CLINICAL STUDY PROTOCOL

TSUMURA USA

Tsumura USA, Inc.

Protocol Title: Randomized, Double-Blind, Placebo-Controlled, Phase 2 Trial to Evaluate the Safety and Efficacy of TU-100 as an Adjunct to an Enhanced Recovery after Surgery (ERAS) Protocol in Subjects Undergoing Bowel Resection

Protocol Number: TU100P2T4

IND Number:	72103
Name of Investigational Product	Daikenchuto (TU-100)
Phase of Development:	Phase 2
Indication:	Postoperative ileus
Sponsor:	[redacted] [redacted] [redacted]. 202 Carnegie Center Suite 306 Princeton, NJ 08540 [redacted]) [redacted]
Original Protocol Version: **Protocol Amendment Version(s):**	1.0 2.0 3.0 4.0 5.0 6.0
Original Protocol Date: **Protocol Amendment Date(s):**	28-August-2020 01-February-2021 16-April-2021 18-February-2022 27-July-2022 21-February-2022

PROTOCOL APPROVAL SIGNATURES

Protocol Title: Randomized, Double-Blind, Placebo-Controlled, Phase 2 Trial to Evaluate the Safety and Efficacy of TU-100 as an Adjunct to an Enhanced Recovery after Surgery (ERAS) Protocol in Subjects Undergoing Bowel Resection

Protocol Number: TU100P2T4

This study will be conducted in compliance with the clinical study protocol (and amendments), International Council for Harmonisation (ICH) guidelines for current Good Clinical Practice (GCP), and applicable regulatory requirements.

Sponsor Signatory

[redacted]
President
Tsumura USA, Inc.

JUN KOSAKA *Electronically signed by: JUN KOSAKA Reason: I am the approver Date: Feb 24, 2023 15:58 EST*

Signature

Date (DD-Mmm-YYYY)

Contract Research Organization Signatory

[redacted]
Medical Director, Medical Management
Syneos Health

Electronically signed by: Ryan O'Connor Reason: I am the approver Date: Feb 27, 2023 09:10 EST

Signature

Date (DD-Mmm-YYYY)

INVESTIGATOR SIGNATURE PAGE

Protocol Title: Randomized, Double-Blind, Placebo-Controlled, Phase 2 Trial to Evaluate the Safety and Efficacy of TU-100 as an Adjunct to an Enhanced Recovery after Surgery (ERAS) Protocol in Subjects Undergoing Bowel Resection

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Confidentiality and Current Good Clinical Practice (GCP)/E6(R2) Compliance Statement

- I, the undersigned, have reviewed this protocol, including appendices, and I will conduct the study as described in compliance with this protocol (and amendments), GCP, and relevant International Council for Harmonisation (ICH) guidelines.
- I am thoroughly familiar with the appropriate use of the study drug, as described in this protocol and any other information provided by Tsumura USA, Inc. including, but not limited to, the current investigator's brochure.
- Once the protocol has been approved by the independent ethics committee (IEC)/institutional review board (IRB), I will not modify this protocol without obtaining prior approval of Tsumura USA, Inc. and of the IEC/IRB. I will submit the protocol amendments and/or any informed consent form modifications to Tsumura USA, Inc. and the IEC/IRB, and approval will be obtained before any amendments are implemented.
- I ensure that all persons or party assisting me with the study are adequately qualified and informed about the Tsumura USA, Inc. study drug and of their delegated study-related duties and functions as described in the protocol.
- I ensure that source documents and trial records that include all pertinent observations on each of the site's trial subjects will be attributable, legible, contemporaneous, original, accurate, and complete.
- I understand that all information obtained during the conduct of the study with regard to the subjects' state of health will be regarded as confidential. No subject names will be disclosed. All subjects will be identified by assigned numbers on all case report forms, laboratory samples, or source documents forwarded to the Sponsor. Clinical information may be reviewed by the Sponsor or its agents or regulatory agencies. Agreement must be obtained from the subject before disclosure of subject information to a third party.
- Information developed in this clinical study may be disclosed by Tsumura USA, Inc. to other clinical investigators, regulatory agencies, or other health authority or government agencies as required.

<Name>

<Title>

_____
Investigator Signature

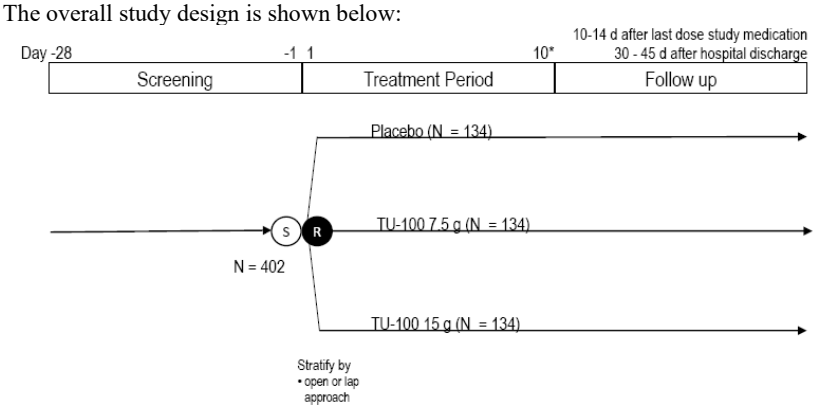
_____
Date (DD-Mmm-YYYY)

_____
Institution

2 SYNOPSIS

Title of Study:	Randomized, Double-Blind, Placebo-Controlled, Phase 2 Trial to Evaluate the Safety and Efficacy of TU-100 as an Adjunct to an Enhanced Recovery after Surgery (ERAS) Protocol in Subjects Undergoing Bowel Resection
Protocol Number:	TU100P2T4
Investigators/Study Sites:	Up to approximately 55 study sites in the United States
Phase of Development:	Phase 2
Objectives:	The primary objective of this study is to compare the effect of TU-100 with placebo, in conjunction with an enhanced recovery pathway for gastrointestinal (GI) recovery, on resolution of postoperative ileus (POI) following bowel resection (BR) as assessed by recovery of upper and lower GI motility. Secondary objectives: • Evaluate the effect of TU-100 compared with placebo on measures of early GI recovery related outcomes including hospital length of stay (see secondary endpoint list) • Evaluate the safety and tolerability of TU100 compared with placebo after daily in-hospital dosing (up to 10 days) • Evaluate TU-100 compared with placebo with regards to the incidence and severity of hepatobiliary adverse events (AEs) Exploratory objective: • Explore the potential effects of TU-100 on other early postoperative surgical outcomes related to its mechanism of action (see exploratory endpoint list)
Study Endpoints:	Primary endpoint: The primary efficacy endpoint is the time to achieve recovery of GI motility as measured by a composite endpoint representing upper (time to first toleration of clear liquids) AND lower (time to first bowel movement [BM] OR absence of distention AND presence of bowel sounds AND flatus) GI recovery. This endpoint is referred to as gastrointestinal recovery (GIR). GIR = Time to first toleration of clear liquids (i.e., time at which the subject transitions from clear liquids to the next diet stage; [full liquids or solids]) AND (Time to first BM) OR (absence of distension + presence of bowel sounds + flatus) Secondary efficacy endpoints: • Time to GI-2 recovery (i.e., time to first toleration of solids AND time to first BM) • Time to ready for discharge (based solely on GI recovery) • Time to discharge order written • Length of hospital stay (based on calendar day of discharge order written) • Proportion of GIR responders o GIR by day • POI-related morbidity (primary POI only) o POI that required readmission within 7 days of discharge, or o Need for postoperative nasal gastric tube (NGT) insertion to manage symptoms of POI (i.e., vomiting/retching, abdominal distension) per protocol guidelines • Time to first toleration of clear liquids, i.e., time at which the subject transitions from clear liquids to the next diet stage; (full liquids or solids) • Time to first BM • Time to absence of distension and presence of bowel sounds and flatus (i.e., time to when all 3 conditions have been achieved) • Time to toleration of solids (i.e., able to eat a meal that requires chewing without a vomiting episode by the time the next consecutive meal is offered) o Note, if the first solid meal is consumed on the day of hospitalization (i.e., the day of surgery), the time to toleration of solids is as stated (e.g., the time to eat solids without a vomiting episode by the time the next consecutive meal is offered). Further, if the first solid meal is consumed on the day of discharge, the time to toleration of solids criteria is met if there is no vomiting prior to discharge. In all cases, the actual time that should be recorded on the electronic

	case report form is the time the meal was consumed by the subject, not the cutoff time for vomiting prior to next consecutive meal or discharge. Safety endpoints: • Incidence of AEs • Clinical laboratory tests (hematology and serum chemistry, especially liver function tests [LFTs]) o Close observation if early signals of possible drug induced liver injury (DILI) are detected and confirmed ▪ Repeating aminotransferases (aspartate aminotransferase [AST], alanine aminotransferase [ALT]), and total bilirubin tests 3 times weekly (the initial retest will occur within 48 hours) until stable or discontinuation of study drug; frequency of retesting can decrease to once a week or less if abnormalities stabilize or the study drug has been discontinued and the subject is asymptomatic. ▪ Obtaining more detailed history of symptoms and prior or concurrent diseases ▪ Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets if not already captured in medical history ▪ Ruling out active hepatitis virus types A, B, C, D, and E; autoimmune or alcoholic hepatitis; nonalcoholic steatohepatitis (NASH); hypoxic/ischemic hepatopathy; and biliary tract disease ▪ Obtaining a history of exposure to environmental chemical agents ▪ Consideration of GI or hepatology consultations • Incidence of independently assessed hepatobiliary AEs • Vital sign measurements (blood pressure, heart rate, respiratory rate, and temperature) • 12-lead electrocardiogram (ECG) test results Exploratory endpoints: • Subject-reported (eDiary) occurrence of nausea, vomiting and/or retching, and abdominal bloating per day • Subject-reported (eDiary) number of episodes of vomiting and/or retching per day • Subject-reported (eDiary) bothersomeness of nausea and abdominal bloating by day • Incidence and severity of nausea, vomiting and/or retching, abdominal bloating based on spontaneous AE reporting from safety database • Postoperative antiemetic rescue • Postoperative complications ▪ Prolonged POI: • Minimal incision BR (laparoscopic or robotically-assisted or hand-assisted): Unresolved POI by postoperative Day 3 • Laparotomy (open) BR: unresolved POI by postoperative Day 5 **NOTE: POI = primary POI (i.e., POI that is not secondary to surgical complication, such as anastomotic leak, abscess formation, or sepsis etc.)** ▪ Recurrent POI (prolongs hospital stay or results in readmission - based on investigator assessment) ▪ Surgical site infection ▪ Anastomotic leak ▪ Early postoperative mechanical small bowel obstruction (EPSBO)

	• Confirmed by the presence of a transition point between dilated and decompressed bowel demonstrated on abdominal imaging o Time to discontinue intravenous (IV) fluids o Time to transition to oral analgesics o Hospital readmission (within 7 days and within 30 days of discharge)
Study Design:	This multicenter, randomized, double-blinded, placebo-controlled study will evaluate the effect of TU-100 on resolution of POI in subjects undergoing open or minimally invasive BR. Straight, hand-assisted, and robotically-assisted laparoscopic approaches are allowed. Subjects undergoing major abdominal resective intestinal surgeries (BRs; e.g., small BR, large BR, rectal resections [low anterior resection], total colectomy with ileo-rectal anastomosis) will be eligible for enrollment. Subjects will be screened up to 28 days before their planned surgery and will be randomized 1:1:1 (TU-100 15 g/day: TU-100 7.5 g/day: placebo) on postoperative Day 1 before the first dose of study medication. Subjects will be stratified based on surgical approach (open versus laparoscopic). After randomization, subjects will receive a total daily dose of TU-100 15g, TU-100 7.5 g, or matching placebo (TID) until hospital discharge or ≤ 10 days (whichever is earlier). All subjects will be treated with study medication as adjunct to an enhanced recovery (i.e., ERAS) pathway for GI recovery, the components of which are standardized, to the extent possible, by the protocol (standard of care). There will be 2 independent committees with additional safety oversight. Each will have a formal charter and their specific roles will be discussed within the protocol. • Data and safety and monitoring board (DSMB; clinical trial safety oversight) • Clinical endpoint adjudication committee (CEAC; for assessment of hepatobiliary AEs) The overall study design is shown below:  • Abbreviations: R = Randomized; S = Surgery. • [a] Treatment will be for a maximum of 10 days while subjects are hospitalized. If a subject is discharged before postoperative Day 10, study medication will be discontinued upon hospital discharge.
Selection of Subjects:	Main Inclusion Criteria: Eligible subjects are males or females ≥ 18 years of age with an American Society of Anesthesiologist Physical Status Score of 1 to 3 who are scheduled for an elective BR. The BR may be open or laparoscopic (straight, hand-assisted, or robotically assisted) and may include small BR, large BR, total colectomy with ileo-rectal anastomosis, or rectal resection. The anticipated hospital stay is ≥ 4 days with at least 3 overnight stays. • Please follow your local testing guidelines for SARS-CoV-2. If testing is mandatory at your institution, a positive result would be exclusionary • Subjects with prior history of COVID-19 must be confirmed recovered per CDC criteria and have a SARS-CoV-2 negative test result • If a subject contracts COVID-19 during the interval between screening and pre-surgery Day -1, he or she may be rescreened once recovered, assuming the subject meets all other inclusion/exclusion criteria and per investigator clinical judgement Main Exclusion Criteria: Subjects are not eligible if they are scheduled for a type of surgery not

	listed in the inclusion criteria, if a surgery is required for an ongoing GI infection, bowel obstruction, or perforated bowel, or if the subject requires a ventral hernia repair through component separation techniques or placement of mesh. Subjects requiring any additional resections beyond the intestine (e.g., hepatectomy, distal pancreatectomy, pancreaticoduodenectomy, gastric resection, uterine resection) or concomitant surgeries (with the exception of biopsies) are not eligible as are subjects requiring resection of the rectum (resection of a portion of the rectum is permitted as part of a low anterior resection). Subjects requiring the formation of a stoma (ileostomy or colostomy) or stoma reversals with anastomosis, or who have a functional colostomy or ileostomy are not eligible. Subjects who use an enteral feeding device (including, but not limited to, nasogastric tube, nasoduodenal tube, or gastric tube) within 30 days of surgery are also not eligible. Subjects who have an ongoing history of short bowel syndrome, chronic constipation (≤3 spontaneous BMs/week), chronic diarrhea (3 or more loose stools per day that last for at least 4 weeks), complete bowel obstruction, or a history of GI dysmotility are not eligible.
Planned Sample Size:	The study is expected to enroll approximately 402 eligible subjects (with expected loss to follow-up or censorship of primary endpoint) with a 1:1:1 randomization ratio over a post-surgery period (up to 10 days). Based on historical evidence, it is assumed that a small proportion of subjects will be lost to follow-up or will have events censored due to required analytical methods. To account for this anticipated loss in contribution of events to the primary endpoint, an additional 36 subjects (approximately 10% of 366) will be enrolled to achieve a final sample size of 402 subjects (134 per group).
Investigational Therapy:	TU-100, an herbal product manufactured by Tsumura & Co (Tsumura; Tokyo, Japan), is a granular formulation of daikenchuto. TU-100 will be provided in sachets of 2.5 gr. Subjects will receive TU-100 TID by mouth (in a solution with water) from Day 1 (the day after surgery) through Day 10 or hospital discharge (whichever occurs earlier).
Reference Therapy:	Placebo will be similar in appearance to TU-100 and will be provided in sachets of 2.5 gr. Subjects will receive placebo by mouth (in a solution with water) from Day 1 (the day after surgery) through Day 10 or hospital discharge (whichever occurs earlier).
Treatment Duration:	Each subject will receive study drug or matching placebo starting on Day 1 through Day 10 or hospital discharge (whichever occurs earlier). All dosing will be in the hospital (no outpatient dosing).
Efficacy:	The primary efficacy variable will be time to Gastrointestinal Recovery (TGIR), which will be the time to recovery of both upper (time to first toleration of liquids), and lower (first BM OR absence of distention AND bowel sounds AND flatus) GIR. Secondary efficacy assessments: • Time to GI-2 recovery (i.e., time to first toleration of solids AND time to first BM) • Time to ready for discharge (based solely on GI recovery) • Time to discharge order written • Length of hospital stay (based on calendar day of discharge order written) • Proportion of GIR responders (GIR by day) • POI-related morbidity (primary POI only) defined as 1) POI that required readmission within 7 days of discharge or 2) Need for postoperative NGT insertion to manage symptoms • Time to first toleration of clear liquids • Time to first BM • Time to absence of distention and presence of bowel sounds and flatus (all 3 achieved) • Time to toleration of solids Exploratory assessments: • Subject-reported (eDiary) occurrence of nausea, vomiting and/or retching, and abdominal bloating per day • Subject-reported (eDiary) number of episodes of vomiting/retching per day • Subject-reported (eDiary) bothersomeness of nausea and abdominal bloating by day • Incidence and severity of nausea, vomiting/retching, abdominal bloating based on spontaneous AE reporting from safety database • Postoperative antiemetic rescue • Postoperative complications, including prolonged or recurrent POI, infection at site, anastomotic leak, or bowel obstruction • Time to discontinue IV fluids • Time to transition to oral analgesics • Hospital readmission (within 7 days and within 30 days of discharge)

