PERIOP-06

A Phase II Study of Perioperative QBECO Site Specific Immunomodulator (Qu Biologics®) in Patients with Metastatic Colorectal Adenocarcinoma within the Liver Undergoing Resection

Protocol Number: 4023

Principal Investigators: Dr. Rebecca Auer
Dr. Paul Karanicolas

Regulatory Sponsor: Qu Biologics

Funding Agency: Qu Biologics

Investigational Product: QBECO Site Specific Immunomodulator (SSI)

Coordinating Centre: Centre for Clinical Trial Support (CCTS)
Sunnybrook Research Institute
C8-2075 Bayview Avenue
Toronto, ON M4N 3M5
Canada
Email: PERIOP-06@sunnybrook.ca

SPONSOR STATEMENT OF COMPLIANCE

This study will comply with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human use Good Clinical Practice E6 (ICH-GCP), World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Participants, as well as applicable regulatory and institutional requirements.

Personnel listed below are authorized to sign the protocol and any subsequent protocol amendments on behalf of the sponsor:

Name:
(Print) _____

Title:
(Print) _____

Signature: _____

Date of Approval:
(yyyy-mmm-dd) _____

PROTOCOL SIGNATURE PAGE

I have read this protocol in its entirety and its appendices. I agree to comply with the requirements of the study protocol and procedures for data recording/reporting and acknowledge my responsibility for the well-being of each research participant, and to ensure that all persons involved in study activities are adequately informed about the protocol, the investigational product, and their trial-related duties. The signature below constitutes the agreement to conduct this study in accordance with the REB approved protocol, GCP, and applicable regulatory requirements, including confidentiality, ethical guidelines and regulations regarding the conduct of research in humans.

Qualified Investigator:

Name:
(Print) _____

Title & Institution:
(Print) _____

Signature: _____

Date of signature:
(yyyy-mmm-dd) _____

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

The following abbreviations describe terms, documents, and study personnel used in the conduct of this study protocol.

ADR	Adverse Drug Reaction
AE	Adverse Event/Adverse Experience
CBC	Complete Blood Count
CEA	Carcinoembryonic Antigen
CIOMS	Council for international Organizations of Medical Sciences
CTCAE	Common Terminology Criteria for Adverse Events
CCTS	Centre for Clinical Trial Support
CRF	Case Report Form
CRLM	Colorectal Liver Metastases
CT	Computed Tomography
ctDNA	Circulating Tumour DNA
DSMB	Data and Safety Monitoring Board
DVT	Deep Vein Thrombosis
FFPE	Formalin-fixed paraffin-embedded
GI	Gastrointestinal
H&E	Hematoxylin and Eosin
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
IBD	Inflammatory Bowel Disease
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICU	Intensive Care Unit
IP	Investigational Product
ICF	Informed Consent Form
IV	Intravenous
GCP	Good Clinical Practice
LSIR	Local Skin Immune Response
MDSC	Myeloid-Derived Suppressor Cell
MedDRA	Medical Dictionary for Regulatory Activities
MODS	Multiple Organ Dysfunction Syndrome
NK	Natural Killer

OS	Overall Survival
PFS	Progression-Free Survival
PHI	Personal Health Information
PHIPA	Personal Health Information Protection Act
PI	Principal Investigator
POD	Postoperative Day
QoR	Quality of Recovery
REB	Research Ethics Board
RCT	Randomized Controlled Trial
SAE	Serious Adverse Event/Serious Adverse Experience
SC	Subcutaneous
SOP	Standard Operating Procedure
SSI	Site Specific Immunomodulator
SUADR	Serious and Unexpected Adverse Drug Reaction
TME	Tumour Microenvironment
VTE	Venous Thromboembolism
WOCBP	Women of Childbearing Potential

PROTOCOL SUMMARY

Protocol Title	A Phase II Study of Perioperative QBECO Site Specific Immunomodulator (Qu Biologics®) in Patients Undergoing Resection of Metastatic Colorectal Adenocarcinoma within the Liver
Protocol Number	4023
Phase	II
Study Design	Multicentre, blinded, randomized, placebo-controlled trial
Study Duration	7 years
Setting	Tertiary referral centres in Canada
Sample Size	115 participants
Main Inclusion Criteria	Adult patients planned to undergo resection of colorectal liver metastases for complete clearance of all visible disease
Primary Outcome:	Progression-free survival
Secondary and Correlative Outcomes:	ctDNA, side-effect profile, quality of recovery, 5-year overall survival, and blood-based measurements of postoperative immune dysfunction
Investigational Product and Planned Use	QBECO is a site specific immunomodulator (SSI) designed to promote innate immune responses in the gastrointestinal tract and related organs, including the liver. QBECO or placebo will be administered according to the following regimen: 0.1mL SC q2days for 11-120 days preoperatively, and 41 days postoperatively.
Statistical Analysis:	Descriptive statistics, time-to-event analysis, and regression modelling

1 KEY ROLES AND CONTACT INFORMATION

Principal Investigators:	Dr. Paul Karanicolas, MD, PhD, FRCSC, FACS Sunnybrook Health Sciences Centre 2075 Bayview Avenue, Room T2-16 Toronto, ON M4N 3M5 Phone: 416-480-4774 Fax: 416-480-6002 Email: paul.karanicolas@sunnybrook.ca Dr. Rebecca Auer, MD, MSc, FRCSC The Ottawa Hospital, General Campus 501 Smyth Road, CCW 1617 Ottawa, ON K1H 8L6 Email: rauer@toh.ca
Regulatory Sponsor:	Qu Biologics 4475 Wayburne Drive, Suite 305 Burnaby, BC V5G 4X4
Academic Research Organization:	Centre for Clinical Trial Support (CCTS) Sunnybrook Research Institute C8-2075 Bayview Avenue Toronto, ON M4N 3M5
Primary Funding Agency:	Qu Biologics 4475 Wayburne Drive, Suite 305 Burnaby, BC V5G 4X4
Additional Supporting Agencies:	Foundation Medicine, Inc. 150 Second Street Cambridge, MA 02141 USA Personal Genome Diagnostics Inc 3600 Boston St., Suite 10 Baltimore, MD 21224 NKMAX 1172 Dolma-ro Bundang-gu, Seongnam-si, Gyeonggi-do 13605 Republic of Korea

Clinical Laboratory:	Dr. Rebecca Auer The Ottawa Hospital, General Campus 501 Smyth Road, CCW 1617 Ottawa, ON K1H 8L6

2 INTRODUCTION

This study document is a protocol for research involving human participants. This study is to be conducted according to Canadian and international standards, and in compliance with the protocol, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice E6 (GCP), World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Participants, as well as applicable regulatory and institutional requirements and research policies.

2.1 Background

Surgical trauma induces a profound suppression of immune function that occurs within hours, lasts for days to weeks, and is directly correlated with the severity of the surgical procedure.[1] Postoperative immune suppression has serious implications in cancer, where it has been shown to contribute to cancer regrowth and metastases.[2–6] The significance of surgery-induced immune suppression in patients with cancer presents a critical target for future therapies.

The mechanism of postoperative immune suppression is largely mediated by natural killer (NK) cell dysfunction. NK cells are innate lymphocytes that play a pivotal role in immune surveillance and clearance of metastases. In response to surgical trauma, the bone marrow is stimulated to rapidly release myeloid cells into the blood.[7,8] This "emergency myelopoiesis" has evolved to protect the host, however, some of these myeloid cells have immunosuppressive properties. Studies from Dr. Auer's lab have demonstrated that these myeloid-derived suppressor cells (MDSCs) are responsible for postoperative immunosuppression, including NK cell dysfunction, and the resulting proliferation of cancer metastases.[3]

In addition to surgical trauma, cancers themselves induce NK cell dysfunction, which is significant and measurable across cancer types.[3,4,9] However, Dr. Auer's lab has shown that the magnitude of cancer-induced NK cell dysfunction pales in comparison to NK cell dysfunction following surgical stress.[9] Most striking is that once suppressed, no amount of stimulation in *in vitro* NK cell assays can recover the defect and restore NK cell effector functions.[2,3] However, preoperative hyperactivation, while unable to reverse the effects of surgery, can attenuate them.[2,3,9,10]

Perioperative trained immunity is hypothesized to prevent or attenuate the effects of postoperative immune suppression. Trained immunity is a process whereby exposure to microbial stimuli induces long lasting epigenetic and functional changes in innate immune cells such as NK cells. These changes occur predominantly in the bone marrow, where microbial modulation of myelopoiesis results in the production of epigenetically modified macrophages better able to address the next immunological threat, whether that be another pathogen or cancer.[11,12] Following immunological training, both myeloid and NK cells have enhanced cytokine production in response to stimuli with associated improved cytotoxicity.[13] Given the postoperative period is associated with the release of MDSCs leading to NK cell dysfunction, preoperative innate immune training may prevent or attenuate the negative immune sequelae of surgery. However, there are no available cancer treatments directed at innate immune training, nor have any available therapies proven effective in alleviating postoperative immune suppression.

2.2 Site Specific Immunomodulators

Qu Biologics is a Canadian biotechnology company that has developed a novel class of immunomodulators, called Site Specific Immunomodulators (SSIs). They are designed, in the context of cancer, to induce trained immunity in organs at risk for micrometastases. The anti-cancer effects of SSIs are mediated through a number of mechanisms. SSIs relieve the immune suppression that dominates the tumour microenvironment (TME), make cancer cells more visible to the immune system through upregulation of stress-induced molecules such as NKG2D ligands, recruit M1 macrophages into the TME, down-regulate inhibitory molecules such as PD-1 and PD-L1, and enhance NK cell cytotoxicity.[14]

QBECO is an SSI formulated from inactivated *E. coli* bacteria that is specifically designed to target pathologies of the gastrointestinal (GI) tract and related organs, such as the liver. This trial will test the hypothesis that in patients undergoing resection of colorectal liver metastases (CRLMs), perioperative treatment with QBECO will attenuate the postoperative immune suppression and will improve progression-free survival (PFS).

2.2.1 SSI Preclinical Data

Preclinical studies found that perioperative administration of an SSI designed to target lung pathologies (QBKPN) attenuated pulmonary metastases and improved NK cell function in the postoperative period (unpublished data from Dr. Auer's lab). The efficacy of QBKPN was not limited to lung cancer cell lines and demonstrated reduced tumour burden in models of lung metastases for melanoma. These unpublished data from Dr. Auer's lab provided proof-of-concept for the use of SSIs in cancer.

With regards to QBECO, preclinical studies have demonstrated reduced formation of colorectal cancer and prolonged survival in murine models of metastatic disease.[15] The immune response following administration of QBECO was directed to the GI and peritoneal regions, corresponding to the endogenous niche for *E. coli*. In a murine model of metastatic colorectal cancer (MC38 in C57BL/6 mice), perioperative QBECO resulted in a significant reduction in hepatic metastases (unpublished data, Dr. Auer's lab).

In terms of preclinical safety of QBECO, no adverse events (AEs) or notable toxicities in mice were observed after 28 days of therapy with 1,168 times the dose, by body weight, to be used in this study. Further details regarding these safety data are provided in the Investigator's Brochure (IB).

2.2.2 SSI Clinical Data

QBECO SSI is an inactivated whole-cell bacterial vaccine. Inactivated whole-cell bacterial vaccines have been in use for almost 150 years, including ongoing use of whole-cell inactivated *B. pertussis* vaccine in millions of infants annually. The side effect profile of this class of drugs is well characterized and understood, including:

- Risk of anaphylaxis: Rare; particularly rare with inactivated whole-cell bacterial vaccines like QBECO that have no excipients.
- Exotoxin related risk: Washing steps (x3) in QBECO manufacturing process eliminates this risk.
- Endotoxin systemic immune activation (e.g., pyrexia) due to high free endotoxin levels: The QBECO manufacturing process results in levels of free endotoxin in QBECO dosing that is orders of magnitude below levels that induce pyrexia/systemic immune activation, as described below.

For example, *B. pertussis* vaccine, administered to infants, has an average free endotoxin dose of approximately 60,000 Endotoxin Units (EU)/ml and is administered as a 5 mL dose = 300,000 EU per dose. The QBECO clinical lot has a free endotoxin dose of approximately 3,350 EU/mL and is administered as a 0.1 mL dose = 335 EU per dose, which is ≈ 900 times lower. Providing further margins of safety, (1) risk of endotoxin systemic immune activation is based on EU/Kg body weight and thus, a 335 EU dose in adults is far safer than a 300,000 EU dose in infants, and (2) QBECO is administered subcutaneously, which is significantly less likely to cause endotoxin systemic immune activation than intramuscular injection, which is the method of administration of the *B. pertussis* vaccine.

