PrECOG Protocol Number: PrE0204

A Multi-Institutional, Single Arm, Two-Stage Phase II Trial of Nab-Paclitaxel and Gemcitabine for First-Line Treatment of Patients with Advanced or Metastatic Cholangiocarcinoma

STUDY CHAIR: Vaibhav Sahai, MD

STUDY CO-CHAIR: Hidayatullah G. Munshi, MD
Halla Nimeiri, MD
Al B. Benson III MD, FACP
Peter O'Dwyer, MD

STATISTICIAN: Paul J. Catalano, ScD

PRECOG STUDY SITE CONTACT: Carolyn Andrews, RN

MEDICAL MONITOR: Katherine M. Smith, MD

(Internal Use Only) Celgene US Ref. ID Number: AX-CL-OTHER-PrECOG-004080

IND #: Exempt

EudraCT #: 2015-002066-24

Version Number: 3.0

Version Date: 1/19/2016

Release/Revision History	
Version 1.0: 5/02/2014	Released to Sites
Version 2.0: 6/04/2015	Released to Sites
Version 3.0: 1/19/2016	Released to Sites

This protocol contains information that is confidential and proprietary

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PrECOG STUDY SITE CONTACT

Carolyn Andrews, RN
Project Manager
PrECOG, LLC
1818 Market Street
Suite 1100
Philadelphia, PA 19103
Phone: 215-789-7001
Email: candrews@precogllc.org

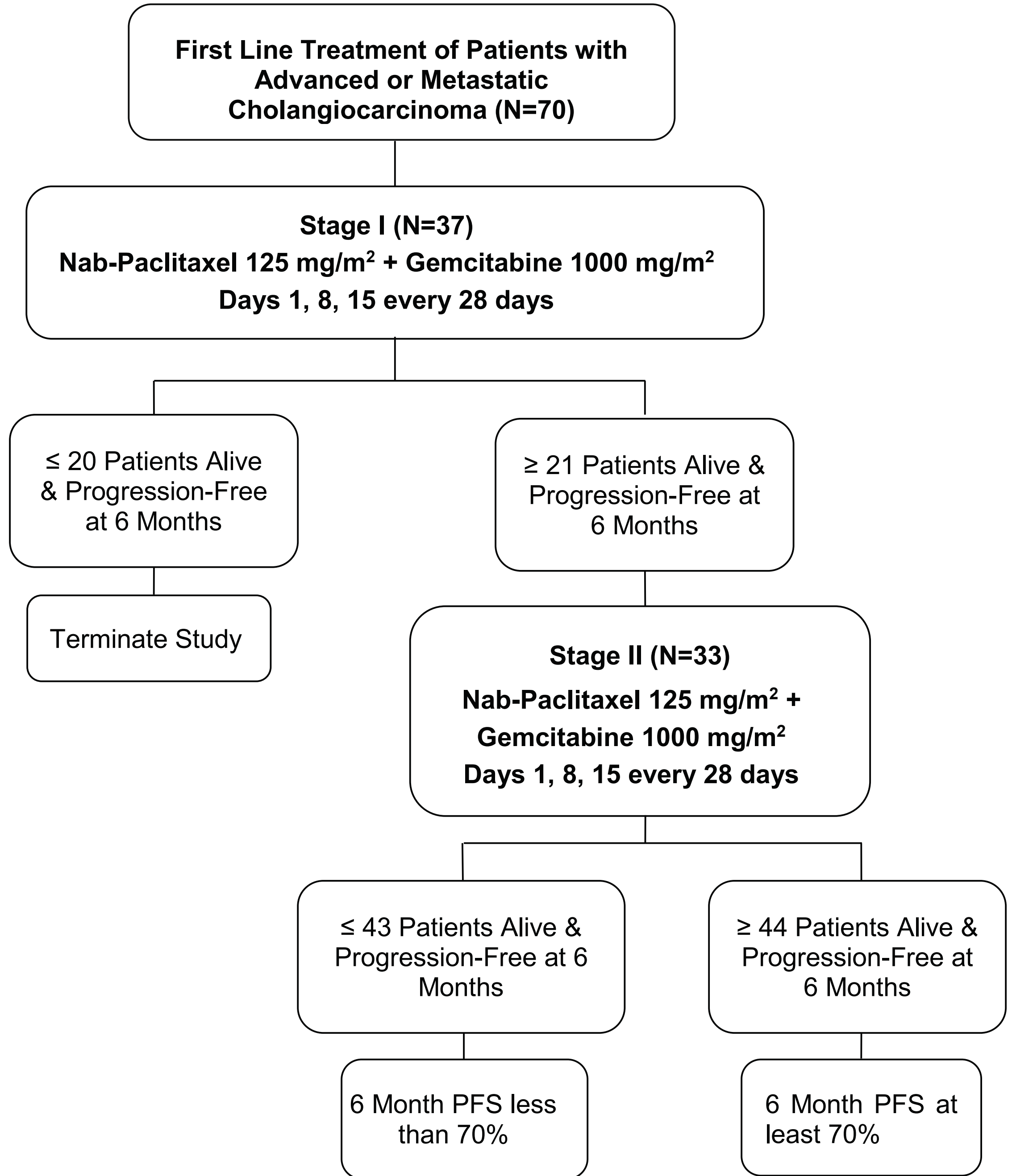
MEDICAL MONITOR

Katherine M. Smith, MD
1000 Continental Drive
Suite 200
King of Prussia, PA 19406
During normal business hours
(8:30 am-5:00 pm EST):
Phone: 610-354-0404
After normal business hours:
Phone: 484-574-2367
Email: ksmith@precogllc.org
SAE Fax: 888-537-9901

Brief Protocol Synopsis

See Protocol Document Sections for complete details

Study Schema



Treatment should begin within 10 working days of registration

List of Abbreviations

ADC	Apparent Diffusion Coefficient
AE	Adverse Event
AJCC	American Joint Committee on Cancer
ALT	Alanine Aminotransaminase (SGPT)
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransaminase (SGOT)
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
C°	Celsius
CA 19-9	Carbohydrate Antigen 19-9
CBC	Complete Blood Count
CCA	Cholangiocarcinoma
CDA	Deoxycytidine Deaminase
CEA	Carcinoembryonic Antigen
CECs	Circulating Endothelial Cells
CFR	Code of Federal Regulations
CI	Confidence Interval
CK	Cytokeratin
CR	Complete Response
CrEL	Cremaphor EL
CT	Computed Tomography
CTC	Circulating Tumor Cell
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DCR	Disease Control Rate
dFdU	Difluorodeoxyuridine
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
ECC	Extra-Hepatic Cholangiocarcinoma
ECM	Extra-Cellular Matrix
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
eDC	Electronic Data Capture
EGFR	Epidermal Growth Factor Receptor
EpCAM	Epithelial Cell Adhesion Molecule
EVCTM	Eudravigilance Clinical Trials Module
F°	Fahrenheit
FDA	Food and Drug Administration

FFPE	Formalin-Fixed Paraffin-Embedded
FGFR2	Fibroblast Growth Factor Receptor 2
FNA	Fine Needle Aspirate
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GED1	Geometrically Enhanced Differential Immunocapture
Gr	Grade
H&E	Hematoxylin and Eosin
HBV	Hepatitis B Virus
Hct	Hematocrit
hENT1	Human Equilibrative Nucleoside Transporter 1
Hgb	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
HUS	Hemolytic-Uremic Syndrome
ICC	Intra-Hepatic Cholangiocarcinoma
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICORG	Ireland Cooperative Oncology Research Group
IHC	Immunohistochemistry
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
IV	Intravenously
kDA	KiloDalton
MR	Magnetic Resonance
mRNA	Messenger Ribonucleic Acid
MTD	Maximum-Tolerated Dose
N	Number
NCI	National Cancer Institute
NS	Normal Saline
ORR	Overall Response Rate
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PDAC	Pancreatic Ductal Adenocarcinoma
PFS	Progression-Free Survival
PI	Principal Investigator

pl	picoliter
PR	Partial Response
PS	Performance Status
PT	Prothrombin Time
RECIST	Response Evaluation Criteria in Solid Tumors
RR	Response Rate
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event
SD	Stable Disease
siRNA	Small Interfering Ribonucleic Acid
SPARC	Secreted Protein Acidic and Rich in Cysteine
SRM	Study Reference Manual
SUSAR	Suspected Unexpected Serious Adverse Reaction
TGF-β	Transforming Growth Factor-β
TMA	Tissue Microarray
TTP	Time to Progression
ULN	Upper Limit of Normal
US	Ultrasonography
WBC	White Blood Count
WOCBP	Women of Childbearing Potential

1. Introduction- Background and Rationale

1.1 Cholangiocarcinoma – Disease Overview

Cholangiocarcinomas (CCAs) develop as a result of malignant transformation of the biliary tract mucosa and are anatomically classified as intrahepatic, perihilar or extra-hepatic. CCAs accounts for 10-15% of all primary liver cancer cases worldwide, and its incidence is rising [1]. Advanced CCAs are aggressive tumors with median survival time after diagnosis of less than 12 months [2], and five-year overall survival (OS) of ~5% despite systemic chemotherapy [3]. The options for systemic chemotherapy for patients with advanced CCA remains limited with only a few meaningful improvements made over the past few decades (Table 1). Valle et al randomly assigned 410 patients with locally advanced or metastatic biliary tract cancer to receive either cisplatin followed by gemcitabine or gemcitabine alone in the Phase III ABC-02 trial [2]. Patients on the cisplatin/gemcitabine arm showed an improvement in OS (11.7 versus 8.1 months; hazard ratio (HR), 0.64; 95% confidence interval (CI), 0.52 to 0.80; p>0.001) compared to the gemcitabine alone arm. This led to the Food and Drug Administration (FDA) approval for gemcitabine/cisplatin combination as first line chemotherapy regimen for patients with advanced CCA.

1.2 Role of Gemcitabine and Paclitaxel in CCA

The support for gemcitabine as an anchor drug in advanced CCA comes from a retrospective review of 304 patients with advanced CCAs who received gemcitabine, a cisplatin-based regimen, or a fluoropyrimidine-based regimen. Significantly, patients receiving a gemcitabine-based regimen were shown to have a lower risk of death [4]. Although gemcitabine has been used as the backbone chemotherapy in a number of Phase I/II clinical trials in combination with other cytotoxic and/or biological therapeutic agents in advanced CCA patients, overall there was not a significant improvement in efficacy (Table 1).

Gemcitabine (2’,2’-difluorodeoxycytidine) is a pyrimidine nucleoside analogue with clinical activity in multiple solid tumors, including pancreatic, breast, lung and bladders cancers. Due to the singular importance of this drug in CCA, significant efforts have been dedicated to improve the therapeutic ratio (relationship between efficacy and toxicity) and select a cohort of patients that seem to derive the most benefit. Amongst other biomarkers, human equilibrative nucleoside transporter 1 (hENT1) seems to have the most promising data. hENT1 is a transmembrane glycoprotein that mediates the intracellular uptake of gemcitabine. High expression of hENT1 has been correlated with improved OS in retrospective studies in patients with CCA [5].

Interestingly, Park et al studied in a Phase II trial the combination of gemcitabine with liposomal paclitaxel in 35 patients with advanced CCAs (Table 1). An overall response rate (ORR) of 35.6% was seen with median progression-free survival (PFS) of 4.07 and OS of 12.3 months [6]. These data are comparable to the results of the gemcitabine and cisplatin regimen studied in the ABC-02 trial and suggest that the combination regimen of gemcitabine with paclitaxel is an effective regimen. Moreover, in-vitro data suggests that there is synergistic activity when paclitaxel is administered prior to gemcitabine to increase apoptosis [7]. Furthermore, Tuveson et al also showed in a mouse model of pancreatic cancer that the gemcitabine/nab-paclitaxel combination has synergistic activity by action of paclitaxel which leads to an increase in intra-tumoral gemcitabine by decreasing the level of primary gemcitabine metabolizing enzyme, deoxycytidine deaminase (CDA) [8].

1.3 Role of Nab-Paclitaxel

Paclitaxel is a hydrophobic molecule and is dissolved in a solvent, cremophor EL (CrEL), which has been associated with significant risk of hypersensitivity reactions and neuropathy. Moreover, CrEL is also known to impair free drug delivery to the tumor, limiting clinical effectiveness of paclitaxel [9]. Nab-paclitaxel (Abraxane®, Celgene, Summit, NJ) was developed as a solvent-free, albumin-bound colloidal formulation of paclitaxel for

intravenous (IV) use allowing for shorter infusion time without pre-medications or specialized infusion sets. Comparative intra-tumoral and anti-tumoral activity of nab-paclitaxel has been demonstrated to be greater than CrEL-paclitaxel in multiple tumor types using preclinical models [10, 11]. In nude mice bearing human xenograft breast tumors, nab-paclitaxel treated mice showed more complete regressions, longer time to recurrence, longer doubling time and prolonged survival compared to paclitaxel [12]. Moreover, pharmacokinetic modeling has shown that nab-paclitaxel has lower risk for neutropenia when compared to equivalent dosing of paclitaxel due to shorter time of free paclitaxel concentration in serum [13, 14]. In a Phase III clinical trial in patients with metastatic breast cancer, nab-paclitaxel demonstrated higher response rates, better safety and side-effect profile compared to conventional paclitaxel, and improved survival in patients receiving it as second line therapy [15].

In pancreatic cancer, Von Hoff et al. published pre-clinical in vivo data showing that the aggregate tumor regression response in individual xenografts were 22/90 (24%), 34/95 (36%), and 53/96 (55%) for gemcitabine, nab-paclitaxel, and gemcitabine plus nab-paclitaxel, respectively [16]. The combination of nab-paclitaxel with gemcitabine was studied in a Phase I/II trial in patients with metastatic pancreatic cancer and showed promising clinical activity with improvement in median OS to 12.2 months [16] from 5.65 months with single agent gemcitabine [17]. The maximum-tolerated dose (MTD) for the combination was defined as gemcitabine 1000 mg/m^2 IV and nab-paclitaxel 125 mg/m^2 IV on days 1, 8, 15 every 28 days [16]. This led to the MPACT International Multi-Center Phase III Trial conducted in 861 patients comparing nab-paclitaxel plus gemcitabine to gemcitabine monotherapy as first line treatment of metastatic pancreatic adenocarcinoma. Results significantly favored the nab-paclitaxel plus gemcitabine arm of the trial with a median OS of 8.5 months (95% CI=7.89, 9.53) versus 6.7 months (95% CI=6.01, 7.23) in the gemcitabine arm. The HR of nab-paclitaxel plus gemcitabine/gemcitabine (HRA+G/G) was 0.72 (95% CI=0.617, 0.835) indicating a 28% overall reduction in the risk of death for patients receiving the combination of nab-paclitaxel and gemcitabine, $p<0.0001$, stratified log rank test [18]. The updated results from this trial were presented at the 2014 Gastrointestinal Cancers Symposium. The gemcitabine/nab-paclitaxel combination resulted in a statistically significant improvement in OS (8.7 versus 6.6 months; HR, 0.72; $p<0.0001$) compared to gemcitabine alone in patients with advanced pancreatic cancer [9]. Nab-paclitaxel has also shown anti-tumor activity in other advanced cancer types, including breast [15], lung [20], and melanoma [21] and has received FDA approval for use in breast, non-small cell lung and pancreatic cancers.

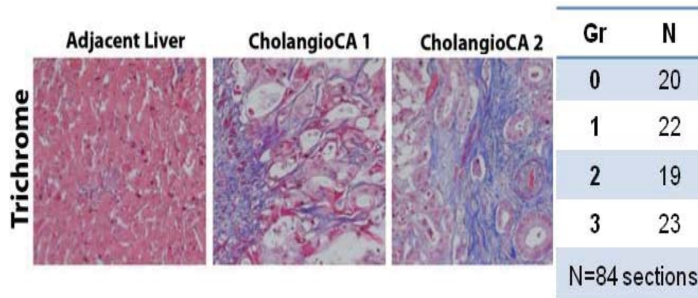
1.4 Why is CCA so Difficult to Treat?

CCA has proven to be a highly challenging cancer to treat despite a multitude of available cytotoxic and biological agents. There are a host of genetic alterations in CCA resulting from increased genomic instability, which not only contributes to tumor invasion and metastasis, but also to the development of resistance to standard therapies. In addition, CCA harbors a dense, fibroblastic stroma similar to that present in pancreatic cancer that has been associated with poor prognosis and increased metastasis. This desmoplastic stroma can up-regulate mesenchymal markers, serve as a barrier to chemotherapy delivery, and activate the cell signaling pathways through epigenetic regulation to increase proliferation and decrease apoptosis [22].