Safety:	Safety measures include the following • Incidence of AEs • Clinical laboratory tests (hematology, serum chemistry including liver transaminases, and LFTs, and close observation for possible DILI) • Incidence of independently assessed hepatobiliary AEs • Vital sign measurements (blood pressure, heart rate, respiratory rate, and body temperature) • 12-lead ECG test results
Pharmacokinetics:	Sparse sampling will be conducted to evaluate the pharmacokinetics (PK) of TU-100 constituents (e.g., HAS, HBS).
Statistical Methods and Planned Analyses:	Populations: Safety analysis set: All randomized subjects who receive at least 1 dose of study medication. The Safety Analysis Set will be used primarily for the analysis of safety data. Subjects will be classified based upon the treatment received. Full analysis set (FAS): All randomized subjects who receive at least 1 dose of study medication, who underwent an elective BR surgery as specified in the protocol, and who provided at least 1 post-surgery assessment of the primary endpoint (GIR) during the treatment period. The FAS will be used primarily for the analysis of efficacy data. Participants will be analyzed according to the treatment group to which they are randomized. Per-protocol analysis (PP) set: A subset of the FAS who: • Had a protocol-specified surgery • Did not take prohibited medications during the treatment period that could affect the primary endpoint • Had otherwise complied with the protocol without any other major protocol deviation (any exceptions for inclusion/exclusion criteria granted by the Sponsor/medical monitor will not be considered protocol violations) PK analysis set: All subjects who have PK data available for at least 1 dose of TU-100. The primary efficacy variable is time to TGIR, a composite endpoint defined as the following: TGIR = maximum (time to first toleration of liquids; time to first BM OR [absence of distention AND bowel sounds AND flatus]) Time to an event is defined as the elapsed time, in hours, from the end of surgery to the time of the first event, to be calculated as: Time to event (hours) = (time of the event − time of end of surgery) The primary analysis of TGIR will be based on the Cox proportional hazard model (Cox PH) that includes the main effects for treatment and surgical approach (open versus laparoscopic). The estimates of time to event for the quartiles (25%, 50%, 75%), hazard ratios and their corresponding one-sided 95% confidence intervals (CIs), the Wald Chi-square one-sided *P* values from the Cox analysis will be summarized. The ties in the data will be handled by the Breslow method. A secondary analysis will be applied for TGIR using the Cox PH model including main effects for treatment, surgical approach (open versus laparoscopic), and covariates such as surgical type, gender, age (years), post-operation opioid consumption (mg morphine equivalents), thoracic epidural use, and surgery duration (hours). Backward method will be used to select statistically significant covariates (P <0.1). Adjusted *P* value and hazard ratio (HR) will be provided after the final model is selected. In addition, TGIR will be assessed for TU-100 versus placebo at each dose level using a stratified log-rank with the one stratum used for randomization: surgical approach (open versus laparoscopic). The Kaplan-Meier estimate of the median TGIR and the corresponding 1-sided 95% CIs calculated using the Greenwood's formula will be presented. Plot of the Kaplan-Meier estimate of the survival distribution function over time will be presented. The restricted mean survival time (RMST) at time t* using the Kaplan-Meier estimate of the survival function will also be provided, where t* is the minimum of the largest observed time (event or censored) in each of the 2 groups. Furthermore, the difference in RMST, as well as the ratio of RMST between 2 groups will be evaluated. Similar analyses will be applied for other time to event efficacy endpoints. A secondary efficacy variable, time to ready for discharge (based solely on the treating medical team's determination of GI recovery), which is defined as the time to the assessment date of "ready for hospital discharge." Length of stay is based on the time to the calendar day of discharge order written from the calendar day of surgery. Treatment effect on other numeric variables will be analyzed using an analysis of variance (ANOVA) if normally distributed, or the Wilcoxon rank sum test otherwise. Treatment effect on categorical variables will be analyzed using the stratified Miettinen and Nurminen (M&N) method (stratified by surgical approach), or the logistic regression model if appropriate.

3 TABLE OF CONTENTS

3.1 List of In-text Tables

3.2 List of In-text Figures

4 LIST OF ABBREVIATIONS

Abbreviation	Definition
ADR	adverse drug reaction
AE	adverse event
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Classification
BM	bowel movement
BMI	body mass index
BR	bowel resection
CEAC	clinical endpoint adjudication committee
CFR	Code of Federal Regulations
CI	confidence interval
Cox PH	Cox proportional hazard model
CSR	clinical study report
DILI	drug induced liver injury
DSMB	data and safety and monitoring board
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
eDiary	electronic diary
eGFR	estimated glomerular filtration rate
EPSBO	early postoperative mechanical small bowel obstruction
ERAS	enhanced recovery after surgery
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	good clinical practice
GI	gastrointestinal
GIR	gastrointestinal recovery
HBV	hepatitis B virus
HCV	hepatitis C virus
HIPAA	Health Insurance Portability Accountability Act
HIV	human immunodeficiency virus
HR	Hazard ratio
IB	investigator’s brochure
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	independent ethics committee
IND	Investigational New Drug
INR	international normalized ratio

Abbreviation	Definition
IRB	institutional review board
IV	intravenous
IWRS	interactive web response system
LFT	liver function test
LOS	length of stay
MedDRA	Medical Dictionary for Regulatory Activities
NASH	nonalcoholic steatohepatitis
NGT	nasal gastric tube
NPO	nothing by mouth
NSAID	nonsteroidal anti-inflammatory drug
PI	principal investigator
PK	pharmacokinetic
POI	postoperative ileus
PONV	postoperative nausea and vomiting
PP	Per-protocol
QTc	corrected QT interval
QTcF	QT interval with Fridericia correction
RMST	restricted mean survival time
SAE	serious adverse event
SAP	statistical analysis plan
SOC	standard of care
SUSAR	suspected unexpected serious adverse reactions
TAP	transverse abdominis plane [block]
TEAE	treatment-emergent adverse event
TGIR	time to gastrointestinal recovery
TID	3 times per day (i.e., 6 hours ± 1 hour apart)
ULN	upper limit of normal
US	United States
WOCBP	women of childbearing potential

5 INTRODUCTION

5.1 Background on Postoperative Ileus

[redacted]

5.2 Background on TU-100

[redacted]

[redacted]

[redacted]

5.2.1 Nonclinical Studies

[redacted]

[redacted]

[redacted]

[redacted]

[redacted].

5.2.2 Clinical Studies

[redacted]

Efficacy

[redacted]

[redacted]

[redacted]

Safety

[redacted]

[redacted]

[redacted]

[redacted]

- [redacted]
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  - [redacted]
- [redacted]

[redacted].

5.3 Clinical Risks/Benefits of TU-100

Risks

Evaluation of the clinical trial safety database for TU-100 involving 290 healthy volunteers and subjects with chronic GI disorders or major GI surgery revealed no apparent trends or signals with regard to hepatobiliary disorders, including DILI.

The most commonly reported AEs from completed clinical studies in healthy volunteers were headache and dyspepsia; in subjects with chronic GI disorders (including Crohn's disease, irritable bowel syndrome, and chronic constipation) were headache, nausea, back pain, and flatulence; and in surgical subjects were pruritus, vomiting, abdominal distension, and procedural pain.

Based on the totality of the evidence from post-marketing safety surveillance from Japan, no clear association between TU-100 exposure and serious liver injury is apparent at this time. The incidence of hepatobiliary ADRs is low (0.003%) and consistent over time.

Benefits

TU-100 has been evaluated across multiple patient populations and conditions within the US development program. Primary efficacy endpoints in completed US Phase 2 surgery studies were not met; however, these studies were small and uncontrolled (Study TU100P2T1) and not prospectively designed to evaluate the effect of TU-100 compared with placebo on GI recovery/POI (Studies TU100P2T1 and TU100P2T3). Thus, within this context, the question regarding potential benefit remains unanswered.

Nevertheless, there were signals that TU-100 may have improved GI recovery after colorectal resection including lower antiemetic use for nausea and numerically lower LOS compared with placebo in the Phase 2 trial of subjects undergoing laparoscopic colectomy (TU100P2T3). Moreover, in Japanese investigator-initiated trials, daikenchuto, used with varying dosing paradigms, was associated with accelerated time to first bowel movement (BM) in some patients undergoing open large BR, hepatectomy, and gastrectomy, particularly for those thought to have POI (Katsuno 2015, Kono 2019, Shimada 2015, Yoshikawa 2015). However, due to differences in SOC and dosing regimens used in Japan compared with the US, these data must be interpreted within that context.

Thus, this adequate and well-controlled Phase 2 study is designed *specifically* to directly evaluate the effect of TU-100 compared with placebo on GI recovery and other related key early in-hospital outcomes (e.g., postoperative GI-related complications, hospital LOS) following major abdominal surgery.

Details regarding known or anticipated benefits and risks, as well as reasonably anticipated AEs for TU-100 may be found in the IB.

5.4 Study Rationale

Nonclinical studies performed to date have demonstrated daikenchuto's ability to improve intestinal motility, improve intestinal blood flow, and prevent the incidence of adhesions and anastomotic leaks after colorectal surgery in animal models. It is thought that TU-100 would have the potential to have similar benefits in humans to prevent POI after colorectal surgery.

In completed US Phase 2 surgery studies, although primary efficacy endpoints were not achieved, neither study was prospectively designed to evaluate the effect of TU-100 compared with placebo on GI recovery/POI. However, there were signals that TU-100 may have impacted GI recovery after colorectal resection including lower antiemetic use for nausea and numerically lower LOS compared with placebo in the Phase 2 trial of subjects undergoing laparoscopic colectomy. Moreover, in investigator-initiated Phase 3 trials in large BR, hepatectomy, and gastrectomy conducted in Japan, time to first BM was accelerated in some subjects, particularly those thought to have POI (Katsuno 2015, Kono 2019, Shimada 2015, Yoshikawa 2015).

In clinical trials, TU-100 has been shown to be well tolerated. The most common AEs have been GI-related. In order to systematically evaluate the potential risk for DILI, based on post-marketing surveillance in Japan suggesting the potential for risk of liver injury, close oversight of safety will be implemented.

This adequate and well-controlled Phase 2 study is designed specifically to directly evaluate the effect of TU-100 on GI recovery and other related key early in-hospital outcomes (e.g., postoperative GI-related complications, hospital LOS) following BR.

6 STUDY OBJECTIVES AND ENDPOINTS

6.1 Study Objectives

6.1.1 Primary Objective

The primary objective of this study is to compare the effect of TU-100 with placebo, in conjunction with an enhanced recovery pathway for GI recovery, on resolution of POI following BR as assessed by recovery of upper and lower GI motility.

6.1.2 Secondary Objectives

- Evaluate the effect of TU-100 compared with placebo on measures of early GI recovery related outcomes including hospital LOS (see Section 6.2.2)
- Evaluate the safety and tolerability of TU-100 compared with placebo after daily in-hospital dosing (up to 10 days)
- Evaluate TU-100 compared with placebo with regards to the incidence and severity of hepatobiliary AEs

6.1.3 Exploratory Objectives

- Explore the potential effects of TU-100 on other early postoperative surgical outcomes related to its mechanism of action (see Section 6.2.4)

6.2 Study Endpoints

6.2.1 Primary Efficacy Endpoint

Primary endpoint: The primary efficacy endpoint is the time to achieve recovery of GI motility as measured by a composite endpoint representing upper (time to first toleration of clear liquids) AND lower (time to first BM OR absence of distention AND presence of bowel sounds AND flatus) GI recovery. This endpoint is referred to as gastrointestinal recovery (GIR).

GIR=Time to first toleration of clear liquids (i.e., time at which the subject transitions from clear liquids to the next diet stage; [full liquids or solids])
AND
(Time to first BM) OR (absence of distension + presence of bowel sounds + flatus)

6.2.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints are as follows:

- Time to GI-2 recovery (i.e., time to first toleration of solids AND time to first BM)
- Time to ready for discharge (based solely on GI recovery)
- Time to discharge order written
- Length of hospital stay (based on calendar day of discharge order written)
- Proportion of GIR responders
    - GIR by day
- POI-related morbidity (primary POI only)
    - POI that required readmission within 7 days of discharge, or

    - Need for postoperative NGT insertion to manage symptoms of POI (i.e., vomiting/retching, abdominal distension) per protocol guidelines
- Time to first toleration of clear liquids, i.e., time at which the subject transitions from clear liquids to the next diet stage (full liquids or solids)
- Time to first BM
- Time to absence of distension and presence of bowel sounds and flatus (i.e., time to when all 3 conditions have been achieved)
- Time to toleration of solids (i.e., able to eat a meal that requires chewing without a vomiting episode by the time the next consecutive meal is offered).
    - Note, if the first solid meal is consumed on the day of hospitalization (i.e., the day of surgery), the time to toleration of solids is as stated (e.g., the time to eat solids without a vomiting episode by the time the next consecutive meal is offered). Further, if the first solid meal is consumed on the day of discharge, the time to toleration of solids criteria is met if there is no vomiting prior to discharge. In all cases, the actual time that should be recorded on the eCRF is the time the meal was consumed by the subject, not the cutoff time for vomiting prior to next consecutive meal or discharge.

6.2.3 Safety Endpoints

Safety measures include the following:

- Incidence of AEs
- Clinical laboratory tests (hematology and serum chemistry, especially liver function tests [LFTs])
    - Close observation if early signals of possible DILI are detected and confirmed
        - Repeating aminotransferases (AST, ALT), and total bilirubin tests 3 times weekly (the initial retest will occur within 48 hours) until stable or discontinuation of study drug; frequency of retesting can decrease to once a week or less if abnormalities stabilize or the study drug has been discontinued and the subject is asymptomatic.
        - Obtaining more detailed history of symptoms and prior or concurrent diseases
        - Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets if not already captured in medical history
        - Ruling out active hepatitis virus types A, B, C, D, and E; autoimmune or alcoholic hepatitis; nonalcoholic steatohepatitis (NASH); hypoxic/ischemic hepatopathy; and biliary tract disease
        - Obtaining a history of exposure to environmental chemical agents
        - Consideration of GI or hepatology consultations
        - Incidence of independently assessed hepatobiliary AEs

- Vital sign measurements (blood pressure, heart rate, respiratory rate, and body temperature)
- 12-lead electrocardiogram (ECG) test results

6.2.4 Exploratory Endpoints

Other exploratory endpoints of this study are as follows:

- Subject-reported (electronic diary [eDiary]) occurrence of nausea, vomiting and/or retching, and abdominal bloating per day
- Subject-reported (eDiary) number of episodes of vomiting and/or retching per day
- Subject-reported (eDiary) bothersomeness of nausea and abdominal bloating by day
- Incidence and severity of nausea, vomiting/retching, abdominal bloating based on spontaneous AE reporting from safety database
- Postoperative antiemetic rescue
- Postoperative complications
    - Prolonged POI:
        - Minimal incision BR (laparoscopic or robotically-assisted or hand-assisted): Unresolved POI by postoperative Day 3
        - Laparotomy (open) BR: Unresolved POI by postoperative Day 5

      Note: POI = primary POI (i.e., POI that is not secondary to surgical complication, such as anastomotic leak, abscess formation, or sepsis etc.)
    - Recurrent POI (that prolongs hospital stay or results in readmission – based on investigator assessment)
    - Surgical site infection
    - Anastomotic leak
    - Early postoperative mechanical small bowel obstruction (EPSBO) (Confirmed by the presence of a transition point between dilated and decompressed bowel demonstrated on abdominal imaging)
- Time to discontinue intravenous (IV) fluids
- Time to transition to oral analgesics
- Hospital readmission (within 7 days and within 30 days of discharge)

7 INVESTIGATIONAL PLAN

7.1 Description of Overall Study Design and Plan

This multicenter, randomized, double-blinded, placebo-controlled study will evaluate the effect of TU-100 on resolution of POI in subjects undergoing open or minimally invasive BR. Straight, hand-assisted, and robotically-assisted laparoscopic approaches are allowed. Subjects undergoing major abdominal resective intestinal surgeries (BRs; e.g., small BR, large BR, rectal resections [low anterior resection], total colectomy with ileo-rectal anastomosis) will be eligible for enrollment.

Subjects will be screened up to 28 days before their planned surgery and will be randomized 1:1:1 (TU-100 15 g/day: TU-100 7.5 g/day: placebo) on postoperative Day 1 before the first dose of study medication. Subjects will be stratified based on surgical approach (open versus laparoscopic). After randomization, subjects will receive a total daily dose of TU-100 15 g, TU-100 7.5 g, or matching placebo TID until hospital discharge or $\leq$ 10 days (whichever is earlier). All subjects will be treated with study medication as adjunct to an enhanced recovery (i.e., ERAS) pathway for GI recovery, the components of which are standardized, to the extent possible, by the protocol (SOC) (See Section 7.2).

Efforts will be made to obtain 134 subjects in each of the 3 treatment groups for a total of 402 subjects.

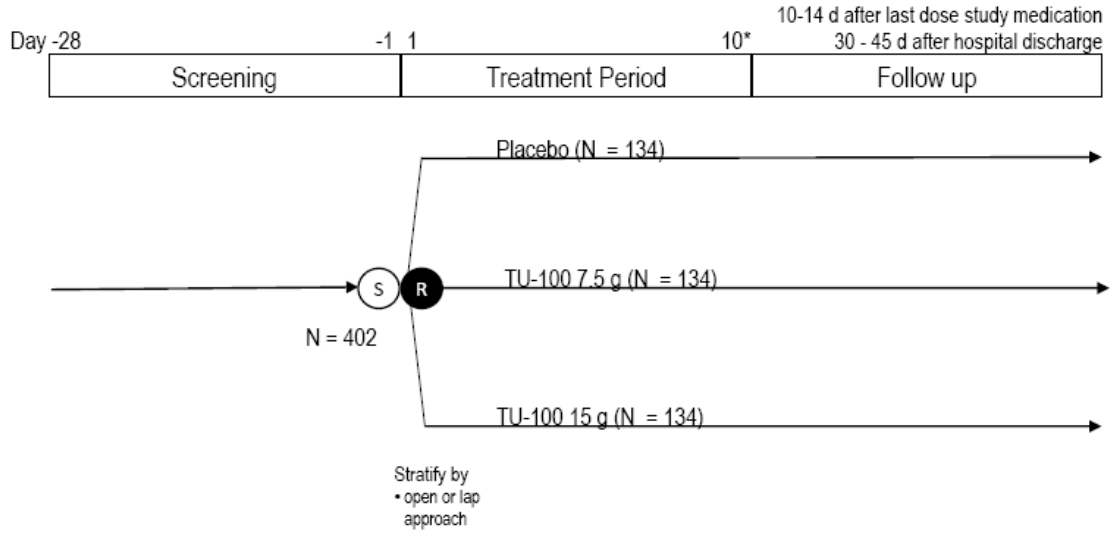
There will be 2 independent committees with additional safety oversight. Each will have a formal charter and their specific roles will be discussed within this protocol.

