GEORGETOWN UNIVERSITY | Lombardi Comprehensive Cancer Center

Phase II Study of Fluorouracil, Leucovorin, and Nanoliposomal Irinotecan in Previously Treated Advanced Biliary Tract Cancer (NAPOLI-2)

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GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

5-FU	5-fluorouracil
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
AUC	Area under the curve
β-hCG	β-human chorionic gonadotropin
BTC	Biliary tract cancer
BUN	Blood urea nitrogen
CBC	Complete blood count
CDKN2A	Cyclin-dependent kinase inhibitor 2A (gene)
CI	Confidence interval
CNS	Central nervous system
CR	Complete response
CRF	Case report form
CT	Computed tomography
ctDNA	Circulating tumor DNA
CYP	Cytochrome P450
DCR	Disease control rate
DDC	Duration of disease control
DLT	Dose-limiting toxicity
DNA	Deoxyribonucleic acid
DSMB	Data and safety monitoring review board
ECOG	Eastern Cooperative Oncology Group
ECG	Electrocardiogram
EORTC	European Organisation for Research and Treatment of Cancer
FDA	US Food and Drug Administration
FdUMP	5-Fluoro-2′-deoxyuridine 5′-monophosphate
FOLFIRI	Leucovorin, fluorouracil, and irinotecan
FOLFOX	Leucovorin, fluorouracil, and oxaliplatin
FUTP	5-fluorouridine 5'-triphosphate
GEM	Gemcitabine
GGT	Gamma-glutamyl transpeptidase
HIV	Human immunodeficiency virus
HR	Hazard ratio
IFL	Irinotecan, fluorouracil (bolus), and leucovorin
IHC	Immunohistochemistry
IND	Investigational new drug
INR	International normalized ratio
IV	Intravenously
LCCC	Lombardi Comprehensive Cancer Center
LD	Longest diameter
LFT	Liver profile
LLN	Lower limit of normal
LV	Leucovorin
MRI	Magnetic resonance imaging
mOS	Median overall survival
mPFS	Median progression-free survival
NGS	Next-generation Sequencing
ORR	Overall response rate

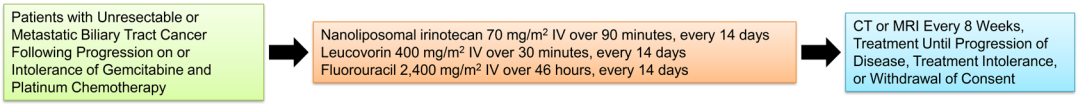
OS	Overall survival
PCR	Polymerase chain reaction
PD	Progression of disease
PFS	Progression-free survival
$PFS_{4\ months}$	Progression-free survival rate at 4 months
PI	Principal investigator
PK	Pharmacokinetics
PO	By mouth
PR	Partial response
PS	Performance Status
PT	Prothrombin time
PTT	Partial thromboplastin time
QD	Once daily
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic acid
RP2D	Recommended Phase II Dose
RR	Response rate
SAE	Significant adverse event
SD	Stable disease
SRS	Stereotactic radiosurgery
TS	Thymidylate synthase
TTP	Time to progression
UGT1A1	UDP glucuronosyltransferase family 1 member A1
ULN	Upper limit of normal
XELOX	Capecitabine and oxaliplatin

STUDY SYNOPSIS

Title	Phase II Study of Fluorouracil, Leucovorin, and Nanoliposomal Irinotecan in Previously Treated Advanced Biliary Tract Cancer (NAPOLI-2)
Short Title	Fluorouracil, Leucovorin, and Nanoliposomal Irinotecan in Biliary Cancer
Protocol Number	2018-0877
Clinicaltrials.gov number:	pending
Phase	Phase II
Investigational Agents	1) Fluorouracil 2) Leucovorin 3) Nanoliposomal irinotecan
Indication	Advanced biliary tract cancer following progression on or intolerance to gemcitabine and platinum chemotherapy
Concept and Scientific Rationale	Gallbladder carcinoma, intra-, and extra-hepatic cholangiocarcinoma comprise biliary tract cancers, rare but aggressive malignancies that frequently present with locally advanced unresectable or metastatic disease. Surgical resection of the primary tumor is the only curative treatment option. An estimated 12,190 people will be diagnosed with and 3,790 will die from biliary tract cancers in the US in 2018.[1] Based on the ABC-02 trial, the standard of care for locally advanced of metastatic biliary tract cancer is gemcitabine and cisplatin.[2] Despite being responsive to multiple chemotherapy agents, there is no established second-line treatment regimen, and patients are strongly encouraged to enroll in clinical trials. Few prospective trials of patients who have disease progression or intolerance of gemcitabine and platinum chemotherapy have been conducted. The largest trial comparing second-line mFOLFOX (leucovorin, fluorouracil, and oxaliplatin) versus best supportive care alone following progression on gemcitabine and platinum is currently ongoing (NCT01926236). Other second-line agents with at least modest activity include fluorouracil, irinotecan, oxaliplatin, S-1, and sunitinib (see Table 1 below). Nanoliposomal irinotecan contains irinotecan free base encapsulated in liposome nanoparticles. The liposome shelters the irinotecan from conversion to its active metabolite (SN-38), and it has been shown to increase the intratumoral levels of SN-38 compared with standard irinotecan.[3] Given the lack of an effective second-line regimen, we propose the approved second-line regimen in metastatic pancreatic adenocarcinoma: fluorouracil, leucovorin, and nanoliposomal irinotecan. In the NAPOLI-1 study, patients with metastatic pancreatic adenocarcinoma previously treated with gemcitabine-based therapies had an overall survival of 6.1 months with fluorouracil, leucovorin, and nanoliposomal irinotecan vs. 4.2 months with fluorouracil and leucovorin (P = 0.012).[4] Fluorouracil and irinotecan have known activity in biliary tract cancers. Patients who progress on first-line therapy and maintain a good performance status are in need of established treatment options.

Study Duration	36 months
Study Centers	1) Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC 2) Siteman Cancer Center, Washington University in St. Louis, St. Louis, MO 3) Indiana University Simon Cancer Center, Indianapolis, IN 4) The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY
Objectives	Primary Objective: To determine the efficacy of fluorouracil, leucovorin, and nanoliposomal irinotecan in advanced biliary tract cancers following progression on or intolerance of gemcitabine and platinum chemotherapy using progression-free survival at 4 months as the primary endpoint. Secondary Objectives: To assess the activity of fluorouracil, leucovorin, and nanoliposomal irinotecan, in patients with advanced biliary tract cancers treated following progression on or intolerance of gemcitabine and platinum chemotherapy in terms of: • Best overall response rate (ORR) • Median progression-free survival (mPFS) • Median overall survival (mOS) • Median time to disease progression (mTTP) • Disease control rate (DCR) (defined as ORR + rate of stable disease at 4 months) • Median duration of disease control (DDC) • Maximum change in tumor marker, CA19-9 • Tolerability and safety • Quality of life assessment Exploratory Objectives: • To assess blood for the analysis of circulating tumor DNA as a surrogate marker of disease burden • To assess archived tumor tissue using next-generation sequencing (NGS) and immunohistochemistry (IHC) in order to elucidate potential mutational biomarkers predictive of response to fluorouracil, leucovorin, and nanoliposomal irinotecan
Number of Subjects	A minimum of 19 and a maximum of 39 evaluable patients
Diagnosis and Main Inclusion and Exclusion Criteria	Key Inclusion Criteria • Pathologically-confirmed biliary tract cancer (cholangiocarcinoma or gallbladder adenocarcinoma), unresectable or metastatic • Disease progression on or intolerance of gemcitabine- and platinum-based chemotherapy • No more than 1 prior line of chemotherapy for unresectable or metastatic disease (adjuvant therapy does not count) • Measurable disease by RECIST v1.1 criteria • ECOG performance status of 0-1 • At least 18 years of age

	• HIV-positive patients are eligible provided:
	  ○ Stable HAART regimen
	  ○ No concurrent prophylactic antibiotics or antifungals
	  ○ CD4 count above 250 and undetectable viral load
	• Adequate bone marrow, hepatic, and renal function
	• Consent to access archived tumor tissue if available (available tissue is not required for enrollment)
	
	<u>Key Exclusion Criteria</u>
	• Ampullary adenocarcinoma
	• Woman who are pregnant or breastfeeding
	• Anti-cancer treatment within 3 weeks prior to enrollment
	• Prior irinotecan or nanoliposomal irinotecan
	• Central nervous system metastases unless stable for at least 4 weeks and at least 2 weeks off corticosteroids
	• Exposure to a strong CYP3A4 inducer, strong CYP3A4 inhibitor, or strong UGT1A1 inhibitor within 2 weeks of study start
	• Known concurrent malignancy or other maligancy within 3 years except for non-melanomatous skin cancers, prostate or cervical cancers following curative therapy, or superficial bladder cancer
	• Bowel obstruction
	• Allergy or hypersensitivity to fluoropyrimidines, irinotecan, or nanoliposomal irinotecan
	• Clinically significant liver disease
	  ○ Patients with resolved hepatitis B infection are eligible if HBsAg testing is negative
	  ○ Patients with resolved hepatitis C infection are eligible if viral RNA PCR is negative
	• Severe infections within 4 weeks prior to enrollment
	• Major surgery within 4 weeks prior to enrollment

Study Design	This is a single arm, open label, multicenter phase II study to evaluate the clinical activity of the combination of fluorouracil, leucovorin, and nanoliposomal irinotecan as second-line treatment in patients with advanced biliary tract cancers following gemcitabine and platinum chemotherapy. Patients with advanced biliary tract cancers who have adequate performance status and adequate hepatic and renal function will be eligible. Patients may have received adjuvant chemotherapy and/or radiation therapy prior to enrolling in the trial, but a minimum of 6 months between adjuvant chemotherapy and this current therapy are required. Patients may continue on study as long as they are tolerating treatment and do not have progression of disease by RECIST v1.1 criteria. Response assessments will occur using imaging (CT or MRI) every 8 weeks. **Figure 1**. Study Schema  For patients homozygous for the *UGT1A1*28* allele, the initial dose will be 50 mg/m^2 then increased to 70 mg/m^2 after the first cycle in the absence of drug-related toxic effects.

Statistical Design, Feasibility, and Trial Duration	This phase II trial will assess the efficacy of fluorouracil, leucovorin, and nanoliposomal irinotecan in patients with advanced biliary tract cancer who progressed on gemcitabine and platinum chemotherapy. The median progression-free survival (mPFS) reported for patients receiving second-line 5-FU doublet chemotherapy is around 3 months, and retrospective data on second-line FOLFIRI/XELIRI show a $PFS_{4\ months}$ of around 30%.[5,6] This new treatment combination of fluorouracil, leucovorin, and nanoliposomal irinotecan would be of great interest in this patient population if it could increase the $PFS_{4\ months}$ to 50% or higher. Thus, the primary endpoint of this trial is $PFS_{4\ months}$ based on central radiology review. We will use a two-stage Simon Minimax phase II design. Using a one-sided α of 0.05 and 80% power, 39 patients will be required to detect a difference in $PFS_{4\ months}$ between 30% and 50%.[7] All patients meeting the eligibility criteria who have signed a consent form, begun treatment, and are not lost to follow-up or who are not taken off study for reasons other than progression or death before 4 months will be considered evaluable for $PFS_{4\ months}$. 19 patients will be needed for the first stage. If 6 or fewer of the 19 patients are alive and progression-free at 4 months, we will conclude that this treatment is insufficiently active in this population and terminate the study. If 7 or more of these 19 patients are alive and progression-free at 4 months, we will continue the study to the second stage and accrue 20 more patients for a total of 39 evaluable patients. If 16 or fewer of these 39 patients are alive and progression-free at 4 months, we will conclude that this treatment is insufficiently active in this population. If 17 or more of these 39 patients are alive and progression-free at 4 months, this will be considered adequate evidence of efficacy of this treatment and may be recommended for further testing in subsequent studies. Assuming a drop-out rate of 20%, a maximum of 50 patients will be accrued. All patients who receive at least 1 dose of the study treatment will be eligible for safety analysis. Patients will be replaced for the primary efficacy analysis if they come off study for reasons other than disease progression prior to the 4 month efficacy endpoint. We will substitute patients who screen fail or screen and do not begin study treatment. Between the Lombardi Comprehensive Cancer Center at Georgetown University, Siteman Cancer Center at Washington University in St. Louis, Indiana University Simon Cancer Center, and The Tisch Cancer Institute at Icahn School of Medicine at Mount Sinai, over 80 new cases of biliary tract cancer are seen annually, at least one-third of which have metastatic disease. Since most patients with metastatic disease eventually progress on first-line chemotherapy, most patients seen in the medical oncology clinic with metastatic disease will be eligible for this trial. Therefore, recruitment is anticipated to take 32 months (1.4 patients per month), and with each patient followed for a minimum of 4 months, the anticipated time to complete follow-up of all patients for the primary endpoint will be 36 months.

1.0 BACKGROUND AND JUSTIFICATION

1.1 Biliary Tract Cancer (BTC)

Gallbladder carcinoma, intra-, and extra-hepatic cholangiocarcinoma comprise BTCs, rare but aggressive malignancies that frequently present with locally advanced unresectable or metastatic disease. Surgical resection of the primary tumor is the only curative treatment option. An estimated 12,190 people will be diagnosed with and 3,790 will die from biliary tract cancers in the US in 2018.[1]

1.2 First-Line Treatment of BTC

Based on the ABC-02 trial, the standard of care for locally advanced of metastatic biliary tract cancer is gemcitabine and cisplatin.[2] In this trial, 410 patients were randomly assigned 1:1 to gemcitabine 1000 mg/m^2 and cisplatin 25 mg/m^2 days 1 and 8 every 21 days for 8 cycles or gemcitabine monotherapy 1000 mg/m^2 days 1, 8, and 15 every 28 days for 6 cycles. Patients in the combination arm had a median OS (mOS) of 11.7 months vs. 8.1 months in the monotherapy arm (hazard ratio [HR] 0.64, 95% confidence interval [CI] 0.52-0.80; P < 0.001).[2] Despite being responsive to multiple chemotherapy agents, there is no established second-line treatment regimen, and patients are strongly encouraged to enroll in clinical trials.

