 toxicity range, delay dosing for a two-fold drug-related increase in AST, ALT or Bilirubin, or for AST, ALT or bilirubin values 8x ULN (whichever is lower).
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.
- Grade 2 or 3 diarrhea or colitis
- Grade 2 pneumonitis
- Grade 2 or 3 hypophysitis
- Grade 2 adrenal insufficiency
- Grade 3 hyperglycemia
- Grade 3 rash or suspected Stevens-Johnson syndrome or toxic epidermal necrolysis
- New-onset moderate or severe neurologic signs or symptoms

Subjects who require delay of nivolumab should be re-evaluated weekly or more frequently if clinically indicated. It is recommended to monitor elevations in AST or ALT approximately every 3 days till levels peak or begin to decline. Nivolumab dosing can be resumed when re-treatment criteria are met (Section 8.2.1.4). Tumor assessments for all subjects should continue as per protocol even if dosing is delayed.

8.2.2.1 Treatment Discontinuation Criteria for Nivolumab

Discontinuation or delay of nivolumab is upon investigator's decision.
Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis, eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment.
- Any Grade 3 non-skin, adverse event lasting > 7 days or recurs, with the following exceptions for laboratory abnormalities, drug-related uveitis, pneumonitis, bronchospasm, neurologic toxicity, hypersensitivity reactions, infusion reactions, and endocrinopathies:
- Grade 3 drug-related uveitis, pneumonitis. bronchospasm, neurologic toxicity, hypersensitivity reaction, or infusion reaction of any duration require discontinuation.
- Any Grade 4 adverse event or laboratory abnormality
- Any event that leads to delay in dosing lasting > 6 weeks from the previous dose requires discontinuation, with the following exceptions:

- Dosing delays to allow for prolonged steroid tapers to manage adverse events are allowed.

- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the participant with continued study treatment.
- Grade 4 pneumonitis
- Grade 4 hypophysitis
- Grade 4 hyperglycemia
- Grade 4 rash or confirmed SJS or TEN
- Immune-mediated encephalitis
- Life-threatening grade 4 adverse reaction
- Other adverse reaction which require 10mg per day or greater prednisone or equivalent for more than 12 weeks
- Other grade 2 or 3 adverse reactions lasting 12 weeks or longer

Treatment is not need to be permanently discontinued for the followings:

- Grade 3 drug-related endocrinopathies, adequately controlled with only physiologic hormone replacement do not require discontinuation. Adrenal insufficiency requires discontinuation regardless of control with hormone replacement.
- Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation **except**:
  - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
  - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation
  - ◊ AST or ALT > 10 x ULN for > 2 weeks
  - ◊ AST or ALT > 15 x ULN irrespective of duration
  - ◊ Total bilirubin > 5 x ULN for those participants with normal total bilirubin at entry or > 8 x ULN for participants with elevated bilirubin at study entry, irrespective of duration.
  - Grade 4 neutropenia $\leq$ 7 days
  - Grade 4 lymphopenia or leukopenia or asymptomatic amylase or lipase
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset.

Tumor assessments should continue as per protocol even if dosing is delayed.

8.2.2.2 Criteria to Resume Dosing for Nivolumab

Participants may resume treatment with nivolumab when the AE(s) resolve to Grade $\leq$ 1 or baseline value, with the following exceptions:

- Participants may resume treatment in the presence of Grade 2 fatigue.
- Participants who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity.
- Participants with baseline Grade 1 AST, ALT, or total bilirubin who require dose delays for reasons other than a drug-related hepatic event may resume treatment in the presence of Grade 2 AST, ALT, or total bilirubin.
- Participants who require dose delays for drug-related increased in AST, ALT, or total bilirubin may resume treatment when hepatic parameters are at baseline or Grade 1.

- Participants with AST, ALT, or total bilirubin values meeting discontinuation criteria should have treatment permanently discontinued.
- Drug-related pulmonary toxicity, diarrhea, or colitis must have resolved to baseline before treatment is resumed.

8.3 Concomitant Therapy

All medications taken within 28 days before the administration of the first dose of study drug and all concomitant therapy administered during the study until 100 days after last dose of study drug will be collected.

8.3.1 Prohibited Medication

The following are prohibited during the study (unless utilized to treat a drug-related adverse event):

- Antineoplastic systemic therapy (ie, chemotherapy, hormonal therapy, immunotherapy), Palliative radiotherapy may be permitted for symptomatic control of pain from bone metastasis, provided that the radiotherapy does not affect target lesion, and the reason for the radiotherapy does not reflect progressive disease.
- Investigational agents other than nivolumab
- Immunosuppressive agents
- Immunosuppressive doses of systemic corticosteroids (Corticosteroids equivalent to > 10 mg/day of prednisone)
- Live vaccines within 30 days prior to the first dose of study drug and while participating in the study.
- Prophylactic G-CSF during cycle 1 and 2 is not permitted.

Strong P-gp inhibitor/inducers are to be avoided unless deemed to be absolutely necessary. In such cases, patients should be monitored closely.

8.4 Duration of Treatment

In case of no treatment related adverse events and PD, subjects will receive 9 cycles of Nivolumab, Trastuzumab, Gemcitabine and Cisplatin. From 10th cycle, unless are there adverse events to discontinue Nivolumab or Trastuzumab, IPs are to administered for maximum 2 years. Gemcitabine and Cisplatin could be either continued or discontinued from 10th cycle under the investigator's decision.