- Data and safety monitoring board (DSMB; clinical trial safety oversight)
- Clinical endpoint adjudication committee (CEAC; for assessment of hepatobiliary AEs)

This study follows the FDA guidance on the conduct of clinical trials and statistical considerations for clinical trials during the COVID-19 pandemic as necessary.

Figure 1 presents the study design.

Figure 1. Study Design



- Abbreviations: R = Randomized; S = Surgery.
- [a] Treatment will be for a maximum of 10 days while subjects are hospitalized. If a subject is discharged before postoperative Day 10, study medication will be discontinued upon hospital discharge.

7.2 Discussion of Study Design

The clinical development program is designed to explore the benefit of TU-100 given after BR surgery to prevent the occurrence of POI and as adjunct to ERAS. Previous studies with TU-100 showed that it was generally well tolerated. In completed Phase 2 studies in the US, while primary efficacy endpoints were not achieved, it should be noted that these studies were not designed to evaluate the effect of TU-100 compared with placebo on GI recovery or POI; therefore, the question regarding potential benefit after BR remains unanswered. However, there were signals that TU-100 had an impact on GI recovery after colorectal resection, including less need for nausea medication and a lower number of adverse symptoms.

The in-hospital observation period is up to 10 days after GI surgery. TU-100 is administered in addition to the routine standard of ERAS care (see Section 10.2.1) and provides a multimodal approach in order to enhance recovery after surgery. Treatment with TU-100 should begin immediately after randomization to provide its effect on GI recovery and ERAS. Hospitalization of subjects provides a controlled setting to assure compliance with study drug administration and allows accurate monitoring of efficacy and safety measures by study staff.

The following provides a rationale for the duration of in-hospital dosing:

- The most favorable benefit-risk profile for TU-100 was expected to occur within the period of highest risk for delayed GI recovery and associated complications, i.e., during the subject's hospital stay.
- Based on TU-100's mechanism of action and the clinical presentation of POI, it is anticipated that the optimal dosing duration is beginning the day after surgery and

continuing through the early postoperative period, during which GI motility should be resuming.

- Discontinuing TU-100 at discharge is appropriate as surgical patients are routinely not discharged before achieving GI recovery milestones. Continuing dosing after discharge would offer no further substantive benefit to the subject and cause unnecessary drug exposure.
- In-hospital dosing allows for close oversight of subjects for both safety and efficacy.

7.3 End of Study

A subject will have fulfilled the requirements for study completion if/when the subject has completed all study periods, including Discharge Follow-up Visit on the 30 days (+15 days) after discharge as indicated in the Schedule of Assessments (Table 1).

The end of the study will be the last subject's last visit or the last subject's scheduled visit/assessment as indicated in the Schedule of Assessments (Table 1).

8 SELECTION OF STUDY POPULATION

Section 7.1 provides information regarding number of subjects planned to be randomized. All subjects must pass the screening eligibility before and after surgery. All subjects must pass the randomization eligibility criteria before randomization on Day 1 in order to be randomized. The screening eligibility criteria are outlined in Section 8.1 and Section 8.2. Randomization eligibility criteria are included in Section 8.4.

8.1 Inclusion Criteria

To be considered eligible to participate in this study, a subject must meet all the inclusion criteria listed below:

1. Male or female ≥ 18 years of age
2. Women of childbearing potential (WOCBP) or men whose sexual partners are WOCBP; must be able and willing to use at least 1 highly effective method of contraception during the study and for 30 days after the last dose of study drug. A female subject is considered to be a WOCBP following menarche and until she is in a postmenopausal state for 12 months or otherwise permanently sterile (for which acceptable methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy). Highly effective methods of contraception include intrauterine contraceptive device; intrauterine hormone-releasing system; combined hormonal or progestogen-only contraception associated with inhibition of ovulation (oral, intravaginal, or transdermal); bilateral tubal ligation or occlusion; vasectomy; condom in combination with contraceptive cream, jelly, or foam; or abstinence. Male subjects must agree to not donate sperm during the study period after drug is given until the 30-day follow-up.
3. American Society of Anesthesiologists Physical Status Score of 1 to 3
4. Scheduled for an elective BR as listed below via open or laparoscopic (straight, hand-assisted, robotically assisted) approach
    a) Small BR
    b) Large BR (partial colectomy)
    c) Total colectomy with ileo-rectal anastomosis
    d) Rectal resection (low anterior resection)
5. Based on investigator assessment, subject's hospital stays anticipated to be ≥ 4 calendar days (i.e., at least 3 overnight stays). It is anticipated that the subject will not be discharged until they have achieved upper and lower GI recovery per the protocol definition.
6. Eastern Cooperative Oncology Group performance status of 0 or 1
7. Ability to understand the study procedures, have agreed to participate in the study program, and have voluntarily provided informed consent

8.2 Exclusion Criteria

To be considered eligible to participate in this study, a subject must not meet any of the exclusion criteria listed below:

1. Scheduled for a BR that is not listed in this protocol (Inclusion Criterion #4)
2. Requires emergency surgery or surgery in the presence of ongoing GI infection, bowel obstruction, or perforated bowel
3. Requires ventral hernia repair through component separation techniques or placement of mesh
4. Requires any additional resections beyond the intestine (e.g., hepatectomy, distal pancreatectomy, pancreaticoduodenectomy, gastric resection, uterine resection), or concomitant surgeries (with the exception of biopsies)
5. Requires resection of the rectum (Note: Resection of a portion of the rectum is permitted as part of a low anterior resection)
6. Requires the formation of a stoma (ileostomy or colostomy) or stoma reversals with anastomosis
7. History of previous surgeries, illness, or behavior that in the opinion of the investigator might confound the study results or pose additional risk in administering the study procedures
8. Have a functional colostomy or ileostomy
9. Ongoing history of short bowel syndrome, chronic constipation (≤3 spontaneous BMs per week), chronic diarrhea (3 or more loose stools per day that last for at least 4 weeks), and history of GI dysmotility or use of enteral feeding device (including, but not limited to, nasogastric tube, nasoduodenal tube, or gastric tube) within 30 days of surgical procedure preoperatively
10. Complete bowel obstruction
11. Received radiation therapy to the abdomen or pelvis within 2 months of scheduled surgery
12. Received chemotherapy within 1 month of scheduled surgery
13. Chemotherapy or radiation-induced bowel dysfunction
14. Diagnosed with advanced or metastatic colon cancer (Stage IV by tumor, node, and metastasis classification)
15. Hepatic impairment (defined as Child-Pugh A, B, or C)
16. Active hepatitis
17. Decompensated cirrhosis, i.e., history of hepatic encephalopathy, ascites, and variceal bleeding
18. Abnormal coagulation parameters (e.g., international normalized ratio [INR] > 1.5 × the ULN) and not on Coumadin
19. Abnormal liver tests

a) ALT or AST > ULN
b) Alkaline phosphatase (ALP) > ULN
c) Total bilirubin or direct bilirubin > ULN

Note: Subjects with an elevated indirect bilirubin (< 2 × ULN) and who meet all other liver test criteria (ALT, AST, ALP, and direct bilirubin) are eligible for participation in the study (e.g., patients with known Gilbert's disease)

20. Positive COVID-19 test result within 72 hours prior to surgery
21. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection (including known human immunodeficiency virus [HIV]), diabetes, symptomatic congestive heart failure and ejection fraction < 35%, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with protocol requirements
22. Chronic pain syndrome unrelated to the planned surgery requiring consistent management with analgesics and/or other non-pharmacologic modalities
23. Myocardial infarction within 3 months
24. Moderate to severe renal impairment (i.e., estimated glomerular filtration rate [eGFR < 60 mL/min/1.73m^2] or estimated creatinine clearance < 60 mL/min)
25. Corrected QT interval (QTc) > 500 msec
26. Diabetic gastroparesis
27. Compromised immune system, either from treatment with corticosteroids or other immunosuppressive agent within 2 weeks of surgery or from immunosuppressive disease (e.g., known HIV)
28. Immunosuppressive therapy

    Note: Subjects with inflammatory bowel disease who are receiving immunosuppressive therapy, including biologics or corticosteroids, are allowed to continue their therapy. Perioperative use of immunomodulators (e.g., azathioprine, methotrexate) in these patients is also permitted

29. Pregnant (identified by a positive serum pregnancy test) or lactating, or are not postmenopausal (no menses for at least 1 year) and are of childbearing potential and not using an accepted method of birth control (i.e., surgical sterilization, intrauterine contraceptive device, oral contraceptive, diaphragm, or condom in combination with contraceptive cream, jelly, or foam or abstinence)
30. Participated in another investigational drug/biologic or medical device study within 30 days of surgery or will be enrolled in another investigational drug or medical device study, or any study in which active subject participation is required outside routine hospital data collection during the course of the study
31. Illicit drug use or alcohol abuse based on medical history, or currently engaged in illicit drug use or alcohol abuse. Alcohol abuse is defined as 5 or more drinks in one sitting or 15 or more drinks in a week for men and 4 or more drinks in one sitting or 8 or more

drinks in a week for women. A drink is considered a 1.5-oz shot, 12 oz of beer, or 5 oz of wine. However, patients with a medical history of illicit drug use or alcohol abuse ≥ 5 years prior to the time of screening and who have recovered and have been drug/alcohol free for at least that period of time (i.e., 5 years) can be enrolled.

32. Use of supplemental ginger or Zanthoxylum fruit within 72 hours or supplemental ginseng within 120 hours before randomization
33. Has a history of allergic reactions to ginseng, ginger, Zanthoxylum fruit, or lactose
34. Has clinical symptoms of lactose intolerance after ingesting milk or milk-containing products (e.g., abdominal pain, flatulence, diarrhea)
35. Unwilling or unable to comply with procedures described in this protocol or is otherwise unacceptable for enrollment in the opinion of the investigator
36. Use of any prohibited medications (See Section 9.6.1)

8.3 Retesting and Rescreening

8.3.1 Retesting During the Screening Period

If the results of the blood tests (hematology, chemistry) are < 1.5 × ULN, not associated with an acute or chronic clinical condition or comorbidity and considered by the principal investigator (PI) to be non-clinically significant, a new sample can be collected for a retest. Exclusionary liver function test levels may be retested once within 14 days of the original screening date, but only with the documented approval of the Sponsor's medical monitor. Only 1 retest will be allowed within the screening period. If the results of the retest fall within the normal range (i.e., DO NOT meet protocol exclusion criterion #19 for abnormal liver tests) the patient may be considered for randomization assuming all other protocol-specified criteria are met including the PI's clinical judgement. The PI should contact the medical monitor should he/she have any questions or issues for discussion in these cases prior to patient randomization.

8.3.2 Rescreening

Individuals who sign the informed consent form (ICF) to participate in the study but who do not subsequently meet all the requirements as outlined in the inclusion and exclusion criteria, and therefore do not enroll (screen failures) may be rescreened as long as it is before undergoing surgery. If the subject ends up extending the screening visit out of the time of the allowed window, he/she may be rescreened. The number of times an individual may rescreen is not restricted.

Also, rescreening may be considered by PIs or surgeons, keeping in mind the expected delays in elective surgery that may occur in response to addressing increased burden on healthcare institutions associated with local/regional rates of COVID-19 infection requiring hospitalization. If a subject ends up extending the screening visit out of the allowed screening window, he/she may be rescreened.

8.4 Randomization Criteria

In order to be randomized, a subject **MUST** meet the following criteria:

1. Underwent an eligible surgery (Inclusion Criterion #4)
2. NGT tube has been removed, if inserted intraoperatively
3. Is able to receive oral medications

Additionally, in order to be randomized, a subject **MUST NOT** have achieved both upper and lower GI components that comprise the primary efficacy endpoint **before Day 1 Randomization**, as listed below:

Upper GI

4. Transitioned from clear liquids to full liquids and/or solid food

Lower GI

5. Had first BM

OR

Achieved all 3 events below:

a) Absence of abdominal distension; AND

b) Experienced first flatus after surgery; AND

c) Presence of bowel sounds after surgery

8.5 Study Treatment Discontinuation, Study Withdrawal, and Termination of Subjects

- If a subject discontinues study treatment or is withdrawn/terminated from the study for any reason, the study site must immediately notify the medical monitor. The date the subject is withdrawn from the study and/or study medication and the primary reason for discontinuation, regardless of if the subject withdrawn/terminated from the entire study or only from study medication, must be clearly documented on the electronic case report form (eCRF).
- If a subject discontinues study medication, he or she should be encouraged to continue in the study, and efficacy and safety procedures should be conducted as described in this protocol. These patients should continue with subsequent study visits and assessments as specified in the protocol including follow-up visits. Study treatment discontinuation does not imply withdrawal/termination from the study.
- If a subject is withdrawn or terminated from the study before completing the protocol-specified duration of treatment, safety assessments listed under Study Withdrawal/Termination visit in the Schedule of Assessments (Table 1) are to be completed at the time of withdrawal/termination (including all protocol-required laboratory tests). The patient will be followed for the duration of the follow-up period as specified in the protocol Section 10.2.4.
- Note that if a subject withdraws consent for further participation in the study, no further study assessments are to be performed unless the subject agrees to Study Withdrawal/Termination assessments.

In the event that a subject is withdrawn/terminated prematurely from the study because of a TEAE or serious TEAE, the TEAE or serious TEAE will be followed up until it resolves (returns

to normal or baseline values) or stabilizes, or until it is judged by the investigator to no longer be clinically significant. Once a subject is withdrawn from the study, the subject may not re-enter the study.

A subject may voluntarily withdraw or be withdrawn from the study at any time for reasons including, but not limited to, the following:

- AE or SAE
- Subject withdrawal of consent: at any time, a subject's participation in the study may be terminated at his/her request or on the basis of the investigator's clinical judgement. The reason for subject withdrawal will be noted on the eCRF.
- Investigator or the Sponsor deems withdrawal necessary at any time if either one determine that it is not in the subject's best interest to continue. This includes stopping criteria (see Section 8.5.2).
- Intercurrent illness: a condition, injury, or disease unrelated to the primary diagnosis that became apparent during treatment and necessitated the subject's termination from the study
- General or specific changes in the subject's condition that renders him/her ineligible for further treatment according to the inclusion/exclusion criteria
- Subject fails to adhere to the protocol requirements (e.g., drug noncompliance, failure to return for defined number of visits)
- Lost to follow-up: the subject stopped coming for visits, and study personnel were unable to contact the subject
- Pregnancy, as indicated in Section 12.6.6.
- NGT is inserted postoperatively
- Subject remains or reverts to nothing by mouth (NPO) status and is no longer able to receive oral medications

Additionally, the Sponsor could have stopped the study at any time for safety, regulatory, legal, or other reasons aligned with Good Clinical Practice (GCP). This study could have been terminated at the discretion of the Sponsor or any regulatory agency. An investigator could have elected to discontinue or stop the study at his or her study site for any reason, including safety or low enrollment.

8.5.1 Close Observation and Clinical Management Plan

If early signs of possible DILI are detected in a subject, close observation should be initiated, and Sponsor should be notified within 24 hours (See protocol Section 12.6.8).

Clinical signs and symptoms of possible DILI or severe hepatoxicity that may trigger close observation include, but are not limited to, the following:

- Persistent fatigue, weakness, nausea/vomiting, anorexia, pruritis, vague abdominal pain or right upper quadrant pain or tenderness, fever, rash, eosinophilia (eosinophils > 5%) in the absence of more likely causes
- Peripheral edema, ascites, encephalopathy, GI bleeding

- ALT > 3-fold ULN in subjects with normal baseline levels or 2-fold increases above baseline in subjects with abnormal levels prior to drug exposure

Close observation includes:

- Repeating liver enzyme and serum bilirubin tests 2 or 3 times weekly (the initial retest will occur within 48 hours). Frequency of retesting can decrease to once a week or less if abnormalities stabilize or the study drug has been discontinued and the subject is asymptomatic.
- Obtaining a more detailed history of symptoms and prior or concurrent diseases
- Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Ruling out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; NASH; hypoxic/ischemic hepatopathy; and biliary tract disease
- Obtaining a history of exposure to environmental chemical agents
- Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Considering gastroenterology or hepatology consultations

Note that if additional testing is conducted, this information should be recorded in the eCRF.

Note: for any subject in whom a concern for hepatotoxicity or DILI develops (i.e., any subject that meets criteria for close observation), a narrative summary will be provided to the chairman of the DSMB by the Sponsor (via the CRO DSMB manager) for review by the board. The initial narrative summary will be provided within 24 to 48 hours of Sponsor notification of identifying a subject that meets the close observation criteria and updated versions provided as additional information becomes available and/or is requested by the DSMB chairman.