1.3 Second- and Later-Line Treatment of BTC

Few prospective trials of patients who have disease progression or intolerance of gemcitabine and platinum chemotherapy have been conducted. The largest trial comparing second-line mFOLFOX (leucovorin, fluorouracil, and oxaliplatin) versus best supportive care alone following progression on gemcitabine and platinum is currently ongoing (NCT01926236). Other second-line agents with at least modest activity include fluorouracil, irinotecan, oxaliplatin, S-1, and sunitinib (see Table 1 below).

Table 1. Second-line Clinical Trials in Advanced Biliary Tract Cancer

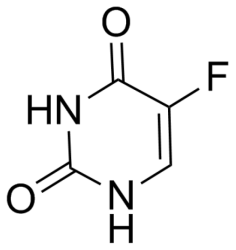
1st Author (year)	Regimen	GP (%)	N	ORR (%)	PFS or TTP (mo)	OS (mo)
Zheng (2018)[6]	XELIRI vs. Iri	100%	64	13% vs. 7%	3.7 vs. 2.4	10.1 vs. 7.3
Cereda (2016)[8]	Cape 2000 mg/m^2 D1-14 + MMC 6 mg/m^2 D1 vs. Cape alone q 21 days	100%	57	3% vs. 0%	2.3 vs. 2.1	8.1 vs. 9.5
Brieau (2015)[5]	FOLFIRI/XELIRI (retrospective)	100%	61	12%	2.6	6.1
Brieau (2015)[5]	5FU + LV + Cis (retrospective)	100%	37	14%	NR	NR
Brieau (2015)[5]	Cape or 5FU + LV (retrospective)	100%	37	11%	3.3	5.6
Brieau (2015)[5]	FOLFOX/XELOX (retrospective)	100%	20	10%	NR	NR
Brieau (2015)[5]	Sunitinib 37.5 mg daily (retrospective)	100%	9	11%	4.6	8.4
Hwang (2014)[9]	Ox 85 mg/m^2 D1 + LV 30 mg/m^2 D1-2 + 5FU 1500 mg/m^2 D1-2 (mFOLFOX3) q 14 days	93%	30	7%	1.6	4.4
He (2013)[10]	Ox 85 mg/m^2 D1 + LV 200 mg/m^2 D1-2 + 5FU 400 mg/m^2 D1-2 + 600 mg/m^2 D1-2 (FOLFOX4) q 14 days	100%	37	22%	3.1	6.9
Yi (2012)[11]	Sunitinib 37.5 mg daily	68%	56	9%	1.7	4.8
Sasaki (2012)[12]	S-1 80 mg/m^2 D1-28 q 6 weeks (refractory to Gem monotherapy only)	0%	22	23%	5.4	13.5
Oh (2011)[13]	Gem 1250 mg/m^2 D1 and D8 q 3 weeks (refractory to 5FU monotherapy only)	0%	32	7%	1.6	4.1

5FU = 5-fluorouracil; Cape = capecitabine; Cis = cisplatin; FOLFIRI = 5-fluorouracil, leucovorin, and irinotecan; FOLFOX = 5-fluorouracil, leucovorin, and oxaliplatin; Gem = gemcitabine; GP = patients receiving gemcitabine and platinum first-line chemotherapy; Iri = irinotecan. LV = leucovorin; MMC = mitomycin C; NR = not reported; ORR = objective response rate; OS = overall survival; Ox = oxaliplatin; PFS = progression-free survival; TTP = time to progression; XELIRI = capecitabine and irinotecan; XELOX = capecitabine and oxaliplatin;.

1.4 Fluorouracil and Leucovorin

Fluorouracil (5-FU) has been the backbone of chemotherapy regimens for gastrointestinal malignancies dating back to the 1950s. 5-FU is FDA-approved for use in colorectal, breast, gastric, and pancreatic adenocarcinoma.[14]

Figure 1. Chemical Structure of Fluorouracil



5-FU is a nucleoside metabolic inhibitor that is converted to FdUMP which inhibits thymidylate synthase (TS), disrupting DNA synthesis. Leucovorin (folinic acid) stabilizes the ternary complex of FdUMP, TS, and tetrahydrofolate, further potentiating TS inhibition.[15] The 5-FU metabolite FUTP is incorporated into RNA, and another, FdUTP, is incorporated into DNA, further disrupting RNA and DNA synthesis resulting in cell death in rapidly dividing cells.

5-FU must be permanently discontinued in patients with early onset or unusually severe toxicity which may indicate complete or near absence of dipyrimidine dehydrogenase (DPD) activity. Fluorouracil carries a risk of cardiotoxicity, hyperammonemic encephalopathy, neurologic toxicity, diarrhea, palmar-plantar erythrodyesthesia (hand-foot syndrome), myelosuppression, mucositis, increased risk of INR with warfarin, and embryofetal toxicity.

1.5 FOLFIRI Regimen

The active metabolite of irinotecan (SN-38) is a potent topoisomerase I inhibitor that induces DNA damage and cell death (see section 1.7.1.2). Irinotecan is FDA-approved for the first-line treatment of metastatic colorectal cancer (mCRC) and for treatment of mCRC following progression on or recurrence following 5-FU-based therapy.[16] Saltz and colleagues demonstrated that the addition of irinotecan 125 mg/m^2 IV in combination with 5-FU 425 mg/m^2 IV bolus and LV 20 mg/m^2 given weekly for 4 weeks every 6 weeks (IFL regimen) was superior to 5-FU and LV at the same doses given daily days 1-5 every 4 weeks in patient with mCRC.[17] André and colleagues also demonstrated the efficacy of bolus 5-FU 400 mg/m^2 IV, LV 400 mg/m^2 IV, irinotecan 180 mg/m^2 IV, and infusional 5-FU 2400 mg/m^2 IV over 46 hours every 2 weeks (FOLFIRI regimen).[18] Fuchs and colleagues later showed that irinotecan, LV, and infusional 5-FU (FOLFIRI) was superior to irinotecan, LV, and bolus 5-FU (IFL), both with and without bevacizumab.[19] FOLFIRI is an established regimen for mCRC in the first- or second-line settings.[20]

As mentioned above, there are no phase III data to guide treatment selection of patients with advanced BTCs other than the ABC-02 trial which demonstrated the superior of gemcitabine and cisplatin to gemcitabine monotherapy in the first-line setting.[2] Thus, regimens taken from mCRC are frequently used in refractory advanced BTC, including fluoropyrimidine (5-FU or capecitabine) combinations with oxaliplatin (FOLFOX or XELOX) and irinotecan (FOLFIRI or XERLIRI, see Table 1).

Brieau and colleagues looked retrospectively at 196 patients with advanced BTC treated with different second-line regimens following progression on gemcitabine plus oxaliplatin or

gemcitabine plus cisplatin.[5] This study included 61 patients treated with FOLFIRI or XELIRI, and then had a mPFS of 2.6 months and a mOS of 6.1 months.

More recently, Zheng and colleagues prospectively randomized 64 patients who had disease progression on first-line gemcitabine/platinum doublet to irinotecan 180 mg/m^2 IV every 14 days or the same in combination capecitabine 1000 mg/m^2 by mouth twice daily days 1-10 (XELIRI).[6] mPFS was 3.7 months with XELIRI vs. 2.4 months with irinotecan (HR 0.54, 95% CI 0.30-0.98; P = 0.036), and mOS was 10.1 vs. 7.3 months (HR 0.63, 95% CI 0.35-1.12; P = 0.107), indicating that XELIRI prolongs PFS compared with irinotecan monotherapy in this patient population.[6] Grade 3-4 adverse events in the XELIRI arm included neutropenia (27%), thrombocytopenia (7%), anemia (7%), diarrhea (3%), nausea (10%), vomiting (3%), and hand-foot syndrome (7%).

Therefore, the retrospective Brieau and prospective Zheng data indicate that fluoropyrimidine and irinotecan combinations are active and safe in second-line advanced BTC.

1.6 Fluorouracil, Leucovorin, and Nanoliposomal Irinotecan Regimen in Pancreatic Cancer (NAPOLI-1)[4]

In the NAPOLI-1 trial, patients with metastatic pancreatic ductal adenocarcinoma previously treated with gemcitabine-based chemotherapy were randomized 1:1:1 to receive nanoliposomal irinotecan monotherapy (120 mg/m^2 IV every 3 weeks), LV 200 mg/m^2 IV and 5-FU 2400 mg/m^2 IV over 24 hours weekly for 4 weeks of each 6-week cycle, or the combination with nanoliposomal irinotecan 70 mg/m^2 IV, LV 400 mg/m^2 IV, and 5-FU 2400 mg/m^2 IV over 46 hours every 2 weeks. mOS was significantly longer in the combination arm compared to the 5-FU/LV arm (6.1 vs. 4.2 months; HR 0.67, 95% CI 0.49-0.92; P = 0.012). Common grade 3-4 adverse events in the combination arm included neutropenia (27%), anemia (9%), hypokalemia (3%), diarrhea (13%), nausea (8%), vomiting (11%), fatigue (14%), and decreased appetite (4%).

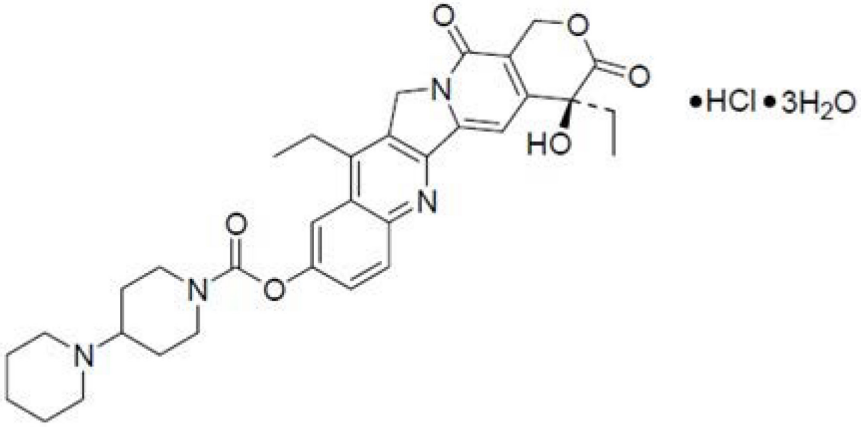
Nanoliposomal irinotecan contains irinotecan free base encapsulated in liposome nanoparticles. The liposome shelters the irinotecan from conversion to its active metabolite (SN-38), and it has been shown to increase the intratumoral levels of SN-38 compared with standard irinotecan (see section 1.7).[3] Given the lack of an effective second-line regimen in BTC, we propose the approved second-line regimen in metastatic pancreatic adenocarcinoma based on the NAPOLI-1 study: nanoliposomal irinotecan 70 mg/m^2 IV, LV 400 mg/m^2 IV, and 5-FU 2400 mg/m^2 IV over 46 hours every 2 weeks.

1.7 Nanoliposomal Irinotecan[21]

1.7.1 Description

Nanoliposomal irinotecan is formulated with irinotecan hydrochloride trihydrate, a topoisomerase inhibitor, into a liposomal dispersion for intravenous use. The chemical name of irinotecan hydrochloride trihydrate is (S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate. The empirical formula is C33H38N4O6·HCl·3H2O and the molecular weight is 677.19 g/mole. The molecular structure is:

Figure 3. Chemical Structure of Nanoliposomal Irinotecan



Nanoliposomal irinotecan is a sterile, white to slightly yellow opaque isotonic liposomal dispersion. Each 10 mL single-dose vial contains 43 mg irinotecan free base at a concentration of 4.3 mg/mL. The liposome is a unilamellar lipid bilayer vesicle, approximately 110 nm in diameter, which encapsulates an aqueous space containing irinotecan in a gelated or precipitated state as the sucrose octasulfate salt. The vesicle is composed of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) 6.81 mg/mL, cholesterol 2.22 mg/mL, and methoxy-terminated polyethylene glycol (MW 2000)-distearoylphosphatidyl ethanolamine (MPEG-2000-DSPE) 0.12 mg/mL. Each mL also contains 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES) as a buffer 4.05 mg/mL and sodium chloride as an isotonicity reagent 8.42 mg/mL.

1.7.2 Clinical Pharmacology

1.7.2.1 Mechanism of Action

Irinotecan liposome injection (Onivyde™) is a topoisomerase 1 inhibitor encapsulated in a lipid bilayer vesicle or liposome. Topoisomerase 1 relieves torsional strain in DNA by inducing single-strand breaks. Irinotecan and its active metabolite SN-38 bind reversibly to the topoisomerase 1-DNA complex and prevent re-ligation of the single-strand breaks, leading to exposure time-dependent double-strand DNA damage and cell death. In mice bearing human tumor xenografts, irinotecan liposome administered at irinotecan HCl-equivalent doses 5-fold lower than irinotecan HCl achieved similar intratumoral exposure of SN-38.

1.7.2.2 Pharmacokinetics
The plasma pharmacokinetics of total irinotecan and total SN-38 were evaluated in patients with cancer who received nanoliposomal irinotecan, as a single agent or as part of combination chemotherapy, at doses between 50 and 155 mg/m^2 and 353 patients with cancer using population pharmacokinetic analysis.

The pharmacokinetic parameters of total irinotecan and total SN-38 following the administration of nanoliposomal irinotecan 70 mg/m^2 as a single agent or part of combination chemotherapy are presented in Table 2.

Table 2. Summary of Mean (± Standard Deviation) Total Irinotecan and Total SN-38

Dose (mg/m^2)	Total Irinotecan					Total SN-38		
	C_{max} [µg/mL] (n=25)	$AUC_{0-\infty}$ [h·µg/mL] (n=23)	$t_{1/2}$ [h] (n=23)	CL [L/h] (n=23)	V_d [L] (n=23)	C_{max} [ng/mL] (n=25)	$AUC_{0-\infty}$ [h·ng/mL] (n=13)	$t_{1/2}$ [h] (n=13)
70	37.2 (8.8)	1364 (1048)	25.8 (15.7)	0.20 (0.17)	4.1 (1.5)	5.4 (3.4)	620 (329)	67.8 (44.5)

C_{max}: Maximum plasma concentration
$AUC_{0-\infty}$: Area under the plasma concentration curve extrapolated to time infinity
$t_{½}$: Terminal elimination half-life
CL: Clearance
V_d: Volume of distribution

Over the dose range of 50 to 155 mg/m^2, the C_{max} and AUC of total irinotecan increases with dose. Additionally, the C_{max} of total SN-38 increases proportionally with dose; however, the AUC of total SN-38 increases less than proportionally with dose.

