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Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study

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Keywords:	indocyanine green-fluorescence imaging, liver tumour, hepatectomy

Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study

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Keywords: indocyanine green-fluorescence imaging, liver tumour, hepatectomy

ABSTRACT

Introduction: In vivo fluorescence imaging techniques using indocyanine green to identify liver tumours and hepatic segment boundaries have been recently developed. The purpose of this study is to evaluate the efficacy of fusion indocyanine green (ICG)-fluorescence imaging for navigation during hepatectomy.

Methods and analysis: This will be an exploratory single-arm clinical trial; patients with liver tumours will undergo hepatectomy using the ICG-fluorescence imaging system. In total, 110 patients with liver tumours scheduled for elective hepatectomy will be included in this study. Preoperatively, ICG will be intravenously injected at a dose of 0.5 mg/kg body weight within 2 days. Intraoperatively, to detect liver tumours, the hepatic surface will be initially observed using the ICG-fluorescence imaging system. After identifying and clamping the portal pedicle corresponding to the hepatic segments, including the liver tumours to be resected, additional ICG will be injected intravenously at a dose of 0.5 mg/kg body weight to identify the boundaries of the hepatic segments. The primary outcome measure will be considered to represent the success or failure of the ICG-fluorescence imaging system in identifying hepatic segments. The secondary outcomes will be the success or failure in identifying liver tumours, liver function indicators, operative time, blood loss, rate of postoperative complications, and recurrence-free survival. The findings obtained through this study are expected to help establish the utility of ICG-fluorescence imaging systems and

therefore contribute to prognostic outcome improvements in patients who will undergo hepatectomy for various causes.

Ethics and dissemination: The protocol has been approved by the Kobe University Clinical Research Ethical Committee. The findings of this study will be disseminated widely through peer-reviewed publications and conference presentations.

Trial registration number: This study is registered at the UMIN Clinical Trials Registry: UMIN0000180139 and Japan Registry of Clinical Trials: jRCT1051180070. The Registration Data Set is available at https://jrct.niph.go.jp/.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study is expected to address the clinical utility of real-time navigation during hepatectomy using indocyanine green (ICG)-fluorescence imaging systems.
- Efficacy and safety of hepatectomy using ICG-fluorescence imaging systems is expected to be clarified through the analysis of associations between the success rate in identifying hepatic segments and clinical outcomes, including liver function indicators, operative time, blood loss, rate of postoperative complications, and recurrence-free survival.
- This is an exploratory single-arm study, the results of which will be compared against historical data from our facility.

INTRODUCTION

Hepatectomy remains the mainstay treatment for hepatocellular carcinoma (HCC) and metastatic liver tumours and is commonly performed in patients with preserved liver function.[1-3] Vascular invasion is a poor prognostic factor in HCC, and anatomical resection of the cancer-bearing portal regions is a theoretically effective procedure for the treatment of HCC and metastatic liver tumours complicated by invasion of the Glisson's capsule.[4]

To perform anatomical resection safely and precisely, the liver's anatomical boundaries must be visually recognized. Particularly, the hepatic veins are considered to indicate the absolute boundaries of hepatic segments and can easily be identified by intraoperative ultrasonography. However, due to the three-dimensional shape of the hepatic segment, the hepatic veins are not sufficient for guiding anatomical resection. Under such conditions, the role of intraoperative navigation in hepatectomy allows for a real-time identification of three-dimensional structures, including tumours and hepatic segment boundaries.

Several techniques for identifying hepatic segments have been reported so far.[5-9] Recently, in vivo fluorescence imaging techniques for the identification of biological structures intraoperatively have been developed. Among the various fluorophores used, indocyanine green (ICG) receives a substantial amount of attention because of its well-known pharmacokinetic and safety profile, making it a potentially valuable clinical tool.[10] For example, it is well known that ICG rapidly and completely binds to plasma proteins—among

which albumin is the principal carrier—following intravenous injection. Also, ICG is excreted in bile in an unconjugated form and is not cleared by extrahepatic mechanisms. Furthermore, single or repeated intravenous injections or infusions rarely cause unfavourable adverse effects. Taking advantage of these characteristics and development of concomitant fluorescence imaging techniques, ICG-fluorescence imaging systems are widely used for detecting sentinel lymph nodes and arterial blood flow, and their effectiveness has been recognized.[11,12] Also, the potential utility of this approach to identify liver tumours and hepatic segment boundaries, as well as to detect intraoperative bile leakage has recently been demonstrated.[7,13-19]

The ICG-fluorescence imaging system was initially introduced for use during open hepatectomy. Similar fluorescence imaging systems have been recently developed for use during laparoscopic hepatobiliary surgery. Several reports have demonstrated the efficacy of such systems during laparoscopic cholecystectomy and hepatectomy.[20] However, whether the hepatic boundaries visualised by ICG-fluorescence imaging systems are clinically precise and useful has not been adequately assessed. For example, there may be minor deviations because due to the confluence of communicating vessel branches between hepatic segments and the injected ICG likely passes through the hepatic segments and the tumour to be removed. The evidence regarding the efficacy of ICG-fluorescence imaging systems is not fully established, and further investigation is required.

The purpose of this study is to evaluate the efficacy of the ICG-fluorescence imaging system during hepatectomy for patients with liver tumours by analysing the detection rate of hepatic boundaries and tumours. In addition, we assess the precision of the detected hepatic boundaries by evaluating the postoperative clinical data.

METHODS AND ANALYSIS

Study design

This prospective study is a single-arm, exploratory clinical trial. Patients with liver tumours will undergo hepatectomy using the ICG-fluorescence imaging system. This study will be performed at Kobe University.

Target population

From 2018 to 2021, patients with liver tumours treated at Kobe University will be enrolled. The inclusion criteria are as follows: male or female patients with liver tumours, aged 20 years and older, scheduled for elective hepatectomy, preserved liver function, ability to understand the nature of the study procedures, and willingness to participate and give voluntary written consent. Liver functional reserve will be assessed by serum biochemical data (albumin level, total bilirubin level, and prothrombin time) and ICG retention for 15 minutes (ICG-R15). The patients will be categorized according to the severity of liver disease

based on Child-Pugh stages and the liver damage classification, defined by the LCSGJ.[21,22] Preserved liver function is defined as ICG-R15 <15% and Child-Pugh classification A or B.

The exclusion criteria are as follows: liver or renal insufficiency, known ICG hypersensitivity, pregnancy or breastfeeding, and inability to understand the nature of the study procedure.

Intervention

ICG is injected intravenously at a dose of 0.5 mg/kg body weight within 2 days preoperatively. Intraoperatively, we will initially observe the hepatic surface using a fusion ICG-fluorescence imaging system to detect liver tumours. After identifying and clamping the portal pedicle corresponding to the hepatic segments to be removed, additional ICG is injected intravenously at a dose of 0.5 mg/kg body weight to identify the boundaries of the hepatic segments. Hepatectomy is performed based on the demarcation between fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic segments. The demarcation will also be checked at appropriate intervals during parenchymal resection. Parenchymal resection will be performed using an ultrasonic surgical aspirator (CUSA; Cavitron Lasersonic Corp., Stamford, CT, USA), and a bipolar clamp coagulation system (ERBE, Tubingen, Germany). The fusion ICG-fluorescence images will only be used for the hepatectomy. The Pringle manoeuvre will be performed and a drainage tube will be routinely

inserted around the cut surface of the liver parenchyma.

Sample size calculation

The purpose of the primary analysis of this study is to estimate the success rate, which is defined as the proportion of identifying hepatic segments by the ICG-fluorescence imaging system during hepatectomy. In order to judge the procedure as useful, a success rate of at least 80% is thought to be required. When the expected success rate is 90% and the two-sided 95% confidence interval width is 0.12, the required number of participants is 98. To allow for an approximately 10% dropout, the target sample size of this study has been set to 110.

Outcome measures

Primary endpoint

The primary endpoint is the success and failure of identifying hepatic segments using the ICG-fluorescence imaging system. We evaluate the identification of hepatic segments in two points: observation of the liver surface and the hepatic transection surface. We assume that identification is successful when fulfilling the following two criteria:

(1) Hepatic surface

Identification of hepatic segments by the ICG-fluorescence imaging system is considered successful when the demarcation between fluorescing and non-fluorescing areas is consistent

with the ischemic demarcation area observed by clamping the portal pedicle.

(2) Hepatic transection surface

Hepatic parenchymal resection is performed based on the demarcation between fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic segments. We divide the time taken to perform parenchymal resection into three equal intervals, and the identification of hepatic segment boundaries is evaluated at each interval. Identification of hepatic segments is considered successful when we can identify the hepatic segments at more than two intervals.

Secondary endpoints

The secondary endpoints are the success and failure of identifying liver tumours by the ICG-fluorescence imaging system, liver function indicators (alanine transaminase, albumin, total bilirubin, international normalized ratio of prothrombin time, platelet count), the operative time, the blood loss, the rate of postoperative complications, and recurrence-free survival. Recurrence-free survival time is defined as the time from enrolment until first recurrence after the surgical intervention. Patients without recurrence will be censored at the date of last confirmation of recurrence-free status. Patients lost to follow-up without a diagnosis of recurrence and those who die will be censored at the date of last confirmation of recurrence-free status.