8.5 Treatment of Discontinuation

Upon treatment discontinuation all end of treatment evaluations and tests will be conducted. All participants who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the participant's medical records and the appropriate CRF.

Reasons for treatment discontinuation should be classified as follows:

- Death
- Progressive disease
- Toxicity; treatment related or unrelated

- Investigator judgment
  - The Investigator may discontinue a participant if, in his/her judgment, it is in the best interest of the participant to do so.
- Noncompliance
- Participant voluntary withdrawal
  - A participant may withdraw from the study at any time, for any reason. If a participant discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal.
  -

8.6 Diet/Activity/Other Considerations

8.6.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

8.6.2 Contraception

For this trial, male subjects will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

(1) postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 150 days after the last dose of study drug by complying with one of the following:

(1) practice abstinence[†] from heterosexual activity;

OR

(2) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are[‡]:

Single method (one of the following is acceptable):

• intrauterine device (IUD)

• vasectomy of a female subject's male partner

• contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

• diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)

• cervical cap with spermicide (nulliparous women only)

• contraceptive sponge (nulliparous women only)

• male condom or female condom (cannot be used together)

• hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

†Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the subject's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and ERCs/IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 150 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

9.0 STUDY PROCEDURES

9.1 Participant Registration

Participants are histologically or cytologically confirmed diagnosis of biliary tract cancer including Intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer with HER2 positive patients. HER2 positive is defined as either IHC 3+ or IHC 2+ in combination with ISH + (or FISH), as assessed by local review on primary or metastatic tumor. ISH positivity is defined as either a ratio of ≥2 for the number of HER2 gene copies to the number of signals for CEP17; or ERBB2 gene copy number ≥ 6.0 in NGS.

9.2 Baseline evaluation

Subjects, who have signed the informed consent form, must undergo a series of assessments (screening/baseline assessments) to identify whether they are eligible for participation to the trial. These assessments must be completed at least 28 days prior to the start day of drug administration.

All consented subjects will undergo a screening visit and the following assessments/clinical procedures will be performed:

- Demographics and Medical History
- Disease Details/Treatment & Pathological Findings

- Prior and Concomitant Medication Review
- Review of Adverse Events
- Physical Examination (e.g., Full Physical Examination, ECOG Performance Status)
- Vital Signs (e.g., Body temperature, Pulse rate, Respiration rate, Weight, Blood Pressure, etc.)
- Clinical Laboratory Test (includes hematology, coagulation, blood chemistry, urinalysis, pregnancy test, tumor markers)
- Quality of Life Assessment- patient reported outcomes (PRO) surveys
- EKG
- Echo or MUGA
- Chest X-ray
- Tumor Imaging Test
- Tumor Tissue and Blood Sampling for exploratory endpoints (Optional/ Under Subject's Consent)
- Blood Test, including thyroid function test

All results will be reviewed by the investigator, or a qualified designee, to confirm whether the subject meets all the inclusion criteria. If a subject was not allocated to any of the above exams, the reason for it must be verified/recorded.

9.3 Evaluations Performed at Each Cycle

A series of assessments will be performed throughout the trial as mentioned in table 2. The following tests and procedures will be performed before each drug administration.

Mandatory tests and procedures before administration of the drug at day 1 of each cycle include:

- Prior and Concomitant Medical Review
- Review Adverse Events
- Physical Examination (e.g., Directed Physical Examination, ECOG Performance Status)
- Vital Signs (e.g., Body Temperature, Pulse rate, Respiration rate, Weight, Blood pressure, etc.)
- Clinical Laboratory Test (hematology, coagulation*, blood chemistry, urinalysis*, pregnancy test*)

* * No additional test is required if a screening assessment was performed within 3 days since the first date of drug administration.

Additional tests that are required every 3 cycles of treatment include:

- Tumor Marker (CEA, CA 19-9)
- Quality of Life Assessment- patient reported outcomes (PRO) surveys
- EKG
- Tumor Imaging Test
- Thyroid function test

* These assessments will be performed regardless of delays in treatment cycles. However, if a treatment cycle is delayed, tumor imaging test will also be delayed accordingly to align with the treatment cycle.

Additional tests that are required every 12 weeks (± 7 days) from the date of first administration are as follows:

- Echo or MUGA

** Patients who achieve CR or PR for the first time must undergo confirmatory tumor response assessment at the next scheduled evaluation, or at least 4 weeks after the initial response assessment.

Some patients may experience an initial transient radiologic worsening of disease within the first few months after starting immunotherapy, followed by subsequent tumor response. Therefore, if the investigator considers the observed radiologic PD to be possible pseudoprogression related to immunotherapy, treatment may be continued until PD is confirmed based on repeat tumor assessment performed within 4–8 weeks from the date of initial PD observation, in accordance with iRECIST.