Contents of the narrative summary will be comparable to the information provided in each case submitted to the CEAC for review/adjudication. Tsumura will be responsible for collecting the information and developing the narrative summary for submission to the DSMB. Based on their findings, the DSMB will make recommendations to the Sponsor regarding management of the case at the patient, site, and/or study level(s) based on their responsibilities as defined in their charter (e.g., study drug continuation, additional clinical and/or diagnostic information/testing, need for medical specialty consultation [e.g., gastroenterology/hepatology]).

Note: if transaminase elevations persist, subjects will be evaluated for other causes of transaminase elevations including tests of hepatic function (e.g., viral hepatitis, nonalcoholic fatty liver disease/NASH, ischemic hepatitis, thyroid disorders, occult celiac disease). The type and extent of testing will be determined via recommendations by the DSMB and PI (to include recommendations from clinical consults should they be performed). This may include, but is not limited to, diagnostic imaging procedures and liver biopsy. Recommended testing will be shared with the Sponsor's medical director.

If no other cause for persistent transaminase elevation is found, then the subject will be monitored closely, and the study drug will be discontinued.

8.5.2 Stopping Criteria

Stopping criteria for subject with elevated liver tests and relevant clinical signs and symptoms:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks
- ALT or AST > 3 × ULN and total bilirubin > 2 × ULN or INR > 1.5
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (eosinophils > 5%)

9 TREATMENTS

9.1 Details of Study Treatments

All dosing of study drug will be in the hospital (no outpatient dosing).

All study medication will be blinded.

9.1.1 TU-100 (Daikenchuto)

TU-100, an herbal product manufactured by Tsumura (Tokyo, Japan), is a granular formulation of daikenchuto. It consists of ginseng, Zanthoxylum fruit, and processed ginger, along with safe and suitable excipients of maltose, lactose, and magnesium stearate. TU-100 will be provided in sachets of 2.5 gr. Subjects will receive study drug TID (3 times per day [i.e., 6 hours ± 1 hour apart]) by mouth from Day 1 (the day after surgery) through Day 10 or hospital discharge (whichever occurs earlier).

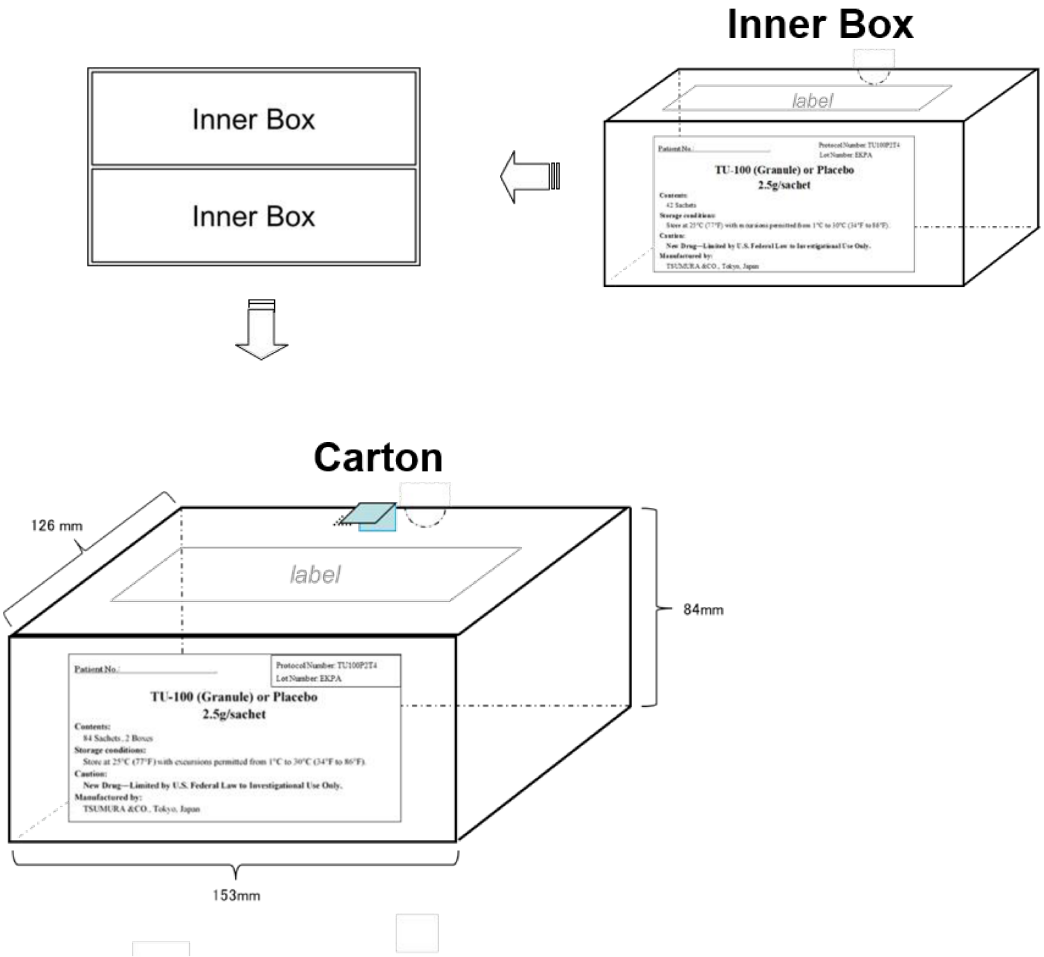
TU-100 drug product is supplied in air-tight containers (heat-sealed in aluminum packages/sachets). TU-100 packages should be stored at room temperature (25ºC [77ºF]; excursions permitted from 1ºC to 30ºC [34ºF to 86ºF]). TU-100 has a confirmed 3-year stability when stored at ambient room temperature.

In order to maintain the blind, all subjects will receive 2 sachets TID for a total of 6 sachets a day. Subjects randomized to receive TU-100 15 g/day will be administered 2 sachets of TU-100 2.5 g TID. Subjects randomized to receive TU-100 7.5 g/day will be administered 1 sachet of TU-100 2.5 g and 1 placebo sachet TID. Subjects randomized to placebo will receive 2 sachets of placebo TID. Placebo and TU-100 will be identical in appearance.

Sachets are provided to the study sites in cartons, each containing 2 inner boxes of 42 sachets each (a total of 84 sachets per 1 carton will be dispensed for each subject). ONE SACHET FROM EACH INNER BOX WILL BE PULLED FOR EACH DOSING. The label of each carton will contain the unique number, the name of the Sponsor, and any other information required by local regulations (Figure 2). The pharmacy or study staff will prepare the solution of TU-100 by mixing the 2 sachets in approximately 30 mL (1 ounce) of lukewarm water in a suitable dosing container, which is large enough for mixing the study drug in the water, then administering this oral solution to the subject. When deemed necessary, a low-volume rinse of approximately 15 mL of this dosing container should be prepared following the administration, with the subject drinking the rinse.

All drug supplies will be provided by the Sponsor.

Figure 2. Packaging of TU-100



9.1.2 Placebo

Placebo is provided as a granular formulation similar in appearance to TU-100 and will be packaged in sachets identical to TU-100. Placebo will contain excipients only.

9.2 Dosage Schedule

Depending on their randomized assignment, subjects will receive either study drug or placebo TID (3 times per day [i.e., 6 hours ± 1 hour apart]) by mouth from Day 1 (the day after surgery) through Day 10 or hospital discharge (whichever occurs earlier).

All subjects will be administered 2 sachets/doses TID. Subjects randomized to receive TU-100 15 g/day will be administered 2 sachets/dose TID of TU-100 (every 6 hours [± 1 hour]) for a total of 6 sachets/day, and subjects randomized to receive TU-100 7.5 g/day will be administered 1 sachet/dose TID of TU-100 and 1 sachet/dose TID of placebo for a total of 6 sachets/day. Subjects randomized to placebo will be administered 2 sachets/dose TID of matching placebo for a total of 6 sachets/day.

One sachet from each inner box assigned will be pulled for each dosing. Study drug or matching placebo is to be taken orally as a solution. Two sachets (1 from each inner box) will be mixed together to be dissolved in approximately 30mL (1 ounce) of lukewarm water immediately before consumption and taken. When deemed necessary, a low-volume rinse (approximately 15 mL) of the dosing container should be prepared following the administration, with the subject drinking the rinse also.

9.3 Measures to Minimize Bias: Study Treatment Assignment and Blinding

9.3.1 Method of Study Treatment Assignment

Subjects will be randomized in a 1:1:1 ratio to TU-100 15 g/day, TU-100 7.5 g/day, or placebo on Day 1 before the first dose of study drug. Subject randomization will be stratified based on surgical approach, i.e., whether surgery was open or laparoscopic.

Subjects who sign an ICF will be assigned a subject number sequentially at screening. The first 3 numbers of a subject number indicate the study center and the last 4 digits indicate the order in which the subject presented to be screened (for example, Subject XXX-XXXX).

9.3.2 Blinding

This is a randomized, double-blind, placebo-controlled study. Investigators, study staff, Sponsor designees, CEAC, and subjects will be blinded to randomized study treatment for the duration of the study. The pharmacist or designee performing the preparation and administration will also be blinded. A designated, independent statistical group who prepare analyses and reports for the DSMB will be unblinded.

According to the randomization schedule as indicated in the Schedule of Assessments (Table 1) and in accordance with the pharmacy manual, the investigator or designee will obtain the study drug number from the interactive web response system (IWRS) for the subject, and the number will be provided to the pharmacist or designee at the study center who is responsible for the preparation of study treatment. TU-100, placebo granules, and prepared study treatments will be identical in appearance and labeled in a blinded manner. Each sachet will be labeled with the content, caution, and name of manufacturer. No other study site personnel, subjects, Sponsor personnel, or Sponsor designees will be unblinded to treatment assignment throughout the duration of the study unless unblinding is required. If an investigator becomes unblinded to a given subject's study treatment, that subject will be terminated from the study unless there are ethical reasons for that subject not to be withdrawn/terminated; approval from the Sponsor's medical monitor must be obtained in such instances.

In the event that emergency unblinding is required for a given subject because of AEs or concerns for the subject's safety or wellbeing, the investigator may break the randomization code for the subject via the IWRS, by which system the unblinding will be captured. The investigator is responsible for notifying the medical monitor and/or Sponsor of such an event as soon as possible. The unblinding and its cause will also be documented on the eCRF.

9.4 Dosage Modification

No dose modification is allowed during study treatment.

9.5 Accountability and Compliance

The pharmacist or other designated individual will maintain records of the inventory at the study site, the distribution to and use by each subject, and the return of materials to the Sponsor for storage or disposal. These records should include dates, quantities, batch/serial numbers, expiration dates, in-clinic temperature log, and unique code numbers assigned to the product and study subjects.

All doses will be given by study site personnel or a floor nurse (if properly trained on study protocol requirements) while the subject is hospitalized. Study site personnel will record compliance of the subject with the subject's assigned regimen.

Investigators will maintain records that adequately document that the subjects were provided with the correct study treatment kits and reconcile the products received from the drug dispensing center. Study drug will not be returned to the Sponsor until accountability has been fully monitored.

Administration of study drug will be supervised by study site personnel to ensure compliance until a floor nurse is properly trained on study protocol requirements.

9.6 Prior and Concomitant Medications

9.6.1 Prior and Concomitant Medications

Restricted prior therapies and medications are provided in the Exclusion Criteria in Section 8.2. All prior therapies taken in the previous 30 days before screening will be recorded on the eCRF.

All medications and other treatments taken by the subject during the study, including those treatments initiated before the start of the study, must be recorded on the eCRF.

Medications taken by or administered to the subject for the time period before study drug administration will be recorded on the eCRF. Note, laxatives used for the preoperative mechanical bowel preparation are permitted in the preoperative period including polyethylene glycol, magnesium preparations, sodium phosphate, and bisacodyl. Also note, oral antibiotics used for preoperative mechanical bowel preparation are permitted in the preoperative period including aminoglycosides, metronidazole, and erythromycin.

After the first study drug administration, medication to treat minor treatment-emergent illness(es) is generally permitted**;** however, the following therapies are expressly prohibited throughout the study: alvimopan, methylnaltrexone, naloxone (except for treatment of known or suspected opioid overdose), and prophylactic or routine use of metoclopramide, along with *routine* use of laxatives and stool softeners, prophylactic use of prokinetics (e.g., metoclopramide, cisapride, domperidone, erythromycin used as a prokinetic agent), corticosteroids (except for preoperative multimodal antiemetic prophylaxis in patients at-risk, i.e. ≥2 risk factors), or other immunosuppressive therapy in patients without pre-existing inflammatory bowel disease, illicit drugs, antidiarrheal medications (except for the treatment of an AE such as diarrhea), another

investigational drug/biologic, and use of supplemental ginger, ginseng, or Zanthoxylum fruit. Radiation therapy and chemotherapy is prohibited.

Note: As part of the study ERAS protocol, gabapentinoids may be administered at a single low dose preoperatively; however, they should not be used in the postoperative setting as part of multimodal acute pain management unless indicated for postoperative neuropathic pain.

Acute postoperative neuropathic pain is generally characterized by 1 or more of the following signs or symptoms in the absence of other likely causative factors (e.g., postoperative complications such as surgical site infection):

- Pain that is difficult to manage and does not respond to traditional analgesic agents (e.g., opioids, nonsteroidal anti-inflammatory drugs [NSAIDs])
- Hyperalgesia (postoperative pain disproportionately higher in intensity than what would be expected)
- Dysesthesia (constant burning, tingling, throbbing pain)
- Allodynia (pain associated with a stimulus that would not be expected to cause pain [e.g., light touch or pressure])

These symptoms may be limited to or extend beyond the region of the surgical incision/site and may be persistent despite standard-of-care pain management.

Refer to Section 10.2.1.1 for the standardized ERAS pathway for preoperative, intraoperative, and postoperative drugs/treatments that are allowed and to be used to enhance GI recovery.

Concomitant medications allowed during the study include oral antibiotics, pain medications (acetaminophen, NSAIDs, opioids [used sparingly]), anesthesia/analgesia, and antiemetic prophylaxis in at-risk subjects. Based on the potential impact of opioids on GI recovery/POI, more details on concomitant opioid use may be required on the eCRF.

Any prohibited medication or therapy that is taken by or administered to the subject during the course of the study must be recorded on the eCRF. The entry must include the dose, regimen, route, indication, and dates of use.

The use of supplemental ginger or Zanthoxylum fruit within 72 hours or supplemental ginseng within 120 hours before randomization is prohibited.

10 STUDY PROCEDURES

Table 1 outlines the timing of procedures and assessments to be performed throughout the study. Section 12.5 specifies laboratory assessment samples to be obtained. See Section 11 and Section 12 for additional details regarding efficacy assessments and safety assessments, respectively. Details for PK analytical methodology are presented in Section 13.

Table 1. Schedule of Assessments

Procedure	Screening Day -28 to -1[a]	Surgery Day -1		Randomization Day 1	Day 2 to 10[b]	Hospital Discharge or Study Withdrawal/Termination	Follow-up Contact[c]	30 days (+15 days) After Discharge Follow-up Visit[d]
		Pre-surgery	Post-surgery					
Informed consent	X							
Study screening eligibility/ inclusion/exclusion criteria	X	X	X					
Randomization Eligibility Criteria				X				
Demographics, medical history, including previous abdominal surgeries	X	X						
Medication history/ concomitant medications	X[e]	X	X	X	X	X	X	X
COVID-19 screening questions[f]	X	X						
COVID-19 test	X							
Pregnancy test[g]	X	X						
Physical examination[h]	X					X		X
Vital signs[i]	X			X		X		X

Procedure	Screening Day -28 to -1[a]	Surgery Day -1		Randomization Day 1	Day 2 to 10[b]	Hospital Discharge or Study Withdrawal/Termination	Follow-up Contact[c]	30 days (+15 days) After Discharge Follow-up Visit[d]
		Pre-surgery	Post-surgery					
12-lead ECG[j]	X			X		X		
Serology[k]: HBV, HCV	X							
Hematology, serum chemistry, urinalysis[l]	X			X		X		
LFTs[m]	X	X		X	X	X		X
Record surgery start and stop time			X					
Record estimated blood loss			X					
Record need for blood transfusion			X					
Record total intraoperative IV fluid type and volume								
Record NGT removal, if used intraoperatively			X					
Record information on postoperative NGT insertion, if used				X	X	X		
Randomization				X				

Procedure	Screening Day -28 to -1[a]	Surgery Day -1		Randomization Day 1	Day 2 to 10[b]	Hospital Discharge or Study Withdrawal/Termination	Follow-up Contact[c]	30 days (+15 days) After Discharge Follow-up Visit[d]
		Pre-surgery	Post-surgery					
Administration of study medication TID every 6 hours[n]				X	X	X		
Perform GI assessments twice daily[o]				X	X	X		
Subject eDiary assessments[p]				X	X	X		
Ready for discharge based solely on GI recovery (when applicable)				X	X	X		
Hospital discharge order written						X		
Time to discontinue IV fluids and transition to oral liquids (when applicable)				X	X	X		
Time to transition to oral analgesics (when applicable)				X	X	X		
Monitoring of AEs		X	X	X	X	X	X	X

Procedure	Screening Day -28 to -1[a]	Surgery Day -1		Randomization Day 1	Day 2 to 10[b]	Hospital Discharge or Study Withdrawal/Termination	Follow-up Contact[c]	30 days (+15 days) After Discharge Follow-up Visit[d]
		Pre-surgery	Post-surgery					
Monitoring of prespecified postoperative complications			X	X	X	X	X	X
Review of data for hepatobiliary AEs[q]			X	X	X	X	X	X
Hospital readmission assessment							X	X
PK sampling[r]				X	X	X		

Abbreviations: AE = adverse event; BM = bowel movement; CAEC = clinical endpoint adjudication committee ; ECG = electrocardiogram; eCRF = electronic case report form; GI = gastrointestinal; HBV = hepatitis B virus; HCV = hepatitis C virus; INR = International Normalized Ratio; IV = intravenous; LFTs = liver function tests; NGT = nasal gastric tube; PCR = polymerase chain reaction; PK = pharmacokinetic; PT = prothrombin time; RBC = red blood cells; TID = 3 times per day (i.e., 6 hours ± 1 hour apart).

