Neoadjuvant chemotherapy in patients with biliary tract carcinomas

A Nordic phase II study
Version 1.6 (15.06.15)

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Sponsors signature: ________________________________________________

Investigators signature: ____________________________________________
**STUDY SUMMARY**

<table>
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<th>Protocol title:</th>
<th>Preoperative chemotherapy in patients with biliary tract carcinomas (BTC)</th>
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<tr>
<td>Protocol version:</td>
<td>Version 1.5</td>
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</table>
| Principal investigators: | Bengt Isaksson  
Lars Lundell  
Michael Bau Mortensen  
Per Pfeiffer  
Magnus Rizell |
| Protocol Phase: | Phase II |
| Indication: | Resectable intra- or extrahepatic biliary tract carcinomas |
| Objectives: | Primary objective:  
1. 2 year survival for all patients starting chemotherapy  
Secondary objectives:  
2. R0 resection rate  
3. Response rate  
4. TTP/PFS  
5. OS  
6. Complication rate (according to Clavien at 1 month after surgery)  
7. In-hospital 30 and 90 days mortality |
| Study design: | Phase II (Simon two-stage)  
Neoadjuvant chemotherapy is administered for 3 months before surgery. The primary objective is 2 year survival but for calculation of number of patients R0 resection-rate will be used.  
The sample size is based on Simon’s two stages Mini-max design. This design ensures early study termination if there is insufficient effect.  
A resection rate less than 60% after 3 months of preoperative chemotherapy is not clinically acceptable. Assuming a significance level at 0.05 (α = 0.05) and a power at 90% (β = 0.10) it can be calculated, that 26 patients should be included in the first part of the study. The enrolment will continue until 26 patients have completed 3 months of chemotherapy and have been re-evaluated for resection by CT scan (and EUS, if available). If 15 or less out of the first 26 consecutive patients are being resected we will reject our hypotheses and close the study after the first stage of accrual. If 16 or more patients are resected, an additional 19 patients will be accrued in the second stage. If 32 out of 45 patients are resected, a true resection rate of
80% cannot be excluded, and we will conclude that the treatment is effective enough to continue with future studies.

To ensure 45 evaluable patients we will include a total of 50 patients.

**Planned sample size:** Maximum 50 patients recruited in order to obtain 45 evaluable patients (First stage 26; second stage 19 evaluable patients).

**Total number of centers:** Expected 5

**Selection criteria:**
- Histologically, cytologically or radiographically proven biliary carcinoma
- Resectable disease
- >18 years of age
- WHO performance status 0-1
- Adequate haematological, renal, and hepatic function
- No prior chemotherapy
- Written informed consent

**Study medication(s):** Chemotherapy administered for 3 months according to local guidelines, with a 2 drug regimen including platinum (cisplatin or oxaliplatin) and gemcitabine. 4 weeks (± 1 week) after resection patients will be evaluated for 2-3 months of adjuvant therapy depending on patients performance and histopathological report.

**Main parameters of safety:** Adverse events, graded according to the NCI CTC, version 4.0 pre-operatively, and according to Clavien classification postoperatively.

**Study procedures:** Patients are screened, and staged. If judged resectable at a hepatobiliary MDT, patients can be registered.

When a patient has consented to participate in this study, chemotherapy will start within 14 days from registration.

Patients will attend study specific visit at least every 4th week during chemotherapy and radiological evaluation will be performed after 12 weeks.

Before surgery patients will be re-evaluated regarding resectability at the surgical department (EUS and/or LAP and/or CT and/or MR and/or PET/CT).

After resection, 30 day complication rate will be gathered and a visit will take part 4-6 weeks postoperatively, where R0 resection rate is determined histopathologically.

4 weeks (± 1 week) after resection patients will be evaluated for 2-3 months of adjuvant therapy depending on patients performance and histopathological report.

Local relapse and systemic spread is radiologically controlled at 6, 12, 18 and 24 months after surgery. Survival status will be registered at 2 years.
### Informed Consent
Written informed consent must be obtained prior to the patient undergoing any study-specific procedures.