To continue study treatment under suspicion of pseudoprogression, all of the following criteria must be met:

- No symptoms or signs indicating clinically significant disease progression

- No deterioration in ECOG performance status

- No need for intensified management, including increased analgesic use, radiotherapy, or other palliative interventions

9.4 Evaluation Performed at End of Treatment

If subjects are deemed unsuitable for continuing the trial, subject will complete an End of treatment visit, no later than 30 days from the last dose of the study drug. Reasons for treatment discontinuation must be recorded in the CRF, and the end-of-treatment visit should include the following assessments:

- Prior and Concomitant Medical Review
- Review Adverse Effects
- Conditions of anti-cancer therapy after trial
- Survival Status
- Physical Examination (e.g., Directed Physical Examination, ECOG Performance Status)
- Vital Signs (e.g., Body Temperature, Pulse rate, Respiration rate, Weight, Blood pressure, etc.)
- Clinical Laboratory Test (hematology, blood chemistry, tumor biomarkers)
- Quality of Life Assessment- patient reported outcomes (PRO) surveys
- Tumor Tissue Sampling (Optional/ Under Subject's Consent)
- Blood Test

Subjects, who are discontinuing the trial for reasons other than the progression of the disease, must undertake a tumor imaging test every 2 months- since their last tumor imaging test- until disease progression is confirmed.

9.5 Post-Treatment Follow-Up Evaluations

9.5.1 Survival Follow-up

For subjects discontinuing the study treatment due to documented radiographic progression, obtain survival status via phone call or medical chart review, including information about subsequent anticancer therapies (including best response) every 3 months until death, loss of follow-up, or withdrawal of consent, whichever comes first.

For subjects discontinuing the study treatment prior to documented radiographic progression, tumor assessments should continue on the schedule approximately every 3 months or at the current scan interval at the time of treatment discontinuation until radiographic evidence of disease progression, the start of a subsequent anticancer therapy, or decision to no longer treat (e.g., supportive care only), whichever is first. At that time, survival status (And subsequent anticancer therapy information, including best response, if appropriate) will be collected every 3 months until death, loss of follow-up, or withdrawal of consent, whichever comes first.

9.6 Schedule of Procedures and Observations

See Table. Schedule of Activity

* For patients receiving only nivolumab + trastuzumab from Cycle 10 onward, the CxD8 visit may be omitted. However, if gemcitabine or cisplatin is continued beyond Cycle 10, CxD8 assessments must be performed prior to treatment administration for safety monitoring.

** Any decisions regarding conversion surgery, study drug interruption, discontinuation, or dose skipping will be made in discussion with the main (principal) center.

9.7 Exploratory Objectives

Additional biomarker research will be conducted to identify factors that are important in determining the treatment response of Nivolumab-Trastuzumab-Gem/Cis therapy. This trial will collect subjects' blood samples (a total of 20cc whole blood specimen) and tumor tissue samples: from primary or metastatic lesions (including peritoneal metastasis). Samples will be collected pre/post treatment, and only from those who have given their consent.
The volume/amount of samples collected is as follows:
1) Blood Specimens (mandatory): before, C2D1, C4D1 and after treatment; whole blood 10cc * 2 (a total of 20cc)
2) Tumor Tissue Sample (optional): For central confirmation, either two unstained slides for HER2 IHC and SISH or one HER2 IHC-stained slide plus one unstained slide will be collected at screening for confirmation by the central lab. For additional NGS analysis, 10–20 unstained slides or a tumor block will be collected at pre-treatment, C2D1 (optional), and end of study (optional). (The number of slides can be negotiated with the main (principal) institution if there are insufficient slides)

A comprehensive genomic profiling of the subject's tumor sample- either archival tissue or those collected pre/post treatment- will be done via immunohistochemistry, in situ hybridization, and next-generation sequencing (NGS), etc. Blood samples, including whole blood specimen and blood plasma (PBMC, peripheral blood mononuclear cell) will be used to identify biomarkers that predict treatment responses.

RNA-sequencing, gene penal sequencing, whole-exome sequencing, and whole genome sequencing, based on NGS, will be used for genetic analysis of the subject's tumor tissue sample. Tumor tissues and blood specimen will be stored in the biobank in Severance Hospital for up to 15 years since the termination of trial, and will be destroyed thereafter. These samples will be coded and will not include any personal information (e.g., name or resident registration number).

Among HER2-positive tumors, protein overexpression is typically assessed visually by pathologists, and relatively high inter-observer agreement has been reported among experienced pathologists. However, discrepancies in interpretation can still occur even among expert pathologists, particularly for cases with

intermediate HER2 expression such as immunohistochemistry (IHC) 2+, which is known to have relatively low concordance. Reducing inter-observer variability is therefore important for accurately assessing HER2 expression and for guiding treatment decisions regarding HER2-targeted therapy.
Lunit SCOPE HER2 is a model trained on large-scale digitized pathology data that analyzes image characteristics of individual cases and quantitatively assesses HER2 protein expression on HER2 IHC-stained slides. In a previous study involving patients with biliary tract cancer treated with trastuzumab plus FOLFOX, HER2 overexpression detected by Lunit SCOPE HER2 was shown to distinguish patient prognosis (Kim et al., JCO PO 2025). In addition, Lunit SCOPE IO is a model that enables spatial and quantitative analysis of tumor-infiltrating lymphocytes (TILs) using hematoxylin and eosin (H&E)-stained slides (Bang et al., CCR 2025). Previous studies across multiple tumor types have demonstrated correlations between HER2 gene expression or amplification and quantitatively assessed TIL features analyzed by this model.
In this study, we aim to exploratorily evaluate the association between HER2 protein overexpression and spatial and quantitative TIL classification—analyzed using Lunit SCOPE HER2 and Lunit SCOPE IO, respectively—and treatment response to combined HER2-targeted therapy and immunotherapy in patients who provide separate written informed consent for this analysis.
- To evaluate the association between HER2 overexpression assessed from HER2 IHC slides using Lunit SCOPE HER2 and treatment response
- To evaluate the association between immune phenotype classification assessed from H&E slides using Lunit SCOPE IO and treatment response

9.8 Quality of Life assessment via PRO

The subject's quality of life will be assessed via a self-reported survey (PRO) EORTC-QLQ-C30 which will be collected at various times throughout the trial: before treatment, every 3cycles of treatment, and after treatment. Subjects will be given sufficient time to understand the survey questions and the collected data will be used for interpretation by the investigators.