Table 1: Clinical Trials for Advanced/Metastatic CCA from 2008-2012							
Study	**Type**	**Drug Combination**	**N**	**First or Second Line Therapy**	**RR (DCR) %**	**Median PFS (months)**	**Median OS (months)**
Lee et al [23] 2008	Phase II	Gemcitabine + Cisplatin	39	First	17.1 (45.7)	-	8.6
Nehls et al [24] 2008	Phase II	Capecitabine + Oxaliplatin	ECC =47	First	27 (76)	-	12.8
			ICC =18		0 (33)	-	5.2
Koeberle et al [25] 2008	Phase II	Gemcitabine + Capecitabine	44	First	57 (75)	-	13.2
Kim et al [26] 2009	Phase II	Gemcitabine + Oxaliplatin	40	First	15 (52.5)	-	8.5
Valle et al [27] 2009	Phase II	Gemcitabine	86	First	22.6 (58.1)	6 month PFS= 45.5%	-
		Gemcitabine + Cisplatin			27.8 (74.9)	6 month PFS= 57.1%	-
Valle et al [2] 2010	Phase II/III	Gemcitabine	410	First	71.8	5.0 6 month PFS= 42.5%	8.1
		Gemcitabine + Cisplatin			81.4	8.0 6 month PFS= 59.3%	11.7
Jang et al [28] 2010	Phase II	Gemcitabine + Oxaliplatin	53	First	18.9 (69.8)	4.8	8.3
Zhu et al [29] 2010	Phase II	Gemcitabine + Oxaliplatin + Bevacizumab	35	First	14 (40)	7.0	12.7
Halim et al [30] 2011	Phase II	Gemcitabine + Oxaliplatin	40	First	27.5 (65)	4.0	12.0
Malka et al [31] 2012	Phase II	Gemcitabine + Oxaliplatin	150	First	29 (77)	5.3 4 month PFS= 53%	12.4
		Gemcitabine + Oxaliplatin + Cetuximab			23 (87)	6.0 4 month PFS= 63%	11.0
Jensen et al 2012	Phase II	Gemcitabine + Oxaliplatin + Panitumumab	46	First	33 (86)	8.3 6 month PFS= 74%	10.0
Park et al [6] 2012	Phase II	Gemcitabine + Liposomal Paclitaxel	35	First	35.6 (55.6)	4.07	12.3

1.5 Fibrosis in Cholangiocarcinoma

The majority of the CCAs (>90%) are adenocarcinomas and are further classified as sclerosing, nodular and papillary subtypes. Sclerosing or scirrhous CCAs are characterized by an intense desmoplastic reaction [32] (Figure 1) and are associated with low resectability and cure rates [33]. The majority of the CCAs are scirrhous, with 281 out of 294 (95.6%) reported to be sclerosing or scirrhous subtype in a retrospective study [33]. This desmoplastic reaction is composed of bands of fibrous stroma or collagen surrounding the malignant cells and is a characteristic feature of the majority of CCA tumors. Collagen type I and III are the most common extra-cellular matrix (ECM) components that have been described in CCA [34].



Gr	N
0	20
1	22
2	19
3	23
N=84 sections	

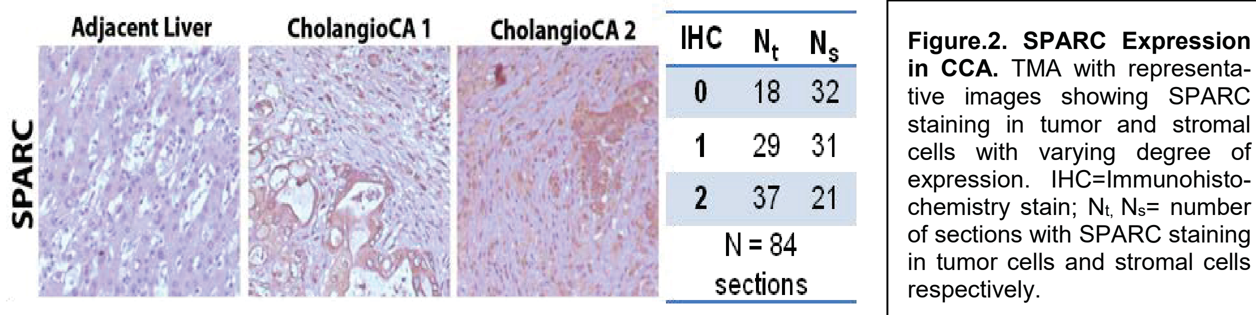
Figure.1. Fibrosis and CCA. Human CCA tissue microarray (TMA) containing 42 specimens were trichrome stained to assess for fibrosis. Representative trichrome staining (blue=fibrosis) of adjacent liver demonstrating no fibrosis compared to varying degree of fibrosis in CCA specimens. Gr=grade of fibrosis; N=number of tumor sections

1.6 Role of SPARC in Fibrosis

SPARC (secreted protein acidic and rich in cysteine, also known as osteonectin or BM-40) is a matricellular glycoprotein known to be involved in diverse biological processes, including collagen fibrilogenesis, morphogenesis, proliferation, migration, angiogenesis and apoptosis [35]. It is a collagen binding protein and has been demonstrated to affect both collagen extracellular matrix assembly and activity of transforming growth-factor (TGF-β) [36]. TGF-β is known to stimulate collagen synthesis and, therefore, SPARC has the capacity to regulate fibrosis through the TGF-β pathway. Interestingly, TGF-β is also known to stimulate SPARC production and silencing of SPARC through small interfering ribonucleic acid (siRNA) has been shown to decrease TGF-β-induced fibrotic response [37]. The up-regulation of SPARC expression in fibrotic human liver has been associated with increased levels of type I collagen [38]. A reduction in SPARC expression through siRNA showed corresponding decrease in both TGF-β expression and fibrosis in rat livers treated with thioacetamide to induce fibrosis [39]. Similarly, multiple models have shown association between SPARC expression and collagen deposition that becomes more apparent in injury [40]. In malignancy, SPARC expression by tumor cells and surrounding stroma results in extensive remodeling and redistribution of the extracellular matrix components promoting cell invasion and metastasis [41, 42]. SPARC increases cell invasiveness and expression of SPARC by fibroblastic cells in the stromal compartment of pancreatic cancer is strongly associated with poor patient outcome [43].

1.7 SPARC Expression in Cholangiocarcinoma

Although SPARC expression in human pancreatic cancers has been clearly established, less is known about SPARC in CCAs. We have found that there is a robust expression of SPARC in both tumor cells and the surrounding stroma in CCAs compared to adjacent normal liver (n=42 specimens) (Figure 2).



IHC	N_t	N_s
0	18	32
1	29	31
2	37	21
N = 84 sections		

Figure.2. SPARC Expression in CCA. TMA with representative images showing SPARC staining in tumor and stromal cells with varying degree of expression. IHC=Immunohistochemistry stain; N_t, N_s= number of sections with SPARC staining in tumor cells and stromal cells respectively.

1.8 Interaction of SPARC with Nab-Paclitaxel and Gemcitabine

Nab-paclitaxel is an albumin-bound formulation and SPARC is known to bind albumin. Albumin plays an important role in endothelial transcytosis of protein bound and unbound plasma constituents mainly by binding to a cell-surface 60 kDa glycoprotein receptor (gp60) on the endothelial cell membrane. This leads to activation of caveolin-1, a major component of membrane vesicles, resulting in receptor mediated internalization of the albumin-drug complex into caveolae (small invaginations of plasma membrane). Subsequently, caveolae transports the albumin-drug conjugate to the extracellular space, including the tumor interstitium. Patient derived xenograft mice show depletion of this desmoplastic stroma accompanied by dilation of blood vessels in the tumor milieu when exposed to nab-paclitaxel compared to vehicle or gemcitabine alone. This reduction in tumor stroma and the accompanying increase in vascularization facilitate an increase in the intra-tumoral delivery and intracellular concentration of gemcitabine [8, 16].

The biomarker analysis of the Phase I/II gemcitabine plus nab-paclitaxel regimen in patients with advanced pancreatic cancer showed that the patients with high-SPARC expression have a significant increase in OS compared with patients in the low-SPARC group (median OS, 17.8 versus 8.1 months, respectively; p=0.0431) [16]. Furthermore, the SPARC level remained a significant predictor for the OS in a multivariate Cox regression model after adjusting for clinical covariates and baseline carbohydrate antigen 19-9 (CA 19-9) level. Additionally, only the stromal SPARC was significantly correlated with OS (p=0.013), but not SPARC in tumor cells (p=0.15) [16]. Similarly, in peri-ampullary cancers, SPARC has been shown to be over-expressed in stroma (90%) compared to chronic pancreatitis (62%) and normal pancreas (0%). Tumors expressing SPARC were more likely to have higher T-staging (p=0.056), nodal metastases (p=0.06) and decreased OS (p=0.013) than those lacking SPARC expression [7].

1.9 Imaging of CCA

Ultrasonography (US) is the initial screening imaging modality for evaluating biliary dilatation in patients with jaundice. However, US is relatively less accurate in the estimation of tumor spread in the abdomen and the determination of tumor resectability [44]. Both computed tomography (CT) and magnetic resonance (MR) imaging provide adequate assessment at diagnosis and follow-up of CCAs. Contrast-enhanced CT is relatively fast, provides complete abdominal assessment and widely available and has become the non-invasive diagnostic test of choice for detailed evaluation and staging of cholangiocarcinomas [44]. However, gadolinium-enhanced MR imaging is considered the optimal imaging modality for suspected CCAs [45]. It provides information regarding liver and biliary anatomy, local extent of tumor, extent of duct involvement by tumor, degree of vascular involvement, presence of lymph node enlargement, liver metastases, and adjacent organ invasion. CCA is a hypovascular tumor, and following the IV administration of gadolinium, the classic enhancement pattern of CCA is hypointense on T1-weighted, and hyperintense on T2-weighted imaging relative to liver parenchyma with early rim enhancement followed by progressive centripetal heterogeneous enhancement of the remainder of the lesion with

extracellular MR contrast agents [46, 47]. Apparent diffusion coefficient (ADC) values are calculated from tri-directional gradients (b-values) and lesions with the lowest ADC value (i.e., impeding diffusion to the greatest degree) are more likely to be malignant. The b-values utilized in liver imaging range from 0-800 with b0 serving as a T2-weighted sequence used for lesion conspicuity and anatomic correlation [47].

1.10 Role of Circulating Tumor Cells (CTCs) in CCA

In this emerging era of personalized medicine, there is a great need for individualizing therapy through predictive and prognostic markers. We rely largely on imaging assessment, however, these are not ideal for several reasons, including – absence of decrease in size of the tumor either due to central necrosis, or use of cytostatic therapy, absence of radiographically measurable disease at baseline (such as peritoneal disease, ascites), or the need to wait at least 2-3 months to see measurable response. Epithelial tumor cells overexpress epithelial cell adhesion molecule (EpCAM) which has been exploited to detect these cells from peripheral blood using multiple techniques in various epithelial cancers, including CCA [48-50]. Geometrically enhanced differential immunocapture (GEDI) platform has been utilized in prostate and pancreatic cancer to capture circulating tumor cells using antibody-coated obstacles, specific to EpCAM [51-53]. Ustwani et al showed in a pilot study of 16 patients with advanced CCA and gallbladder carcinoma that CTCs can be successfully measured. Two patients with serial blood collection had correlation of CTC values with change in burden of disease. However, due to small sample size and inability to obtain serial blood samples in all patients, they were unable to determine any prognostic relevance [54].

Furthermore, CTCs can potentially be analyzed to more accurately understand the biology of the tumor as a response to therapy. To date, many somatic gene mutations have been identified and related to the presence and development of CCA. However, genomic analysis requires fresh (preferred) or formalin-fixed paraffin-embedded (FFPE) tissue from core biopsy samples which has been difficult in CCA since diagnosis is mostly based on brush or fine needle aspiration (FNA) biopsies during endoscopic retrograde cholangio-pancreatography. Moreover, it has been challenging to repeat biopsies on progression of disease on clinical trials to identify changes in the genetic signature of these cancers compared to that on diagnosis. Targeted gene expression analysis of CTCs represents a potentially useful alternative for these patients for detection of ‘druggable’ targets. Further development and assessment of this non-invasive marker should lead to more effective and better tailored anticancer treatments for individual patients, thus resulting in improved life expectancy in this rare cancer.

1.11 Role of CDA in CCA

Intracellular gemcitabine is irreversibly deaminated by deoxycytidine deaminase (CDA) to inactivated difluorodeoxyuridine (dFdU). Tuveson et al. showed in a pancreatic ductal adenocarcinoma (PDAC) mouse model that the combination of nab-paclitaxel and gemcitabine increases intra-tumoral gemcitabine levels by decreasing the levels of primary gemcitabine metabolizing enzyme, CDA through post-translational mechanism [8]. CDA metabolizes gemcitabine to an inactive metabolite, 2’,2’-difluorodeoxyuridine. Low plasma CDA activity and CDA mutation 208G>A have been significantly associated with prolonged OS in patients with advanced pancreatic adenocarcinoma [55]. However, to date, role of CDA has not been studied in CCA. Our pre-clinical data shows that there is robust CDA staining in the tumoral cells of CCA (n=42 specimens) (Figure 3).

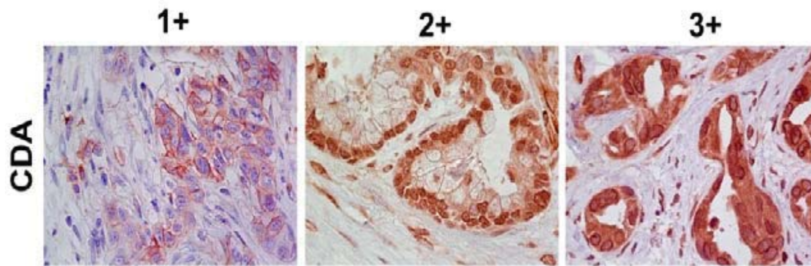


Figure 3. CDA Expression in CCA. Human CCA TMA (n=42 specimens) with representative images showing CDA IHC staining in tumor and stromal cells with varying degree of expression.

1.12 Role of hENT1 in CCA

Gemcitabine is a pyrimidine nucleoside analogue that undergoes complex cellular uptake and metabolism. The drug is transported into the cells mostly by the human equilibrative nucleoside transporter 1 (hENT1) and is rapidly activated via phosphorylation to the active compounds diphosphate and triphosphate which are mainly incorporated into deoxyribonucleic acid (DNA) leading to masked chain termination. Given the short infusion times of gemcitabine (30 minutes) and the short serum half-life of gemcitabine, and excretion as non-toxic metabolites, it was hypothesized that cells with low hENT1 protein expression may be clinically resistant to gemcitabine. Our pre-clinical data shows that there is robust hENT1 staining in the tumoral cells of CCA (n=42 specimens) (Figure 4). Mori et al evaluated messenger RNA (mRNA) hENT1 levels by quantitative reverse polymerase chain reaction (RT-PCR) in CCA and PDAC cell lines treated with gemcitabine and found that the degree of hENT1 mRNA level expression was directly associated with the chemosensitivity of these cell lines [56]. Intra-tumoral hENT1 expression was investigated by immunohistochemistry (IHC) in 105 patients with resected CCA of which 54 received adjuvant chemotherapy with gemcitabine. The median OS in patients with high hENT1 expression was significantly better than those with low hENT1 expression for patients who received adjuvant chemotherapy (p=0.008), but not among patients who did not receive chemotherapy (p=0.894) [5]. In another retrospective study which evaluated 31 patients with advanced unresectable CCA, patients with positive hENT1 expression (67.7%) showed a statistically significant time to progression (TTP) (6.33 versus 2.83 months; p=0.0394) and a trend towards longer median OS (14 versus 7 months; p=0.128) when compared with patients with low hENT1 expression [57]. This suggests the role of intra-tumoral hENT1 expression as both a prognostic and predictive biomarker for patients receiving gemcitabine-based chemotherapy for CCA. However, to date, there are no prospective data for validation in CCA.

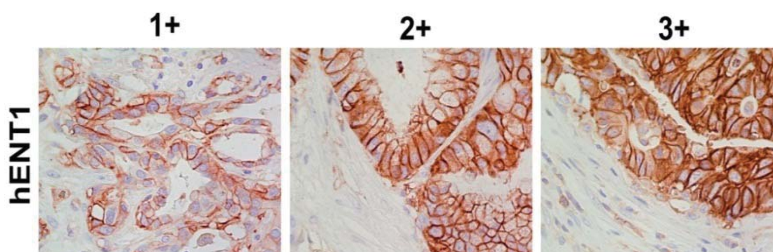


Figure 4. hENT1 expression in CCA. Human CCA TMA (n=42 specimens) with representative images showing hENT1 IHC staining in tumor and stromal cells with varying degree of expression.

1.13 Summary of Rationale for Proposed Study

Pre-clinical murine data suggests that concurrent nab-paclitaxel treatment leads to increased intra-tumoral concentration of gemcitabine. Moreover, inhibition of CDA enzyme by nab-paclitaxel further increases the intracellular concentration of gemcitabine. Significantly, a Phase III clinical trial with gemcitabine and nab-paclitaxel combination showed a significant improvement in OS. Interestingly, the biomarker analysis of the earlier

Phase I/II trial of this combination in a similar patient population showed that high stromal SPARC expression was associated with a statistically and clinically significant improvement in OS. The goal of this clinical trial is to evaluate the efficacy of gemcitabine plus nab-paclitaxel in a Phase II clinical trial in which up to 70 patients with advanced CCA will be enrolled. Specifically, the primary objective will be measurement of the 6-month PFS. Secondary objectives will include measurements of ORR, disease-control rate (DCR), toxicity and OS. We will also examine whether CTC levels with targeted gene expression analysis and stromal SPARC levels correlate with patient outcome and thus serve as prognostic biomarkers. We will evaluate the role of hENT1 and CDA as additional prognostic and predictive biomarkers in CCA. This clinical trial is expected to improve on the dismal prognosis of patients with advanced CCA by establishing the activity of a platinum-free doublet, nab-paclitaxel plus gemcitabine that has shown clear clinical benefit in pancreatic cancer which has close biological parallels to CCA. Findings are also expected to establish CTC, SPARC, hENT1 and CDA as potential prognostic biomarkers for CCA.

1.13.1 Rationale for the Combination of Nab-Paclitaxel and Gemcitabine in CCA

As noted above, nab-paclitaxel is an albumin-bound formulation, and SPARC is known to bound albumin. We have shown that SPARC is expressed in both the tumor as well as surrounding stromal cells in CCA. The cell transports the albumin-drug conjugate to the extracellular space, including the tumor interstitium. This interaction of SPARC with nab-paclitaxel has been shown to concentrate the drug in the tumor vicinity although more recently Neesse et al have shown that this effect may be saturable at administered therapeutic levels of nab-paclitaxel [58, 59]. Nevertheless, nab-paclitaxel has been shown to concentrate more than cremophor-based paclitaxel as well as increase the intra-tumoral concentration of gemcitabine [8, 10, 16]. Moreover, the albumin-bound paclitaxel has less vehicle-related toxicity, shorter plasma exposure and less neurotoxicity compared to cremaphor-based paclitaxel.

