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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

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Manuscripts

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4 **Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence**
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7 **imaging: protocol for a prospective cohort study**
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For peer review only

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

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4 therefore contribute to prognostic outcome improvements in patients who will undergo
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7 hepatectomy for various causes.
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10 **Ethics and dissemination:** The protocol has been approved by the Kobe University Clinical
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13 Research Ethical Committee. The findings of this study will be disseminated widely through
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16 peer-reviewed publications and conference presentations.
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19 **Trial registration number:** This study is registered at the UMIN Clinical Trials Registry:
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22 UMIN0000180139 and Japan Registry of Clinical Trials: jRCT1051180070. The Registration
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25 Data Set is available at <https://jrct.niph.go.jp/>.
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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

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4 which albumin is the principal carrier—following intravenous injection. Also, ICG is excreted
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7 in bile in an unconjugated form and is not cleared by extrahepatic mechanisms. Furthermore,
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10 single or repeated intravenous injections or infusions rarely cause unfavourable adverse
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13 effects. Taking advantage of these characteristics and development of concomitant
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16 fluorescence imaging techniques, ICG-fluorescence imaging systems are widely used for
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19 detecting sentinel lymph nodes and arterial blood flow, and their effectiveness has been
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22 recognized.^{11,12} Also, the potential utility of this approach to identify liver tumours and
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25 hepatic segment boundaries, as well as to detect intraoperative bile leakage has recently been
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27
28 demonstrated.^{7,13-19}

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

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

21 **Study design**

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

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40 From 2018 to 2021, patients with liver tumours treated at Kobe University will be
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43 enrolled. The inclusion criteria are as follows: male or female patients with liver tumours,
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46 aged 20 years and older, scheduled for elective hepatectomy, preserved liver function, ability
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49 to understand the nature of the study procedures, and willingness to participate and give
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52 voluntary written consent. Liver functional reserve will be assessed by serum biochemical
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55 data (albumin level, total bilirubin level, and prothrombin time) and ICG retention for 15
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58 minutes (ICG-R15). The patients will be categorized according to the severity of liver disease
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4 based on Child-Pugh stages and the liver damage classification, defined by the LCSGJ.^{21,22}

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7 Preserved liver function is defined as ICG-R15 <15% and Child-Pugh classification A or B.

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

21 22 **Intervention**

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

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13 The purpose of the primary analysis of this study is to estimate the success rate, which is
14 defined as the proportion of identifying hepatic segments by the ICG-fluorescence imaging
15 system during hepatectomy. In order to judge the procedure as useful, a success rate of at least
16 80% is thought to be required. When the expected success rate is 90% and the two-sided 95%
17 confidence interval width is 0.12, the required number of participants is 98. To allow for an
18 approximately 10% dropout, the target sample size of this study has been set to 110.
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34 **Outcome measures**

35 *Primary endpoint*

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

52 Identification of hepatic segments by the ICG-fluorescence imaging system is considered
53 successful when the demarcation between fluorescing and non-fluorescing areas is consistent
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4 with the ischemic demarcation area observed by clamping the portal pedicle.
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7 (2) Hepatic transection surface
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10 Hepatic parenchymal resection is performed based on the demarcation between
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12 fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic
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14 segments. We divide the time taken to perform parenchymal resection into three equal
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16 intervals. We divide the time taken to perform parenchymal resection into three equal
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18 intervals, and the identification of hepatic segment boundaries is evaluated at each interval.
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20 Identification of hepatic segments is considered successful when we can identify the hepatic
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22 segments at more than two intervals.
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31 *Secondary endpoints*
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34 The secondary endpoints are the success and failure of identifying liver tumours by the
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36 ICG-fluorescence imaging system, liver function indicators (alanine transaminase, albumin,
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38 total bilirubin, international normalized ratio of prothrombin time, platelet count), the
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40 operative time, the blood loss, the rate of postoperative complications, and recurrence-free
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42 survival. Recurrence-free survival time is defined as the time from enrolment until first
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44 recurrence after the surgical intervention. Patients without recurrence will be censored at the
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46 date of last confirmation of recurrence-free status. Patients lost to follow-up without a
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48 diagnosis of recurrence and those who die will be censored at the date of last confirmation of
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50 recurrence-free status.
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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

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4 completed around January 2023.
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7 Participants will be informed about the study during their preoperative visit to our
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9 hospital, and will have ample time to consider participation. Possible complications will be
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11 evaluated in the year following the surgery. The schedules of enrolment, interventions, and
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13 assessments are shown in Table 1.
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18 19 20 21 22 **Statistical analysis** 23

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25 The analysis populations will include the following three sets. Firstly, the full analysis
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27 set (FAS) will consist of all participants that completed the surgery with navigation by ICG-
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29 fluorescence images and have efficacy data available, excluding those without baseline data
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31 or significant protocol violations (e.g., absence of informed consent, enrolment outside the
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33 contract period). Secondly, the per protocol set (PPS) will consist of the FAS participants
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35 completed 1 year of follow-up, excluding those with any of the following significant
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37 protocol violations involving the study method, the inclusion criteria, the exclusion criteria
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39 and concomitant therapy. Lastly, the safety analysis set (SAS) will consist of the participants
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41 who enrolled in this study and were given at least one dose of ICG.
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52 The analysis will be performed after the data lock following completion of study drug
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54 administration to all participants. For all efficacy endpoints, the FAS will be used in the
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56 primary analysis, while the PPS will be used in a reference analysis. Safety will be analysed
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4 using the SAS. The baseline participant characteristics' distribution and summary statistics
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7 will be calculated according to group in each analysis population.
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10 All statistical analyses will be performed as indicated using JMP software, version
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13 13.0.0 (SAS Institute, Inc., Cary, NC, USA).
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16 Interim analyses will not be performed in this study.
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22 *Primary outcome*

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

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

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58 We will perform logistic regression analysis of the success or failure of the ICG
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4 fluorescence imaging system. The following factors will be included in the model: age,
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7 gender, body mass index, viral infection, Child-Pugh classification, cirrhosis, tumour size,
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10 tumour number, tumour location, type of hepatectomy, liver function indicators (alanine
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13 transaminase, albumin, total bilirubin, international normalized ratio and prothrombin time,
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16 platelet count), operative time, blood loss, rate of postoperative complications, and
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19 recurrence-free time.
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25 *Safety analysis*