1.7.2.2.1 Distribution

Direct measurement of irinotecan liposome showed that 95% of irinotecan remains liposome-encapsulated, and the ratios between total and encapsulated forms did not change with time from 0 to 169.5 hours post-dose. The mean volume of distribution is summarized in Table 2.

Plasma protein binding is <0.44% of the total irinotecan in nanoliposomal irinotecan.

1.7.2.2.2 Elimination

Metabolism

The metabolism of irinotecan liposome has not been evaluated. Irinotecan is subject to extensive metabolic conversion by various enzyme systems, including esterases to form the active metabolite SN-38, and UGT1A1 mediating glucuronidation of SN-38 to form the inactive glucuronide metabolite SN-38G. Irinotecan can also undergo CYP3A4-mediated oxidative metabolism to several inactive oxidation products, one of which can be hydrolyzed by carboxylesterase to release SN-38. In the population pharmacokinetic analysis using the results of a subset with UGT1A1*28 genotypic testing, in which the analysis adjusted for the lower dose administered to patients homozygous for the UGT1A1*28 allele, patients homozygous (N=14) and non-homozygous (N=244) for this allele had total SN-38 average steady-state concentrations of 1.06 and 0.95 ng/mL, respectively.

Excretion

The disposition of nanoliposomal irinotecan has not been elucidated in humans. Following administration of irinotecan HCl , the urinary excretion of irinotecan is 11 to 20%; SN-38, <1%; and SN-38 glucuronide, 3%. The cumulative biliary and urinary excretion of irinotecan and its metabolites (SN-38 and SN-38 glucuronide), over a period of 48 hours following administration of irinotecan HCl in two patients, ranged from approximately 25% (100 mg/m^2) to 50% (300 mg/m^2).

1.7.2.2.3 Specific Populations

Age, Gender, and Renal Impairment:

The population pharmacokinetic analysis suggests that age (28 to 87 years) had no clinically meaningful effect on the exposure of irinotecan and SN-38.

The population pharmacokinetic analysis suggests that gender (196 males and 157 females) had no clinically meaningful effect on the exposure of irinotecan and SN-38 after adjusting for body surface area (BSA).

In a population pharmacokinetic analysis, mild-to-moderate renal impairment had no effect on the exposure of total SN-38 after adjusting for BSA. The analysis included 68 patients with moderate (CLcr 30 - 59 mL/min) renal impairment, 147 patients with mild (CLcr 60 - 89 mL/min) renal impairment, and 135 patients with normal renal function (CLcr > 90 mL/min). There was insufficient data in patients with severe renal impairment (CLcr < 30 mL/min) to assess its effect on pharmacokinetics.

Ethnicity: The population pharmacokinetic analysis suggests that Asians (East Asians, N=150) have 56% lower total irinotecan average steady state concentration and 8% higher total SN-38 average steady state concentration than Whites (N=182).

Hepatic Impairment: The pharmacokinetics of irinotecan liposome have not been studied in patients with hepatic impairment. In a population pharmacokinetic analysis, patients with baseline bilirubin concentrations of 1-2 mg/dL (N=19) had average steady state concentrations for total SN-38 that were increased by 37% compared to patients with baseline bilirubin concentrations of <1 mg/dL (N=329); however, there was no effect of elevated ALT/AST concentrations on total SN-38 concentrations. No data are available in patients with bilirubin >2 mg/dL.

1.7.2.2.4 Drug Interactions

In a population pharmacokinetic analysis, the pharmacokinetics of total irinotecan and total SN38 were not altered by the co-administration of fluorouracil/leucovorin.

Following administration of irinotecan HCl, dexamethasone, a moderate CYP3A4 inducer, does not alter the pharmacokinetics of irinotecan.

In vitro studies indicate that irinotecan, SN-38 and another metabolite, aminopentane carboxylic acid (APC), do not inhibit cytochrome P-450 isozymes.

*1.7.2.3 Pharmacogenomics (UGT1A1*28)*

Individuals who are homozygous for the UGT1A1*28 allele are at increased risk for neutropenia from irinotecan HCl. In NAPOLI-1, patients homozygous for the UGT1A1*28 allele (N=7) nitiated nanoliposomal irinotecan at a reduced dose of 50 mg/m^2 in combination with 5-FU/LV. The frequency of Grade 3 or 4 neutropenia in these patients [2 of 7 (28.6%)] was similar to the frequency in patients not homozygous for the UGT1A1*28 allele who received a starting dose of nanoliposomal irinotecan of 70 mg/m^2 [30 of 110 (27.3%)].

1.7.3 **Nonclinical Toxicology - Carcinogenesis, Mutagenesis, Impairment of Fertility**

No studies have been performed to assess the potential of irinotecan liposome for carcinogenicity, genotoxicity or impairment of fertility. Intravenous administration of irinotecan hydrochloride to rats once weekly for 13 weeks followed by a 91-week recovery period resulted in a significant linear trend between irinotecan HCl dosage and the incidence of combined uterine horn endometrial stromal polyps and endometrial stromal sarcomas. Irinotecan HCl was clastogenic both in vitro (chromosome aberrations in Chinese hamster ovary cells) and in vivo (micronucleus test in mice). Neither irinotecan nor its active metabolite, SN-38, was mutagenic in the in vitro Ames assay.

Dedicated fertility studies have not been performed with irinotecan liposome injection. Atrophy of male and female reproductive organs was observed in dogs receiving irinotecan liposome injection every 3 weeks at doses equal to or greater than 15 mg/kg, (approximately 3 times the clinical exposure of irinotecan following administration to nanoliposomal irinotecan dosed at 70 mg/m^2) for a total of 6 doses. No significant adverse effects on fertility and general reproductive performance were observed after intravenous administration of irinotecan HCl in doses of up to 6 mg/kg/day to rats; however, atrophy of male reproductive organs was observed after multiple daily irinotecan HCl doses both in rodents at 20 mg/kg (approximately 0.007 times the clinical irinotecan exposure following nanoliposomal irinotecan administration at 70 mg/m^2) and in dogs at 0.4 mg/kg (0.0007 times the clinical exposure to irinotecan following administration of nanoliposomal irinotecan).

1.7.4 Supply, Storage, and Handling

1.7.4.1 Supply

Nanoliposomal irinotecan is available in a single-dose vial containing 43 mg irinotecan free base at a concentration of 4.3 mg/mL.

1.7.4.2 Storage and Handling

Store nanoliposomal irinotecan at 2ºC to 8ºC (36°F to 46°F). Do NOT freeze. Protect from light.

Nanoliposomal irinotecan is a cytotoxic drug. Follow applicable special handling and disposal procedures.

1.7.5 Preparation and Administration

Withdraw the calculated volume of nanoliposomal irinotecan from the vial. Dilute nanoliposomal irinotecan in 500 mL 5% Dextrose Injection, USP or 0.9% Sodium Chloride Injection, USP and mix diluted solution by gentle inversion.

Protect diluted solution from light.

Administer diluted solution within 4 hours of preparation when stored at room temperature or within 24 hours of preparation when stored under refrigerated conditions [2ºC to 8ºC (36ºF to 46ºF)]. Allow diluted solution to come to room temperature prior to administration.

Do NOT freeze.

Infuse diluted solution intravenously over 90 minutes. Do not use in-line filters. Discard unused portion.

1.7.6 Warnings and Precautions

1.7.6.1 Severe neutropenia resulting in fatal neutropenic sepsis occurred in 1 of 117 patients receiving nanoliposomal irinotecan, 5-FU, and LV in the NAPOLI-1 trial and 1 of 147 patients receiving nanoliposomal irinotecan monotherapy (0.8%). For patients receiving nanoliposomal irinotecan, 5-FU, and LV in the NAPOLI-1 trial, grade 3-4 neutropenia was higher in Asian patients compared to White patients (55% vs. 18%) as was neutropenic fever/neutropenic sepsis (6% vs. 1%).

1.7.6.2 Severe diarrhea can occur with nanoliposomal irinotecan. All patients will receive prophylactic atropine prior to drug infusion. Diarrhea typically occurs in an early onset phase within 24 hours of chemotherapy (sometimes accompanied by other cholingeric

reactions) and/or a late onset diarrhea more than 24 hours from chemotherapy. Grade 3-4 diarrhea occurred in 13% of patients receiving nanoliposomal irinotecan, 5-FU, and LV in the NAPOLI-1 trial, compared to 4% in patients receiving 5-FU and LV. Nanoliposomal irinotecan should be held for grade 2-4 diarrhea. Loperamide should be started for late onset diarrhea of any severity. Nanoliposomal irinotecan can be resumed at a reduced dose following recovery to grade 1 diarrhea.

1.7.6.3 Irinotecan can cause interstitial lung disease (ILD). Nanoliposomal irinotecan should be held in patients with new or progressive dyspnea, cough, or fever. Nanoliposomal irinotecan should be permanently discontinued in patients who have a confirmed diagnosis of ILD.

1.7.6.4 Irinotecan can cause severe hypersensitivity reactions including anaphylaxis; nanoliposomal irinotecan should be permanently discontinued in patients who experience a severe hypersensitivity reaction.

1.7.6.5 Irinotecan is embryotoxic and teratogenic and cause fetal harm to pregnant women. Women of child-bearing potential must use effective contraception during treatment with nanoliposomal irinotecan during treatment and for one month following the final dose.

1.7.7 Drug Interactions

Strong CYP3A4 inducers have the potential to reduce exposure to irinotecan or its active metabolite, SN-38, and thus should be avoided if possible. These drugs include rifampin, phenytoin, carbamazepine, rifabutin, rifapentine, Phenobarbital, and St. John’s wort. Substitute non-enzyme inducing therapies at least 2 weeks prior to initiation of nanoliposomal irinotecan.

Strong CYP3A4 or UGT1A1 inhibitors have the potential to increase exposure to irinotecan or its active metabolite, SN-38, and thus should be avoided if possible. These drugs include ketoconazole, clarithromycin, indinavir, itraconazole, lopinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telaprevir, voriconazole, atazanavir, or gemfibrozil.

2.0 STUDY OBJECTIVES

2.1 Primary Objective:

To determine the efficacy of fluorouracil, leucovorin, and nanoliposomal irinotecan in advanced biliary tract cancers following progression on or intolerance of gemcitabine and platinum chemotherapy using progression-free survival at 4 months as the primary endpoint.

2.2 Secondary Objectives:

To assess the activity of fluorouracil, leucovorin, and nanoliposomal irinotecan, in patients with advanced biliary tract cancers treated following progression on or intolerance of gemcitabine and platinum chemotherapy in terms of:

2.2.1 Best overall response rate (ORR)
2.2.2 Median progression-free survival (mPFS)
2.2.3 Median overall survival (mOS)
2.2.4 Median time to disease progression (mTTP)
2.2.5 Disease control rate (DCR) (defined as ORR + rate of stable disease at 4 months)
2.2.6 Median duration of disease control (DDC)
2.2.7 Maximum change in tumor marker, CA19-9

2.2.8 To assess the tolerability and safety of fluorouracil, leucovorin, and nanoliposomal irinotecan, in patients with advanced biliary tract cancers treated following progression on or intolerance of gemcitabine and platinum chemotherapy

2.2.9 To assess the changes in quality of life over the treatment course of patients with biliary tract cancers treated following progression on or intolerance of gemcitabine and platinum chemotherapy with fluorouracil, leucovorin, and nanoliposomal irinotecan

2.3 Exploratory Objectives:

2.3.1 To assess blood for the analysis of circulating tumor DNA as a surrogate marker of disease burden

2.3.2 To assess tumor tissue for potential predictive biomarkers of response to fluorouracil, leucovorin, and nanoliposomal irinotecan using NGS and IHC

2.4 Primary Endpoint:

PFS at 4 months in a patient with advanced biliary tract cancer treated with fluorouracil, leucovorin, and nanoliposomal irinotecan following progression on or intolerance of gemcitabine and platinum chemotherapy, defined as positive if a patient does not have evidence of disease progression (PD) at 4 months, as measured by expert radiologists using RECIST v1.1 criteria

2.5 Secondary Endpoints:

2.5.1 Best response in a patient, defined as the largest percent decrease in tumor size from baseline and categorized as a complete response (CR), partial response (PR), stable disease (SD), or PD by imaging studies, measured by expert radiologists using RECIST v1.1 criteria. Overall response rate (ORR) is the proportion of patients obtaining a CR or PR as the best response. Disease control rate (DCR) is the proportion of patients obtaining a CR, PR, or SD at 4 months. Duration of disease control (DDC) is the median length of time of patients maintaining a CR, PR, or SD. Maximum change in tumor marker (CA19-9) is defined as maximum decrease in CA19-9 compared to baseline at any point on the study.

2.5.2 PFS in a patient with advanced biliary tract cancer treated with fluorouracil, leucovorin, and nanoliposomal irinotecan following progression on or intolerance of gemcitabine and platinum chemotherapy, defined as the time from Cycle 1, Day 1 to PD (as measured by expert radiologists using RECIST v1.1 criteria), death from any cause, or last follow-up, as determined by the investigator

2.5.3 OS in a patient with advanced biliary tract cancer treated with fluorouracil, leucovorin, and nanoliposomal irinotecan following progression on or intolerance of gemcitabine and platinum chemotherapy, defined as the time from Cycle 1, Day 1 to death from any cause or last follow-up

2.5.4 TTP in a patient with advanced biliary tract cancer treated with fluorouracil, leucovorin, and nanoliposomal irinotecan following progression on or intolerance of gemcitabine and platinum chemotherapy, defined as the time from Cycle 1, Day 1 to PD (as measured by expert radiologists using RECIST v1.1 criteria), as determined by the investigator

2.5.5 Adverse events in a patient with advanced biliary tract cancer treated with fluorouracil, leucovorin, and nanoliposomal irinotecan following progression on or intolerance of gemcitabine and platinum chemotherapy, defined by CTCAE v5.0 as determined by the investigator

2.5.6 Quality of life changes as measured the by EORTC QLQ-C30 questionnaire

2.6 Exploratory Endpoints:

2.6.1 Correlation of dynamics of circulating tumor DNA change compared with change in CA19-9

2.6.2 NGS profile of archived tumor tissue, in particular the presence of *TP53*, *ARID1A*, *KRAS*, *IDH1*, and *CDKN2A* mutations, and TOPO1 expression by IHC

2.7 Indication:

Patients with advanced biliary tract cancer following progression on or intolerance of gemcitabine and platinum chemotherapy

3.0 SUBJECT POPULATION

3.1 Subject Population, Number of Subjects and Feasibility

(See Appendix B for Study Eligibility Checklist)

3.1.1 Subject Population

Patients with advanced biliary tract cancers following progression on or intolerance of gemcitabine and platinum chemotherapy and who have an adequate performance status (PS), bone marrow, hepatic, and renal function

3.1.2 Number of Subjects

50 patients to get 39 evaluable patients

3.1.3 Feasibility

Between the Lombardi Comprehensive Cancer Center at Georgetown University, Siteman Cancer Center at Washington University in St. Louis, Indiana University Simon Cancer Center, and The Tisch Cancer Institute at Icahn School of Medicine at Mount Sinai, over 80 new cases of biliary tract cancer are seen annually, at least one-third of which have metastatic disease. Since most patients with metastatic disease eventually progress on first-line chemotherapy, most patients seen in the medical oncology clinic with metastatic disease will be eligible for this trial. Therefore, recruitment is anticipated to take 32 months (1.4 patients per month), and with each patient followed for a minimum of 4 months, the anticipated time to complete follow-up of all patients for the primary endpoint will be 36 months.