Data collection

Three experienced surgeons will judge the intraoperative identification hepatic segment boundaries. The success rate of their identification is used as the end point. The entire surgical procedure, including ICG-fluorescence imaging, will be digitally recorded and analyzed by an additional expert panel consisting of three highly experienced surgeons to confirm the identification of hepatic segment boundaries. A flow chart of the study procedure is presented in Figure 1.

Postoperative complications will be graded according to the extended Clavien-Dindo classification of surgical complications, which was published by the Japan Clinical Oncology Group and more precisely described the original criteria of the Clavien-Dindo classification.[23,24]

Follow-up visits will be carried out at two weeks after hospital discharge, and every three months thereafter. Follow-up evaluation will be performed using routine blood tests, including liver function tests, coagulation function tests, and serum AFP level; abdominal ultrasonography; and abdominal enhanced computed tomography.

Study timeline

Data will be collected from April 2018 until January 2022, and analysis is expected to be

completed around January 2023.

Participants will be informed about the study during their preoperative visit to our hospital, and will have ample time to consider participation. Possible complications will be evaluated in the year following the surgery. The schedules of enrolment, interventions, and assessments are shown in Table 1.

Statistical analysis

The analysis populations will include the following three sets. Firstly, the full analysis set (FAS) will consist of all participants that completed the surgery with navigation by ICG-fluorescence images and have efficacy data available, excluding those without baseline data or significant protocol violations (e.g., absence of informed consent, enrolment outside the contract period). Secondly, the per protocol set (PPS) will consist of the FAS participants completed 1 year of follow-up, excluding those with any of the following significant protocol violations involving the study method, the inclusion criteria, the exclusion criteria and concomitant therapy. Lastly, the safety analysis set (SAS) will consist of the participants who enrolled in this study and were given at least one dose of ICG.

The analysis will be performed after the data lock following completion of study drug administration to all participants. For all efficacy endpoints, the FAS will be used in the primary analysis, while the PPS will be used in a reference analysis. Safety will be analysed

using the SAS. The baseline participant characteristics' distribution and summary statistics will be calculated according to group in each analysis population.

All statistical analyses will be performed as indicated using JMP software, version 13.0.0 (SAS Institute, Inc., Cary, NC, USA).

Interim analyses will not be performed in this study.

Primary outcome

The primary objective of this study is to estimate the success rate, which is defined as the proportion of identifying hepatic segments by the ICG-fluorescence imaging system. The point estimate of the rate and the 95% confidence interval (CI) will be calculated.

Secondary outcomes

The point estimate and 95% CI of the success rate of tumour detection by the ICG-fluorescence imaging system will be calculated. For analysis of other secondary outcomes, we will conduct a test using historical data collected at our facility as the control group. No multiplicity adjustment will be performed in the analysis of secondary efficacy endpoints.

Exploratory analysis

We will perform logistic regression analysis of the success or failure of the ICG

fluorescence imaging system. The following factors will be included in the model: age, gender, body mass index, viral infection, Child-Pugh classification, cirrhosis, tumour size, tumour number, tumour location, type of hepatectomy, liver function indicators (alanine transaminase, albumin, total bilirubin, international normalized ratio and prothrombin time, platelet count), operative time, blood loss, rate of postoperative complications, and recurrence-free time.

Safety analysis

The safety endpoint of this study is the frequency of adverse events. A table will be prepared to summarize the endpoint. For estimation of the rates of adverse events, a two-sided 95% CI will be calculated.

Data monitoring

Monitoring will be performed in order to periodically check whether the study is being conducted safely in accordance with the protocol and whether the data are properly collected. The following items are reviewed every six months: informed consent, obtained and signed; participant retention; study implementation system; study safety and data; and study progress.

Patient and Public involvement

There were no patient and public involvement in planning of this study.

ETHICS AND DISSEMINATION

Is there scientific and clinical value in conducting this study?

We can evaluate the efficacy and safety of hepatectomy using ICG-fluorescence imaging systems by analysing the association between the success rate of identifying hepatic segments and clinical outcomes. This study will help determine whether the boundaries detected by ICG-fluorescence imaging systems during hepatectomy are valid and useful.

The findings obtained through this study will help establish the utility of ICG-fluorescence imaging systems and therefore the study is expected to contribute to the improvement of prognostic outcomes in patients who undergo hepatectomy due to various causes.

Ethical approval

This study was approved by the Kobe University Clinical Research Ethical Committee. Possible protocol amendments will be sent to the Kobe University Clinical Research Ethical Committee.

Consideration of participants' human rights, safety, and disadvantages

The principal investigator and sub-investigators will comply with the principals of the protection of participants' privacy rights. Study personnel will make the utmost of effort to protect the participants' personal information and privacy, and will not divulge any personal information learned from this study without due reasons, even outside working hours. In this study, a list of subject identification codes will be prepared to link the subject source data with the study database or study-related documents. Limited participant information, such as sex and date of birth, may be used to identify participants or verify the list of subject identification codes, within the range of all applicable laws and regulations.

All effort will be taken to ensure than participants will not be personally identifiable from publications arising from this study.

Foreseeable disadvantages (burdens and risks)

The administration of ICG will be the only additional invasive intervention performed in each patient. ICG administration rarely causes anaphylactic reactions (<1:10,000). Patients with terminal renal insufficiency seem to be more prone for such an anaphylactic reaction. The estimated mortality rate due to anaphylactic reaction is reported as <1 per 330,000.[25-28]

To minimize the risk of adverse events and disadvantages that may occur in this study, the inclusion and exclusion criteria have been carefully discussed. All adverse events occurring in this study will be monitored to ensure that they are within the expected range. If

any serious or unexpected adverse events occur, the event will be carefully examined and reviewed, and necessary countermeasures will be taken. Participation in this study may require increased hospital visits, test frequency, and blood sampling volume, compared to routine medical care. In the event of tumour progression, severe organ dysfunction, physical weakening, etc., during the preoperative treatment or during the waiting period for surgical resection, the planned surgical resection may not be possible.

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AUTHOR STATEMENT

H. Gon, S. Komatsu, S. Murakami, M. Kido, M. Tanaka, K. Kuramitsu, M. Awazu, and T. Fukumoto all made substantial contributions to the conception and design of the study. H. Gon, S. Komatsu, and S. Murakami drafted the manuscript. All authors provided critical review and final approval of the present manuscript.

FUNDING STATEMENT

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

DATA SHARING STATEMENT

This is a research protocol. That means the data for this study are being retrieved at this moment. All authors have access to these data, and these data will be published as described in the protocol, coordinated by H. Gon and S. Komatsu.

COMPETING INTERESTS STATEMENT

None declared.

FIGURE LEGENDS

Figure 1. Flowchart of the study procedures. ICG, indocyanine green.

Table 1. Schedule of enrollment, interventions, and assessments.

STUDY PERIOD						
	Within 14 days before registration	Before surgery	Day of surgery	After surgery	Day of discharge	Every 3 months after discharge
ENROLLMENT						
Eligibility screen	X					
Informed consent	X					
Background Blood test	X					
INTERVENTIONS						
ICG-fluorescence imaging technique			X			
ASSESSMENTS						
Primary outcome			X	X		
Blood test		X	X	X	X	X
Postoperative complication			X	X	X	
Adverse event			X	X	X	
Abdominal ultrasonography						X
Abdominal enhanced CT						X

CT, computed tomography; ICG, indocyanine green.

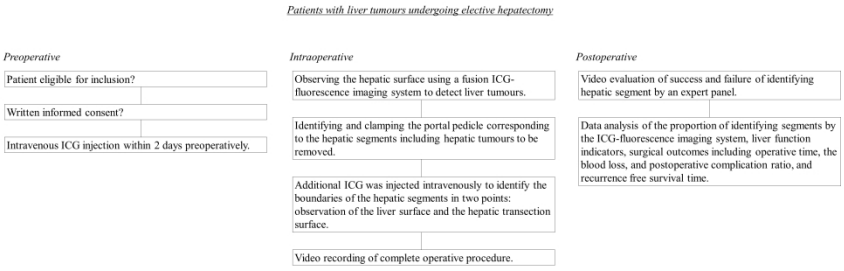

Figure 1. Flowchart of the study procedures.

Figure 1

1200x900mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	#3	Date and version identifier	4
Funding	#4	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	21,22
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-8
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8,9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	16
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10,11

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12,13
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16,17
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14,15
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	16
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant	N/A

		parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12,13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16,17
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	N/A
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study

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Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study

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Keywords: indocyanine green-fluorescence imaging, liver tumour, hepatectomy

ABSTRACT

Introduction: In vivo fluorescence imaging techniques using indocyanine green to identify liver tumours and hepatic segment boundaries have been recently developed. The purpose of this study is to evaluate the efficacy of fusion indocyanine green (ICG)-fluorescence imaging for navigation during hepatectomy.