### Screening
Baseline screening includes:
- Informed consent
- Eligibility assessment
- Medical history
- WHO performance status
- Tumor assessment
- Concomitant treatments
- Physical examination
- Vital signs
- Pregnancy test
- Haematology
- Blood chemistry

### Assessments during chemotherapy

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<td>WHO performance status</td>
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<td>Adverse events according to CTC</td>
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<tr>
<td>Routine blood test for administration of chemotherapy</td>
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### Assessments during Post-Treatment Follow-up:

- At 30 days postoperative visit: WHO performance, concomitant treatment, physical exam, vital signs, haematology, blood chemistry, complication rate according to Clavien.
- At 6, 12, 18 and 24 months after surgery: radiological (CT scan) and clinical follow-up
- Chemotherapy use (number of courses and dose-intensity)
- End of study: survival, disease progression

### Analysis plan
When 26 patients have been evaluated, the decision whether to end or continue the study will be taken.

### Duration of the Study
It is expected that the first patients will be enrolled Q3 2015.
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1 BACKGROUND

Malignant tumors of the biliary tract (gallbladder and bile ducts) are rare, with approximately 700 new cases diagnosed annually in the Nordic Countries. Patients with biliary tract carcinomas (BTC) have a poor prognosis, with a 5-year survival rate less than 10%. Surgical resection offers a chance of cure but given the proximity to the the porta hepatis and the intra- and extrahepatic complex anatomy, fewer than 1/3 of patients are diagnosed with resectable disease. And even after seemingly radical resection, the relapse rates are very high. Because of the high rate of recurrence, a strategy with postoperative adjuvant radiotherapy and/or chemotherapy is logical and may improve long-term outcome.

However, data supporting adjuvant therapy are very limited and literature consists mainly of uncontrolled series, and even though many institutions recommend adjuvant therapy there is a lack of consensus regarding the optimal chemotherapy regimen.

A recent meta-analysis (Horgan et al, JCO 2012) found a non-significant improvement in overall survival with adjuvant therapy (radiotherapy alone, chemotherapy, or both) compared with surgery alone for the entire group of biliary tract cancers \( (p = 0.06) \). Horgan et al concluded that adjuvant therapy should be considered in prospective studies and that this conclusion adhered in part to the results of the only phase III randomized trial performed to date (Takada et al, Cancer 2002). The authors even recommended against a no-treatment arm in future randomized trials but this statement was questioned by others (Bariani et al, JCO 2012, comments).

Recent data in the metastatic setting seems to support the idea of peri-operative chemotherapy, since the chemo-sensitivity of combination therapy in BTC provided response rates around 30%, tumor control rates of 80-90% and OS of 9-12 months (Sharma et al, JCO 2010; Valle et al, NEJM 2010; Lee et al, Lancet Oncol 2012; Okusaka et al, BJC 2009).

Even though the tumor burden is much lower in patients who are candidates for neoadjuvant therapy, there is a still risk of 10 - 20% that the disease will progress during pre-operative chemotherapy and in some patients (around 10%) the disease may become non-resectable. However, it is expected that neoadjuvant therapy will increase the chance of cure and that this benefit will outweigh a possible harm.

1.1 DEFINITIONS

BTC are subdivided according to their anatomical location in:
- Intrahepatic BTC (C22.1)
- Perihilar BTC (from the cystic duct to second-order biliary ducts) (C24.0)
- Distal BTC (from the ampulla of Vater to the cystic duct) (C24.8, C24.9)
- Gallbladder cancer (C23.9)

For differential diagnostic reasons preoperative identification of true CCC within the Ampulla of Vater is considered impossible.

1.2 DIAGNOSIS

Preoperative biopsy verification of BTC may be impossible due to the nature and/or the location of the tumor. Percutane biopsies should be avoided, and EUS-FNA should only be used in selected cases where the needle track is part of the planned resection.

The diagnostic strategy may vary between institutions, but the following diagnostic procedures should be considered (Razumilava et al, CGH 2013):

1.2.1 Intrahepatic BTC

CT/MRI identified mass in patient without history of/or suspected extrahepatic primary cancer. HCC should be excluded based on patient history, biomarkers and CT (e.g. no wash out of contrast during venous phase).

Resectability assessment: Only single lesions, but local resectability follows standard recommendations for hepatic lesions (segments/lobe resection). No extrahepatic lesions.