QBECO manufacture and formulation is based on other inactivated whole cell bacterial products that are or were commercially available for more than 40 years, such as Mixed Respiratory Vaccine (Bayer Corporation, Hollister-Stier Laboratories, and Stallergenes) and Polyvaccinum Forte (Biomed, Poland). As with QBECO SSI, these inactivated whole-cell bacterial vaccines were manufactured by cultivation, inactivation, washing with saline, and resuspension in saline, with a similar concentration, dose (up to 0.4 mL, which is 4x higher than the PERIOP-06 QBECO dose), dose frequency, and treatment duration, and were used for the treatment of relatively benign conditions such as rhinitis, sinusitis, bronchitis, and cystitis, providing historic precedent of safety. The free endotoxin level of one of these commercially used products, Polyvaccinum Forte, which contains inactivated whole-cell *E. coli* in addition to other inactivated bacterial species, was tested, using the endotoxin assay (Endosafe [Charles River]) used in QBECO release and stability testing, and was found to be 24,000 EU/mL, which is ≈ 7x higher than the free endotoxin level of PERIOP-06 QBECO clinical lot (i.e., 3,350 EU/mL), thus, affording QBECO clinical lot additional significant margins of safety.

The half-life of free endotoxin in humans is 4-5 minutes with 100% cleared after 30 minutes and thus, there is no cumulative effect on the level of free endotoxin with the every second day dosing schedule used in this study.[16]

Over 360 patients have been treated with SSIs in Phase I/II trials and compassionate use programs. SSIs have been well tolerated by patients with >90% compliance rates and favorable side effect profiles to date that are similar to those of traditional whole-cell inactivated vaccines.

QBKPN SSI has been administered to a total of 111 patients with advanced lung cancer or lung metastases, including 105 patients in a compassionate use program and 6 patients in an open-label, single-arm Phase 1/2 clinical study of 6 adults with recurrent lung adenocarcinoma.[14] A retrospective case-matched analysis (n = 7 matched cases) of patients with advanced lung adenocarcinoma in the compassionate use program found a 14-month median survival advantage associated with QBKPN treatment. In the Phase 1/2 study (n = 6), all patients demonstrated stable tumour burden during the 12 weeks of QBKPN treatment and there was a trend towards decreased proportion of circulating immunosuppressive PD-1 and PD-L1 positive myeloid cells, indicative of immune activation. No serious adverse events (SAEs) attributed to QBKPN treatment occurred in the total of 111 participants treated.

QBECO SSI has been administered to a total of 208 patients, including 121 patients in compassionate use programs in oncology (n = 109), Crohn's disease (n = 10), and ulcerative colitis (n = 2), and 87 patients in Phase 1/2 inflammatory bowel disease (IBD) clinical trials in Crohn's disease (n = 76) and ulcerative colitis (n = 11).

In the oncology compassionate use program, QBECO SSI was well tolerated with no severe adverse events attributed to QBECO treatment in the 109 patients treated. A retrospective case-matched analysis (n = 9 matched cases) of patients with advanced colorectal adenocarcinoma in the compassionate use program found a 6-month median survival advantage associated with QBECO treatment.

In IBD, it was hypothesized that QBECO treatment would activate a gastrointestinal (GI) tract-specific innate immune response, leading to restoration of productive innate immunity, re-establishment of barrier function, clearance of bacterial infection, restoration of autophagy, and reduced IBD activity. The first IBD trial (n = 11 subjects with moderate-to-severe ulcerative colitis treated with QBEC0 SSI every second day for up to 52 weeks) found 8 of 11 subjects (73%) experienced clinical improvement as measured by Mayo score (≥ 3 point decrease), 6 of 11 subjects (55%) experienced endoscopic improvement, and 6 of 11 subjects (55%) experienced histological improvement.[17] The second IBD trial (n = 68 subject randomized placebo controlled study in subjects with moderate-to-severe Crohn's diseases treated with QBECO or placebo every second day for up to 16 weeks) found greater improvement in Crohn's disease activity index (CDAI) score in QBECO treated subjects than in subjects treated with placebo.[18] The third IBD trial (n = 20 subject open-label study in subjects with moderate-to severe Crohn's disease treated for up to 52 weeks) demonstrated evidence of endoscopic and histological response, as well as clinical symptom response and maintenance over the 52-week treatment period. In these three IBD trials, QBECO was again well tolerated, with the majority of AEs being mild to moderate. Local skin immune response at the site of injection and mild transient fatigue were the most commonly reported AEs. Safety data from these clinical trials are available in the IB, along with a detailed description of the pharmacology, efficacy, and safety of QBECO SSI.

Collectively, these data support the safety and potential efficacy of SSI treatment to overcome the immune suppression that enables cancer growth and metastases.

2.3 Rationale

This multicentre, blinded, phase II, randomized, placebo-controlled trial will evaluate the effect of QBECO on PFS in patients undergoing resection of CRLMs. This trial is motivated by the promising preclinical and clinical data supporting the safety and efficacy of QBECO in attenuating postoperative immunosuppression and the resulting proliferation of cancer.

Patients undergoing resection of CRLMs were chosen as the study population due to their survival characteristics and potential to benefit from the intervention. Approximately 25-30% of patients diagnosed with colorectal adenocarcinoma develop liver metastases during the course of their disease.[19–21] Surgery is the only potentially curative treatment for patients with liver metastases, however following liver resection, 75% of patients will still experience a recurrence of their cancer.[22] Three-year PFS is estimated to be approximately 42% in patients undergoing CRLM resection.[22] Neoadjuvant and adjuvant systemic therapies have demonstrated improvements in PFS for patients with resectable CRLMs, however a clear overall survival (OS) benefit has not been shown.[22–24] The high incidence of disease progression, yet noteworthy potential for cure, make patients undergoing resection of CRLM an ideal population for investigation. If this trial demonstrates improved cancer outcomes, SSIs have the potential to be transformative in treating all patients undergoing cancer surgery.

Given the clinical safety data to date in the 208 subjects treated with QBECO, the broad safety margins in non-clinical toxicology studies demonstrating no notable toxicity using doses up to 1,168 times (by body

weight) the QBECO dose of this study, the preclinical data demonstrating that QBECO treatment reduces metastases in a peri-operative colorectal cancer liver metastases model, and the urgent need to improve outcomes in stage 4 colorectal cancer patients undergoing resection of liver metastases for which there are no current adjuvant treatments that improve overall survival, it is likely that QBECO has a favorable risk-benefit profile in this study population.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of this randomized controlled trial (RCT) is to determine if QBECO administered perioperatively can improve PFS in adult patients undergoing resection of CRLMs for complete clearance of metastatic disease.

3.2 Secondary Objectives

Secondary objectives will be to:

1. Determine the effect of QBECO on the frequency and kinetics of clearance (and recurrence) of circulating tumor DNA (ctDNA) in the postoperative period and further evaluate the use of ctDNA as part of ongoing surveillance.

2. Determine the side-effect profile of perioperative QBECO. This will include an assessment of AEs and postoperative complications, and whether the administration of QBECO delays or prevents patients from proceeding to surgery.

3. Determine the effect of QBECO on patient-reported quality of recovery (QoR).

4. Determine the effect of QBECO on 5-year OS.

3.3 Correlative Objectives

Correlative objectives may be to:

1. Determine the effect of QBECO on immune phenotype and dysfunction in the perioperative period, as measured by phenotypic and functional NK cell and myeloid cell assays, such as cytokine secretion, cytotoxicity, and flow cytometry.

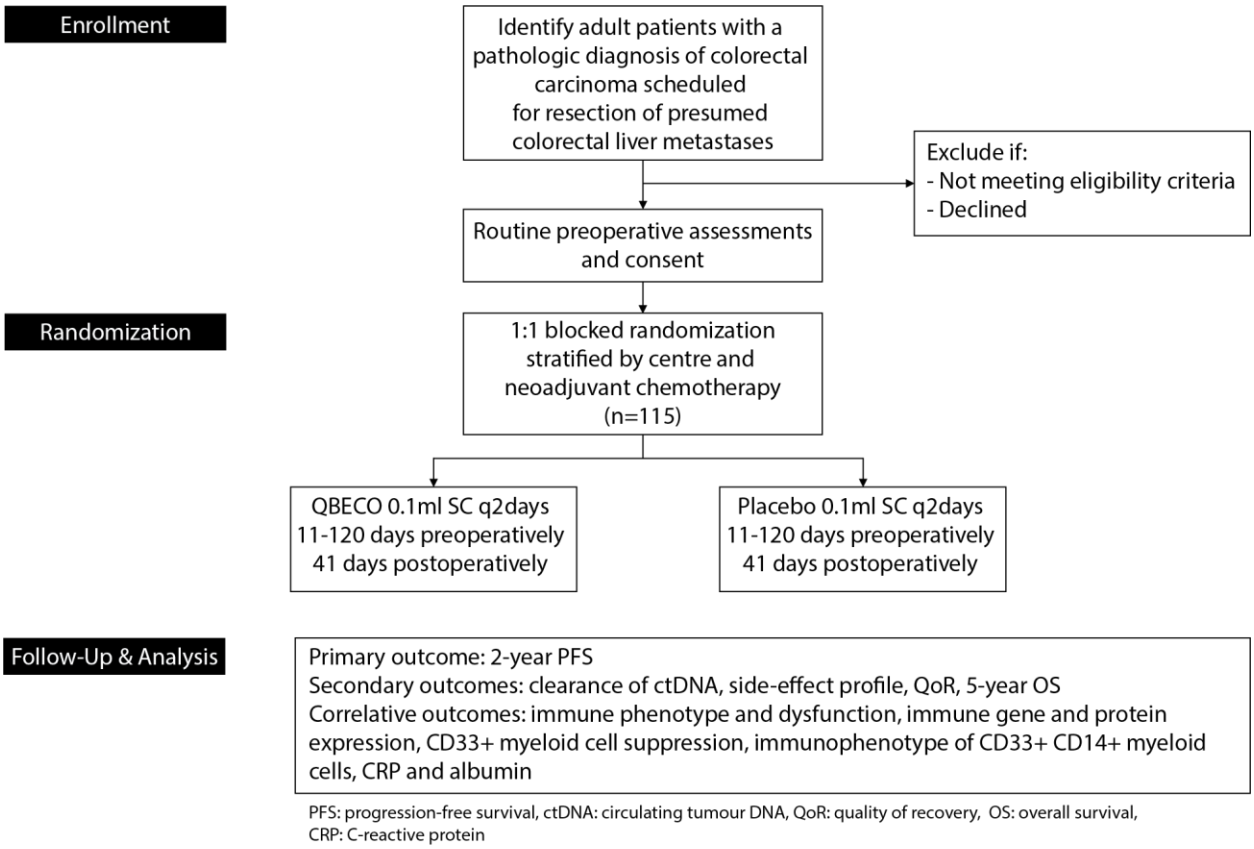
2. Determine the effect of QBECO on immune gene and protein expression in resected hepatic metastases and in the blood during the perioperative period using techniques such as immunohistochemistry, RNA sequencing, extracellular and intracellular flow cytometry and mass spectrometry.

4 STUDY DESIGN

4.1 General Design

This will be a multicentre, phase II, blinded, parallel group RCT. Approximately 115 participants will be randomized 1:1 to receive QBECO or placebo. Participants will be followed for 5 years after surgery. The trial design schematic is presented below:

Figure 1: Study Schema



PFS: progression-free survival, ctDNA: circulating tumour DNA, QoR: quality of recovery, OS: overall survival, CRP: C-reactive protein

4.2 Primary Outcome/Endpoint

The primary outcome will be the PFS rate. PFS will be defined as the time from enrollment to the first event that is either locoregional recurrence (of the resected primary colorectal adenocarcinoma or the resected ± intraoperatively ablated metastatic disease), distant metastases, or death from any cause. *Section 5* further defines PFS and its measurement.

4.3 Secondary Outcomes/Endpoints

The following list constitutes the secondary outcomes and includes a brief rationale for their measurement.

1. **Clearance of ctDNA.** A substantial body of research has demonstrated associations between ctDNA and cancer progression.[25,26] Timing of measurement will be at baseline (which will occur within 28 days prior to the first dose of Investigational Product (IP) preoperatively on the day of surgery, and postoperatively. A sample of fresh tumour tissue will be obtained from a representative hepatic tumour and from the primary colorectal tumour, if resection occurs at the same operation as the liver resection, along with a sample of normal adjacent tissue (one of each type per surgical specimen) to tailor the ctDNA assay to the individual participant's tumour profile. If fresh tumour tissue is not available from the resected specimen at the time of the surgery, archival tissue will be requested. If there is insufficient material from the fresh and archival tissue to undertake the ctDNA assay, tumour tissue from a prior resection or biopsy of the participant's colon cancer will be requested. If disease progression is detected, ctDNA will also be measured at that time.