Methods and analysis: This will be an exploratory single-arm clinical trial; patients with liver tumours will undergo hepatectomy using the ICG-fluorescence imaging system. In total, 110 patients with liver tumours scheduled for elective hepatectomy will be included in this study. Preoperatively, ICG will be intravenously injected at a dose of 0.5 mg/kg body weight within 2 days. Intraoperatively, to detect liver tumours, the hepatic surface will be initially observed using the ICG-fluorescence imaging system. After identifying and clamping the portal pedicle corresponding to the hepatic segments, including the liver tumours to be resected, additional ICG will be injected intravenously at a dose of 0.5 mg/kg body weight to identify the boundaries of the hepatic segments. The primary outcome measure will be the success or failure of the ICG-fluorescence imaging system in identifying hepatic segments. The secondary outcomes will be the success or failure in identifying liver tumours, liver function indicators, operative time, blood loss, rate of postoperative complications, and recurrence-free survival. The findings obtained through this study are expected to help establish the utility of ICG-fluorescence imaging systems and therefore contribute to

prognostic outcome improvements in patients undergoing hepatectomy for various causes.

Ethics and dissemination: The protocol has been approved by the Kobe University Clinical Research Ethical Committee. The findings of this study will be disseminated widely through peer-reviewed publications and conference presentations.

Trial registration number: This study is registered at the UMIN Clinical Trials Registry: UMIN0000180139 and Japan Registry of Clinical Trials: jRCT1051180070. The Registration Data Set is available at https://jrct.niph.go.jp/.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study is expected to address the clinical utility of real-time navigation during hepatectomy using indocyanine green (ICG)-fluorescence imaging systems.
- The efficacy and safety of hepatectomy using ICG-fluorescence imaging systems is expected to be clarified through the analysis of associations between the success rate in identifying hepatic segments and clinical outcomes, including liver function indicators, operative time, blood loss, rate of postoperative complications, and recurrence-free survival.
- This is an exploratory single-arm study, the results of which will be compared against historical data from our facility.

INTRODUCTION

Hepatectomy remains the mainstay treatment for hepatocellular carcinoma (HCC) and metastatic liver tumours and is commonly performed in patients with preserved liver function.[1-3] Vascular invasion is a poor prognostic factor in HCC, and anatomical resection of the cancer-bearing portal regions is a theoretically effective procedure for the treatment of HCC and metastatic liver tumours complicated by invasion of the Glisson's capsule.[4]

To perform anatomical resection safely and precisely, the liver's anatomical boundaries must be visually recognized. Particularly, the hepatic veins are considered to indicate the absolute boundaries of hepatic segments and can easily be identified by intraoperative ultrasonography. However, due to the three-dimensional shape of the hepatic segment, the hepatic veins are not sufficient for guiding anatomical resection. Under such conditions, intraoperative navigation in hepatectomy allows for the real-time identification of three-dimensional structures, including tumours and hepatic segment boundaries.

Several techniques for identifying hepatic segments have been reported thus far.[5-9] Recently, in vivo fluorescence imaging techniques for the identification of biological structures intraoperatively have been developed. Among the various fluorophores used, indocyanine green (ICG) receives a substantial amount of attention because of its well-known pharmacokinetic and safety profile, making it a potentially valuable clinical tool.[10] For example, it is well known that ICG rapidly and completely binds to plasma proteins - among

which albumin is the principal carrier - following intravenous injection. Also, ICG is excreted in bile in an unconjugated form and is not cleared by extrahepatic mechanisms. Furthermore, single or repeated intravenous injections or infusions rarely cause unfavourable adverse effects. Taking advantage of these characteristics and the development of concomitant fluorescence imaging techniques, ICG-fluorescence imaging systems are widely used for detecting sentinel lymph nodes and arterial blood flow, and their effectiveness has been recognized.[11,12] Also, the potential utility of this approach to identify liver tumours and hepatic segment boundaries, as well as to detect the bile duct tree intraoperatively, has recently been demonstrated.[7,13-19]

The ICG-fluorescence imaging system was initially introduced for use during open hepatectomy. Similar fluorescence imaging systems have been recently developed for use during laparoscopic hepatobiliary surgery. Several reports have demonstrated the efficacy of such systems during laparoscopic cholecystectomy and hepatectomy.[20] However, whether the hepatic boundaries visualised by ICG-fluorescence imaging systems are clinically precise and useful has not been adequately assessed. For example, there may be minor deviations due to the confluence of communicating vessel branches between hepatic segments; the injected ICG likely passes through the hepatic segments and the tumour to be removed. Evidence regarding the efficacy of ICG-fluorescence imaging systems is not fully established, and further investigation is required.

The purpose of this study is to evaluate the efficacy of the ICG-fluorescence imaging system during hepatectomy for patients with liver tumours by analysing the detection rate of hepatic boundaries and tumours. In addition, we assess the precision of the detected hepatic boundaries by evaluating the postoperative clinical data.

METHODS AND ANALYSIS

Study design

This prospective study is a single-arm, exploratory clinical trial. Patients with liver tumours will undergo hepatectomy using the ICG-fluorescence imaging system. This study will be performed at Kobe University.

Target population

From 2018 to 2020, patients with liver tumours treated at Kobe University will be enrolled. The inclusion criteria are as follows: male or female patients with liver tumours, aged 20 years and older, scheduled for elective hepatectomy, preserved liver function, ability to understand the nature of the study procedures, and willingness to participate and give voluntary written consent. Liver functional reserve will be assessed by serum biochemical data (albumin level, total bilirubin level, and prothrombin time) and ICG retention for 15 minutes (ICG-R15). The patients will be categorized according to the severity of liver disease

based on Child-Pugh stages and the liver damage classification, defined by the Liver Cancer Study Group of Japan.[21,22] Preserved liver function is defined as ICG-R15 <15% and Child-Pugh classification A or B.

The exclusion criteria are as follows: liver or renal insufficiency, known ICG hypersensitivity, pregnancy or breastfeeding, and inability to understand the nature of the study procedure.

Intervention

ICG is injected intravenously at a dose of 0.5 mg/kg body weight within 2 days preoperatively. Intraoperatively, we will initially observe the hepatic surface using a fusion ICG-fluorescence imaging system (PINPOINT, Stryker Japan K.K.) to detect liver tumours. Among several methods for identifying liver segments with fluorescence imaging, we will use the negative staining technique to identify the liver segments in this study.[23] After identifying and clamping the portal pedicle corresponding to the hepatic segments to be removed, additional ICG is injected intravenously at a dose of 0.5 mg/kg body weight to identify the boundaries of the hepatic segments.[24] Hepatectomy is performed based on the demarcation between fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic segments. The demarcation will also be checked at appropriate intervals during parenchymal resection. Parenchymal resection will be performed using an ultrasonic surgical

aspirator (CUSA; Cavitron Lasersonic Corp., Stamford, CT, USA), and a bipolar clamp coagulation system (ERBE, Tubingen, Germany). The fusion ICG-fluorescence images will only be used for the hepatectomy. The Pringle manoeuvre will be performed and a drainage tube will be routinely inserted around the cut surface of the liver parenchyma.

Sample size calculation

The purpose of the primary analysis of this study is to estimate the success rate, which is defined as the proportion hepatic segments identified by the ICG-fluorescence imaging system during hepatectomy. In order to judge the procedure as useful, a success rate of at least 80% is thought to be required. When the expected success rate is 90% and the two-sided 95% confidence interval width is 0.12, the required number of participants is 98. To allow for an approximately 10% dropout, the target sample size of this study has been set to 110.

Outcome measures

Primary endpoint

The primary endpoint is the success and failure of identifying hepatic segments using the ICG-fluorescence imaging system. We will evaluate the identification of hepatic segments at two points: observation of the liver surface and the hepatic transection surface. We assume that identification is successful when fulfilling the following two criteria:

(1) Hepatic surface

Identification of hepatic segments by the ICG-fluorescence imaging system is considered successful when the demarcation between fluorescing and non-fluorescing areas is consistent with the ischemic demarcation area observed by clamping the portal pedicle.

(2) Hepatic transection surface

Hepatic parenchymal resection is performed based on the demarcation between fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic segments. We divide the time taken to perform parenchymal resection into three equal intervals by reviewing the recorded videos after surgery, and the identification of hepatic segment boundaries is evaluated at each interval. Identification of hepatic segments is considered successful when we can identify the hepatic segments for more than 80% of the process during parenchymal resection at more than two intervals.

Secondary endpoints

The secondary endpoints are the success and failure of identifying liver tumours by the ICG-fluorescence imaging system, liver function indicators (alanine transaminase, albumin, total bilirubin, international normalized ratio of prothrombin time, platelet count), the operative time, the blood loss, the rate of postoperative complications, and recurrence-free survival. Successful identification of liver tumours is determined when any isolated

fluorescence signals are detected, also considering liver tumours diagnosed by other modalities, including preoperative imaging and IOUS, and finally confirmed by pathological examination. The fluorescence pattern is considered according to the preoperative diagnosis because liver lesions have differing fluorescence patterns on the basis of their tumour biology.[25] If we identify lesions with isolated fluorescence signal on fusion-fluorescence imaging that were not identified by preoperative imaging, we evaluate the lesions by intraoperative ultrasound sonography, and, if necessary, frozen section biopsies are performed to determine whether additional hepatectomy is required. The recurrence-free survival is analysed for each liver tumour, including primary liver cancer and liver metastases. Recurrence-free survival time is defined as the time from enrolment until first recurrence after the surgical intervention. Patients without recurrence will be censored at the date of last confirmation of recurrence-free status. Patients lost to follow-up without a diagnosis of recurrence and those who die will be censored at the date of last confirmation of recurrence-free status.