TNM stage (if possible): AJCC/UICC , 7th edition (intrahepatic BTC follows the TNM staging system for primary hepatic tumors)

Biomarker(s): serum CA 19-9, AFP, and CEA

1.2.2 Perihilar BTC (Cholangiocarcinomas)

90% have painless jaundice and 50% have systemic symptoms.

Bile duct dilatation with or without associated mass on CT (three-phased) and/or MRI/MRCP (3D liver acq.) and MR angio

ERC with biopsy/brushing (+/- Fish), perhaps SpyGlass,

If not possible: PTC (+ biopsy)

EUS (EUS-FNA in selected cases)

TNM stage (if possible): AJCC/UICC , 7th edition.
Resectability assessment: The UK and Rochester resection guidelines and the 2011 classification are used to ensure an uniformed approach to resectability assessment (Malhi & Gores, Hepatol 2006; DeOliveira et al, Hepatology 2011; Khan et al, Gut 2002).

- Type I and II: En-bloc resection of the extrahepatic bile ducts and gall bladder, regional lymphadenectomy and R-en-Y reconstruction
- Type III: As above but including right or left hepatectomy
- Type IV: As above but including extended right or left hepatectomy
- Segment 1 liver resection must be considered in type II-IV
- “Unilobar disease is considered resectable, even with ipsilateral encasement of the hepatic artery or portal vein branch, and/or involvement of ipsilateral secondary biliary radicals with associated lobar atrophy”
- “Bilobar involvement precludes curative surgery, as both the right and left lobes of the liver cannot be removed. This can manifest as bilateral portal vein branch involvement, involvement of the main portal vein, and bilateral involvement of secondary biliary radicals. Furthermore, unilobar involvement or atrophy with contralateral vascular involvement also represents disease advanced beyond resectability”

Biomarker(s): serum CA 19-9, serum IgG4

A false positive diagnosis of perihilar BTC will occur in 10-12% of the cases when following this diagnostic approach (Lundell & Mortensen, private communication).

1.2.3 Distal BTC
(Ampullary/peri-ampullary lesions should be excluded)

EUS (FNA from the duodenum/antrum)

Bile duct dilatation with or without associated mass on CT and/or MRI/MRCP

ERC with biopsy/brushing (+/- Fish),

If not possible: PTC (+ biopsy)

Resectability: Managed by pancreaticoduodenectomy with standart lymph node dissection.

TNM stage (if possible): AJCC/UICC, 7th edition.

Biomarker(s): serum CA 19-9, serum IgG4

1.2.4 Gallbladder cancer

CT/MRI

EUS
Resectability: Local resectability follows standard recommendations.

TNM stage (if possible): AJCC/UICC, 7th edition.

2 STUDY RATIONALE

There is definitely a need for improved outcome in patients with resectable BTC.

During the last decade, our therapeutic tools for dealing with BTC have gradually improved. Several new chemotherapeutic agents with activity against gastro-intestinal tumours have entered the market. The aim of this study is primarily to demonstrate that neoadjuvant therapy is effective and that combination chemotherapy can be safely administered in patients with resectable BTC.

- Many patients develop recurrence even after R0 resection.
- Adjuvant chemotherapy increase the chance of cure after R0 resection of adenocarcinoma in most cases of the gastrointestinal tract (CRC, gastric cancer, pancreatic cancer)
- Adjuvant chemotherapy may add to survival after R0 resection for BTC.
- Neoadjuvant therapy may be more effective than adjuvant therapy as seen in many adenocarcinomas of the GI tract (e.g. rectal cancer, gastric cancer)
- Combination chemotherapy prolongs survival in patients with metastatic BTC.

3 AIM OF THE STUDY

The overall and long term objectives are to improve outcome for patients with BTC — carcinomas of the gallbladder, intrahepatic, perihilar, or distal bile ducts. (ICD-10 C22.1, C23, C24.0, C24.8, C24.9).

Standard therapy is initial surgery in patients with resectable BTC but there is increasingly evidence that neoadjuvant therapy (oncologic therapy before surgery in patients with resectable disease) can increase long-term outcome, but there is also a risk of progression during neoadjuvant therapy and a risk of increased surgical morbidity after pre-operative therapy.