The biomarker analysis of the Phase I/II gemcitabine plus nab-paclitaxel regimen in patients with advanced pancreatic cancer showed that the patients with high-SPARC expression had a significant increase in OS compared with patients in the low-SPARC group (median OS, 17.8 versus 8.1 months, respectively; p=0.0431) [13]. Furthermore, the SPARC level remained a significant predictor for OS in a multivariate Cox regression model after adjusting for clinical covariates and baseline CA 19-9 level. Additionally, only the stromal SPARC was significantly correlated with OS (p=0.013), but not SPARC in tumor cells (p=0.15) [13]. Similarly, in peri-ampullary cancer, SPARC has been shown to be over-expressed in stroma (90%) compared to chronic pancreatitis (62%) and normal pancreas (0%). Tumors expressing SPARC were more likely to have higher T-staging (p=0.056), nodal metastasis (p=0.06) and decreased OS (p=0.013) than those lacking SPARC expression [6].

The goal of this study is to evaluate the efficacy of gemcitabine plus nab-paclitaxel in a Phase II clinical trial of patients with advanced CCA. This is based on the hypothesis that nab-paclitaxel binds to SPARC through its interaction with albumin, leading to an increase in intra-tumoral concentration of gemcitabine through decreased CDA enzyme. The proposed research is innovative because it aims to utilize a targeted approach to deliver cytotoxic chemotherapy to target the peri-tumoral stroma of CCA and thus increase the intra-tumoral concentration of chemotherapeutic agents. Our contribution here is expected to improve on the OS of patients with advanced CCA through the use of the synergistic combination of nab-paclitaxel and gemcitabine to specifically target the SPARC protein in the peri-tumoral stroma. This contribution is significant because it aims to improve on the current abysmal prognosis for these patients and provide critical data to further develop pharmacologic strategies to target the desmoplastic stroma in order to

increase chemotherapy responsiveness of CCAs. The research will be of added significance because what is learned here will be applicable to other malignancies characterized by extensive fibrosis and resistance to current therapeutics.

1.13.2 Rationale for the Study Design and Treatment Plan

This will be an open-label, single-arm, two-stage Phase II clinical trial to evaluate the efficacy and safety of the combination of nab-paclitaxel and gemcitabine in patients with advanced CCA. The primary objective will be to determine the efficacy of nab-paclitaxel and gemcitabine as measured by improvement in 6-month PFS rate. The secondary objectives will be to measure clinical efficacy (median OS, PFS, TTP, best ORR, DCR), describe the safety and toxicity profile of the drug combination and correlate the maximum change in CA 19-9 with survival. We will also examine whether CTC values and stromal SPARC levels correlate with patient outcome, and thus serve as prognostic biomarkers. Furthermore, we will evaluate the role of CDA and hENT1 as additional prognostic and/or predictive biomarkers in CCA. This clinical trial is expected to improve on the dismal prognosis of patients with advanced CCA by targeting the albumin-binding SPARC protein by nab-paclitaxel plus gemcitabine. The proposed research is innovative because it aims to utilize a targeted approach to deliver cytotoxic chemotherapy to target the peri-tumoral stroma of CCA, and thus increase the intra-tumoral concentration of chemotherapeutic agents.

2. Study Objectives

2.1 Primary Objective

To determine the efficacy of gemcitabine and nab-paclitaxel in patients with advanced CCA as measured by improvement in 6-month PFS.

2.2 Secondary Objectives

2.2.1 To evaluate clinical efficacy by assessment of median OS, PFS, TTP as well as best ORR and disease control rate (DCR).

2.2.2 Describe the safety and toxicity profile of the combination of nab-paclitaxel and gemcitabine in this population.

2.2.3 Correlate the change in CA 19-9 with ORR, DCR, median PFS, TTP and OS.

2.3 Exploratory Objectives

2.3.1 Correlate change in Circulating Tumor Cells (CTCs) obtained from whole blood (optional) to ORR, DCR and median PFS, TTP and OS. Perform targeted gene expression analysis on CTCs and correlate with CCA pathology, PFS, TTP, ORR and OS.

2.3.2 Correlate stromal SPARC (high versus low) expression by immunohistochemistry (IHC) and fibrosis (low, intermediate and high) by trichrome staining with ORR, DCR and median PFS, TTP and OS.

2.3.3 Correlate CDA (high versus low) and hENT1 (high versus low) expression by IHC with ORR, DCR and median PFS, TTP and OS.

2.3.4 Optional specimen banking of patient blood specimens (including serum, plasma and buffy coat) as well as fixed left-over tissue specimens when available from all enrolled patients in this trial for possible future molecular, pharmacogenomic, and/or proteomic testing.

3. Selection of Patients

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist must be photocopied, completed and maintained in the patient's chart.

In calculating days of tests and measurements, the day a test or measurement is done is considered Day 0. Therefore, if a test is done on a Monday, the Monday four weeks later would be considered Day 28.

PrECOG Patient No. ____________________

Patient's Initials (F, M, L) ____________________

Physician Signature and Date ______________________________

NOTE: All questions regarding eligibility should be directed to the Medical Monitor or Study Site Contact.

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration by the treating physician.

_____ 3.1 Patients must have a histologically-confirmed diagnosis of cholangiocarcinoma American Joint Committee on Cancer (AJCC) Stage II, III, or IV CCA (intra-hepatic, extra-hepatic and perihilar) that is not eligible for curative resection, transplantation, or ablative therapies. Tumors of mixed histology are not allowed.

_____ 3.2 Must have radiographically measurable disease (RECIST Version 1.1) in at least one site not previously treated with radiation, chemoembolization, radioembolization, or other local ablative procedures (i.e. must have at least one measurable target lesion, either within the liver or in a measurable metastatic site); a new area of tumor progression within or adjacent to a previously-treated lesion, if clearly measurable by a Radiologist, is acceptable. Appropriate imaging should have been completed ≤ 4 weeks prior to registration.

_____ 3.3 May have received prior radiation, chemoembolization, radioembolization, or other local ablative therapies, or hepatic resection if completed ≥ 4 weeks prior to registration AND if patient has recovered to ≤ grade 1 toxicity.

NOTE: Measurable disease (as required above) must still be present.

_____ 3.4 May have received prior radiation for bone or brain metastases if patient is now asymptomatic and has completed all radiation and steroid therapy (if applicable) ≥ 2 weeks prior to registration.

_____ 3.5 Age ≥ 18 years.

_____ 3.6 Child-Pugh score of A or B with ≤ 7 points (Appendix I).

_____ 3.7 ECOG performance status of 0-1 (Appendix II).

_____ 3.8 Ability to understand and willingness to sign IRB-approved informed consent.

_____ 3.9 Willing to provide archived tissue, if available, from a previous diagnostic biopsy.

_____ 3.10 Must be able to tolerate CT and/or MRI with contrast.

_____ 3.11 Adequate organ function as measured by the following criteria, obtained ≤ 2 weeks prior to registration:

- Absolute Neutrophil Count (ANC) ≥ 1500/mm^3

  ANC: ________________ Date of Test: __________

- Hemoglobin >9.0 g/dL

  Hemoglobin: ____________ Date of Test: ____________

- Platelets >100,000/mm^3

  Platelets: _______________ Date of Test: ____________

- Serum Creatinine ≤ 1.5x Upper Limit Normal (ULN)

  Serum Creatinine: ________ ULN: ________ Date of Test: ___________

- Creatinine Clearance ≥ 50 ml/min

  Creatinine Clearance: ______ Date of Test: ____________

- Albumin ≥ 2.8 g/dL

  Albumin: _________________ Date of Test: ____________

- Total Bilirubin ≤ 1.5 mg/dL or ≤ 1.5x ULN

  Total Bilirubin: ___________ ULN: ________ Date of Test: ___________

- AST/ALT ≤ 2.5x ULN (≤ 5x ULN in patients with liver metastases)

  AST/ALT: ______________ ULN: _______ Date of Test: ___________

- INR <1.5x the ULN [INR ≥ 1.5 is allowed if anticoagulation is used. Patient must be on a stable dose of anticoagulant (i.e. Coumadin) for ≥ 2 weeks at time of randomization.]

  INR: ___________________ ULN: _______ Date of Test: ___________

_____ 3.12 Women must not be pregnant or breastfeeding since nab-paclitaxel and/or gemcitabine may harm the fetus or child. All females of childbearing potential (not surgically sterilized and between menarche and 1 year post menopause) must have a blood test to rule out pregnancy within 2 weeks prior to registration.

Is the patient a woman of childbearing potential? _____________________ (yes/no)

If yes, Date of Test: _______________ Results: ____________________________

_____ 3.13 Women of child-bearing potential (not surgically sterilized and between menarche and 1 year post menopause) and men must agree to use 2 methods of adequate contraception (hormonal plus barrier or 2 barrier forms) OR abstinence prior to study entry, for the duration of study participation, and for 6 months following the last dose of study therapy. Method of contraception must be documented.

NOTE: Should a female patient receiving study drug or a female partner of a male patient receiving study drug become pregnant or suspect she is pregnant during the study or within 6 months after the last dose of study drug, the patient must inform the study doctor immediately.

_____ 3.14 Must not have received prior systemic cytotoxic chemotherapy or targeted therapy *for this cancer*.

NOTE: Prior systemic cytotoxic therapy for other diagnoses is permitted if the last dose was >6 months prior, any prior toxicity has recovered to ≤ grade 1, and treatment was not discontinued for toxicity.

_____ 3.15 Must not be receiving treatment with other investigational agents and must not have received any other investigational agent's ≤ 4 weeks prior to registration.

_____ 3.16 Must not have a pre-existing >grade 2 peripheral neuropathy.

_____ 3.17 Must not be receiving immunosuppressive medications, including systemic corticosteroids, aside from the following exceptions: used for adrenal replacement, appetite stimulation, therapy for asthma, bronchitis exacerbation (≤ 2 weeks), anti-emesis, or pre-medication for procedures (i.e. CT scan).

_____ 3.18 No known Hepatitis B, Hepatitis C, or Human Immunodeficiency Virus (HIV) seropositivity. The risk for potential toxicities secondary to Hepatitis B, Hepatitis C or HIV (e.g., increased risk for fatal opportunistic infection) may confound the toxicity profile of the chemotherapy regimen. Testing is not required in absence of clinical suspicion.

_____ 3.19 Must not have undergone liver transplantation.

_____ 3.20 Must not have symptomatic brain or bone metastases (Section 3.4 for additional requirements).

_____ 3.21 Must not have serious non-healing wound, ulcer, bone fracture, or abscess.

_____ 3.22 Must not have undergone a major surgical procedure <4 weeks prior to registration.

_____ 3.23 Must not have possible histories of pneumonitis or pneumonitis risk factors (Section 5.3.5).

_____ 3.24 Must not have an active second malignancy other than non-melanoma skin cancer or cervical carcinoma in situ.

NOTE: Patients with history of malignancy are not considered to have a “currently active” malignancy if they have completed therapy and are now considered by their physician to be at less than 30% risk for relapse.

_____ 3.25 Must have no ongoing or active, uncontrolled infections (afebrile for ≥ 48 hours off antibiotics).

_____ 3.26 Must have no evidence of significant, uncontrolled concomitant diseases including, but not limited to: symptomatic congestive heart failure, unstable angina pectoris, uncontrolled cardiac arrhythmia, myocardial infarction within preceding 12 months, uncontrolled peripheral vascular disease, cerebrovascular accident within preceding 12 months, pulmonary disease impairing functional status or requiring oxygen, connective tissue disease including lupus.

_____ 3.27 Must not have any history of allergic reaction(s) attributed to compounds of similar composition to nab-paclitaxel or gemcitabine or any of their excipients.

_____ 3.28 Must not require prohibited medications with the potential for serious interactions with protocol therapy, and who cannot have therapeutic substitution (Section 5.4).

_____ 3.29 Must not have a psychiatric illness, other significant medical illness, or social situation which, in the investigator’s opinion, would limit compliance or ability to comply with study requirements.

4. Registration Procedures

4.1 Ethics

This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki and will be consistent with applicable US regulatory requirements and International Conference on Harmonization/Good Clinical Practice (ICH/GCP).

The study will be conducted in compliance with the protocol. The protocol and any amendments and the patient informed consent will receive Institutional Review Board (IRB) approval prior to initiation of the study.

Freely given written informed consent must be obtained from every patient or their legally acceptable representative prior to clinical trial participation, including informed consent for any screening procedures conducted to establish patient eligibility for the trial.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s). This trial will not use the services of investigators or study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure, debarment). Investigators are responsible for the conduct of the study at their study site.

4.2 Regulatory Requirements

Before a site may enter patients, protocol-specific regulatory and other documents must be submitted to PrECOG as noted in study materials. Detailed information regarding document submission and control is provided to each site in separate study materials.

Once required documents are received, reviewed, and approved by PrECOG or their representative, a Study Reference Manual (SRM) will be forwarded to the site. Any changes to site regulatory documents must be submitted by the investigator to the responsible party in a timely manner. Initial study drug shipment will not occur until the regulatory packet is complete. No patients will begin protocol therapy without formal registration as per the process below.

4.3 Patient Registration

Patients must not start protocol treatment prior to registration.

Patients must meet all of the eligibility requirements listed in Section 3 prior to registration. Treatment should begin ≤ 10 working days from study entry (date of registration).

An eligibility checklist is included in Section 3. A confirmation of eligibility assessment by the investigator and/or site will be performed during the registration process.

Upon determination that a subject meets eligibility criteria, the subject will be registered in the study by site personnel via an electronic data capture (eDC) system. Confirmation of registration will be displayed in the eDC system.

Full information regarding registration procedures and guidelines can be found in the SRM provided to your site. Correspondence regarding patient registration must be kept in the study records.

4.4 Research Blood and Tissue Samples

Mandatory biologic samples for correlative studies: Procurement of archived tissue, if available, from a previous diagnostic biopsy is mandatory for enrollment. If not available, this will not preclude participation in the trial, nor will additional biopsies be performed for research purposes only.

Optional blood samples (whole blood) will be requested from the patient to test for Circulating Tumor Cells (CTCs).

In addition, blood samples (including serum, plasma and buffy coat) and fixed left over tissue specimens will be banked, when available from all enrolled patients for possible future molecular, pharmacogenomic, and/or proteomic testing. These specimens are optional. See Section 11.0 for more information.

5. Treatment Plan

5.1 Overview

This is an open label, single-arm, two-stage, multi-institutional Phase II trial to evaluate the efficacy and safety of the combination of nab-paclitaxel and gemcitabine as first-line therapy for patients diagnosed with histologically-confirmed advanced CCA (intra-, extra-hepatic and perihilar). All patients will receive nab-paclitaxel at a dose of 125 mg/m^2 IV over 30 minutes, followed by gemcitabine at a dose of 1000 mg/m^2 IV over 30 minutes on days 1, 8, and 15 of each cycle (1 cycle=28 days). Radiographic assessment will be performed at baseline and every 8 weeks to evaluate response to treatment by RECIST Version 1.1 guidelines. Patients may continue to receive treatment until disease progression or development of unacceptable toxicity. The primary endpoint will be PFS at 6 months. A maximum of 70 patients will be enrolled.

5.2 Treatment Administration

Patients will receive both study drugs by IV infusion on days 1, 8, and 15 of each cycle (1 cycle=28 days). Patients may receive premedication with diphenhydramine (25-50 mg IV) 30 minutes prior to nab-paclitaxel or per institutional standards. (Premedication to prevent hypersensitivity reaction is generally not needed prior to the administration of nab-paclitaxel but may be needed in patients who have had prior hypersensitivity reactions to nab-paclitaxel.) Nab-paclitaxel will be administered first, at a dose of 125 mg/m^2 IV over a period of 30 minutes; gemcitabine will be administered second, at a dose of 1000 mg/m^2 over a period of 30 minutes.

Calculate the patient's body surface area (BSA) according to standard institutional methods. BSA will be calculated on Cycle 1 Day 1 and recalculated per the site's standard of care, or if body weight changes by more than 10%. Actual heights and weights should be used to calculate surface areas (no downward adjustment to "ideal" weight). This principle applies to individuals whose calculated surface area is 2.2 m^2 or less. In those rare cases where a patient's surface area is greater than 2.2 m^2, the actual surface area or 2.2 may be used. Dosing BSA may be capped if the treating physician believes it is in the best interest of an obese patient.

Table 2: Regimen Description

Drug	Pre-Medication	Dose	Route	Cycle/Schedule
Nab-Paclitaxel	Premedication may occur with diphenhydramine 25-50 mg IV 30 minutes prior to infusion, or per institutional standards	125 mg/m^2 in an empty sterile, IV bag	IV over 30 minutes	Days 1, 8 and 15 every 28 days 1 cycle=28 days
Gemcitabine		1000 mg/m^2 per commercial package insert	IV over 30 minutes after completion of nab-paclitaxel	

Patients will be monitored by infusion center nursing staff every 15 minutes during infusion or per institutional standards, and treatment will be interrupted for any evidence of an infusion reaction. If hypersensitivity symptoms develop, the infusion will be stopped. Patients will be treated with additional H1 antagonist, as well as an H2 antagonist (such as famotidine 20 mg or ranitidine 50 mg IV) at the discretion of the treating investigator. If hypersensitivity symptoms are mild (grade 1 or 2) and resolve over 30-60 minutes of observation with administration of additional H1 and H2 blockers, the study drug infusion may resume with caution at a reduced rate (over approximately 60 minutes), at the discretion of the treating investigator. Additional supportive care measures should be available at each study site infusion center in case of severe hypersensitivity reaction. Patients who have had severe hypersensitivity reactions should not be re-challenged.

5.3 Dose Delays & Modifications

All toxicities should be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE V4.0). A copy of the CTCAE V4.0 can be downloaded from the CTEP website (http://www.ctep.cancer.gov).

A 3 day window is allowed for scheduled therapy, tests and/or results except as noted below for CBC with differential and platelet count. Delays due to holidays, weekends, bad weather or other unforeseen circumstances will be permitted.

All dose reductions are permanent unless otherwise noted.

CBC with differential and platelet count must be drawn on day of or day before treatment (Day 1, Day 8, and Day 15), and results known prior to treatment administration.