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28 The safety endpoint of this study is the frequency of adverse events. A table will be
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30 prepared to summarize the endpoint. For estimation of the rates of adverse events, a two-sided
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34 95% CI will be calculated.
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40 **Data monitoring**

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

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4 There were no patient and public involvement in planning of this study.
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10 **ETHICS AND DISSEMINATION**

11 12 13 **Is there scientific and clinical value in conducting this study?**

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16 We can evaluate the efficacy and safety of hepatectomy using ICG-fluorescence imaging
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18 systems by analysing the association between the success rate of identifying hepatic segments
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20 and clinical outcomes. This study will help determine whether the boundaries detected by
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22 ICG-fluorescence imaging systems during hepatectomy are valid and useful.
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28 The findings obtained through this study will help establish the utility of ICG-
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30 fluorescence imaging systems and therefore the study is expected to contribute to the
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32 improvement of prognostic outcomes in patients who undergo hepatectomy due to various
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34 causes.
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43 **Ethical approval**

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46 This study was approved by the Kobe University Clinical Research Ethical Committee.
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49 Possible protocol amendments will be sent to the Kobe University Clinical Research Ethical
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52 Committee.
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58 **Consideration of participants' human rights, safety, and disadvantages**

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4 The principal investigator and sub-investigators will comply with the principals of the
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7 protection of participants' privacy rights. Study personnel will make the utmost of effort to
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10 protect the participants' personal information and privacy, and will not divulge any personal
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13 information learned from this study without due reasons, even outside working hours. In this
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15
16 study, a list of subject identification codes will be prepared to link the subject source data
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19 with the study database or study-related documents. Limited participant information, such as
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21
22 sex and date of birth, may be used to identify participants or verify the list of subject
23
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25 identification codes, within the range of all applicable laws and regulations.
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28 All effort will be taken to ensure than participants will not be personally identifiable
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31 from publications arising from this study.
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37 **Foreseeable disadvantages (burdens and risks)**

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40 The administration of ICG will be the only additional invasive intervention performed in
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43 each patient. ICG administration rarely causes anaphylactic reactions (<1:10,000). Patients
44
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46 with terminal renal insufficiency seem to be more prone for such an anaphylactic reaction.
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49 The estimated mortality rate due to anaphylactic reaction is reported as <1 per 330,000.²⁵⁻²⁸
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52 To minimize the risk of adverse events and disadvantages that may occur in this study,
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55 the inclusion and exclusion criteria have been carefully discussed. All adverse events
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58 occurring in this study will be monitored to ensure that they are within the expected range. If
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4 any serious or unexpected adverse events occur, the event will be carefully examined and
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7 reviewed, and necessary countermeasures will be taken. Participation in this study may
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10 require increased hospital visits, test frequency, and blood sampling volume, compared to
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13 routine medical care. In the event of tumour progression, severe organ dysfunction, physical
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16 weakening, etc., during the preoperative treatment or during the waiting period for surgical
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19 resection, the planned surgical resection may not be possible.
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10 2016;6:e011668.
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16 **AUTHOR STATEMENT**

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19 H. Gon, S. Komatsu, S. Murakami, M. Kido, M. Tanaka, K. Kuramitsu, M. Awazu, and
20
21
22 T. Fukumoto all made substantial contributions to the conception and design of the study. H.
23
24
25 Gon, S. Komatsu, and S. Murakami drafted the manuscript. All authors provided critical
26
27
28 review and final approval of the present manuscript.
29
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33

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36
37 This research received no specific grant from any funding agency in the public,
38
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40 commercial or not-for-profit sectors.
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46 **DATA SHARING STATEMENT**

47
48
49 This is a research protocol. That means the data for this study are being retrieved at this
50
51
52 moment. All authors have access to these data, and these data will be published as described
53
54
55 in the protocol, coordinated by H. Gon and S. Komatsu.
56
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4 **COMPETING INTERESTS STATEMENT**
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7 None declared.
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13 **FIGURE LEGENDS**
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16 **Figure 1.** Flowchart of the study procedures. ICG, indocyanine green.
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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.

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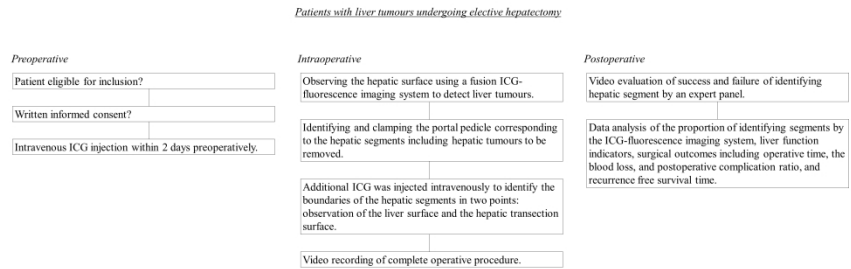


Figure 1. Flowchart of the study procedures.

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	<u>#1</u>	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	<u>#2a</u>	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	<u>#2b</u>	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	<u>#3</u>	Date and version identifier	4
Funding	<u>#4</u>	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	<u>#5a</u>	Names, affiliations, and roles of protocol contributors	21,22
Roles and responsibilities: sponsor contact information	<u>#5b</u>	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	<u>#5c</u>	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	<u>#5d</u>	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