10.0 SAFETY EVALUATION

10.1 Adverse Events

10.1.1 Definition

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite').
An AE is considered "unexpected" if it is not listed in the investigator brochure is not listed at the specificity or severity that has been observed.

10.1.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

10.1.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.
However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

10.1.1.3 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).
If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

- AST or ALT > 10 x ULN for > 2 weeks,
- AST or ALT > 15 x ULN irrespective of duration,
- Total bilirubin > 8 x ULN irrespective of duration for subjects with elevated bilirubin at study entry or > 5 x ULN for those with normal T bilirubin at entry,
- Concurrent AST or ALT > 3 x ULN and Total bilirubin > 5 x ULN for subjects entering treatment with a normal bilirubin and up to 8 x ULN for subjects with elevated bilirubin
- Grade 4 neutropenia < 7 days
- Grade 4 lymphopenia or leukopenia
- Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis.
- Isolated Grade 4 electrolyte imbalances or abnormalities that are not associated with clinical sequelae and are corrected with supplementation and appropriate management within 72 hours of their onset.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as “hyperkalemia.”
Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

10.1.1.4 Preexisting Medical Conditions (Baseline Conditions)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

10.1.2 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 5 of the NCI Common Terminology Criteria for

Adverse Events (CTCAE) will be utilized for AE reporting.
AEs not covered by specific terminology listed should be reported with common medical terminology, and documented according to the grading scales provided in the CTCAE Version 5.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Related:** There is a relationship between suspect drug and adverse event.
- **Not related:** There is no relationship between suspect drug and adverse event.

10.2 Serious Adverse Events

10.2.1 Definition

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in ANY of the following:

- Death.
- A life-threatening adverse event (experience). Any AE that places a participant or participants, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does NOT include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.

Other medically significant situation; an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization).

10.2.2 Reporting Adverse Events

All SAEs occurring from the date the participant received study drug and until 30 days after the last intervention or a new treatment is started, whichever comes first, will will be collected in this study. Principal investigator will provide all SAEs to OPKR by using SAE reporting form(Attachement1). The report is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

- Reporting timeline: Within 24 hours of becoming aware of SAE
- **Reporting contact point:** [redacted]

10.3 Suspected Unexpected Serious Adverse Reaction (SUSAR)

10.3.1 Definition

A Serious adverse reaction that is not consistent with the investigator's brochure' description including severity, modality.

10.3.2 Reporting of Suspected Unexpected Serious Adverse Reaction (SUSAR)

Sponsor (investigator) should bear the responsibility to report Suspected Unexpected Serious Adverse Reaction

(SUSAR) to MFDS as per Korea Good Clinical Practice. Sponsor should report all SUSAR to MFDS and if needed, also to IRB within following timeline.

10.4 Follow-Up for Serious Adverse Events

All SAEs should be followed to their resolution, until the study participant is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

10.5 Reporting Pregnancy

If pregnancy occur, it must be reported to OPKR.
The Principal Investigator shall report pregnancy event within twenty-four (24) hours of becoming aware of the pregnancy by using the Pregnancy reporting form (Attachment 2).
As for the pregnancy, the Principal Investigator shall follow up the pregnancy until delivery or the end of pregnancy.

Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies).
Other appropriate pregnancy follow-up procedures should be considered if indicated.
Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be recorded as much as possible.

- Reporting timeline: Within 24 hours of becoming aware of pregnancy
- Reporting contact point: [redacted]

10.6 Unanticipated Problems

10.6.1 Definition

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
  - The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant privacy or confidentiality of data.
  - The characteristics of the participant population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).

Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed Serious per Section 9.2.

10.6.2 Reporting Unanticipated Problems

In case of life-threatening or death: Report should be done within 7 days investigator has recognized the event. In this case, detailed information should be done within 8 days after initial report.
Other serious and unanticipated adverse events: Report should be done within 15 days investigator has recognized the event.

11.0 STATISTICAL METHODOLOGY

11.1 Efficacy

11.1.1 Efficacy Endpoint

The primary endpoint of phase Ib study is to assess dose limiting toxicity (DLT) of the first cycle, and to define recommended phase II dose (RP2D). Definition of DLT is as follows:

Hematologic DLT

- Grade 4 neutropenia lasting for ≥ 7 days in duration
- Grade 4 neutropenia with fever > 38.5 C and/or infection requiring antibiotic or anti-fungal treatment
- Grade 4 thrombocytopenia

Non-hematologic DLT

- Grade 3 or greater toxicity lasting > 48 hours other than anorexia, nausea, diarrhea, and alopecia (despite appropriate supportive care)

The primary efficacy objective of phase II study is to evaluate the anti-tumor activity of trastuzumab, nivolumab and chemotherapy in subjects with advanced biliary tract cancer. Objective response rates per RECIST 1.1 as assessed will be used as the primary response rate efficacy endpoint. Standard RECIST criteria may not provide a complete response assessment of immunotherapeutic agents such as nivolumab. When feasible, subjects should not be discontinued until progression is confirmed. This allowance to continue treatment despite initial radiologic progression takes into account the observation that some subjects can have a transient tumor flare in the first few months after the start of immunotherapy, but with subsequent disease response. A secondary objectives including PFS, OS, DCR and DOR will be also evaluated.