3.1 Primary endpoint

2 year survival for all patients starting chemotherapy
3.2 Secondary endpoints
Surgical morbidity and mortality
Number of patients with progression during chemotherapy
Number of patients with R0 resection
Acute and late toxicity
Search for predictive factors (tumour-markers).

4 STUDY DESIGN
This is an open multicenter phase II trial.

5 STUDY POPULATION
Inclusion and exclusion criterias
- Biliary tract carcinoma (ICD-10: C22.1, C23, C24.0, C24.8, C24.9).
  A. Cytologically or histologically verified or
  B. Clinical/radiologically as defined in section 1.2
- BTC which is considered to be resectable by the treating surgeon i.e. T1-3N0-1M0.
  The patient must be evaluated at the local HBP-MDT conference.
- The patient is operable (i.e. no co-morbidity which can preclude anaesthesia or surgery).
- No sign of M1 disease.
- WHO performance status 0-1.
- Age ≥ 18 years.
- Adequate haematological, renal, and hepatic function: WBC > 3.0 x 10^9/l, platelets > 100 x 10^9/l, creatinine <1.5 x UNL (Upper normal limit), bilirubin <3.0 x UNL, PP % 0,5 – 1,3, APTT < 1,5 x UNL
- Patients with obstruction of bile duct or gut must be drained before start of therapy.
- Oral and written informed consent must be obtained prior to registration with planned date of first treatment within 14 days from registration.
- No prior radiotherapy to abdominal cavity.
- No pregnancy or breast-feeding. Fertile patients must use adequate contraceptives.
- No sign of other severe uncontrolled concomitant illness (e.g. clinically significant cardiac disease or myocardial infarction within 12 months).
- No prior chemotherapy for BTC.
- No prior chemotherapy or other oncologic therapy within 12 months.
- No contraindications towards Oxaliplatin, Gemcitabine and/or Cisplatin, respectively (see Appendix 2)

6 NUMBER OF PATIENTS & STATISTICS
To ensure 45 evaluable patients we will include a total of 50 patients.

6.1 Definition of populations to be analyzed
**Intention-to-treat-population:** consist of all eligible patients who received at least one course of chemotherapy.

**Eligible population:** Consist of the patients who do not have major deviations from inclusion and exclusion criterias.

6.2 Statistical methods
Non-parametric statistical methods will be used.

PFS and OS will be analyzed in the intention-to-treat population and will be estimated using Kaplan-Meier’s.

Toxicities will be graded using the NCI CTC, version 4.0.

Response rate will be calculated in patients with measurable disease according to RECIST.

7 STUDY PROCEDURES
7.1 Pretreatment work-up
The investigator will perform clinical and laboratory assessments to confirm that the subject meets all entry criteria. This must include documentation in the subject’s medical record of the following:

**Before inclusion**
- Inclusion criteria
- CT scan within 4 weeks before registration
- Informed consent

**Within 2 week before inclusion**
Medical history (with registration of symptoms) and physical examination.

Body surface area.

Performance status.

Blood counts: haemoglobin (Hb), absolute neutrophil count (ANC), platelet count (Pl).

Liver chemistry: bilirubin, ALAT, LDH, alkaline phosphatase

Renal chemistry: creatinine, albumin.

Other blood tests: CRP, CA 19.9, AFP, CEA, PP %, APTT.

Serum tumour markers (appendix 1).

If the subject’s characteristics comply with all the clinical and laboratory criteria necessary for registration, the local investigator or sub-investigator should send registration form to Clinical research Unit, OUH, which then return confirmation of study entry including the patient’s registration number.

### 7.2 Evaluation during neoadjuvant therapy

**After first course of chemotherapy**

- Nadir hematology

**Before each course of chemotherapy (day 1 and 8 in each cycle)**

- Platelet count, absolute neutrophil count and other blood tests according to clinical practice in treating department

**After each course of chemotherapy**

- Toxicity

**After 6 weeks of chemotherapy**

- New referral to department of surgery

**After 12 weeks of chemotherapy**

- CT scan
7.3 Evaluation before surgery
- 2-4 weeks after last course of chemotherapy the patient will be re-evaluated at the department of surgery

7.4 Evaluation after surgery
- Status of resection (R0, R1 or R2) and pathological report will be evaluated at the local MDT and further adjuvant therapy will be discussed (further adjuvant monotherapy for 3 months is recommended in most cases).
- Patients will be followed with CT scan every 6 months for 2 years.