2. **Side-effect profile of QBECO.** This will include an evaluation of AEs and postoperative complications in the treatment and control groups. AEs will be measured through the procedures specified in *Section 9.* A postoperative complication will be defined as any deviation from the normal postoperative course.[27] All clearly related signs, symptoms, and abnormal diagnostic procedures should be recorded in the source document and should be grouped under one diagnosis. The deviation must be possibly or probably related to the surgery and must occur postoperatively. The modified Clavien-Dindo system will be used to define and classify postoperative complications.[27] Complications of particular interest will be evaluated individually for associations with the intervention and include the following:

    - Surgical site infection. This will be stratified according to the Centers for Disease Control and Prevention National Healthcare Safety Network definitions of superficial incisional, deep incisional, organ/space surgical site infection.[28]
    - Postoperative hemorrhage requiring transfusion, radiologic intervention, or surgical intervention.
    - Bile leak. This will be defined and graded according to the International Study Group of Liver Surgery classification.[29]
    - Post hepatectomy liver failure. This will also be defined and graded according to the International Study Group of Liver Surgery classification.[30]
    - Venous thromboembolism (VTE). This will be defined as either a deep vein thrombosis (DVT) or pulmonary embolism. DVT may be diagnosed by either ultrasonography or contrast venography. Pulmonary embolism may be diagnosed by either a high probability V/Q scan, positive pulmonary angiogram, or a CT demonstrating an intraluminal filing defect in a pulmonary vessel.

3. **Quality of recovery,** as measured by the QoR-40. The QoR-40 is a validated tool designed to measure the early postoperative health status of patients.[31,32] The tool has been widely used as a patient-reported outcome measure of recovery after surgery and has been translated into multiple languages.[33–36] Timing of measurement will be at baseline (which will occur within 28 days prior to the first IP dose on postoperative day (POD) 4 (±1), and 6 weeks postoperatively. The survey may be administered in person or virtually by telephone or video call.

4. **Five-year overall survival**. This will be measured from date of enrollment to the date of death.

4.4 Correlative Outcomes

The following list constitutes correlative outcomes. These outcomes will be optional depending on funding and trial results.

1. **Immune phenotype and dysfunction as measured by functional assays.** Dr. Auer's lab has demonstrated significantly impaired NK cell function, including cytokine secretion and cytotoxicity in response to surgical stress.[9] The laboratory has also demonstrated that innate immune cells, including myeloid cells, have a differential cytokine secretion profile following surgery. SSIs have been shown to induce a proinflammatory cytokine profile in innate immune cells. We may use cytokine release assays to determine the effect of surgery on immune cell cytokine secretion and cytotoxicity assays to measure tumour cell killing. Timing of measurement will be at baseline (which will occur within 28 days prior to the first IP dose preoperatively on the day of surgery, and postoperatively. If disease progression is detected, the outcome may also be measured at that time.

2. **Immune gene and protein expression as measured by mRNA expression levels and protein levels**. Immune cells from the blood and/or the tumour microenvironment may be evaluated using RNA sequencing, immunohistochemistry, or mass spectrometry to determine the effect of SSI on gene expression at baseline and in response to stimulation. Flow cytometry for intracellular and extracellular proteins may be performed on frozen or fixed lymphocytes for immunoprofiling. Plasma will also be collected and may also be analyzed. Timing of measurement will be at baseline (which will occur within 28 days prior to the first IP dose, preoperatively on the day of surgery, and postoperatively. If disease progression is detected, the outcome will also be measured at that time.

3. **C-reactive protein (CRP) and serum albumin.** There is evidence to suggest that markers of systemic inflammation are associated with worse outcome in advanced cancers.[37,38] Specifically, elevated preoperative CRP and hypoalbuminemia may be prognostic of worse survival after resection of CRLM.[39] Alternatively, these levels may be increased with SSI administration and be associated with a better prognosis and postoperative recovery. Preoperative CRP and Albumin may be measured in this trial and assessed for associations with survival and postoperative outcomes.

Participants enrolled at The Ottawa Hospital will be evaluated for the same secondary and correlative outcomes as the other study sites with the potential for additional measurement. Participants at The Ottawa Hospital will also be evaluated for the additional correlative outcome on CD33+ cells, which can only be evaluated when the cells are processed fresh. These assay is described below:

4. **CD33+ myeloid cell suppression.** CD33+ myeloid cells contribute to postoperative immune suppression and NK cell suppression.[3] An NK cell killing assay will be performed to measure CD33+ suppression of NK cells, when possible. This will be measured for participants enrolled at the Ottawa Hospital. This outcome will not be measured for participants from other centres. Timing of measurement will be at baseline (which will occur within 28 days prior to the first IP dose and postoperatively. If disease progression is detected, the outcome will also be measured at that time.

5 DEFINING AND MEASURING PFS

As PFS is the primary outcome of this study, it is vital that it is precisely defined and documented.

5.1 Definitions

PFS will be defined as the time from enrollment to the first event that is either locoregional recurrence (of the resected primary colorectal adenocarcinoma or the resected ± intraoperatively ablated metastatic disease), distant metastases, or death from any cause. The identification of a new primary colorectal adenocarcinoma or other primary cancer will not be considered an event. Loss to follow-up will be censored. This definition of PFS was chosen to align with a consensus agreement on the definition of relapse-free survival in the context of adjuvant colorectal cancer studies.[40] "Progression" was selected as a more appropriate label for events than "relapse" given participants in this trial will have metastatic disease at the time of enrollment.

The following additional occurrences will be considered events at the date of surgery: (1) the identification of extrahepatic metastases at the time of the operation that are not resected or (2) if the participant does not undergo resection or ablation of all known disease during the operation (unless the rationale for this is a complete clinical response). Events will not include the intraoperative identification of previously undocumented liver metastases if the metastases are resected or ablated at the time of surgery.

Distinguishing a new primary colorectal adenocarcinoma from a recurrent primary will be at the discretion of the medical care team and investigators.

5.2 Measuring PFS

PFS will be measured from the date of enrollment.

Disease recurrence is defined as the clinical presence of cancer that has either been confirmed by biopsy, has had treatment initiated, or is documented in the treating physician's notes. The date of disease recurrence will be defined as the date of the first suspicious record of the malignant lesion but the date cannot be before the date of surgery.

For participants who do not undergo resection or ablation of all known disease (Including any extrahepatic metastases identified intraoperatively, the event date will be the date of surgery (unless the rationale is a complete clinical response).

For participants who, in retrospect, had extra-hepatic disease present before surgery that was not recognized, the date of progression will be defined as the date that the lesion in question progressed in size or became apparent to the clinical team

6 PARTICIPANT SELECTION AND WITHDRAWAL

We will enroll 115 participants as explained by the sample size calculations in *Section 11.2*. Participant recruitment is anticipated to take approximately 2 years. The subsequent sections describe the inclusion and exclusion criteria that must be met at the time of enrollment. Participants will not be excluded on the basis of any neoadjuvant chemotherapy or planned adjuvant chemotherapy.

6.1 Inclusion Criteria

Each participant must meet all the following inclusion criteria:

1. Adults aged 18 years or older at time of enrollment.
2. Pathologic diagnosis of colorectal carcinoma with clinical diagnosis of liver metastases.
3. Planned to undergo resection of liver lesions for complete clearance of all visible metastatic disease. This will include those who may undergo synchronous resection of the primary colorectal cancer and/or those who may receive a combination of surgery and ablation to treat all lesions.
4. Computed Tomography (CT) of the chest, abdomen, and pelvis with intravenous (IV) contrast within 6 weeks prior to enrollment.
5. MRI of the liver within 6 weeks prior to enrollment *OR* within 6 weeks prior to starting neoadjuvant chemotherapy OR during neoadjuvant chemotherapy (for patients treated with chemotherapy).
6. Planned to receive the last dose of neoadjuvant chemotherapy at least 25 days prior to surgery (for patients treated with neoadjuvant chemotherapy).
7. Agree to comply with the contraceptive requirements of the protocol when applicable (see Appendix B).
8. Willing and able to either perform subcutaneous injections according to the study protocol, or receive the injections from a caregiver delegated by the participant.
9. Able to provide informed consent or has a substitute decision maker capable of providing consent on their behalf.

6.2 Exclusion Criteria

All participants meeting any of the following exclusion criteria at baseline will be excluded from participation in this study:

1. Prior or current evidence of extrahepatic metastases. Patients with small (< 1.0 cm) indeterminate pulmonary nodules may be included at the investigator's discretion.
2. Prior hepatic arterial infusion or embolization. Prior portal vein embolization, ablation, or liver resection are permitted.
3. Patients with any invasive cancer history other than colorectal cancer in the last 5 years. In situ disease (e.g., melanoma in situ, ductal carcinoma in situ of the breast) or non-melanoma skin cancers are permitted.
4. Patients with a documented history of clinically severe autoimmune disease or a syndrome that requires systemic steroids or immunosuppressive agents. This includes patient requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalent, or depot corticosteroids in the 6 weeks before enrollment) or immunosuppressant drugs (such as azathioprine, tacrolimus, cyclosporine, etc.) within the 14 days prior to enrollment or a reasonable

expectation that the patient may require such treatment during the course of the study. Inhaled or topical or inter-articular steroids, and adrenal replacement steroid doses ≤ 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease. Steroids used for premedication prior to chemotherapy or as part of a chemotherapy regimen are allowed.

5. Patients with known active human immunodeficiency virus (HIV), Hepatitis B, or Hepatitis C infections.
6. Pregnant patients or those who are nursing an infant.

6.3 Participant Recruitment

Participants will be recruited at each of the participating sites. A member of the circle of care will introduce the study to potential participants and ask if they are willing to discuss the study with a member of the research team. A member of the research team will obtain informed consent. The participant will be considered enrolled after providing informed consent.

Patients must have an anticipated date of surgery of at least 11 days from enrollment, since participants with anticipated dates of surgery less than 11 days from enrollment would not be able to receive the minimum of 6 preoperative doses of IP if enrolled.

6.3.1 Randomization Procedures

Randomization will occur using a secure web-based system. Participants will be randomized 1:1 in permuted blocks to receive either QBECO or placebo according to the dosing schedule outlined in *Section 7.1.3*. Randomization will be stratified according to centre and whether the participant received or is receiving neoadjuvant chemotherapy in advance of their planned liver resection.

6.3.2 Blinding and Unblinding Procedures

Blinded stakeholders will include participants, clinicians, data collectors, outcome adjudicators, and data analysts.[41] The study blind may only be broken if the safety of the participant is at risk and the treatment plan relies on knowledge of study allocation. If knowledge of the treatment assignment is absolutely necessary, the blind may be broken only with the permission of a Principal Investigator. If unblinding occurs, the circumstances around the unblinding will be documented.

6.4 Discontinuation of IP and Participant Withdrawal

6.4.1 Discontinuation of IP and Study Procedures

The clinical scenarios in which participants will discontinue IP and study procedures are described below and summarized in Appendix C.

At their own discretion, participants may discontinue IP for any reason at any time. These participants will continue all study related procedures and visits if they are agreeable..

Investigators may also discontinue the IP for reasons including, but not limited to, safety or behavioral concerns. These patients continue all study related procedures and visits.

IP should be held postoperatively if a participant has an unplanned admission to an Intensive Care Unit (ICU) due to the development of multiple organ dysfunction syndrome (MODS). MODS is defined as the

development of a physiologic derangement involving 2 or more organ systems arising as a result of a physiologic insult.[42,43] Whether a participant meets the definition of MODS will be at the discretion of the treating clinicians. Treatment should be resumed when the Qualified/Sub-Investigator feels it is safe to do so. Other study related procedures will continue as scheduled

If a participant does not undergo surgery for any reason other than an apparent clinical complete response to neoadjuvant therapy (i.e., progression, the participant is too unwell, participant preference, or >120 days have elapsed since starting IP), the participant will discontinue IP as soon as possible and be monitored for AEs for 30 days after the last dose. PFS and OS will be followed as scheduled. ctDNA will be measured once within 14 days of the decision to not undergo surgery. Quality of recovery and correlative outcomes will not be assessed.

If a participant does not undergo surgery due to an apparent clinical complete response to neoadjuvant therapy, the participant will continue taking IP for 41 days after the decision to not operate. Quality of recovery and correlative outcomes will not be assessed; however, the participant will continue to follow all other study related procedures and visits with timing defined based on the decision to not operate.

If a participant undergoes surgery without a liver resection or ablation due to an apparent clinical complete response, the participant will continue IP and all other study procedures as scheduled.

