Clinical Protocol

ACME ABC TRIAL

Study Title	**Australian Comprehensive Molecular Evaluation of Advanced Biliary Cancer Trial (ACME ABC)**
Short Title	**ACME ABC**
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Sponsor for Protocol Development	**Australasian Gastro-Intestinal Trials Group (AGITG)**
Lead Clinical Site	**Monash University** **Melbourne VIC 3168** **Australia**
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COMPLIANCE STATEMENT

This trial will be conducted in accordance with the protocol, International Conference on Harmonization (ICH) and Good Clinical Practice (GCP). In addition, the trial will be conducted in compliance will all applicable laws and regulatory requirements relevant to the use of new therapeutic agents in Australia and any other participating country. Agreement of the investigator(s) to conduct and administer this trial in accordance with the protocol and associated regulations will be documented in the trial agreements with the Sponsor and other forms required by national authorities in the country where the trial site is located.

The Investigator(s) is responsible for ensuring the privacy, safety and welfare of the patients during and after the trial.

The Principal Investigator at each site has the overall responsibility for the conduct and administration of the trial at their site, and for conduct with the trial site management, the Independent Ethics Committee (IEC) / Institutional Review Board (IRB), and local authorities.

This protocol has been developed with funding from the Victorian Cancer Agency

Protocol History

Version No	Date	Author	Reason
1.0	23rd March 2022	Daniel Croagh	Initial

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Signature Page

The signatures below confirm that the following protocol has been agreed upon and mutually accepted by the listed Principal Investigators.

Principal Investigators agree to conduct this clinical study in compliance with the approved protocol version and adhere to principles outlined within the NHMRC's National Statement on Ethical Conduct of Research in Humans, the Therapeutical Goods Administration's Clinical Trial Handbook, Good Clinical Practice, internal SOP's and any other regulatory requirements for the safe and ethical conduct of this research.

By signing this page, I agree to ensure that the confidential information pertained in this document will not be used for any other purpose other than the evaluation and conduct of the clinical study without consent from appropriate parties.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and/or on behalf of Study Sponsor_____

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Signature Date_____

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Printed Name Position / Role

Site Principal Investigator

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Signature Date

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1. Protocol synopsis

The **A**ustralian **C**omprehensive **M**olecular **E**valuation of **A**dvanced **B**iliary **C**ancer (**ACME-ABC)** Trial

1.1 Background and rationale

Biliary Cancer (BC) remains a rare but important cause of cancer related mortality worldwide. Although surgical resection is the only potentially curative treatment, most patients present with advanced disease and are not candidates for this. Patients with advanced biliary cancer (ABC), whether that is locally advanced or metastatic, are offered palliative chemotherapy. Current chemotherapy has improved median survival to between 1 and 2 years. Recently, novel therapeutic strategies have been devised based on the molecular and genetic characteristics that are present within subsets of patients with ABC.

There are a number of barriers to the implementation of routine molecular screening in ABC. First, the predominant method of obtaining tissue to establish the diagnosis of ABC include percutaneous or endoscopic biliary brushings or biopsies and endoscopic ultrasound guided fine needle aspiration (EUS-FNA) cytology. These techniques only provide a limited amount of tissue, which may make molecular characterisation difficult. Second, this tissue is usually fixed in formalin and embedded in paraffin for subsequent histological analysis. This process leads to the loss and or degradation of DNA and RNA, which further hampers genetic analysis. Third, the best strategy for the molecular characterisation of ABC biopsy material is unclear and potentially expensive. Options include targeted sequencing, whole exome or whole genome sequencing and transcriptomic analysis. Fourth, the interpretation of results providing by sequencing may be challenging and difficult particularly as biopsy tissue contains both malignant and non-malignant cells in varying amounts which can make assessment of these results difficult. Fifth, access to novel treatments may not be straightforward which may limit the current usefulness of this approach. Finally, patients with ABC tend to progress rapidly, providing a limited window of opportunity for the application of targeted therapies.

The primary aim of this study is to examine the feasibility and potential benefit of introducing routine comprehensive molecular profiling of ABC using either fresh frozen or archival tissue obtained at the time of routine diagnostic tests such as ERCP or EUS.

We have recently developed a technique for the isolation of DNA and RNA from an additional fresh frozen EUS-FNA biopsy taken at the time of diagnostic EUS for pancreatic and biliary cancer (Monash Health HREC Ref: 15450A). We have also demonstrated that it is possible to use this tissue to perform comprehensive molecular profiling of pancreatic cancer using the TSO500 gene panel and this is currently being tested in a clinical trial (ACTRN12620000762954). We now wish to extend these techniques to patients with ABC.

1.2 Aim

The aim of this study is to assess the feasibility and usefulness of routine comprehensive molecular analysis of tissue obtained by endoscopic biopsy for patients with ABC.

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1.3 Primary Endpoints

1. To determine the proportion of patients with ABC that can have comprehensive molecular profiling using endoscopic biopsies, either EUS-FNA or direct cholangioscopic biopsies.

1.4 Secondary endpoints

1. To compare the relative utility of EUS-FNA and direct cholangioscopic biopsy material for the comprehensive molecular profiling of ABC.
2. To determine the proportion of participants with ABC that can have treatment recommendations based on comprehensive molecular profiling.
3. To determine the number of participants with ABC that have changes in their treatment based on comprehensive molecular profiling.
4. To determine if comprehensive molecular profiling of endoscopic biopsy supernatant material is feasible for patients with ABC
5. To assess the sensitivity of whole genome sequencing to provide a genetic diagnosis of ABC.
6. To determine the quantity and quality of DNA and RNA that can be obtained from the various types of ABC biopsy material.

1.5 Tertiary endpoints

1. To investigate the potential correlations between comprehensive molecular profiling and stage, prognosis and response to treatment in patients with ABC.
2. To investigate the potential for peripheral blood to provide sufficient circulating tumour DNA (ctDNA) to allow for comprehensive molecular profiling of ABC.
3. To assess overall survival of patients who receive targeted therapy.

1.6 Hypotheses

1. It will be possible to perform comprehensive molecular profiling in at least 50% of patients with ABC using EUS-FNA or direct cholangioscopic biopsies.
2. EUS-FNA and direct cholangioscopic supernatant material will have equivalent utility in allowing comprehensive molecular profiling of patients with ABC.
3. It will be possible to make treatment recommendations in at least 50% of patients with ABC who have had comprehensive molecular profiling.
4. Some patients with a targetable phenotype will be able to access targeted therapy.
5. A successful molecular diagnosis of ABC will be able to be made in 80% of participants with supernatant from EUS-FNA or endoluminal biopsy.

1.7 Interventions

Patients with suspected ABC who are of good performance status are candidates for entry into this trial. Once a clinical diagnosis has been established and after discussion with the treating team, patients will be reviewed and fully informed of the nature of this study.