Objective Response Rate (ORR): a confirmed complete response (CR) or partial response (PR) per RE CIST 1.1. ORR is the proportion of participants with objective response.

PFS: Time from enrollment to the first documented PD per RECIST 1.1 or death from any cause whi chever occurs first.

OS: Time from enrollment to the date of death due to any cause.

11.2 Safety

The primary safety objective of this trial is to characterize the safety and tolerability of nivolumab in combination with gemcitabine, cisplatin, and trastuzumab in subjects with HER2 positive biliary tract cancer. The primary safety analysis will be based on subjects who experienced toxicities as defined by NCI CTCAE 5.0 criteria. Safety will be assessed by quantifying the toxicities and grades experienced including serious adverse events (SAEs).

11.3 Sample size determination

Phase Ib

Approximately 3-6 patients evaluable for DLT are planned based on modified 3+3 design. [Dose level 0, Dose level -1] For each dose, 3 patients would be enrolled for the DLT for the first 3 weeks. RP2D will be the highest dose of therapy observed to cause a DLT in 1 patient out of 3 patients, or fewer than 2 patients out of 6 patients. The study will begin with dose level 0. If one of the first three subjects experiences a DLT, then up to three

additional subjects will be enrolled at that dose level. If two or more subjects experience DLT, the dose would be de-escalated to dose level -1.

If a final DLT occurs despite working to Level -1, the study is temporarily suspended to further review the minimum data and consider changes in the trial design.

Phase II

Phase II study will be open to 38 patients at the dose of RP2D chosen at Phase Ib study.

According to Simon's two-stage minimax design to have an 80% power with a type I error of 10%. Comparing with the benchmark response of 27% among patients with advanced BTC in TOPAZ-1 trial (ORR for durvalumab+Gem/Cis: 26.7%), ORR under 27% implies not enough response rate so no further evaluation might be done (P0=27%), ORR over 45% implies enough response rate, considering further investigation (P1=45%). Interim study after 16 patient's response showing less than 5 patients' response discontinue this study. Considering drop-out rate of 10%, 38 patients will be enrolled in phase II part.

Accordingly, about 41-44 patients will be enrolled to this Ib/II sequential study. The patients will be treated until disease progression, unacceptable adverse events or patient's withdrawal of the study.

11.4 Population for Analysis

All patients who receive the RP2D, including those enrolled in phase Ib, will be included in the full analysis set (FAS) for efficacy and safety analyses.

11.4.1 Efficacy Analysis Population

The primary efficacy analysis will be performed in the full analysis set (FAS), defined as all patients who received at least one dose of study treatment and had at least one baseline tumor assessment.

11.4.2 Safety Analysis Population

All subjects who have received at least one dose of the study drug are included. These subjects will be analyzed to assess the safety of their treatment. The severity of adverse events will be decided by the investigator, following the CTCAE, Version 5.0 guidelines. Safety analysis will include the following criteria:

- Laboratory and non-laboratory adverse events will follow the CTCAE 5.0. Maximum severity for each system organ class and all CTCAE > grade 3 will be investigated, despite its association with the study drug

- Progressive free survival (PFS) is defined as the time from day 1 of cycle 1 to the point, where disease progression is first proven or death, whichever comes first. In accordance with the RECIST 1.1 criteria, disease progression will be defined based on the records from the investigators. Subjects who are alive and have not progressed at the time of the analysis will be censored at the time of their last tumor assessment, or enrollment + day 1 in case of no subsequent response evaluation after baseline visit. Kaplan-Meier plots and estimates of the quartiles and their corresponding 95% CIs will be computed.

- In accordance with the RECIST 1.1 criteria, the overall response rate (ORR) will be calculated as the fraction of subjects with complete/partial response in total ITT patients. The ORR will be calculated as the percentage of patients who achieved a confirmed complete response (CR) or partial response (PR). DCR was the proportion of subjects with confirmed CR, PR, and stable disease for at least 4 weeks. 95% confidence interval will be calculated using the Clopper-Perason method.

Changes in data analysis methods will be the sole reason for the revision of this document.

Further exploratory analysis will be performed when deemed appropriate.

11.5 Pre-planned statistical and sensitivity analyses to be conducted

- Objective response rate (ORR): The point estimate of ORR will be accompanied by a two-sided 95% exact binomial CI using the Clopper-Pearson method.

- PFS and OS: PFS and OS distribution will be estimated using Kaplan-Meier method. Median survival time as well as 25th and 75th percentiles along with 95% CI will be constructed based on Brookmeyer and Crowley method using log-log transformation. Rates at fixed timepoints will be derived from Kaplan-Meier estimate and corresponding two-sided 95% confidence interval will be derived based on Greenwood formula for variance deviation and log-log transformation applied on the survival function. Timepoints of PFS and OS rates include 3, 6, 9 ,12 and 18 months. The median of the reverse OS Kaplan-Meier distribution will be used as the estimate of the median follow-up time. Survival distributions will be estimated using the Kaplan–Meier method, and differences between subgroups will be assessed using the log-rank test. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) will be estimated using Cox proportional hazards models to explore the association between baseline covariates and survival outcomes, as well as for subgroup analyses.