Data collection

Three experienced surgeons will judge the intraoperative identification of hepatic segment boundaries. The entire surgical procedure, including ICG-fluorescence imaging, will be digitally recorded and analysed by an additional expert panel consisting of three highly

experienced surgeons, different from those performing the surgeries, to confirm the identification of hepatic segment boundaries. The success rate of their identification is used as the end point. A flow chart of the study procedure is presented in Figure 1.

Postoperative complications will be graded according to the extended Clavien-Dindo classification of surgical complications, which was published by the Japan Clinical Oncology Group and more precisely described the original criteria of the Clavien-Dindo classification.[26,27]

Follow-up visits will be carried out at two weeks after hospital discharge, and every three months thereafter. Follow-up evaluation will be performed using routine blood tests, including liver function tests, coagulation function tests, serum tumour maker levels depending on the type of liver tumour, abdominal ultrasonography, and abdominal enhanced computed tomography.

Study timeline

Data will be collected from February 2018 until January 2020, and analysis is expected to be completed around January 2022.

Participants will be informed about the study during their preoperative visit to our hospital, and will have ample time to consider participation. Possible complications will be evaluated in the year following the surgery. The schedules of enrolment, interventions, and

assessments are shown in Table 1.

Statistical analysis

The analysis populations will include the following three sets. Firstly, the full analysis set (FAS) will consist of all participants that completed the surgery with navigation by ICG-fluorescence images and have efficacy data available, excluding those without baseline data or significant protocol violations (e.g., absence of informed consent, enrolment outside the contract period). Secondly, the per protocol set (PPS) will consist of the FAS participants completing 1 year of follow-up, excluding those with any significant protocol violations involving the study method, the inclusion criteria, the exclusion criteria, and concomitant therapy. Lastly, the safety analysis set (SAS) will consist of the participants who enrolled in this study and were given at least one dose of ICG.

The analysis will be performed after the data lock following completion of study drug administration to all participants. For all efficacy endpoints, the FAS will be used in the primary analysis, while the PPS will be used in a reference analysis. Safety will be analysed using the SAS. The baseline participant characteristics' distribution and summary statistics will be calculated according to group in each analysis population.

All statistical analyses will be performed as indicated using JMP software, version 13.0.0 (SAS Institute, Inc., Cary, NC, USA).

Interim analyses will not be performed in this study.

Primary outcome

The primary objective of this study is to estimate the success rate, which is defined as the proportion of hepatic segments identified by the ICG-fluorescence imaging system. The point estimate of the rate and the 95% confidence interval (CI) will be calculated.

Secondary outcomes

The point estimate and 95% CI of the success rate of tumour detection by the ICG-fluorescence imaging system will be calculated. For analysis of other secondary outcomes, we will conduct a test using historical data collected at our facility as the control group. No multiplicity adjustment will be performed in the analysis of secondary efficacy endpoints.

Exploratory analysis

We will perform logistic regression analysis regarding the success or failure of identifying liver segments using the ICG fluorescence imaging system. The following factors will be included in the model to evaluate the association between the proportion of successful cases of liver segment identification and clinical variables: age, sex, body mass index, viral infection, Child-Pugh classification, cirrhosis, tumour size, tumour number, tumour location,

type of hepatectomy, liver function indicators (alanine transaminase, albumin, total bilirubin, international normalized ratio, prothrombin time, platelet count), operative time, blood loss, rate of postoperative complications, and recurrence-free time.

Safety analysis

The safety endpoint of this study is the frequency of adverse events. A table will be prepared to summarize the endpoint. For estimation of the rates of adverse events, a two-sided 95% CI will be calculated.

Data monitoring

Monitoring will be performed in order to periodically check whether the study is being conducted safely in accordance with the protocol and whether the data are properly collected. The following items are reviewed every six months: informed consent, obtained and signed; participant retention; study implementation system; study safety and data; and study progress.

Patient and Public involvement

There was no patient and/or public involvement in planning of this study.

ETHICS AND DISSEMINATION

Is there scientific and clinical value in conducting this study?

Whereas the conventional pedicle clamping method can only detect hepatic boundaries from the hepatic surface, the ICG-fluorescence imaging system can detect both the hepatic surface and transection surface during parenchymal resection. We can evaluate the efficacy and safety of hepatectomy using ICG-fluorescence imaging systems by analysing the association between the success rate of identifying hepatic segments and clinical outcomes. This study will help determine whether the boundaries detected by ICG-fluorescence imaging systems during hepatectomy are valid and useful.

The findings obtained through this study will help establish the utility of ICG-fluorescence imaging systems and therefore the study is expected to contribute to the improvement of prognostic outcomes in patients who undergo hepatectomy due to various causes.

Ethical approval

This study was approved by the Kobe University Clinical Research Ethical Committee. Possible protocol amendments will be sent to the Kobe University Clinical Research Ethical Committee.

Consideration of participants' human rights, safety, and disadvantages

The principal investigator and sub-investigators will comply with the principals of the protection of participants' privacy rights. Study personnel will make the utmost of effort to protect the participants' personal information and privacy, and will not divulge any personal information learned from this study without due reasons, even outside working hours. In this study, a list of subject identification codes will be prepared to link the subject source data with the study database or study-related documents. Limited participant information, such as sex and date of birth, may be used to identify participants or verify the list of subject identification codes, within the range of all applicable laws and regulations.

All effort will be taken to ensure than participants will not be personally identifiable from publications arising from this study.

Foreseeable disadvantages (burdens and risks)

The administration of ICG will be the only additional invasive intervention performed in each patient. ICG administration rarely causes anaphylactic reactions (<1:10,000). Patients with terminal renal insufficiency seem to be more prone for such an anaphylactic reaction. The estimated mortality rate due to anaphylactic reaction is reported as <1 per 330,000.[28-31]

To minimize the risk of adverse events and disadvantages that may occur in this study, the inclusion and exclusion criteria have been carefully discussed. All adverse events occurring in this study will be monitored to ensure that they are within the expected range. If

any serious or unexpected adverse events occur, the event will be carefully examined and reviewed, and necessary countermeasures will be taken. Participation in this study may require increased hospital visits, test frequency, and blood sampling volume, compared to routine medical care. In the event of tumour progression, severe organ dysfunction, physical weakening, etc., during the preoperative treatment or during the waiting period for surgical resection, the planned surgical resection may not be possible.

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AUTHOR STATEMENT

H. Gon, S. Komatsu, S. Murakami, M. Kido, M. Tanaka, K. Kuramitsu, D. Tsugawa, M. Awazu, H. Toyama, and T. Fukumoto all made substantial contributions to the conception and design of the study. H. Gon, S. Komatsu, and S. Murakami drafted the manuscript. All authors provided critical review and final approval of the present manuscript.

FUNDING STATEMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

DATA SHARING STATEMENT

This is a research protocol. That means the data for this study are being collected currently. All authors have access to these data, and these data will be published as described in the protocol, coordinated by H. Gon and S. Komatsu.

COMPETING INTERESTS STATEMENT

None declared.

FIGURE LEGENDS

Figure 1. Flowchart of the study procedures. ICG, indocyanine green.

Table 1. Schedule of enrolment, interventions, and assessments.

STUDY PERIOD						
	Within 14 days before registration	Before surgery	Day of surgery	After surgery	Day of discharge	Every 3 months after discharge
ENROLMENT						
Eligibility screen	X					
Informed consent	X					
Background Blood test	X					
INTERVENTIONS						
ICG-fluorescence imaging technique			X			
ASSESSMENTS						
Primary outcome			X	X		
Blood test		X	X	X	X	X
Postoperative complication			X	X	X	
Adverse event			X	X	X	
Abdominal ultrasonography						X
Abdominal enhanced CT						X

CT, computed tomography; ICG, indocyanine green.

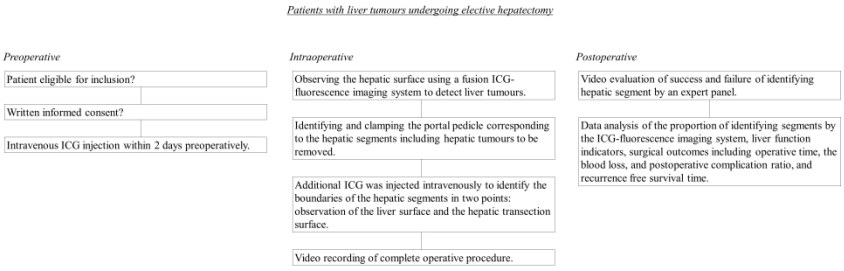

Figure 1. Flowchart of the study procedures.

Figure 1

1200x900mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	#3	Date and version identifier	4
Funding	#4	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	21,22
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-8
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8,9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	16
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10,11

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12,13
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16,17
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14,15
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	16
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant	N/A

		parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12,13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16,17
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	N/A
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

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Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study

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Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study

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ABSTRACT

Introduction: In vivo fluorescence imaging techniques using indocyanine green to identify liver tumours and hepatic segment boundaries have been recently developed. The purpose of this study is to evaluate the efficacy of fusion indocyanine green (ICG)-fluorescence imaging for navigation during hepatectomy.