Informed consent will encompass the following;

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1. The nature and purpose of this study, comprehensive molecular profiling of ABC using biopsy obtained via EUS biopsy or direct cholangioscopic biopsies.
2. The supernatant from routine biopsies will also be assessed for molecular profiling.
3. The fact that additional biopsies (EUS-FNA or direct cholangioscopic) will be obtained
4. The fact that insufficient material may be available for comprehensive molecular profiling.
5. The nature of the TS0500 gene panel and the fact that it is not a NATA accredited test.
6. The process of the molecular tumour board review and dissemination of results to the participant's treating team.
7. The possibility that analysis of the biopsy may indicate a possible germline mutation (~5%). This may require further testing for confirmation. If a germline mutation is confirmed the patients will be offered referral for genetic counselling. The discovery of germline mutation may also have implications for the patient's relatives.
8. The fact that participants and the treating doctor will be informed if a potentially targetable molecular phenotype is revealed.
9. That when a targeted treatment is identified and deemed appropriate by the treating team, an attempt will be made to access this treatment. This may involve;
    a. Access through the PBS
    b. Referral for entry into clinical trials that provide access to targeted therapies. E.g. the MoST study
    c. Direct application to the relevant pharmaceutical company for compassionate access under the special access scheme.
10. That targetable molecular phenotypes may be revealed, for which the appropriate therapy may not be able to be obtained. We hope that at least 5% of patients who are able to have comprehensive molecular profiling will be able to access targeted therapy.

DNA and RNA will be extracted from the fresh frozen biopsies and supernatant and the quantity and quality of this material will be assessed. In those patients with adequate DNA (and RNA if also adequate), whole genome sequencing (WGS) will be performed at the VCCC under the supervision of Sean Grimmond.

The results of comprehensive molecular profile and bioinformatical analysis (PierianDx reports or equivalent) will be reviewed by a Molecular Tumour Board (MTB). The MTB will meet every 3 months or as required and the outcome of this meeting will be conveyed to the patients treating medical oncologist, who will communicate the results to the participants.

After WGS and MTB review, patients will be offered enrolment in the MoST study to facilitate access to relevant clinical trials. Participants will be followed to assess for uptake of targeted therapies and overall survival as per the MoST study protocol.

Establishing a cytological or histological diagnosis of ABC can be challenging, particularly in hilar cancer. Genetic analysis of fresh frozen biopsy material has the potential to provide a "genetic" diagnosis of ABC in the absence of a cytological diagnosis. A genetic diagnosis will be based on molecular tumour board assessment of the mutation profile revealed by whole genome sequencing. The tissue may also be assessed with a custom designed NanoString panel, which has previously been developed to establish a "genetic" diagnosis of pancreatic based on RNA expression. The utility of this signature will also be assessed in this cohort.

Peripheral blood will be stored for potential germ line testing if a germline mutation is suspected and for future ctDNA analysis.

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1.8 Assessments

The following assessments will be performed:

1. DNA and RNA extraction and quantification from additional biopsy and supernatant specimen
2. Comprehensive molecular profiling with TSO500 (or equivalent) and NanoString gene panels (if sufficient DNA/RNA)
3. Peripheral blood banking obtained on the day of tissue biopsy acquisition
4. Germline testing (peripheral blood) if a germline mutation is suspected
5. MTB review
6. Communication of MTB outcome to treating team
7. MoST follow up to review uptake of targeted therapy
8. MoST follow up to determine overall survival

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2. Research Plan

2.1 Study Design

This is a prospective cohort study with a target accrual of 50-75 patients.

2.2 Study Procedures

Patients with suspected diagnosis of ABC and who have good performance status are candidates for entry into the trial. If participants are later found to have a benign condition, they will be excluded from the assessment of primary and relevant secondary outcomes. They will be included however in the assessment of the utility of CMP to provide a genetic diagnosis of ABC. Potential participants will have been identified and reviewed at MDT and have been found to be unsuitable for surgical resection either because the tumour is locally advanced, metastatic or recurrent. Potential participants will be contacted and offered an appointment to consider the trial and provide informed consent.

Informed consent will provide permission for additional biopsies to be taken at subsequent endoscopic interventions for the routine diagnosis and management of cholangiocarcinoma. In addition, supernatant from routine diagnostic biopsies that is generated during the processing of routine biopsies and normally discarded will also be stored. DNA and RNA will be extracted from the additional biopsies. This will be performed using the Qiagen AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, or similar) and will be quantified using the Nanodrop spectrophotometer (ThermoScientific) and Qubit Fluorometer (Life Technologies). The quality of genomic material will be assessed using a Bioanalyser (Agilent) and DNA TapeScreen (Agilent).

If there is sufficient genetic material, comprehensive molecular profiling using WGS and ongoing follow up will be performed at the Victorian Comprehensive Cancer (VCCC) under the supervision of Sean Grimmond. After molecular tumour board (MTB) review participants with successful WGS will be enrolled in the MoST study at this point for access to the clinical trials platform and for ongoing follow up.

DNA and RNA will also be extracted from the supernatant generated from routine diagnostic biopsies. This utility of this material will be assessed with respect to its capacity for comprehensive molecular profiling at Monash Health Translational Precinct. This is predominantly for research purposes with the primary focus to be CMP of the tissue biopsy and then also whether supernatant provides adequate DNA and RNA for CMP in its' own right. If the supernatant provides useful information, then this will passed onto the investigators at the MoST study. It is note that the analysis of supernatant material is a non NATA accredited test.

Peripheral blood will be stored for future ctDNA analysis and possible comprehensive molecular profiling.

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2.3 Outcome and measurements

1. Proportion of patients with ABC that can have comprehensive molecular profiling of the tumour using a fresh frozen EUS-FNA biopsy material
2. Proportion of patients with ABC that can have comprehensive molecular profiling of the tumour using fresh frozen endoluminal biopsy material obtained by direct cholangioscopic biopsy at either ERCP or PTC
3. Proportion of patients with a molecular characterisation of ABC that can have treatment recommendations made on the basis of comprehensive molecular profiling
4. Proportion of patients with a targetable phenotype that have targeted treatment
5. Sensitivity of and specificity of a molecular diagnosis of ABC as defined by the presence of one or more mutations that have previously been described in cholangiocarcinoma, for example in The Cancer Genome Atlas (TCGA)
6. Sensitivity and specificity of a molecular diagnosis of ABC using a NanoString signature previously validated in pancreatic cancer
7. Time taken between enrolment and MTB to assess the feasibility of CMP in ABC
8. The utility of supernatant material for CMP.
9. Assessment of peripheral blood as a potential source of comprehensive molecular profiling in ABC
10. Assessment of overall survival of patients who receive targeted therapy

2.4 Recruitment Strategy

This trial will be open to all patients at participating sites who meet the inclusion and exclusion criteria.

Endoscopic Ultrasound and direct cholangioscopic examinations are specialised procedures and are normally performed at tertiary referral hospitals. This results in a concentration of biliary cancer patients at a limited number of sites which will facilitate recruitment for this trial. A research coordinator for the trial will be placed at each of the participating sites (where resources are available) who will work with the treating clinicians and screen the EUS/ERCP/PTC lists, and/or multi-disciplinary meetings (MDTs) to identify patients that have been recently diagnosed with suspected advanced biliary cancer who could be potential candidates for the study. We have previously sought advice from the Monash Health Ethics committee regarding the need for a waiver of consent for this screening procedure for a similar study in pancreatic cancer and it was determined that this was not required (due to the necessity to identify the potential participants in a time critical way).