- [a] Exceptional measure for COVID-19: Depending on the COVID-19 situation, measures below may be considered for the screening:
- Consider performing laboratory testing, urine pregnancy tests, and safety laboratory tests at a local hospital, or regional independent laboratory located near the subject.
- Telephone conversation or video conferencing may be used for obtaining information on demography, medical and surgical history, and prior and concomitant medications.
- Local at-home services performed by trained non-study personnel may be used for measurement of vital signs, physical examinations, and ECG.
- [b] Study drug is to be administered up to Day 10 or hospital discharge, whichever is earlier.
- [c] Subjects are to be contacted via telephone (or visited in-person if still in the hospital) 10 to 14 days after the last dose of study medication. Need for hospital readmission will be determined.
- [d] Depending on the COVID-19 situation or if there are any reasonable restrictions, measures below may be considered for the 30-day follow-up visit.
- If subject is unable to come to clinic for in-person visit, then consider performing LFT laboratory tests (if needed) at a local hospital, or regional independent laboratory located near the subject. Telephone conversation or video conferencing may be used for other procedures at this visit. Additionally, local at-home services performed by trained non-study personnel may be used for measurement of vital signs and physical examinations.

- [e] Medications taken within 30 days before screening are to be recorded on the eCRF.
- [f] Additional screening questions for COVID-19 will be asked by the investigator or site staff: 1) Has the subject traveled to countries known to be COVID-19 hotspots as well as US cities with high COVID-19 case densities, within the last 14 days before screening? 2) Does the subject currently or did the subject have flu-like symptoms within the past 72 hours, e.g., fever, cough, shortness of breath? 3) Has the subject had any close contact with any person exhibiting flu-like symptoms within the past 14 days? and 4) Has the subject been exposed to anyone who has been diagnosed with COVID-19 within the past 14 days?
- [g] A serum pregnancy test must be performed during screening unless done within 28 days of the screening visit. A urine pregnancy test may be performed on the day before or the day of surgery. A local laboratory should be used for screening and pre-surgery Day 1 pregnancy tests. If a serum pregnancy test has been performed within 28 days before surgery, but before consent is obtained, serum pregnancy test need not be repeated unless deemed appropriate by the investigator.
- [h] Physical examinations are to include measurement of weight; height is to be measured at the screening visit only. If a physical examination has been performed within 28 days before surgery, but before consent is obtained, the full physical examination need not be repeated unless deemed appropriate by the investigator. A review of the physical examination with revision or addition of any changes, if required, will be performed after the consent has been signed.
- [i] Vital signs to include blood pressure, heart rate, respiratory rate, and body temperature. On Day 1, vital signs should be taken before study drug administration to represent baseline values.
- [j] Additional ECGs may be conducted as clinically warranted. On Day 1, this should be done before study drug administration to represent baseline ECG. If a 12-lead ECG has been performed within 28 days before surgery, but before consent is obtained, the 12-lead ECG need not be repeated unless deemed appropriate by the investigator.
- [k] If the subject has a previous test result for HCV and/or has previously tested positive for HCV, an HCV RNA (PCR) test should be done, with a negative result confirmed. If the subject has a history of HBV or has previously tested positive for HBV, a hepatitis B surface antibody test needs to be performed and negative results confirmed. If these tests have been performed within 28 days before surgery, but before consent is obtained, the HBV/ HCV test need not be repeated unless deemed appropriate by the investigator.
- [l] Blood and urine samples will be obtained. Tests for routine hematology tests will include red blood cell count, hemoglobin, hematocrit, platelet count, and white blood cell count with differential. Tests for routine serum chemistry include glucose, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), blood urea nitrogen or urea, creatinine, creatinine clearance, total protein, and albumin. A urinalysis includes appearance, pH, glucose, ketones (by dipstick), RBCs (by dipstick), specific gravity, and microscopy if needed. If these tests have been performed within 28 days before surgery, but before consent is obtained, the hematology, serum chemistry, urinalysis tests need not be repeated unless deemed appropriate by the investigator. On Day 1, this should be taken prior to study drug administration to represent baseline values. A local laboratory should be used for screening and before pre-surgery Day -1. A central laboratory should be used after randomization on Day 1 and Hospital Discharge/Study Termination.
- [m] Samples for routine clinical liver function tests will include alkaline phosphatase, aspartate transaminase, alanine transaminase, total and direct bilirubin, prothrombin time and INR. If these tests have been performed within 28 days before surgery, but before consent is obtained, the LFTs need not be repeated unless deemed appropriate by the investigator. On Day-1 LFT do not have to be repeated if done within 14 days of Surgery and the LFT were within normal limits. On Day 1, this should be taken before study drug administration to represent baseline values. Liver functions tests to include PT/INR and will be obtained during screening, pre-surgery, Randomization Day 1, Day 4, Day 7, and Day 10 (if remaining in the hospital), and the day of hospital discharge. Liver function tests are needed at the 30-day follow-up visit only if LFTs were elevated at discharge and trending down. If a subject remains in the hospital for an AE of elevated tests, then levels will be followed until resolution. A local laboratory should be used for screening and before pre-surgery Day -1. A central laboratory should be used after randomization on Day 1 and future visits.
- [n] Subjects will be randomized 1:1:1 (TU-100 15 g: TU-100 7.5 g: placebo) and stratified by surgical approach (open versus laparoscopic). Study drug will be administered TID (3 times per day [i.e., 6 hours ± 1 hour apart]).

- [o] GI assessments are to be performed twice daily, morning and afternoon with assessments separated by at least 6 hours.
- [p] Subjects will record the following items in their eDiaries on all postoperative in-hospital study days: first flatus (record in real time), first BM (record in real time), nausea occurrence and bothersomeness (24-hour reflection), vomiting/retching occurrence/number of episodes (24-hour reflection), abdominal bloating occurrence and bothersomeness (24-hour reflection).
- [q] Blinded review and assessment of hepatobiliary AEs by an independent CEAC will be performed on an ongoing basis during the conduct of the study.
- [r] PK samples will be drawn only at designated sites at the following times below, and at early termination (not needed at hospital discharge). If a subject is terminating early on a day scheduled for a blood draw, a second sample on the day of termination should be taken if possible. If a subject is terminating early on a day not scheduled for a blood draw, at least 1 sample should be obtained that day if possible.
- 1 hour (± 30 min) after first dose on Day 1
- 4 hours (± 1 hour) after the first dose on Day 1
- Predose on Day 3 (if not discharged yet)
- 2 hours (± 1 hour) on Day 3 (if not discharged yet)
- Predose on Day 5 (if not discharged yet)
- 2 hours (± 1 hour) on Day 5 (if not discharged yet)

10.1 Informed Consent

Before performing any study-related procedures, the investigator (or designee) will obtain written informed consent from the subject.

In the event that rescreening occurs (allowed as long as the subject did not undergo surgery), the individual is required to sign a new ICF and must be assigned a new identification number.

10.2 Study Procedures

Assessments and their timing are to be performed as outlined in the Schedule of Assessments (Table 1). Section 12.5 specifies laboratory assessment samples to be obtained. Assessments and procedures scheduled at a visit where study drug is administered should be performed before administration of treatment unless otherwise indicated in the Schedule of Assessments (Table 1).

Efficacy assessments are described in Section 11 and include GI assessments, eDiary assessments, time to ready for discharge, time to writing of hospital discharge order, time to discontinuation of IV fluids (transitioning to oral liquids), blood loss, need for blood transfusion, NGT removal time, postoperative NGT insertion, time to transition to oral analgesics, and postoperative complications. Safety assessments are described in Section 12 and include vital signs, physical examinations, ECGs, laboratory assessments (hematology, chemistry and LFTs) and AEs including hepatobiliary AEs. PK sampling is described in Section 13. The investigator may, at his/her discretion, arrange for a subject to have an unscheduled assessment, especially in the case of AEs that require follow-up or are considered by the investigator to be possibly related to the use of study drug. The unscheduled visit page in the eCRF must be completed. Study treatment discontinuation and study withdrawal/termination procedures are described in Section 8.5. Subjects are to be contacted for follow-up visits by telephone (or visit in-person, if still in the hospital) 10 to 14 days after the last dose of study drug.

10.2.1 Standard Enhanced Recovery After Surgery Protocol

All subjects will be managed with an ERAS protocol as per hospital SOC. However, elements within ERAS protocols intended to manage GI recovery will be standardized and compliance measured to the extent possible within this trial. Although alvimopan (approved for the acceleration of upper and lower GI recovery after surgeries that include partial BR with primary anastomosis) is used in ERAS pathways to accelerate GI recovery, use of alvimopan in this trial is prohibited, as its use would confound results. Methylnaltrexone, naloxone (except for treatment of known or suspected opioid overdose), and prophylactic or routine use of metoclopramide are also prohibited. Other hospital-specific ERAS elements not listed below will be allowed (Carmichael 2017, Lassen 2012, Gustafsson 2012, Kelliher 2015, Melloul 2016, Pecorelli 2016, Pedziwiatr 2018, Warner 2017). ERAS measures intended to manage GI recovery that will be standardized per protocol include Section 10.2.1.1, Section 10.2.1.2, and Section 10.2.1.3.

10.2.1.1 Preoperative Elements

- Mechanical bowel preparation plus oral antibiotics should be used.
    - Note, laxatives and oral antibiotics used for mechanical bowel preparation are

permitted in the preoperative period (See Section 9.6.1)

- Preemptive, multimodal antiemetic prophylaxis (i.e., ondansetron and dexamethasone) must be used for all at-risk subjects (i.e., ≥ 2 risk factors [female gender at birth, nonsmoker, history of motion sickness or postoperative nausea and vomiting (PONV), postoperative opioid use]).
- A multimodal, opioid-sparing, pain management plan should be planned as appropriate (e.g., combination of acetaminophen and NSAIDs, gabapentin administered at a low dose).
    - **Note**: although an opioid-sparing approach should be used for postoperative pain management, opioid analgesics use is not prohibited.
- Alvimopan, methylnaltrexone, naloxone (except for treatment of known or suspected opioid overdose), and prophylactic or routine use of metoclopramide are prohibited for the purposes of this protocol in order to reduce confounding of the effect of TU-100 on the primary outcome measure.

10.2.1.2 Intraoperative Elements

- Excess fluid administration and volume overload should be avoided.
- Transverse abdominis plane (TAP) blocks (with bupivacaine or bupivacaine liposome injectable) are permitted.
- Epidural anesthesia should not be used for the purposes of this protocol in order to reduce confounding of the effect of TU-100 on the primary outcome measure.

10.2.1.3 Postoperative Elements

- A multimodal, opioid-sparing, pain management plan should be implemented as appropriate (e.g., combination of acetaminophen and NSAIDs).
    - Gabapentinoids (i.e., gabapentin, pregabalin) should not be used unless indicated for postoperative neuropathic pain. (See Section 9.6.1)
    - **Note**: although an opioid-sparing approach should be used for postoperative pain management, opioid analgesics use is not prohibited.
- Indwelling NGTs for *prophylaxis* cannot be used.
- Protocol guidelines for selective postoperative nasogastric intubation for the management of clinical events should be followed, as shown below.
    - Occurrence of one or more of the following events:
        - ≥ 2 low-volume (e.g., < 300 cc) episodes of emesis
        - ≥ 1 high-volume (e.g., ≥ 300 cc) episode of emesis
        - Moderate to severe abdominal distension
- IV fluids are to be discontinued as soon as the subject can tolerate oral liquids and maintain adequate hydration.

- Begin oral feeding early unless otherwise contraindicated.
- Subjects must be mobilized early, starting from Day 1 unless otherwise contraindicated.
- For the purposes of this protocol, in order to reduce confounding of the effect of TU-100 on the primary outcome measure, the following interventions are prohibited:
    - Alvimopan, methylnaltrexone, naloxone (except for treatment of known or suspected opioid overdose)
    - Routine use of laxatives and stool softeners
    - Prophylactic use of prokinetics (e.g., metoclopramide, cisapride, domperidone, erythromycin used as a prokinetic agent)
    - Sham feeding (e.g., gum chewing)

10.2.2 Screening Period

Screening (Day -28 to Day -1 [day of surgery])

Subjects will be consented and screened for eligibility. Screening will include a brief interview (including demographics, medical history [including previous abdominal surgeries and acute or chronic hepatic conditions], and prior medication), serum pregnancy test, vital signs, physical examination, laboratory testing (including hematology, chemistry, LFTs, serology [hepatitis B virus (HBV) and hepatitis C virus (HCV)], and urinalysis) and ECG. Subjects who do not satisfy all eligibility criteria will be recorded as screening failures.

Additional screening questions for COVID-19 will be asked by the investigator or site staff:

1) Has the subject traveled to countries known to be COVID-19 hotspots as well as US cities with high COVID-19 case densities, within the last 14 days before screening?
2) Does the subject currently have or did the subject have flu-like symptoms within the past 72 hours, e.g., fever, cough, shortness of breath?
3) Has the subject had any close contact with any person exhibiting flu-like symptoms within the past 14 days?
4) Has the subject been exposed to anyone who has been diagnosed with COVID-19 within the past 14 days?

Retesting during the screening period

If the results of the blood tests (hematology, chemistry) are < 1.5 × ULN, not associated with an acute or chronic clinical condition or comorbidity and considered by the PI to be non-clinically significant, a new sample can be collected for a retest. Exclusionary liver function test levels may be retested once within 14 days of the original screening date, but only with the documented approval of the Sponsor's medical monitor. Only 1 retest will be allowed within the screening period. If the results of the retest fall within the normal range (i.e., DO NOT meet protocol exclusion criterion #19 for abnormal liver tests) the patient may be considered for randomization assuming all other protocol-specified criteria are met including the PI's clinical judgement. The PI should contact the medical monitor should he/she have any questions or issues for discussion in these cases prior to patient randomization.

Exceptional measure for COVID-19

- Please follow your local testing guidelines for SARS-CoV-2. If testing is mandatory at your institution, a positive result would be exclusionary
- Subjects with prior history of COVID-19 must be confirmed recovered per CDC criteria and have a SARS-CoV-2 negative test result
- If a subject contracts COVID-19 during the interval between screening and pre-surgery Day -1 (and falls out of the screening window), he or she may be rescreened once recovered, assuming the subject meets all other inclusion/exclusion criteria and per investigator clinical judgement

Depending on the COVID-19 situation (and/or to reduce burden of patient travel as needed, e.g., need to travel long distances to the site), measures below may be considered for the screening:

- Consider performing laboratory testing, urine pregnancy tests, and safety laboratory tests at a local hospital, or regional independent laboratory located near the subject.
- Telephone conversation or video conferencing may be used for obtaining information on demography, medical and surgical history, and prior and concomitant medications.
- Local at-home services performed by trained non-study personnel may be used for measurement of vital signs, physical examinations, and ECG.

If physical examination, screening day laboratory tests, 12-lead ECG, and serum pregnancy test have been performed within 28 days before surgery, but before consent is obtained, the full physical examination, screening laboratory tests, 12-lead ECG, and serum pregnancy test will be reviewed and need not be repeated unless deemed appropriate by the investigator. A review of the physical examination with revision or addition of any changes, if required, will be performed after the consent has been signed.

The Schedule of Assessments is located in Table 1.

Day -1 (Day of surgery)

LFTs will be assessed pre-surgery, unless performed within 14 days of Day-1and results were within normal limits.
The following procedures will be completed on the day of surgery:

- Capture additional medical history since screening
- Record concomitant medications (pre- and post-surgery)
- Perform a urine pregnancy test for WOCBP only
- Monitor prespecified postoperative complications
- Record surgery start (as noted from operating room records) and stop dates and times (time of the last suture or staple)
- Record need for blood transfusion
- Record estimated blood loss
- Record total intraoperative IV fluid type and volume

- Record NGT removal, if used intraoperatively

All subjects will be managed with an ERAS protocol as per hospital SOC. However, elements within ERAS protocols intended to manage GI recovery will be standardized and compliance measured to the extent possible within this trial (see Sections 10.2.1).

Screened subjects should be instructed to call ahead before they come for scheduled surgery if they have COVID-19 symptoms or are sick to help the PI team reschedule their surgery and associated study procedures.

10.2.3 Treatment Period

Day 1 (postoperative Day 1, day of randomization)
On the day after surgery, eligible subjects will be randomly assigned (1:1:1) to receive TU-100 15 g/day, 7.5 g/day, or placebo if subjects meet randomization criteria as described in Section 8.4. Subjects will be stratified based on surgical approach (open versus laparoscopic).

Samples for routine clinical laboratory tests (including hematology, chemistry, LFTs, and urinalysis) should be taken before study drug administration to represent baseline values.
An ECG should be performed on Day 1.

Day 1 (postoperative Day 1) up to Day 10 (postoperative Day 10) or hospital discharge (whichever occurs first)
After randomization, subjects will be provided with an eDiary and given instructions for use. The diary will be used by subjects to record both clinical (e.g., time and occurrence of flatus or BM) and subject-related outcomes (e.g., symptoms associated with delayed GI recovery, such as nausea, vomiting/retching, abdominal bloating). Daily entries in the subject eDiary will be made at the hospital from the day of randomization throughout the entire hospitalization period.