If a participant undergoes surgery without a complete resection or ablation of all known disease due to progression, unresectable disease, or any reason other than a clinical complete response, the participant should discontinue IP as soon as possible. PFS, OS, the side-effect profile, and quality of recovery will be measured as scheduled. ctDNA and correlative outcomes will be measured once postoperatively.

If a participant undergoes surgery with the resection of extrahepatic metastases discovered intraoperatively, and no known disease is left unresected or unablated, the participant should continue IP and all other study procedures as scheduled.

If a participant is found to have additional hepatic or extrahepatic metastases during follow-up that in retrospect were present prior to surgery and were not removed at the time of surgery, the participant will discontinue IP as soon as possible. These participants will be monitored for AEs until 30 days after the last dose was administered. All other study procedures will continue as scheduled.

If the final pathology from the liver resection is not consistent with metastatic colorectal carcinoma, the participant will discontinue IP as soon as possible and be monitored for AEs for 30 days after the last dose. PFS, OS, ctDNA, and correlative outcomes will not continue to be assessed. Quality of recovery will be evaluated as scheduled. . Note, if the medical care team and investigators believe the pathology result represents a pathological complete response to neoadjuvant therapy participants will continue to take IP until 41 days postoperatively and follow all study procedures.

6.4.2 Withdrawal

Participants can choose to withdraw from the study at any time for any reason. As discussed above, participants who choose to discontinue IP or placebo will continue all study related procedures and visits if they are agreeable. For participants who withdraw and no longer wish to take part in any study related activities (including medical record review for long term outcomes), they will stop the IP as soon as

possible. If participants received any doses of IP or placebo prior to being withdrawn, they will be followed by study personnel for 30 days after the last dose to monitor for AEs. Withdrawn participants will be contacted by the research team to arrange an early termination visit, as detailed in *Section 8.6*. Every effort will be made to obtain permission to document the reason for withdrawal. Any data collected prior to the withdrawal may be retained and used by the sponsor. Participants who withdraw from the study prior to receiving study treatment will not receive any further study specific tests and will not be included in any analysis. These withdrawn participants will be replaced with another participant.

7 INTERVENTIONS

7.1 Investigational Product

QBECO is a suspension of inactivated *E. coli* cells in physiological saline (0.9%). The concentration of cells in the suspension is normalized to an optical density (OD) of 5.0 ± 1.0 per mL when measured at 600 nm. The product has been developed by Qu Biologics as part of a novel class of microbial based immunotherapies, termed SSIs. QBECO is designed to activate a site specific innate immune response in the GI tract and related organs, including the liver.

7.1.1 Acquisition, Formulation and Packaging

7.1.1.1 Acquisition and Formulation

The IP and placebo will be provided by the manufacturer, Qu Biologics. These will be formulated by the manufacturer in an injectable sodium chloride suspension and will require no additional preparation.

7.1.1.2 Packaging

The IP and placebo will be packaged in 3 mL, EZ-Fill Borosilicate vials that conform to USP Type I requirements with 13-mm "Flurotec" (fluorinated polymer)-coated, bromobutyl rubber stoppers and TrueEdge flip off seals. Each vial will contain 2.3 mL of IP or placebo.

QBECO SSI is a parenteral preparation of inactivated whole-cell bacteria in normal saline. The suspension should be agitated gently before each use to ensure uniform distribution. A multi-dose vial adapter is supplied with each study drug vial, allowing for repeated puncture and withdrawal. For first use of each vial, the multi-dose vial adapter is inserted and then will remain in place. Prior to each dose, the rubber area of the multi-dose vial adapter is swabbed with an alcohol swab and then the Luer-Lok syringe is attached, and the dose withdrawn. The Luer-Lok syringe is then detached from the multi-dose vial adapter and the needle attached to the syringe. A maximum of 10 doses (i.e., 20 days of treatment) can be withdrawn from each vial.

7.1.2 Treatment Assignment Procedures

Study intervention (QBECO vs. Placebo) will be assigned by the randomization process described in *Section 6.3.1*.

7.1.3 Dosage and Administration

Participants will receive either QBECO or placebo, 0.1 mL delivered via subcutaneous injection (SC) once every 2 days (q2days), for 11 to 120 days preoperatively and 41 days postoperatively.

The first preoperative study treatment dose must not be given within 14 days following completion of neoadjuvant chemotherapy (the last dose of chemotherapy must be at least 14 days prior to the first dose of IP). Chemotherapy can cause abnormalities in laboratory tests of hematologic and immune function, which would complicate the ability to determine differences in efficacy or toxicity that are related to the IP. Hematologic and immune function generally returns to baseline by 14 days following completion of chemotherapy. Participants are intended to receive a minimum of 6 doses of the study treatment

preoperatively (minimum of 11 days of treatment). Therefore, the last dose of neoadjuvant chemotherapy must be at least 25 days prior to surgery.

The last preoperative dose is intended to be given 1 day before surgery. Given the q2day dosing regimen, some participants will be scheduled to receive their last dose 2 days prior to surgery. These participants will be asked to administer an additional dose 1 day before surgery (back-to-back doses).

Participants will receive the maximum number of preoperative doses within the constraints of the criteria listed above. The timeline from consent for surgery to operation ranges from approximately 14-28 days, which would allow 7-14 doses of the IP preoperatively. Participants with delayed operations will continue the IP or placebo until surgery for a maximum of 120 days. In the unlikely event that surgery is delayed beyond the 120-day maximum, the participant will be withdrawn as specified in *Section 6.4.1*.

Postoperative treatment with IP will start on POD1 and continue every 2 days until POD41.

7.1.4 Procedures for Intervention Training

Study treatment will be self-administered by the participant. Participants will receive SC injection teaching by a study team member or health care professional before receiving the IP or placebo. Participants may delegate one or more individuals who can assist with injections.

7.1.5 Dose Modification

A local skin immune response (LSIR) (i.e., pink/red erythema at the injection site) may occur within the first 24 hours after injection. The LSIR may be mildly tender to the touch but not painful and slightly raised but not swollen. The LSIR is comparable to the skin wheal associated with a positive tuberculosis skin test and is indicative of a local innate immune response.

In the event of a local skin immune response involving erythema approximately 7.0 cm or greater and/or interfering with normal functions of daily life, QBECO SSI dose will be reduced to 0.05 mL. If a local skin immune response involving erythema approximately 7.0 cm or greater and/or interfering with normal functions of daily life occurs following the reduced 0.05 mL dose, QBECO SSI dose will be further reduced to 0.02 mL. If the LSIR still meets the above criteria after 2 additional doses at the 0.02 mL dose, treatment will be stopped.

In the unlikely event of a skin reaction involving edema, induration and/or erythema approximately 10 cm or greater, treatment will be discontinued until the skin reaction resolves, at which time treatment can be continued with a 0.02 mL dose.

Between study visits, participants will be advised to contact the research team if they experience a LSIR meeting the above criteria. The dose may be held until an in person assessment is able to be performed at the investigators discretion if required. Any dose modifications must be approved by a study investigator.

Subjects receiving QBECO SSI may experience mild, transient fatigue that will resolve within a few days of initiating treatment. Study treatment does not need to be modified or discontinued in this context.

7.1.6 Prior and Concomitant Medications/Treatments

Drug interactions are not expected to occur with study IP. For participants receiving neoadjuvant chemotherapy, the last dose of chemotherapy must be at least 14 days prior to the first dose of IP and at least 25 days prior to surgery to ensure that hematologic and immune function are not compromised by chemotherapy while participants receive IP.

Adjuvant chemotherapy, if planned, should likewise not be administered until completion of the IP.

7.1.7 Assessment of Participant Compliance

Compliance will be assessed according to the Schedule of Procedures in *Section 8*. Should a participant be found non-compliant with administering the IP or placebo, every reasonable effort will be made to improve compliance, such as offering additional injection teaching. Non-compliant participants will still be included as per the principles of intention-to-treat analysis and will be encouraged to continue the usual follow-up schedule.

7.1.8 Receiving, Storage, Dispensing and Return

7.1.8.1 Receipt of Investigational Product

The IP manufacturer, Qu Biologics, will ship IP and placebo to participating centres. Products will be maintained at appropriate storage temperatures of 2-8° C throughout transport.

Upon receipt of the IP or placebo, an inventory must be performed and a receipt log filled out and signed by the research team member accepting the shipment. Any damaged or unusable product in a given shipment will be documented in the study files. The site must notify the Coordinating Centre of any damaged or unusable product that was supplied to the site. The Coordinating Centre will subsequently notify the sponsor.

7.1.8.2 Storage and Stability

Study IP will be stored at 2-8° C in a secure facility at each participating site, accessible to authorized study personnel. Participants will be instructed to store vials in the refrigerator to maintain the appropriate storage temperature once dispensed.

7.1.8.3 Dispensing of Investigational Product

IP or placebo and injection supplies will be dispensed to participants by authorized study personnel. The dispensing study personnel will provide instructions for safe storage and use.

While admitted to hospital, site leads will be responsible for ensuring the study drug is available to the nursing team, stored correctly, and that administration orders are entered into the participant's medical chart if needed. Study drug may be administered by the nursing staff or the participant, based on local site arrangements and requirements. Upon discharge, study personnel will be responsible for revisiting administration instructions, as needed.

The IP and placebo will be reconciled by the research team at regular intervals, in addition to reconciliations performed during monitoring. Reconciliation will include verification of IP or placebo kit

assignment, inventory, and dispensing documentation. Participants will keep diaries to document the dates of IP administration.

7.1.8.4 Return and/or Destruction of Investigational Product

Participants will be informed about the need to return all unused IP or placebo to the dispensing personnel. At the completion of the study, there will be a final reconciliation of IP shipped, used, and remaining. This reconciliation will be documented. Any discrepancies will be investigated, resolved, and documented prior to return or destruction of unused product. Documentation of product destroyed on site will be retained in the study files.

8 STUDY SCHEDULE AND PROCEDURES

Participants will be followed from screening until 5 years after enrollment. Data may be collected in person, via phone, email, or online survey as applicable. Please see below for the schedule of study assessments:

Table 1. Schedule of Procedures: Up to Day of Surgery

	Prior to surgery			Day of surgery
	Prior to randomization	Before starting IP, after chemotherapy (if applicable)	Q2days, 11-120 days pre-op	
Eligibility Assessment and Informed Consent	x			
Demographic Information	x			
Routine Bloodwork		x		
Pregnancy Test[b]	X			
Intervention			x	
Compliance Assessment				X[a]
Imaging[c]	x			
CEA and CBC		x		
PFS and OS Assessment				
ctDNA		x		x
Side-effect profile[d]				X[a]
QoR Survey		x		
Correlative Endpoints		x		x

IP: investigational product, POD: post-operative day, ctDNA: circulating tumour DNA, CEA: carcinoembryonic antigen, CBC: complete blood count, OS: overall survival, PFS: progression free survival, QoR: quality of recovery

* Blood samples will be drawn only for participants who are still in hospital. A protocol deviation will not be required for blood samples that were unable to be obtained on the day of discharge

a. Compliance assessment and side effect profile can also be collected the day before surgery via telephone if unable to collect the information on the day of surgery

b. When appropriate (see *Section 8.4*)

c. For specific imaging requirements, see *Section 8.3*

d. Adverse events will be monitored at each contact with a participant until at least 30-days following the last treatment dose. This time may be rounded to the next visit. Extended follow-up applies in the event of pregnancy (*Section 9.6*)

e. Following recurrence, no additional imaging or laboratory evaluations will be performed, however participants will continue to be followed for OS

f. Data can also be collected upon review of medical records if available. If data is not available in participants medical records, study centre to perform outlined tests in table above

g. ± 30 days of site's awareness

Table 2. Schedule of Procedures: After Surgery

	Q2 days, POD1 -41	POD1 *	POD4* (± 1 day)	6 weeks post-op (± 10 days)	3 months post-op (± 14 days)	6 months post-op (± 28 days)	9 months post-op (± 28 days)	12 months post-op (± 28 days)	15 months post-op (± 28 days)	18 months post-op (± 28 days)	21 months post-op (± 28 days)	24 months post-op (± 28 days)	30 months post-op (± 28 days) [f]	36 months post-op (± 28 days) [f]	42 months post-op (± 28 days) [f]	48 months post-op (± 28 days) [f]	54 months post-op (± 28 days) [f]	5 years post-op (± 28 days)	'Disease Progression [e](±30 days) [g]
Eligibility Assessment and Informed Consent																			
Demographic Information																			
Routine Bloodwork		x	x	x															
Pregnancy Test[b]																			
Intervention	x	x	x	x															
Compliance Assessment					x														
Imaging[c]					x	x	x	x	x	x	x	x	X	X	X	X	X	x	
CEA and CBC					x	x	x	x	x	x	x	x	X	X	X	X	X	x	
PFS and OS Assessment[e]					x	x	x	x	x	x	x	x	X	X	X	X	X	x	
ctDNA		x	x	x		x		x		x		x							x
Side-effect profile[d]				x	x														
QoR Survey			x	x															
Correlative Endpoints		x	x	x		x		x		x		x							x

IP: investigational product, POD: post-operative day, ctDNA: circulating tumour DNA, CEA: carcinoembryonic antigen, CBC: complete blood count, OS: overall survival, PFS: progression free survival, QoR: quality of recovery

* Blood samples will be drawn only for participants who are still in hospital. A protocol deviation will not be required for blood samples that were unable to be obtained on the day of discharge

a. Compliance assessment and side effect profile can also be collected the day before surgery via telephone if unable to collect the information on the day of surgery

b. When appropriate (see *Section 8.4*)

c. For specific imaging requirements, see *Section 8.3*

d. Adverse events will be monitored at each contact with a participant until at least 30-days following the last treatment dose. This time may be rounded to the next visit. Extended follow-up applies in the event of pregnancy (*Section 9.6*)

e. Following recurrence, no additional imaging or laboratory evaluations will be performed, however participants will continue to be followed for OS

f. Data can be collected upon review of medical records if available. If data is not available in participants medical records, study centre to perform outlined tests in table above

g. ± 30 days of site's awareness

8.1 Prior to Surgery

Participating sites will pre-screen patients for eligibility according to the inclusion and exclusion criteria in *Section 6.1* and *6.2* and site approvals. Should a patient be identified as a potential candidate for enrollment, a member of the circle of care will obtain permission from the patient to introduce a research team member. The research team will obtain informed consent and then confirm eligibility. If eligibility cannot be confirmed due to missing imaging, these will be obtained by the surgeon prior to enrollment.