Once the potential participants for the study are identified, the research coordinator will communicate directly with the treating clinician involved in the patient's care who will liaise with the patient to offer potential involvement in the study. If the patients are willing to participate in the study, their contact details will be passed on to the study investigators who will inform and consent them for enrolment in the study.

The study investigator from Monash Health will contact the potential participant to conduct screening with respect to the inclusion and exclusion criteria. If they are considered eligible for the trial, they will be provided with a copy of the PICF and they will be offered a tele-health appointment to discuss the nature of the trial and provide informed consent for entry into the trial. The research

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coordinator will then liaise with the treating clinician (endoscopist) to arrange for an additional biopsy to be taken at the time of EUS or direct cholangioscopic examination and for supernatant from routine diagnostic biopsies to be retained by the pathology department for subsequent collection.

It is possible that some patients who are potentially eligible for this study may have already consented to participating in biobanking of ABC biopsies at the time of diagnostic biopsy. The Pancreatic and Biliary Cancer Biobank (PCB) was established for the purpose of banking biopsy material from patients with either pancreatic or biliary tract cancer. Consent for biobanking is obtained by one of the principal investigators or trial co-ordinators of the biobank. Most commonly this is the doctor performing the biopsy procedure. Biopsy procedures are usually day procedures and consent for the biobanking of material is usually performed immediately after consent for the actual biopsy procedure has been obtained, on the day of the procedure. An endoscopic procedure may on occasion precede the MDT discussion for the patient. This is particularly the case if a biliary stent is required to treat jaundice. Given that patients can only be included in his study after MDT has determined that they have locally advanced, metastatic or recurrent biliary cancer, the use of previously biobanked material (endobiliary biopsy only for locally advanced disease and either endobiliary or FNA biopsy material for those with metastatic or recurrent disease) will be permitted to the allow these patients to enter the trial. Patients with localised disease who have had an EUS-FNA prior to MDT discussion will not be permitted to enter the trial even if biobanked material is available. This is because EUS-FNA could in certain circumstances be deemed to be inappropriate for patients with operable disease because of the risk of tumour seeding from this transluminal biopsy technique.

2.5 Feasibility

This will be a multicentre study seeking to enrol 50-75patients. This project will be open in at least 5 sites around Australia each of which is hoped to recruit 10-15 patients over a 2 year period.

2.6 Health Funding

The costs of patient enrolment and biobanking are currently being met by many individual centres throughout Australia such as the Department of Surgery at Monash Health, however this is not universally available. The costs required to expand the biobank to facilitate a National Programme and the costs of establishing additional MTBs where required will be met in part by the AGITG ideas grant (100,000 AUD). The cost of patient follow-up will also be met by this grant but will also be met in part by the enrolment in patients in the MoST study. The costs of the comprehensive molecular profiling by WGS will be met primarily by the VCCC program dedicated to the study of rare tumours project. Additional support for this study to further defray the costs outlined above may be sort from relevant funding agencies.

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3. Background

3.1 Advanced Biliary Cancer

Biliary cancer arises from the biliary epithelium and therefore includes intra and extra hepatic cholangiocarcinoma and gall bladder cancer. Extrahepatic cholangiocarcinoma can affect the bile ducts close to the liver (hilar cholangiocarcinoma) or the distal bile ducts. Although rare it remains a leading cause of cancer related deaths in Australia and around the world. The incidence varies wildly, and it is particularly common in Southeast Asia. Median 5-year survival for all stages of the disease is extremely poor and measured in the order of months. Complete surgical resection provides the only chance of cure for BC, but the 5-year survival rates post resection even with adjuvant chemotherapy remains low. Most patients (>80%) are not candidates for surgical resection. Even with modern chemotherapy, median survival in this group is at best between 1 and 2 years at best. It is clear from these statistics that BC has, unlike many other cancers, seen little progress in the past 20 years. It deserves to be a medical and public health priority in Australia, as well as globally.

3.2 Molecular profiling in Cholangiocarcinoma

Recent advances in next-generation sequencing technologies have revealed a high degree of tumour heterogeneity among biliary tract cancer patients (Cao, et al., 2020). In particular, the genetic landscape of BTC is influence by the aetiology of the tumour and the location of the tumour (Churi, et al., 2014). Mutations in *IDH1/2* and *FGFR* are found almost exclusively in intrahepatic BTC whereas mutations in *KRAS* are found predominantly in extrahepatic BTC. This has led to increasing interest in the application of targeted therapy based on the molecular characterisation of patients with BTC. For example, the United States Food and Drug Administration (FDA) recently granted accelerated approval of pemigatinib for patients with an *FGFR2* fusion after failure of first line therapy.

A major obstacle, however, to personalised therapy for ABC has been the difficulty in isolating high-quality tumour-derived genetic material (genomic DNA and/or RNA) in sufficient quantity and quality for subsequent molecular profiling. One recent and ongoing attempt to address this is the COMPASS-B-MUHC trial. In this trial, patients with inoperable metastatic biliary tract cancer will undergo percutaneous biopsies to provide tissue for whole genome sequencing. However, this technique will only be available to patients who are amenable to percutaneous biopsy. Many patients with locally advanced biliary tract cancer will not be amenable to percutaneous core biopsy.

Accordingly, there is an urgent and unmet clinical need to incorporate robust isolation of high-quality genetic material in a timely manner for the vast majority of ABC patients. Endoscopic, and percutaneous biopsies are relatively non-invasive techniques which are routinely used to sample tumour tissue in ABC patients for cytological or histological diagnosis. Although endoscopic biopsies (and FNA cytology) have been used to provide tissue for genetic analysis of ABC, their clinical utility is still in its infancy. Furthermore, issues relating to insufficient tumour cellularity, poor quality DNA, and contamination by surrounding non-tumour tissue have, until now, collectively hampered its broader use in the clinical setting.