Methods and analysis: This will be an exploratory single-arm clinical trial; patients with liver tumours will undergo hepatectomy using the ICG-fluorescence imaging system. In total, 110 patients with liver tumours scheduled for elective hepatectomy will be included in this study. Preoperatively, ICG will be intravenously injected at a dose of 0.5 mg/kg body weight within 2 days. To detect liver tumours intraoperatively, the hepatic surface will be initially observed using the ICG-fluorescence imaging system. After identifying and clamping the portal pedicle corresponding to the hepatic segments, including the liver tumours to be resected, additional ICG will be injected intravenously at a dose of 0.5 mg/kg body weight to identify the boundaries of the hepatic segments. The primary outcome measure will be the success or failure of the ICG-fluorescence imaging system in identifying hepatic segments. The secondary outcomes will be the success or failure in identifying liver tumours, liver function indicators, operative time, blood loss, rate of postoperative complications, and recurrence-free survival. The findings obtained through this study are expected to help establish the utility of ICG-fluorescence imaging systems and therefore contribute to

prognostic outcome improvements in patients undergoing hepatectomy for various causes.

Ethics and dissemination: The protocol has been approved by the Kobe University Clinical Research Ethical Committee. The findings of this study will be disseminated widely through peer-reviewed publications and conference presentations.

Trial registration number: This study is registered at the UMIN Clinical Trials Registry: UMIN000031054 and Japan Registry of Clinical Trials: jRCT1051180070. The Registration Data Set is available at https://jrct.niph.go.jp/.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study is expected to address the clinical utility of real-time navigation during hepatectomy using indocyanine green (ICG)-fluorescence imaging systems.
- The efficacy and safety of hepatectomy using ICG-fluorescence imaging systems are expected to be clarified through the analysis of associations between the success rate in identifying hepatic segments and clinical outcomes, including liver function indicators, operative time, blood loss, rate of postoperative complications, and recurrence-free survival.
- This is an exploratory single-arm study, the results of which will be compared against historical data from our facility.

INTRODUCTION

Hepatectomy remains the mainstay of treatment for hepatocellular carcinoma (HCC) and metastatic liver tumours and is commonly performed in patients with preserved liver function.[1-3] Vascular invasion is a poor prognostic factor in HCC, and anatomical resection of the cancer-bearing portal regions is a theoretically effective procedure for the treatment of HCC and metastatic liver tumours complicated by invasion of the Glisson's capsule.[4]

To perform anatomical resection safely and precisely, the liver's anatomical boundaries must be visually recognized. Particularly, the hepatic veins are considered to indicate the absolute boundaries of hepatic segments and can easily be identified by intraoperative ultrasonography. However, due to the three-dimensional shape of the hepatic segment, the hepatic veins are not sufficient for guiding anatomical resection. Under such conditions, intraoperative navigation in hepatectomy allows for the real-time identification of three-dimensional structures, including tumours and hepatic segment boundaries.

Several techniques for identifying hepatic segments have been reported thus far.[5-9] Recently, in vivo fluorescence imaging techniques for the identification of biological structures intraoperatively have been developed. Among the various fluorophores used, indocyanine green (ICG) receives a substantial amount of attention because of its well-known pharmacokinetic and safety profile, making it a potentially valuable clinical tool.[10] For example, it is well known that ICG rapidly and completely binds to plasma proteins - among

which albumin is the principal carrier - following intravenous injection. Also, ICG is excreted in bile in an unconjugated form and is not cleared by extrahepatic mechanisms. Furthermore, single or repeated intravenous injections or infusions rarely cause unfavourable adverse effects. Taking advantage of these characteristics and the development of concomitant fluorescence imaging techniques, ICG-fluorescence imaging systems are widely used for detecting sentinel lymph nodes and arterial blood flow, and their effectiveness has been recognized.[11, 12] Moreover, the potential utility of this approach to identify liver tumours and hepatic segment boundaries, as well as to detect the bile duct tree intraoperatively, has recently been demonstrated.[7, 13-19]

The ICG-fluorescence imaging system was initially introduced for use during open hepatectomy. Similar fluorescence imaging systems have been recently developed for use during laparoscopic hepatobiliary surgery. Several reports have demonstrated the efficacy of such systems during laparoscopic cholecystectomy and hepatectomy.[20] However, whether the hepatic boundaries visualised by ICG-fluorescence imaging systems are clinically precise and useful has not been adequately assessed. For example, there may be minor deviations due to the confluence of communicating vessel branches between hepatic segments; the injected ICG likely passes through the hepatic segments and the tumour to be removed. Evidence regarding the efficacy of ICG-fluorescence imaging systems is not fully established, and further investigation is required.

The purpose of this study is to evaluate the efficacy of the ICG-fluorescence imaging system during hepatectomy for patients with liver tumours by analysing the rate of detection of hepatic boundaries and tumours. In addition, we assess the precision of the detected hepatic boundaries by evaluating the postoperative clinical data.

METHODS AND ANALYSIS

Study design

This prospective study is a single-arm, exploratory clinical trial. Patients with liver tumours will undergo hepatectomy using the ICG-fluorescence imaging system. This study will be performed at Kobe University.

Target population

From 2018 to 2020, patients with liver tumours treated at Kobe University will be enrolled. The inclusion criteria are as follows: male or female patients with liver tumours, aged 20 years and older, scheduled for elective hepatectomy, have preserved liver function, able to understand the nature of the study procedures, and willing to participate and give voluntary written consent. Liver functional reserve will be assessed by serum biochemical data (albumin level, total bilirubin level, and prothrombin time) and ICG retention for 15 minutes (ICG-R15). The patients will be categorized according to the severity of liver disease

based on Child-Pugh stages and the liver damage classification, defined by the Liver Cancer Study Group of Japan.[21, 22] Preserved liver function is defined as ICG-R15 <15% and a Child-Pugh classification of A or B.

The exclusion criteria are as follows: has liver or renal insufficiency, or known ICG hypersensitivity, pregnant or breastfeeding, or unable to understand the nature of the study procedure.

Intervention

ICG is injected intravenously at a dose of 0.5 mg/kg body weight within 2 days preoperatively. Intraoperatively, we will initially observe the hepatic surface using a fusion ICG-fluorescence imaging system (PINPOINT; Stryker, Kalamazoo, MI, US) to detect liver tumours. Among several methods for identifying liver segments with fluorescence imaging, we will use the negative staining technique to identify the liver segments in this study.[23] After identifying and clamping the portal pedicle corresponding to the hepatic segments to be removed, additional ICG is injected intravenously at a dose of 0.5 mg/kg body weight to identify the boundaries of the hepatic segments.[24] Hepatectomy is performed based on the demarcation between fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic segments. The demarcation will also be checked as continuously as possible during parenchymal resection. Parenchymal resection will be performed using an

ultrasonic surgical aspirator (CUSA; Cavitron Lasersonic Corp., Stamford, CT, USA), and a bipolar clamp coagulation system (ERBE, Tubingen, Germany). The fusion ICG-fluorescence images will only be used for the hepatectomy. The Pringle manoeuvre will be performed and a drainage tube will be routinely inserted around the cut surface of the liver parenchyma.

Sample size calculation

The purpose of the primary analysis of this study is to estimate the success rate, which is defined as the proportion of hepatic segments identified by the ICG-fluorescence imaging system during hepatectomy. In order to judge the procedure as useful, a success rate of at least 80% is thought to be required. When the expected success rate is 90% and the two-sided 95% confidence interval width is 0.12, the required number of participants is 98. To allow for an approximately 10% dropout, the target sample size of this study has been set to 110.

Outcome measures

Primary endpoint

The primary endpoint is the success and failure of identifying hepatic segments using the ICG-fluorescence imaging system. We will evaluate the identification of hepatic segments at two points: observation of the liver surface and the hepatic transection surface. We assume that identification is successful when fulfilling the following two criteria:

(1) Hepatic surface

Identification of hepatic segments by the ICG-fluorescence imaging system is considered successful when the demarcation between fluorescing and non-fluorescing areas is consistent with the ischemic demarcation area observed by clamping the portal pedicle.

(2) Hepatic transection surface

Hepatic parenchymal resection is performed based on the demarcation between fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic segments. We divide the time taken to perform parenchymal resection into three equal intervals by reviewing the recorded videos after surgery, and the identification of hepatic segment boundaries is evaluated at each interval. Identification of hepatic segments is considered successful when we can identify the hepatic segments in more than 80% of the transected area during parenchymal resection at more than two intervals.

Secondary endpoints

The secondary endpoints are the success and failure of identifying liver tumours by the ICG-fluorescence imaging system, liver function indicators (alanine transaminase, albumin, total bilirubin, international normalized ratio of prothrombin time, platelet count), the operative time, the blood loss, the rate of postoperative complications, and recurrence-free survival. Successful identification of liver tumours is determined when any isolated

fluorescence signals are detected, also considering liver tumours diagnosed by other modalities, including preoperative imaging and IOUS, and finally confirmed by pathological examination. The fluorescence pattern is considered according to the preoperative diagnosis because liver lesions have differing fluorescence patterns on the basis of their tumour biology.[25] If we identify lesions with isolated fluorescence signal on fusion-fluorescence imaging that were not identified by preoperative imaging, we evaluate the lesions by intraoperative ultrasound sonography, and, if necessary, frozen section biopsies are performed to determine whether additional hepatectomy is required. The recurrence-free survival is analysed for each case of liver tumour, including primary liver cancer and liver metastases. Recurrence-free survival time is defined as the time from enrolment until first recurrence after the surgical intervention. Patients without recurrence will be censored at the date of last confirmation of recurrence-free status. Patients lost to follow-up without a diagnosis of recurrence and those who die will be censored at the date of last confirmation of recurrence-free status.