8 CHEMOTHERAPY
Combination chemotherapy will be administered for 3 months according to local guidelines, with a 2 drug regimen including platinum (cisplatin or oxaliplatin) and gemcitabine (Fiteni et al, 2014). 4 weeks (± 1 week) after resection patients will be evaluated for 2-3 months of adjuvant therapy depending on patients performance and histopathological report. The following regimens may be used, but each center must plan one regimen for the entire study:
- GemCis (gemcitabine and cisplatin)
- GemOx (gemcitabine and oxaliplatin)

GemCis (gemcitabine and cisplatin)
Cisplatin 25 mg/m² iv will followed by gemcitabine 1000 mg/m² iv, each administered on days 1 and 8 every 3 weeks for four cycles.

GemOx (gemcitabine and oxaliplatin)
Gemcitabine 1000 mg/m² iv. will be followed by oxaliplatin 100 mg/m² iv. Gemcitabine 1000 mg/m² (30-100 min intravenous infusion) will be administered on day 1 and 8. Oxaliplatin
100 mg/m² will be administered as a 30-120 min intravenous infusion on day 1. Treatment will be repeated every 3 weeks for 4 cycles.

9 DOSE ADJUSTMENTS

Hematological toxicities:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Current cycle, day 1</th>
<th>Any time during cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia 2-3</td>
<td>ANC ≥ 0.5 x 10⁹/l</td>
<td>Delay treatment until toxicity has resolved to grade 0-1. No dose modification</td>
</tr>
<tr>
<td>Neutropenia 4 or Febrile neutropenia</td>
<td>ANC &lt; 0.5 x 10⁹/l</td>
<td>Delay treatment until toxicity has resolved to grade 0-1 (ANC ≥ 1.5 x 10⁹/l)</td>
</tr>
<tr>
<td>Thrombopenia 2-3</td>
<td>T ≥ 25 x 10⁹/l</td>
<td>Delay treatment until toxicity has resolved (T ≥ 100 x 10⁹/l)</td>
</tr>
<tr>
<td>Thrombopenia 4</td>
<td>T &lt; 25 x 10⁹/l</td>
<td>Delay treatment until toxicity has resolved (T ≥ 100 x 10⁹/l)</td>
</tr>
</tbody>
</table>

Non-hematological toxicities:

<table>
<thead>
<tr>
<th>Grade</th>
<th>At day 1 of cycle</th>
<th>At any time during previous cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity grade 2 (excluding nausea, emesis, alopecia)</td>
<td>Delay treatment until toxicity has resolved.</td>
<td>No dose modification.</td>
</tr>
<tr>
<td>Toxicity grade 3-4 (excluding nausea III)</td>
<td>Delay treatment until toxicity has resolved, and then continue treatment at 80% of prior dose</td>
<td>Delay treatment until toxicity has resolved, and then continue treatment at 80% of previous dose.</td>
</tr>
</tbody>
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Dose modifications for abnormal liver enzyme results (bilirubin, alkaline phosphatase, ASAT, ALAT) are left to the discretion of the individual investigator.

10 ADVERSE EVENTS

Definitions

10.1 Adverse events (or adverse experience) (AE):

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally asso-
associated with the use of a medicinal product, whether or not considered related to the medicinal product. Due to regulatory requirements, events occurring during pre- and post-treatment periods should also be designated as AEs. Therefore, safety surveillance - reporting of (S)AEs - commences at the time when the subject is enrolled into the study (date of signature of the informed consent) until the End of Study Visit has been performed. Therefore events occurring in the period between the signed informed consent and beginning of the study drug administration are to be designated as AEs. This procedure complies with requirements by some authorities.

10.2 Serious adverse event or reaction/experience (SAE):

A serious AE (experience) or reaction is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the subject 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 in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect or
- Is an important medical event

In addition laboratory value changes may require reporting unless otherwise specified in the protocol.

End organ toxicity as a medically significant event or clinically laboratory change, in which a patient not necessarily may be hospitalised or disabled, but is found clinically significant to demand further monitoring.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in cases of important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room, or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse, or malignant tumors when they are histologically different from the primary tumor.