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Our laboratory has recently optimised protocols for the simultaneous extraction of genomic DNA and RNA from pancreatic and biliary cancer EUS-FNA biopsies (n = 350 pancreatic ductal adenocarcinoma [PDAC] patients). Real-time quantitative Polymerase Chain Reaction (qPCR) demonstrated high expression of epithelial-specific KRT7 and KRT19 genes confirming the accuracy of the technique (Berry W, 2017). Genome wide transcriptome analysis (n= 97 PDAC patients) was then performed with RNAseq and this was found to be consistent with data from the TCGA which was derived from genomic analysis of resection specimens. Comparison with transcriptomic analyses of non-malignant biopsies has allowed us to develop a diagnostic signature of pancreatic cancer. This has been validated using a NanoString gene panel encompassing 190 genes in a separate cohort of EUS-FNA biopsies from malignant and non-malignant lesions (Lundy et al Clinical Cancer Research, Accepted)

Whole exome sequencing using EUS-FNA material has also been performed in our laboratory (n=48), although bioinformatic interpretation proved difficult and time consuming partly due to the variability in tumour cellularity between specimens which makes mutation calling challenging. However, another more targeted sequencing approach, known as comprehensive molecular profiling, has been developed to phenotype a wide variety of haematological and solid tumours. This is now recommended in a wide variety of settings (Jennings LJ, 2017). An example of this is the TSO500 gene panel which is complemented by a commercially available bioinformatic support package (PeirianDx Report). Using this we have established an accurate and efficient technique to identify clinically relevant and targetable mutations in PDAC using tissue obtained from EUS-FNA biopsies (n=48) (Lundy et al submitted). Collectively, our novel data strongly suggest that high quality genetic material can be obtained from EUS-FNA biopsies in sufficient quantity for subsequent clinically relevant comprehensive molecular profiling and we are currently assessing this in a prospective clinical trial (Masoumi Moghaddam et al Endoscopic Ultrasound, in press. ACTRN12620000762954). We now plan to extend this concept to patients with biliary tract cancer and to biopsies obtained at the time of routine diagnosis whether that be EUS or cholangioscopic via ERCP or PTC. Further, we wish to test whether the cell free fluid that is left over after normal processing of endoscopic biopsies contains sufficient for CMP given that this material has recently been found to contain useful quantities of genetic material (Roy-Chowdhuri et al. Modern Pathology 2018 31:1036-45.) .

3.3 Precision therapy options in Cholangiocarcinoma

A number of actionable phenotypes have been identified in ABC. These include:

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Mutation	Frequency	Potential Treatment	Availability	TS0 500
FGFR2 Fusion		Pemigatinib	FDA approved	+
FGFR1-3 Fusion		Infigratinib Debio 1347	NCT03773302 – Open at Monash NCT03834220 – Registered at Peninsula Private	+
MSI	1%	Checkpoint inhibitor	TGA approved	+
TMB high	Rare	Checkpoint inhibitor	TGA approved	+
IDH1	4.9-36%	Ivosidenib	Approved for AML by TGA	+
IDH2		Enasidenib	Approved for AML by TGA	+
NTRK fusion	0.75%	Entrectinib	NCT02568267 – Open at Austin Hospital	
ERBB2/3 amplifications	11-17%		Approved	+
STK11	Rare	Everolimus	TGA approved	+
ROS1 fusion (Lim SM, 2017)	1.1%	Crizotinib	TGA approved	

3.4 Molecular Tumour Board

There is minimal data on the use of molecular tumour boards in BT cancer. However, "the complexity and vast amounts of data generated through molecular profiling techniques, like next-generation sequencing, make expert review an absolute requirement in order to translate molecular profiles into clinical benefit for our patients" (van de Haar J, 2019). The presence of a molecular pathologist in addition to the curated report from PierianDx or equivalent will greatly facilitate the interpretation of comprehensive molecular profiling. Therefore, we have established a hepatopancreaticobiliary molecular tumour board within the Monash Partners Comprehensive Cancer Consortium to assess the results of comprehensive molecular profiling.

3.5 Accessing targeted treatments options in ABC

There is limited data on accessing targeted therapies in ABC. Furthermore, in studies that do investigate the comprehensive molecular profiling of malignancy, patients are unlikely to receive targeted therapy despite up to 30% of patients demonstrating a targetable phenotype. Often only a small fraction of these patients actually receives such a therapy. In a recent study of a molecular tumour board in the United Kingdom, comprising 895 patients, although 20% had actionable mutations, only 7% received such therapies (Moore DA, 2019). However, there have been some recent initiatives focused on providing targeted treatments to patients with cancer including the MoST study (ACTRN12616000908437).

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3.6 Circulating tumour DNA in ABC

Liquid biopsies may eventually provide the most efficient way of performing comprehensive molecular profiling. Blood will be stored in the PCB to allow this to be tested in the future. Comprehensive molecular profiling of the matched primary tumour is likely to greatly facilitate the interpretation of comprehensive molecular profiling of peripheral blood.

4. Aims and Objectives

4.1 General Aim

The aim of this study is to assess the utility of minimally invasive biopsies, routinely employed for the diagnosis of ABC, to provide material for comprehensive molecular profiling of ABC with a view to guiding precision therapy.

The primary objective of the study is to determine the proportion of patients with ABC that can have comprehensive molecular profiling using fresh frozen biopsy material. Furthermore, the study will compare the relative utility of EUS-FNA and direct cholangioscopic biopsies in providing material that is suitable for CMP.

The secondary objectives (endpoints) include:

1. To determine the proportion of participants with ABC in which comprehensive molecular profiling reveals a targetable phenotype
2. To determine the number of participants with ABC that are administered targeted treatments
3. To assess the sensitivity and specificity of a molecular diagnosis of ABC based on analysis of the TSO-500 gene panel and NanoString signature, previously validated in pancreatic cancer.
4. To assess the amount and quality of DNA/RNA obtained from various fresh frozen ABC biopsy specimens.
5. To assess the utility of the supernatant from routine EUS-FNA and cholangioscopic biopsies for CMP in ABC
6. To correlate overall survival with the outcome of the TSO500 and NanoString gene panels
7. To assess the capacity for ctDNA to allow comprehensive molecular profiling of ABC

4.2 Study Design

A prospective cohort study involving moderate-to-high volume hepatopancreaticobiliary cancer units in Australia with a target accrual of 50-75 patients. Accrual may continue beyond this point depending on funding availability.

4.3 Subject Population

Patients must meet all the inclusion criteria and none of the exclusion criteria to be eligible for this trial. There will be no exceptions made to these eligibility requirements at the time of registration. All enquiries about eligibility should be addressed by contacting the coordinating trial staff or coordinating Principal Investigators prior to patient registration.

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4.4 Target Population / Recruitment

Adult patients with ABC or suspected ABC and good performance status are eligible for this trial. ABC is defined as locally advanced, metastatic, or recurrent biliary tract cancer. The planned recruitment for this trial will be 24 months. Patients will be followed up for up to 2 years following enrolment date.

4.4.1 Inclusion criteria

1. Males or females with suspected advanced biliary tract cancer as determined by MDT review that is either:
    a. Locally advanced
    b. Metastatic
    c. Recurrent
2. Study participants either:
    a. Are scheduled for an endoscopic procedure for the purposes of tissue diagnosis or biliary stenting
    b. Have previous consented to biobanking of endoscopic biopsy material*
3. Study participants must be ≥ 18 years of age at time of screening
4. ECOG Performance Status 0-2
5. Suitability for chemotherapy
6. Able to give signed informed consent
7. Life expectancy of at least 3 months from the time of screening as judged by screening investigator

4.4.2 Exclusion criteria

1. Patients considered to have operable or potentially operable BC
2. Patients with hepatocellular cancer or liver metastatic disease in which the primary malignancy is not BC.
3. Evidence of systemic disease (cardiovascular, respiratory, renal, hepatic, etc.) that would preclude chemotherapy.
4. Serious medical or psychiatric conditions that might compromise protocol-based management as judged by investigator

*Only endobiliary (direct cholangioscopic) biopsy material is acceptable for patients with localised disease. Both endobiliary and EUS-FNA are acceptable for patients with metastatic or recurrent disease.