Once eligibility is confirmed, the research team will randomize the participant.

Demographic information including but not limited to age, sex and ethnicity will be collected once consent is received. Baseline blood-based outcome measures and routine preoperative bloodwork will be drawn prior to starting the IP or placebo. The blood-based outcome measures are described in *Section 4.3* and *4.4*. Routine preoperative bloodwork will include the following: complete blood count (CBC), serum chemistry, serum creatinine, liver enzymes and liver function tests (LFTs), coagulation profile (INR), serum carcinoembryonic antigen (CEA), and complete blood count (CBC). All bloodwork will be drawn within 28 days prior to starting the IP.

8.2 Day of Surgery

Research staff will record any missed preoperative doses of the study drug, as well as any AEs that a participant may have experienced. This information can also be collected the day before surgery via telephone.

Select blood-based outcome measures will be re-evaluated prior to skin incision on the day of surgery. A sample of fresh tumour tissue will be obtained from a representative hepatic tumour and from the primary colorectal tumour, if resection occurs at the same operation as the liver resection, along with a sample of normal adjacent tissue (one of each type per surgical specimen); to be used for creating the ctDNA assay and molecular profiling of the tumour microenvironment. The ideal size for each sample will be > $1cm^3$. The surgical specimens will be handled fresh and fixed in 10% buffered formalin and then transferred to 70% ethanol. Subsequently, the fixed specimen will be shipped to Ottawa for processing and for tumor cellularity assessment. If no suitable tumour tissue is found (i.e., < 30% tumour cellularity or insufficient DNA extraction), archival formalin-fixed paraffin-embedded (FFPE) tissue from the participant will be obtained through pathology. If FFPE tissue is not available, tissue slides (20 unstained and 2 H&E stained slides) will be obtained instead. Tissue from a prior resected or biopsied specimen from the participant may also be sought if archival tissue from the resected specimen is still inadequate.

A study site may opt out of fresh specimen procurement. In this case, archival slides from the surgical resection of the liver, that meet acceptable tumor cellularity (> 30% cellularity) will be requested.

Some participants enrolled at the Ottawa Hospital may also undergo one additional blood draw for measurement of ctDNA postoperatively, on the day of surgery.

8.3 After Surgery

The participant will continue to receive the IP or placebo for 41 days postoperatively, starting on POD1.

Postoperative bloodwork values will be recorded on POD1, POD4±1 if they are still in hospital including: CBC, serum chemistry, serum creatinine, liver enzymes and LFTs, and coagulation profile. The participant will only be required to undergo research bloodwork on POD1 and 4±1 if they are still in hospital at these time periods. At 6-weeks post-operatively, blood-based outcome measures and routine bloodwork will be drawn. The blood-based outcome measures are described in Section 4.3 and 4.4. Routine bloodwork will include the following: CBC, serum chemistry, serum creatinine, liver enzymes and LFTs, and coagulation profile (INR).

Visits may be virtual (i.e., by phone, email, online survey, review of medical records as applicable) after 2 years.

If the participant is identified as having disease progression, select blood-based outcome measures will also be redrawn at that time of sites awareness. Participants will stop taking the IP and no further study-protocolled imaging or laboratory investigations will be required after disease progression, however the participant will continue to be followed for OS.

For assessment of disease progression, serial CT scans of the chest, abdomen, and pelvis (or CT chest and MRI abdomen), and serum CEA and CBC should be performed every 3 months for 2 years postoperatively, then every 6 months for 5 years postoperatively. After 2 years, clinicians may follow their routine imaging and biochemical surveillance regimens. Imaging will ideally be performed at the treating study centre, however if this is not possible, outside images will be accepted.

8.4 Pregnancy Testing

Women of childbearing potential (WOCBP) must undergo a pregnancy test prior to randomization and within 30 days prior to starting the IP or placebo. Both male and female patients will be informed of contraceptive requirements and must agree to these requirements as part of confirming eligibility. The definition of WOCBP and the specific contraceptive requirements are detailed in Appendix B.

8.5 Pregnancy Visit and Follow-Up

WOCBP and fertile male participants will be required to use a highly effective contraceptive while receiving IP or placebo, as specified in Appendix B. Participants who become pregnant after commencing the IP or placebo will be asked to discontinue the product however will remain enrolled.

The following groups will be followed until 6 to 8 weeks beyond the date of delivery or the termination of the pregnancy:

- Participants who become pregnant while receiving IP
- Partners of male participants who become pregnant while the male participant receives IP. Follow-up will be contingent upon investigators obtaining informed consent from the pregnant female partner to record pregnancy information.

AEs during this extended follow-up period will be defined and recorded according to *Section 9.6*.

The study sponsor will be informed of pregnant participants and pregnant partners of male participants within 24 hours of when investigators are first notified.

8.6 Early Termination Visit

Participants who withdraw from the study prior to study completion will be asked to complete an early termination visit with a study team member, with the exception of participants who withdrew prior to receiving IP (these participants will not complete any further study procedures and will be replaced). At this visit, the following data will be recorded: compliance with IP injections (if applicable), reason for discontinuation of IP (if applicable), reason for withdrawal, disease progression status, and AEs. Participants who discontinue the study drug will also be asked if they would agree to ongoing follow-up as per the study protocol.

8.7 Final Study Visit

The final study visit will be at 5 years postoperatively, at which time disease progression and OS will be documented. At the conclusion of the trial, survival data may be abstracted from the participant's medical record beyond 5 years for the sake of time-to-event analysis, however this would not necessitate a formal appointment.

8.8 Protocol Deviations

It is the responsibility of the investigators to ensure that only investigative procedures, as outlined in this protocol, are performed on study participants; the occurrence of deviations from the protocol or standard operating procedures (SOPs) are limited; and compliance with the regulations is maintained. Planned deviations from the protocol must not be implemented without prior agreement from the sponsor and approval from the local research ethics boards (REBs), as required, unless to eliminate an immediate hazard to a participant.

Planned or unplanned deviations may occur on the part of the participant, the investigators, or the study research team. Following a deviation, corrective/preventative actions are to be developed and implemented in a timely manner. Protocol deviations will be documented and reported as required and assessed where necessary during analysis.

8.9 Handling Procedures for Blood and Tissue Derived Outcome Measures

Biologic materials will be stored appropriately for transport and disposed of according to institutional standards following analysis. A correlative sample manual of Standard Operative Procedures will be provided to each site. All correlative samples will be sent to the lab of Dr. Rebecca Auer at the Ottawa Hospital Research Institute. Foundation Medicine (*www.foundationmedicine.com*) will provide materials for the measurement of ctDNA, and NKMAX *(www.nkmax.com/eng/)* will provide testing kits for one of the NK cell functional assays, if these are being performed.

9 ASSESSMENT OF SAFETY

The safety of research participants is foremost and should always be considered throughout the conduct of research.

9.1 Definitions

9.1.1 Adverse Events

An AE is any untoward medical occurrence in a participant who has been administered a pharmaceutical product and does not necessarily need to have a causal relationship with the product.

To be considered an AE, the medical occurrence must occur within the AE recording period. The recording period is after the first administered dose of IP and ≤ 30-days after the last administered dose. Events starting outside this period will not be considered AEs.

Postoperative complications will be recorded as AEs if they occur within the AE recording period. Postoperative complications have been defined in *Section 4.3.* Depending on severity, a postoperative complication may qualify as a SAE.

The following events will not be considered AEs and therefore do not require recording:

- Manifestations of pre-existing diseases unless, at the discretion of the investigators, the disease or condition worsens in severity or frequency during the recording period.
- Common events occurring within the postoperative period. The postoperative period will be measured from the time of surgery to either the time of discharge or POD28, whichever occurs later. However, a common event within the postoperative period may be recorded as an AE if (1) at the discretion of the investigators, the event has a severity or duration that is not in keeping with the normal postoperative course *AND* (2) the event occurs within the AE recording period. Common events within the postoperative period include but are not limited to:
    - Abdominal pain
    - Nausea
    - Vomiting
    - Obstipation
    - Fatigue
    - Headache
    - Pruritus
    - Weight loss (less than 15% of the pre-surgical weight)
    - Bleeding per rectum if a bowel resection was performed at the time of surgery
    - Urinary retention
    - Difficulty ambulating
- Elective medical or surgical procedures

9.1.2 Serious Adverse Events

A SAE or reaction is any untoward occurrence that meets at least one of the following criteria:

- An event that results in death.
- An event that is life-threatening. This is defined as events where the participant is at risk of death; it does not refer to an event which hypothetically might have caused death if it were more severe.
- An event that requires inpatient hospitalization or prolongation of existing hospitalization. Prolonged hospitalization will be defined as lasting $\geq$ 21 days from the date of surgery.
- Results in persistent or significant disability/incapacity.
- A congenital abnormality in a child of a participant or partner of a male participant who delivered after the start of IP.
- A spontaneous abortion in a participant or partner of a male participant who delivered after the start of IP.

9.1.3 Unexpected Adverse Event

An unexpected AE is any AE that is not identified in nature, severity, or frequency in the current IB.

9.1.4 Adverse Drug Reaction and Unexpected Adverse Drug Reaction

An ADR is any noxious or unintended response to a medicinal product related to any dose.

The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

The expression “causal relationship” is meant to convey that in general there are facts, evidence, or arguments to suggest a reasonable causal relationship.

All serious and unexpected ADRs will have expedited reporting to regulatory agencies in accordance with the ICH-GCP and local regulatory requirements.

9.2 Assessment of an Adverse Event

9.2.1 Relationship (Causality/Relatedness)

The causality assessment is the determination, according to the investigator’s clinical judgment, of the existence of a reasonable possibility that the IP caused or contributed to an AE.

If the investigator or delegated sub-investigator is unsure about whether the study drug caused or is related to the AE, then the event will be handled as “related” for reporting purposes. If the causality assessment is "unknown but not related” to the study drug, this should be clearly documented in the source documents.

9.2.2 Expectedness

Events are classified as unforeseen or unexpected if the nature, severity, or frequency is not consistent with the risk information set out in the IB.

9.2.3 Seriousness

Events are classified as serious if they are associated with effects threatening the life or physiological functions of a participant. Refer to the definition for “Serious Adverse Events” in *Section 9.1.2*.

9.2.4 Severity

The term "severe" is often used to describe the intensity (severity) of a specific event (e.g., mild, moderate, or severe myocardial infarction). However, the event itself may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on participant/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. The terms “serious” and “severe” are not synonymous. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

To assess the severity of an AE the investigators will use the NCI Common Terminology Criteria for Adverse Events (CTCAE v5.0).

9.3 Adverse Event Recording

Investigations into potential AEs should be done during each contact with a participant within the AE recording period. The recording period is after the first administered dose of IP and ≤ 30-days after the last administered dose. The last AE assessment may be rounded to the next visit if there is not a scheduled encounter 30-days after the last dose of IP.