- Duration of response (DOR): DOR is defined for patients with an objective response as the time from the first documented objective response to documented disease progression as determined by the investigator with use of RECIST or death from any cause, whichever occurs first. DOR will be estimates using Kaplan-Meier methodology for subjects who achieve complete response or partial response. Median values along with two-sided 95% CIs will be calculated.

- Disease control rate (DCR): Summary statistics using DCR, DCR-24w, and DCR-32w as assessed by the Investigator according to RECIST 1.1.

- Time to symptom, function, or HRQoL deterioration of key PRO endpoints using EORTC QLQ-C30: Kaplan-Meier method, log-rank test

- Improvement rates for EORTC QLQ-C30: Logistic regression

- Missing values will be treated as missing, and all analyses will be performed using the original raw data without imputation or adjustment for missing data.

12.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

12.1 Investigational Product

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Clinical Supplies of nivolumab will be provided by ONO Phama Korea.

Clinical Supplies of gemzar and trastuzumab will be provided by Boryung Pharmaceutical, Korea.

Product Descriptions

Product Name & Potency	Dosage Form
Nivolumab 100mg, 10ml/vial	Clear to opalescent, colorless to pale-yellow solution in a single-dose vial
Trastuzumab (Samfenet®) 150mg/vial, 440mg/vial	Colorless transparent vial containing white to light yellow freeze-dried powder
Gemcitabine Hydrochloride (Gemzar®) 1g/vial, 200mg/vial	Freeze-dried white to off-white lumps or powders filled in colorless transparent vials for injection

12.2 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

12.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, designee are not blinded to treatment. Drug identity (name, strength) is included in the label text

12.4 Storage and Handling Requirements

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

12.5 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies received from ONO pharma Korea or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial. Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

13.0 ADMINISTRATIVE AND REGULATORY DETAILS

13.1 Confidentiality

13.1.1 Confidentiality of Data

By signing this protocol, the investigator affirms to the investigator that information furnished will be maintained in confidence, and such information will be divulged to the institutional review board, ethics review committee (IRB) or similar or expert committee; affiliated institution and employees, only under an appropriate understanding of confidentiality with such board or committee, affiliated institution and employees. Data generated by this trial will be considered confidential by the investigator, except to the extent that it is included in a publication as provided in the Publications section of this protocol.

13.1.2 Confidentiality of Subject Records

By signing this protocol, the investigator agrees that IRB, or regulatory authority representatives may consult trial documents in order to verify worksheet/case report form data. By signing the consent form, the subject agrees to this process. If trial documents will be photocopied during the process of verifying worksheet/case report form information, the subject will be identified by unique code only

By signing this protocol, the investigator agrees to treat all subject data used and disclosed in connection with this trial in accordance with all applicable privacy laws, rules and regulations.

13.2 Compliance with Financial Disclosure Requirements

Not applicable to this study.

13.3 Compliance with Law, Audit and Debarment

13.4 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

13.5 Institutional Review Board

IRB shall perform its duties following the MFDS regulations of the respective country. Prior to initiating the study, the investigator will both provide and receive a written confirmation from the IRB for the following appropriate materials: protocol, informed consent, recruiting materials (e.g., promotional materials), and other written information provided to the patients. Moreover, the investigator will provide the IRB with all appropriate material, including the investigator's brochure, product manual, and other written information provided to the patients. Appropriate reports on the progress of the study (e.g., safety update, protocol amendment, administrative letter, etc.) will be made to the IRB.

13.6 Informed Consent

It will be the investigator's responsibility to prepare the informed consent, which complies with FDA, ICH, regulations in Japan, GCP, and local regulations. Clinical trial physicians must confirm that the consent form includes a clear elaboration of the objectives of the trial, potential risks, and other essential information about the trial. Once the subject signs the informed consent form, insurance for clinical trials should be provided in accordance with appropriate regulations of the relevant country. Adequate insurance coverage should comply with GCP or regulations of the relevant country. Information about the confirmation of insurance coverage should be recorded on the Investigator Site File.

14.0 MONITORING

Monitoring and auditing of the trial must comply with the GCP regulation. It is mandatory that investigators or a qualified designee have access to original source documents (e.g., protocol signed by the investigator, protocol amendment, institution related information, and informed consent form) with permissions from appropriate regulatory authorities.

The institution should ensure that the trial is equipped with appropriate facilities and professionals. Moreover, the institution should put great effort to maintain the safety of the subjects. Sub-investigators must be fully aware of all adverse events/ precautions written in the trial protocol. Thus, if an adverse event occurs, they should be capable of instantly pausing and giving appropriate intervention to the subject. Then, they should report the circumstance to the institutional review board and the chief investigator.

It will be the principal investigator and the monitor's responsibility to inspect the case report forms at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, correctness and accuracy of all case report form entries. Also, the auditor (Dr. Han Sang Kim) will monitor and perform regular reviews every 12 months to assess whether the trial is being conducted in compliance with the GCP guidelines. Specifically, the auditor will review the data completeness, reliability of the CRF, protocol deviation/violation, inclusion/exclusion criteria, efficacy endpoints, safety information. Monitoring results will be officially recorded and reported to IRB.