1	Background and	<u>#6a</u>	Description of research question and justification for undertaking	6-8
2	rationale		the trial, including summary of relevant studies (published and	
3			unpublished) examining benefits and harms for each intervention	
4				
5				
6	Background and	<u>#6b</u>	Explanation for choice of comparators	N/A
7	rationale: choice of			
8	comparators			
9				
10				
11	Objectives	<u>#7</u>	Specific objectives or hypotheses	8
12				
13				
14	Trial design	<u>#8</u>	Description of trial design including type of trial (eg, parallel	8
15			group, crossover, factorial, single group), allocation ratio, and	
16			framework (eg, superiority, equivalence, non-inferiority,	
17			exploratory)	
18				
19				
20				
21	Study setting	<u>#9</u>	Description of study settings (eg, community clinic, academic	8
22			hospital) and list of countries where data will be collected.	
23			Reference to where list of study sites can be obtained	
24				
25				
26	Eligibility criteria	<u>#10</u>	Inclusion and exclusion criteria for participants. If applicable,	8,9
27			eligibility criteria for study centres and individuals who will	
28			perform the interventions (eg, surgeons, psychotherapists)	
29				
30				
31	Interventions:	<u>#11a</u>	Interventions for each group with sufficient detail to allow	9
32	description		replication, including how and when they will be administered	
33				
34				
35	Interventions:	<u>#11b</u>	Criteria for discontinuing or modifying allocated interventions for	16
36	modifications		a given trial participant (eg, drug dose change in response to	
37			harms, participant request, or improving / worsening disease)	
38				
39				
40	Interventions:	<u>#11c</u>	Strategies to improve adherence to intervention protocols, and any	N/A
41	adherence		procedures for monitoring adherence (eg, drug tablet return;	
42			laboratory tests)	
43				
44				
45	Interventions:	<u>#11d</u>	Relevant concomitant care and interventions that are permitted or	N/A
46	concomitant care		prohibited during the trial	
47				
48				
49	Outcomes	<u>#12</u>	Primary, secondary, and other outcomes, including the specific	10,11
50			measurement variable (eg, systolic blood pressure), analysis metric	
51			(eg, change from baseline, final value, time to event), method of	
52			aggregation (eg, median, proportion), and time point for each	
53			outcome. Explanation of the clinical relevance of chosen efficacy	
54			and harm outcomes is strongly recommended	
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1	Participant timeline	<u>#13</u>	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
2				
3				
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6	Sample size	<u>#14</u>	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
7				
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11	Recruitment	<u>#15</u>	Strategies for achieving adequate participant enrolment to reach target sample size	10
12				
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15	Allocation: sequence generation	<u>#16a</u>	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
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25	Allocation concealment mechanism	<u>#16b</u>	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
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32	Allocation: implementation	<u>#16c</u>	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
33				
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35				
36	Blinding (masking)	<u>#17a</u>	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
37				
38				
39				
40				
41	Blinding (masking): emergency unblinding	<u>#17b</u>	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
42				
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46	Data collection plan	<u>#18a</u>	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
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1	Data collection plan:	<u>#18b</u>	Plans to promote participant retention and complete follow-up,	N/A
2	retention		including list of any outcome data to be collected for participants	
3			who discontinue or deviate from intervention protocols	
4				
5				
6	Data management	<u>#19</u>	Plans for data entry, coding, security, and storage, including any	16,17
7			related processes to promote data quality (eg, double data entry;	
8			range checks for data values). Reference to where details of data	
9			management procedures can be found, if not in the protocol	
10				
11				
12				
13	Statistics: outcomes	<u>#20a</u>	Statistical methods for analysing primary and secondary outcomes.	13-15
14			Reference to where other details of the statistical analysis plan can	
15			be found, if not in the protocol	
16				
17				
18	Statistics: additional	<u>#20b</u>	Methods for any additional analyses (eg, subgroup and adjusted	14,15
19	analyses		analyses)	
20				
21				
22	Statistics: analysis	<u>#20c</u>	Definition of analysis population relating to protocol non-	N/A
23	population and		adherence (eg, as randomised analysis), and any statistical	
24	missing data		methods to handle missing data (eg, multiple imputation)	
25				
26				
27	Data monitoring:	<u>#21a</u>	Composition of data monitoring committee (DMC); summary of	15
28	formal committee		its role and reporting structure; statement of whether it is	
29			independent from the sponsor and competing interests; and	
30			reference to where further details about its charter can be found, if	
31			not in the protocol. Alternatively, an explanation of why a DMC is	
32			not needed	
33				
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37	Data monitoring:	<u>#21b</u>	Description of any interim analyses and stopping guidelines,	14
38	interim analysis		including who will have access to these interim results and make	
39			the final decision to terminate the trial	
40				
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43	Harms	<u>#22</u>	Plans for collecting, assessing, reporting, and managing solicited	15
44			and spontaneously reported adverse events and other unintended	
45			effects of trial interventions or trial conduct	
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48	Auditing	<u>#23</u>	Frequency and procedures for auditing trial conduct, if any, and	N/A
49			whether the process will be independent from investigators and the	
50			sponsor	
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53	Research ethics	<u>#24</u>	Plans for seeking research ethics committee / institutional review	16
54	approval		board (REC / IRB) approval	
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57	Protocol amendments	<u>#25</u>	Plans for communicating important protocol modifications (eg,	N/A
58			changes to eligibility criteria, outcomes, analyses) to relevant	
59				
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parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)

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4	Consent or assent	<u>#26a</u>	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
5			12,13
6			
7			
8	Consent or assent: ancillary studies	<u>#26b</u>	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
9			N/A
10			
11	Confidentiality	<u>#27</u>	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
12			16,17
13			
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17	Declaration of interests	<u>#28</u>	Financial and other competing interests for principal investigators for the overall trial and each study site
18			22
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21	Data access	<u>#29</u>	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
22			N/A
23			
24			
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26	Ancillary and post trial care	<u>#30</u>	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
27			15
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29			
30	Dissemination policy: trial results	<u>#31a</u>	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
31			N/A
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38	Dissemination policy: authorship	<u>#31b</u>	Authorship eligibility guidelines and any intended use of professional writers
39			N/A
40			
41			
42	Dissemination policy: reproducible research	<u>#31c</u>	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
43			N/A
44			
45			
46	Informed consent materials	<u>#32</u>	Model consent form and other related documentation given to participants and authorised surrogates
47			N/A
48			
49			
50	Biological specimens	<u>#33</u>	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
51			N/A
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BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-030233.R1
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Complete List of Authors:	Gon, Hidetoshi; Kobe University Graduate School of Medicine School of Medicine, Surgery; Komatsu, Shohei; Kobe University Graduate School of Medicine School of Medicine Murakami, Sae; Kobe University Hospital, Kido, Masahiro; Kobe University Graduate School of Medicine School of Medicine Tanaka, Motofumi; Kobe University Graduate School of Medicine School of Medicine Kuramitsu, Kaori; Kobe University Graduate School of Medicine School of Medicine Tsugawa, Daisuke; Kobe University Graduate School of Medicine School of Medicine Awazu, Masahide; Kobe University Graduate School of Medicine School of Medicine Toyama, Hirochika; Kobe University Graduate School of Medicine School of Medicine Fukumoto, Takumi; Kobe University Graduate School of Medicine School of Medicine
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Gastroenterology and hepatology
Keywords:	indocyanine green-fluorescence imaging, liver tumour, hepatectomy