4.5 Patient Screening

Patients may be referred for consideration of the trial by the local treating team or be identified by the RA by screening of endoscopy or MDT lists at participating sites. If patients have already provided tissue for biobanking at the time of diagnostic biopsy the treating clinician may also be contacted by the trial co-ordinator and or investigators so that their patients can be afforded the opportunity of participating in this trial. The research coordinator will document the inclusion and exclusion criteria along with patient contact details. This will be returned to the trial co-ordinator. The co-ordinator will collate the patient's information including the cross-sectional imaging which demonstrates ABC. These results will be reviewed by a principal investigator who must be satisfied

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that the patient has ABC. The patient will then be contacted by investigators at Monash Health to discuss participation in the trial and to obtain informed consent.

4.6 Patient Consent

Patients who satisfy the inclusion and exclusion criteria will be provided with a participant information and consent form and an appointment with the co-ordinating principal investigator or sub-investigator.

Patients will meet with the principal or sub-investigator to discuss the nature of the trial and address any questions or concerns prior to providing informed consent. Consent may be obtained using telehealth.

Informed consent will encompass the following:

1. The nature and purpose of this study, comprehensive molecular profiling of ABC using fresh frozen or supernatant biopsy material.
2. The fact that additional biopsies will be obtained at the time of scheduled endoscopic procedure (or that biopsy material will be retrieved from the PCB if available).
3. The procedure for extraction of genetic material from previously fresh frozen, biobanked and supernatant biopsy material.
4. The fact that insufficient material may be available for comprehensive molecular profiling
5. That the participants will be referred to the MoST study for NGS, MDT review, assessment of clinical trial opportunities with MoST and ongoing follow up.
6. That NGS and MTB review may be performed prior to entry into the MoST study (e.g., by the VCCC) and MoST will be provided with these results for consideration of trials and ongoing follow up.
7. The nature of the TS0500 gene panel or equivalent and the fact that this may not be a NATA accredited test.
8. The process of the molecular tumour board review and dissemination of results to the participant's treating team.
9. The possibility that analysis of the biopsy material may indicate a possible germline mutation (~5%). This may require further testing for confirmation. If a germline mutation is confirmed the patients will be offered referral for genetic counselling. The discovery of germline mutation may also have implications for the patient's relatives.
10. The fact that participants and the treating doctor will be informed if a potentially targetable molecular phenotype is revealed.
11. That when a targeted treatment is identified and deemed appropriate by the treating team, an attempt will be made to access this treatment. This may involve:
    a. Access through the MoST trial platform
    b. Access through the Pharmaceutical Benefits Scheme
    c. Direct application to the relevant pharmaceutical company for compassionate access under the special access scheme
12. That targetable molecular phenotypes may be revealed, for which the appropriate therapy may not be able to be obtained. We hope that at least 10% of patients who are able to have comprehensive molecular profiling will be able to access targeted therapy.

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If the potential participant requires more time to consider their potential participation, a further appointment will be scheduled. Interpreting services will be provided as required. Written informed consent must be signed and dated by the subject, and signed and dated by the Investigator contemporaneously, prior to any study-specific investigations being performed.

4.7 Patient Registration

Participants must meet all the inclusion criteria and none of the exclusion criteria to be eligible for this study. There will be no exceptions or waivers made to these eligibility requirements at the time of registration. Registration should only occur after all screening assessments have been performed and the responsible investigator has both verified the subject's eligibility and signed the completed registration form. All enquiries about eligibility should be addressed by contacting the Coordinating Principal Investigator prior to registration.

Basic demographic data including the postcode of home address, year of birth, Aboriginal or Torres Strait origin, country of birth, what language did you speak as a child and what is the language you mainly speak at home will be collected in line with Cancer Australia reporting requirements

Once the registration process has been completed the participant will be assigned an individual study number at their local site to label their specimen and written confirmation of registration will be provided to the site through the study coordinators Once a patient/participant has been registered as eligible, this cannot be retracted. Participants have the right to withdraw their consent and be removed from the study at any point during the process.

Participants must be registered before comprehensive molecular profiling is performed.

5. Treatment Plan

5.1 Pre-treatment criteria

Prior to study enrolment, participants must undergo the following evaluations.

- Participants must have had a diagnosis of ABC or presumed ABC as determined by MDT review.
- Patients with non-diagnostic biopsies may still be included if they are deemed to have likely ABC by MDT review.
- It is noted that establishing a tissue diagnosis in this condition can be challenging and one of the secondary endpoints for this study is to assess the utility of a molecular diagnosis of ABC in this setting.
- The patients must have an adequate performance status (ECOG 0/1/2) and life expectancy of at least 3 months.

Patients who meet the inclusion and exclusion criteria will be enrolled in this trial and who undergo successful CMP will subsequently offered entry into the MoST study.

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5.2 Tissue acquisition

Study participants will be monitored to identify upcoming endoscopic procedures scheduled for the diagnosis and or management of ABC. These procedures include both EUS and cholangioscopy (via ERCP or PTC) for the purposes of diagnosis and ERCP for the purpose of biliary drainage.

At the time of the endoscopic procedure additional biopsies will be obtained depending on the nature of the procedure. If cholangioscopy is being performed to establish a diagnosis, additional biopsies will be obtained and immediately frozen, if an EUS-FNA is being performed an additional EUS-FNA will be obtained and frozen, and if ERCP without cholangioscopy is being performed for biliary stenting an EUS will be added to the procedure for the purpose of obtaining fresh tissue for CMP.

Blood (25mls in total) will be drawn for future germline analyses and exploratory studies at the time of the tissue biopsy via EUS or ERCP. The liquid media (Hanks' solution) that the tissue biopsy samples are placed into generates the supernatant. The supernatant will also be retrieved from the pathology department.

The specimens and peripheral blood will be transported to Monash Health for DNA and RNA extraction and long-term storage of residual supernatant and DNA/RNA after performance of CMP.

5.3 Molecular analyses

5.3.1 DNA/RNA extraction

DNA will be extracted from the cell free supernatant by QIAsymphony using Qiagen circulating DNA Kit (Qiagen, Germantown, MD) as per the manufacturer's guidelines. RNA will also be extracted although it is unclear whether supernatant will contain sufficient RNA to perform the TSO500 gene panel.

Irrespective of the source of the genomic material, the quality and quantity of DNA will be determined on a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Qualitative analysis of extracted supernatant DNA will be performed using Agilent High Sensitivity D1000 ScreenTape on the Agilent 2200 TapeStation system (Agilent, Santa Clara, CA) according to manufacturer's instructions. RNA will be quantified and assessed for fragmentation using a NanoDrop and Qubit Fluorometer (Life Technologies), and the Aligent Bioanalyzer (Aligent Technologies).