Subjects will record the following items in their eDiaries on all postoperative in-hospital study stays (Day 1 up to Day 10)

- First flatus (record in real time)
- First BM (record in real time)
- Nausea occurrence and bothersomeness (24-hour reflection)
- Vomiting/retching occurrence/number of episodes (24-hour reflection)
- Abdominal bloating occurrence and bothersomeness (24-hour reflection)

The following procedures will be performed on all postoperative in-hospital study stays (Day 1 up to Day 10)

- Administer study medication by mouth TID (3 times per day [i.e., 6 hours ± 1 hour apart]) until hospital discharge or for a maximum of 10 days
    - **Note**: Dosing will be in-hospital only. Dosing will occur approximately every 6 hours (± 1 hour) and will be administered by study coordinator or floor nurse
    - Record time of dosing

- Perform GI assessments (twice daily, morning and afternoon; assessments must be separated by at least 6 hours)
- Record time of GI assessment, and also:
    - Record time of first:
        - Clear liquid toleration
        - Solid diet toleration
        - Absence of distention and presence of bowel sounds and flatus (i.e., time of when all 3 conditions have been achieved)
    - Record time subject is ready for hospital discharge (based solely upon recovery of GI function as determined by the treating medical team)
- Time of discontinue IV fluids and transition to oral liquids
- Time of transition to oral analgesics
- Record concomitant medications
- If an NGT is inserted, record date, time, and reason for insertion
- Monitor AEs
- Review of hepatobiliary AEs
- Monitor prespecified postoperative complications (See Section 11.7)
- Collect LFT samples on Day 4, Day 7, and Day 10 (if subject remains in the hospital) per Table 1.
- Collect blood samples for PK analyses only at designated sites at:
    - 1 hour (± 30 minutes) after the first dose on Day 1
    - 4 hours (± 1 hour) after the first dose of Day 1
    - Predose on Day 3*
    - 2 hours (± 1 hour) on Day 3*
    - Predose on Day 5*
    - 2 hours (± 1 hour) on Day 5*

      *if not discharged yet

In addition, PK samples should be taken at early termination. If a subject is terminating early on a day scheduled for a blood draw, a second sample on the day of termination should be taken if possible. If a subject is terminating early on a day not scheduled for a blood draw, at least 1 sample should be obtained that day if possible.

All subjects will be managed with an ERAS protocol as per hospital SOC. However, elements within ERAS protocols intended to manage GI recovery will be standardized and compliance measured to the extent possible within this trial. (Section 10.2.1).

Hospital discharge or study withdrawal/termination

Subjects will record the following items in their eDiaries on hospital discharge/study termination day:

- First flatus (record in real time)
- First BM (record in real time)
- Nausea occurrence and bothersomeness (24-hour reflection)
- Vomiting/retching occurrence/number of episodes (24-hour reflection)
- Abdominal bloating occurrence and bothersomeness (24-hour reflection)

The following procedures will be performed at the hospital discharge/study termination visit:

- Perform physical examination, including weight. Any clinically relevant physical abnormalities (with the exception of surgical scars) that were not present at screening should be recorded as AEs
- Record vital signs (blood pressure, heart rate, respiratory rate, and temperature)
- Obtain 12-lead ECG
- Collect blood samples for hematology and serum chemistry, LFTs and a urine sample for urinalysis
- Collect blood samples for PK testing at early termination (not necessary for normal hospital discharge). If a subject is terminating early on a day scheduled for a blood draw, a second sample on the day of termination should be taken if possible. If a subject is terminating early on a day not scheduled for a blood draw, at least 1 sample should be obtained that day if possible. Record time of blood sampling.
- Perform GI assessments
    - Record time of GI assessment
    - Record time of first:
        - Clear liquid toleration
        - Solid diet toleration
        - Absence of distention and presence of bowel sounds and flatus (i.e., time of when all 3 conditions have been achieved)
    - Record time subject is ready for hospital discharge (based solely upon recovery of GI function as determined by the treating medical team)
- Review records relating to hospital discharge: date and time that discharge order was written (order must be written the same day that discharge is expected to occur)
- Record concomitant medications
- Monitor AEs
- Monitor prespecified postoperative complications

10.2.4 Follow-up Period

Follow-up contact (10 to 14 days after last dose of study drug)

All subjects will be contacted via telephone (or in-person if still in the hospital) within 10 to 14 days after the last dose of study medication for follow-up that will include:

- Record concomitant medications
- Monitor AEs
- Review of data for hepatobiliary AEs
- Monitor prespecified postoperative complications
- Determine whether hospital readmission occurred and reason for readmission

A certified letter will be sent to any subject not contacted after 3 attempts by telephone.

Follow-up visit (30 days + 15 days after hospital discharge)

All subjects are to return for a follow-up visit 30 days (+ 15 days). Procedures to be conducted at this visit include:

- Perform physical examination, including weight. Any clinically relevant physical abnormalities (with the exception of surgical scars) that were not present at screening should be recorded as AEs.
- Record vital signs (blood pressure, heart rate, respiratory rate, and temperature)
- Record concomitant medications
- Monitor AEs
- Monitor prespecified postoperative complications
- Determine whether hospital readmission occurred and reason for readmission
- If elevated at discharge and trending down, then liver tests will be completed at the 30-day follow-up visit. If a subject remains in the hospital for an AE of elevated tests, then levels will be followed until resolution.

If a patient does not return for a scheduled visit, all necessary measures should be taken to contact the patient and document the patient's outcome.

Reasonable efforts should be made to contact subjects who are lost to follow-up. A certified letter will be sent to any subject not contacted after 3 attempts by telephone. These efforts must be documented in the subject's file.

Exceptional measure for COVID-19

Depending on the COVID-19 situation (and/or to reduce burden of patient travel as needed), measures below may be considered for the 30-day follow-up visit.

- Window for the 30 day follow-up can be extended to +15 days after hospital discharge.
- If subject is unable to come to clinic for in-person visit, then performing LFT laboratory tests (if needed) at a local hospital, or regional independent laboratory located near the subject will be considered. Local laboratory results should be documented in subject's file.

- Telephone conversation or video conferencing may be used for other procedures at this visit. Additionally, local at-home services performed by trained non-study personnel may be used for measurement of vital signs and physical examinations.

11 EFFICACY ASSESSMENTS

The Schedule of Assessments (Table 1) outlines the efficacy assessments to be performed throughout the study and their timing.

11.1 Surgery Data Collection

During the surgery, the following information should be collected and recorded on the eCRF:

- Surgery start and stop times (stop time is defined as the time of the last stitch or staple)
- Any estimated blood loss
- Need for blood transfusion
- Total intraoperative IV fluid type and volume
- NGT removal time, if used

11.2 Gastrointestinal Assessments

Gastrointestinal assessments will be performed twice daily in the morning and afternoon, starting on randomization Day 1, and continuing until hospital discharge or study termination. Assessments must be separated by at least 6 hours. Gastrointestinal assessments will include:

- Recording time of first:
    - Clear liquid toleration (i.e., time at which the subject transitions from clear liquids to the next diet stage [full liquids or solids])
    - Solid diet toleration (i.e., able to eat a meal that requires chewing without a vomiting episode by the time the next consecutive meal is offered)
        - Note, if the first solid meal is consumed on the day of hospitalization (i.e., the day of surgery), the time to toleration of solids is as stated (e.g., the time to eat solids without a vomiting episode by the time the next consecutive meal is offered). Further, if the first solid meal is consumed on the day of discharge, the time to toleration of solids criteria is met if there is no vomiting prior to discharge. In all cases, the actual time that should be recorded on the eCRF is the time the meal was consumed by the subject, not the cutoff time for vomiting prior to next consecutive meal or discharge.
    - Absence of distention + presence of bowel sounds + flatus (time of when all 3 conditions have been achieved)
- Record time subject is ready for hospital discharge (based solely upon recovery of GI function as determined by the treating medical team)

11.3 Electronic Diary Assessments

Subjects will record in their eDiaries on all postoperative in-hospital days. They should record the following:

- First flatus (record in real time)
- First BM (record in real time)

- Nausea occurrence and bothersomeness (a 24-hour reflection)
- Vomiting/retching occurrence/number of episodes (a 24-hour reflection)
- Abdominal bloating occurrence and bothersomeness (a 24-hour reflection)

The investigator will assess eDiary entries at study visits starting on Randomization Day 1 and continuing daily until hospital discharge or study termination.

11.4 Time to Writing of Discharge Order

The time when the discharge order is written should be noted on the eCRF by the investigator.

11.5 Time to Discontinuation of IV Fluids

The time of discontinuation of IV fluids and the transition to oral liquids should be noted on the eCRF by the investigator.

11.6 Time to Transition of Oral Analgesics

The time when the subject is able to transition from IV to oral analgesics should be noted on the eCRF by the investigator.

11.7 Prespecified Postoperative Complications

Any postoperative complications should be recorded on the eCRF by the investigators. These prespecified complications include:

- Need for blood transfusion information (recorded as part of in-surgery information)
- Readmission to hospital within 7 days and 30 days of discharge
- POI-related morbidity as evidenced by:
    - POI that required readmission within 7 days of discharge, or
    - Need for postoperative NGT insertion to manage symptoms of POI (i.e., vomiting/retching, abdominal distension) per protocol guidelines
- Other postoperative complications including:
    - Prolonged POI
        - Minimal incision BR (laparoscopic or robotically-assisted or hand-assisted): Unresolved POI by postoperative Day 3
        - Laparotomy (open) BR: Unresolved POI by postoperative Day 5

      Note: POI = primary POI (i.e., POI that is not secondary to surgical complication, such as anastomotic leak, abscess formation, or sepsis)
    - Recurrent POI (prolongs hospital stay or results in readmission – based on investigator assessment)
    - Surgical site infection
    - Anastomotic leak
    - EPSBO

- Confirmed by the presence of a transition point between dilated and decompressed bowel demonstrated on abdominal imaging

12 SAFETY ASSESSMENTS

Safety assessments (vital signs, physical examinations, ECG recording, AEs, clinical laboratory results [routine hematology and biochemistry]) are to be performed at protocol-specified visits, as specified in the Schedule of Assessments (Table 1).

12.1 Medical History

Medical history will be recorded at screening. Investigators should document the occurrence, signs, and symptoms of the subject's ongoing pre-existing conditions present at the time when informed consent is given and up to the time of first dosing, significant surgeries (i.e., cardiac, vascular, or general thoracic surgery), and GI-related conditions/surgeries. Medical history will include alcohol consumption, illicit drug use, and smoking history, if applicable.

Illnesses first occurring or detected during the study and/or worsening of a concomitant illness during the study are to be documented as AEs on the eCRF in accordance with Section 12.6. All changes not present at baseline or described in the past medical history and identified as clinically noteworthy must be recorded as AEs.

Additionally, demographic data will be collected for all subjects and include age at study entry, gender, race, ethnicity, weight, and height, according to applicable regulations.

12.2 Vital Signs

Vital signs (body temperature, respiratory rate, heart rate, and systolic and diastolic blood pressure measurements) will be evaluated at the visits indicated in the Schedule of Assessments (Table 1). All vital signs will be measured after the subject has been resting in a sitting position for at least 5 minutes. Blood pressure measurements are to be taken in the same arm for the duration of the study. Day 1 (Postoperative Day 1) vital signs should be taken before study drug administration.

Vital sign measurements will be repeated if clinically significant or machine/equipment errors occur. Out-of-range blood pressure, respiratory rate, or heart rate measurements will be repeated at the investigator's discretion. Any confirmed, clinically significant vital sign measurements must be recorded as AEs.

12.3 Physical Examination

A complete physical examination (head, eyes, ears, nose and throat; heart; lungs; abdomen; skin; cervical and axillary lymph nodes; and neurological and musculoskeletal systems) will be performed at screening (Day -28 to Day -1). Body weight (without shoes) will be recorded at screening and at the follow-up visit (30 days + 15 days after hospital discharge) and height (without shoes) will be recorded at screening only. Physical examinations will be performed by a physician.

If a physical examination has been performed within 28 days before surgery, but before consent is obtained, the full physical examination does not need to be repeated unless deemed appropriate by the investigator; however, this should be assessed.

Symptom-driven, limited physical examinations will be performed as clinically indicated at any study visit.

12.4 Electrocardiograms

A 12-lead, resting ECG will be obtained at the visits indicated in the Schedule of Assessments (Table 1).

At screening, the investigator will examine the ECG traces for signs of cardiac disease that could exclude the subject from the study. If an ECG has been performed within 28 days before surgery, but before consent is obtained, it does not need to be repeated unless deemed appropriate by the investigator but should be reviewed by the site PI.

The ECG on Day 1 should be performed before study drug administration.

An assessment of normal or abnormal will be recorded; if the ECG is considered abnormal, the abnormality will be documented on the eCRF. Electrocardiograms will be repeated if clinically significant abnormalities are observed, or artifacts are present.

12.5 Laboratory Assessments

Laboratory assessment samples (Table 2) are to be obtained at designated visits as detailed in the Schedule of Assessments (Table 1).

If laboratory assessments have been performed within 28 days before surgery, but before consent is obtained, they do not need to be repeated unless deemed appropriate by the investigator, but should be reviewed by the site PI.

The blood samples taken on Day 1 should be taken before study drug administration. The calculation of eGFR is necessary for screening of subject eligibility per entry criteria (see Section 8.2).

A urine pregnancy test may also be performed on the day before or the day of surgery.

Table 2. Laboratory Assessments

Hematology	**Serum Chemistry**	**Liver Function Tests**
Hct Hb Platelet count RBC count WBC count with differential	Total protein Albumin BUN or urea Creatinine Creatinine clearance Electrolytes (sodium, potassium, calcium, chloride, and bicarbonate) Glucose	ALT ALP AST Total and direct bilirubin **Coagulation** PT INR
	Serology	**Urinalysis**
	HBV HCV	Appearance pH Protein Glucose Ketones (dipstick) RBCs (dipstick) Specific gravity Microscopy (if needed)
Pregnancy test: A serum pregnancy test will be performed on all women of childbearing potential at screening. A urine pregnancy test may be performed on the day before or the day of surgery.		
eGFR calculation[a]: GFR = $141 \times \min(Scr/\kappa,1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times$ 1.159 [if black], *where* Scr = serum creatinine (mg/dL), κ = 0.7 for females and 0.9 for males, α = -0.329 for females and -0.411 for males, min = minimum of Scr/κ or 1, and max = maximum of Scr/κ or 1.		

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration; eGFR = estimated glomerular filtration rate; Hb = hemoglobin; HBV = hepatitis B virus; Hct = hematocrit; HCV = hepatitis C virus; INR = international normalized ratio; PT = prothrombin time; RBC = red blood cell; WBC = white blood cell.

- [a] eGFR calculation is known as the CKD-EPI formula (Levy, 2009).

Blood taken at screening or pre-surgery on Day -1 will be analyzed at a local laboratory facility. Blood taken post-surgery on Day 1 (Postoperative Day 1, before study drug administration), and Day 2 through Day 10 will be analyzed at the central laboratory facility. All laboratory reports must be reviewed, signed, and dated by the investigator. A legible copy of all reports must be filed with both the subject's eCRF and medical record (source document) for that visit. Any laboratory test result considered by the investigator to be clinically significant should be considered an AE (clinically significant AEs include those that require an intervention). Clinically significant abnormal values occurring during the study will be followed up until repeat test results return to normal, stabilize, or are no longer clinically significant.

12.6 Adverse Events

12.6.1 Adverse Events

An AE is any symptom, physical sign, syndrome, or disease that either emerges during the study or, if present at screening, worsens during the study, regardless of the suspected cause of the event. All medical and psychiatric conditions (except those related to the indication under study) present at screening will be documented on the medical history eCRF. Changes in these conditions and new symptoms, physical signs, syndromes, or diseases should be noted on the AE eCRF during the rest of the study. Clinically significant laboratory abnormalities should also be recorded as AEs.

Subjects will be instructed to report AEs at each study visit. All AEs are to be followed up until resolution or a stable clinical endpoint is reached. Each AE is to be documented on the eCRF with reference to date of onset, duration, frequency, severity, relationship to study drug, action taken with study drug, treatment of event, and outcome. Furthermore, each AE is to be classified as being serious or nonserious. Changes in AEs and resolution dates are to be documented on the eCRF.

For the purposes of this study, the period of observation for collection of AEs extends from the time the subject gives informed consent until the follow-up visit. Follow-up of the AE, even after the date of therapy discontinuation, is required if the AE persists until the event resolves or stabilizes at a level acceptable to the investigator.

When changes in the intensity of an AE occur more frequently than once a day, the maximum intensity for the event should be noted. If the intensity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates).

The DSMB will be periodically reviewing safety data.

The CEAC will be adjudicating prespecified AEs that may be suggestive of hepatotoxicity. Those events will be determined by the CEAC and clinical research organization/Sponsor before study initiation and will be contained in the CEAC charter. The anticipated AEs determined by the Sponsor will be included in the ICF. These prespecified AEs should be reported only in the adjudication database and not the general clinical trial safety database to avoid double reporting and mitigate discordance across these datasets. Data related to these events required for adjudication (e.g., signs, symptoms, laboratory results, imaging study results, consult reports) will also be determined by the CEAC before study initiation and systematically collected on eCRFs and provided as a case package to CEAC.

Specific guidelines for classifying AEs by intensity and relationship to study drug are given in Table 3 and Table 4.

Table 3. Classification of Adverse Events by Intensity

MILD: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
MODERATE: An event that is sufficiently discomforting to interfere with normal everyday activities.
SEVERE: An event that prevents normal everyday activities.