The following adjustments in Table 3 should be made to the appropriate drug (depending on the attribution of toxicity) based on the dose reduction guidelines. In addition, please note:

If more than one toxicity requiring dose reduction occurs, use lowest dose level required. All dose reductions are permanent unless otherwise noted.

If multiple toxicities are seen, the dose administered in subsequent cycle should be based on the most severe toxicity experienced in the current cycle.

If a Grade 4 adverse event (AE) recurs after the dose has been reduced twice, the patient will be discontinued from the treatment unless, at the discretion of the Principal Investigator (PI), there is evidence of continuing benefit to the patient.

Patient's not experiencing resolution of their neutropenia in 28 days, despite uninterrupted Granulocyte-Colony Stimulating Factor (G-CSF) treatment, will be discontinued from treatment.

Day 1 Dose Missed: If the dose held or missed was to be given on Day 1 of the next cycle that next cycle will not be considered to start until the day the first dose is actually administered.

Day 8 and/or 15 Dose Missed: Held doses of either drug on Day 8 and/or Day 15 will be considered omitted. For example, if gemcitabine is held on Cycle 2, Day 8, the next dose of gemcitabine the following week will be considered Cycle 2, Day 15, rather than delaying the cycle and giving the day 8 dose. Another example, if the Cycle 3, Day 15 dose is held, the next dose would be on Day 28 or Cycle 4, Day 1 (Day 21 will remain the rest week and patient will remain on a 28 day schedule).

Regardless of any reason for holding the study treatment, the maximum allowable length of treatment interruption (between a missed scheduled dose and the next one) is 4 weeks.

For any toxicity (regardless of grade), despite optimal supportive care, that is felt by the treating investigator to represent a risk to the patient's safety, additional dose reduction, treatment delay, or treatment discontinuation is permitted at the discretion of the treating investigator.

If toxicity during treatment is predominantly attributed to nab-paclitaxel or gemcitabine, and dose reduction is also poorly tolerated, and it is determined that it is not in the best interest of the patient to continue combination therapy then therapy may continue with either drug alone upon approval from the study PI only.

Up to 3 dose level reductions of nab-paclitaxel and up to 2 dose level reductions of gemcitabine are allowed. Patients should be taken off study if further dose reduction is required.

Table 3: Dose Modification Schedule		
Nab-Paclitaxel		
Current Dose	**Percentage Decrease**	**Modified Dose**
125 mg/m^2	20%	100 mg/m^2
100 mg/m^2	20%	80 mg/m^2
80 mg/m^2	20%	65 mg/m^2
65 mg/m^2	100%	Discontinue
Gemcitabine		
Current Dose	**Percentage Decrease**	**Modified Dose**
1000 mg/m^2	20%	800 mg/m^2
800 mg/m^2	20%	640 mg/m^2
640 mg/m^2	100%	Discontinue

5.3.1 Day 1 of New Cycle Based on Worst Type of Toxicity Observed in the Prior Cycle

For any toxicity not mentioned in the table below, especially laboratory values of little or no clinical significance, dose reductions need not be done and will be at treating investigators' discretion or per institutional standards.

If more than one grade 4 toxicity is seen concurrently as a result of therapy (e.g., diarrhea and concurrent neutropenic fever) patients should be taken off treatment, unless continuation is approved by the PI.

Table 4: Day 1 Dose Modifications for Hematologic and Non-Hematologic Toxicity for Nab-Paclitaxel and Gemcitabine		
Hematologic Toxicity	**Dose Adjustment for Nab-Paclitaxel and/or Gemcitabine**	
ANC ≥ 1500/mm^3 **AND** **Platelets ≥ 100,000/mm^3**	Treat as scheduled	
ANC < 1500/mm^3 **OR/AND** **Platelets < 100,000/mm^3**	Hold both nab-paclitaxel and gemcitabine up to a maximum of 28 days until ANC ≥ 1500/mm^3 AND platelets ≥ 100,000/mm^3 then resume at next lower dose level as detailed in Table 3. If not resolved, then discontinue all treatment.	
Toxicity	**Nab-Paclitaxel**	**Gemcitabine**
Peripheral Neuropathy		
Grade 0 or 1	Maintain dose level	Maintain dose level
Grade 2	Decrease dose by 1 level	Maintain dose level
Grade 3	Hold for up to a maximum of 28 days until toxicity resolves to Grade ≤ 2, then resume at next lower dose level as detailed in Table 3. If not resolved by then, discontinue.	May continue

Grade 4	Discontinue	May continue
Cutaneous Toxicity		
Grade 0 or 1	Maintain dose level	Maintain dose level
Grade 2 or 3[a]	Decrease dose by 1 level	Decrease dose by 1 level
Grade 4	Discontinue	Discontinue
[a] If patient continues to experience these reactions, despite dose reduction, treatment should be discontinued.		
Diarrhea		
Grade 0, 1, or 2	Maintain dose level	Maintain dose level
Grade 3 or 4	Decrease dose by 1 level	Decrease dose by 1 level
Mucositis		
Grade 0, 1, or 2	Maintain dose level	Maintain dose level
Grade 3 or 4	Decrease dose by 1 level	Decrease dose by 1 level
Vomiting		
Grade 0 or 1	Maintain dose level	Maintain dose level
Grade 2 or 3	Maintain dose level; add antiemetics	Maintain dose level; add antiemetics
Grade 4	Decrease dose by 1 level; add antiemetics	Decrease dose by 1 level; add antiemetics
Hyperbilirubinemia		
Grade 0, 1 **Normal to ≤ 1.5x ULN**	Maintain dose level	Maintain dose level
Grade 2-4 **>1.5x ULN**	Hold both nab-paclitaxel and gemcitabine up to a maximum of 28 days until toxicity resolves to Grade ≤ 1, then resume at same dose as before. If not resolved, then discontinue all treatment.	
Other Non-Hematologic Toxicities- Dose reduce IF investigator deems appropriate and clinically necessary.		
Grade ≤ 2	Maintain dose level as Day 1 of previous cycle	
Grade 3 (except alopecia, nausea [unless maximally treated, including prophylaxis], pulmonary embolism and deep vein thrombosis [Section 5.3.4])	Hold both nab-paclitaxel and gemcitabine up to a maximum of 28 days until toxicity resolves to Grade ≤ 2, then resume at next lower dose level as detailed in Table 3. If not resolved, then discontinue all treatment.	
Grade 4 (except hyperbilirubinemia as above, if considered reversible through intervention)	Discontinue. Subjects may only continue treatment for transient Grade 4 toxicity after discussion with Sponsor.	

5.3.2 Day 8 and 15 of Each Cycle

Administer nab-paclitaxel and gemcitabine if the following criteria are met on day 8 and 15 of each cycle (Table 5 and 6).

Table 5: Day 8 and Day 15 Dose Modifications for Hematologic Toxicity for Nab-Paclitaxel and Gemcitabine					
***Note - Use table below by correlating Day 8 counts of current cycle, then choose Day 15 results from that row.**					
DAY 8			**DAY 15**		
Blood Counts	**Dose Adjustment for Nab-Paclitaxel**	**Dose Adjustment for Gemcitabine**	**Blood Counts**	**Dose Adjustment for Nab-Paclitaxel**	**Dose Adjustment for Gemcitabine**
ANC >1000/mm^3 AND Platelets ≥ 75,000/mm^3	No change in dose	No change in dose	**ANC >1000/mm^3 AND Platelets ≥ 75,000/mm^3**	No change	No change
			ANC 500-1000/mm^3 OR Platelets 50,000–74,999/mm^3	Decrease dose given on Day 8 by 1 level + consider GCSF (filgrastim)[a]	Decrease dose given on Day 8 by 1 level + consider GCSF (filgrastim)[a]
			ANC <500/mm^3 OR Platelets <50,000/mm^3	Hold treatment + consider GCSF (filgrastim)[a]	Hold treatment + consider GCSF (filgrastim)[a]
ANC >1000/mm^3 AND Platelets 50,000-74,999/mm^3	No change in dose	Decrease dose by 1 level	**ANC >1000/mm^3 AND Platelets ≥ 75,000/mm^3**	No change	Return to prior full dose
			ANC 500-1000/mm^3 OR Platelets 50,000–74,999/mm^3	Decrease dose given on Day 8 by 1 level + consider GCSF (filgrastim)[a]	Same dose as given on Day 8 + consider GCSF (filgrastim)[a]
			ANC <500/mm^3 OR Platelets <50,000/mm^3	Hold treatment + consider GCSF (filgrastim)[a]	Hold treatment + consider GCSF (filgrastim)[a]

Table 5: Day 8 and Day 15 Dose Modifications for Hematologic Toxicity for Nab-Paclitaxel and Gemcitabine

***Note - Use table below by correlating Day 8 counts of current cycle, then choose Day 15 results from that row.**

DAY 8			**DAY 15**		
Blood Counts	**Dose Adjustment for Nab-Paclitaxel**	**Dose Adjustment for Gemcitabine**	**Blood Counts**	**Dose Adjustment for Nab-Paclitaxel**	**Dose Adjustment for Gemcitabine**
ANC 500-1000/mm^3 AND/OR Platelets >50,000/mm^3	Decrease dose by 1 level	Decrease dose by 1 level	**ANC >1000/mm^3 AND Platelets ≥ 75,000/mm^3**	Return to prior full dose[a]	Return to prior full dose[a]
			ANC 500-1000/mm^3 OR Platelets 50,000–74,999/mm^3	Same dose as given on Day 8 + consider GCSF (filgrastim)[a]	Same dose as given on Day 8 + consider GCSF (filgrastim)[a]
			ANC <500/mm^3 OR Platelets <50,000/mm^3	Hold treatment + consider GCSF (filgrastim)[a]	Hold treatment + consider GCSF (filgrastim)[a]
ANC <500/mm^3 AND/OR Platelets <50,000/mm^3	Hold Treatment	Hold Treatment	**ANC >1000/mm^3 AND Platelets ≥ 75,000/mm^3**	Decrease dose given on Day 1 by 1 level[a]	Decrease dose given on Day 1 by 1 level[a]
			ANC 500-1000/mm^3 OR Platelets 50,000–74,999/mm^3	Decrease dose given on Day 1 by 1 level + consider GCSF (filgrastim)[a]	Decrease dose given on Day 1 by 1 level + consider GCSF (filgrastim)[a]
			ANC <500/mm^3 OR Platelets <50,000/mm^3	Hold + consider GCSF (filgrastim)[a]	Hold + consider GCSF (filgrastim)[a]

Any Day During Cycle Treatment Dose Modification		
	Nab-Paclitaxel	**Gemcitabine**
Febrile Neutropenia (Grade 3 or 4)	Hold treatment for a maximum 28 days. On resuming treatment decrease to next lower dose and do not re-escalate during treatment.	Hold treatment for a maximum 28 days. On resuming treatment decrease to next lower dose and do not re-escalate during treatment.

Table 5: Day 8 and Day 15 Dose Modifications for Hematologic Toxicity for Nab-Paclitaxel and Gemcitabine					
*Note - Use table below by correlating Day 8 counts of current cycle, then choose Day 15 results from that row.					
DAY 8			DAY 15		
Blood Counts	**Dose Adjustment for Nab-Paclitaxel**	**Dose Adjustment for Gemcitabine**	**Blood Counts**	**Dose Adjustment for Nab-Paclitaxel**	**Dose Adjustment for Gemcitabine**
Recurrent Febrile Neutropenia (Grade 3 or 4)	Decrease to next lower dose and do not re-escalate throughout the rest of the treatment.		Decrease 2 dose levels (to 640 mg/m^2) and do not re-escalate throughout the rest of the treatment.		

[a] G-CSF (filgrastim) is optional if descent affects platelets only. G-CSF use per institutional standards.

Table 6: Day 8 and Day 15 Dose Modifications for Non-Hematologic Toxicity for Nab-Paclitaxel and Gemcitabine	
Non-Hematologic Toxicity	**Dose Adjustment for Nab-Paclitaxel and/or Gemcitabine**
Grade ≤ 2 (includes peripheral neuropathy)	Treat as scheduled or at next lower dose level as detailed in Table 3.
Grade 2-4 Hyperbilirubinemia	Hold both nab-paclitaxel and gemcitabine up to a maximum of 28 days until toxicity improves to Grade ≤ 1, then resume at same dose as before. If toxicity has not improved, then discontinue all treatment.
Grade 3 Peripheral Neuropathy	Hold nab-paclitaxel dose until toxicity improves to Grade ≤ 2 until a maximum of 28 days, then resume treatment at same dose or next lower dose level, as detailed in Table 3. Continue gemcitabine dosing as scheduled.
Other Non-Hematologic Toxicities - Dose reduce IF the investigator deems appropriate and clinically necessary.	
All other Grade 3 (except alopecia, nausea or vomiting [unless maximally treated, including prophylaxis], pulmonary embolism and deep vein thrombosis [Section 5.3.4])	Hold both nab-paclitaxel and gemcitabine until toxicity improves to Grade ≤ 2 until a maximum of 28 days, then resume at next lower dose level as detailed in Table 3.
Grade 4 (except hyperbilirubinemia as above, if considered reversible through intervention)	Discontinue all treatment. Subjects may only continue treatment for transient Grade 4 toxicity after discussion with Sponsor.

5.3.3 Subjects with Abnormal Hepatic Function

Nab-paclitaxel and gemcitabine should only be administered if hepatic function is within the parameters as detailed above. Hepatic toxicity may occur but it is uncommon. Therefore, hepatic dysfunction that occurs while the subject is on study should prompt an evaluation to determine the cause, including the possibility of metastatic disease and hepatotoxicity from concurrent medications, alcohol use, or other factors.

5.3.4 Pulmonary Embolism and Deep-Vein Thrombosis

In the event of a pulmonary embolism or deep-vein thrombosis, subjects must be started on low molecular weight heparin or similar anticoagulation therapy to resume nab-paclitaxel and gemcitabine. Grade 4 events must be improved to Grade ≤ 3 within 21 days to continue study treatment.

5.3.5 Interstitial Pneumonitis

While participating in this study, subjects should be carefully monitored to prevent or minimize the occurrence of interstitial pneumonitis. Careful pre-study screening with continuous on-study monitoring for signs and symptoms is required. Should a subject develop symptoms of pneumonitis during this study, the timely initiation of appropriate management is required. Recommended guidelines are as follows:

1. Before enrollment, evaluate candidate subjects for familial, environmental, or occupational exposure to opportunistic pathogens, and do not enroll those with a history of slowly progressive dyspnea and unproductive cough, or of conditions such as sarcoidosis, silicosis, idiopathic pulmonary fibrosis, pulmonary hypersensitivity pneumonitis, or multiple allergies.
2. During study treatment, provide close attention to episodes of transient or repeated dyspnea with unproductive persistent cough or fever. Radiographic evaluation with chest x-rays and CT scans (normal or high resolution) may be indicated to evaluate for infiltrates, ground-glass opacities, or honeycombing patterns. Pulse oximetry and pulmonary function tests can show respiratory and ventilation compromise.
3. Infections should be ruled out with routine immunological/microbiological methods. Transbronchial lung biopsy is not recommended, given its limited value and risk of pneumothorax and hemorrhage, and should be reserved for cases with unclear etiology.
4. Administration of study treatment should be interrupted upon diagnosis of interstitial pneumonitis and subjects permanently discontinued from further study treatment. After ruling out an infectious etiology, intravenous high-dose corticosteroid therapy should be instituted without delay, with appropriate premedication and secondary pathogen coverage. Subjects with an added immunological agent may also require immune modulation with azathioprine or cyclophosphamide. Appropriate ventilation and oxygen support should be used when required.

5.3.6 Prophylaxis Against Sepsis

In the metastatic pancreatic cancer Phase 3 study (CA046), an increase in cases of non-neutropenic sepsis was observed with the combination of nab-paclitaxel and gemcitabine. An exploratory analysis suggested that the presence of biliary stents may have increased the risk of sepsis in that population. Investigators were to provide oral broad spectrum antibiotics to subjects who were then to initiate these antibiotics at the first occurrence of fever. Subjects enrolled in this clinical trial may not have the same risk of sepsis as metastatic pancreatic cancer patients. Subjects

should be advised that there could be an increased risk of serious infection and they should contact their physician for evaluation when they develop a fever. Fever or similar symptoms should be fully evaluated as an early sign of a serious infection. Broad spectrum antibiotics such as fluoroquinolones may be provided to subjects to treat or as prophylaxis for infection at the discretion of the treating physician.

5.3.7 Hypersensitivity Reactions

Hypersensitivity reactions are infrequent with nab-paclitaxel. If they do occur, minor symptoms such as flushing, skin reactions, dyspnea, hypotension, or tachycardia may require temporary interruption of the infusion. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema, or generalized urticaria require immediate discontinuation of nab-paclitaxel administration and aggressive symptomatic therapy.

Subjects who develop a severe hypersensitivity reaction to nab-paclitaxel should not be re-challenged.

5.4 Concurrent Therapies

5.4.1 Required and/or Permitted

The following concomitant medications or treatments are required and/or permitted as noted:

- Hormonal birth control is permitted while on study.
- Treatment with low molecular weight heparin, unfractionated heparin or other newer anticoagulants *is permitted* if patients are on a stable dose without evidence of clinically significant bleeding for at least 2 weeks prior to registration.
- Patients on Coumadin should be carefully monitored and dose adjusted if needed for their anticoagulation.
- Premedication with diphenhydramine 25-50 mg IV prior to study drug infusion is permitted.
- Treatment with additional H1 antagonist and an H2 antagonist (such as famotidine or ranitidine) is permitted if hypersensitivity symptoms develop, at the discretion of the treating investigator.
  - Patients who are receiving any medications or substances *known to affect* or with *potential to affect* the activity or pharmacokinetics of nab-paclitaxel and/or gemcitabine are discouraged but may be eligible per the discretion of the local investigator.

NOTE: Efforts should be made to switch patients who are taking medications or substances that are known to *inhibit* (e.g., ketoconazole and other imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or *induce* (e.g., rifampicin, carbamazepine, phenytoin, efavirenz, nevirapine) either CYP2C8 or CYP3A4 isoenzymes.