The quantity and quality of DNA and RNA recovered from each source/technique will be reported as part of this study.

5.3.2 NanoString gene panel

A minimum input of 50ng of total RNA will be used for processing of NanoString assays. Samples will be prepared using an nCounter GX Custom Codeset (201 genes), as per manufacturer's instructions.

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Briefly, prepared samples will be input into nCounter hybridisation reactions containing nanoString Reporter and Capture probes, and left to hybridise at 65°C for 16 hours on a thermal cycler before a temperature ramp down to 4°C. Samples will then be loaded onto nCounter SPRINT cartridges and processed on the nCounter SPRINT Profiler (NanoString Technolgies) in the Monash Biochemistry Imaging Facility.

5.3.3 Next Generation Sequencing (NGS)

NGS will be performed either at the VCCC (according to SOP) under the supervision of Professor Sean Grimmond).

5.3.4 Peripheral blood for germline mutation testing.

25mls of peripheral blood stored in the PCB may be used to assess for germline mutations if these are suspected based on family history or the results of comprehensive molecular profiling of the tumour. This will occur on the recommendation of the MTB. Blood will also be banked for later analysis to determine whether targeted or comprehensive molecular profiling of advanced biliary cancer can be performed using liquid biopsy

5.3 MTB review

MTB review will follow return of the NGS following variant curation and bioinformatic analysis. This will be performed at the VCCC under the direction of Sean Grimmond according to SOP.

5.4 Follow up

All participants who have successful CMP will be referred to the MoST study for potential access to clinical trials and ongoing follow up. It is expected that the vast majority of participants will provide consent to participate in MoST in which case there will be no need for further follow-up outside this programmed.

If participants refuse to participate in MoST they will be followed up at 12, 24 and 36 months. Follow up will be conducted by review of the medical record at their local site by the research coordinator at each participating site. The participants' medical record will be reviewed to assess for the therapies that have been administered. Overall survival will also be recorded. The patients' treating team may be contacted for further information if the medical record is insufficient.

5.5 Data storage

Data for this project will be stored securely at Monash University. Monash University implements a defence in depth approach to information security and employs a multitude of controls to protect our infrastructure and data. These controls are regularly audited to ensure they meet global best practices and are aligned with ISO 27001 security practices. Data collected will be stored on

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university managed secure and resilient infrastructure located in Australia that complies with all applicable data protection and privacy obligations.

6. Investigator Roles and Responsibilities

6.1 Participant screening and recruitment

Participant screening will be performed by the RA or study investigator at each site. The RA will forward the potential participants contact details to the study investigators at Monash Health at liaising with the participant's treating clinician. The RA will collate the participants details in relation to stage and MDT review. The study investigators at Monash Health will assess the suitability of the participant the inclusion and exclusion criteria and consent the participants for entry into the trial (potentially by telehealth for interstate patients)

6.2 Collection of additional biopsy specimens and peripheral blood

Study investigators are each site will be responsible for collection or additional biopsies at the time of routine endoscopic procedure. The study coordinator will be responsible for transport and storage of the additional biopsies at -80°C. The study coordinator will also be responsible for collecting the supernatant from routine biopsies from the pathology department prior to it being discarded and for storage of this material.

6.3 Retrieval and transport of biopsy specimens to Monash Health

The local study co-ordinator will arrange for additional snap frozen biopsies (and supernatant) to be transported on dry ice to Monash Medical Centre where specimens will be stored in Biobbank Victoria prior to DNA/RNA extraction.

6.4 DNA and RNA extraction

DNA and RNA extraction from the additional biopsy material will be performed at Monash Health / Monash University. ctDNA extraction from peripheral blood will be performed at Monash Health / Monash University.

NanoString expression analysis (secondary and tertiary endpoints) will be performed at Monash Health / Monash University by study investigators.

Excess supernatant, excess blood and excess DNA/RNA/ctDNA will be stored indefinitely in the PCB housed at Biobank Victoria.

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6.5 Comprehensive Molecular Profiling

Participants will have CMP performed on both the additional biopsies and supernatant if available.

With respect to the additional biopsy, participants may have CMP performed under the MoST trial according to standard protocols. Alternatively, the CMP may be performed either at the VCCC according to standard protocol or at the MHTP using the TSO-500 gene panel.

With respect to the supernatant, CMP will generally be performed by the MHTP. The results of the supernatant CMP will be provided to MoST if it reveals information beyond that of the additional biopsy.

6.6 Molecular Tumour Board Review

The Molecular Tumour Board will be conducted at the VCCC under the oversight of Sean Grimmond according to SOP.

6.7 Follow-up

The follow-up for this study will be performed by MoST according to standard protocols. Those who decline to be enrolled in MoST will have annual follow-up for 3 years.

6.8 Data Management

Basic demographic data including the postcode of home address, year of birth, Aboriginal or Torres Strait origin, country of birth, what language did you speak as a child and what is the language you mainly speak at home will be collected in line with Cancer Australia reporting requirements. The outcome of the DNA/RNA extraction and molecular analyses at the Monash will be stored on a secure REDcap database. Only sub-investigators and the principal coordinator will have access to the data stored on the REDCap database.The outcome of the comprehensive molecular profiling and the molecular tumour board review will be stored on a secure servers at the VCCC. The follow up data will be stored by MoST.

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7. Schedule of Assessments

	Screening visit	Scheduled endoscopic procedure for routine care	Molecular characterisation of tumour	Follow up (MoST Study)
Written Informed consent	**X**			
Inclusion / Exclusion Criteria	**X**			
ECOG Status	**X**			
Chemotherapy history	**X**			
Fresh Tissue acquisition at Endoscopy (or from Biobank[1])/ Retrieval of supernatant from pathology		**X**		
Peripheral blood for translational studies [2]		**X**		
Transport of Tissue and blood to Monash University		**X**		
DNA/RNA extraction			**X**[3]	
TSO500 gene panel			**X**[4]	
Varian curation and MTB review			**X**[4]	
Nanostring gene panel			**X**[3]	
Peripheral blood for germline assessment			**X/-**	
Treatment History Survival				**X**

Schedule of Assessment Footnotes:

1. Refer to PCB study manual for details on collection, handling and storage of tumour tissue for research purposes.

2. Refer to Laboratory manual for the collection, handling and processing of translational laboratory samples.

3. Performed at Monash Health / Monash University (Hudson Institute)

4. These steps may be performed under the auspices of the MoST study or by the VCCC or MHTP prior to referral to the MoST study.

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8. Outcomes, Endpoints, and other measures

8.1 Primary Endpoints

The overarching aim of this prospective cohort study is to determine the proportion of patients with ABC that can have comprehensive molecular profiling using fresh frozen endoscopic biopsy material obtained at the time of a routine diagnostic procedure.