Evaluation Criteria:

- whether the research facilities comply with the trial objectives
- Compliance with the protocol and trial flow chart
- Assurance that the protocol amendments receive approval from IRB and the principal investigator is notified to the IRB
- Assurance of the data accurateness
- Assurance of instant/accurate data report to the principal investigator and IRB

15.0 DATA HANDLING

To enable evaluations and/or audits from regulatory authorities, the Investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, eg, CRFs and hospital records), all original signed informed consent forms, copies of all CRFs, source documents, and detailed records of treatment disposition. The records should be retained by the Investigator according to ICH, local regulations, or as specified in the Clinical Study Agreement, whichever is longer.

16.0 SAMPLE IDENTIFICATION AND RETENSION SPECIMEN

The residual (excess) samples (DNA, RNA) will be stored at the site according to the regulations, up to the retention period specified by the subject in the consent form. Residual samples can be analyzed for research purposes using the next generation gene analysis technique.
However, privacy and de-identification will be thorough. The collected samples are recognized by coded numbers and the participation of this clinical study only collects personal information, such as the subject's name, but that information is not directly used or required by the study. Therefore, the collected information is properly managed under the Privacy Act.

Upon expiration of the retention period determined by subject, the human-derived material shall be destroyed in accordance with the standards and methods per the [Wastes Control Act] Article 13.

17.0 APPENDICES

17.1 Gemcitabine-based chemotherapy regimens

Gemcitabine + Cisplatin

Gemcitabine + Cisplatin + Nab-paclitaxel

Gemcitabine + Cisplatin + S-1

Gemcitabine + S-1

Gemcitabine + Capecitabine

Gemcitabine + Nab-paclitaxel

Gemcitabine monotherapy

Gemcitabine + Cisplatin + Durvalumab

Gemcitabine + Cisplatin + Pembrolizumab

17.2 ECOG Performance Status Scores

Description	Status
Fully active, able to carry on all pre-disease performance without restriction.	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	1
Ambulatory and capable of all self-care but unable to carry out any work activities.	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	3
Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	4
Dead	5

17.3 Common Terminology Criteria for Adverse Events V5.0 (CTCAE)

The description and grading scales found in the CTEP Version 5 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 5 of the CTCAE is identified and located at:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

17.3 EORTC QLQ-C30

ENGLISH

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ____

Your birthdate (Day, Month, Year): ____

Today's date (Day, Month, Year): 31 ____

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

ENGLISH

During the past week:	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

18.0 REFERENCE

1. Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. N Engl J Med. 2010 Apr 8;362(14):1273–81.

2. Shroff RT, Javle MM, Xiao L, Kaseb AO, Varadhachary GR, Wolff RA, et al. Gemcitabine, cisplatin, and nab-paclitaxel for the treatment of advanced biliary tract cancers: A phase 2 clinical trial. JAMA Oncol. 2019 Jun 1;5(6):824–30.

3. Kim RD, Chung V, Alese OB, El-Rayes BF, Li D, Al-Toubah TE, et al. A phase 2 multi-institutional study of nivolumab for patients with advanced refractory biliary tract cancer. JAMA Oncol. 2020 Jun 1;6(6):888–94.

4. Ueno M, Ikeda M, Morizane C, Kobayashi S, Ohno I, Kondo S, et al. Nivolumab alone or in combination with cisplatin plus gemcitabine in Japanese patients with unresectable or recurrent biliary tract cancer: a non-randomised, multicentre, open-label, phase 1 study. Lancet Gastroenterol Hepatol. 2019 Aug;4(8):611–21.

5. Piha-Paul SA, Oh D-Y, Ueno M, Malka D, Chung HC, Nagrial A, et al. Efficacy and safety of pembrolizumab for the treatment of advanced biliary cancer: Results from the KEYNOTE-158 and KEYNOTE-028 studies. Int J Cancer. 2020 Oct 15;147(8):2190–8.

6. Lee C-K, Cheon J, Kim EJ, Kim CG, Kim S, Kim C, et al. Characterization of hyperprogressive disease in patients with advanced biliary tract cancer treated with anti-PD-1 inhibitor: A multicenter retrospective study. J Clin Oncol. 2021 Jan 20;39(3_suppl):339–339.

7. Oh D-Y, Ruth He A, Qin S, Chen L-T, Okusaka T, Vogel A, et al. Durvalumab plus gemcitabine and cisplatin in advanced biliary tract cancer. NEJM Evidence [Internet]. 2022 Jul 26;1(8). Available from: http://dx.doi.org/10.1056/evidoa2200015

8. Valle JW, Lamarca A, Goyal L, Barriuso J, Zhu AX. New Horizons for precision medicine in biliary tract cancers. Cancer Discov. 2017 Sep;7(9):943–62.

9. Lee C-K, Chon HJ, Cheon J, Lee MA, Im H-S, Jang J-S, et al. Trastuzumab plus FOLFOX for gemcitabine/cisplatin refractory HER2-positive biliary tract cancer: A multi-institutional phase II trial of the Korean Cancer Study Group (KCSG-HB19-14). J Clin Oncol. 2022 Jun 1;40(16_suppl):4096–4096.

10. Chaganty BKR, Qiu S, Gest A, Lu Y, Ivan C, Calin GA, et al. Trastuzumab upregulates PD-L1 as a potential mechanism of trastuzumab resistance through engagement of immune effector cells and stimulation of IFNγ secretion. Cancer Lett. 2018 Aug;430:47–56.