Data collection

Three experienced surgeons will judge the intraoperative identification of hepatic segment boundaries. The entire surgical procedure, including ICG-fluorescence imaging, will be digitally recorded and analysed by an additional expert panel consisting of three highly

experienced surgeons, different from those performing the surgeries, to confirm the identification of hepatic segment boundaries. The success rate of their identification is used as the end point. A flow chart of the study procedure is presented in Figure 1.

Postoperative complications will be graded according to the extended Clavien-Dindo classification of surgical complications, which was published by the Japan Clinical Oncology Group and more precisely described in the original criteria of the Clavien-Dindo classification.[26, 27]

Follow-up visits will be carried out at two weeks after hospital discharge, and every three months thereafter. Follow-up evaluation will be performed using routine blood tests, including liver function tests, coagulation function tests, serum tumour maker levels depending on the type of liver tumour, abdominal ultrasonography, and abdominal enhanced computed tomography.

Study timeline

Data will be collected from February 2018 to January 2020, and analysis is estimated to be completed by January 2022.

Participants will be informed about the study during their preoperative visit to our hospital, and will have ample time to consider participation. Possible complications will be evaluated in the year following the surgery. The schedules of enrolment, interventions, and

assessments are shown in Table 1.

Statistical analysis

The analysis populations will include the following three sets. Firstly, the full analysis set (FAS) will consist of all participants who completed the surgery with navigation by ICG-fluorescence images and have efficacy data available, excluding those who have missing baseline data or have had significant protocol violations (e.g., absence of informed consent, enrolment outside the contract period). Secondly, the per protocol set (PPS) will consist of the FAS participants completing 1 year of follow-up, excluding those with any significant protocol violations involving the study method, the inclusion criteria, the exclusion criteria, and concomitant therapy. Lastly, the safety analysis set (SAS) will consist of the participants who enrolled in this study and were given at least one dose of ICG.

The analysis will be performed after the data lock following completion of study drug administration to all participants. For all efficacy endpoints, the FAS will be used in the primary analysis, while the PPS will be used in a reference analysis. Safety will be analysed using the SAS. The baseline distribution of participant characteristics and summary statistics will be calculated according to group in each analysis population.

All statistical analyses will be performed as indicated using JMP software, version 13.0.0 (SAS Institute, Inc., Cary, NC, USA).

Interim analyses will not be performed in this study.

Primary outcome

The primary objective of this study is to estimate the success rate, which is defined as the proportion of hepatic segments identified by the ICG-fluorescence imaging system. The point estimate of the rate and the 95% confidence interval (CI) will be calculated.

Secondary outcomes

The point estimate and 95% CI of the success rate of tumour detection by the ICG-fluorescence imaging system will be calculated. For analysis of other secondary outcomes, we will conduct a test using historical data collected at our facility as the control group. No multiplicity adjustment will be performed in the analysis of secondary efficacy endpoints. We will estimate the recurrence-free survival by the Kaplan Meier method. The recurrence-free survival will also be analysed by univariate COX proportional hazard model for each clinical variable. Multivariate Cox proportional hazard models will be adopted to analyse the risk factors of recurrence-free survival. The following variables will be included in the multivariate model: the success or failure of identifying liver segments using the ICG fluorescence imaging system and other variables for which the p-value is under 0.05 in the univariate analysis.

Safety analysis

The safety endpoint of this study is the frequency of adverse events. A table will be prepared to summarize the endpoint. For estimation of the rates of adverse events, a two-sided 95% CI will be calculated.

Data monitoring

Monitoring will be performed in order to periodically check whether the study is being conducted safely in accordance with the protocol and whether the data are properly collected. The following items are reviewed every six months: informed consent, obtained and signed; participant retention; study implementation system; study safety and data; and study progress.

Patient and Public involvement

There was no patient and/or public involvement in planning of this study.

ETHICS AND DISSEMINATION

Is there scientific and clinical value in conducting this study?

Whereas the conventional pedicle clamping method can only detect hepatic boundaries from the hepatic surface, the ICG-fluorescence imaging system can detect both the hepatic

surface and transection surface during parenchymal resection. We can evaluate the efficacy and safety of hepatectomy using ICG-fluorescence imaging systems by analysing the association between the success rate of identifying hepatic segments and clinical outcomes. This study will help to determine whether the boundaries detected by ICG-fluorescence imaging systems during hepatectomy are valid and useful.

The findings obtained through this study will help to establish the utility of ICG-fluorescence imaging systems and therefore the study is expected to contribute to the improvement of prognostic outcomes in patients who undergo hepatectomy due to various causes.

Ethical approval

This study was approved by the Kobe University Clinical Research Ethical Committee. Possible protocol amendments will be sent to the Kobe University Clinical Research Ethical Committee.

Consideration of participants' human rights, safety, and disadvantages

The principal investigator and sub-investigators will comply with the principals of the protection of participants' privacy rights. Study personnel will make the utmost effort to protect the participants' personal information and privacy, and will not divulge any personal

information learned from this study without due reasons, even outside working hours. In this study, a list of subject identification codes will be prepared to link the subject source data with the study database or study-related documents. Limited participant information, such as sex and date of birth, may be used to identify participants or verify the list of subject identification codes, within the range of all applicable laws and regulations.

All effort will be taken to ensure that participants will not be personally identifiable from publications arising from this study.

Foreseeable disadvantages (burdens and risks)

The administration of ICG will be the only additional invasive intervention performed in each patient. ICG administration rarely causes anaphylactic reactions (<1:10,000). Patients with terminal renal insufficiency seem to be more prone to such an anaphylactic reaction. The estimated mortality rate due to anaphylactic reaction is reported as <1 per 330,000.[28-31]

To minimize the risk of adverse events and disadvantages that may occur in this study, the inclusion and exclusion criteria have been carefully discussed. All adverse events occurring in this study will be monitored to ensure that they are within the expected range. If any serious or unexpected adverse events occur, the event will be carefully examined and reviewed, and necessary countermeasures will be taken. Participation in this study may require increased hospital visits, test frequency, and blood sampling volume, compared to

routine medical care. In the event of tumour progression, severe organ dysfunction, physical weakening, etc., during the preoperative treatment or during the waiting period for surgical resection, the planned surgical resection may not be possible.

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AUTHOR STATEMENT

H. Gon, S. Komatsu, S. Murakami, M. Kido, M. Tanaka, K. Kuramitsu, D. Tsugawa, M. Awazu, H. Toyama, and T. Fukumoto all made substantial contributions to the conception and design of the study. H. Gon, S. Komatsu, and S. Murakami drafted the manuscript. All authors provided critical review and final approval of the present manuscript.

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This research received no specific grant from any funding agency in the public,

commercial, or not-for-profit sectors.

DATA SHARING STATEMENT

This is a research protocol. That means the data for this study are being collected currently. All authors have access to these data, and these data will be published as described in the protocol, coordinated by H. Gon and S. Komatsu.

COMPETING INTERESTS STATEMENT

None declared.

FIGURE LEGENDS

Figure 1. Flowchart of the study procedures. ICG, indocyanine green.

Table 1. Schedule of enrolment, interventions, and assessments.

STUDY PERIOD						
	Within 14 days before registration	Before surgery	Day of surgery	After surgery	Day of discharge	Every 3 months after discharge
ENROLMENT						
Eligibility screen	X					
Informed consent	X					
Background Blood test	X					
INTERVENTIONS						
ICG-fluorescence imaging technique			X			
ASSESSMENTS						
Primary outcome			X	X		
Blood test		X	X	X	X	X
Postoperative complication			X	X	X	
Adverse event			X	X	X	
Abdominal ultrasonography						X
Abdominal enhanced CT						X

CT, computed tomography; ICG, indocyanine green.

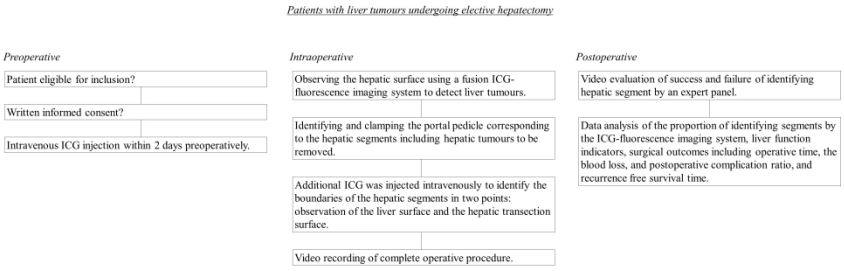

Figure 1. Flowchart of the study procedures.

Figure 1

1200x900mm (96 x 96 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	#3	Date and version identifier	4
Funding	#4	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	21,22
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-8
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8,9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	16
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10,11

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12,13
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16,17
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14,15
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	16
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant	N/A

		parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12,13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16,17
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	N/A
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study

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Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study

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ABSTRACT

Introduction: In vivo fluorescence imaging techniques using indocyanine green to identify liver tumours and hepatic segment boundaries have been recently developed. The purpose of this study is to evaluate the efficacy of fusion indocyanine green (ICG)-fluorescence imaging for navigation during hepatectomy.