10.3 Events not to be treated as SAEs

Progression of disease is not to be regarded as a SAE. Due to the seriousness of the disease in this study, certain conditions defined, as SAEs will be excluded from expedited reporting on a SAE report form: Elective hospitalization and surgery for treatment of disease.
The following will not be considered serious for this study:
An event that results in hospitalization or prolongs an existing hospitalization if the only reason for the hospitalization or prolongation is for the following:

- Hospitalization is secondary to expected chemotherapy morbidity as
  - Myelosuppression
  - Fever
  - Nausea and vomiting
- Hospitalization is secondary to expected cancer morbidity as
  - Weight loss
  - Fatigue
  - Electrolyte disturbances
  - Pain management
  - Anxiety
  - Admission for palliative care
  - Admission of chemotherapy
  - Transfusion of blood product
  - Administration of study procedure
  - Placement of permanent intravenous catheter
  - Hospice placement for terminal care

These events will be recorded on the data collection form. Any patient’s death must be recorded on the data collection form.

**10.4 Recording of Adverse Events**

All AEs must be documented in the appropriate section of the CRF. The following aspects must be recorded for each event in the CRF:

- A description of the AE in medical terms, not as reported by the subject
- The date of onset (start date)
- The date of recovery (stop date)

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination.

All AE’s occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization or until it has been determined that study treatment or participation is not the cause. SAE’s which are still on-going at the end of the study period must be followed up to determine the final outcome. Any SAE, which occurs after the study period and is considered to be possibly related to study treatment or study participation should be recorded and reported immediately.

The grade as assessed by the investigator according to the definitions in NCI-CTC, version 4.0: grade 1 – 4:
• Grade 1 = mild
• Grade 2 = moderate
• Grade 3 = severe
• Grade 4 = life-threatening or disabling
• Grade 5 = death related to AE (only for NCI-CTC version 3.0)

The causal relationship to chemotherapy as assessed by the investigator; the decisive factor in the documentation is the temporal relation between the AE and chemotherapy. The following judgments of the causality to chemotherapy or study procedures are to be used:

Not Related: There is not a temporal relationship to study drug administration (too early, too late, or study drug not taken), or there is a reasonable causal relationship between another drug, concurrent disease, or circumstance and the AE.

Not Likely: There is a temporal relationship to study drug administration, but there is not a reasonable causal relationship between the study drug and the AE.

Possible: There is a reasonable causal relationship between the study drug and the AE. Dechallenge information (information referring to withdrawal of drug) is lacking or unclear.

Probable: There is a reasonable causal relationship between the study drug and the AE. The event responds to dechallenge (withdrawal of study drug). Rechallenge is not required.

Certain/Definite: There is a reasonable causal relationship between the study drug and the AE. The event responds to dechallenge and recurs with rechallenge, when clinically feasible.

10.5 Reporting of SAE and SUSAR
In the case of a Serious Adverse Event the investigator must immediately (within 1 working day) SEND (preferably by fax or by e-mail) SAE-CRF to sponsor at OUH. Any deadly or life-threatening SAE that is both unexpected and suspected to be related to treatment (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC and as per national regulatory requirements in participating countries (i.e. as soon as possible, and in no case later than seven days after knowledge by the investigator of such an event, and relevant follow-up information is subsequently communicated within 15 days total). Unexpected means that the AE is not described in paragraph 4.8 of each SPC. The principal investigator at each site is responsible for SUSAR reporting as described above, and also for informing the international coordinating investigator (by delegation of the sponsor), as well as informing the sponsor. Any SUSAR grade 3 or less will be reported to the regulatory authorities and relevant ethics committee within 15 days of the event having been brought to the attention of the investi-
gator. Additional relevant information regarding the event will be sent to the authorities as soon as possible.

Death on Study
Any death occurring between the study inclusion and 30 days following the last dose will be reported to the Regulatory Authorities and ethics committees according to local rules. Death occurring during the study follow-up period (i.e. later than 30 days after the last dose) need only to be reported as serious adverse event if it is thought that there is a possible relation to study drug(s) (possible, probable). All deaths should be reported on the death report form section on the CRF regardless of cause.

10.6 Follow-up
Patients withdrawn from the study treatment due to any AE will be followed at least until the outcome is determined even if it implies that the follow-up continues after the patient has left the trial, and where appropriate until the end of the planned period of follow-up.