SCHOLARONE™
Manuscripts

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4 **Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence**
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7 **imaging: protocol for a prospective cohort study**
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13 Hidetoshi Gon, MD, PhD^{a*}; Shohei Komatsu, MD, PhD^{a*}; Sae Murakami, MD, PhD^b;

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4 2566 words
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10 Keywords: indocyanine green-fluorescence imaging, liver tumour, hepatectomy
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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. 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

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4 prognostic outcome improvements in patients undergoing hepatectomy for various causes.
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7 **Ethics and dissemination:** The protocol has been approved by the Kobe University Clinical
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10 Research Ethical Committee. The findings of this study will be disseminated widely through
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13 peer-reviewed publications and conference presentations.
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16 **Trial registration number:** This study is registered at the UMIN Clinical Trials Registry:
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19 UMIN0000180139 and Japan Registry of Clinical Trials: jRCT1051180070. The Registration
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22 Data Set is available at <https://jrct.niph.go.jp/>.
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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

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

31 The ICG-fluorescence imaging system was initially introduced for use during open
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34 hepatectomy. Similar fluorescence imaging systems have been recently developed for use
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37 during laparoscopic hepatobiliary surgery. Several reports have demonstrated the efficacy of
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40 such systems during laparoscopic cholecystectomy and hepatectomy.²⁰ However, whether the
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43 hepatic boundaries visualised by ICG-fluorescence imaging systems are clinically precise and
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46 useful has not been adequately assessed. For example, there may be minor deviations due to
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49 the confluence of communicating vessel branches between hepatic segments; the injected ICG
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52 likely passes through the hepatic segments and the tumour to be removed. Evidence regarding
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55 the efficacy of ICG-fluorescence imaging systems is not fully established, and further
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58 investigation is required.
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4 The purpose of this study is to evaluate the efficacy of the ICG-fluorescence imaging
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7 system during hepatectomy for patients with liver tumours by analysing the detection rate of
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10 hepatic boundaries and tumours. In addition, we assess the precision of the detected hepatic
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13 boundaries by evaluating the postoperative clinical data.
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19 **METHODS AND ANALYSIS**

21 **Study design**

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

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40 From 2018 to 2020, patients with liver tumours treated at Kobe University will be
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43 enrolled. The inclusion criteria are as follows: male or female patients with liver tumours,
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45
46 aged 20 years and older, scheduled for elective hepatectomy, preserved liver function, ability
47
48
49 to understand the nature of the study procedures, and willingness to participate and give
50
51
52 voluntary written consent. Liver functional reserve will be assessed by serum biochemical
53
54
55 data (albumin level, total bilirubin level, and prothrombin time) and ICG retention for 15
56
57
58 minutes (ICG-R15). The patients will be categorized according to the severity of liver disease
59
60

1
2
3
4 based on Child-Pugh stages and the liver damage classification, defined by the Liver Cancer
5
6
7 Study Group of Japan.^{21,22} Preserved liver function is defined as ICG-R15 <15% and Child-
8
9
10 Pugh classification A or B.

11
12
13 The exclusion criteria are as follows: liver or renal insufficiency, known ICG
14
15
16 hypersensitivity, pregnancy or breastfeeding, and inability to understand the nature of the
17
18
19 study procedure.
20
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24

25 **Intervention**

26
27
28 ICG is injected intravenously at a dose of 0.5 mg/kg body weight within 2 days
29
30
31 preoperatively. Intraoperatively, we will initially observe the hepatic surface using a fusion
32
33
34 ICG-fluorescence imaging system (PINPOINT, Stryker Japan K.K.) to detect liver tumours.
35
36
37 Among several methods for identifying liver segments with fluorescence imaging, we will use
38
39
40 the negative staining technique to identify the liver segments in this study.²³ After identifying
41
42
43 and clamping the portal pedicle corresponding to the hepatic segments to be removed,
44
45
46 additional ICG is injected intravenously at a dose of 0.5 mg/kg body weight to identify the
47
48
49 boundaries of the hepatic segments.²⁴ Hepatectomy is performed based on the demarcation
50
51
52 between fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the
53
54
55 hepatic segments. The demarcation will also be checked at appropriate intervals during
56
57
58 parenchymal resection. Parenchymal resection will be performed using an ultrasonic surgical
59
60

1
2
3
4 aspirator (CUSA; Cavitron Lasersonic Corp., Stamford, CT, USA), and a bipolar clamp
5
6
7 coagulation system (ERBE, Tübingen, Germany). The fusion ICG-fluorescence images will
8
9
10 only be used for the hepatectomy. The Pringle manoeuvre will be performed and a drainage
11
12
13 tube will be routinely inserted around the cut surface of the liver parenchyma.
14
15
16
17
18

19 **Sample size calculation**

20
21
22 The purpose of the primary analysis of this study is to estimate the success rate, which is
23
24 defined as the proportion hepatic segments identified by the ICG-fluorescence imaging
25
26
27 system during hepatectomy. In order to judge the procedure as useful, a success rate of at least
28
29
30 80% is thought to be required. When the expected success rate is 90% and the two-sided 95%
31
32
33 confidence interval width is 0.12, the required number of participants is 98. To allow for an
34
35
36 approximately 10% dropout, the target sample size of this study has been set to 110.
37
38
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42

43 **Outcome measures**

44 *Primary endpoint*

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46
47
48 The primary endpoint is the success and failure of identifying hepatic segments using the
49
50
51 ICG-fluorescence imaging system. We will evaluate the identification of hepatic segments at
52
53
54 two points: observation of the liver surface and the hepatic transection surface. We assume
55
56
57 that identification is successful when fulfilling the following two criteria:
58
59
60