Traditionally, biopsy material is either spread and fixed on cytology slides (cytology) or processed into formalin fixed paraffin embedded blocks for routine histological analysis (histology). However, this material is rarely suitable for comprehensive molecular profiling. Adequate RNA, in particular, is difficult to obtain from archival tissue which is important due the relatively high prevalence of targetable fusion genes in ABC. Therefore, in this cohort study we are exploring a novel approache to tissue acquisition for CMP, the use of additional snap frozen biopsies taken at the time of routine endoscopic procedures.

Therefore, the primary end point of this study is:

1. To determine the proportion of patients with ABC that can have comprehensive molecular profiling using fresh frozen biopsy material.

The biopsy procedure can be performed in one of two ways, either EUS-FNA (transluminal biopsy) or direct cholangioscopic biopsy (endoluminal). We also aim to compare the relative efficacy of these two approaches, (although obviously not in the same patient), if a sufficient number of patients are enrolled to allow a meaningful comparison between these two groups.

8.2 Secondary Endpoints

The secondary aims of this trial are to assess the potential clinical benefit provided by comprehensive molecular analysis. These include:

1. To determine the proportion of participants with ABC that can have treatment recommendations based on comprehensive molecular profiling
2. To determine the number of participants with ABC that have changes in their treatment based on comprehensive molecular profiling
3. To assess the utility of biopsy supernatant (cell free DNA/RNA that is normally discarded during the processing of routine biopsies) for CMP
4. To assess the sensitivity and specificity of a molecular diagnosis of ABC (as defined above).
5. To assess the quantity and quality of DNA and RNA derived from various types of fresh frozen and supernatant specimens.

8.3 Tertiary Endpoints

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The tertiary or exploratory end points are related to potential impacts of comprehensive molecular profiling and targeted therapy on overall survival. This trial is not powered to address these questions, rather it is an attempt to examine any potential impact to guide future studies. In addition, blood will be banked for later analysis to determine whether targeted or comprehensive molecular profiling of advanced biliary cancer can be performed using liquid biopsy. It is not intended that this will be performed as part of this trial.

8.4 Follow up

To minimise patient burden and to harness a platform that is already in existence, follow up for this trial will be conducted through the MoST study. If not followed up through MoST, then the participants will receive follow-up review at 12, 24 and 36 months

9. Adverse Events

The overall risk of death and complications from advanced biliary cancer is quite high and the risks of complications from endoscopic procedures is also quite significant (predominantly pancreatitis and cholangitis related to ERCP). This project involves the use of an additional biopsy taken during a routine endoscopic procedure for the diagnosis or management of patients with advanced biliary cancer. An additional biopsy does expose the participants to a very small risk of complications. With respect to EUS-FNA an additional pass of the FNA needle poses a very small risk of bleeding. With respect to endobiliary biopsy, there may be a slight increase in the risk of cholangitis due to the additional time required to take additional biopsies for comprehensive molecular profiling. Overall, the additional risk associated with the additional biopsies in this study is so negligible that it will be impossible to distinguish from the inherent risk of the procedure itself.

There is also a risk of psychological harms related to this project. These include the fact that the patient's biopsy material may not be adequate for comprehensive molecular profiling. Comprehensive molecular profiling may also reveal the likelihood of a germline mutation, which may have implications for both the patient and their family. However, screening for germline mutations is now recommended for cancer patients when there is a suspicion of an inherited cancer syndrome and it could therefore be considered part of routine clinical practice. Patients and their families will be offered access to genetic counselling if this occurs. There is also a risk of psychological harm if a patient is discovered to have a molecular phenotype that is amenable to personalised therapy, but this therapy is unavailable to them. The MTB will assist in advocating on behalf of patients and their clinicians to facilitate access to novel targeted therapies, however it is not possible to guarantee access to novel therapies.

If a potentially targetable phenotype is revealed by the molecular characterisation of ABC, it is possible that introduction of a targeted therapy may be associated with the risk of adverse events. However, this is outside the scope of this trial. As far as possible it is hoped that novel targeted therapies will be administered within the context of a separate clinical trial (e.g. the MoST study).

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9.1 Definitions

Adverse Event:

Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device.

NOTE: this definition includes events related to the additional endoscopic procedure if this is performed solely to obtain genetic material for the purposes of comprehensive molecular profiling.

Serious Adverse Event (SAE):

A serious adverse event as defined by ICH GCP is any untoward medical occurrence that at any dose meets any of the following conditions:

- Results in death;
- Is life-threatening (The subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalisation or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect;
- Medically significant events.

Important medical events that may not result in death or be life-threatening.

Hospitalisation may be considered serious when, based upon appropriate medical judgement,

They may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Hospitalisation:

Adverse events reported from studies associated with hospitalisations and prolongations of hospitalisations are considered as a serious untoward event. Any initial admission, irrespective of time frame, to a healthcare facility meets this criterion of 'serious'. Hospital admissions to note however, include the following circumstances:

- Rehabilitation Facilities
- Hospice facilities
- Nursing homes
- Routine emergency room admissions

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- Same day surgeries (as outpatient / same day procedures)

Hospitalisation or prolongation of hospitalisation with the following reasons will not be reported as a serious adverse event:

- Admission for treatment of a pre-existing condition not associated with the development of a new adverse event or with a worsening of the pre-existing condition (e.g., for work-up of persistent pre-treatment lab abnormality);
- Social admission (e.g., patient has no place to sleep);
- Administrative admission (e.g., for yearly physical exam);
- Protocol-specified admission during a study (e.g., for a procedure required by the study protocol);
- Optional admission not associated with a precipitating clinical adverse event (e.g., for elective cosmetic surgery);
- Pre-planned treatments or surgical procedures should be noted in the baseline documentation for the entire protocol and/ or for the individual patient;
- Admission exclusively for the administration of blood products.
- Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an adverse event. For example, an acute appendicitis that begins during the adverse event reporting period should be reported as the adverse event, and the resulting appendectomy should be recorded as treatment of the adverse event.

10. Study Management and Administration

Study Management

10.1 Training of Site Personnel

The Coordinating Principal Investigator (CPI) and relevant Principal Investigator will ensure that all appropriate training relevant to the clinical study is provided to all staff involved. Any new information regarding study management and safety updates will be provided to all staff involved immediately.

The Principal Investigator will maintain a record of all individuals involved in the study (Medical,

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Nursing and other staff on a Delegation of Authority log). Delegation logs will be maintained in Investigator Site Files and will be kept up to date by site staff for site monitoring and in the event of an audit.

10.2 Monitoring of the Clinical Study

During site initiation for the clinical study, the CPI / Study Delegates will review clinical investigation plan with site staff. This is an investigator-initiated study and therefore a Source Data Verification plan will be proposed to verify key points of the clinical study. Site staff will be required to redact and send requested information as per the Source Data Verification plan for remote monitoring. This will include, but not be limited to pathology reports and imaging reports.

10.3 Quality Assurance and Standardisation

The clinical study will incorporate a central review of radiologic imaging if there is any concern regarding the operability of patients with ABC on a patient to patient basis depending on the outcome of the MDT meeting.

Resectability will be defined according to NCCN Guidelines Version 5.2020 Hepatobiliary Cancers.

Access to biobank specimens will be provided according to SOP.