10.7 Safety instructions specific to the trial
Adverse events will be recorded for all Patients (including those withdrawing from the study treatment because of toxicity) for 28 days following the last dose of study drug. Adverse events related to study drug(s) that are observed, either during study treatment, or prior to the twenty-eighth day following the last dose of study drug(s), will be followed until resolution or stabilization.

11 STATISTICS
The primary efficacy variable is percentage of patients alive at 2 years calculated from the day of registration.

Secondary objectives are to determine:
- OS (deaths of all causes)
- Overall RR (investigator evaluated) according to the RECIST criteria version 1.1 in patients with measurable disease.
- Safety and toxicity of the treatment
- To evaluate the relations between treatment effect and potentially predictive and prognostic tumor biomarkers.

Efficacy analysis
OS and DFS will be calculated from the date of registration to the date of documented progressive disease, according to the RECIST criteria, or death.
The sample size is based on Simon’s two stages Mini-max design. This design ensures early study termination if there is insufficient effect.

A resection rate less than 60% after 3 months of preoperative chemotherapy is not clinically acceptable. Assuming a significance level at 0.05 (α = 0.05) and a power at 90% (β = 0.10) it can be calculated, that 26 patients should be included in the first part of the study. The enrolment will continue until 26 patients have completed 3 months of chemotherapy and have been re-evaluated for resection by CT scan and EUS. If 15 or less out of the first 26 consecutive patients are being resected we will reject our hypotheses and close the study after the first stage of accrual. If 16 or more patients are resected, an additional 19 patients will be accrued in the second stage. If 32 out of 45 patients are resected, a true resection rate of 80% cannot be excluded, and will be concluded that the treatment is effective enough to continue with future studies.

To ensure 45 evaluable patients we will include a total of 50 patients.

We will use non-parametric methods for calculation of patient characteristics, side effects and disease control. DFS (and PFS) and OS will be calculated and reported as median survival (Kaplan-Meier method).

12 ETHICS

The study will be conducted in compliance with the protocol and in accordance with the ethical principles put forward in the second Declaration of Helsinki and in accordance with GCP rules. The rights, safety and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society. Study personnel involved in conducting this trial will be qualified by education, training and experience to perform their respective tasks.

In this study we will treat patients with resectable disease with pre-operative chemotherapy. Preoperative chemotherapy has so far only been sparsely studied in patients with BTC. Previous studies have shown a very low risk of progression during 3 months of chemotherapy. Even though the tumor burden is much lower in patients who are candidates for neoadjuvant therapy, there is a still risk of 10 - 20% that the disease will progress during pre-operative chemotherapy and in some patients (around 10%) the disease may become non-resectable. However, it is expected that neoadjuvant therapy will increase the chance of cure and that this benefit will outweigh a possible harm.

The primary objective of this study is to investigate the effect of chemotherapy.

Informed consent
Before inclusion in study the patients will be informed about the planned aim of the study and risk of possible adverse events. Every patient shall give informed consent after having been informed about the clinical investigation. This will be documented by the dated signature of the patient. Information will further be supplied from the ministry of Science and the Ethical Committee: “Your rights as a study person in a biomedical investigation.”
Information of the Patient
Participation in the investigation is voluntary. If the patient denies participation this will not have any consequences or lack of opportunities for any other treatment of the patient. If the patient denies participation in the study this will not in any way, result in consequences or the lack of opportunities for any other possible treatment option.

Rights and responsibilities
At any time the patient have the right to withdraw from the investigation without this will have any influence on further treatment.
The patient must be aware that personal information will be examined closely under audit of relevant authorized personal, but that this personal information will be handled strictly confidential and will not be published in a medical periodical. In this case the patient is guaranteed to remain anonymous.
Data that may identify the patient will be found in the hospital records. Material from the patient will only include what is mentioned in the section on biomarkers.

13 TIME SCHEDULE
The trial is expected to start after approval of the protocol has been granted from the Ethics Committee and the Danish Health and Medicines Authority, respectively. Target recruitment period is estimated to be 12 months and follow-up period/end date is estimated to be one year from last infusion.