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3
4 (1) Hepatic surface
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6

7 Identification of hepatic segments by the ICG-fluorescence imaging system is considered
8
9
10 successful when the demarcation between fluorescing and non-fluorescing areas is consistent
11
12
13 with the ischemic demarcation area observed by clamping the portal pedicle.
14
15

16 (2) Hepatic transection surface
17
18

19 Hepatic parenchymal resection is performed based on the demarcation between
20
21
22 fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic
23
24
25 segments. We divide the time taken to perform parenchymal resection into three equal
26
27
28 intervals by reviewing the recorded videos after surgery, and the identification of hepatic
29
30
31 segment boundaries is evaluated at each interval. Identification of hepatic segments is
32
33
34 considered successful when we can identify the hepatic segments for more than 80% of the
35
36
37 process during parenchymal resection at more than two intervals.
38
39
40
41
42

43 *Secondary endpoints*
44
45

46 The secondary endpoints are the success and failure of identifying liver tumours by the
47
48
49 ICG-fluorescence imaging system, liver function indicators (alanine transaminase, albumin,
50
51
52 total bilirubin, international normalized ratio of prothrombin time, platelet count), the
53
54
55 operative time, the blood loss, the rate of postoperative complications, and recurrence-free
56
57
58 survival. Successful identification of liver tumours is determined when any isolated
59
60

1
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3
4 fluorescence signals are detected, also considering liver tumours diagnosed by other
5
6
7 modalities, including preoperative imaging and IOUS, and finally confirmed by pathological
8
9
10 examination. The fluorescence pattern is considered according to the preoperative diagnosis
11
12
13 because liver lesions have differing fluorescence patterns on the basis of their tumour
14
15
16 biology.²⁵ If we identify lesions with isolated fluorescence signal on fusion-fluorescence
17
18
19 imaging that were not identified by preoperative imaging, we evaluate the lesions by
20
21
22 intraoperative ultrasound sonography, and, if necessary, frozen section biopsies are performed
23
24
25 to determine whether additional hepatectomy is required. The recurrence-free survival is
26
27
28 analysed for each liver tumour, including primary liver cancer and liver metastases.
29
30
31 Recurrence-free survival time is defined as the time from enrolment until first recurrence after
32
33
34 the surgical intervention. Patients without recurrence will be censored at the date of last
35
36
37 confirmation of recurrence-free status. Patients lost to follow-up without a diagnosis of
38
39
40 recurrence and those who die will be censored at the date of last confirmation of recurrence-
41
42
43 free status.
44
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48

49 **Data collection**

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51
52 Three experienced surgeons will judge the intraoperative identification of hepatic
53
54
55 segment boundaries. The entire surgical procedure, including ICG-fluorescence imaging, will
56
57
58 be digitally recorded and analysed by an additional expert panel consisting of three highly
59
60

1
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3
4 experienced surgeons, different from those performing the surgeries, to confirm the
5
6
7 identification of hepatic segment boundaries. The success rate of their identification is used as
8
9
10 the end point. A flow chart of the study procedure is presented in Figure 1.
11
12

13 Postoperative complications will be graded according to the extended Clavien-Dindo
14
15
16 classification of surgical complications, which was published by the Japan Clinical Oncology
17
18
19 Group and more precisely described the original criteria of the Clavien-Dindo
20
21
22 classification.^{26,27}
23
24

25 Follow-up visits will be carried out at two weeks after hospital discharge, and every
26
27
28 three months thereafter. Follow-up evaluation will be performed using routine blood tests,
29
30
31 including liver function tests, coagulation function tests, serum tumour maker levels
32
33
34 depending on the type of liver tumour, abdominal ultrasonography, and abdominal enhanced
35
36
37 computed tomography.
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43 **Study timeline**

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46 Data will be collected from February 2018 until January 2020, and analysis is expected
47
48
49 to be completed around January 2022.
50
51

52 Participants will be informed about the study during their preoperative visit to our
53
54
55 hospital, and will have ample time to consider participation. Possible complications will be
56
57
58 evaluated in the year following the surgery. The schedules of enrolment, interventions, and
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60

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4 assessments are shown in Table 1.
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9

10 **Statistical analysis**

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12
13 The analysis populations will include the following three sets. Firstly, the full analysis
14 set (FAS) will consist of all participants that completed the surgery with navigation by ICG-
15
16 set (FAS) will consist of all participants that completed the surgery with navigation by ICG-
17
18 fluorescence images and have efficacy data available, excluding those without baseline data
19
20 or significant protocol violations (e.g., absence of informed consent, enrolment outside the
21
22 contract period). Secondly, the per protocol set (PPS) will consist of the FAS participants
23
24 completing 1 year of follow-up, excluding those with any significant protocol violations
25
26 involving the study method, the inclusion criteria, the exclusion criteria, and concomitant
27
28 therapy. Lastly, the safety analysis set (SAS) will consist of the participants who enrolled in
29
30 this study and were given at least one dose of ICG.
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40 The analysis will be performed after the data lock following completion of study drug
41
42 administration to all participants. For all efficacy endpoints, the FAS will be used in the
43
44 primary analysis, while the PPS will be used in a reference analysis. Safety will be analysed
45
46 using the SAS. The baseline participant characteristics' distribution and summary statistics
47
48 will be calculated according to group in each analysis population.
49
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54
55 All statistical analyses will be performed as indicated using JMP software, version
56
57 13.0.0 (SAS Institute, Inc., Cary, NC, USA).
58
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4 Interim analyses will not be performed in this study.
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10 *Primary outcome*
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13 The primary objective of this study is to estimate the success rate, which is defined as the
14 proportion of hepatic segments identified by the ICG-fluorescence imaging system. The point
15 estimate of the rate and the 95% confidence interval (CI) will be calculated.
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25 *Secondary outcomes*
26
27

28 The point estimate and 95% CI of the success rate of tumour detection by the ICG-
29 fluorescence imaging system will be calculated. For analysis of other secondary outcomes, we
30 will conduct a test using historical data collected at our facility as the control group. No
31 multiplicity adjustment will be performed in the analysis of secondary efficacy endpoints.
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34
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42