11. Padmanabhan R, Kheraldine HS, Meskin N, Vranic S, Al Moustafa A-E. Crosstalk between HER2 and PD-1/PD-L1 in breast cancer: From clinical applications to mathematical models. Cancers (Basel). 2020 Mar 10;12(3):636.

12. Janjigian YY, Maron SB, Chatila WK, Millang B, Chavan SS, Alterman C, et al. First-line pembrolizumab and trastuzumab in HER2-positive oesophageal, gastric, or gastro-oesophageal junction cancer: an open-label, single-arm, phase 2 trial. Lancet Oncol. 2020 Jun;21(6):821–31.

13. Rha SY, Lee C-K, Kim HS, Kang B, Jung M, Kwon WS, et al. A multi-institutional phase Ib/II trial of first-line triplet regimen (Pembrolizumab, Trastuzumab, Chemotherapy) for HER2-positive advanced gastric and gastroesophageal junction cancer (PANTHERA Trial): Molecular profiling and clinical update. J Clin Oncol. 2021 Jan 20;39(3_suppl):218–218.

14. Janjigian YY, Kawazoe A, Yañez P, Li N, Lonardi S, Kolesnik O, et al. The KEYNOTE-811 trial of dual PD-1 and HER2 blockade in HER2-positive gastric cancer. Nature. 2021 Dec;600(7890):727–30.

15. Park S, Jiang Z, Mortenson ED, Deng L, Radkevich-Brown O, Yang X, et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. Cancer Cell. 2010 Aug 9;18(2):160–70.

16. Taylor C, Hershman D, Shah N, Suciu-Foca N, Petrylak DP, Taub R, et al. Augmented HER-2 specific immunity during treatment with trastuzumab and chemotherapy. Clin Cancer Res. 2007 Sep 1;13(17):5133–43.

17. zum Büschenfelde CM, Hermann C, Schmidt B, Peschel C, Bernhard H. Antihuman epidermal growth factor receptor 2 (HER2) monoclonal antibody trastuzumab enhances cytolytic activity of class I-restricted HER2-specific T lymphocytes against HER2-overexpressing tumor cells. Cancer Res. 2002 Apr 15;62(8):2244–7.

18. Varadan V, Gilmore H, Miskimen KLS, Tuck D, Parsai S, Awadallah A, et al. Immune signatures following single dose trastuzumab predict pathologic response to PreoperativeTrastuzumab and chemotherapy in HER2-positive early breast cancer. Clin Cancer Res. 2016 Jul 1;22(13):3249–59.

19. Triulzi T, Forte L, Regondi V, Di Modica M, Ghirelli C, Carcangiu ML, et al. HER2 signaling regulates the tumor immune microenvironment and trastuzumab efficacy. Oncoimmunology. 2019;8(1):e1512942.

20. Triulzi T, Regondi V, De Cecco L, Cappelletti MR, Di Modica M, Paolini B, et al. Early immune modulation by single-agent trastuzumab as a marker of trastuzumab benefit. Br J Cancer. 2018 Dec;119(12):1487–94.

21. Stagg J, Loi S, Divisekera U, Ngiow SF, Duret H, Yagita H, et al. Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. Proc Natl Acad Sci U S A. 2011 Apr 26;108(17):7142–7.

22. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. Nat Med. 2009 Oct;15(10):1170–8.

23. Tesniere A, Schlemmer F, Boige V, Kepp O, Martins I, Ghiringhelli F, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene. 2010 Jan 28;29(4):482–91.

24. Müller P, Kreuzaler M, Khan T, Thommen DS, Martin K, Glatz K, et al. Trastuzumab emtansine (T-DM1) renders HER2+ breast cancer highly susceptible to CTLA-4/PD-1 blockade. Sci Transl Med. 2015 Nov 25;7(315):315ra188.

25. Javle M, Borad MJ, Azad NS, Kurzrock R, Abou-Alfa GK, George B, et al. Pertuzumab and trastuzumab for HER2-positive, metastatic biliary tract cancer (MyPathway): a multicentre, open-label, phase 2a, multiple basket study. Lancet Oncol. 2021 Sep;22(9):1290–300.

26. Ohba A, Morizane C, Ueno M, Kobayashi S, Kawamoto Y, Komatsu Y, et al. Multicenter phase II trial of trastuzumab deruxtecan for HER2-positive unresectable or recurrent biliary tract cancer: HERB trial. Future Oncol. 2022 Jun;18(19):2351–60.

27. Wang C, Thudium KB, Han M, Wang X-T, Huang H, Feingersh D, et al. In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates. Cancer Immunol Res. 2014 Sep;2(9):846–56.

28. Li B, VanRoey M, Wang C, Chen T-HT, Korman A, Jooss K. Anti-programmed death-1 synergizes with granulocyte macrophage colony-stimulating factor--secreting tumor cell immunotherapy providing therapeutic benefit to mice with established tumors. Clin Cancer Res. 2009 Mar 1;15(5):1623–34.

29. Janjigian YY, Shitara K, Moehler M, Garrido M, Salman P, Shen L, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and

oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. Lancet. 2021 Jul;398(10294):27–40.

Attachment 1.

SAE Reporting Form_v1.docx

Attachment 2.

Pregnancy Reporting Form_v1