3.2 Inclusion Criteria

1. Pathologically-confirmed biliary tract cancer (cholangiocarcinoma or gallbladder adenocarcinoma)

2. Disease progression on or intolerance of gemcitabine- and platinum-based chemotherapy

3. No more than 1 prior line of chemotherapy for unresectable or metastatic disease (adjuvant therapy does not count)

4. Measurable disease by RECIST v1.1 criteria (see Section 7.0)

5. ECOG performance status of 0-1 (see Table 3)

6. At least 18 years of age

7. HIV-positive patients are eligible provided:

    a. Patients are on a stable HAART regimen that has not changed in at least 6 months
    b. Patients are not on concurrent prophylactic antibiotics or antifungals
    c. The CD4 count above 250 cells/μL and the viral load is undetectable

   NOTE: Testing only required for those with known or suspected infection.

8. Subjects with no brain metastases or a history of previously treated brain metastases who have been treated by surgery or stereotactic radiosurgery (SRS) at least 4 weeks prior to enrollment and have a baseline MRI that shows no evidence of active intracranial disease and at least 2 weeks off corticosteroids

9. Consent to access archived tumor tissue if available (available tissue is not required for enrollment)

10. Bone marrow function: absolute neutrophil count (ANC) ≥ 1,500/mm^3; Platelets ≥ 100 × $10^{9/}$L; hemoglobin ≥ 9.0 g/dL. Patients may have a transfusion of red blood cells to meet the hemoglobin requirement.

11. Renal function: serum creatinine ≤ 1.5 × upper normal limit of institution's normal range or creatinine clearance ≥ 30 mL/min for subjects with creatinine levels above institutional normal

12. Hepatic function: AST and ALT ≤ 3.0 × the upper normal limit of institution's normal range. Total bilirubin ≤ 1.5 × the upper normal limit of institution's normal range. For subjects with liver metastases, AST and ALT < 5 × the upper normal limit of institution's normal range, and total bilirubin >1.5 – 3.0 x the upper normal limit of institution's normal range are acceptable as long as there is no persistent nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia.

13. Partial Thromboplastin Time (PTT) must be ≤ 1.5 × upper normal limit of institution's normal range and INR (International Normalized Ratio) < 1.5. Subjects on anticoagulant (such as warfarin) will be permitted to enroll as long as the INR is in the acceptable therapeutic range as determined by the investigator.

14. Patients must have fully recovered from all effects of surgery. Patients must have had at least two weeks after minor surgery and four weeks after major surgery before starting therapy. Minor procedures requiring "Twilight" sedation such as endoscopies or mediport placement may only require a 24-hour waiting period, but this must be discussed with an investigator.

15. Women of childbearing potential must have a negative serum pregnancy test within 14 days prior to initiation of treatment and/or postmenopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential

16. Patient is capable of understanding and complying with parameters as outlined in the protocol and able to sign and date the informed consent, approved by the Institutional Review Board (IRB), prior to the initiation of any screening or study-specific procedures

Table 3. ECOG Performance Status

Grade	ECOG
0	Fully Active, able to carry on all pre-disease performance without restriction.
1	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.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

3.3 Exclusion Criteria

1. Ampullary adenocarcinoma
2. Women who are pregnant or breastfeeding
3. Anti-cancer treatment within 3 weeks prior to enrollment
4. Prior irinotecan or nanoliposomal irinotecan
5. Known concurrent malignancy or other maligancy within 3 years except for non-melanomatous skin cancers, prostate or cervical cancers following curative therapy, superficial bladder cancer, or other noninvasive or indolent malignancy either that has undergone potentially curative treatment or not requiring active treatment as determined by the Principal Investigator
6. Bowel obstruction
7. Allergy or hypersensitivity to fluoropyrimidines, irinotecan, or nanoliposomal irinotecan
8. Exposure to a strong CYP3A4 inducer, strong CYP3A4 inhibitor, or strong UGT1A1 inhibitor within 2 weeks of study start
9. Clinically significant liver disease
    a. Patients with resolved hepatitis B infection are eligible if HbsAg testing is negative
    b. Patients with resolved hepatitis C infection are eligible if viral RNA PCR is negative

NOTE: Testing only required for those with known or suspected infection.

10. Severe infections within 4 weeks prior to enrollment
11. Major surgery within 4 weeks prior to enrollment
12. Psychiatric illness or social situation that would limit compliance with study requirements
13. Patient has any other concurrent severe and/or uncontrolled medical condition that would, in the investigator's judgment, cause unacceptable safety risks, contraindicate patient participation in the clinical study or compromise compliance with the protocol (e.g. chronic pancreatitis, chronic active hepatitis, active untreated or uncontrolled fungal, bacterial or viral infections other than described above, etc.).
14. Clinically significant, uncontrolled heart disease and/or cardiac repolarization abnormalities, including any of the following:
    a. History of acute coronary syndromes (including myocardial infarction, unstable angina, coronary artery bypass grafting, coronary angioplasty, or stenting) or symptomatic pericarditis within 6 months prior to screening
    b. History of documented congestive heart failure (New York Heart Association functional classification III-IV)

c. Documented cardiomyopathy

d. Clinically significant cardiac arrhythmias (e.g. ventricular tachycardia), complete left bundle branch block, high-grade AV block (e.g. bifascicular block, Mobitz type II and third-degree AV block)

e. Long QT syndrome or family history of idiopathic sudden death or congenital long QT syndrome, or any of the following:

f. Risk factors for Torsades de Pointes (TdP) including uncorrected hypokalemia or hypomagnesemia, history of cardiac failure, or history of clinically significant/symptomatic bradycardia.

g. Concomitant use of medication(s) with a known risk to prolong the QT interval and/or known to cause Torsades de Pointes that cannot be discontinued (within 5 half-lives or 7 days prior to starting study drug) or replaced by safe alternative medication

h. Systolic blood pressure (SBP) >160 mmHg or <90 mmHg at screening

3.4 Prior and Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins and/or herbal supplements) that the subject is receiving at Screening up to the Final Visit must be recorded in source documents and the case report forms (CRFs). The reason for use, date(s) of administration (including start and end dates), and dosage information (including dose and frequency) must be recorded. Any change in concomitant therapy during the study period must be similarly recorded. Questions regarding prior or concomitant therapy should be directed to one of the investigators.

3.4.1 Prior Anti-Cancer Therapy

For purposes of this protocol, anti-tumor treatment may be defined as, but is not limited to, anti-cancer agents (cytotoxic chemotherapy, immunotherapy, or biologic therapy), radiotherapy, and investigational agents. An investigational agent is any drug or therapy not currently approved for use in humans.

3.4.2 Prior Surgery

Patients must have fully recovered from all effects of surgery. Patients must have had at least two weeks after minor surgery and four weeks after major surgery before starting therapy. Minor procedures requiring “Twilight” sedation such as endoscopies or mediport placement may only require a 24 hour waiting period, but this must be discussed with an investigator.

3.4.3 Supportive Care

Subjects should receive best supportive care and treatment of symptoms during the study as appropriate, including transfusion of blood and blood products, oxygen therapy, nutritional support, intravenous fluids, and treatment with appropriate medications (antibiotics, antiemetics, antidiarrheals, and analgesics, etc.). Medications, including steroids, which are given for supportive care, such as appetite stimulation, may be given concurrently.

3.4.3.1 Bisphosphonates and denosumab

Bisphosphonates and denosumab are permitted for the treatment of osteoporosis and prevention of skeletal related events for patients with bone metastases. Chronic concomitant bisphosphonate/denosumab therapy for the prevention of bone metastasis is not permitted.

3.4.3.2 Hematopoietic growth factors

Hematopoietic growth factors may be used according to ASCO guidelines.

3.4.3.3 Palliative radiotherapy

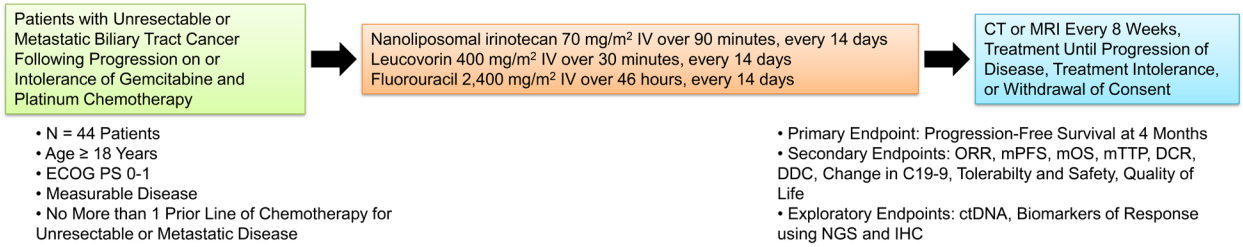
Palliative radiation is permitted if done solely for bone pain relief. It should not be delivered to a target lesion and it should not encompass more than 25% of irradiated bone marrow. No dose modification of study treatment is needed during palliative radiotherapy.

4.0 TRIAL DESIGN AND DETAILED STUDY PROCEDURES

4.1 Treatment Plan Overview

This is a single arm, open label, multicenter phase II study to evaluate the clinical activity of the combination of fluorouracil, leucovorin, and nanoliposomal irinotecan as second-line treatment in patients with advanced biliary tract cancers following gemcitabine and platinum chemotherapy (see Figure 1). Patients with advanced biliary tract cancers who have adequate performance status and adequate hepatic and renal function will be eligible. Patients may have received adjuvant chemotherapy and/or radiation therapy prior to enrolling in the trial, but a minimum of 6 months between adjuvant chemotherapy and this current therapy are required. Patients may continue on study as long as they are tolerating treatment and do not have progression of disease by RECIST v1.1 criteria. Response assessments will occur using imaging (CT or MRI) every 8 weeks.

Figure 1. Study Schema (Expanded)



4.2 Detailed Study Plan

4.2.1 Screening

Study activities are detailed in Table 4, and a study activity checklist in Table 5. Screening will occur within 14 days prior to administration of the first doses of fluorouracil, leucovorin, and nanoliposomal irinotecan on Cycle 1 Day 1. Signed informed consent will be obtained from the subject or the subject's legally acceptable representative before any study-specific procedures are undertaken. For procedures performed at screening and repeated, the later procedure performed prior to dosing, will serve as a baseline for clinical assessment. A complete history and physical will be obtained at the screening visit. Additionally, labs will be reviewed/ordered during the screening visit, prior to the initiation of therapy.

Patients who pass screening will again undergo a full evaluation on Cycle 1 Day 1, including a physical examination, vital signs, performance status, chemistry, hematology, medication review, and adverse event evaluation. If any abnormality is identified at subject assessment on Cycle 1 Day 1, prior to initiating therapy, the patient will be deemed a screen failure and will not start treatment.

All patients who receive at least 1 dose of the study treatment will be eligible for safety analysis. Patients will be replaced for the primary efficacy analysis if they come off study for reasons other than disease progression prior to the 4 month efficacy endpoint. We will substitute patients who screen fail or screen and do not begin study treatment.

The screening procedures include the following listed below.

4.2.1.1 Informed Consent

Signed informed consent will be obtained from the subject or the subject's legally acceptable representative before any study-specific procedures are undertaken.

4.2.1.2 Medical History

The following information will be collected during the Screening Period:

1) Complete medical history, including documentation of any clinically significant medical conditions
2) History of tobacco and alcohol use
3) Presence and severity of any symptoms/conditions associated with metastatic pancreatic cancer
4) Detailed oncology history, including:
    a. Date of primary cancer diagnosis
    b. Pathology (histology or cytology) of primary tumor
    c. Metastasis information (including the location)
    d. Surgical history
    e. Anti-cancer and radiation treatments administered (including dates and type of modality)
5) At each visit, the subject's medical history will be reviewed and any changes from baseline will be recorded in the case report form (CRF). On C1D1, any changes observed from the screening assessments, prior to dosing, will be recorded in the subject's medical history. All medications (prescription or over-the-counter, including vitamins and/or herbal supplements) will be recorded beginning with the screening visit and continuing up through the date of the off study visit.

4.2.1.3 Demographics

Age, gender, race and ethnicity will be recorded.

4.2.1.4 Review subject eligibility criteria

4.2.1.5 Review previous and concomitant medications

4.2.1.6 Physical exam including vital signs, height, and weight

A complete physical examination will be performed at the screening visit. Body weight will be recorded during every physical exam. The subject will wear lightweight clothing and no shoes during weighing. Height will be measured at the screening visit only; the subject will not wear shoes.

4.2.1.7 Vital sign determinations

Vital sign determinations of heart rate, blood pressure and body temperature will be obtained at the screening visit. If possible, blood pressure and heart rate measurements should not immediately follow scheduled blood collections.

4.2.1.8 Performance Status

Performance status will be evaluated prior to study entry according to Table 3.

4.2.1.9 Hematology

Hematology samples (CBC with differential) will be collected and assessed using a certified laboratory. The Investigator will review, initial and date all laboratory results. Any laboratory value outside the reference range stated in the inclusion criteria will preclude the patient from study participation.