43 *Exploratory analysis*
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46 We will perform logistic regression analysis regarding the success or failure of
47 identifying liver segments using the ICG fluorescence imaging system. The following factors
48 will be included in the model to evaluate the association between the proportion of successful
49 cases of liver segment identification and clinical variables: age, sex, body mass index, viral
50 infection, Child-Pugh classification, cirrhosis, tumour size, tumour number, tumour location,
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4 type of hepatectomy, liver function indicators (alanine transaminase, albumin, total bilirubin,
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7 international normalized ratio, prothrombin time, platelet count), operative time, blood loss,
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9
10 rate of postoperative complications, and recurrence-free time.
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16 *Safety analysis*

17
18 The safety endpoint of this study is the frequency of adverse events. A table will be
19
20 prepared to summarize the endpoint. For estimation of the rates of adverse events, a two-sided
21
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25 95% CI will be calculated.
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31 **Data monitoring**

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34 Monitoring will be performed in order to periodically check whether the study is being
35
36
37 conducted safely in accordance with the protocol and whether the data are properly collected.
38
39
40 The following items are reviewed every six months: informed consent, obtained and signed;
41
42
43 participant retention; study implementation system; study safety and data; and study progress.
44
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49 **Patient and Public involvement**

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51
52 There was no patient and/or public involvement in planning of this study.
53
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58 **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

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4 The principal investigator and sub-investigators will comply with the principals of the
5
6
7 protection of participants' privacy rights. Study personnel will make the utmost of effort to
8
9
10 protect the participants' personal information and privacy, and will not divulge any personal
11
12
13 information learned from this study without due reasons, even outside working hours. In this
14
15
16 study, a list of subject identification codes will be prepared to link the subject source data
17
18
19 with the study database or study-related documents. Limited participant information, such as
20
21
22 sex and date of birth, may be used to identify participants or verify the list of subject
23
24
25 identification codes, within the range of all applicable laws and regulations.
26
27

28 All effort will be taken to ensure than participants will not be personally identifiable
29
30
31 from publications arising from this study.
32
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37 **Foreseeable disadvantages (burdens and risks)**

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39

40 The administration of ICG will be the only additional invasive intervention performed in
41
42
43 each patient. ICG administration rarely causes anaphylactic reactions (<1:10,000). Patients
44
45
46 with terminal renal insufficiency seem to be more prone for such an anaphylactic reaction.
47
48

49 The estimated mortality rate due to anaphylactic reaction is reported as <1 per 330,000.²⁸⁻³¹
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51

52 To minimize the risk of adverse events and disadvantages that may occur in this study,
53
54
55 the inclusion and exclusion criteria have been carefully discussed. All adverse events
56
57
58 occurring in this study will be monitored to ensure that they are within the expected range. If
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4 any serious or unexpected adverse events occur, the event will be carefully examined and
5
6
7 reviewed, and necessary countermeasures will be taken. Participation in this study may
8
9
10 require increased hospital visits, test frequency, and blood sampling volume, compared to
11
12
13 routine medical care. In the event of tumour progression, severe organ dysfunction, physical
14
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16 weakening, etc., during the preoperative treatment or during the waiting period for surgical
17
18
19 resection, the planned surgical resection may not be possible.
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46 **AUTHOR STATEMENT**

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49 H. Gon, S. Komatsu, S. Murakami, M. Kido, M. Tanaka, K. Kuramitsu, D. Tsugawa, M.
50
51
52 Awazu, H. Toyama, and T. Fukumoto all made substantial contributions to the conception
53
54
55 and design of the study. H. Gon, S. Komatsu, and S. Murakami drafted the manuscript. All
56
57
58 authors provided critical review and final approval of the present manuscript.
59
60

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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.

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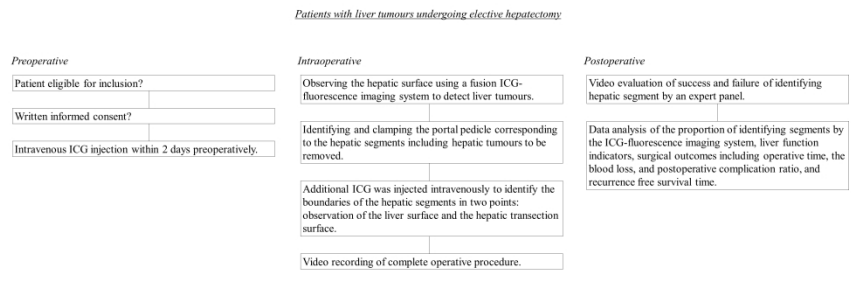


Figure 1. Flowchart of the study procedures.

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	<u>#1</u>	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	<u>#2a</u>	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	<u>#2b</u>	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	<u>#3</u>	Date and version identifier	4
Funding	<u>#4</u>	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	<u>#5a</u>	Names, affiliations, and roles of protocol contributors	21,22
Roles and responsibilities: sponsor contact information	<u>#5b</u>	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	<u>#5c</u>	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	<u>#5d</u>	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