DNA/RNA extraction will be performed according to SOP.

The quantity and quality of DNA and RNA extracted will be assessed prior to comprehensive molecular profiling with the TSO500 gene panel.

CMP will be performed within MoST or by the VCCC (outsourced) or within the MHTP according to SOP.

10.4 Changes to Clinical Trial Protocol

Any substantial changes to the Clinical trial protocol will be required to undergo protocol amendment procedure. The amendment will require approval by relevant ethics committees and any other national regulatory authorities as applicable, before the implementation and adherence to new trial requirements. Monash University will distribute any subsequent amendments and new versions of the clinical trial protocol for independent ethics committee approval as per local regulatory requirements.

10.5 Protocol Deviations

Deviations from this clinical trial protocol are not permitted with rationale of patient safety and wellbeing or considered to be clinically appropriate. All protocol deviations should be recorded and from site initiation through to study close-out visit. All protocol deviations must be reported to Monash University and/or CPI. Approval may be obtained from CPI / Monash University for deviations; however, investigators will still be required to report and submit deviations to the ethics committee as per local guidelines.

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10.6 Ethical Considerations

It is mandatory that all considerations regarding the protection of human research participants be carried out in accordance with the clinical study protocol, Good Clinical Practice (GCP), ISO 14155: 2011, the ethical principles that have their origin in the Declaration of Helsinki, and all other applicable regulatory requirements by local guidance and law.

10.7 Informed Consent

Monash University will provide to Investigators a participant informed consent form that complies with the ISO 14155 guidelines and regulatory requirements and is considered appropriate for this study.

The Principal Investigator (according to applicable regulatory requirements) or a person designated by the Principal Investigator and under the Investigator's responsibility must comply with the terms of the informed consent process and must fully inform study participants of all pertinent aspects of the clinical study.

- Ensure each study participant is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study prior to any study procedures
- Ensure each study participant is notified that they are free to discontinue from the study at any time
- Ensure that each study participant is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each study participant and/or study participant's legally authorised representative provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the study participant
- Ensure that the Informed consent process, and version number is documented in the study participant source documentation, and notes that the study participant was given a copy of the Information and Consent forms to keep.
- Ensure that any incentives for study participants who participate in the study as well as any provisions for study participants harmed as a consequence of study participation are described in the informed consent form that is approved by an Ethics Committee.

10.8 Audits and Inspections

Authorised representatives of Monash University, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study related activities and

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documents, to determine whether these activities were conducted, and data were recorded, Analysed, and accurately reported according to the CIP, Good Clinical Practices (GCP), ISO 14155 and any applicable regulatory requirements. The Investigator will contact Monash University or CPI immediately if contacted by a regulatory agency about an inspection at the centre.

10.9 Discontinuation of Study

Monash University maintain and reserve the right to discontinue this study under conditions specified in the clinical study agreement.

10.10 Clinical Study Insurance

Clinical Study insurance will not be obtained for the study as comprehensive molecular profiling is recommended as part of routine care by the National Comprehensive Cancer Network (NCCN) guidelines.

Insurance and indemnity

The Australasian Gastro-Intestinal Trials Group (AGITG) as sponsor certifies that it has a liability insurance policy which covers the liability of each participating institution's Principal Investigator. This insurance policy is in accordance with local laws and requirements. The insurance of the Sponsor does not relieve the sites, investigators, or manufacturers of the study interventions of any obligation to maintain their own liability insurance policy as required by applicable law.

Conflict of interest

Any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this study will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed appropriately. Investigators are responsible for providing information on financial interests during the study

Publication Policy

The AGITG is committed to the responsible publishing of data from its clinical research program. The AGITG will manage the timely publication of results at a suitable journal and/or meeting. The authors of any publication will be involved in this process.

The AGITG will assess authorship eligibility according to the guidelines issued by the International Committee of Medical Journal Editors (Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication, October 2004). In line with these recommendations, authorship credit will be based on 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. All 3 above points should be met to qualify for authorship.

The lead author for any publication must be the Principal Investigator/Study Chair or a major contributor to the study and must have participated sufficiently in the work to take public

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responsibility for appropriate portions of the content. Publications and abstracts must be presented to investigators for review and approved prior to submission.

The Trial Management Committee (TMC) will appoint a Writing Committee to draft manuscript(s) based on the trial data. Manuscript(s) will be submitted to peer-reviewed journal(s). The first publication will be the report of the full trial results based on the main protocol using the study group name, with subsequent publications of data subsets in individual names based on contribution. The Writing Committee will develop a publication plan, including authorship, target journals and expected dates of publication. All publications must receive prior written approval from the TMC prior to submission.

10.11 Final Study Report and Publication Plan

At the conclusion of the study, a final study report will be compiled for submission to regulatory authorities. The report will be signed by the Coordinating Investigator(s), in addition to those compiling the document.

It is planned to publish the outcome of the study, and the Coordinating Investigator(s) will be nominated as the lead author for the study, along with any accompanying entities responsible for research conducted through this clinical study.

11. Statistical Considerations

This is a prospective cohort study to investigate feasibility of minimally invasive biopsy material to provide sufficient DNA/RNA to allow for comprehensive molecular phenotyping of advanced biliary cancer.

The co-primary endpoints of the study are to determine the proportion of patients with ABC that can have successful molecular analysis using either fresh frozen EUS-FNA or cholangioscopic biopsy material. We postulate that a significant proportion of patients who can have successful molecular phenotyping using fresh frozen could also have CMP from supernatant (cell free) biopsy material. The former may be more effective in enabling CMP but the latter has the benefit of posing no additional risk to the patient and may be more widely applicable as it does not required immediate storage on an additional biopsy.

We expect that 50 matched patients should be sufficient to establish the proportion of patients who can comprehensive molecular profiling with an additional biopsy and with cell free DNA/RNA obtained from routine diagnostic biopsies (“the supernatant”). Given that the expect incidence of targetable mutations in ABD is 30-40%, We have with a reasonable level of confidence that 50 patients will be sufficient to confirm this.

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Appendices:

Appendix I – List of Abbreviations

Abbreviation	Term
ABC	Advanced Biliary Cancer
BTC	Biliary Tract Cancer
CIP	Clinical Investigation Plan
CPI	Coordinating Principal Investigator
CRA	Clinical Research Associate
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DICOM	Digital Imaging and Communications in Medicine
ECOG	Eastern Cooperative Oncology Group
HREC	Human Research Ethics Committee
EUS	Endoscopic Ultrasound
FFPE	Formalin Fixed Paraffin Embedded
FNA	Fine Needle Aspiration
GCP	Good Clinical Practice
IB	Investigator Brochure
ICF	Informed Consent Form
ISO	International Standards Organization
MDT	Multidisciplinary Team
mL	Millilitres
mm	Millimetres
NATA	National Association of Testing Authorities
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
PI	Principal Investigator
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SIV	Site Initiation Visit
SoC	Standard of Care
TSO500	Trusight Oncology 500
UGICR	Upper Gastrointestinal Cancer Registry
WHO	World Health Organization

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