4.2.1.10 Serum chemistries

Comprehensive metabolic panel (CMP) to include: albumin, alkaline phosphatase, ALT, AST, BUN, creatinine, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), glucose, and total bilirubin. Any laboratory value outside the reference range stated in the inclusion criteria will preclude the patient from study participation.

4.2.1.11 Pregnancy Test

For female subjects of childbearing potential, a serum pregnancy test will be performed at the screening visit within 14 days of C1D1 and a urine pregnancy test will be done at the C1D1 visit prior to the first dose of study drug. Subjects considered not of childbearing potential must be documented as being surgically sterile or post-menopausal (for at least 1 year). The test results must be reviewed and determined to be negative prior to dosing. If the urine pregnancy test is positive at C1D1, it should be confirmed by a serum pregnancy test. The test may be repeated at the discretion of the investigator at any time during the study. Should a female study subject become pregnant or suspect she is pregnant while participating in this study, she should inform the treating Investigator immediately.

4.2.1.12 Tumor assessment

Only patients with unresectable or metastatic biliary tract cancer not eligible for resection will be considered for entry. Subjects must have measurable disease, defined as at least 1 unidimensionally measurable lesion as defined by RECIST v1.1.

4.3 Patient Study Number Assignment and Sample Labeling

4.3.1.1 Patient Study Number Assignment

Patients will be de-identified and assigned with a 5 digit study label (X-XXX-X):

- The first number will be the site from which the patient was enrolled (single digit)
    - 1 = Georgetown
    - 2 = TBD
    - 3 = Siteman (Washington University in St. Louis)
    - 4 = Indiana
    - 5 = Mount Sinai
- The second number will be the site-assigned patient number (three digit)
- The third number will indicate the collection timing (see 4.3.1.2)

4.3.1.2 Labeling of Serial Blood Samples for ctDNA

Serial blood samples will be labeled in a similar fashion, except that the collection timing will be labeled by week number (e.g. week 0 = pretreatment; 8 = week 8; 16 = week 16, etc.)

4.4 Detailed Patient Assessments

Subject assessments (physical examinations, vital signs, performance status, chemistry, hematology, medication review, and adverse event evaluations) will be conducted at screening, C1D1, and D1 of every subsequent cycle (every 14 days ± 3 days). The radiologic first response assessment will occur at 8 weeks (± 7 days), and subsequent radiologic response assessments every 8 weeks thereafter (± 7 days). Details are provided in Table 4, and a checklist highlighting the important events for screening through the completion of the trial is provided below, as Table 5.

4.4.1 Physical Examinations

A complete physical examination will be performed at the screening visit, on C1D1, and D1 of every subsequent cycle (every 14 days ± 3 days). Any significant physical examination findings after the administration of the first doses of fluorouracil, leucovorin, and nanoliposomal irinotecan

will be recorded as adverse events. Body weight will be recorded during every physical exam. The subject will wear lightweight clothing and no shoes during weighing. Weight will be measured on the same scale at each visit. Height will be measured at the screening visit only; the subject will not wear shoes.

4.4.2 Medical History

At each visit, the subject's medical history will be reviewed and any changes from baseline will be recorded as an adverse event. On C1D1, any changes observed from the screening assessments, prior to dosing, will be recorded in the subject's medical history. All medications (prescription or over-the-counter, including vitamins and/or herbal supplements) will be recorded beginning with the screening visit and continuing up through the date of the final visit.

4.4.3 Vital Signs

Vital sign determinations of body temperature (in degrees Celsius), heart rate, respiratory rate, blood pressure and pain (using a 0-10 Numeric Ranking Scale) will be obtained at the screening visit, on each day the subject is seen by the treating physician, and at the final visit. If possible, blood pressure and heart rate measurements should not immediately follow scheduled blood collections.

4.4.4 Clinical Laboratory Tests

All subjects will undergo the laboratory assessments outlined in Table 4.

1) A complete blood count (CBC) with differential will be collected at the screening visit, on C1D1, and D1 of every subsequent cycle (every 14 days ± 3 days).
2) Serum chemistries (total protein, albumin, total bilirubin, AST, ALT, alkaline phosphatase, sodium, potassium, chloride, bicarbonate, BUN, creatinine, and calcium) will be collected at the screening visit, on C1D1, and D1 of every subsequent cycle (every 14 days ± 3 days).
3) PT/INR and aPTT will be collected at the screening visit (this may be repeated during the course of the study at the discretion of the investigator but is not required).
4) Additionally, a tumor marker (serum CA19-9 or CEA level) and research blood samples for ctDNA will be sampled on Cycle 1 Day 1 and every 8 weeks thereafter.

Laboratory samples for this study will be assessed using the certified laboratory at the investigators' institutions, or at a clinical laboratory such as Quest or LabCorp and these data will be used for all data analysis. The Principal Investigator or sub-investigator will review, initial and date all laboratory results. Any laboratory value outside the reference range that is considered clinically significant by the investigator will be followed as appropriate. Clinically significant laboratory values will be recorded as adverse events if they meet the criteria as specified in Section 6.0.

4.4.4 UGT1A1 Genotype Testing

During screening, all subjects will undergo serum blood testing for UGT1A1 genotype as individuals are homozygous for the UGT1A1*28 allele are at increased risk of neutropenia from nanoliposomal irinotecan and are started at a lower initial dose (50 mg/m^2 IV).

4.4.6 Collection and Processing of ctDNA

For collection of ctDNA on C1D1, every 8 weeks thereafter (± 7 days), and at the end of study, 4 mL of patient blood will be collected via venipuncture (venipuncture is preferred, however if an indwelling catheter must be used, 3 catheter volumes of blood must be collected and discarded prior to collection of the sample) into a 10 mL red-top SST tube. Immediately after collection, the tubes will be inverted 8 to 10 times to ensure good mixing of blood and placed in an ice bath. The date and time of collection for each sample will be recorded. The complete process of centrifugation, transfer to polypropylene tubes and freezing should be accomplished within 60

minutes from blood draw. The processing of samples should be performed as described in the **Lab Manual**.

4.4.7 Collection and Processing of Archived Tumor Tissue

Archived tumor tissue is not required for this study; however, if patients have archived tumor tissue available then it will be made available for trial purposes. Archived tumor tissue will be collected and analyzed for sample adequacy. The tumor sample will then be sent for NGS and IHC testing (TOPO1 and others) at a CLIA-certified laboratory.

4.4.8 Adverse Event Evaluation

The Principal Investigator or sub-investigators will assess adverse events, laboratory data and vital signs throughout the study. Adverse events will be assessed by NCI CTCAE Version 5.0. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf. The investigators will monitor each subject for clinical and laboratory evidence of adverse events on a routine basis throughout the study. The investigator will assess and record any adverse event in detail including the date of onset, event diagnosis (if known) or sign/symptom, severity, time course, duration and outcome, relationship of the adverse event to study drug, and any action(s) taken. For serious adverse events not considered "probably related" to study drug, the investigator will provide an "Other" cause of the event. For adverse events to be considered intermittent, the events must be of similar nature and severity. Adverse events, whether in response to a query, observed by site personnel, or reported spontaneously by the subject will be recorded. All adverse events will be followed to a satisfactory conclusion.

4.4.9 Imaging and Response Evaluation

Patients will undergo a baseline tumor evaluation with imaging studies within 28 days prior to C1D1. Imaging studies should include a diagnostic CT scan of the chest, abdomen, and pelvis with PO and IV contrast (unless medically contraindicated). If a patient has an allergy to IV contrast, appropriate pre-medication can be given to prevent a contrast reaction. Patients may undergo other modalities such as an MRI instead of a CT scan at the treating physician's discretion if appropriate (such as severe allergy to CT contrast, extremity tumors, bone metastases requiring bone scans, etc.).

Response evaluation will occur every 8 weeks (± 7 days, regardless of treatment cycle) and at the final visit, if not performed within the last 28 days. Tumor response and/or disease progression will be assessed by the modality(ies) used prior to treatment. Patients will continue to remain on study as long as there is no evidence of PD and the therapy is adequately tolerated.

4.4.10 Removal of Subjects from Therapy or Assessment

4.4.10.1 Reasons for Removal of Subjects from Therapy or Assessment

Each subject has the right to withdraw from study treatment at any time. In addition, the investigator may discontinue a subject from the study treatment at any time for any reason if the investigator considers it necessary, including the occurrence of an adverse event or noncompliance with the protocol. Each subject will be withdrawn from the study if any of the following occur:

1) The subject experiences either clinical or radiographic progressive disease.
2) The subject requires radiotherapy (except for palliative radiation for bone relief as outlined in section 3.4.3.3) or alternate antineoplastic agents during the study period.
3) The investigator believes it is in the best interest of the subject.
4) Clinically significant deterioration of the subject's medical status as determined by the investigator.
5) Subject becomes pregnant or begins breastfeeding during the treatment portion of the study.

6) The subject or subject's legally acceptable representative decides to withdraw consent for any reason.
7) Any other medical reason that the study investigator deems appropriate.

4.4.10.2 Discontinuation of Individual Subjects

When a subject discontinuation from the study (without reaching a protocol-defined endpoint) is planned, the investigator will notify the PI as soon as possible (provided, in each case, subject care and safety are not compromised). When a subject discontinues the study, a final visit will be conducted (preferably prior to the initiation of another anticancer therapy). However, these procedures should not interfere with the initiation of any new treatments or therapeutic modalities that the investigator feels are necessary to treat the subject's condition. Following discontinuation of the study drug, the subject will be treated in accordance with the investigator's best clinical judgment. At the final visit, the reason(s) for the discontinuation from the study will be recorded and a physical examination, body weight, vital signs measurement, laboratory analyses, performance status, tumor assessment, and an assessment of adverse events will be performed as soon as possible after discontinuation from the study. All subjects will have one final visit, which does not need to be performed for subjects who have had a visit greater than 30 days after discontinuation of the study drugs. If a subject is discontinued from the study with an ongoing adverse event or an unresolved clinically significant laboratory result, personnel will attempt to provide follow-up until a satisfactory clinical resolution of the laboratory result or adverse event is achieved. In the event that a positive result is obtained on a pregnancy test for a subject during the study, the administration of study drug to that subject must be discontinued immediately.

4.4.10.3 Discontinuation of Entire Study

The investigators may terminate this study provided that written notice is submitted at a reasonable time in advance of the intended termination. The following procedures for discontinuation will be followed:

1) If the investigators have decided to prematurely discontinue the study, the investigators will promptly notify in writing the IRB of the decision and give detailed reasons for the discontinuation.
2) The PI must promptly notify enrolled subjects of the premature discontinuation and administer appropriate treatments such as replacement of protocol therapy by other appropriate regimens.

4.4.11 Longitudinal Outcomes Assessment

Subsequent therapies will be administered at the discretion of the treating physician. Patients will be followed after progression until death (or up to 24 months) to assess overall survival. Information pertaining to survival and post-treatment therapy will be collected approximately every 12 weeks (Month 3, 6, 9, 12, 15 and 18) beginning after the final visit, for a period up to 24 months. Overall survival after 24 months will be captured if available until the subject's death, the subject withdrawals consent, or the study is terminated, whichever occurs first.

4.4.12 Quality of Life Assessment

Patients will be complete quality of life questionnaires using the EORTC QLQ-C30 questionnaire on C1D1, every 8 weeks (± 7 days), and at the end of treatment.

4.4.13 Protocol Deviations

The investigator should not implement any deviation from the protocol without prior review and agreement by the Sponsor and in accordance with the IRB and local regulations, except when necessary to eliminate an immediate hazard to study subjects.

Table 4. Study Activities

	Screening[e]	C1D1	C2D1 and Every Even-Numbered Cycle ± 3 Days	C3D1 and Every Odd-Numbered Cycle ± 3 Days	Every 8 Weeks ± 7 Days	End of Treatment	Follow Up (Up to 24 Months)
Informed Consent	X						
Demographics	X						
Medical History	X						
ECOG PS[a]	X	X	X	X		X	
Concomitant Medications Collection	X	X	X	X		X	
Adverse Events Assessment	X	X	X	X		X	
Vital Signs	X	X	X	X		X	
Height	X						
Weight	X	X	X	X		X	
History and Physical Exam	X	X	X	X		X	
CT or MRI Scan Tumor Evaluation and Assessment[b]	X				X	X[f]	
β-hCG[c]	X	X		X			
CBC with differential	X	X	X	X		X	
CMP[d]	X	X	X	X		X	
PT/INR and aPTT	X						
UGT1A1 Testing	X						
CA19-9	X	X		X	X	X	
Research Serum Sample for ctDNA		X			X	X	
Quality of Life Questionnaire		X			X	X	
Dispense 5-FU, LV and nanoliposomal irinotecan		X	X	X			
Survival		X	X	X	X	X	X

[a]Eastern Cooperative Oncology Group Performance Status

[b]Screening scans should be within 28 days of starting treatment

[c]Serum (screening) and urine (C1D1) pregnancy tests (for women of childbearing potential only)

[d]Comprehensive metabolic panel: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, AST, ALT, alkaline phosphatase, total bilirubin, total protein, and albumin

[e]The screening visit must take place within 14 days of C1D1

[f]Unless performed within 28 days of the off-study date

Table 5. Study Activities Checklist

Screening

Informed Consent	_____
Demographics	_____
Medical History	_____
ECOG PS	_____
Concomitant Medications Collection	_____
Adverse Events Assessment	_____
Vital Signs	_____
Height	_____
Weight	_____
History and Physical Exam	_____
CT or MRI Scan	
Tumor Evaluation and Assessment	_____
β-hCG	_____
CBC with Differential	_____
Comprehensive Metabolic Panel	_____
PT/INR and aPTT	_____
UGT1A1 Testing	_____
CA19-9	_____

Cycle 1 Day 1

ECOG PS	_____
Concomitant Medications Collection	_____
Adverse Events Assessment	_____
Vital Signs	_____
Weight	_____
History and Physical Exam	_____
β-hCG	_____
CBC with Differential	_____
Comprehensive Metabolic Panel	_____
CA19-9	_____
Research Serum Sample for ctDNA	_____
Quality of Life Questionnaire	_____
Dispense 5-FU, LV, and Nanoliposomal Irinotecan	_____
Survival	_____

Cycle 2 Day 1 and Every Even-Numbered Cycle (± 3 Days)

ECOG PS	_____
Concomitant Medications Collection	_____
Adverse Events Assessment	_____
Vital Signs	_____
Weight	_____
History and Physical Exam	_____
CBC with Differential	_____
Comprehensive Metabolic Panel	_____
Dispense 5-FU, LV, and Nanoliposomal Irinotecan	_____
Survival	_____

Cycle 3 Day 1 and Every Odd-Numbered Cycle (± 3 Days)

ECOG PS _____
Concomitant Medications Collection _____
Adverse Events Assessment _____
Vital Signs _____
Weight _____
History and Physical Exam _____
β-hCG _____
CBC with Differential _____
Comprehensive Metabolic Panel _____
CA19-9 _____
Dispense 5-FU, LV, and Nanoliposomal Irinotecan _____
Survival _____

Every 8 Weeks (± 7 Days)

CT or MRI Scan
Tumor Evaluation and Assessment _____
CA19-9 _____
Research Serum Sample for ctDNA _____
Quality of Life Questionnaire _____

End of Treatment

ECOG PS _____
Concomitant Medications Collection _____
Adverse Events Assessment _____
Vital Signs _____
Weight _____
History and Physical Exam _____
CT or MRI Scan
Tumor Evaluation and Assessment _____
CBC with Differential _____
Comprehensive Metabolic Panel _____
CA19-9 _____
Research Serum Sample for ctDNA _____
Quality of Life Questionnaire _____
Survival _____

Follow Up (Up to 24 Months)

Survival _____

5.0 DOSAGES AND DISPENSATION OF DRUGS

5.1 Dispensation of Study Drug and Administration

The study drugs are defined as 5-FU, LV, and nanoliposomal irinotecan. Due to the infusional 5-FU, study drugs must be administered via central line (mediport, PICC, or other). Treatment cycles are every 14 days ± 3 days.