1	Background and	<u>#6a</u>	Description of research question and justification for undertaking	6-8
2	rationale		the trial, including summary of relevant studies (published and	
3			unpublished) examining benefits and harms for each intervention	
4				
5				
6	Background and	<u>#6b</u>	Explanation for choice of comparators	N/A
7	rationale: choice of			
8	comparators			
9				
10				
11	Objectives	<u>#7</u>	Specific objectives or hypotheses	8
12				
13				
14	Trial design	<u>#8</u>	Description of trial design including type of trial (eg, parallel	8
15			group, crossover, factorial, single group), allocation ratio, and	
16			framework (eg, superiority, equivalence, non-inferiority,	
17			exploratory)	
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20				
21	Study setting	<u>#9</u>	Description of study settings (eg, community clinic, academic	8
22			hospital) and list of countries where data will be collected.	
23			Reference to where list of study sites can be obtained	
24				
25				
26	Eligibility criteria	<u>#10</u>	Inclusion and exclusion criteria for participants. If applicable,	8,9
27			eligibility criteria for study centres and individuals who will	
28			perform the interventions (eg, surgeons, psychotherapists)	
29				
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31	Interventions:	<u>#11a</u>	Interventions for each group with sufficient detail to allow	9
32	description		replication, including how and when they will be administered	
33				
34				
35	Interventions:	<u>#11b</u>	Criteria for discontinuing or modifying allocated interventions for	16
36	modifications		a given trial participant (eg, drug dose change in response to	
37			harms, participant request, or improving / worsening disease)	
38				
39				
40	Interventions:	<u>#11c</u>	Strategies to improve adherence to intervention protocols, and any	N/A
41	adherence		procedures for monitoring adherence (eg, drug tablet return;	
42			laboratory tests)	
43				
44				
45	Interventions:	<u>#11d</u>	Relevant concomitant care and interventions that are permitted or	N/A
46	concomitant care		prohibited during the trial	
47				
48				
49	Outcomes	<u>#12</u>	Primary, secondary, and other outcomes, including the specific	10,11
50			measurement variable (eg, systolic blood pressure), analysis metric	
51			(eg, change from baseline, final value, time to event), method of	
52			aggregation (eg, median, proportion), and time point for each	
53			outcome. Explanation of the clinical relevance of chosen efficacy	
54			and harm outcomes is strongly recommended	
55				
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1	Participant timeline	<u>#13</u>	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
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5				
6	Sample size	<u>#14</u>	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
7				
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11	Recruitment	<u>#15</u>	Strategies for achieving adequate participant enrolment to reach target sample size	10
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14				
15	Allocation: sequence generation	<u>#16a</u>	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
16				
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25	Allocation concealment mechanism	<u>#16b</u>	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
26				
27				
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32	Allocation: implementation	<u>#16c</u>	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
33				
34				
35				
36	Blinding (masking)	<u>#17a</u>	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
37				
38				
39				
40				
41	Blinding (masking): emergency unblinding	<u>#17b</u>	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
42				
43				
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45				
46	Data collection plan	<u>#18a</u>	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
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1	Data collection plan:	<u>#18b</u>	Plans to promote participant retention and complete follow-up,	N/A
2	retention		including list of any outcome data to be collected for participants	
3			who discontinue or deviate from intervention protocols	
4				
5				
6	Data management	<u>#19</u>	Plans for data entry, coding, security, and storage, including any	16,17
7			related processes to promote data quality (eg, double data entry;	
8			range checks for data values). Reference to where details of data	
9			management procedures can be found, if not in the protocol	
10				
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12				
13	Statistics: outcomes	<u>#20a</u>	Statistical methods for analysing primary and secondary outcomes.	13-15
14			Reference to where other details of the statistical analysis plan can	
15			be found, if not in the protocol	
16				
17				
18	Statistics: additional	<u>#20b</u>	Methods for any additional analyses (eg, subgroup and adjusted	14,15
19	analyses		analyses)	
20				
21				
22	Statistics: analysis	<u>#20c</u>	Definition of analysis population relating to protocol non-	N/A
23	population and		adherence (eg, as randomised analysis), and any statistical	
24	missing data		methods to handle missing data (eg, multiple imputation)	
25				
26				
27	Data monitoring:	<u>#21a</u>	Composition of data monitoring committee (DMC); summary of	15
28	formal committee		its role and reporting structure; statement of whether it is	
29			independent from the sponsor and competing interests; and	
30			reference to where further details about its charter can be found, if	
31			not in the protocol. Alternatively, an explanation of why a DMC is	
32			not needed	
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36				
37	Data monitoring:	<u>#21b</u>	Description of any interim analyses and stopping guidelines,	14
38	interim analysis		including who will have access to these interim results and make	
39			the final decision to terminate the trial	
40				
41				
42				
43	Harms	<u>#22</u>	Plans for collecting, assessing, reporting, and managing solicited	15
44			and spontaneously reported adverse events and other unintended	
45			effects of trial interventions or trial conduct	
46				
47				
48	Auditing	<u>#23</u>	Frequency and procedures for auditing trial conduct, if any, and	N/A
49			whether the process will be independent from investigators and the	
50			sponsor	
51				
52				
53	Research ethics	<u>#24</u>	Plans for seeking research ethics committee / institutional review	16
54	approval		board (REC / IRB) approval	
55				
56				
57	Protocol amendments	<u>#25</u>	Plans for communicating important protocol modifications (eg,	N/A
58			changes to eligibility criteria, outcomes, analyses) to relevant	
59				
60				

1		parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
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3			
4	Consent or assent	<u>#26a</u> Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12,13
5			
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7			
8	Consent or assent: ancillary studies	<u>#26b</u> Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
9			
10			
11	Confidentiality	<u>#27</u> 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
12			
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17	Declaration of interests	<u>#28</u> Financial and other competing interests for principal investigators for the overall trial and each study site	22
18			
19			
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21	Data access	<u>#29</u> Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
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23			
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26	Ancillary and post trial care	<u>#30</u> Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
27			
28			
29			
30	Dissemination policy: trial results	<u>#31a</u> 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
31			
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38	Dissemination policy: authorship	<u>#31b</u> Authorship eligibility guidelines and any intended use of professional writers	N/A
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41			
42	Dissemination policy: reproducible research	<u>#31c</u> Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
43			
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45			
46	Informed consent materials	<u>#32</u> Model consent form and other related documentation given to participants and authorised surrogates	N/A
47			
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49			
50	Biological specimens	<u>#33</u> 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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Manuscripts

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4 **Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence**
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7 **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

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4 prognostic outcome improvements in patients undergoing hepatectomy for various causes.
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7 **Ethics and dissemination:** The protocol has been approved by the Kobe University Clinical
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10 Research Ethical Committee. The findings of this study will be disseminated widely through
11
12
13 peer-reviewed publications and conference presentations.
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15

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

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4 which albumin is the principal carrier - following intravenous injection. Also, ICG is excreted
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7 in bile in an unconjugated form and is not cleared by extrahepatic mechanisms. Furthermore,
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10 single or repeated intravenous injections or infusions rarely cause unfavourable adverse
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13 effects. Taking advantage of these characteristics and the development of concomitant
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16 fluorescence imaging techniques, ICG-fluorescence imaging systems are widely used for
17
18
19 detecting sentinel lymph nodes and arterial blood flow, and their effectiveness has been
20
21
22 recognized.[11, 12] Moreover, the potential utility of this approach to identify liver tumours
23
24
25 and hepatic segment boundaries, as well as to detect the bile duct tree intraoperatively, has
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27
28 recently been demonstrated.[7, 13-19]