5.4.2 Not Permitted

- Other investigational agents.
- Immunosuppressive medications, including systemic corticosteroids (unless used for adrenal replacement, appetite stimulation, therapy for asthma, bronchitis exacerbation ≤ 2 weeks, to manage side-effects of chemotherapy, or pre-medication for procedures [i.e. CT scan]).
- No concurrent radiation

5.5 Supportive Care

5.5.1 All supportive measures consistent with optimal patient care will be given throughout the study.

5.5.2 The administration of hematopoietic colony stimulating factors and transfusion of blood products according to institutional practice is at the discretion of the investigator.

5.6 Duration of Therapy

Patients will receive protocol therapy unless:

1. Disease progression per RECIST Version.1.1 guidelines or clinical progression.
2. Toxicities considered unacceptable by either the patient or the investigator, despite optimal supportive care and dose modifications.
3. Development of an inter-current illness that prevents further administration of study treatment.
4. Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued.
5. Patient withdraws consent or is unable to comply with study procedures.

5.7 Duration of Follow-Up

Patients will be followed for adverse events for 30 days after their last dose of study medication.

After treatment discontinuation, follow-up for survival and initiation of any other anti-cancer therapies will be documented every 3-6 months for up to 2 years from treatment discontinuation or until death, whichever comes first or 3 years after first date of treatment initiation for those that remain on treatment. The study will end 2 years after the final patient discontinues treatment or after all patients are deceased, whichever comes first. If a patient continues to do well on the study drug combination, follow-up will cease 3 years after first date of treatment but patient may continue to receive the combination treatment from the local investigator.

If a patient is removed from treatment for reason(s) other than progression, follow with regular tumor assessments per standard of care until progression.

For patients who are registered but do not receive any protocol therapy, baseline and follow-up information per Section 8 will be collected.

5.8 Criteria for Removal from Study Treatment

A genuine effort will be made to determine the reason(s) why a patient fails to return for the necessary visits or is discontinued from the trial, should this occur. It will be documented whether or not each patient completed the clinical study. If for any patient study treatment or observations were discontinued, the reason will be recorded on the appropriate electronic case report form. Reasons that a patient may discontinue treatment in a clinical study are considered to constitute one of the following:

1. Recurrence of disease or documented progression of disease (unless continued treatment with study drug is deemed appropriate at the discretion of the investigator).
2. Intercurrent illness that prevents further administration of treatment per investigator discretion.
3. Unacceptable adverse events.
4. Treatment interruption of more than 4 weeks.

5. Investigator and/or patient discontinue nab-paclitaxel and/or gemcitabine.
6. Pregnancy.
7. Develops a second malignancy (except for non-melanoma skin cancer or cervical carcinoma in-situ) that requires treatment, which would interfere with this study.
8. The patient may choose to withdraw from the study at any time for any reason.
9. General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator.
10. Severe non-compliance to protocol as judged by the investigator.
11. Lost to follow-up.
12. Death.
13. For patients who continue to do well on the study drug combination, follow-up will cease 3 years after first date of treatment.
14. Closure of study by PrECOG.

Any patient who receives at least one dose of study drug (nab-paclitaxel or gemcitabine) will be included in the safety analysis. Patients who discontinue study treatment early should be followed for response assessments, if possible. Follow-up will continue per Section 8, as applicable.

5.9 Inevaluable Patients

Patients enrolled on the study and who did not receive even one dose of nab-paclitaxel and gemcitabine on the study will be considered inevaluable and additional patients will need to be enrolled.

6. Adverse Event Reporting

6.1 Collection of Safety Information

Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient administered a medicinal product in a clinical investigation and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (e.g., including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product (investigational or marketed), whether or not considered related to the product (investigational or marketed).

After informed consent, but prior to initiation of study treatment (nab-paclitaxel and gemcitabine), only AEs/SAEs caused by a protocol-mandated intervention will be collected (e.g., SAEs related to invasive procedures such as biopsies). After the initiation of study treatment, all identified AEs and SAEs must be recorded and described on the appropriate page of the electronic Case Report Form (eCRF). If known, the diagnosis of the underlying illness or disorder should be recorded, rather than individual symptoms. The following information should be documented for all AEs: date of onset and resolution, severity of the event; the investigator's opinion of the relationship to investigational product (see definitions below); treatment required for the AE treatment required for the AE; cause of the event (if known); and information regarding resolution/outcome.

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the eCRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more-frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5x the ULN associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event eCRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the eCRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7.0 mEq/L should be recorded as "hyperkalemia".

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the eCRF unless their severity, seriousness, or etiology changes.

Severity

The categories and definitions of severity used for clinical trials AEs are defined in the NCI's Common Terminology Criteria (CTCAE) V4.0 (http://www.ctep.cancer.gov).

Attribution

The following categories and definitions of causal relationship or attribution to study drug should be used to assess Adverse Events:

- Definite: There is a reasonable causal relationship between the study drug and the event. The event response to withdrawal of study drug (de-challenge) and recurs with re-challenge, if clinically feasible.
- Probable: There is a reasonable causal relationship between the study drug and the event. The event responds to de-challenge. Re-challenge is not required.
- Possible: There is a reasonable causal relationship between the study drug and the event. De-challenge information is lacking or unclear.

- Unlikely: There is doubtful causal relationship between the study drug and the event.
- Unrelated: There is clearly not a causal relationship between the study drug and the event or there is a causal relationship between another drug, concurrent disease, or circumstances and the event.

Categories 'definite', 'probable' and 'possible' are considered study drug related. Categories 'unlikely' and 'unrelated' are considered not study drug-related.

The development of a new cancer should be regarded as an AE. New cancers are those that are not the primary reason for administration of study treatment and have been identified after inclusion of the patient into the clinical study.

AEs related to nab-paclitaxel and/or gemcitabine should be followed for 30 days after last dose of study therapy (nab-paclitaxel and/or gemcitabine) until ≤ grade 1 or stabilization, and reported as SAEs if they become serious.

6.2 Handling of Serious Adverse Events (SAEs)

6.2.1 SAE Definitions

A ***serious AE*** is any untoward medical occurrence occurring after initiation of study treatment or that at any dose:

- results in death,
- is life-threatening (defined as an event in which the study patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or causes prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above).

Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

6.3 SAE Reporting Requirements

Serious adverse events (SAE) are defined above. The investigator should inform PrECOG of any SAE within 24 hours of being aware of the event. The date of awareness should be noted on the report. This must be documented on the PrECOG SAE form. This form must be completed and supplied to PrECOG within 24 hours/1 business day at the latest on the following working day. The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up PrECOG SAE report form. A final report to document resolution of the SAE is required. A copy of the fax transmission confirmation of the SAE report to PrECOG should be attached to the SAE and retained with the patient records.

All US SAEs should be faxed to:

888-537-9901

as per the instructions found in study materials provided to the investigator site.

Katherine M Smith, M.D.
Medical Monitor
During normal business hours
(8:30 am-5:00 pm EST):
Phone: 610-354-0404
After normal business hours:
Phone: 484-574-2367
Email: ksmith@precogllc.org

Manager, Clinical Safety
During normal business hours
(8:30 am-5:00 pm EST):
Phone: 610-354-0404
After normal business hours:
Cell: 484-574-2367

Reporting Requirements for European Site SAE and SUSAR (Suspected Unexpected Serious Adverse Reaction):

- SUSARs will be reported to EVCTM (Eudravigilance Clinical Trials Module), national competent authorities and ethics committees as per the requirements of the European clinical trial directive 2001/20/EC and 'CT-3' guidance.
- An annual development safety update report will be prepared by Ireland Cooperative Oncology Research Group (ICORG) and submitted to the competent authorities and concerned ethics committees of the European countries where the trial has been approved.

All European SAEs should be faxed to:

Mary Stapleton
Pharmacovigilance Manager
ICORG Pharmacovigilance Unit
+353 1 6697 869

ICORG will acknowledge receipt of the SAE within one business day by email to the Investigator and will forward SAEs to PrECOG within 24 hours of ICORG's Awareness Date.

PrECOG will notify the Celgene Corporation of all SAE's within 24 hours of PrECOG's Awareness Date as discussed above. Relevant follow-up information will be provided to Celgene as soon as it becomes available.

Investigators should also report event(s) to their IRB as required.

Collection of complete information concerning SAEs is extremely important. Full descriptions of each event will be followed. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report.

All SAEs, regardless of causality, must be collected which occur within 30 days of last dose of study treatment. This includes all deaths within 30 days of last dose of nab-paclitaxel and/or gemcitabine regardless of attribution. In addition, the Investigator should notify PrECOG or designee of any SAE that may occur after this time period which they believe to be definitely, probably or possibly related to investigational product.

NOTE: After study closure, study-drug related SAEs should be reported voluntarily by the treating physician to the manufacturer.

6.4 Reporting of Other Second Primary Cancers

New cancers are those that are not the primary reason for administration of study treatment and have been identified after inclusion of the patient into the clinical study.

All cases of new primary cancers that occur during or after protocol treatment must be reported to PrECOG on a Second Primary Cancer form within 30 days of diagnosis, regardless of relationship to protocol treatment. Secondary primary malignancies should also be reported as a SAE. The SAE form is not for use for reporting recurrence or development of metastatic disease. A copy of the pathology report, if applicable, should be sent, if available.

NOTE: Once data regarding survival and remission status are no longer required by the protocol, no follow-up data should be submitted.

6.5 Procedures in Case of Pregnancy

Prior to study enrollment, women of childbearing potential (WOCBP) and male patients with a female partner of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy, documented in the informed consent. In addition, all WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

Pregnancy of a female patient or the female partner of a male patient occurring while the patient is receiving study drug or within 3 months after the patient's last dose of study drug will be reported to PrECOG on a Pregnancy Form within 24 hours of the investigator's knowledge of the pregnancy.

All reports of congenital abnormalities/birth defects and spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth including health of the newborn or congenital abnormality) must be followed and documented on the Pregnancy Form even if the subject was discontinued from the study treatment. Should pregnancy occur during a subject's participation, the subject will immediately be discontinued from the treatment and followed per protocol.

The study-specific Pregnancy Form can be found in the Study Reference Manual.

7. Measurement of Effect

7.1 Primary Endpoint Assessment

PFS will be calculated in months from date of first dose of protocol therapy to date of documented disease progression or death from any cause, whichever comes first. PFS will be censored at the date of last disease evaluation for patients who have not experienced progression or death. Eligible patients who receive at least one dose of nab-paclitaxel and gemcitabine will be included in the analysis of PFS.

7.2 Secondary Endpoint Assessment

7.2.1 Clinical Efficacy

OS is defined as the time from enrollment until death or last patient contact, whichever comes first. A patient who dies will be considered to have experienced an event; otherwise the patient will be considered censored at last patient contact. PFS will be calculated in months from date of first dose of protocol therapy to date of documented disease progression or death from any cause, whichever comes first. TTP will be calculated in months from date of first dose of protocol therapy to date of removal from study for progression. For the patients removed from the study for reasons other than progression (such as toxicity or other reasons without clinical or radiographic evidence of tumor progression), disease status will continue to be followed per Section 8 and TTP will be censored at the date of last disease evaluation. Patients who withdraw consent prior to progression will be censored at their last disease evaluation prior to withdrawal (unless patient agrees to continued follow-up). Eligible patients who receive at least one dose of nab-paclitaxel and gemcitabine will be included in the analyses.

Stable disease (SD), complete response (CR), partial response (PR) and progressive disease (PD) will be defined as per the response evaluation criteria in solid tumors (RECIST) Version 1.1 guidelines (Section 7.4) to calculate ORR (PR + CR) and DCR (SD + PR + CR) [60].

Radiographic assessment will be completed with either CT chest/abdomen/pelvis with contrast or CT chest with contrast plus MR imaging abdomen and pelvis with and without contrast at baseline and every 8 weeks to evaluate response to treatment by RECIST Version 1.1 guidelines. MR scan is preferred and the imaging protocol for MR would be the typical institutional imaging protocol, with the following caveats:

1. Must include T1-weighted pre-contrast 3D GRE sequence with whole liver/whole tumor coverage.
2. Must include T1-weighted post-contrast 3D GRE with identical parameters and coverage to #1 above.
3. Must include T2-weighted FSE/SSFSE sequence with coverage as above.
4. Optional but encouraged: diffusion-weighted imaging with at least two b-values (encouraged b=0 and b=400 or b=800) and ADC maps.
5. Additional sequences per institutional protocol, including dynamic post-contrast, in/opposed phase, and others.
6. Must use extracellular contrast agent (no Eovist® or MultiHance® due to conflicting hepatocyte uptake mechanism) [47].

7.2.2 Safety Endpoints

A patient will be considered for safety endpoints if the patient is eligible and one of the following occurs: (1) receives one dose of protocol therapy; (2) death on protocol

therapy for a reason considered possibly, probably or likely related to protocol therapy; or (3) are removed from protocol therapy because of an adverse experience possibly, probably or likely related to one of the agents. Review will be performed by Data Safety and Monitoring Board (DSMB) approximately every 6 months. Refer to Section 10.2.1 for the Toxicity Monitoring Plan.

7.2.3 CA 19-9 Response

In patients with baseline carbohydrate antigen 19-9 (CA 19-9) ≥ 40 U/ml, CA 19-9 response will be measured by the percent change from baseline value to the value at the time of best CA 19-9 response. The proportion of patients with ≥ 50% decline from baseline will be measured and the median time to maximum change in CA 19-9 level will be calculated. The maximum change in CA 19-9 will be examined for association with absolute TTP, PFS, OS and ORR by log-rank test [16].

7.3 Exploratory Endpoint Assessment

7.3.1 Immunohistochemical Staining of CCA Tumors

Immunohistochemical staining of CCA tumors will be performed for SPARC, CDA and hENT1. The specimens will represent pre-treatment, de-identified diagnostic pathology specimens that have been obtained in the course of standard biopsy or surgery. Procurement of archived tissue, if available, from a previous diagnostic biopsy is mandatory for enrollment. If not available, this will not preclude participation in the trial, nor will additional biopsies be performed for research purposes only.

The difference in PFS, TTP, ORR, DCR and OS between the low- and high-‘antigen’ groups will be assessed by the log-rank test, and a multivariate Cox regression model will be used to assess the independent predictive power of the antigen levels. If there is limited tissue specimen availability, then we would prioritize immunohistochemical staining as (1) SPARC, (2) fibrosis, (3) CDA and (4) hENT1.

Table 7: SPARC, hENT1, CDA Scoring	
SPARC analysis by IHC [61] using anti-SPARC antibody ON1-1 (Invitrogen, Camarillo, CA)	
Scoring Criteria for Expression in Stromal Fibroblasts	
Intensity	0 (absent) 1+ (weak) 2+ (moderate) 3+ (strong)
Extent	≥ 25% <25%
Scoring Guide	
Low	No staining, or Only seen at 40x magnification in <25% of stroma
High (2+)	Staining seen at 10-20x magnification in >25% of stroma

High (3+)	Staining seen at 2-4x magnification in >50% of stroma
hENT1 analysis by IHC (method #1) [62] using anti-hENT1 antibody, SP120 (Ventana, Tucson, AZ)	
Scoring Guide for Expression in Tumoral Cells (membranous staining for hENT1)	
Low	>50% of tumor is negative for stain, or Unequivocal membrane staining visible only at 20x, 40x
High	>50% of tumor is stained, and Unequivocal membrane staining at 4-10x
hENT1 analysis by IHC (method #2) [63] using anti-hENT1 antibody, SP120 (Ventana, Tucson, AZ)	
Scoring Guide for Expression in Tumoral Cells (membranous staining for hENT1)	
Intensity	0 (absent) 1+ (weak) 2+ (moderate) 3+ (strong)
Extent of Staining	Percentage of tumor cells stained
*Scoring Guide using H score (= intensity * extent of staining)*	
Low	Mean H-score < Median H-score
High	Mean H-score > Median H-score
CDA analysis by IHC [63, 64} using anti-CDA antibody	
Scoring Criteria for Expression in Tumoral Cells (cytosolic staining for CDA)	
Intensity	0 (absent) 1+ (weak) 2+ (moderate) 3+ (strong)
Extent of Staining	Percentage of tumor cells stained
*Scoring Guide using H score (= intensity * extent of staining)*	
Low	Mean H-score < Median H-score
High	Mean H-score > Median H-score

7.3.2 Tumor Fibrosis Evaluation

Available tissue (as noted in Section 7.3.1) will be stained with trichrome to evaluate for fibrosis.

The degree of fibrosis will be visually estimated by a blinded gastrointestinal pathologist as per standard practice. A separate 3x2 contingency table will be

generated relating the grade of fibrosis (low, intermediate, high) with staining (low versus high) for each tumoral and stromal SPARC. Results of the contingency tables will indicate whether there is a direct or inverse relationship between fibrosis and staining level for SPARC. A Spearman correlation coefficient between fibrosis and SPARC staining will be calculated and tested for significance using a t-test with n-2 degrees of freedom.

7.3.3 Optional Specimens for Testing and Banking

Blood specimens (whole blood) will be requested from the patient to test for Circulating Tumor Cells [CTCs] (Section 11.0). Correlation of change in CTCs to ORR, DCR and median PFS, TTP and OS will be completed. Targeted gene expression analysis will be performed on CTCs for correlation with CCA pathology, PFS, TTP, ORR and OS.

In addition, blood specimens (including serum, plasma and buffy coat) as well as fixed left-over tissue specimens (Section 11.0) will be banked, when available, from all enrolled patients in this trial for possible future molecular, pharmacogenomic, and/or proteomic testing. Such testing would be indicated if there is a subset of patients with sustained radiographic response and/or prolonged disease control to suggest the existence of underlying predictive biomarkers. These specimens are optional and will be banked.

If the patient consents, blood specimens will be obtained prior to Cycle 1 Day 1, Cycle 1 Day 8, Cycle 3 Day 1, and Off Treatment.

7.4 Solid Tumor Response Criteria (RECIST Version 1.1)

7.4.1 Malignant Disease Evaluation

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline Version 1.1 [65]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in RECIST.