A research infusion nurse will access the patient's line. Treatment will then be administered in the following order (substitutions for prophylactic medications are allowed at the treating investigator's discretion):

Table 6. Drug Administration

Drug	Indication	Dose/Route	Duration
Ondansetron	Nausea prophylaxis	8 mg IV	10 minutes
Dexamethasone	Nausea prophylaxis	10 mg IV	15 minutes
Atropine	Diarrhea prophylaxis	0.5 mg IV	IV push
Nanoliposomal Irinotecan	Treatment	70 mg/m^2 IV*	90 minutes
Leucovorin	Treatment	400 mg/m^2 IV	30 minutes (can be administered simultaneously with nanoliposomal irinotecan via a Y-connector)
Fluorouracil	Treatment	2400 mg/m^2 IV	46 hours

*The starting dose for patients who are homozygous for the UGT1A1*28 allele is 50 mg/m^2 IV for the first cycle. The dose may be escalated to 70 mg/m^2 in subsequent cycles at the discretion of the treating investigator.

The patient will return to the research infusion clinic after 46 hours for disconnection of the 5-FU infusional pump.

5.2 Packaging, Labeling, Storage, and Handling of Nanoliposomal Irinotecan

Nanoliposomal irinotecan comes in a single-dose vial containing 43 mg irinotecan free base at a concentration of 4.3 mg/mL (NDC: 69171-398-01). It must be stored at 2-8ºC (36-46ºF), not frozen, and protected from the light. As it is a cytotoxic drug, follow applicable special handling and disposal procedures (OSHA Hazardous Drugs. *OSHA.* http://www.osha.gov/SLTC/hazardousdrugs/index.html)

5-FU and LV should be stored and handled as per their respective FDA labels and following protocols and guidelines of the treating institution.[14,15]

6.0 SAFETY VARIABLES AND TOXICITY ASSESSMENT

The Principal Investigator or sub-investigators will assess adverse events, laboratory data and vital signs throughout the study. Adverse events will be assessed by NCI CTCAE Version 5.0. http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

6.1 Adverse Event Assessment

The investigators will monitor each subject for clinical and laboratory evidence of adverse events on a routine basis throughout the study. The investigator will assess and record any adverse event in detail including the date of onset, event diagnosis (if known) or sign/symptom, severity, time course, duration and outcome, relationship of the adverse event to study drug, and any action(s) taken. For serious adverse events not considered "probably related" to study drug, the investigator will provide an "Other" cause of the event. For adverse events to be considered intermittent, the events must be of similar nature and severity. Adverse events, whether in response to a query, observed by personnel, or reported spontaneously by the subject will be recorded. All adverse events will be followed to a satisfactory conclusion.

6.2 Study Monitoring

6.2.1 Data Safety Monitoring Committee at Georgetown

The Georgetown Lombardi Comprehensive Cancer Center will be responsible for the data and safety monitoring of this multi-site trial. As this study is an investigator initiated study phase II study, it is considered a high-risk study which requires real-time monitoring by the PI and study team and review every 4 months by the LCCC Data and Safety Monitoring Committee (DSMC).

The Principal Investigator and the Co-Investigators will review the data including safety monitoring at their weekly institution based disease group meetings, on monthly disease group teleconferences, and monthly teleconferences between study sites.

Severe Adverse Events (SAEs) are required to be reported to the IRB per IRB reporting guidelines. Based on SAEs, the IRB retains the authority to suspend further accrual pending more detailed reporting and/or modifications to further reduce risk and maximize the safety of participating patients.

Progress on the trial and the toxicities experienced will be reviewed by the LCCC Data and Safety Monitoring Committee every 4 months from the time the first patient is enrolled on the study. Results of the DSMC meetings will be forwarded to the IRB with recommendations regarding need for study closure.

DSMC recommendations should be based not only on results for the trial being monitored as well as on data available to the DSMC from other studies. It is the responsibility of the PI to ensure that the DSMC is kept apprised of non-confidential results from related studies that become available. It is the responsibility of the DSMC to determine the extent to which this information is relevant to its decisions related to the specific trial being monitored.

A written copy of the DSMC recommendations will be given to the trial PI and the IRB. If the DSMC recommends a study change for patient safety or efficacy reasons the trial PI must act to implement the change as expeditiously as possible. In the unlikely event that the trial PI does not concur with the DSMC recommendations, then the LCCC Associate Director of Clinical Research must be informed of the reason for the disagreement. The trial PI, DSMC Chair, and the LCCC AD for Clinical Research will be responsible for reaching a mutually acceptable decision about the study and providing details of that decision to the IRB. Confidentiality must be preserved during these discussions. However, in some cases, relevant data may be shared with other

selected trial investigators and staff to seek advice to assist in reaching a mutually acceptable decision.

If a recommendation is made to change a trial for reasons other than patient safety or efficacy the DSMC will provide an adequate rationale for its decision. If the DSMC recommends that the trial be closed for any reason, the recommendation will be reviewed by the Associate Director for Clinical Research at G-LCCC. Authority to close a trial for safety reasons lies with the IRB, with the above described input from DSMC and the AD for Clinical Research.

6.2.2 Study Monitoring with Ipsen

The Study Chair and, when available, the responsible research coordinator, will hold a phone conference with Ipsen monthly to discuss trial management, safety concerns, response assessment and correlative studies. More frequent conferences may be held as necessary.

6.3 Adverse Event and Toxicity Definitions

6.3.1 Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore 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 the event is considered causally related to the use of the product. Such an event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental or intentional overdose, drug abuse, or drug withdrawal. Any worsening of a pre-existing condition or illness is considered an adverse event. Laboratory abnormalities and changes in vital signs are considered to be adverse events only if they result in discontinuation from the study, necessitate therapeutic medical intervention, and/or if the investigator considers them to be adverse events.

An elective surgery/procedure scheduled to occur during a study will not be considered an adverse event if the surgery/procedure is being performed for a pre-existing condition and the surgery/procedure has been pre-planned prior to study entry. However, if the pre-existing condition deteriorates unexpectedly during the study (e.g., surgery performed earlier than planned), then the deterioration of the condition for which the elective surgery/procedure is being done will be considered an adverse event.

6.3.2 Serious Adverse Events

If an adverse event meets any of the following criteria, it is to be reported to the DSMC as a serious adverse event (SAE) within 24 hours of the study personnel being made aware of the serious adverse event.

1) **Death of Subject** An event that results in the death of a subject.
2) **Life-Threatening** An event that, in the opinion of the investigator, would have resulted in immediate fatality if medical intervention had not been taken. This does not include an event that would have been fatal if it had occurred in a more severe form.
3) **Hospitalization or**
4) **Prolongation of Hospitalization** An event that results in an admission to the hospital for any length of time or prolongs the subject's hospital stay. This does not include an emergency room visit or admission to an outpatient facility.
5) **Congenital Anomaly** An anomaly detected at or after birth, or any anomaly that results in fetal loss.
6) **Persistent or Significant Disability/Incapacity** An event that results in a condition that substantially interferes with the activities of daily living of a study subject. Disability is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle).

7) **Important Medical Event Requiring Medical or Surgical Intervention to Prevent Serious Outcome** An important medical event that may not be immediately life-threatening or result in death or hospitalization, but based on medical judgment may jeopardize the subject and may require medical or surgical intervention to prevent any of the outcomes listed above (i.e., death of subject, life-threatening, hospitalization, prolongation of hospitalization, congenital anomaly, or persistent or significant disability/incapacity). Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
8) **Spontaneous Abortion** Miscarriage experienced by study subject.
9) **Elective Abortion** Elective abortion performed on study subject.

6.3.3 Adverse Event Severity

The study investigator will rate the severity of each adverse event according to the NCI CTCAE Version 5.0. For adverse events not captured by the NCI CTCAE Version 5.0, the following should be used:

1) **Grade 1 (Mild)** The adverse event is transient and easily tolerated by the subject.
2) **Grade 2 (Moderate)** The adverse event causes the subject discomfort and interrupts the subject's usual activities.
3) **Grade 3-4 (Severe or Life Threatening)** The adverse event causes considerable interference with the subject's usual activities and may be incapacitating or life-threatening.
4) **Grade 5 (Death)** The adverse event resulted in death of the subject.

6.3.4 Relationship to Study Drug

The investigator will use the following definitions to assess the relationship of the adverse event to the use of study drug:

1) **Definitely Related** An adverse has a clear temporal relationship to study drug and/or recurs on re-challenge and an Other cause of event is extremely unlikely.
2) **Probably Related** An adverse event has a strong temporal relationship to study drug or recurs on re-challenge and an Other cause of event is unlikely or significantly less likely.
3) **Possibly Related** An adverse event has a strong temporal relationship to the study drug and an Other cause of event is equally or less likely compared to the potential relationship to study drug.
4) **Probably Not Related** An adverse event has little or no temporal relationship to the study drug and/or a more likely Other cause of event exists.
5) **Not Related** An adverse event is due to an underlying or concurrent illness or effect of another drug and is not related to the study drug (e.g., has no temporal relationship to study drug or has a much more likely Other cause of event).

If an investigator's opinion of possibly, probably not, or not related to study drug is given, an Other cause of event must be provided by the investigator for the serious adverse event.

6.4 Adverse Event Collection Period

All adverse events reported from the time of study drug administration until 30 days following discontinuation of study drug administration have elapsed will be collected, whether elicited or spontaneously reported by the subject. In addition, serious adverse events will be collected from the time the subject signed the study-specific informed consent.

6.5 Adverse Event Reporting

In the event of a serious adverse event, whether related to study drug, study procedure such as a biopsy, or even if not directly related to any study intervention, the investigator will notify the IRB after being made aware of the serious adverse event, if the SAE meets the requirements of reporting per local IRB guidelines. Furthermore, all serious adverse events must be reported by

facsimile within 24 hours to Ipsen at [redacted]. Forms can be obtained from, and returned to e-mail: [redacted] and [redacted]

The SAE report should comprise a full written summary, detailing relevant aspects of the adverse events in question. Where applicable, information from relevant hospital case records and autopsy reports should be included. Follow-up information should be forwarded to Ipsen within 24 hours.

SAEs brought to the attention of the investigator at any time after cessation of nanoliposomal irinotecan and considered by the investigator to be related or possibly related to nanoliposomal irinotecan must be reported to Ipsen if and when they occur. Additionally, in order to fulfill international reporting obligations, SAEs that are related to study participation (e.g., procedures, invasive tests, change from existing therapy) or are related to a concurrent medication will be collected and recorded from the time the subject consents to participate in the study until he/she is discharged.

SAEs will be reported to the FDA if it meets FDA reporting guidelines for reporting of Serious and Unexpected Suspected Adverse Reactions. Report may be faxed to the FDA to 1-800 (332)-0178 or online: www.fda.gov/medwatch/report.htm

6.6 Pregnancy

In the event of a positive pregnancy test result, study drugs will be immediately discontinued. The investigator must report the positive pregnancy test within 72 hours of the study personnel becoming aware of the pregnancy to the IRB and to Ipsen. Patients should also notify the investigator if it is determined after completion of the study that they become pregnant either during the treatment phase of the study or within five days after the treatment period. Information regarding a pregnancy occurrence in a study subject and the outcome of the pregnancy will be collected, and the status of the mother and child should be reported to the IRB and to Ipsen after delivery. Pregnancy in a study subject is not considered an adverse event but does require discontinuation of the subject from the study. However, the medical outcome of an elective or a spontaneous abortion, stillbirth or congenital anomaly is considered a serious adverse event and must be reported to the IRB and to Ipsen within 72 hours of study personnel becoming aware of the event. Male subjects should also notify the investigators if the subject's partner should become pregnant during the study, this should also be reported within 72 hours of study personnel awareness.

6.7 Toxicity Management and Dose Adjustments

6.7.1 Dose Reduction and Delays

Dose modifications for nanoliposomal irinotecan should be made according the FDA label as outlined in Table 7. 5-FU modifications correspond to modifications to nanoliposomal irinotecan in 25% increments as suggested in the FDA label for nanoliposomal irinotecan.[21]. LV dosing will not be modified (it will always be 400 mg/m^2).