14 PUBLICATIONS
The Vancouver declaration should be followed in all publications based on this study and we plan that the study will be published in international peer-reviewed journals. The manuscript will be prepared by the Sponsor-Investigator who will also decide who will be the first author. Co-authors are an oncologist and a surgeon from those centers that have included at least 10% of the patients. The protocol committee writes the first and final version. In publication of tumor biological sub-studies the researchers are the main authors and co-authors are the protocol committee and representatives from each center participating in the tumor biological collection including at least 10% of the patients.

15 ECONOMY
This study is initiated and conducted by doctors in Denmark and Sweden. There is no economic benefit for neither the participating departments nor the hospital employees.
16 REFERENCES

APPENDIX 1 Nordic-BTC-01 – Tumormarkers

Purpose
Blood sampling for bio-banking will be performed during the trial. These samples will later be analysed for potential prognostic and/or predictive markers for the disease or treatment. Serum, plasma and blood cells will be collected.

Procedure for blood-sampling:
Blood is drawn from a peripheral vein and divided into
a) 10 ml blood for serum
b) 10 ml blood for EDTA-plasma
c) 10 ml blood for Na-citrate
All samples should be processed and stored within 2 hours of collection.

PLASMA AND BUFFY COAT LAYER COLLECTION: EDTA
NOTE: Processing of all EDTA plasma tubes can be combined.
1. Manually and gently invert EDTA blood tube 5 times
2. Centrifuge EDTA tubes. Speed 2000 g, 10 mins, Temperature: 4ºC
3. Using a sterile transfer pipette, carefully aspirate plasma from tube and combine in 5 Sarstedt vials.
4. Store
5. Record processing details on Sample Processing Information Sheet

BUFFY COAT LAYER:
The second whitish layer containing a mixed population of white blood cells is the Buffy Coat.

Using a sterile transfer pipette, carefully aspirate the Buffy Coat layer from the tube(s).
It is recommended that 1-2 mm of plasma and 1-2 mm of red cell layers are included in the buffy coat aspirate to ensure collection of all the buffy coat layer.

Aliquot buffy coat into DNAse free sarstedt vials.

PLASMA COLLECTION: Na Citrate Procedure
1. Manually and gently invert Na Citrate blood tubes 5 times
2. Centrifuge Na Citrate tubes. Speed 2000 g, 10 mins, Temperature: 4ºC
3. Aliquot plasma into 5 Sarstedt vials
4. Record processing details on Sample Processing Information sheet
SERUM COLLECTION

Procedure
1. Invert Serum tube and allow to clot at room temperature for a minimum of 30 minutes
2. Centrifuge 2000g, Spin: 10 minutes. Temperature: 4°C
3. Aliquot serum into 5 Sarstadt vials
4. Store
5. Record processing details on Sample Processing Information Sheet

Record procedure
The vials are labeled with the patient number, date and code for timing. It should be clearly marked what container is in question, EDTA Serum etc. By text and color.

Blood sampling frequency
Patients will be asked to donate blood for biobanking
1) Before initiation of chemotherapy
2) Before surgery

Should the patient refuse to donate blood to the biobank he/she can still participate in the study.

Initial analysis
Blood samples can only be used after the approval of the protocol committee. Initial analysis include YKL-40 and TIMP-1. Other analysis will not be performed before accept from the local ethical committee is obtained.
18 APPENDIX 2 Nordic-BTC-01 – Contraindications

Gemcitabine:
- Hypersensitivity to the active substance or to any of the excipients.

Cisplatin:
- With hypersensitivity to cisplatin or other platinum compounds or to any the excipients
- With renal dysfunction (creatinine clearance < 60 ml/min)
- In dehydrated condition (pre- and post-hydration is required to prevent serious renal dysfunction)
- With myelosuppression
- With a hearing impairment
- Withy neuropathy caused by cisplatin
- Who are breastfeeding
- In combination with liver vaccines, including yellow fever vaccine
- In combination with phenytoin in prophylactic use

Oxaliplatin:
- Have a known history of hypersensitivity to oxaliplatin
- Are breast feeding
- Have myelosuppression prior to starting first course, as evidenced by baseline neutrophils < 2 x 10^9/L and/or platelet count of < 100 x 10^9/L
- Have a peripheral sensitive neuropathy with functional impairment prior to first course
- Have a severely impaired renal function (creatinine clearance less than 30 ml/min)