Methods and analysis: This will be an exploratory single-arm clinical trial; patients with liver tumours will undergo hepatectomy using the ICG-fluorescence imaging system. In total, 110 patients with liver tumours scheduled for elective hepatectomy will be included in this study. Preoperatively, ICG will be intravenously injected at a dose of 0.5 mg/kg body weight within 2 days. To detect liver tumours intraoperatively, the hepatic surface will be initially observed using the ICG-fluorescence imaging system. After identifying and clamping the portal pedicle corresponding to the hepatic segments, including the liver tumours to be resected, additional ICG will be injected intravenously at a dose of 0.5 mg/kg body weight to identify the boundaries of the hepatic segments. The primary outcome measure will be the success or failure of the ICG-fluorescence imaging system in identifying hepatic segments. The secondary outcomes will be the success or failure in identifying liver tumours, liver function indicators, operative time, blood loss, rate of postoperative complications, and recurrence-free survival. The findings obtained through this study are expected to help establish the utility of ICG-fluorescence imaging systems and therefore contribute to

prognostic outcome improvements in patients undergoing hepatectomy for various causes.

Ethics and dissemination: The protocol has been approved by the Kobe University Clinical Research Ethical Committee. The findings of this study will be disseminated widely through peer-reviewed publications and conference presentations.

Trial registration number: This study is registered at the UMIN Clinical Trials Registry: UMIN000031054 and Japan Registry of Clinical Trials: jRCT1051180070. The Registration Data Set is available at https://jrct.niph.go.jp/.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study is expected to address the clinical utility of real-time navigation during hepatectomy using indocyanine green (ICG)-fluorescence imaging systems.
- The efficacy and safety of hepatectomy using ICG-fluorescence imaging systems are expected to be clarified through the analysis of associations between the success rate in identifying hepatic segments and clinical outcomes, including liver function indicators, operative time, blood loss, rate of postoperative complications, and recurrence-free survival.
- This is an exploratory single-arm study, the results of which will be compared against historical data from our facility.

INTRODUCTION

Hepatectomy remains the mainstay of treatment for hepatocellular carcinoma (HCC) and metastatic liver tumours and is commonly performed in patients with preserved liver function.[1-3] Vascular invasion is a poor prognostic factor in HCC, and anatomical resection of the cancer-bearing portal regions is a theoretically effective procedure for the treatment of HCC and metastatic liver tumours complicated by invasion of the Glisson's capsule.[4]

To perform anatomical resection safely and precisely, the liver's anatomical boundaries must be visually recognized. Particularly, the hepatic veins are considered to indicate the absolute boundaries of hepatic segments and can easily be identified by intraoperative ultrasonography. However, due to the three-dimensional shape of the hepatic segment, the hepatic veins are not sufficient for guiding anatomical resection. Under such conditions, intraoperative navigation in hepatectomy allows for the real-time identification of three-dimensional structures, including tumours and hepatic segment boundaries.

Several techniques for identifying hepatic segments have been reported thus far.[5-9] Recently, in vivo fluorescence imaging techniques for the identification of biological structures intraoperatively have been developed. Among the various fluorophores used, indocyanine green (ICG) receives a substantial amount of attention because of its well-known pharmacokinetic and safety profile, making it a potentially valuable clinical tool.[10] For example, it is well known that ICG rapidly and completely binds to plasma proteins - among

which albumin is the principal carrier - following intravenous injection. Also, ICG is excreted in bile in an unconjugated form and is not cleared by extrahepatic mechanisms. Furthermore, single or repeated intravenous injections or infusions rarely cause unfavourable adverse effects. Taking advantage of these characteristics and the development of concomitant fluorescence imaging techniques, ICG-fluorescence imaging systems are widely used for detecting sentinel lymph nodes and arterial blood flow, and their effectiveness has been recognized.[11, 12] Moreover, the potential utility of this approach to identify liver tumours and hepatic segment boundaries, as well as to detect the bile duct tree intraoperatively, has recently been demonstrated.[7, 13-19]

The ICG-fluorescence imaging system was initially introduced for use during open hepatectomy. Similar fluorescence imaging systems have been recently developed for use during laparoscopic hepatobiliary surgery. Several reports have demonstrated the efficacy of such systems during laparoscopic cholecystectomy and hepatectomy.[20] However, whether the hepatic boundaries visualised by ICG-fluorescence imaging systems are clinically precise and useful has not been adequately assessed. For example, there may be minor deviations due to the confluence of communicating vessel branches between hepatic segments; the injected ICG likely passes through the hepatic segments and the tumour to be removed. Evidence regarding the efficacy of ICG-fluorescence imaging systems is not fully established, and further investigation is required.

The purpose of this study is to evaluate the efficacy of the ICG-fluorescence imaging system during hepatectomy for patients with liver tumours by analysing the rate of detection of hepatic boundaries and tumours. In addition, we assess the precision of the detected hepatic boundaries by evaluating the postoperative clinical data.

METHODS AND ANALYSIS

Study design

This prospective study is a single-arm, exploratory clinical trial. Patients with liver tumours will undergo hepatectomy using the ICG-fluorescence imaging system. This study will be performed at Kobe University.

Target population

From 2018 to 2020, patients with liver tumours treated at Kobe University will be enrolled. The inclusion criteria are as follows: male or female patients with liver tumours, aged 20 years and older, scheduled for elective hepatectomy, have preserved liver function, able to understand the nature of the study procedures, and willing to participate and give voluntary written consent. Liver functional reserve will be assessed by serum biochemical data (albumin level, total bilirubin level, and prothrombin time) and ICG retention for 15 minutes (ICG-R15). The patients will be categorized according to the severity of liver disease

based on Child-Pugh stages and the liver damage classification, defined by the Liver Cancer Study Group of Japan.[21, 22] Preserved liver function is defined as ICG-R15 <15% and a Child-Pugh classification of A or B.

The exclusion criteria are as follows: has liver or renal insufficiency, or known ICG hypersensitivity, pregnant or breastfeeding, or unable to understand the nature of the study procedure.

Intervention

ICG is injected intravenously at a dose of 0.5 mg/kg body weight within 2 days preoperatively. Intraoperatively, we will initially observe the hepatic surface using a fusion ICG-fluorescence imaging system (PINPOINT; Stryker, Kalamazoo, MI, US) to detect liver tumours. Among several methods for identifying liver segments with fluorescence imaging, we will use the negative staining technique to identify the liver segments in this study.[23] After identifying and clamping the portal pedicle corresponding to the hepatic segments to be removed, additional ICG is injected intravenously at a dose of 0.5 mg/kg body weight to identify the boundaries of the hepatic segments.[24] Hepatectomy is performed based on the demarcation between fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic segments. The demarcation will also be checked as continuously as possible during parenchymal resection. Parenchymal resection will be performed using an

ultrasonic surgical aspirator (CUSA; Cavitron Lasersonic Corp., Stamford, CT, USA), and a bipolar clamp coagulation system (ERBE, Tubingen, Germany). The fusion ICG-fluorescence images will only be used for the hepatectomy. The Pringle manoeuvre will be performed and a drainage tube will be routinely inserted around the cut surface of the liver parenchyma.

Sample size calculation

The purpose of the primary analysis of this study is to estimate the success rate, which is defined as the proportion of hepatic segments identified by the ICG-fluorescence imaging system during hepatectomy. In order to judge the procedure as useful, a success rate of at least 80% is thought to be required. When the expected success rate is 90% and the two-sided 95% confidence interval width is 0.12, the required number of participants is 98. To allow for an approximately 10% dropout, the target sample size of this study has been set to 110.

Outcome measures

Primary endpoint

The primary endpoint is the success and failure of identifying hepatic segments using the ICG-fluorescence imaging system. We will evaluate the identification of hepatic segments at two points: observation of the liver surface and the hepatic transection surface. We assume that identification is successful when fulfilling the following two criteria:

(1) Hepatic surface

Identification of hepatic segments by the ICG-fluorescence imaging system is considered successful when the demarcation between fluorescing and non-fluorescing areas is consistent with the ischemic demarcation area observed by clamping the portal pedicle.

(2) Hepatic transection surface

Hepatic parenchymal resection is performed based on the demarcation between fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic segments. We divide the time taken to perform parenchymal resection into three equal intervals by reviewing the recorded videos after surgery, and the identification of hepatic segment boundaries is evaluated at each interval. Identification of hepatic segments is considered successful when we can identify the hepatic segments in more than 80% of the transected area during parenchymal resection at more than two intervals.

Secondary endpoints

The secondary endpoints are the success and failure of identifying liver tumours by the ICG-fluorescence imaging system, liver function indicators (alanine transaminase, albumin, total bilirubin, international normalized ratio of prothrombin time, platelet count), the operative time, the blood loss, the rate of postoperative complications, and recurrence-free survival. Successful identification of liver tumours is determined when any isolated

fluorescence signals are detected, also considering liver tumours diagnosed by other modalities, including preoperative imaging and IOUS, and finally confirmed by pathological examination. The fluorescence pattern is considered according to the preoperative diagnosis because liver lesions have differing fluorescence patterns on the basis of their tumour biology.[25] If we identify lesions with isolated fluorescence signal on fusion-fluorescence imaging that were not identified by preoperative imaging, we evaluate the lesions by intraoperative ultrasound sonography, and, if necessary, frozen section biopsies are performed to determine whether additional hepatectomy is required. The recurrence-free survival is analysed for each case of liver tumour, including primary liver cancer and liver metastases. Recurrence-free survival time is defined as the time from enrolment until first recurrence after the surgical intervention. Patients without recurrence will be censored at the date of last confirmation of recurrence-free status. Patients lost to follow-up without a diagnosis of recurrence and those who die will be censored at the date of last confirmation of recurrence-free status.