Investigations may be done through specific questioning and by examination as appropriate. Information on all AEs should be recorded promptly in the source document and assessed by an investigator in a timely manner allowing sufficient time to meet required reporting timelines for SAEs and serious and unexpected adverse drug reactions (SUADRs) if needed. AE case report forms (CRFs) should be completed using source documents by a delegated research team member in a timely manner. All clearly related signs, symptoms, and abnormal diagnostic procedures should be recorded in the source document and should be grouped under one diagnosis. Each diagnosed AE should then be categorized in accordance with Medical Dictionary for Regulatory Activities (MedDRA) classifications/the revised NCI CTCAE v5.0.

9.4 Reporting of SAEs and Unanticipated Events

9.4.1 Investigator reporting: Notifying the Research Ethics Board

SAEs and unanticipated events should be recorded and reported to the REB in accordance with local reporting requirements and timelines.

9.4.2 Investigator reporting: Notifying the Coordinating Centre

The investigators are responsible for reporting SAEs and SUADRs to the Coordinating Centre within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded and reported to the Coordinating Centre within 24 hours of site awareness.
- SAEs other than death and immediately life-threatening events, regardless of relationship, will be reported to the Coordinating Centre within 72 hours of site awareness.

AE information will be entered into the CRF in a timely manner and no later than 15 days from the time the investigator becomes aware of the event.

SAE information will be entered into the CRF in a timely manner from the time the investigator becomes aware of the event.

Events that are assessed to be serious, unexpected, and related, or cannot be ruled out as related, to the IP are considered SUADRs. Reporting for SUADRs should include a description of the event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. The minimum information required includes at least one identifiable participant, one identifiable reporter, one serious reaction, and one suspect product.

Additionally, a Suspect Adverse Reaction Report (Council for International Organizations of Medical Sciences (CIOMS) I Form, see Appendix A) must be completed by the investigator and forwarded to the Coordinating Centre within 24 hours of site awareness. Information on other possible causes of the event, such as concomitant medications and illnesses should also be provided as soon as is made available.

9.4.3 Coordinating Centre Reporting of SUADRs: Notifying Health Canada

The regulatory sponsor is responsible for reporting SUADRs to regulatory authorities in accordance with local expedited reporting requirements and timelines. These activities have been delegated to the Coordinating Centre. In addition, the Coordinating Centre will complete the ADR Expedited Reporting Summary Form and submit this form in conjunction with the completed CIOMS Form to the appropriate Health Canada directorate.

9.4.4 Coordinating Centre Reporting of SUADRs: Notifying Sites

The Coordinating Centre is responsible for distributing blinded expedited reports of SUADRs to each investigator for submission to local REBs within 15 days of Coordinating Centre awareness.

9.4.5 *Coordinating Centre Reporting of SAEs and SUADRs: Notifying the Sponsor*

The Coordinating Centre will report all SAEs and SUADRS to the regulatory sponsor (Qu Biologics) within 5 days of Coordinating Centre awareness for fatal and life-threatening events and 10 days of awareness for other non-fatal events. SUADRS will be sent to the regulatory sponsor prior to submission to Health Canada.

9.5 Duration of Follow-up for Adverse Events

AEs occurring after the first administered dose of the IP and 30-days after the last administered dose will be collected. AEs recorded during this period will be followed through to resolution, or until the event is assessed as chronic or stable.

9.6 Pregnancy and Adverse Events

Extended monitoring for AEs will be required for the following groups:

- Participants who become pregnant while receiving IP
- Partners of male participants who become pregnant while the male participant receives IP These participants and partners will be followed until 6 to 8 weeks after delivery or termination of the pregnancy. For partners, follow-up is contingent upon investigators

receiving informed consent collect relevant health and pregnancy information. The AE recording period for these participants and partners will therefore be extended to the last pregnancy-related follow-up encounter.

Any pregnancy complication, elective termination, or congenital malformation will be considered AEs. AEs may also be considered SAEs if they meet the definition specified in *Section 9.1.2.* Spontaneous abortions and congenital malformations will be considered SAEs and will be reported as such.

9.7 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be established for this trial. The role of the DSMB will be to review safety data over the study duration. The specific responsibilities, procedures, and meeting format of the DSMB is detailed in the DSMB Charter.

10 SITE MONITORING, auditing, and inspecting

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure the safety of human study participants and the protection of their rights and well-being. Monitoring also verifies that collected study data is accurate, complete, and verifiable by source documentation and that the study is conducted in accordance with the protocol and operating procedures.

Monitoring for this study is the responsibility of the sponsor. The delegated monitor will evaluate study processes and documentation based on the approved protocol/amendment(s), Part C, Division 5 of the Food and Drug Regulations, the ICH-GCP, and institutional policies.

The extent and nature of monitoring is outlined in the Monitoring Plan. The monitoring plan specifies the frequency of monitoring, monitoring procedures, level of site monitoring activities (e.g., the percentage of participant data to be reviewed), and distribution of monitoring reports. Monitoring activities may be performed in person and/or remotely. Reports of findings identified during monitoring activities will be provided to sites detailing any required actions. Documentation of monitoring activities and findings will be provided to the site study team and the study principal investigators (PIs). The institution and/or local REB reserves the right to conduct independent audits as necessary.

The investigators are responsible for ensuring monitors and/or quality assurance reviewers are given access to all study-related documents noted above and study related facilities (e.g., pharmacy, diagnostic laboratory, etc.), and have adequate space to conduct the monitoring visit or audit.

10.2 Auditing and Inspecting

The investigators will provide direct access to source data/documents for the purposes of study-related monitoring, audits, and inspections by the coordinating centre, REBs, the sponsor, and applicable regulatory bodies. The investigator will permit the review of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data, etc.) and will ensure access to applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

We hypothesize that participants receiving QBECO will experience a 65% improvement in the percentage with PFS compared to those receiving placebo.

Sample Size Considerations

Given a predicted 40% at 2-years in participants undergoing potentially curative resection of colorectal liver metastases,[22] a 65% improvement in the percentage surviving progression free represents a PFS of approximately 1.65*40% = 65% at 2 years. Under the assumption of exponential survival, these two-year PFS percentages imply an annual rate of events in the control group of 0.458 and a rate in the QBECO group is 0.215; the corresponding hazard ratio (HR) for treatment is 0.47. The table below shows a power to detect this hazard ratio of 0.47 (in a one-sided test, of the null hypothesis that HR=1 with alpha=0.05), and hazard ratios of 0.5 to 0.6 when the analysis is done once a pre-set number of study events (progression or death) has been observed. We choose 54 events as our target, which gives 87% power for a 65% increase in survival at 2 years (HR=0.47) and 82% power for HR=0.5.

	Power (%)			
Number of Events	HR = 0.47	HR = 0.5	HR = 0.55	HR = 0.6
51	85	80	69	57
52	86	80	70	58
53	86	81	70	58
54	87	82	71	59
55	88	82	72	60
56	88	83	72	61

It is anticipated that 58 participants can be recruited per year. Under the assumed event rates above, assuming an enrollment of 116 patients over two years, it is expected that the trial will reach 54 events at approximately 3 years after the first patient is enrolled. These calculations are based on an expected 10% of patients not undergoing liver resection due to progression of disease and 5% being lost to follow-up or withdrawing for other reasons after surgery but before reaching the study endpoint (so that there are sufficient events in participants who undergo liver resection for an analysis in just that subset – the sensitivity analysis in section 11.3). Projected enrolment will be approximately 20 participants for each of the participating sites.

Planned Safety Analyses

Two planned safety analyses of all safety data will be performed once 20 participants have had 6 weeks of follow-up and once 50 participants have had 6 weeks of follow-up. The DSMB will review the results of these safety analyses. Additional safety analyses may be reviewed by the DSMB as needed.

11.2 Stopping Rules

This study will be stopped prior to its completion if: (1) the intervention is associated with AEs that call into question the safety of the intervention; (2) difficulty in study recruitment or retention will significantly

impact the ability to evaluate the study endpoints; (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) other situations occur that might warrant stopping the trial.

11.3 Final Analysis Plan

The cohorts for analysis will be defined as follows and are summarized according to clinical scenario in Appendix C::

1. **Primary analysis cohort:** This will be an intention-to-treat (ITT) cohort for the analysis of the primary and secondary outcomes. This cohort will analyze all enrolled participants who received at least one dose of IP according to treatment allocation. Withdrawn participants who received at least one dose of IP will be also included in this cohort, unless they withdraw consent to the use of their data.

2. **Correlative analysis cohort:** This will be the cohort for the analysis of the correlative outcomes. This cohort will analyze all enrolled participants who undergo surgery, even if the surgery does not involve a liver resection or not all disease is resected or ablated

3. **Sensitivity analysis cohort:** This will be a per-protocol cohort and only include participants who meet all of the following criteria. Note that withdrawn participants will be included if they meet the below criteria and do not withdraw consent for use of their data:

    - Receive a minimum of 80% of the prescribed preoperative doses of IP and at least 90% of the postoperative doses.

    - Undergo liver resection with or without ablation of all visible disease and pathology confirms CRLM or a pathologic complete response; ***or***, undergoes surgery without a resection or ablation due to an apparent clinical complete response; ***or***, does not undergo surgery due to an apparent clinical complete response.

    - No preoperative extrahepatic metastases are identified at any point.

4. **Safety analysis cohort:** This cohort will include all enrolled participants who receive at least one dose of IP/placebo

Descriptive statistics will be used to summarize variables in each treatment arm.

OS and PFS of participants in each treatment arm will be evaluated using the Kaplan-Meier method and compared using the log-rank test. Participants will be censored at the time of last follow-up. Cox regression will be used to estimate hazard ratios and identify independent predictors of OS and PFS. The proportional hazards assumption will be assessed for all Cox regression models. Other outcomes will be evaluated using multivariable regression models appropriate for the type and distribution of the data.

The amount and patterns of missing data and patients lost to follow-up will be evaluated. AEs, disease progression, or death may account for missing postoperative assessments. Participants with missing covariates will be excluded from multivariable models.

Additional hypothesis generating subgroup analyses will be performed according to the following disease and treatment characteristics:

- Synchronous vs. metachronous disease. Synchronous liver metastases will be defined as those identified before resection of the primary colorectal carcinoma, or those that in retrospect were present prior to resection of the primary, which is consistent with prior definitions.[44,45] Metachronous metastases will be defined as those identified following resection of the primary.
- Combination of surgery and ablation vs. surgery only.
- Progression identified at the time of surgery or prior to surgery.
- Some visible disease (primary or metastases) not resected or ablated at the time of surgery.
- Neoadjuvant vs. no neoadjuvant chemotherapy.
- Minor vs. major liver resection. Major resection will be defined as resection of 3 or more hepatic segments.[46]

12 DATA HANDLING AND RECORD KEEPING

12.1 Confidentiality

Information about study participants will be kept confidential and managed according to the requirements of the Personal Health Information Protection Act of 2004 (PHIPA) and the REB. PHIPA outlines the rules for the collection, use and disclosure of personal health information (PHI). The Act requires each participant to consent to the collection, use and access of PHI, unless consent is waived by the REB. Where consent is required, each participant must be informed of the following:

- What PHI will be collected
- Who will have access to their PHI information and why
- Who will use or disclose their PHI
- The rights of a participant to revoke their authorization for use of their PHI.

If a participant revokes authorization to collect or use their PHI, the investigator may use all information collected prior to the revocation of participant authorization. For participants that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e., that the participant is alive) at the end of the scheduled study period.

12.2 Source Documents

Source data/documents are original documents, data, and records in a clinical study that are necessary for the reconstruction and evaluation of the study. Examples of these original documents and data records include, but are not limited to:

- Worksheets
- Hospital records
- Medical records
- Memorandum
- Participants' diaries or evaluation checklists
- Pharmacy dispensing records
- Recorded data from automated instruments (i.e., ECGs)
- Copies or transcriptions certified after verification as being accurate and complete
- Participant files and records kept at the pharmacy
- Entries entered directly into the printed CRF

Each participating site will maintain appropriate medical and research records for this study, in addition to regulatory and institutional requirements for the protection of participant confidentiality. If electronic source documents are printed, they should be signed and dated by the investigator to confirm content and subsequently filed with other source documents.

The investigators and research team members listed on the Task Delegation Log (TDL) will have access to participant medical records and will collect only the information needed for the study. Sponsor delegated monitors, representatives of institutional committees, and regulatory authority representatives of the country in which the study is being conducted will also have access to examine records for the purposes of quality assurance reviews, audits, and evaluation of study safety.

12.3 Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study personnel under the supervision of the investigators. All source documents and applicable laboratory reports should be reviewed as needed and used to ensure that data collected for the purposes of the study are accurate and complete. Contemporaneous review of laboratory results and the assessment of clinical significance for those results considered out of range should be documented by means of dated signature by the reviewing investigator. Study personnel, including data entry team members, should use source documents to complete CRFs.