To assess objective response, it is necessary to estimate the overall tumor burden at baseline to which subsequent measurements will be compared. Measurable disease is defined by the presence of at least one measurable lesion.

All measurements should be recorded in metric notation by use of a ruler or calipers. The same method of assessment and the same technique should be used to characterize each identified lesion at baseline and during follow-up. All baseline evaluations should be performed as closely as possible to the beginning of treatment and **never more than four weeks** before registration.

The term evaluable in reference to measurability will not be used because it does not provide additional meaning or accuracy.

At baseline, tumor lesions will be characterized as either measurable or non-measurable.

7.4.1.1 Measurable

Measurable tumor lesions are those that can be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- ≥ 10 mm by CT scan (irrespective of scanner type) and MRI (*no less than double the slice thickness and a minimum of 10 mm*)
- ≥ 10 mm caliper measurement by clinical exam (when superficial)

- ≥ 20 mm by chest x-ray (if clearly defined and surrounded by aerated lung)

If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

7.4.1.2 Malignant Lymph Nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis (perpendicular to longest diameter) when assessed by CT scan.

7.4.1.3 Non-Measurable

All other lesions (or sites of disease), including small lesions not meeting the criteria in 7.4.1.1 and 7.4.1.2, are considered non-measurable lesions. This includes lymph nodes measured at ≥ 10 to <15 mm in the short axis. **NOTE:** Lymph nodes measured at <10 mm in the short axis are considered normal.

Lesions considered to be non-measurable include the following: leptomeningeal disease, ascites, pleural/pericardial effusions, inflammatory breast disease, lymphangitic involvement of the skin or lung, and abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

NOTE: ‘Cystic lesions’ that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

‘Cystic lesions’ thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lytic bone lesions, with an identifiable soft tissue component, evaluated by CT or MRI, can be considered as measurable lesions if the soft tissue component otherwise meets the definition of measurability in Section 7.4.1.1. Blastic bone lesions are non-measurable.

Tumor lesions that are situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

7.4.2 Definitions of Response

7.4.2.1 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 (those with the longest diameters), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

The sum of the target lesions (longest diameter for non-nodal lesions, short axis for nodal lesions) will be calculated and reported as the baseline sum. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum of the diameters/axes will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Complete Response (CR)

The disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (the sum may not be "0" if there are target nodes). To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed ≥ 4 weeks after the criteria for response are first met.

Partial Response (PR)

At least a 30% decrease in the sum of the diameters/axes of target lesions, taking as reference the baseline sum diameters/axes. To be assigned a status of partial response, changes in tumor measurements must be confirmed by repeat assessments performed ≥ 4 weeks after the criteria for response is met.

Progressive Disease (PD)

At least a 20% increase in the sum of the diameters/axes of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm over the nadir. (**NOTE:** the appearance of one or more new lesions is also considered progression).

Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters/axes while on study. (**NOTE:** a change of 20% or more that does not increase the sum of the diameters by 5 mm or more is coded as stable disease).

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of ≥ 4 weeks.

7.4.2.2 Non-Target Lesions

All other lesions or sites of disease including any measurable lesions over and above the 5 target lesions and lymph nodes measured at ≥ 10 to <15 mm in the short axis 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 unequivocal progression of each should be noted throughout follow-up.

Complete Response (CR)

The disappearance of all non-target lesions and normalization of tumor marker levels, if applicable. All lymph nodes must be non-pathological in size (<10 mm short axis). To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed ≥ 4 weeks after the criteria for response are first met.

NOTE: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD

The persistence of one or more non-target lesion(s) and/or the maintenance of tumor marker levels above the normal limits. To be assigned a status of Non-CR/Non-PD, measurements must have met the Non-CR/Non-PD criteria at least once after study entry at a minimum interval of ≥ 4 weeks.

Progressive Disease (PD)

The appearance of one or more new lesion(s) and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

When the patient also has measurable disease, there must be an overall level of substantial worsening in non-target disease such that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest "increase" in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient only has non-measurable disease, the increase in overall disease burden should be comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden from "trace" to "large", an increase in nodal disease from "localized" to "widespread", or an increase sufficient to require a change in therapy.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances.

7.4.3 Evaluation of New Lesions

The appearance of new lesions constitutes Progressive Disease (PD).

7.4.4 Symptomatic Deterioration

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.

7.5 Evaluation of Patient's Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence or non-protocol therapy (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).

To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed ≥ 4 weeks after the criteria for response are first met.

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of ≥ 4 weeks.

Table 8: Overall Response for All Possible Combinations of Tumor Response

Target Lesions	Non-Target Lesions	New Lesion	Overall Response	Remarks
CR	CR	No	CR	Confirmation at ≥ 4 weeks
CR	Non-CR/Non-PD*	No	PR	Confirmation at ≥ 4 weeks
CR	Not Evaluated	No	PR	Confirmation at ≥ 4 weeks
PR	Non-PD*/Not Evaluated	No	PR	Confirmation at ≥ 4 weeks
SD	Non-PD*/Not Evaluated	No	SD	Documented at least once ≥ 4 weeks from study entry
Not All Evaluated	Non-PD	No	Not Evaluable	
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD**	Yes or No	PD*	
Any	Any	Yes	PD	

* PD in non-target lesions should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Please refer to Section 7.4.2.2 Non-Target Lesions-Progressive Disease for further explanation.
** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

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. However, the patient will be considered inevaluable for survival analysis.

7.5.1 Methods of Measurement

Imaging based evaluation is preferred to evaluation by clinical examination. The same imaging modality should be used throughout the study to measure disease (preferred but not mandated).

7.5.1.1 Clinical Lesions

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm in diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

7.5.1.2 CXR

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

7.5.1.3 CT and MRI

This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

7.5.1.4 PET-CT

At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

7.5.1.5 Ultrasound

Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

7.5.1.6 Endoscopy, Laparoscopy

The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

7.5.1.7 Tumor Markers

Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

7.5.1.8 Cytology, Histology

These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions

in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

8. Study Parameters

1. All pre-study scans should be done ≤ 4 weeks prior to registration.
2. All other pre-study assessments should be done ≤ 2 weeks prior to registration.

Procedures	Screening	Cycle 1* (1 cycle=28 Days)				Cycle 2 and Subsequent Cycles*				Every 2 Cycles* (every 8 weeks)	Off Treatment[10]	Follow-Up[12]
		Day 1	Day 8	Day 15	Day 21 BREAK	Day 1	Day 8	Day 15	Day 21 BREAK			
Written Informed Consent	X											
Disease Characteristics[1]	X											
Medical/Surgical History	X											
Assessment of Baseline Signs & Symptoms	X											
Height	X											
Physical Exam including Weight	X	X	X			X					X	
Vital Signs (Temperature, Pulse, Blood Pressure)	X	X	X	X		X					X	
Body Surface Area (BSA)	X	X				X						
Performance Status	X	X				X					X	
CBC/Differential/Platelets[2]	X	X	X	X		X	X	X			X	
Chemistry[3]	X	X	X	X		X					X	
CA 19-9 & CEA	X									X[8]	X	
PT/INR	X											
Serum Pregnancy Test[4]	X											

Procedures	Screening	Cycle 1* (1 cycle=28 Days)				Cycle 2 and Subsequent Cycles*				Every 2 Cycles* (every 8 weeks)	Off Treatment[10]	Follow-Up[12]
		Day 1	Day 8	Day 15	Day 21 BREAK	Day 1	Day 8	Day 15	Day 21 BREAK			
MRI or CT (Abdomen & Pelvic) with Contrast	X									X[9]	X[10]	
Chest CT with Contrast	X									X[9]	X[10]	
Archived Tissue Procurement (Mandatory)[5]	X											
Research Blood Specimens (Optional)[6]		X	X							X[6]	X[6]	
Treatment Administration[7]		X	X	X		X	X	X				
Concomitant Medication Review	X	X	X	X		X	X	X			X	
Adverse Events Assessment		X	X	X		X	X	X			X[11]	
Survival Status												X

* **Scheduled Visits:** +/- 3 day window for therapy/tests/visits during therapy except as noted in footnote #2 for CBC with differential and platelet count. Delay due to holidays, weekends, bad weather or other unforeseen circumstances will be permitted.

1 Record date of diagnosis, primary tumor type, histology, stage.

2 CBC with differential and platelet count which includes WBC, ANC, Platelets, Hgb, and Hct. Required prior to each dose of chemotherapy (on day of or day before treatment on days 1, 8 and 15), and results known prior to treatment administration.

3 Albumin, BUN/creatinine, sodium, potassium, chloride, glucose, calcium, alkaline phosphatase, AST, ALT, total bilirubin, and total protein.

4 Required for females of child-bearing potential.

5 Pre-treatment, diagnostic pathology specimens obtained in the course of standard biopsy or surgery. Formalin-Fixed Paraffin-Embedded (FFPE) blocks or up to 15 FFPE slides plus H&E slide will be required. Procurement of tissue will be mandatory for enrollment, but if additional tissue from initial biopsy is not available, repeat biopsy will not be required. **Optional:** Any leftover tissue banked for future research. See Section 11.1 for details.

6 **Optional:** Collect one (1) 10 ml CellSave purple/yellow top tube (must be shipped within 72 hours of collection), one (1) 10 ml blood in red top tube and one (1) 6 ml EDTA tube prior to Cycle 1, Day 1, Cycle 1 Day 8, Cycle 3, Day 1 (only) and at Off Treatment. See Section 11.2 & 11.3 for details.

7 Patients will receive nab-paclitaxel and gemcitabine by IV infusion on days 1, 8, and 15 of each 28 day cycle (1 cycle=28 days). Patients may receive premedication with diphenhydramine (25-50 mg IV) 30 minutes prior to infusion. Nab-paclitaxel will be administered first, at a dose of 125 mg/m^2 IV over a period of 30 minutes; gemcitabine will be administered second, at a dose of 1000 mg/m^2 over a period of 30 minutes. See Section 5.2 for dosing instructions and Section 5.3 for dose delays/modifications.

8 Beginning with Cycle 3, every 8 weeks (after every even-numbered cycle and before initiation of every odd-numbered cycle).

9 Beginning with Cycle 3, radiographic response will be assessed with imaging every 8 weeks (after every even-numbered cycle and before initiation of every odd-numbered cycle).

10 Thirty (30) days +/- 7 days after last dose of chemotherapy. If more than 4 weeks since last scan, repeat MRI and/or CT. If patient is removed from treatment for reason(s) other than progression, follow with regular tumor assessments per standard of care until progression or start of new treatment.

11 Patients will be followed for adverse events for 30 days after their last dose of study medication.

12 Every 3-6 months for up to 2 years from treatment discontinuation or 3 years after first date of treatment for those who remain on treatment. Initiation of any other anti-cancer therapies will also be documented.

9. Drug Formulation and Procurement

9.1 Nab-Paclitaxel

9.1.1 Other Names

Abraxane®

9.1.2 Classification

Antimicrotubular, Cytotoxic

9.1.3 Mode of Action

Nab-paclitaxel is a microtubule inhibitor that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. Paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

9.1.4 Storage and Stability

Store the vials in original cartons at 20ºC to 25ºC (68ºF to 77ºF). Retain in the original package to protect from bright light. Unopened vials of nab-paclitaxel are stable until the date indicated on the package when stored between 20ºC to 25ºC (68ºF to 77ºF) [excursions permitted between 15ºC to 30ºC (59ºF to 86ºF)], in the original package. Neither freezing nor refrigeration adversely affects the stability of the product.

Stability of Reconstituted Suspension in the Vial
Reconstituted nab-paclitaxel in the vial should be used immediately, but may be refrigerated at 2ºC to 8ºC (36ºF to 46ºF) for a maximum of 8 hours if necessary. If not used immediately, each vial of reconstituted suspension should be replaced in the original carton to protect it from bright light. Discard any unused portion.

Stability of Reconstituted Suspension in the Infusion Bag
The suspension for infusion when prepared as recommended in an infusion bag should be used immediately but may be stored at ambient temperature (approximately 25ºC) and lighting conditions for up to 4 hours. Discard any unused portion.

9.1.5 Dose Specifics

Recommended dosage of nab-paclitaxel is 125 mg/m^2. Refer to Section 5.3 Dose Delays & Modifications for details.

9.1.6 Preparation

Nab-paclitaxel is a cytotoxic drug and, as with other potentially toxic paclitaxel compounds, caution should be exercised in handling nab-paclitaxel. The use of gloves is recommended. If nab-paclitaxel (lyophilized cake or reconstituted suspension) contacts the skin, wash the skin immediately and thoroughly with soap and water. Following topical exposure to paclitaxel, events may include tingling, burning and redness. If nab-paclitaxel contacts mucous membranes, the membranes should be flushed thoroughly with water.

Reconstitution of Nab-Paclitaxel

- Aseptically, reconstitute each vial of nab-paclitaxel by injecting 20 ml of 0.9% Sodium Chloride Injection, USP or equivalent into each vial.
- **Slowly** inject the 20 ml of 0.9% Sodium Chloride Injection, USP, over a minimum of 1 minute, using the sterile syringe directing the solution flow onto the **inside wall** of the vial.

- **DO NOT INJECT** the 0.9% Sodium Chloride Injection, USP directly onto the lyophilized cake as this will result in foaming.
- Once the injection is complete, allow the vial to sit for a **minimum of 5 minutes** to ensure proper wetting of the lyophilized cake/powder.
- **Gently** swirl and/or invert the vial **slowly** for at least **2 minutes** until complete dissolution of any cake/powder occurs. **Avoid** generation of foam.
- Each ml of reconstituted product will contain 5 mg of nab-paclitaxel.

1. The reconstituted suspension should be milky and homogeneous without visible particulates. If unsuspended powder is visible, the vial should be **gently** inverted again to ensure complete resuspension, prior to use.
2. Inject the calculated dosing volume of reconstituted nab-paclitaxel into an empty sterile, IV bag.
3. The use of in-line filters is not recommended.

Refer to Abraxane® Package Insert for full prescribing information.

9.1.7 Route of Administration

Recommended dosage of nab-paclitaxel is 125 mg/m^2 intravenously over 30 minutes on Days 1, 8, and 15 of each 28-day cycle and should precede administration of gemcitabine.

NOTE: It is not recommended to use filter needles in the preparation of, or in-line filters during the administration of nab-paclitaxel. In any event, filters of pore size less than 15 microns (15 µm) must not be used.

Following administration of nab-paclitaxel, the intravenous line should be flushed with sodium chloride 0.9% solution for injection to ensure administration of the complete dose, according to local practice.

Nab-paclitaxel will be reconstituted by appropriate study personnel. The Investigator will calculate the body surface area (BSA) of the patient in order to determine the total amount of nab-paclitaxel to be administered. BSA will be calculated on Cycle 1 Day 1 and recalculated per the site's standard of care, or if body weight changes by more than 10%. Actual heights and weights should be used to calculate surface areas (no downward adjustment to "ideal" weight). This principle applies to individuals whose calculated surface area is 2.2 m^2 or less. In those rare cases where a patient's surface area is greater than 2.2 m^2, the actual surface area or 2.2 may be used. Dosing BSA may be capped if the treating physician believes it is in the best interest of an obese patient.

9.1.8 Incompatibilities

Nab-paclitaxel should be prepared in normal saline ONLY and infusion should not be mixed with other chemicals.

9.1.9 Availability

Nab-paclitaxel will be supplied by Celgene Corporation.

For injectable suspension: lyophilized powder containing 100 mg of paclitaxel in single-use vial for reconstitution.

The initial supply of nab-paclitaxel will be sent directly to the site upon site activation. As needed, nab-paclitaxel may be requested by the PI (or their authorized designees) at each participating institution. The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, disposition, and return/destruction (site's drug destruction policy must be reviewed and approved by PrECOG before any study drug can be destroyed at a site) of nab-paclitaxel.

9.1.10 Agent Ordering

PrECOG will be responsible for ordering drug for re-supply to the site. Requests for shipments of nab-paclitaxel will be coordinated between PrECOG and Celgene.

9.1.11 Agent Accountability

Nab-paclitaxel will be stored in a secure location. Only authorized pharmacy and study staff will have access to this agent. Drug accountability will be performed by PrECOG.

9.1.12 Side Effects

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The most common adverse reactions (≥ 20%) of nab-paclitaxel in combination with gemcitabine for pancreatic cancer from the phase III MPACT trial are as listed in Table 9 [66]. Details of the adverse effects are as listed in the package insert.

Table 9: Toxicity Profile of Nab-Paclitaxel + Gemcitabine

Preferred Term	**Nab-Paclitaxel + Gemcitabine**
Grade ≥ 3 Hematologic AEs, %	
Neutropenia Leukopenia Thrombocytopenia Anemia	38 31 13 13
Patients who received growth factors, %	26
Febrile neutropenia, %	3
Grade ≥ 3 Non-Hematologic Treatment Related AEs in >5% of Patients, %	
Fatigue Peripheral neuropathy Diarrhea	17 17 6

In addition, patients are at increased risk for pneumonitis and sepsis.

Nab-paclitaxel is a Pregnancy Category D drug.

9.1.13 Nursing/Patient Implications

Use caution when handling cytotoxic drugs. Closely monitor the infusion site for extravasation and infiltration. Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration. Limiting the infusion of nab-paclitaxel to 30 minutes, as directed, reduces the likelihood of infusion-related reactions. Severe events such as phlebitis, cellulitis, induration, necrosis, and fibrosis have been reported as part of the continuing surveillance of paclitaxel injection safety. In some cases the onset of the injection site reaction in paclitaxel injection patients either occurred during a prolonged infusion or was delayed by a week to ten days. Recurrence of skin reactions at a site of previous extravasation following administration of paclitaxel injection at a different site, i.e., “recall”, has been reported.

Premedication with diphenhydramine (25-50 mg IV) 30 minutes prior to each infusion may be given. Severe and sometimes fatal hypersensitivity reactions have been reported with nab-paclitaxel. The use of nab-paclitaxel in patients who previously exhibited hypersensitivity to paclitaxel injection or human albumin has not been studied.