Table 4. Classification of Adverse Events by Relationship to Study Drug

UNRELATED: This category applies to those AEs that are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
POSSIBLY: This category applies to those AEs for which a connection with the study drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possibly related if or when it meets 2 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; or (3) it follows a known pattern of response to the test drug.
PROBABLY: This category applies to those AEs that the investigator feels with a high degree of certainty are related to the test drug. An AE may be considered probably related if or when it meets 3 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject's (3) it disappears or decreases on cessation or reduction in dose (note that there are exceptions when an AE does not disappear upon discontinuation of the drug yet drug-relatedness clearly exists; for example, as in bone marrow depression, fixed drug eruptions, or tardive dyskinesia); or (4) it follows a known pattern of response to the test drug.
DEFINITELY: This category applies to those AEs that the investigator feels are incontrovertibly related to test drug. An AE may be assigned an attribution of definitely related if or when it meets all of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it disappears or decreases on cessation or reduction in dose and recurs with re-exposure to drug (if rechallenge occurs); and (4) it follows a known pattern of response to the test drug.

- Abbreviation: AE = adverse event.

12.6.2 Adverse Events of Special Interest

12.6.3 Serious Adverse Events

An SAE is any untoward medical occurrence, in the view of either the investigator or Sponsor, that:

- results in death,
- is life-threatening,
- results in inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, and/or
- is a congenital anomaly/birth defect.

Other important medical events that may not be immediately life-threatening or result in death or hospitalization, based upon appropriate medical judgement, are considered SAEs if they are thought to jeopardize the subject and/or require medical or surgical intervention to prevent one of the outcomes defining an SAE. SAEs are critically important for the identification of significant safety problems; therefore, it is important to take into account both the investigator's and the Sponsor's assessment. If either the Sponsor or the investigator believes that an event is serious, the event must be considered serious and evaluated by the Sponsor for expedited reporting.

12.6.4 Serious Adverse Event Reporting

Only SAEs should be reported to the Sponsor. An SAE occurring from the time informed consent is obtained, during the study, and up to 30 days + 15 days after discharge must be reported to the Syneos Health Safety and Pharmacovigilance group and will be communicated to the Sponsor.

Any such SAE due to any cause, whether or not related to the study drug, must be reported within 24 hours of occurrence or when the investigator becomes aware of the event. Notification can be made using the dedicated fax line or email for the Syneos Health pharmacovigilance group.
Health Safety and Pharmacovigilance fax number: 1-877-464-7787 or 1-800-652-9037
Syneos Health Safety and Pharmacovigilance email address: safetyreporting@syneoshealth.com

If the investigator contacts the Syneos Health pharmacovigilance group by telephone, then a written report must follow within 24 hours and is to include a full description of the event and sequelae in the format detailed in the SAE reporting form.

The event must also be recorded on the standard AE eCRF. Preliminary reports of SAEs must be followed up by detailed descriptions later on, including clear and anonymized photocopies of hospital case reports, consultant reports, autopsy reports, and other documents when requested and applicable. SAE reports must be made whether or not the investigator considers the event to be related to the investigational drug.

Appropriate remedial measures should be taken to treat the SAE, and the response should be recorded. Clinical, laboratory, and diagnostic measures should be employed as needed in order to determine the etiology of the problem. The investigator must report all additional follow-up evaluations to the Syneos Health Safety and Pharmacovigilance group within 24 hours of becoming aware of the additional information or as soon as is practicable. All SAEs will be followed up until the investigator and Sponsor agree the event is satisfactorily resolved.

Any SAE that is not resolved by the end of the study or upon discontinuation of the subject's participation in the study is to be followed up until it either resolves, stabilizes, returns to baseline values (if a baseline value is available), or is shown to not be attributable to the study drug or procedures.

12.6.5 Suspected Unexpected Serious Adverse Reactions

Adverse events that meet all of the following criteria will be classified as suspected unexpected serious adverse reactions (SUSARs) and reported to the appropriate regulatory authorities in accordance with applicable regulatory requirements for expedited reporting:

- serious
- unexpected (i.e., the event is not consistent with the safety information in the IB)
- there is at least a reasonable possibility that there is a causal relationship between the event and the study treatment

The investigator will assess whether an event is causally related to study treatment. The Sponsor (or Syneos Health) will consider the investigator's assessment and determine whether the event meets the criteria for being reportable as a 7-day or 15-day safety report. SUSARs that are fatal or life-threatening must be reported to the regulatory authorities and the independent ethics committee/institutional review board (IEC/IRBs; where required) within 7 days after the Sponsor (or Syneos Health) first has knowledge of them, with a follow-up report submitted within a further 8 calendar days. Other SUSARs must be reported to the relevant regulatory authorities and the IEC/IRB within 15 calendar days after the Sponsor (or Syneos Health) first has knowledge of them.

The Sponsor (or Syneos Health) is responsible for reporting SUSARs and any other events required to be reported in an expedited manner to the regulatory authorities and for informing investigators of reportable events, in compliance with applicable regulatory requirements within specific timeframes. Investigators will notify the relevant IEC/IRB of reportable events within the applicable timeframes.

12.6.6 Pregnancy

Women of childbearing potential must have a negative pregnancy test at screening. After administration of study drug, any known cases of pregnancy in female subjects will be reported until the subject completes or withdraws from the study. The pregnancy will be reported immediately by faxing/emailing a completed pregnancy report to the Sponsor (or designee) within 24 hours of knowledge of the event. The pregnancy will not be processed as an SAE; however, the investigator will follow-up with the subject until completion of the pregnancy and must assess the outcome in the shortest possible time but not more than 30 days after completion of the pregnancy. The investigator should notify the Sponsor (or designee) of the pregnancy outcome by submitting a follow-up pregnancy report. If the outcome of the pregnancy involved spontaneous or therapeutic abortion (any congenital anomaly detected in an aborted fetus is to be documented), stillbirth, neonatal death, or congenital anomaly, the investigator will report the event as an SAE by faxing/emailing a completed pregnancy report form to the Sponsor (or designee) within 24 hours of knowledge of the event.

If the investigator becomes aware of a pregnancy occurring in the partner of a subjects participating in the study, the pregnancy should be reported to the Sponsor (or designee) within 24 hours of knowledge of the event. Information regarding the pregnancy must only be submitted after obtaining written consent from the pregnant partner. The investigator will arrange counseling for the pregnant partner by a specialist to discuss the risks of continuing with the pregnancy and the possible effects on the fetus.

Upon discontinuation from the study, only those procedures that would not expose the subject to undue risk will be performed. The investigator should also be notified of pregnancy occurring during the study but confirmed after completion of the study. In the event that a subject is subsequently found to be pregnant after inclusion in the study, any pregnancy will be followed to term, and the status of mother and child will be reported to the Sponsor after delivery.

12.6.7 Overdose

In ongoing and/or future studies, an unintended overdose is defined as a subject taking more than their assigned dose, e.g., subject receiving the contents of 3 instead of 2 sachets.

In the event that an overdose occurs, the subject should be closely monitored for the following signs/symptoms, including but not limited to:

- Headache*
- Nausea*
- Hepatic function abnormal**
- Diarrhea**

*Considered the most frequent AEs in US studies; **Considered the most frequent AEs in Japanese post-marketing research

Should any of these signs/symptoms occur, the subject should be monitored and treated as deemed clinically appropriate and/or necessary by the PI or assigned designee. An overdose of study drug is considered an AE and should be reported accordingly.

12.6.8 Subjects Who Meet Close Observation Criteria Reporting

If a subject meets the criteria for close observation based early signs of potential DILI or hepatotoxicity (See protocol Section 8.5.1) from the day of randomization, during the study, and up to 30 days + 15 days after discharge, this must be reported to the Sponsor. This must be reported within 24 hours of occurrence or when the investigator becomes aware of the subject meeting close observation criteria.

Notifications can be made using the dedicated email address for potential DILI reporting:

DILIreporting@mail.tsumura.co.jp

When reporting a subject who meets close observation criteria reporting to the dedicated email, the site should include the key clinical signs and symptoms of possible DILI or severe hepatoxicity that triggered close observation, as appropriate. **Note**: clinical signs and symptoms may include, but are not limited to, the following (also See Section 8.5.1):

- Persistent fatigue, weakness, nausea/vomiting, anorexia, pruritis, vague abdominal pain or right upper quadrant pain or tenderness, fever, rash, eosinophilia (eosinophils > 5%) in the absence of more likely causes
- Peripheral edema, ascites, encephalopathy, GI bleeding
- ALT > 3-fold ULN in subjects with normal baseline levels or 2-fold increases above baseline in subjects with abnormal levels prior to drug exposure

The Sponsor will create narrative summary and will contact the site to obtain relevant information for this summary. The Sponsor will provide the narrative summary to the DSMB.

13 PHARMACOKINETICS

13.1 Pharmacokinetic Sampling

13.1.1 Blood Samples

Sparse sampling will be conducted to evaluate PK of TU-100 constituents (e.g., HAS, HBS). A PK sample will be drawn at the time points below only at the designated sites as indicated in the Schedule of Assessments (Table 1).

- 1 hour (± 30 minutes) after the first dose on Day 1
- 4 hours (± 1 hour) after the first dose on Day 1
- Predose on Day 3*
- 2 hours (± 1 hour) on Day 3*
- Predose on Day 5*
- 2 hours (± 1 hour) on Day 5*

*if not discharged yet

In addition, PK samples should be drawn at early termination. If a subject is terminating early on a day scheduled for a blood draw, a second sample on the day of termination should be taken if possible. If a subject is terminating early on a day not scheduled for a blood draw, at least 1 sample should be obtained that day if possible.

Pharmacokinetic samples will be reserved for potential future additional analyses.

The actual date and time of each blood sample collection will be recorded. The timing of PK samples may be altered, and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

Details of PK blood sample collection, processing, storage, and shipping procedures are provided in a separate laboratory manual.

13.2 Pharmacokinetic Analytical Methodology

The concentration of study drug will be determined from the plasma samples using a validated analytical method. Details of the method validation and sample analysis will be included with the final clinical study report (CSR).

14 STATISTICAL ANALYSIS

14.1 General Considerations

A statistical analysis plan (SAP) will be prepared after the protocol is approved. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all study objectives. The SAP will serve as a compliment to the protocol and supersedes it in case of differences.

The statistical evaluation will be performed using Statistical Analysis Software (SAS)® version 9.4 or higher (SAS Institute, Cary, NC). All summaries will be presented by cohort and overall for each part, when appropriate. Summary statistics will be presented by dose group. For continuous variables, data will be summarized with the number of subjects (N), mean, standard deviation, median, minimum, and maximum by treatment group. For categorical variables, data will be tabulated with the number and proportion of subjects for each category by treatment group.

The precision of the measurement units for each continuous variable will be used to determine the number of decimal places used to report these summary statistics. Minimum and maximum values will be reported with the same precision as the units of measure. The mean and median will be reported to 1 greater decimal place, and the standard deviation will be reported to 2 additional decimal places. Any values that require transformation to standard units (metric or international [SI]) will be converted with the appropriate corresponding precision. Percentages will be presented to 1 decimal place. For statistical analyses, baseline is defined as the most recent measurement before initiation of first study drug administration.

The SAP will provide specific details on the analytical methods and data displays. Any change to the data analysis methods described in the protocol will require an amendment ONLY if it changes a principal feature of the protocol. Any other change to the data analysis methods described in the protocol, and the justification for making the change, will be described in the CSR. Additional exploratory analyses of the data will be conducted as deemed appropriate.

14.2 Determination of Sample Size

The study is expected to enroll approximately 402 eligible subjects (with expected loss to follow-up or censorship of primary endpoint) with 1:1:1 randomization ratio over a post-surgery period (up to 10 days). The study is sized to achieve approximately 87% power to detect a hazard ratio (HR; TU-100 versus placebo) of 1.428 for GIR, which translates into an improvement in median time to GIR from 48 hours (2.0 days) for placebo (reference) to 34 hours (1.4 days) for TU-100 at a 0.05 significance level of one-sided log-ranked test for equality of time to GIR survival curve.

Based on historical evidence, it is assumed that a small proportion of subjects will be lost to follow-up or will have events censored due to required analytical methods. To account for this anticipated loss in contribution of events to the primary endpoint, an additional 36 subjects (approximately 10% of 366) will be enrolled to achieve a final sample size of 402 subjects (134 per group).

14.3 Analysis Sets

Safety analysis set
The Safety Analysis Set will consist of all randomized subjects who receive at least 1 dose of study medication. The Safety Analysis Set will be used primarily for the analysis of safety data. Subjects will be classified based upon the treatment received.

Full analysis set (FAS)
The FAS will include all randomized subjects who receive at least 1 dose of study medication, who underwent an elective BR surgery as specified in the protocol, and who provided at least 1 post-surgery assessment of the primary endpoint (GIR) during the treatment period. The FAS will be used primarily for the analysis of efficacy data. Participants will be analyzed according to the treatment group to which they are randomized.

Per-protocol analysis (PP) set
The PP set is a subset of the FAS who:

- Had a protocol-specified surgery
- Did not take prohibited medications during the treatment period that could affect the primary endpoint
- Had otherwise complied with the protocol without any other major protocol deviation (any exceptions for inclusion/exclusion criteria granted by the Sponsor/medical monitor will not be considered protocol violations)

Details of the evaluability criteria will be determined before study unblinding and specified in the SAP.

PK analysis set: The PK Analysis set will include all subjects who have available PK data for at least 1 dose of TU-100.

14.4 Handling of Missing Data and Outliers

For incomplete date in safety data such as prior and concomitant medication and AEs, the following rules will be applied.

For partial start date:

- If the month and year are provided and day is missing, and the month and year match the month and the year of the first dose date of TU-100, the day of the first dose date of TU-100 will be imputed.
- If the year is provided and the month and day are missing and the year matches the year of the first dose date of TU-100, the month and day of the first dose date of TU-100 will be imputed. Otherwise, January will be used.
- If the start date is completely missing, the start date will not be imputed.
- If the stop date is complete and the imputed start date is after the actual stop date, then the start date will be imputed as the stop date.

For partial stop dates:

- If the month and year of stop are provided, but the day is missing, then the last day of the month will be used.
- If the year of stop is provided, but the month and day are missing, then December 31st of that year will be used.
- If the stop date is completely missing, then the date of last study visit will be used.

For laboratory test results:

- Results of a nonnumeric qualifier (e.g., "< x" – below the lower limit of quantification or "> x" – above the upper limit of quantification) will be displayed in the data listings as shown in the data, but the numerical value of "x" will be used for calculation of summary statistics.

Missing values for other data points will remain as missing unless otherwise specified. Missing values will not be imputed and only observed values will be used in data analyses and presentations unless otherwise stated. Detailed missing handling rules will be discussed in the SAP.

14.5 Efficacy Analyses

For efficacy endpoints, the analysis on FAS will be considered as the primary analysis while the analysis on the PP population will be considered confirmatory.

14.5.1 Statistical Hypotheses and Tests

The null hypothesis for the primary efficacy endpoint is that at least 1 dose regimen of TU-100 is superior to placebo with respect to the improvement in time to GIR during the post-surgery observation period (up to 10 days), which translates into a greater HR (TU-100 versus placebo).

Symbolically, the hypotheses for each dose level of TU-100 compared with placebo can be written as:

H_0: HR = 1.428
H_A: HR > 1.428

All analyses will be performed based on 1-sided tests at the significance level of 0.05 for the primary endpoint. Nominal *P* values will be reported without any adjustment for multiple endpoints in the study.

14.5.2 Analysis of Primary Efficacy Endpoint

The primary efficacy variable is time to gastrointestinal recovery (TGIR), a composite endpoint defined as the following:

TGIR = maximum (time to first toleration of liquids; time to first BM OR [absence of distention AND bowel sounds AND flatus])

Time to an event is defined as the elapsed time, in hours, from the end of surgery to the time of the first event, to be calculated as:

Time to event (hours) = (time of the event − time of end of surgery)

When TGIR is greater than 10 days (240 hours), it will be censored at 10 days. Censoring rules for other secondary time-event endpoints will be discussed in the SAP.

The primary analysis of TGIR will be based on the Cox proportional hazard model (Cox PH) that includes the main effects for treatment and surgical approach (open versus laparoscopic). The estimates of time to event for the quartiles (25%, 50%, 75%), hazard ratios and their corresponding one-sided 95% confidence intervals (CIs), the Wald Chi-square one-sided *P* values from the Cox analysis will be summarized. The ties in the data will be handled by the Breslow method.

A secondary analysis will be applied for TGIR using the Cox PH model including main effects for treatment, surgical approach (open versus laparoscopic), and covariates such as surgical type, gender, age (years), post-operation opioid consumption (mg morphine equivalents; APPENDIX 2), thoracic epidural use, and surgery duration (hours). Backward method will be used to select statistically significant covariates ($P < 0.1$). Adjusted *P* value and HR will be provided after the final model is selected.

In addition, TGIR will be assessed for TU-100 versus placebo at each dose level using a stratified log-rank with the one stratum used for randomization: surgical approach (open versus laparoscopic). The Kaplan-Meier estimate of the median TGIR and the corresponding 1-sided 95% CIs calculated using the Greenwood's formula will be presented. Plot of the Kaplan-Meier estimate of the survival distribution function over time will be presented. The restricted mean survival time (RMST) (Uno 2015) at time t* using the Kaplan-Meier estimate of the survival function will also be provided, where t* is the minimum of the largest observed time (event or censored) in each of the 2 groups. Furthermore, the difference in RMST, as well as the ratio of RMST between 2 groups will be evaluated. Similar analyses will be applied for other time to event efficacy endpoints.