As part of the safety plan for this study, the investigator will review individual study participant records to ensure that appropriate mechanisms to protect the safety of study participants are being followed, that protocol requirements are being adhered to, and that data is accurate, complete, and secure. Participant records include, but are not limited to: consent forms, CRFs, data forms, laboratory specimen records, inclusion/exclusion forms, and medical charts. All study data will be collected by a member of the study research team and recorded in accordance with applicable procedures.

12.4 Data Capture and Case Report Forms

The study CRF is the primary data collection instrument for the study. Electronic/Paper CRFs will be used to collect data for this study. CRFs are to be completed by data capture personnel and signed off by an investigator in a timely manner. Good documentation practices should be implemented according to standard operating procedures. All data requested on the CRF must be recorded and verifiable by source document.

12.5 Records Retention

It is the responsibility of the REBs, investigators, and regulatory sponsor to retain study essential documents as per local regulatory requirements and GCP guidelines.

Essential study documents must be maintained in a secure and confidential manner for participating Canadian sites for a period of 15 years. For the purposes of this study, the start date of the retention period is the date of the final report of the trial. Exceptions may be made for sites that close prematurely, wherein the start date for the retention period will be the date of notification to Health Canada of the sites closure. Sites conducting this study outside of Canada must maintain study records for the required retention period as stipulated by local regulatory authorities. All study records are then to be destroyed according to local and national policy and requirements. It is the investigators' responsibilities to request authorization for destruction at the completion of the retention period and/or for the sponsor to inform the investigators/institution when these documents may be destroyed.

12.6 Clinical Trial Registration

In accordance with Health Canada's Notice "Registration and Disclosure of Clinical Trial Information, November 30, 2007", the sponsor will be responsible for registering the study on Clinicaltrials.gov, a publicly available registry that conforms to international standards for registries.

13 QUALITY CONTROL AND QUALITY ASSURANCE

As per ICH-GCP and local regulations, the sponsor is responsible for ensuring the implementation and maintenance of systems that support quality assurance and quality control.

The study must be conducted in compliance with the study protocol and all data collected must be accurate and verifiable by source documents. For the purpose of monitoring and auditing by the coordinating centre and/or Sponsor, and inspection by regulatory authorities, the site will provide direct access to all study related source data/documents. The sponsor will verify that the study is conducted and data has been collected, documented (recorded), and reported in compliance with the protocol, GCP, and applicable regulatory requirements.

Data for the study will be centrally stored and managed by the Centre for Clinical Trial Support (CCTS). To ensure the quality of study data, quality assurance and control systems will be implemented using validated electronic quality control checks within the electronic data capture system. These verification measures will identify missing data, inconsistencies, and/or data anomalies. Both electronic and manual queries will be generated for resolution and review by sites.

Access to secure and validated electronic systems used for the purposes of this study will be controlled by the sponsor. Access will only be granted to individual research team members upon review of training and qualification and authorization by delegation of the investigators.

Quality assurance and control measures will be implemented to ensure training for specific trial–related tasks beyond the usual scope of practice.

14 ETHICS CONSIDERATIONS

14.1 Ethical Standard

The investigators will ensure this study is conducted in accordance with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Participants of Research, and codified in the Tri-Council Policy Statement and/or the ICH E6.

14.2 Research Ethics Board

The protocol, informed consent form (ICF), recruitment materials, and all participant materials will be submitted to the REB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the REB before the changes are implemented in the study, unless to eliminate an immediate hazard.

14.3 Consent

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. A consent form describing in detail the study procedures and risks will be reviewed with and given to each participant. Consent forms will be REB-approved, and the participant is required to read and review the document or have the document read to him or her. The investigators or a designee will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent document prior to any study-related assessments or procedures. Participants will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to participants for their records.

Prior to involvement in any study-related activities, consent must be obtained in writing for each participant using the current REB approved informed consent from. It is the responsibility of the investigator to ensure that all advertisements and written information, including the ICF, disseminated to participants has been approved by the local REB prior to use. The ethics approved ICF and any other written information, must be provided to each participant, allowing ample time to ask and have answered any questions prior to making a decision regarding participation. Neither an investigator nor study staff should unduly influence or coerce a participant to participate in the study.

The ICF will be signed and dated by the participant and individual obtaining consent. The consent process will be documented in the clinical or research record.

The original ICF, in its entirety, will be maintained by the site, and a complete copy of the signed ICF provided to the participant. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The provision of consent is an ongoing process and should be maintained throughout the duration of the study. Participants may withdraw consent at any time throughout the course of the study.

15 PUBLICATION/DATA SHARING POLICY

Authorship on study publications will adhere to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals of the International Committee of Medical Journal Editors. These requirements state "Authorship credit should be based on:

1. Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data.
2. Drafting the article or revising it critically for important intellectual content.
3. Final approval of the version to be published.

Authors should meet conditions 1, 2, and 3." Where journal policies permit, all study site investigators who played a contributing role in the trial, including to its accrual, will be included in an Acknowledgement section.

16 REFERENCES

1. Tsuchiya Y, Sawada S, Yoshioka I, et al. Increased surgical stress promotes tumor metastasis. *Surgery*. 2003;133(5):547-555. doi:10.1067/msy.2003.141

2. Tai LH, Zhang J, Scott KJ, et al. Perioperative influenza vaccination reduces postoperative metastatic disease by reversing surgery-induced dysfunction in natural killer cells. *Clin Cancer Res*. 2013;19(18):5104-5115. doi:10.1158/1078-0432.CCR-13-0246

3. Tai LH, Alkayyal AA, Leslie AL, et al. Phosphodiesterase-5 inhibition reduces postoperative metastatic disease by targeting surgery-induced myeloid derived suppressor cell-dependent inhibition of Natural Killer cell cytotoxicity. *Oncoimmunology*. 2018;7(6):e1431082. doi:10.1080/2162402X.2018.1431082

4. Tai LH, de Souza CT, Bélanger S, et al. Preventing postoperative metastatic disease by inhibiting surgery-induced dysfunction in natural killer cells. *Cancer Res*. 2013;73(1):97-107. doi:10.1158/0008-5472.CAN-12-1993

5. Seth R, Tai LH, Falls T, et al. Surgical stress promotes the development of cancer metastases by a coagulation-dependent mechanism involving natural killer cells in a murine model. *Ann Surg*. 2013;258(1):158-168. doi:10.1097/SLA.0b013e31826fcbdb

6. Ananth AA, Tai LH, Lansdell C, et al. Surgical Stress Abrogates Pre-Existing Protective T Cell Mediated Anti-Tumor Immunity Leading to Postoperative Cancer Recurrence. *PLoS One*. 2016;11(5):e0155947. doi:10.1371/journal.pone.0155947

7. Gabrilovich DI. Myeloid-Derived Suppressor Cells. *Cancer Immunol Res*. 2017;5(1):3-8. doi:10.1158/2326-6066.CIR-16-0297

8. Manz MG, Boettcher S. Emergency granulopoiesis. *Nat Rev Immunol*. 2014;14(5):302-314. doi:10.1038/nri3660

9. Angka L, Martel AB, Kilgour M, et al. Natural Killer Cell IFNγ Secretion is Profoundly Suppressed Following Colorectal Cancer Surgery. *Ann Surg Oncol*. 2018;25(12):3747-3754. doi:10.1245/s10434-018-6691-3

10. Zhang J, Tai LH, Ilkow CS, et al. Maraba MG1 virus enhances natural killer cell function via conventional dendritic cells to reduce postoperative metastatic disease. *Mol Ther*. 2014;22(7):1320-1332. doi:10.1038/mt.2014.60

11. Mitroulis I, Ruppova K, Wang B, et al. Modulation of Myelopoiesis Progenitors Is an Integral Component of Trained Immunity. *Cell*. 2018;172(1-2):147-161.e12. doi:10.1016/j.cell.2017.11.034

12. Kaufmann E, Sanz J, Dunn JL, et al. BCG Educates Hematopoietic Stem Cells to Generate Protective Innate Immunity against Tuberculosis. *Cell*. 2018;172(1-2):176-190.e19. doi:10.1016/j.cell.2017.12.031

13. Netea MG, Joosten LAB, van der Meer JWM. Hypothesis: stimulation of trained immunity as adjunctive immunotherapy in cancer. *J Leukoc Biol*. 2017;102(6):1323-1332. doi:10.1189/jlb.5RI0217-064RR

14. Bazett M, Costa AM, Bosiljcic M, et al. Harnessing innate lung anti-cancer effector functions with a novel bacterial-derived immunotherapy. *Oncoimmunology*. 2018;7(3):e1398875. doi:10.1080/2162402X.2017.1398875

15. Kalyan S, Bazett M, Sham HP, et al. Distinct inactivated bacterial-based immune modulators vary in their therapeutic efficacies for treating disease based on the organ site of pathology. *Sci Rep*. 2020;10(1):5901. doi:10.1038/s41598-020-62735-z

16. Faraj TA, McLaughlin CL, Erridge C. Host defenses against metabolic endotoxaemia and their impact on lipopolysaccharide detection. *Int Rev Immunol*. 2017;36(3):125-144. doi:10.1080/08830185.2017.1280483

17. Sham HP, Bazett M, Bosiljcic M, et al. Immune Stimulation Using a Gut Microbe-Based Immunotherapy Reduces Disease Pathology and Improves Barrier Function in Ulcerative Colitis. *Front Immunol*. 2018;9:2211. doi:10.3389/fimmu.2018.02211

18. Sutcliffe S, Kalyan S, Pankovich J, et al. Novel Microbial-Based Immunotherapy Approach for Crohn's Disease. *Frontiers in Medicine*. 2019;6:170. doi:10.3389/fmed.2019.00170

19. Hackl C, Neumann P, Gerken M, Loss M, Klinkhammer-Schalke M, Schlitt HJ. Treatment of colorectal liver metastases in Germany: a ten-year population-based analysis of 5772 cases of primary colorectal adenocarcinoma. *BMC Cancer*. 2014;14:810. doi:10.1186/1471-2407-14-810

20. Manfredi S, Lepage C, Hatem C, Coatmeur O, Faivre J, Bouvier AM. Epidemiology and Management of Liver Metastases From Colorectal Cancer. *Ann Surg*. 2006;244(2):254-259. doi:10.1097/01.sla.0000217629.94941.cf

21. Engstrand J, Nilsson H, Strömberg C, Jonas E, Freedman J. Colorectal cancer liver metastases – a population-based study on incidence, management and survival. *BMC Cancer*. 2018;18:78. doi:10.1186/s12885-017-3925-x

22. Nordlinger B, Sorbye H, Glimelius B, et al. Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. *Lancet*. 2008;371(9617):1007-1016. doi:10.1016/S0140-6736(08)60455-9

23. Portier G, Elias D, Bouche O, et al. Multicenter randomized trial of adjuvant fluorouracil and folinic acid compared with surgery alone after resection of colorectal liver metastases: FFCD ACHBTH AURC 9002 trial. *J Clin Oncol*. 2006;24(31):4976-4982. doi:10.1200/JCO.2006.06.8353

24. Kanemitsu Y, Shimizu Y, Mizusawa J, et al. Hepatectomy Followed by mFOLFOX6 Versus Hepatectomy Alone for Liver-Only Metastatic Colorectal Cancer (JCOG0603): A Phase II or III

Randomized Controlled Trial. *J Clin Oncol*. Published online September 14, 2021:JCO2101032. doi:10.1200/JCO.21.01032

25. Oxnard GR, Paweletz CP, Kuang Y, et al. Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA. *Clin Cancer Res*. 2014;20(6):1698-1705. doi:10.1158/1078-0432.CCR-13-2482

26. Goldberg SB, Narayan A, Kole AJ, et al. Early Assessment of Lung Cancer Immunotherapy Response via Circulating Tumor DNA. *Clin Cancer Res*. 2018;24(8):1872-1880. doi:10.1158/1078-0432.CCR-17-1341

27. Dindo D, Demartines N, Clavien PA. Classification of Surgical Complications: A New Proposal With Evaluation in a Cohort of 6336 Patients and Results of a Survey. *Annals of Surgery*. 2004;240(2):205-213. doi:10.1097/01.sla.0000133083.54934.ae

28. *Surgical Site Infection Event (SSI)*. National Healthcare Safety Network, Centers for Disease Control and Prevention; 2022.