Data collection

Three experienced surgeons will judge the intraoperative identification of hepatic segment boundaries. The entire surgical procedure, including ICG-fluorescence imaging, will be digitally recorded and analysed by an additional expert panel consisting of three highly

experienced surgeons, different from those performing the surgeries, to confirm the identification of hepatic segment boundaries. When we perform an open hepatectomy, the video will be captured by another surgeon using the scope of a fusion ICG-fluorescence imaging system. When we perform a laparoscopic hepatectomy, the ICG-fluorescence images can be accessed through the laparoscope. The success rate of their identification is used as the end point. A flow chart of the study procedure is presented in Figure 1.

Postoperative complications will be graded according to the extended Clavien-Dindo classification of surgical complications, which was published by the Japan Clinical Oncology Group and more precisely described in the original criteria of the Clavien-Dindo classification.[26, 27]

Follow-up visits will be carried out at two weeks after hospital discharge, and every three months thereafter. Follow-up evaluation will be performed using routine blood tests, including liver function tests, coagulation function tests, serum tumour maker levels depending on the type of liver tumour, abdominal ultrasonography, and abdominal enhanced computed tomography.

Study timeline

Data will be collected from February 2018 to January 2020, and analysis is estimated to be completed by January 2022.

Participants will be informed about the study during their preoperative visit to our hospital, and will have ample time to consider participation. Possible complications will be evaluated in the year following the surgery. The schedules of enrolment, interventions, and assessments are shown in Table 1.

Statistical analysis

The analysis populations will include the following three sets. Firstly, the full analysis set (FAS) will consist of all participants who completed the surgery with navigation by ICG-fluorescence images and have efficacy data available, excluding those who have missing baseline data or have had significant protocol violations (e.g., absence of informed consent, enrolment outside the contract period). Secondly, the per protocol set (PPS) will consist of the FAS participants completing 1 year of follow-up, excluding those with any significant protocol violations involving the study method, the inclusion criteria, the exclusion criteria, and concomitant therapy. Lastly, the safety analysis set (SAS) will consist of the participants who enrolled in this study and were given at least one dose of ICG.

The analysis will be performed after the data lock following completion of study drug administration to all participants. For all efficacy endpoints, the FAS will be used in the primary analysis, while the PPS will be used in a reference analysis. Safety will be analysed using the SAS. The baseline distribution of participant characteristics and summary statistics

will be calculated according to group in each analysis population.

All statistical analyses will be performed as indicated using JMP software, version 13.0.0 (SAS Institute, Inc., Cary, NC, USA).

Interim analyses will not be performed in this study.

Primary outcome

The primary objective of this study is to estimate the success rate, which is defined as the proportion of hepatic segments identified by the ICG-fluorescence imaging system. The point estimate of the rate and the 95% confidence interval (CI) will be calculated.

Secondary outcomes

The point estimate and 95% CI of the success rate of tumour detection by the ICG-fluorescence imaging system will be calculated. For analysis of other secondary outcomes, we will conduct a test using historical data collected at our facility as the control group. No multiplicity adjustment will be performed in the analysis of secondary efficacy endpoints. We will estimate the recurrence-free survival by the Kaplan Meier method. The recurrence-free survival will also be analysed by univariate COX proportional hazard model for each clinical variable. Multivariate Cox proportional hazard models will be adopted to analyse the risk factors of recurrence-free survival. The following variables will be included in the

multivariate model: the success or failure of identifying liver segments using the ICG fluorescence imaging system and other variables for which the p-value is under 0.05 in the univariate analysis.

Safety analysis

The safety endpoint of this study is the frequency of adverse events. A table will be prepared to summarize the endpoint. For estimation of the rates of adverse events, a two-sided 95% CI will be calculated.

Data monitoring

Monitoring will be performed in order to periodically check whether the study is being conducted safely in accordance with the protocol and whether the data are properly collected. The following items are reviewed every six months: informed consent, obtained and signed; participant retention; study implementation system; study safety and data; and study progress.

Patient and Public involvement

There was no patient and/or public involvement in planning of this study.

ETHICS AND DISSEMINATION

Is there scientific and clinical value in conducting this study?

Whereas the conventional pedicle clamping method can only detect hepatic boundaries from the hepatic surface, the ICG-fluorescence imaging system can detect both the hepatic surface and transection surface during parenchymal resection. We can evaluate the efficacy and safety of hepatectomy using ICG-fluorescence imaging systems by analysing the association between the success rate of identifying hepatic segments and clinical outcomes. This study will help to determine whether the boundaries detected by ICG-fluorescence imaging systems during hepatectomy are valid and useful.

The findings obtained through this study will help to establish the utility of ICG-fluorescence imaging systems and therefore the study is expected to contribute to the improvement of prognostic outcomes in patients who undergo hepatectomy due to various causes.

Ethical approval

This study was approved by the Kobe University Clinical Research Ethical Committee. Possible protocol amendments will be sent to the Kobe University Clinical Research Ethical Committee.

Consideration of participants' human rights, safety, and disadvantages

The principal investigator and sub-investigators will comply with the principals of the protection of participants' privacy rights. Study personnel will make the utmost effort to protect the participants' personal information and privacy, and will not divulge any personal information learned from this study without due reasons, even outside working hours. In this study, a list of subject identification codes will be prepared to link the subject source data with the study database or study-related documents. Limited participant information, such as sex and date of birth, may be used to identify participants or verify the list of subject identification codes, within the range of all applicable laws and regulations.

All effort will be taken to ensure that participants will not be personally identifiable from publications arising from this study.

Foreseeable disadvantages (burdens and risks)

The administration of ICG will be the only additional invasive intervention performed in each patient. ICG administration rarely causes anaphylactic reactions (<1:10,000). Patients with terminal renal insufficiency seem to be more prone to such an anaphylactic reaction. The estimated mortality rate due to anaphylactic reaction is reported as <1 per 330,000.[28-31]

To minimize the risk of adverse events and disadvantages that may occur in this study, the inclusion and exclusion criteria have been carefully discussed. All adverse events occurring in this study will be monitored to ensure that they are within the expected range. If

any serious or unexpected adverse events occur, the event will be carefully examined and reviewed, and necessary countermeasures will be taken. Participation in this study may require increased hospital visits, test frequency, and blood sampling volume, compared to routine medical care. In the event of tumour progression, severe organ dysfunction, physical weakening, etc., during the preoperative treatment or during the waiting period for surgical resection, the planned surgical resection may not be possible.

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AUTHOR STATEMENT

H. Gon, S. Komatsu, S. Murakami, M. Kido, M. Tanaka, K. Kuramitsu, D. Tsugawa, M. Awazu, H. Toyama, and T. Fukumoto all made substantial contributions to the conception and design of the study. H. Gon, S. Komatsu, and S. Murakami drafted the manuscript. All authors provided critical review and final approval of the present manuscript.

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This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

DATA SHARING STATEMENT

This is a research protocol. That means the data for this study are being collected currently. All authors have access to these data, and these data will be published as described in the protocol, coordinated by H. Gon and S. Komatsu.

COMPETING INTERESTS STATEMENT

None declared.

FIGURE LEGENDS

Figure 1. Flowchart of the study procedures. ICG, indocyanine green.

Table 1. Schedule of enrolment, interventions, and assessments.

STUDY PERIOD						
	Within 14 days before registration	Before surgery	Day of surgery	After surgery	Day of discharge	Every 3 months after discharge
ENROLMENT						
Eligibility screen	X					
Informed consent	X					
Background Blood test	X					
INTERVENTIONS						
ICG-fluorescence imaging technique			X			
ASSESSMENTS						
Primary outcome			X	X		
Blood test		X	X	X	X	X
Postoperative complication			X	X	X	
Adverse event			X	X	X	
Abdominal ultrasonography						X
Abdominal enhanced CT						X

CT, computed tomography; ICG, indocyanine green.

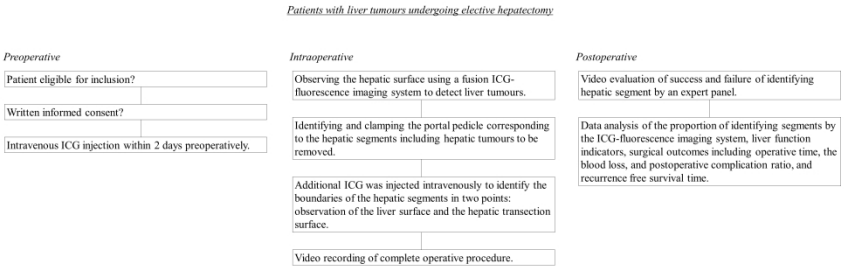

Figure 1. Flowchart of the study procedures.

Figure 1

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	#3	Date and version identifier	4
Funding	#4	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	21,22
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-8
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8,9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	16
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10,11

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12,13
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16,17
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	14,15
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	15
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	16
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant	N/A

		parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12,13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16,17
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	22
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	N/A
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A