The effect of some additional potential covariates on treatment effect may also be evaluated using appropriate methods. Sensitivity analyses for TGIR will be described in the SAP.

14.5.3 Analyses of Secondary and Exploratory Efficacy Endpoints

Similar analysis for the primary efficacy endpoint will be applied for other time to event efficacy endpoints.

A secondary efficacy variable, time to ready for discharge (based solely on the treating medical team's determination of GI recovery), which is defined as the time to the assessment date of "ready for hospital discharge." Length of stay is based on the time to the calendar day of discharge order written from the calendar day of surgery.

Treatment effect on other numeric variables will be analyzed using an ANOVA if normally distributed, or the Wilcoxon rank sum test otherwise. Treatment effect on categorical variables will be analyzed using the stratified Miettinen and Nurminen (M&N) method (stratified by surgical approach) (Miettinen 1985), or the logistic regression model if appropriate. Potential covariates will be identified and tested.

14.5.4 Multiplicity Adjustments

No multiplicity adjustments are planned.

14.6 Safety Analyses

14.6.1 Exposure to Study Medication

The total number of doses and total number of days on study drug will be summarized by treatment group. Compliance will also be summarized. Since the subjects are inpatient subjects, the compliance will only measure the compliance up to the study participation. That is, the compliance rate will not be impacted by premature discontinuation of the study.
Exposure to study medication will be summarized descriptively.

14.6.2 Adverse Events

The incidence of all TEAEs (events with onset dates on or after the start of the study drug) will be tabulated by treatment received. All reported AEs will be classified by system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of TEAEs will be included in incidence tables. All AEs will be listed by subject, along with information regarding onset, duration, relationship and severity to study drug, action taken with study drug, treatment of event, and outcome. An overview of TEAEs, which includes subject incidence of TEAEs, treatment-related TEAEs, TEAEs by severity, SAEs, deaths, AEs leading to discontinuation, and independently adjudicated hepatobiliary AEs will be presented. Events with missing onset dates will be included as treatment-emergent. If a subject experiences more than 1 occurrence of the same AE, the occurrence with the greatest severity and the closest association with the study drug will be used in the summary tables.

14.6.3 Prior or Concomitant Medications

Medications will be coded by the World Health Organization Drug Dictionary to Anatomical Therapeutic Classification (ATC) and preferred drug name. Prior and concomitant medications will be summarized by ATC level and preferred drug name and listed. Post-operation opioid consumption (mg morphine equivalents; APPENDIX_02) will be summarized descriptively. Summary tables will be provided for concomitant medications initiated during the study period.

14.6.4 Clinical Laboratory Parameters

Clinical safety laboratory tests will be conducted by local or central laboratories. Summary statistics for actual values and for changes from baseline will be tabulated for hematology and chemistry (with a focus on liver transaminases and LFTs) results by treatment group. Clinical laboratory data and vital signs will be summarized using descriptive statistics, including mean values and mean change from baseline values, as well as numbers of subjects with values outside limits of the normal range at each time point. Shift tables for clinical laboratory parameters will be provided to assess the changes in laboratory value from baseline to post-baseline visits. Normal reference laboratory range will be used to determine the number and percentage of subjects who had shifts. Subjects with clinical laboratory values outside of the normal reference will be listed. The number and percentage as well as the listing of subjects who meet the criteria of Hy's law (with ALT or AST values ≥ 3 times the ULN [3 × ULN] and total bilirubin ≥ 2 times the ULN [≥ 2 x ULN]) will be summarized by post-baseline time point and treatment group.

14.6.5 Vital Sign Measurements

Descriptive summary statistics for vital sign parameters and changes from baseline will be tabulated by treatment group. Variables summarized include systolic blood pressure (mmHg), diastolic blood pressure (mmHg), pulse rate (bpm), respiratory rate (per minute), and body temperature.

14.6.6 12-Lead Electrocardiograms

The observed values and change from baseline for each ECG measurement day will be summarized with descriptive statistics. Electrocardiogram variables include heart rate, PR, QT, QTc, RR intervals, and QRS duration. In addition, QT interval with Fridericia correction (QTcF) will be calculated.

An outlier analysis will be performed including all maximum individual postdose measurements (not the mean data), including all repeat and unscheduled readings. All incidences of maximum QTcF (>450 and ≤ 480, >480 and ≤ 500, and >500 ms), and all incidences of maximum change from baseline in QTc and QTcF (>30 and ≤ 60 and >60 ms) will be flagged on the listing. More details on the analyses will be included in the SAP.

14.7 Baseline Characteristics and Other Summaries

Descriptive summaries of demographic and baseline characteristics will be tabulated for both Safety Analysis Set, PK Analysis Set, and FAS. Descriptive statistics or a frequency table, whichever is appropriate, will be provided for age, age group (<65 years or ≥65 years), height, weight, body mass index (BMI), and BMI distribution (<30 kg/m^2 or ≥30 kg/m^2). Surgical approach (open versus laparoscopic), surgery duration (hours), time from first dose of study medication to the scheduled start of surgery (hours), type of surgery, primary indication for surgery, and thoracic epidural use will be summarized. Medical history findings will be coded according to MedDRA. Findings will be tabulated by preferred term within system organ class and summarized using number and frequencies.

The number and percentage of subjects who are randomized, treated, complete study treatment, and complete the study will be summarized. The same data will be summarized by site as well. Reasons for discontinuing the study will also be summarized.

Subjects in each analysis population, and those excluded from each analysis population and reasons for exclusion will be summarized using number and frequency. Individual subject listings will also be provided.

14.7.1 Analysis of Adjudicated Hepatobiliary Adverse Events

The independent CEAC adjudicated hepatobiliary AE database will be the primary data source for determining the type and incidence of hepatobiliary AEs. The incidence of the adjudicated hepatobiliary AEs and the difference in these events across treatment groups will be summarized. This analysis will serve as the primary evidence base for determining the risk for DILI in TU-100 and placebo treated subjects.

14.8 Pharmacokinetic/Pharmacodynamic Analysis

Plasma concentrations of the HAS and HBS (the major constituents of TU-100) will be summarized for each treatment group. Plasma concentrations of other constituents of TU-100 may also be summarized based on the ability to detect their presence in the collected blood samples. Exploratory population PK/pharmacodynamic analyses may be conducted to understand the exposure response relationships between TU-100 and efficacy and safety data. Data may be used for future population PK analyses which, if conducted, will be reported separately.

14.9 Interim Analysis

No interim analysis is planned.

14.10 Data Independent Committees

There will be 2 independent committees. Each will have a formal charter and their specific roles will be discussed within the protocol.

- DSMB for clinical trial safety oversight
- CEAC for assessment of hepatobiliary AEs

An independent DSMB will be established to conduct safety reviews on periodic safety data cuts and will follow an approved DSMB charter. The DSMB will communicate back to Tsumura about their assessments. Detailed information on the role of the DSMB and frequency of meetings will be provided in the DSMB charter separate from this protocol. The DSMB will be responsible for safety review and oversight of aggregate data from participants. They are not responsible for review of individual participant efficacy data (i.e., GIR determination).

The CEAC blinded to treatment assignment is distinct from the DSMB and will only focus on assessment of hepatobiliary AEs. The CEAC adjudicated hepatobiliary AE database will be used to determine the risk for hepatobiliary AEs. Detailed information on the role of the CEAC and frequency of meetings will be provided in the CEAC charter separate from this protocol.

15 STUDY MANAGEMENT

15.1 Approval and Consent

15.1.1 Regulatory Guidelines

This study will be conducted in accordance with the accepted version of the Declaration of Helsinki and/or all relevant regulations, as set forth in Parts 50, 56, 312, Subpart D, of Title 21 of the US Code of Federal Regulations (CFR), in compliance with International Council for Harmonisation (ICH) and GCP guidelines and according to the appropriate regulatory requirements in the countries where the study was conducted.

This study follows the FDA guidance on the conduct of clinical trials and statistical considerations for clinical trials during the COVID-19 pandemic as necessary.

15.1.2 Institutional Review Board/Independent Ethics Committee

Conduct of the study must be approved by an appropriately constituted IEC/IRB. Approval is required for the study protocol, protocol amendments (if applicable), IB, ICFs, recruitment material, and subject information sheets and other subject-facing material.

15.1.3 Informed Consent

For each study subject, written informed consent will be obtained before any protocol-related activities. As part of this procedure, the PI or designee must explain orally and in writing the nature of the study, its purpose, procedures, expected duration, alternative therapy available, and the benefits and risks involved in study participation. The subject should be informed that he/she may withdraw from the study at any time, and the subject will receive all information that is required by local regulations and guidelines for ICH. The PI will provide the Sponsor or its representative with a copy of the IEC-/IRB-approved ICF before the start of the study.

15.2 Data Handling

Any data to be recorded directly on the eCRFs (to be considered as source data) will be identified at the start of the study. Data reported on the eCRF that are derived from source documents should be consistent with the source documents, or the discrepancies must be explained. See also Section 15.3.

Subjects will be given eDiaries in which to record events specified in Section 11.3 on postoperative in-hospital days. The eDiary entries are considered source data. Data will be transferred from the eDiaries into the clinical database for analyses.

Clinical data will be entered by site personnel on eCRFs for transmission to the Sponsor. Data on eCRFs transmitted via the web-based data system must correspond to and be supported by source documentation maintained at the study site, unless the study site makes direct data entry to the databases for which no other original or source documentation is maintained. In such cases, the study site should document which eCRFs are subject to direct data entry and should have in place procedures to obtain and retain copies of the information submitted by direct data entry. All study forms and records transmitted to the Sponsor must only include coded identifiers such that directly identifying personal information is not transmitted. The primary method of data transmittal is via the secure, internet-based electronic data capture (EDC) system maintained by Syneos Health. Access to the EDC system is available to only authorized users via the study's internet website, where a user unique assigned username and password are required for access.

Any changes made to data after collection will be made through the use of the EDC system. Electronic case report forms (eCRFs) will be considered complete when all missing and/or incorrect data have been resolved.

15.3 Source Documents

Source documents are considered to be all information in original records and certified copies of original records of clinical findings, observations, data, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. The investigator will provide direct access to source documents and/or source data in the facilitation of trial-related monitoring, audits, review by IEC/IRB, and regulatory inspections.

The investigator/institution should maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, not obscure the original entry, and be explained if necessary.

15.4 Record Retention

Study records and source documents must be preserved for at least 15 years after the completion or discontinuation of/withdrawal from the study, at least 2 years after the drug being studied has received its last approval for sale, or at least 2 years after the drug development has stopped, and in accordance with the applicable local privacy laws, whichever is the longer time period.

The investigator agrees to comply with all applicable federal, state, and local laws and regulations relating to the privacy of subject health information, including, but not limited to, the Standards for Individually Identifiable Health Information, 45 CFR, Parts 160 and 164 (the Health Insurance Portability Accountability Act of 1996 [HIPAA] Privacy Regulation). The investigator shall ensure that study subjects authorize the use and disclosure of protected health information in accordance with HIPAA Privacy Regulation and in a form satisfactory to the Sponsor.

15.5 Monitoring

The study will be monitored according to the monitoring plan to ensure that it is conducted and documented properly according to the protocol, GCP, and all applicable regulatory requirements.

Monitoring visits, on-site and remote (telephone) or a combination and contacts will be made at appropriate times during the study. The PI will assure he/she and adequate site personnel are available throughout the study to collaborate with clinical monitors. Clinical monitors must have direct access to source documentation in order to check the completeness, clarity, and consistency of the data recorded on the eCRFs for each subject.

The investigator will make available to the clinical monitor all source documents and medical records necessary to review protocol adherence and eCRFs. In addition, the investigator will work closely with the clinical monitor and as needed, provide them appropriate evidence that the study is being conducted in accordance with the protocol, applicable regulations, and GCP guidelines.

15.6 Quality Control and Quality Assurance

The Sponsor or its designee will perform the quality assurance and quality control activities of this study; however, responsibility for the accuracy, completeness, security, and reliability of the study data presented to the Sponsor lies with the investigator generating the data.

The Sponsor will arrange audits as part of the implementation of quality assurance to ensure that the study is being conducted in compliance with the protocol, standard operating procedures, GCP, and all applicable regulatory requirements. Audits will be independent of and separate from the routine monitoring and quality control functions. Quality assurance procedures will be performed at study sites and during data management to assure that safety and efficacy data are adequate and well documented.

15.7 Protocol Amendment and Protocol Deviation

15.7.1 Protocol Amendment

Amendments to the protocol that entail corrections of typographical errors, clarifications of confusing wording, changes in study personnel, and minor modifications that have no effect on the safety of subjects or the conduct of the study will be classed as administrative amendments and will be submitted to the IEC/IRB for information only. The Sponsor will ensure that acknowledgement is received and filed. Amendments that are classed as substantial amendments must be submitted to the appropriate regulatory authorities and the IEC/IRB for approval and will not be implemented at sites until such approvals are received, other than in the case of an urgent safety measure.

15.7.2 Protocol Deviations

Should a protocol deviation occur, the Sponsor must be informed as soon as possible. Protocol deviations and/or violations and the reasons they occurred will be included in the CSR. Reporting of protocol deviations to the IRB/IEC and in accordance with applicable regulatory authority mandates is an investigator responsibility.

15.8 Ethical Considerations

This study will be conducted in accordance with this protocol, the accepted version of the Declaration of Helsinki and/or all relevant federal regulations, as set forth in Parts 50, 56, 312, Subpart D, of Title 21 of the CFR; EU 536/2014, Annex 1, D, 17 (a); and in compliance with GCP guidelines.

The IEC/IRB will review and approve this protocol and the ICF. All subjects are required to give written informed consent before participation in the study.

15.9 Financing and Insurance

Before the study commences, the Sponsor (or its designee) and the investigator (or the institution, as applicable) will agree on costs necessary to perform the study. This agreement will be documented in a financial agreement that will be signed by the investigator (or the institution signatory) and the Sponsor (or its designee).

The investigator is required to have adequate current insurance to cover claims for negligence and/or malpractice. The Sponsor will provide no-exclusion insurance coverage for the clinical study as required by national regulations.

15.10 Publication Policy/Disclosure of Data

Both the use of data and the publication policy are detailed within the clinical study agreement. Intellectual property rights (and related matters) generated by the investigator and others performing the clinical study will be subject to the terms of a clinical study agreement that will be agreed between the institution and the Sponsor or their designee. With respect to such rights, the Sponsor or its designee will solely own all rights and interests in any materials, data, and intellectual property rights developed by investigators and others performing the clinical study described in this protocol, subject to the terms of any such agreement. In order to facilitate such ownership, investigators will be required to assign all such inventions either to their institution or directly to the Sponsor or its designee, as will be set forth in the clinical study agreement.

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17 APPENDICES

APPENDIX 1 describes the contraception guidelines applicable for this study.
APPENDIX 2 presents an opioid conversion chart.

APPENDIX 1. CONTRACEPTION GUIDELINES

Women of childbearing potential (WOCBP) and men whose sexual partners are WOCBP must use at least 1 highly effective method of contraception during the study and for 30 days after the last dose of study treatment.

A woman is considered to be a WOCBP (fertile) following menarche and until becoming postmenopausal, unless she is permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) 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, a single FSH measurement is insufficient.

A man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy.

Highly effective methods of contraception are those which have a failure rate of < 1% (when implemented consistently and correctly) and include:

- combined (containing estrogen and progestogen) hormonal contraception associated with inhibition of ovulation (administration may be oral, intravaginal, or transdermal)
- progestogen-only hormonal contraception associated with inhibition of ovulation (administration may be oral, injectable, or implantable)
- intrauterine device
- intrauterine hormone-releasing system
- bilateral tubal ligation or occlusion
- vasectomy (provided that the male has a medical assessment of surgical success)
- condom in combination with contraceptive cream, jelly, or foam
- sexual abstinence (defined as refraining from heterosexual intercourse during the entire period of risk in relation to the duration of the clinical trial, in line with the preferred and usual lifestyle of the subject)

All subjects will be strongly advised that they (or the female partners of male subjects) should not become pregnant while on study treatment or for 30 days after the last dose. A female subject will be advised that she must report immediately to the study site for pregnancy testing and appropriate management in the event that she may be pregnant.

Reference

1. [HMA] Heads of Medicines Agencies. Clinical Trial Facilitation Group page. Recommendations related to contraception and pregnancy testing in clinical trials. http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf. September 15, 2014.

APPENDIX 2. OPIOID CONVERSION CHART

Generic Opioid Name	Unit	Route	Conversion Factor	Oral MME Conversion Factor
Morphine	MG	IV	1	3
Morphine	MG	PO	0.333	1
Methadone	MG	IV	1	
Methadone	MG	PO	0.333	3 (variable with dose)
Nalbuphine	MG	IV	1	
Nalbuphine	MG	PO	0.333	1
Buprenorphine	MG	IV	25	
Buprenorphine	MCG	Transdermal		12.6
Fentanyl	MCG	IV	0.125	0.2
Fentanyl	MCG	Transdermal		7.2
Sufentanil	MCG	IV	1.25	
Alfentanyl	MCG	IV	0.02	15
Oxycodone	MG	PO		1.5
Hydromorphone	MG	IV	6.67	17.5
Hydromorphone	MG	PO	2.22	4
Codeine	MG	PO	0.05	0.15
Dihydrocodeine	MG	PO		0.25
Tramadol	MG	PO		0.1
Meperidine	MG	PO	0.03	0.1
Meperidine	MG	IV/IM		0.4
Pentazocine	MG	PO	0.06	
Pentazocine	MG	IV/IM		0.37

- Abbreviations: IV = intravenous; IM = intramuscular; MME = morphine mg equivalent; PO = orally.

PA6

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