29. Koch M, Garden OJ, Padbury R, et al. Bile leakage after hepatobiliary and pancreatic surgery: a definition and grading of severity by the International Study Group of Liver Surgery. *Surgery*. 2011;149(5):680-688. doi:10.1016/j.surg.2010.12.002

30. Rahbari NN, Garden OJ, Padbury R, et al. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). *Surgery*. 2011;149(5):713-724. doi:10.1016/j.surg.2010.10.001

31. Leslie K, Troedel S, Irwin K, et al. Quality of recovery from anesthesia in neurosurgical patients. *Anesthesiology*. 2003;99(5):1158-1165. doi:10.1097/00000542-200311000-00024

32. Myles PS, Weitkamp B, Jones K, Melick J, Hensen S. Validity and reliability of a postoperative quality of recovery score: the QoR-40. *Br J Anaesth*. 2000;84(1):11-15. doi:10.1093/oxfordjournals.bja.a013366

33. Shida D, Wakamatsu K, Tanaka Y, et al. The postoperative patient-reported quality of recovery in colorectal cancer patients under enhanced recovery after surgery using QoR-40. *BMC Cancer*. 2015;15:799. doi:10.1186/s12885-015-1799-3

34. Peng LH, Wang WJ, Chen J, Jin JY, Min S, Qin PP. Implementation of the pre-operative rehabilitation recovery protocol and its effect on the quality of recovery after colorectal surgeries. *Chin Med J (Engl)*. 2021;134(23):2865-2873. doi:10.1097/CM9.0000000000001709

35. Lee JH, Kim D, Seo D, Son JS, Kim DC. Validity and reliability of the Korean version of the Quality of Recovery-40 questionnaire. *Korean J Anesthesiol*. 2018;71(6):467-475. doi:10.4097/kja.d.18.27188

36. Tanaka Y, Wakita T, Fukuhara S, et al. Validation of the Japanese version of the quality of recovery score QoR-40. *J Anesth*. 2011;25(4):509-515. doi:10.1007/s00540-011-1151-2

37. McMillan DC, Crozier JEM, Canna K, Angerson WJ, McArdle CS. Evaluation of an inflammation-based prognostic score (GPS) in patients undergoing resection for colon and rectal cancer. *Int J Colorectal Dis*. 2007;22(8):881-886. doi:10.1007/s00384-006-0259-6

38. Shrotriya S, Walsh D, Bennani-Baiti N, Thomas S, Lorton C. C-Reactive Protein Is an Important Biomarker for Prognosis Tumor Recurrence and Treatment Response in Adult Solid Tumors: A Systematic Review. *PLoS One*. 2015;10(12):e0143080. doi:10.1371/journal.pone.0143080

39. Frühling P, Hellberg K, Ejder P, Strömberg C, Urdzik J, Isaksson B. The prognostic value of C-reactive protein and albumin in patients undergoing resection of colorectal liver metastases. A retrospective cohort study. *HPB*. 2021;23(6):970-978. doi:10.1016/j.hpb.2020.10.019

40. Punt CJA, Buyse M, Köhne CH, et al. Endpoints in adjuvant treatment trials: a systematic review of the literature in colon cancer and proposed definitions for future trials. *J Natl Cancer Inst*. 2007;99(13):998-1003. doi:10.1093/jnci/djm024

41. Karanicolas PJ, Farrokhyar F, Bhandari M. Blinding: Who, what, when, why, how? *Can J Surg*. 2010;53(5):345-348.

42. Marshall JC. *The Multiple Organ Dysfunction Syndrome*. Zuckschwerdt; 2001. Accessed April 22, 2022. http://www.ncbi.nlm.nih.gov/books/NBK6868/

43. Bone RC, Balk RA, Cerra FB, et al. Definitions for Sepsis and Organ Failure and Guidelines for the Use of Innovative Therapies in Sepsis. *Chest*. 1992;101(6):1644-1655. doi:10.1378/chest.101.6.1644

44. Tsai MS, Su YH, Ho MC, et al. Clinicopathological Features and Prognosis in Resectable Synchronous and Metachronous Colorectal Liver Metastasis. *Ann Surg Oncol*. 2007;14(2):786-794. doi:10.1245/s10434-006-9215-5

45. Lochan R, White SA, Manas DM. Liver resection for colorectal liver metastasis. *Surgical Oncology*. 2007;16(1):33-45. doi:10.1016/j.suronc.2007.04.010

46. Bismuth H, Chiche L. Surgery of hepatic tumors. *Prog Liver Dis*. 1993;11:269-285.

47. Soules MR, Sherman S, Parrott E, et al. Executive summary: Stages of Reproductive Aging Workshop (STRAW). *Climacteric*. 2001;4(4):267-272.

APPENDIX A: CIOMS Form

CIOMS FORM

SUSPECT ADVERSE REACTION REPORT	

I. REACTION INFORMATION

1. PATIENT INITIALS (first, last)	1a. COUNTRY	2. DATE OF BIRTH Day | Month | Year	2a. AGE Years	3. SEX F	4-6 REACTION ONSET Day | Month | Year	8-12 CHECK ALL APPROPRIATE TO ADVERSE REACTION
						☐ PATIENT DIED ☐ INVOLVED OR PROLONGED INPATIENT HOSPITALISATION ☐ INVOLVED PERSISTENCE OR SIGNIFICANT DISABILITY OR INCAPACITY ☐ LIFE THREATENING
7 + 13 DESCRIBE REACTION(S) (including relevant tests/lab data)						

II. SUSPECT DRUG(S) INFORMATION

14. SUSPECT DRUG(S) (include generic name)		20 DID REACTION ABATE AFTER STOPPING DRUG? ☐ YES ☐ NO ☐ NA
15. DAILY DOSE(S)	16. ROUTE(S) OF ADMINISTRATION	21. DID REACTION REAPPEAR AFTER REINTRODUCTION? ☐ YES ☐ NO ☐ NA
17. INDICATION(S) FOR USE		
18. THERAPY DATES (from/to)	19. THERAPY DURATION	

III. CONCOMITANT DRUG(S) AND HISTORY

22. CONCOMITANT DRUG(S) AND DATES OF ADMINISTRATION (exclude those used to treat reaction)
23. OTHER RELEVANT HISTORY (e.g. diagnostics, allergics, pregnancy with last month of period, etc.)

IV. MANUFACTURER INFORMATION

24a. NAME AND ADDRESS OF MANUFACTURER		
	24b. MFR CONTROL NO.	
24c. DATE RECEIVED BY MANUFACTURER	24d. REPORT SOURCE ☐ STUDY ☐ LITERATURE ☐ HEALTH PROFESSIONAL	
DATE OF THIS REPORT	25a. REPORT TYPE ☐ INITIAL ☐ FOLLOWUP	

APPENDIX B: Contraceptive Guidance

Participants of Childbearing Potential:

Women of childbearing potential (WOCBP) are participants of female sex who do not meet either of the following criteria:

1. Postmenopausal participants. Menopause will be clinically defined as occurring 12 months after a participant's last menstrual period.[47] An elevated follicle stimulating hormone level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, follicle stimulating hormone levels will be insufficient to confirm a postmenopausal state. Women on hormone replacement therapy or whose menopausal status remains unknown will be assumed to be WOCBP and required to use one of the non-estrogen containing highly effective contraception methods listed in the subsequent section titled "Contraception Guidance".
2. Participants with a prior hysterectomy, bilateral oophorectomy, and/or bilateral salpingectomy. Documentation of these procedures can come from a study personnel's review of medical records, imaging, or medical history interview.

Fertile men are participants of male sex who have not undergone a vasectomy (without subsequent reversal) or bilateral orchiectomy.

Contraception Guidance:

Participants who are WOCBP or fertile men participating in penile-vaginal intercourse must practice at least 1 highly effective method of contraception with their partners from the time of signing the ICF through 2 months after the last dose of IP, OR these participants must agree to completely abstain from penile-vaginal intercourse. Acceptable methods of contraception are listed in Table 2.

Fertile men must also refrain from donating sperm while receiving IP

All participants of male sex with a pregnant or breastfeeding partner must agree to use a male condom or remain abstinent from penile-vaginal intercourse while receiving IP

Table 2. Highly Effective Contraceptive Methods

User Dependent Methods[a]
Combined (estrogen and progesterone containing) hormonal contraception associated with inhibition of ovulation
- Oral - Intravaginal

- Transdermal
Progesterone-only hormonal contraception associated with inhibition of ovulation
- Oral - Injectable
Male Condom
Abstinence
- Considered highly effective only if participant is completely compliant. The reliability needs to be evaluated in relation to the duration of treatment with IP and the preferred and usual lifestyle of the participant
User Independent Methods
Implantable progesterone-only hormonal contraception associated with inhibition of ovulation
Progesterone or Copper IUD
Bilateral tubal occlusion or ligation
Vasectomy
- Considered highly effective provided that the absence of sperm has been confirmed

IUD: intrauterine device

[a] Typical use failure rates may differ from those when used consistently and correctly.

APPENDIX C: Summary of IP Procedures, Study Specific Tests, and Analysis Cohorts According to Clinical Scenario

	Participant	IP	Study Specific Tests	Primary Analysis	Correlative Analysis	Sensitivity Analysis	Safety Analysis
1	Undergoes surgery with resection +/- ablation of all CRLMs and received a minimum of 80% of the prescribed preoperative doses of IP and at least 90% of the postoperative doses.	Continue to end	**PFS/OS:** as scheduled **ctDNA:** as scheduled **Side effect profile:** as scheduled **QoR:** as scheduled **Correlative outcomes:** as scheduled	Yes	Yes	Yes	Yes
2	Stops IP due to toxicity, patient preference, clinician preference, or MODS and received less than 80% of the prescribed preoperative doses of IP or less than 90% of the postoperative doses.	Stop	**PFS/OS:** as scheduled **ctDNA:** as scheduled **Side effect profile:** as scheduled **QoR:** as scheduled **Correlative outcomes:** as scheduled	Yes	Yes	No	Yes
3	Does not undergo surgery due to progression, the patient is too unwell, patient preference, or >120 days have elapsed since starting IP for reasons other than apparent cCR or withdrawal.	Stop at the time of the decision to not undergo surgery, or after 120 days if sooner	**PFS/OS:** as scheduled **ctDNA:** measured once after the decision to not operate (within 14 days of the decision) **Side effect profile:** as scheduled **QoR:** stop **Correlative outcomes:** stop	Yes	No	No	Yes
4	Does not have surgery due to apparent cCR	Continue x additional 41 days from time of decision not to operate	**PFS/OS:** all assessments with timing defined based on decision not to operate **ctDNA:** all assessments with timing defined based on decision not to operate **Side effect profile:** all assessments with timing defined based on decision not to operate **QoR:** stop **Correlative outcomes:** stop	Yes	No	Yes	Yes
5	Has surgery but no resection or ablation due to apparent cCR	Continue to end	**PFS/OS:** as scheduled **ctDNA:** as scheduled	Yes	Yes	Yes	Yes

			Side effect profile: as scheduled **QoR:** as scheduled **Correlative outcomes:** as scheduled				
6	Has surgery but not a complete resection or ablation due to progression, unresectable disease, or any other reason excluding apparent cCR	Stop at the time of surgery	**PFS/OS:** as scheduled **ctDNA:** measured once postoperatively **Side effect profile:** as scheduled **QoR:** as scheduled **Correlative outcomes:** All pre-operative measurements. Postoperative measurements only on POD1, POD4 (+/- 1 day), and post-op 6 weeks (+/- 10 days)	Yes	Yes	No	Yes
7	Has surgery with the resection of extrahepatic metastases discovered at the time of the operation	Continue to end	**PFS/OS:** as scheduled **ctDNA:** as scheduled **Side effect profile:** as scheduled **QoR:** as scheduled **Correlative outcomes:** as scheduled	Yes – and do not count as an event	Yes	No	Yes
8	Has surgery with resection or ablation of all known disease; however, after surgery found to have extrahepatic disease that was present prior to surgery	Stop when progression is determined	**PFS/OS:** as scheduled **ctDNA:** as scheduled **Side effect profile:** as scheduled **QoR:** as scheduled **Correlative outcomes:** as scheduled	Yes – PFS defined as the date of progression	Yes	No	Yes
9	Has surgery with resection or ablation, and surgical pathology is not CRLM (and not cCR)	Stop when the pathology is reviewed by investigators	**PFS/OS:** stop **ctDNA:** stop **Side effect profile:** as scheduled **QoR:** as scheduled **Correlative outcomes:** stop	No	Yes	No	Yes
10	Chooses to withdraw *If participant withdraws from the study before taking any IP, there will be no further study specific tests and will not be part of any analysis. They will also be replaced with another participant	Stop at time of withdrawal	If willing: **PFS/OS:** as scheduled **ctDNA:** as scheduled **Side effect profile:** as scheduled **QoR:** as scheduled **Correlative outcomes:** as scheduled	If willing	If willing	No	Yes
IP: investigational product, PFS: progression free survival, OS: overall survival, ctDNA: circulating tumour DNA, QoR: quality of recovery, cCR: clinical complete response.							