Table 7. Dose Modifications for Nanoliposomal Irinotecan and 5-FU

Toxicity (NCI CTCAE v5.0)	Occurrence	Adjustments in Patients Receiving 70 mg/m^2		Adjustments in Patients Homozygous for UGT1A1*28 without Previous Increase to 70 mg/m^2	
		Nanoliposomal Irinotecan	5-FU	Nanoliposomal Irinotecan	5-FU
Grade 3 or 4 Adverse Reactions or Grade 2 Diarrhea	Withhold nanoliposomal irinotecan. Initiate loperamide for late onset diarrhea of any severity. Administer intravenous or subcutaneous atropine 0.25 to 1 mg (unless clinically contraindicated) for early onset diarrhea of any severity Upon recovery to ≤ Grade 1, resume nanoliposomal irinotecan and 5-FU at:				
	First	50 mg/m^2	1800 mg/m^2	43 mg/m^2	1800 mg/m^2
	Second	43 mg/m^2	1200 mg/m^2	35 mg/m^2	1200 mg/m^2
	Third	Off Study		Off Study	
Grade 1 Diarrhea	Initiate loperamide for late onset diarrhea of any severity, continue treatment at current doses.				
Interstitial Lung Disease	First	Off Study		Off Study	
Anaphylactic Reaction	First	Off Study		Off Study	

6.8 Prohibited Concurrent Medications

The following medications must be discontinued at least 2 weeks prior to C1D1 (strong CYP3A4 inhibitors or inducers, strong UGT1A1 inhibitors):

Atazanavir, Atazanavir/ritonavir, boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir/ritonavir, darunavir/ritonavir, elvitegravir/ritonavir, grapefruit juice, idelalisib, indinavir, indinavir/ritonavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir,ombitasvir/paritaprevir/dasabuvir/ritonavir (VIEKIRA PAK), posaconazole, ritonavir, saquinavir/ritonavir, telaprevir, telithromycin, tipranavir/ritonavir, troleandomycinvoriconazole, carbamazepine, enzalutamide, lumacaftor, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St. John's wort (hypericum perforatum), adenine, propofol, indomethacin, nilotinib, pazopanib, regorafenib, flunitrazepam, erlotinib, sorafenib, enasidenib, pibrentasvir, glecaprevir, rucaparib, ertugliflozin, fostamatinib, dolutegravir, valproic acid, flurbiprofen, eltrombopag, silibinin A, and sodium aurothiomalate.

7.0 EFFICACY ASSESMENT

7.1 Efficacy Variables

Tumor response and/or disease progression will be assessed by a CT scan (or other appropriate modalities such by way of MRI consistent with the modality used prior to treatment) utilizing RECIST v1.1 criteria. Assessments will be performed within 28 days before screening and every 8 weeks thereafter, and at the final visit, if not performed within the last four weeks. If subjects respond to treatment and are able to have their disease resected, the patient's response will be assessed prior to the surgery. The primary *clinical efficacy* endpoint is $PFS_{4\ months}$ as determined by RECIST v1.1 criteria. Analyses of these endpoints are described in Section 13.

7.2 RECIST v1.1 Criteria for Tumor Response

Changes in the measurable lesions over the course of therapy will be evaluated using the RECIST v1.1 criteria.

7.2.1 Definition of Lesions

7.2.1.1 Measurable Disease

The presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

7.2.1.2 Measurable Lesions

Lesions that can be accurately measured in at least one dimension with longest diameter ≥ 20 mm using conventional techniques or ≥ 10 mm with CT scan.

7.2.1.3 Non-measurable Lesions

All other lesions, including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm with a CT scan), bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions and also abdominal masses that are suspicious though may or may not be confirmed and followed by imaging techniques.

All measurements should be taken and recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 14 days before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is required.

7.2.2 Methods of Measurement

CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis. For accurate objective response evaluation, ultrasound should not be used to measure tumor lesions. However, ultrasound is a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. Ultrasound might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination. Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

Evaluation of the primary efficacy endpoint (progression-free survival at 4 months) will be conducted using central radiology review. Investigator-assessed progression will be used to guide clinical decision-making.

7.2.3 Baseline Documentation of "Target" and "Non-Target" Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response. All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

7.2.4 Definitions of Tumor Response-Measurable Disease

7.2.4.1 Complete Response (CR)

The disappearance of all known disease, determined by two observations not less than 4 weeks apart. There can be no appearance of new lesions.

7.2.4.2 Partial Response (PR)

A 30% or more decrease in the sum of the LD of target lesions that have been measured to determine the effect of therapy by two observations not less than 4 weeks apart. There can be no appearance of new lesions or progression of any lesion.

7.2.4.3 Progressive Disease (PD)

At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started, the appearance of one or more new lesions and/or an unequivocal progression of non-target lesions.

7.2.4.4 Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

7.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 8. Response Evaluation.

Note: If subjects respond to treatment and are able to have their disease resected, the patient's response will be assessed prior to the surgery.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Response for this Category Also Requires:
CR	CR	No	CR	>4 wks. confirmation
CR	Non-CR/Non-PD	No	PR	
PR	Non-PD	No	PR	>4 wks. confirmation
SD	Non-PD	No	SD	Documented at least once >4 wks. from baseline
PD	Any	Yes or No	PD	
Any	PD*	Yes or No	PD	no prior SD, PR or CR
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

7.4 Definition of Disease Progression

Disease progression will be defined as:

1) Radiologic progression of disease by RECIST v1.1 criteria.
2) Clinical progression as determined by the investigator, which may be characterized as, but is not limited to:
    a. Increase of at least 2 points in ECOG performance status attributable to cancer progression.
    b. Requirement for radiation (beyond palliative radiation for bone pain relief as outlined in 3.4.3.3), chemotherapy or surgery.
3) Death from disease progression

8.0 CORRELATIVE RESEARCH

8.1 Quality of Life Assessment

Quality of life assessments will take place throughout the course of the study using the EORTC QLQ-30 Questionnaire (see Appendix A) at study start, every 8 weeks on study, and at the end of treatment. Patients will be strongly encouraged to complete the questionnaire while in the clinic as it is vital to capture potential declines in quality of life which can be missed by lack of compliance in completing questionnaires.

Clinical benefit rate will be calculated as previously demonstrated[22] using pain and change in performance status. We will compare the quality of life assessments at baseline, 8 weeks on-study, and at the end of treatment.

8.2 Circulating Tumor DNA

Patient sera will be collected at C1D1, every 8 weeks on-study, and at the end of treatment for an exploratory analysis of ctDNA. Mutations will be evaluated in the circulating DNA specimens using PCR (predominantly focused around *TP53*, *ARID1A*, *KRAS*, *IDH1*, and *CDKN2A* mutations). We will explore the role of ctDNA in both the detection of resistance mechanisms to fluorouracil, leucovorin, and nanoliposomal irinotecan and detection of early evidence of progression of disease, compared with CA19-9 and imaging analyses.

Samples collected must be shipped per **Lab Manual.**

8.3 Mutational Analysis of Tumor Samples

Archived tumor tissue will be used for targeted next generation sequencing using a panel that includes 592 genes commonly altered in cancer with a specific emphasis on genes that are altered in BTCs. This platform will allow >500x coverage across the genes and provide both single nucleotide variant and copy number information. Each common gene mutation will be evaluated for association with PFS.

8.4 Immunohistochemistry Analysis of Tumor Samples

Archived tumor tissue will be used for IHC analysis using TOPO1 and thymidylate synthase (TS) staining. We hypothesize that patients with TOPO1 overexpression and/or low TS expression will have superior PFS compared with those without TOPO1 expression and/or high TS expression as previously demonstrated.[23,24]

9.0 STATISTICAL CONSIDERATIONS

9.1 Primary Objective:

To determine the efficacy of fluorouracil, leucovorin, and nanoliposomal irinotecan in advanced biliary tract cancers following progression on or intolerance of gemcitabine and platinum chemotherapy using progression-free survival at 4 months as the primary endpoint.

9.2 Secondary Objectives:

To assess the activity of fluorouracil, leucovorin, and nanoliposomal irinotecan, in patients with advanced biliary tract cancers treated following progression on or intolerance of gemcitabine and platinum chemotherapy in terms of:

9.2.1 Best overall response rate (ORR)
9.2.2 Median progression-free survival (mPFS)
9.2.3 Median overall survival (mOS)
9.2.4 Median time to disease progression (mTTP)
9.2.5 Disease control rate (DCR) (defined as ORR + rate of stable disease at 4 months)
9.2.6 Median duration of disease control (DDC)
9.2.7 Maximum change in tumor marker, CA19-9

9.2.8 To assess the tolerability and safety of fluorouracil, leucovorin, and nanoliposomal irinotecan, in patients with advanced biliary tract cancers treated following progression on or intolerance of gemcitabine and platinum chemotherapy

9.2.9 To assess the changes in quality of life over the treatment course of patients with biliary tract cancers treated following progression on or intolerance of gemcitabine and platinum chemotherapy with fluorouracil, leucovorin, and nanoliposomal irinotecan

9.3 Exploratory Objectives:

9.3.1 To assess blood for the analysis of circulating tumor DNA as a surrogate marker of disease burden

9.3.2 To assess tumor tissue for potential predictive biomarkers of response to fluorouracil, leucovorin, and nanoliposomal irinotecan using NGS and IHC

9.4 Primary Endpoint:

PFS at 4 months in a patient with advanced biliary tract cancer treated with fluorouracil, leucovorin, and nanoliposomal irinotecan following progression on or intolerance of gemcitabine and platinum chemotherapy, defined as positive if a patient does not have evidence of disease progression (PD) at 4 months, as measured by expert radiologists using RECIST v1.1 criteria

9.5 Secondary Endpoints:

9.5.1 Best response in a patient, defined as the largest percent decrease in tumor size from baseline and categorized as a complete response (CR), partial response (PR), stable disease (SD), or PD by imaging studies, measured by expert radiologists using RECIST v1.1 criteria. Overall response rate (ORR) is the proportion of patients obtaining a CR or PR as the best response. Disease control rate (DCR) is the proportion of patients obtaining a CR, PR, or SD at 4 months. Duration of disease control (DDC) is the median length of time of patients maintaining a CR, PR, or SD. Maximum change in tumor marker (CA19-9) is defined as maximum decrease in CA19-9 compared to baseline at any point on the study.

9.5.2 PFS in a patient with advanced biliary tract cancer treated with fluorouracil, leucovorin, and nanoliposomal irinotecan following progression on or intolerance of gemcitabine and platinum

chemotherapy, defined as the time from Cycle 1, Day 1 to PD (as measured by expert radiologists using RECIST v1.1 criteria), death from any cause, or last follow-up, as determined by the investigator

9.5.3 OS in a patient with advanced biliary tract cancer treated with fluorouracil, leucovorin, and nanoliposomal irinotecan following progression on or intolerance of gemcitabine and platinum chemotherapy, defined as the time from Cycle 1, Day 1 to death from any cause or last follow-up

9.5.4 TTP in a patient with advanced biliary tract cancer treated with fluorouracil, leucovorin, and nanoliposomal irinotecan following progression on or intolerance of gemcitabine and platinum chemotherapy, defined as the time from Cycle 1, Day 1 to PD (as measured by expert radiologists using RECIST v1.1 criteria), as determined by the investigator

9.5.5 Adverse events in a patient with advanced biliary tract cancer treated with fluorouracil, leucovorin, and nanoliposomal irinotecan following progression on or intolerance of gemcitabine and platinum chemotherapy, defined by CTCAE v5.0 as determined by the investigator

9.5.6 Quality of life changes as measured the by EORTC QLQ-C30 questionnaire

9.6 Exploratory Endpoints:

9.6.1 Correlation of dynamics of circulating tumor DNA change compared with change in CA19-9

9.6.2 NGS profile of archived tumor tissue, in particular the presence of *TP53*, *ARID1A*, *KRAS*, *IDH1*, and *CDKN2A* mutations, and TOPO1 expression by IHC

9.7 Study Design and Sample Size Calculation

This is a single arm, open label, multicenter phase II study to evaluate the clinical activity of the combination of fluorouracil, leucovorin, and nanoliposomal irinotecan as second-line treatment in patients with advanced biliary tract cancers following gemcitabine and platinum chemotherapy. Patients with advanced biliary tract cancers who have adequate performance status and adequate hepatic and renal function will be eligible. Patients may have received adjuvant chemotherapy and/or radiation therapy prior to enrolling in the trial, but a minimum of 6 months between adjuvant chemotherapy and this current therapy are required. Patients may continue on study as long as they are tolerating treatment and do not have progression of disease by RECIST v1.1 criteria. Response assessments will occur using imaging (CT or MRI) every 8 weeks.

The median progression-free survival (mPFS) reported for patients receiving second-line 5-FU doublet chemotherapy is around 3 months, and retrospective data on second-line FOLFIRI/XELIRI show a $PFS_{4\ months}$ of around 30%.[5,6] This new treatment combination of fluorouracil, leucovorin, and nanoliposomal irinotecan would be of great interest in this patient population if it could increase the $PFS_{4\ months}$ to 50% or higher. Thus, the primary endpoint of this trial is $PFS_{4\ months}$ based on central radiology review.

We will use a two-stage Simon Minimax phase II design. Using a one-sided α of 0.05 and 80% power, 39 patients will be required to detect a difference in $PFS_{4\ months}$ between 30% and 50%.[7] All patients meeting the eligibility criteria who have signed a consent form, begun treatment, and are not lost to follow-up or who are not taken off study for reasons other than progression or death before 4 months will be considered evaluable for $PFS_{4\ months}$. 19 patients will be needed for the first stage. If 6 or fewer of the 19 patients are alive and progression-free at 4 months, we will conclude that this treatment is insufficiently active in this population and terminate the study. If 7 or more of these 19 patients are alive and progression-free at 4 months, we will continue the

study to the second stage and accrue 20 more patients for a total of 39 evaluable patients. If 16 or fewer of these 39 patients are alive and progression-free at 4 months, we will conclude that this treatment is insufficiently active in this population. If 17 or more of these 39 patients are alive and progression-free at 4 months, this will be considered adequate evidence of efficacy of this treatment and may be recommended for further testing in subsequent studies. Assuming a drop-out rate of 20%, a total of 50 patients will be accrued.

9.8 Analysis Plan

9.8.1 Analytic plan for primary objective

The null hypothesis is that the PFS rate at 4 months is 30% (based on historical controls)[5,6] versus an alternative hypothesis that the rate is 50% or higher. The plan is to use a Simon's Minimax two-stage design with 19 patients enrolled during the first stage. If 6 or more (out of 19) patients are progression free the trial will continue with an additional 20 patients being enrolled during the second stage (for a total of 39 evaluable patients). If 17 or more patients are progression free at 4 months, the trial will declare that the intervention has met the pre-specified criteria for success.