No reports of accidental exposure to nab-paclitaxel have been received. However, upon inhalation of paclitaxel, dyspnea, chest pain, burning eyes, sore throat, and nausea have been reported. Following topical exposure, events have included tingling, burning, and redness.

On an infusion rate basis, an overdose is defined as any rate faster than the protocol-specified rate. For nab-paclitaxel, an infusion completed in less than 25 minutes may increase Cmax by approximately 20%, therefore a nab-paclitaxel infusion completed in less than 25 minutes will meet the infusion rate criterion for an overdose.

There is no known antidote for nab-paclitaxel overdosage. The primary anticipated complications of overdosage would consist of bone marrow suppression, sensory neurotoxicity, and mucositis.

Refer to Abraxane® Package Insert for full prescribing information.

9.2 Gemcitabine

NOTE: Refer to commercial package insert for full prescribing information.

9.2.1 Other Names

Gemzar®

9.2.2 Classification

Gemcitabine (difluorodeoxycytidine) is a pyrimidine antimetabolite, which is an analogue of deoxycytidine. It was initially synthesized as a potential antiviral drug but selected for anticancer development because of its activity in *in-vivo* and *in vitro* tumors. **Gemcitabine is approved for the treatment of patients with cholangiocarcinoma and will be obtained commercially.**

9.2.3 Mode of Action

Gemcitabine kills cells undergoing DNA synthesis and blocks the progression of cells through the G1/S-phase boundary. Gemcitabine is metabolized by nucleoside kinases to diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. Gemcitabine diphosphate inhibits ribonucleotide reductase, an enzyme responsible for catalyzing the reactions that generate deoxynucleoside triphosphates for DNA synthesis, resulting in reductions in deoxynucleotide concentrations, including dCTP. Gemcitabine triphosphate competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP by the action of the diphosphate enhances the incorporation of gemcitabine triphosphate into DNA (self-potentiation). After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands, which eventually results in the initiation of apoptotic cell death.

9.2.4 Storage and Stability

Unopened vials of gemcitabine are stable until the expiration date indicated on the package when stored at controlled room temperature 20° to 25°C (68° to 77°F) [excursions permitted between 15ºC to 30ºC (59ºF to 86ºF)].

9.2.5 Dose Specifics

Recommended dosage of gemcitabine is 1000 mg/m^2. Refer to Section 5.3 Dose Delays and Modifications for details.

9.2.6 Preparation

The recommended diluent for reconstitution of gemcitabine is 0.9% Sodium Chloride Injection without preservatives.

To reconstitute, add 5 ml of 0.9% Sodium Chloride Injection to the 200-mg vial or 25 ml of 0.9% Sodium Chloride Injection to the 1-g vial. These dilutions each yield a gemcitabine concentration of 38 mg/ml, which includes accounting for the displacement volume of the lyophilized powder (0.26 ml for the 200-mg vial or 1.3 ml for the 1-g vial). The total volume upon reconstitution will be 5.26 ml or 26.3 ml, respectively. Complete withdrawal of the vial contents will provide 200 mg or 1 g of gemcitabine, respectively. The appropriate amount of drug may be administered as prepared or further diluted with 0.9% Sodium Chloride Injection to concentrations as low as 0.1 mg/ml.

Reconstituted gemcitabine is a clear, colorless to light straw-colored solution. Inspect visually for particulate matter and discoloration, prior to administration. If particulate matter or discoloration is found, do not administer.

When prepared as directed, gemcitabine solutions are stable for 24 hours at controlled room temperature 20° to 25°C (68° to 77°F). Discard unused portion. Solutions of reconstituted gemcitabine should not be refrigerated, as crystallization may occur.

Refer to commercial package insert for preparation and full prescribing information.

9.2.7 Route of Administration

Recommended dosage of gemcitabine is 1000 mg/m^2 intravenously over 30 minutes on Days 1, 8, and 15 of each 28-day cycle and should follow administration of nab-paclitaxel.

9.2.8 Incompatibilities

Drug incompatibilities of gemcitabine should be per the commercial package insert.

No incompatibilities have been observed with infusion bottles or polyvinyl chloride bags and administration sets.

9.2.9 Availability

Gemcitabine is approved for the treatment of patients with cholangiocarcinoma and will be obtained commercially.

Gemcitabine for injection, USP, is available in sterile single-use vials individually packaged in a carton containing:

- 200 mg white to off-white, lyophilized powder in sterile single-use vial
- 1 g white to off-white, lyophilized powder in sterile single-use vial

Gemcitabine disposal should be according to the manufacturer's recommendation and institutional procedures.

9.2.10 Side Effects

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Please see the commercial package insert for more details on the known precautions, warnings, and adverse reactions of gemcitabine.

Gemcitabine can suppress bone marrow function as manifested by leukopenia, thrombocytopenia and anemia, and myelosuppression is usually the dose-limiting toxicity. Patients should be monitored for myelosuppression during therapy.

Hemolytic-Uremic Syndrome (HUS) has been reported rarely with the use of gemcitabine.

Gemcitabine is a Pregnancy Category D drug. gemcitabine can cause fetal harm when administered to a pregnant woman. Gemcitabine is embryotoxic causing fetal malformations (cleft palate, incomplete ossification) at doses of 1.5 mg/kg/day in mice (about 1/200 the recommended human dose on mg/m^2 basis). Gemcitabine is fetotoxic causing fetal malformations (fused pulmonary artery, absence of gall bladder) at doses of 0.1 mg/kg/day in rabbits (about 1/600 the recommended human dose on mg/m^2 basis). Embryotoxicity was characterized by decreased fetal viability, reduced live litter sizes, and developmental delays. There are no studies of gemcitabine in pregnant women. If gemcitabine is used during pregnancy, or if the patient becomes pregnant while taking gemcitabine, the patient should be apprised of the potential hazard to the fetus.

The most common adverse reactions (≥ 20%) of nab-paclitaxel in combination with gemcitabine for pancreatic cancer from the Phase III MPACT trial [66] are as listed in Table 9.

9.2.11 Nursing/Patient Implications

1. Administer over 30 minutes.
2. If the patient reports burning at the injection site, slow down rate to allow the dose to run in over 1 hour.
3. Rash can be treated with topical therapy or the administration of diphenhydramine and dexamethasone prior to administration.
4. Flu-like symptoms can be treated with acetaminophen.

Overdosage: Myelosuppression, paresthesias, and severe rash were the main toxicities when a single IV infusion dose as high as 5700 mg/m^2 was administered over 30 minutes every 2 weeks in a dose-escalation study.

Please refer to the commercial package insert for full prescribing information.

10. Statistical Considerations

10.1 Study Design & Sample Size Considerations

Using a Simon two-stage design [67], a maximum of 70 patients will be enrolled to attain 67 eligible/evaluable patients (total target sample size has been inflated by 5% to allow for patient ineligibility/inevaluability [Section 5.9]). Patients will be followed until the minimum of the time to progression, time to death or six months if neither progression nor death is observed before the patient's data will be included in the analysis data set. In the first stage, 37 patients will be entered to have 35 evaluable patients. If 20 or fewer are alive and progression-free at 6 months, then the trial will be terminated due to lack of efficacy. If 21 or more are alive and progression-free at 6 months, then an additional 33 patients (to have 32 evaluable) will be enrolled. However, we will continue enrollment in the second stage without waiting for the 6-month follow-up of all patients enrolled in the first-stage, in the absence of significant grade 3 or higher toxicity. If 43 or fewer of 67 are alive and progression-free at 6 months, we will conclude that the 6 month true PFS rate could be as low as 55%. If 44 or more are alive and progression-free at 6 months, then we will conclude that the true 6 month PFS rate is at least 70%. This design has a 20% chance of falsely identifying the therapy is non-interesting when the true treatment success rate is 70% and a 5% chance of falsely concluding that the therapy is interesting when the true success rate is 55%. There is also greater than 0.66 probability that the study will terminate at the first stage if the null hypothesis is true. The baseline 6-month PFS probability is based on existing data from a Phase III cisplatin/gemcitabine combination trial (6-month PFS probability 59.3%) and a Phase II oxaliplatin/gemcitabine combination (4-month PFS probability 53%) trial [2, 30].

10.2 Planned Analyses

Analyses will be performed for all patients having received at least one dose of study drug after the first and second stage of the study. Distributions of PFS, TTP and OS will be estimated using the method of Kaplan-Meier [68] and univariate testing will be done via the log rank test [69]. Descriptive statistics will be performed for all on-study and clinical-demographic data. Chi-square or Fisher's exact tests will be used as appropriate for categorical associations and Wilcoxon rank tests or t-tests for continuous measures. All event times will be calculated relative to the start date of the study drug combination. ORR and DCR will be summarized by count and percent of subjects with each ordinal response analyzed by Cochran-Mantel-Hanzel test [70] for comparison between stratified groups. Correlation of change in CA 19-9 to PFS, TTP, ORR, DCR and OS will be completed as described above. Binary outcome regression modeling will be accomplished via standard logistic regression and time-to-event models (which will be largely exploratory) will be done using the Cox proportional hazards model. The safety data summaries will describe the incidence of adverse events (including severity and relationship to drug or treatment), serious adverse events and those leading to early withdrawal or death, as well as grade 3 or 4 toxicities. These analyses will be largely descriptive. Efficacy and safety analyses will be completed by intention to treat. Statistical analyses will be completed using SAS® Version 9.3.

10.2.1 Toxicity Monitoring Plan

In addition to continuous monitoring of adverse events through expedited reporting and routine tabulations of toxicities being reported through case report forms, the trial will include two formal evaluations of toxicity before the completion of accrual. It is expected that the true proportion of any grade 3 or higher treatment-related adverse event (exclusive of hematologic toxicity, nausea/vomiting, fatigue, alopecia and liver function abnormalities) will be no higher than 30% in this patient population (most events would be expected to have true rates quite lower than 30%). True proportions higher than 30% would be reason to evaluate the regimen for possible modification. Given these assumptions, the following plan will be followed. After 20 patients have been treated through at least 2 cycles, all treatment-related adverse event information will be compiled and tabulated by toxicity grade. If any observed grade 3 or higher

treatment-related adverse event proportion (exclusive of hematologic toxicity, nausea/vomiting, fatigue, alopecia and liver function abnormalities) is higher than 40% (8 or more out of 20 patients), a detailed review of all treatment-related adverse events will be conducted and the study team may decide that treatment modification is necessary. If the true proportion of any adverse event is 50% (or higher) there is at least 87% probability of exceeding the observed adverse event boundary whereas if the true proportion is 30% there is only 11% probability of crossing the toxicity boundary. The above decision boundary (40% or more observed events) will be followed for grade 4 or higher toxicities in the above exclusion list (hematologic toxicity, nausea/vomiting, fatigue, alopecia and liver function abnormalities) in order to evaluate those events that might be expected to have relatively high event rates at grade 3, but not at grade 4.

A similar analysis will be conducted at the completion of the first stage of accrual (37 patients for 35 evaluable patients) after patients have been treated through at least 2 cycles. Again, if the observed proportion of any grade 3 or higher treatment-related adverse event (exclusive of hematologic toxicity, nausea/vomiting, fatigue, alopecia and liver function abnormalities) is 40% or higher (14 or more out of 35 eligible/evaluable patients), a detailed review of adverse events will be conducted with a possible recommendation of the study team to modify the treatment regimen. The above boundary (40% or more observed events) will be followed for grade 4 or higher toxicities in the above exclusion list (hematologic toxicity, nausea/vomiting, fatigue, alopecia and liver function abnormalities). The power for the toxicity analysis at the end of the first stage is 91% (using again an alternative true proportion of 50%) and the type I error is 14% under the null hypothesis of 30%. In order to maintain statistical power for detecting possibly excessive toxicity, no multiplicity adjustment will be used for testing adverse events. Assuming that the trial moves to full accrual of 67 eligible/evaluable patients, a 90% confidence interval on any true adverse event proportion will be no wider than 21 percentage points.

10.2.2 Analyses of Correlative Studies

Assuming the study moves to full accrual, it is expected that at least 90% of patients will provide tissue and at least that proportion of patients, probably higher, will provide blood samples. For purposes of addressing statistical considerations for the correlative studies (exploratory objectives listed in Section 2.3) we focus here on correlating stromal SPARC with objective tumor response as an example to frame the calculations. It is expected that roughly 30% of patients overall will achieve an objective response on treatment. It is further expected based on previous data (Section 1.7) that approximately 38% of patients will stain low (IHC score of 0) for the stromal SPARC assay, while 62% of patients will score high (IHC score of 1 or 2). Given these marginal parameters and the fact that high SPARC will likely be associated with a lower proportion of objective response, this correlative study will have at least 80% power to detect a difference in objective response rates of 36% (absolute difference) between high and low scoring patients (e.g., a response rate of 47% in low scoring patients versus 11% in high scoring patients). If the SPARC high staining rate is closer to 50%, power will be greater than 80%.

Other correlative studies that have positivity rates close to that of the SPARC analyses will have statistical power for correlating with response similar to that discussed above. If a biomarker is associated with a low positivity proportion (e.g., less than 20%) power will be limited and only relatively large differences in response would be detectable. Similarly, for the time-to-event endpoints of PFS, TTP and OS, power will be fairly limited. Log rank tests will be conducted to evaluate the markers for association with these clinical outcomes but these analyses are considered exploratory. For example, there is 80% power to detect an absolute difference of 25% PFS at 6 months (that is, 56% 6-month PFS in a poor prognostic marker group versus 80% PFS at 6 months in

a better prognostic marker group) using a two-sided 0.05 level log rank test to evaluate PFS. Power for OS will be more limited.

11. Laboratory and Pathology Correlative Studies

11.1 Correlative Studies: Mandatory Archival Tumor Tissue - SPARC, CDA, hENT1 IHC and Fibrosis Analysis

11.1.1 Overview

Tumor tissue specimens will represent pre-treatment, de-identified diagnostic pathology specimens that have been obtained in the course of standard biopsy or surgery. Formalin-fixed paraffin-embedded (FFPE) diagnostic tumor tissue block(s) or up to 15 FFPE slides plus H&E slide from a tumor tissue block will be required. Procurement of tissue will be mandatory for enrollment to complete correlative studies, but if additional tissue from initial biopsy is not available, then repeat biopsy will not be required. Any left-over tissue after assays are completed will be banked for future use (optional).

11.1.2 Assay Methodology for IHC

Immunohistochemical staining of CCA tumors will be performed for SPARC, CDA and hENT1 proteins using the following standard method at an investigator-approved laboratory at the University of Michigan.

Specimen Preparation: FFPE tissues are suitable for staining.

Deparaffinization: Deparaffinize slides using xylene or xylene alternative and graded alcohols. Place the slides in a rack, and perform the following washes:

- Xylene: 2 x 3 minutes
- Xylene 1:1 with 100% ethanol: 3 minutes
- 100% ethanol: 2 x 3 minutes
- 95% ethanol: 3 minutes
- 70 % ethanol: 3 minutes
- 50 % ethanol: 3 minutes
- Running cold tap water to rinse

Keep the slides in the tap water until ready to perform antigen retrieval. At no time from this point onwards should the slides be allowed to dry. Drying out may lead to non-specific antibody binding and therefore to high background staining.

Antibody Dilution: If using the concentrate format of this product, dilute the antibody 1:100. The dilutions are estimates; actual results may differ because of variability in methods and protocols.

Antigen Retrieval: Heat induced epitope retrieval method will be followed using microwave method.

- Add the appropriate antigen retrieval buffer to the microwaveable vessel.
- Remove the slides from the tap water and place them in the microwaveable vessel. Place the vessel inside the microwave and follow the specific microwave instruction.
- When complete remove the vessel and run cold tap water into it for 10 minutes. Use care with hot solution.

Primary Antibody Incubation: Incubate for 30 minutes at room temperature.

- SPARC (clone ON1-1; Invitrogen, Camarillo, CA) – 1:250 antibody dilution
- CDA (Abcam, Cambridge, MA) - 1:100 antibody dilution
- hENT1 (SP120 clone; Ventana, Tucson, AZ) – 1:100 antibody dilution

Slide Washing: Slides must be washed in between steps. Rinse slides with PBS/0.05% Tween.

Visualization: Detect the antibody as instructed by the instructions provided with the specific visualization system used.

Analysis: See Section 7.3.1, Table 7.

The difference in PFS, TTP, ORR, DCR and OS between the low- and high-‘antigen’ groups will be assessed by the log-rank test, and a multivariate Cox regression model will be used to assess the independent predictive power of the antigen levels. If there is limited tissue specimen availability, then immunohistochemical staining will be prioritized as (1) SPARC, (2) fibrosis, (3) CDA and (4) hENT1.

11.1.3 Assay Methodology for Trichrome Staining

Slides will be stained with trichrome to evaluate for fibrosis at an investigator-approved laboratory at the University of Michigan. The degree of fibrosis will be visually estimated by a blinded gastrointestinal pathologist as per standard practice.

A separate 3x2 contingency table will be generated relating the grade of fibrosis (low, intermediate, high) with staining (low versus high) for each tumoral and stromal SPARC. Results of the contingency tables will indicate whether there is a direct or inverse relationship between fibrosis and staining level for SPARC. A Spearman correlation coefficient between fibrosis and SPARC staining will be calculated and tested for significance using a t-test with n-2 degrees of freedom.

11.1.4 Pathology Sample Processing and Shipment

Sites should submit FFPE diagnosis tumor tissue blocks or up to 15 FFPE slides (2 sections per slide) plus H&E slide from a tumor tissue block within 3 months of patient registration. Thickness of the sections should be at 4-5 micron.

A copy of the pathology report from initial diagnosis and/or subsequent tumor sampling should be sent when the sample is shipped. Samples should be shipped **Monday-Thursday**. Samples will be shipped ambient via overnight courier.

All samples collected will be labeled with a unique numeric identifier that will be coded for patient privacy protection.

Kits will be supplied. Instructions and shipping address will be provided.