9.8.2 Analytic plan for secondary objectives

9.8.2.1 We plan to determine the ORR using 95% confidence intervals based on our sample size.

9.8.2.2 The nonparametric Kaplan-Meier method will be used to estimate median PFS.

9.8.2.3 The nonparametric Kaplan-Meier method will be used to estimate median OS.

9.8.2.4 The nonparametric Kaplan-Meier method will be used to estimate median TTP.

9.8.2.5 We plan to determine the DCR using 95% confidence intervals based on our sample size.

9.8.2.6 The nonparametric Kaplan-Meier method will be used to estimate median DDC.

9.8.2.7 We plan to estimate the maximum % change in CA19-9 using 95% confidence intervals based on our sample size.

9.8.2.8 Adverse event data will be compiled and tabulated using descriptive statistics.

9.8.2.9 We plan to estimate the maximum % change in clinical benefit rate using 95% confidence intervals based on our sample size.

9.8.3 Analytic Plan for Exploratory Objectives

9.8.3.1 ctDNA analyses are exploratory without formal statistical analysis, but we aim to correlate PFS and change in CA19-9 with ctDNA level and identify mutational mechanisms of resistance by following ctDNA serially.

9.8.3.2 NGS analysis is also exploratory without formal statistical analysis. Using results from these analyses, we will examine whether median PFS is different with patients with specific mutations in *TP53*, *ARID1A*, *KRAS*, *IDH1*, *CDKN2A*, and others, compared to those without these mutations. We will compare the hazard ratio for each mutation (present vs. absent) and compare hazard ratio for progression using a log-rank test.

9.8.3.2 IHC analysis is exploratory without formal statistical analysis. We will explore patients with either TOPO1-high or TS-low tumors vs. patients with either TOPO1-low or TS-high tumors and compare the hazard ratio for progression using a log-rank test (hypothesizing that the former group will have superior PFS).

9.8.3.3 Quality of life analysis is exploratory without formal statistical analysis. We will determine the maximum net change in quality of life score based on the EORTC-QLQ-C30 questionnaire from baseline for each patient.

9.8.3 Safety Assessments

9.8.3.1 Safety Assessments
The safety of fluorouracil, leucovorin, and nanoliposomal irinotecan will be assessed by evaluating study drug exposure, adverse events, serious adverse events, oncology-related events, all deaths, as well as changes in laboratory determinations and vital sign parameters.

9.8.3.2 Duration of Study Drug
A summarization of the number of days and/or cycles subjects were exposed to study drug will be provided.

9.8.3.3 Adverse Events
Analyses of adverse events (and serious adverse events) will include only "treatment-emergent" events, *i.e*., those that have an onset on or after the day of the first dose of study drug. Analyses will not include those that have an onset greater than 30 days after the last dose of study drug. Treatment emergent adverse events will be summarized by system organ class and preferred term according to the MedDRA adverse event coding dictionary. The percentage of subjects experiencing an adverse event at a given severity, NCI CTCAE toxicity grade, and relationship to study drug will be provided.

9.8.3.4 Deaths
The number of subject deaths will be summarized (1) for deaths occurring while the subject was still receiving study drug in this study, (2) for deaths occurring off treatment within 30 days after the last dose of study drug, and (3) for all deaths in this study regardless of the number of days after the last dose of study drug. The relatedness of the deaths to the study drugs will also be provided.

10.0 ETHICAL CONSIDERATIONS

10.1 Institutional Review Board (IRB)

Good Clinical Practice (GCP) requires that the clinical protocol, any protocol amendments, the Investigator's Brochure, the informed consent and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an IRB. IRB approval of the protocol, informed consent and subject information and/or advertising, as relevant, will be obtained prior to the authorization of drug shipment to the study site. Any amendments to the protocol will require IRB approval prior to implementation of any changes made to the study design. Any serious adverse events that meet the reporting criteria, as dictated by local regulations, will be reported to the IRB. During the conduct of the study, the investigator should promptly provide written reports to the IRB of any changes that affect the conduct of the study and/or increase the risk to subjects.

10.2 Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, International Conference on Harmonization (ICH) guidelines, applicable regulations and guidelines governing clinical study conduct and the ethical Principals that have their origin in the Declaration of Helsinki.

10.3 Subject Information and Consent

Prior to the initiation of any screening or study-specific procedures, the investigator or his/her representative will explain the nature of the study to the subject and answer all questions regarding this study. A separate informed consent is also required from subjects who provide blood for genetic testing or fresh biopsy tissue samples for analyses. Each informed consent will be reviewed, signed and dated by the subject and the person who administered the informed consent. A copy of each informed consent will be given to the subject and each original will be placed in the subject's medical record. An entry must also be made in the subject's dated source documents to confirm that informed consent was obtained prior to any study-related procedures and that the subject received a signed copy. Tissue sample collection for analysis will only be performed if the subject has voluntarily consented to participate after the nature of the testing has been explained and the subject has had the opportunity to ask questions. If the subject does not consent to the tissue sample collection, it will not impact the subject's participation in the study.

10.4 Ethical Consideration for Enrollment

Only patients with advanced cancer, for whom no curative therapy exists, will be considered for enrollment. The only treatment options for these patients are enrollment in a Phase I clinical trial, or treatment off protocol, with a non-standard therapy. As described above, the combination of fluorouracil, leucovorin, and nanoliposomal irinotecan is a rational and promising combination for such patients

10.5 Protection of Patient Confidentiality

All patient records, questionnaires, and tissue specimens will be de-identified using a letter and number assigned to their case at the time of enrollment on study. No record or specimen will contain information which could identify the patient. The key which connects patient identifiable information with this assigned number will be held by the Principal investigator. For computer records, the key will be protected by a double password protection system. Any paper records will be contained in a locked cabinet within a locked office to ensure patient’s privacy is protected.

APPENDIX A – 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):

Not at All (1) | A Little Bit (2) | Quite a Bit (3) | Very Much (4)

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 (1) | A Little Bit (2) | Quite a Bit (3) | Very Much (4)

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

During the past week:

Not at All (1) | A Little Bit (2) | Quite a Bit (3) | Very Much (4)

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 (very poor) and 7 (excellent) that best applies to you

29. How would you rate your overall health during the past week?
1 2 3 4 5 6 7
30. How would you rate your overall quality of life during the past week?
1 2 3 4 5 6 7

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APPENDIX B: STUDY ELIGIBILITY CHECKLIST
Phase II Study of Fluorouracil, Leucovorin, and Nanoliposomal Irinotecan in Previously Treated Advanced Biliary Tract Cancer (NAPOLI-2)

Patient Initials: _____ Study ID: _____
Instructions: This form should be completed by the research staff before registering the patient into the trial.
Completed form can be scanned/emailed to [redacted]

INCLUSION CRITERIA (ALL ITEMS MUST BE CHECKED YES)

YES | NO

☐ | ☐ Pathologically-confirmed biliary tract cancer (cholangiocarcinoma or gallbladder adenocarcinoma)

☐ | ☐ Disease progression on or intolerance of gemcitabine- and platinum-based chemotherapy

☐ | ☐ No more than 1 prior line of chemotherapy for unresectable or metastatic disease (adjuvant therapy does not count)

☐ | ☐ Measurable disease by RECIST v1.1 criteria

☐ | ☐ ECOG performance status of 0-1 (see Table 3)

☐ | ☐ At least 18 years of age

☐ | ☐ HIV-negative or HIV-positive provided:

Patients are on a stable HAART regimen that has not changed in at least 6 months
Patients are not on concurrent prophylactic antibiotics or antifungals
The CD4 count above 250 cells/μL and the viral load is undetectable

☐ | ☐ Subjects with no brain metastases or a history of previously treated brain metastases who have been treated by surgery or stereotactic radiosurgery (SRS) at least 4 weeks prior to enrollment and have a baseline MRI that shows no evidence of active intracranial disease and at least 2 weeks off corticosteroids

☐ | ☐ Consent to access archived tumor tissue if available (available tissue is not required for enrollment)

☐ | ☐ Patients must have fully recovered from all effects of surgery. Patients must have had at least two weeks after minor surgery and four weeks after major surgery before starting therapy. Minor procedures requiring "Twilight" sedation such as endoscopies or mediport placement may only require a 24-hour waiting period, but this must be discussed with an investigator.

☐ | ☐ Women of childbearing potential must have a negative serum pregnancy test within 14 days prior to initiation of treatment and/or postmenopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential

☐ | ☐ Patient is capable of understanding and complying with parameters as outlined in the protocol and able to sign and date the informed consent, approved by the Institutional Review Board (IRB), prior to the initiation of any screening or study-specific procedures

LAB VALUES (ALL MUST BE CHECKED YES)

YES | NO

☐ | ☐ Absolute neutrophil count (ANC) ≥ 1,500/mm^3

☐ | ☐ Platelets ≥ 100,000/mm^3

☐ | ☐ Hemoglobin ≥ 9.0 g/dL

☐ | ☐ Serum creatinine ≤ 1.5 X the upper limit of normal of the institution's normal range OR creatinine clearance ≥ 30 mL/min for subjects with creatinine level above the upper limit of normal

☐ | ☐ Hepatic function: AST and ALT $\leq$ 3.0 $\times$ the upper normal limit of institution's normal range. Total bilirubin $\leq$ 1.5 $\times$ the upper normal limit of institution's normal range. For subjects with liver metastases, AST and ALT < 5 $\times$ the upper normal limit of institution's normal range, and total bilirubin >1.5 - 3.0 x the upper normal limit of institution's normal range are acceptable as long as there is no persistent nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia.

☐ | ☐ Prothrombin Time and Partial Thromboplastin Time (PTT) ≤ 1.5 X the upper limit of the institution's normal range

☐ | ☐ INR (International Normalized Ratio) < 1.5, subjects on anticoagulation will be permitted as long as the INR is in an acceptable therapeutic range as determined by the investigator

EXCLUSION CRITERIA (ELIGIBLE PATIENTS MUST ALL BE CHECKED NO)

☐ | ☐ Ampullary adenocarcinoma

☐ | ☐ Women who are pregnant or breastfeeding

☐ | ☐ Anti-cancer treatment within 3 weeks prior to enrollment

☐ | ☐ Prior irinotecan or nanoliposomal irinotecan

☐ | ☐ Known concurrent malignancy or other maligancy within 3 years except for non-melanomatous skin cancers, prostate or cervical cancers following curative therapy, superficial bladder cancer, or other noninvasive or indolent malignancy either that has undergone potentially curative treatment or not requiring active treatment as determined by the Principal Investigator

☐ | ☐ Bowel obstruction

☐ | ☐ Exposure to a strong CYP3A4 inducer, strong CYP3A4 inhibitor, or strong UGT1A1 inhibitor within 2 weeks of study start

☐ | ☐ Allergy or hypersensitivity to fluoropyrimidines, irinotecan, or nanoliposomal irinotecan

☐ | ☐ Clinically significant liver disease: patients with resolved hepatitis B infection are eligible if HbsAg testing is negative, patients with resolved hepatitis C infection are eligible if viral RNA PCR is negative

☐ | ☐ Severe infections within 4 weeks prior to enrollment

☐ | ☐ Major surgery within 4 weeks prior to enrollment

☐ | ☐ Psychiatric illness or social situation that would limit compliance with study requirements

☐ | ☐ Patient has any other concurrent severe and/or uncontrolled medical condition that would, in the investigator's judgment, cause unacceptable safety risks, contraindicate patient participation in the clinical study or compromise compliance with the protocol (e.g. chronic pancreatitis, chronic active hepatitis, active untreated or uncontrolled fungal, bacterial or viral infections other than described above, etc.).

☐ | ☐ Clinically significant, uncontrolled heart disease and/or cardiac repolarization abnormalities, including any of the following:

History of acute coronary syndromes (including myocardial infarction, unstable angina, coronary artery bypass grafting, coronary angioplasty, or stenting) or symptomatic pericarditis within 6 months prior to screening

History of documented congestive heart failure (New York Heart Association functional classification III-IV)

Documented cardiomyopathy

Clinically significant cardiac arrhythmias (e.g. ventricular tachycardia), complete left bundle branch block, high-grade AV block (e.g. bifascicular block, Mobitz type II and third-degree AV block)

Long QT syndrome or family history of idiopathic sudden death or congenital long QT syndrome, or any of the following:

Risk factors for Torsades de Pointes (TdP) including uncorrected hypokalemia or hypomagnesemia, history of cardiac failure, or history of clinically significant/symptomatic bradycardia.

Concomitant use of medication(s) with a known risk to prolong the QT interval and/or known to cause Torsades de Pointes that cannot be discontinued (within 5 half-lives or 7 days prior to starting study drug) or replaced by safe alternative medication

Systolic blood pressure (SBP) >160 mmHg or <90 mmHg at screening

Table 3 (repeated): ECOG Performance Status Scale

Grade	ECOG
0	Fully Active, able to carry on all pre-disease performance without restriction.
1	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.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

APPENDIX C: PATIENT REGISTRATION FORM
Phase II Study of Fluorouracil, Leucovorin, and Nanoliposomal Irinotecan in Previously Treated Advanced Biliary Tract Cancer (NAPOLI-2)

Patient Initials: _____ Study ID: _____
Instructions: This form should be completed by the research staff before registering the patient into the trial.
Completed form can scanned/emailed to [redacted]

1. Date Informed Consent signed: ____/____/_____

2. Start date for treatment: ____/____/_____

3. Prior therapies (date initiated/type):

4. Please fax the following documentation:
   - ☐ Pathology Report
   - ☐ Physicians Note validating:
     - ▪Previous treatments
   - ☐ CT showing RECIST criteria
   - ☐ Laboratory Results
   - ☐ Past Medical History

ON-STUDY CARD

DOB: ____/____/_____

Race: ☐ African American
☐ Asian
☐ Caucasian
☐ Hispanic
☐ Native American
☐ Pacific Islander
☐ Other
☐ Unknown

Gender: ☐ Male ☐ Female ☐ Unknown

Treating Physician: _____
RN: _____

Ethnicity: ☐ Hispanic or Latino
☐ Not Hispanic or Latino
☐ Unknown

Consent Approval Date:
____/____/_____
Screen Failure: ☐ Yes ☐ No
Screening Date: ____/____/_____
On Study Date: ____/____/_____
Protocol Waiver: ☐ Yes ☐ No
Reason: _____

First Treatment Date: ____/____/_____

APPENDIX D: REFERENCES

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