11.2 Correlative Studies: Optional Peripheral Blood - Circulating Tumor Cell (CTC)

11.2.1 Overview

Optional whole blood specimens will be requested for CTCs using CellSave tubes and shipped to the lab within 72 hours of collection (Section 11.3). Correlation of change in CTCs to ORR, DCR and median PFS, TTP and OS will be completed. Targeted gene expression analysis will be performed on CTCs for correlation with CCA pathology, PFS, TTP, ORR and OS.

Research blood samples will be collected prior to Cycle 1, Day 1; Cycle 1, Day 8; Cycle 3, Day 1 and Off Treatment.

11.2.2 Methodology

CTCs from patient venous blood (1 ml) will be isolated using geometrically enhanced differential immunocapture (GEDI), as previously described [52]. GEDI was developed by Dr. Brian Kirby and has been shown to be effective for the capture of circulating prostate and pancreatic cancer cells [51, 53]. In brief, GEDI is a microfluidic platform that utilizes antibody-coated obstacles to capture rare-cells within blood. This allows for anticoagulated whole blood to be applied to the “chip” (size of a microscope slide) in the laboratory using standard syringe pumps. To capture CTCs, GEDI obstacles will be coated with an antibody specific to epithelial cell adhesion molecule (EpCAM), an epithelial cell-specific marker. After washes, captured cells will be stained with the nuclear marker DAPI and fluorescently labeled antibodies to cytokeratin (CK) 8, 18 and/or 19. Using fluorescence microscopy, CK8, 18 and/or 19+/DAPI+/CD45- EpCAM

captured cells with intact cellular morphology will be counted as CTCs by a blinded observer (Figure 5 in pancreatic cancer). Cells will be fixed and stored at -80°C for further analysis.

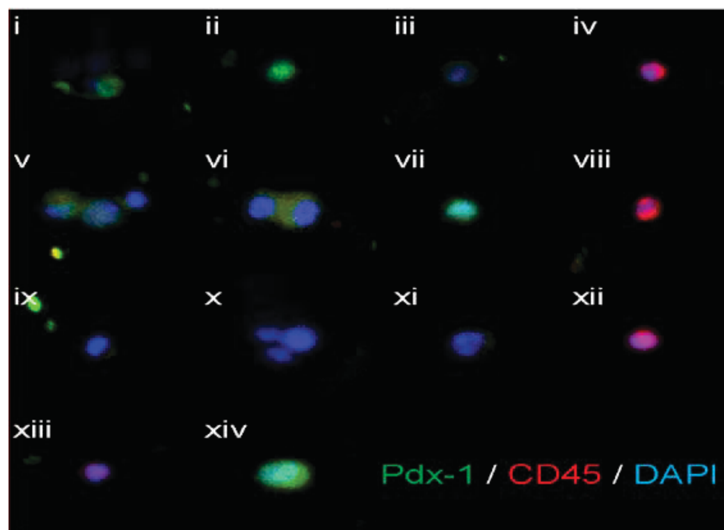


Figure 5. Individual Images of Nucleated Cells Captured on the GEDI Device. Cells were stained for CD45 (red), Pdx-1 (green) and nuclei (DAPI, blue). Cells in i, ii, vii, and xiv contain nuclear Pdx-1 staining, indicating pancreas origin of Circulating Endothelial Cells (CECs). Cells in iv, viii, xii, and xiii are CD45+ leukocytes captured by GEDI but were excluded from analysis.

We will use the RainDrop Picodroplet Digital PCR platform (RainDance Technologies, Billerica, MA) to identify specific gene mutations in the CTCs isolated on the GEDI chip. This microfluidic device partitions samples into 5 picoliter (pl) droplets containing PCR components, resulting in conditions that allow for sensitive and efficient amplification. This platform also features a droplet fluorescence detection device that enables multiplex PCR assays. These assays are highly reproducible [71], sensitive (1 mutant in 2,000,000), and have low false positive rate (5 droplets/sample) [71]. A targeted sequencing protocol (UDT-seq) was developed featuring picodroplet amplification, enabling for analysis of low input samples [72]. Protocols developed by Rhim laboratory, University of Michigan have allowed us to sequence Kras codon 12 from as few as 100 GEDI-captured pancreas cells [Unpublished Data]. We will directly quantify the presence of individual genetic mutations, including Kras codon 12, FGFR2, BRAF, ROS1, EGFR, Her-2/neu, c-MET, c-MYC, PIK3CA, p53, SMAD4, APC and IDH 1/2 mutations in validated picodroplet digital droplet PCR assays using the Rain Drop platform (owned by Rhim laboratory, University of Michigan). We will determine the presence and prevalence (compared to total number of WT PCR transcripts or reads) of mutations in each sample. Mutation detection, identification, filtering and annotation will be made as described before [72]. We will compare samples to determine if CTCs from patients with CCA with a specific pathology are enriched for that individual or for multiple mutations, as well as correlate presence and prevalence of these specific genetic mutations to patient survival and response to chemotherapy. Moreover, we will perform an exploratory analysis to identify change in specific genetic signatures on progression compared with the baseline signature. This will provide a rich repository of mutations that can be targeted in the second line setting to drive responsible development of future clinical trials in this rare cancer. The CTC analysis will not be done in real time and the data will not available to the treating physicians or patients to make clinical decisions.

11.3 Correlative Studies: Optional Specimens for Testing and Banking

A blood specimen (whole blood) will be requested from the patient to test for Circulating Tumor Cells (CTC) (Section 11.2).

In addition, patient blood specimens (serum, plasma and buffy coat) as well as fixed left-over tissue specimens will be banked, when available, from all enrolled patients in this trial for possible future molecular, pharmacogenomic, and/or proteomic testing. Such testing would be indicated if there is a subset of patients with sustained radiographic response and/or prolonged disease control to suggest the existence of underlying predictive biomarkers. The specimens will be banked.

11.3.1 Research Blood Sample Collection

Research blood samples will be collected prior to Cycle 1, Day 1; Cycle 1, Day 8; Cycle 3, Day 1 and Off Treatment.

Whole blood will be collected in one (1) 10 ml CellSave purple/yellow top tube, one (1) 10 ml clotted red top tube for serum and one (1) 6 ml EDTA tube for plasma and buffy coat.

All samples collected will be labeled with a unique numeric identifier that will be coded for patient privacy protection.

Kits and instructions will be supplied.

11.3.2 Research Blood Sample Processing and Shipment

CellSave Purple/Yellow Top Tube for CTC Testing (Whole Blood)

Gently mix the blood sample by inversion 5 times (do not shake). Protect the specimen from light and store at room temperature.

CellSave preservative tube should be shipped to the lab within 72 hours of collection via overnight courier at room temperature. **NOTE:** Samples may be shipped on Friday for Monday delivery.

Shipping labels, supplies and address will be provided.

Clotted Red Top Tube Processing for Serum

Gently mix the blood sample by inversion 5 times (do not shake). Allow the sample to sit at room temperature for 30-60 minutes until a clot has formed. If the blood is not centrifuged immediately after the clotting time (30 to 60 minutes at room temperature), the tubes should be refrigerated (4ºC) for no longer than 4 hours.

Once the clot has formed, the sample is ready for centrifugation. Centrifuge for 15 minutes at room temperature at 1200 RPM. Aliquot and store the resulting serum into two (2) properly labeled polypropylene tubes. Be careful to not disturb the clot. Store the samples in the freezer at ≤ -70°C or colder until they are shipped.

EDTA Tube Processing for Plasma and Buffy Coat

****Process sample within 30 minutes of collection****

- Gently mix blood sample by inversion 10 times (do not shake).
- Place tube immediately on wet ice for 5 minutes.
- Centrifuge at 1200 RPM for 15 minutes at 4°C. If a refrigerated centrifuge is not available, spin sample at room temperature (1200 RPM for 15 minutes). Immediately place the tube on wet ice after centrifugation.

After centrifugation, the plasma layer will be at the top half of the tube. The nucleated cells (WBC) will be in a whitish layer, called the “buffy coat”, just under the plasma and above the red blood cells.

Plasma Preparation:

- Using a transfer pipette take the top two-thirds of the plasma and transfer plasma into a 15 ml conical centrifuge tube, be careful not to disturb the buffy coat layer in the EDTA tube (**NOTE:** see below for buffy coat processing instructions). Centrifuge the 15 ml conical tube at 1200 RPM for 15 minutes at 4°C. If a refrigerated centrifuge is not available, spin sample at room temperature (1200 RPM for 15 minutes). Immediately place the conical tube on wet ice after centrifugation.
- Transfer equal amounts of plasma into two (2) properly labeled polypropylene tubes for cryopreservation being careful not to disturb the small PBMC/pellet.
- Store the two aliquots of plasma samples in the freezer at ≤ -70°C or colder until they are shipped to central lab for banking.

Buffy Coat Preparation:

- From the EDTA tube remove and aliquot the “buffy coat”; be careful not to disturb the layer of red blood cells.
- Store the aliquot of cells in one (1) properly labeled polypropylene tube for cryopreservation.
- Store the sample in the freezer at ≤ -70°C or colder until it is shipped to central lab for banking.

Serum, plasma and buffy coat samples should be batched together and shipped approximately every 3 months. Individual patients should only be included in the shipment if all of their samples have been completed.

Samples should be shipped **Monday-Thursday**. Samples must be shipped on dry ice via overnight courier. Shipping labels, supplies and address will be provided.

12. Administrative

12.1 Protocol Compliance

The study shall be conducted as described in this protocol. All revisions to the protocol must be discussed with, and be prepared by PrECOG and/or representatives. The Investigator should not implement any deviation or change to the protocol or consent without prior review and documented approval from PrECOG and/or representatives and the Institutional Review Board (IRB) of an amendment, except where necessary to eliminate an immediate hazard(s) to study patients.

If a deviation or change to the approved protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB approval, notification will be submitted to the IRB for review and approval as soon as possible afterward. Documentation of approval signed by the chairperson or designee of the IRB(s) should be in the study records. If PrECOG and/or representatives provides an amendment that substantially alters the study design or increases the potential risk to the patient; the consent form must be revised and submitted to the IRB(s) for review and approval; the revised form must be used to obtain consent from patients currently enrolled in the study if they are affected by the Amendment; and the new form must be used to obtain consent from new patients prior to study entry. Information as to who investigators should send correspondence will be provided in additional study documents.

12.2 Institutional Review Board

Before study initiation, the Investigator must have written and dated approval from their respective IRB for the protocol, consent form, patient recruitment materials/process and any other written information to be provided to patients. The Investigator should also provide the IRB with a copy of the Investigator Brochure or product labeling, and any updates.

The Investigator should provide the IRB with reports, updates, and other information (e.g., Safety Updates, amendments, and administrative letters) according to regulatory requirements, IRB or study site procedures.

12.3 Informed Consent Procedures

Investigators must ensure that patients who volunteer for clinical trials or their legally acceptable representative are clearly and fully informed about the purpose, potential risks and other information.

A protocol specific informed consent form (ICF) template will be provided to sites. Preparation of the site-specific consent form is the responsibility of the site Investigator and must include all applicable regulatory and IRB requirements, and must adhere to Good Clinical Practices (GCP) and to the ethical principles that have their origin in the Declaration of Helsinki. All changes to the ICF template will be approved by PrECOG and/or their representatives prior to implementation.

In accordance with the Health Information Portability and Accountability Act (HIPAA), the consent process will also include written authorization by patients to release medical information to allow PrECOG and/or its agents, regulatory authorities, and the IRB of record at the study site for access to patient records and medical information relevant to the study, including the medical history. This will be documented in the informed consent form or other approved form obtained at the time of informed consent per institutional policies. This form should also be submitted to PrECOG and/or its agents for review prior to its implementation.

The Investigator must provide the patient or legally acceptable representative with a copy of the consent form and written information about the study in the language in which the patient is most proficient. The language must be non-technical and easily understood. The Investigator should allow time necessary for patient or patient's legally acceptable representative to inquire about the details of the study, then informed consent must be signed and personally dated by the patient or the patient's legally acceptable representative and by the person who conducted

the informed consent discussion. The patient or legally acceptable representative should receive a copy of the signed informed consent and any other written information provided to study patients prior to patient's participation in the trial. The investigator is responsible for assuring adequate documentation of this process and for storage and maintenance of the original signed consent form for each patient/subject.

The informed consent and any other information provided to patients or the patient's legally acceptable representative, should be revised whenever important new information becomes available that is relevant to the patient's consent, and should receive IRB approval prior to use. The Investigator, or a person designated by the Investigator should inform the patient or the patient's legally acceptable representative of all pertinent aspects of the study and of any new information relevant to the patient's willingness to continue participation in the study. This communication should be documented in the patient record. During a patient's participation in the trial, any updates to the consent form and any updates to the written information will be provided to the patient.

12.4 Safety Communication

Investigators will be notified of all AEs that are serious, unexpected, and definitely, probably, or possibly related to the investigational product. Upon receiving such notices, the Investigator must review and retain the notice with the Investigator Brochure and submit a copy of this information to the IRB according to local regulations. The Investigator and IRB will determine if the informed consent requires revision. The Investigator should also comply with the IRB procedures for reporting any other safety information. All revisions should be submitted to PrECOG and/or agents for review.

12.5 Monitoring

Representatives and agents of PrECOG and, as applicable to the study, the manufacturer of investigational product must be allowed to visit all study site locations periodically to assess the data, quality and study integrity. The purpose of this visit is to review study records and directly compare them with source documents and discuss the conduct of the study with the Investigator, and verify that the facilities remain acceptable. Monitoring of drug accountability will also occur.

The study may be evaluated by other auditors and government inspectors who must be allowed access to electronic Case Report Forms (eCRFs), source documents and other study files. The Investigator must notify PrECOG of any scheduled visits by regulatory authorities, and submit copies of all reports. Information as to who investigators should notify of an audit or where to address questions will be provided in additional study materials.

12.6 Study Records

An Investigator is required to maintain adequate regulatory files with corresponding communication and approvals, accurate histories, observations and other data on each individual treated. Full details of required regulatory documents will be provided in additional study materials. Data reported on the eCRFs must be consistent with the source documents as part of the patient record.

The confidentiality of records that could identify patients must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

A study specific signature record will be maintained to document signatures and initials of all persons at a study site who are authorized to make entries and/or corrections on eCRFs as well as document other study-specific roles.

12.7 Electronic Case Report Form (eCRF) Information

Additional information regarding eCRF instructions, timelines for data entry/ submission and query completion can be found in supplemental materials provided to the site. Sites will be

expected to complete eCRFs as per the schedule provided and submit all relevant data as per the specified timelines. All items recorded on eCRFs must be found in source documents.

The completed eCRF must be promptly reviewed, electronically signed, and dated by the Principal Investigator.

Instructions for management of patients who do not receive any protocol therapy:

If a patient is registered and does not receive any assigned protocol treatment, baseline, Serious Adverse Event and follow-up data will still be entered and must be submitted according to the eCRF instructions. Document the reason for not starting protocol treatment on the appropriate electronic off treatment form.

12.8 Records Retention

FDA Regulations (21CFR 312.62) require clinical investigators to retain all trial-related documentation, including source documents for the periods described below for studies performed under a US Investigational New Drug (IND):

- two years after the FDA approves the marketing application, or
- two years after the FDA disapproves the application for the indication being studied, or
- two years after the FDA is notified by the sponsor of the discontinuation of trials and that an application will not be submitted.

The Investigator must retain investigational product disposition records, copies of eCRFs (or electronic files), and source documents for the maximum period required by applicable regulations and guidelines, or Institution procedures, whichever is longer. The Investigator must contact PrECOG and/or representatives prior to destroying any records associated with the study.

Information as to who investigators should contact for questions will be provided in additional study documents. PrECOG and/or representatives will notify the Investigator when the trial records for this study are no longer needed.

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Appendix I: Child-Pugh Score

Measure	1 point	2 points	3 points
Total Bilirubin mg/dl	<2	2-3	>3
Serum Albumin g/dl	>3.5	2.8-3.5	<2.8
PT Time • **Time** • **INR**	 1-3 <1.8	 4-6 1.8-2.3	 >6 >2.3
Ascites	Absent	Slight	Moderate to Severe
Hepatic Encephalopathy	None	Grade 1-2 (or suppressed with medication)	Grade 3-4 (or refractory)

Points	Class	One Year Survival	Two Year Survival
5-6	A	100%	85%
7-9	B	80%	60%
10-15	C	45%	35%

Source: *R.N.H. Pugh, I.M. Murray-Lyon, J.L. Dawson, M.C. Pietroni, Roger Williams. Transection of the esophagus for bleeding esophageal varices. British Journal of Surgery. Volume 60. Issue 8, pages 646-649, August 1973.*

Appendix II ECOG Performance Status

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

Source: Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair

Appendix III: Investigator's Statement

1. I have carefully read this protocol entitled "A Multi-Institutional, Single Arm, Two-Stage Phase II Trial of Nab-Paclitaxel and Gemcitabine for First-Line Treatment of Patients with Advanced or Metastatic Cholangiocarcinoma", **Version 3.0 dated 1/19/2016 (Protocol Number PrE0204)** and agree that it contains all the necessary information required to conduct the study. I agree to conduct the study as outlined in the protocol.
2. I agree to conduct this study according to the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, the principles of Good Clinical Practice (GCP) as described in 21 Code of Federal Regulations (CFR) and any applicable local requirements.
3. I understand that this trial and any subsequent changes to the trial will not be initiated without approval of the appropriate Institutional Review Board, and that all administrative requirements of the governing body of the institution will be complied with fully.
4. Informed written consent will be obtained from all participating patients in accordance with institutional and Food and Drug Administration (FDA) requirements as specified in Title 21, CFR, Part 50.
5. I understand that my signature on the electronic Case Report Form (eCRF) indicates that I have carefully reviewed each page and accept full responsibility for the contents thereof.
6. I understand that the information presented in this study protocol is confidential, and I hereby assure that no information based on the conduct of the study will be released without prior consent from PrECOG, LLC unless this requirement is superseded by the FDA.

Principal Investigator (PI):

PI Name: ______________________________

Site Name: ______________________________

Signature of PI: ______________________________

Date of Signature: ______________________________
MM DD YYYY