31 The ICG-fluorescence imaging system was initially introduced for use during open
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33
34 hepatectomy. Similar fluorescence imaging systems have been recently developed for use
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37 during laparoscopic hepatobiliary surgery. Several reports have demonstrated the efficacy of
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40 such systems during laparoscopic cholecystectomy and hepatectomy.[20] However, whether
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43 the hepatic boundaries visualised by ICG-fluorescence imaging systems are clinically precise
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46 and useful has not been adequately assessed. For example, there may be minor deviations due
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48
49 to the confluence of communicating vessel branches between hepatic segments; the injected
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51
52 ICG likely passes through the hepatic segments and the tumour to be removed. Evidence
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55 regarding the efficacy of ICG-fluorescence imaging systems is not fully established, and
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58 further investigation is required.
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4 The purpose of this study is to evaluate the efficacy of the ICG-fluorescence imaging
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7 system during hepatectomy for patients with liver tumours by analysing the rate of detection
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10 of hepatic boundaries and tumours. In addition, we assess the precision of the detected hepatic
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13 boundaries by evaluating the postoperative clinical data.
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19 **METHODS AND ANALYSIS**

21 **Study design**

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

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40 From 2018 to 2020, patients with liver tumours treated at Kobe University will be
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43 enrolled. The inclusion criteria are as follows: male or female patients with liver tumours,
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46 aged 20 years and older, scheduled for elective hepatectomy, have preserved liver function,
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48
49 able to understand the nature of the study procedures, and willing to participate and give
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52 voluntary written consent. Liver functional reserve will be assessed by serum biochemical
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55 data (albumin level, total bilirubin level, and prothrombin time) and ICG retention for 15
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57
58 minutes (ICG-R15). The patients will be categorized according to the severity of liver disease
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4 based on Child-Pugh stages and the liver damage classification, defined by the Liver Cancer
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6
7 Study Group of Japan.[21, 22] Preserved liver function is defined as ICG-R15 <15% and a
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9
10 Child-Pugh classification of A or B.

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13 The exclusion criteria are as follows: has liver or renal insufficiency, or known ICG
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16 hypersensitivity, pregnant or breastfeeding, or unable to understand the nature of the study
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18
19 procedure.

20 21 22 23 24 25 **Intervention**

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27
28 ICG is injected intravenously at a dose of 0.5 mg/kg body weight within 2 days
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30
31 preoperatively. Intraoperatively, we will initially observe the hepatic surface using a fusion
32
33
34 ICG-fluorescence imaging system (PINPOINT; Stryker, Kalamazoo, MI, US) to detect liver
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37 tumours. Among several methods for identifying liver segments with fluorescence imaging,
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39
40 we will use the negative staining technique to identify the liver segments in this study.[23]

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43 After identifying and clamping the portal pedicle corresponding to the hepatic segments to be
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46 removed, additional ICG is injected intravenously at a dose of 0.5 mg/kg body weight to
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49 identify the boundaries of the hepatic segments.[24] Hepatectomy is performed based on the
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52 demarcation between fluorescing and non-fluorescing areas, which are assumed to be the
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55 boundaries of the hepatic segments. The demarcation will also be checked as continuously as
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58 possible during parenchymal resection. Parenchymal resection will be performed using an
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4 ultrasonic surgical aspirator (CUSA; Cavitron Lasersonic Corp., Stamford, CT, USA), and a
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6
7 bipolar clamp coagulation system (ERBE, Tübingen, Germany). The fusion ICG-fluorescence
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10 images will only be used for the hepatectomy. The Pringle manoeuvre will be performed and
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13 a drainage tube will be routinely inserted around the cut surface of the liver parenchyma.
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19 **Sample size calculation**

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22 The purpose of the primary analysis of this study is to estimate the success rate, which is
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24 defined as the proportion of hepatic segments identified by the ICG-fluorescence imaging
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26 system during hepatectomy. In order to judge the procedure as useful, a success rate of at least
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28 80% is thought to be required. When the expected success rate is 90% and the two-sided 95%
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30 confidence interval width is 0.12, the required number of participants is 98. To allow for an
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32 approximately 10% dropout, the target sample size of this study has been set to 110.
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43 **Outcome measures**

44 *Primary endpoint*

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48 The primary endpoint is the success and failure of identifying hepatic segments using the
49
50 ICG-fluorescence imaging system. We will evaluate the identification of hepatic segments at
51
52 two points: observation of the liver surface and the hepatic transection surface. We assume
53
54 that identification is successful when fulfilling the following two criteria:
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4 (1) Hepatic surface
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7 Identification of hepatic segments by the ICG-fluorescence imaging system is considered
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10 successful when the demarcation between fluorescing and non-fluorescing areas is consistent
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13 with the ischemic demarcation area observed by clamping the portal pedicle.
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16 (2) Hepatic transection surface
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19 Hepatic parenchymal resection is performed based on the demarcation between
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22 fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic
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24
25 segments. We divide the time taken to perform parenchymal resection into three equal
26
27
28 intervals by reviewing the recorded videos after surgery, and the identification of hepatic
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31 segment boundaries is evaluated at each interval. Identification of hepatic segments is
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34 considered successful when we can identify the hepatic segments in more than 80% of the
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37 transected area during parenchymal resection at more than two intervals.
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43 *Secondary endpoints*
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46 The secondary endpoints are the success and failure of identifying liver tumours by the
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49 ICG-fluorescence imaging system, liver function indicators (alanine transaminase, albumin,
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52 total bilirubin, international normalized ratio of prothrombin time, platelet count), the
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55 operative time, the blood loss, the rate of postoperative complications, and recurrence-free
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58 survival. Successful identification of liver tumours is determined when any isolated
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4 fluorescence signals are detected, also considering liver tumours diagnosed by other
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7 modalities, including preoperative imaging and IOUS, and finally confirmed by pathological
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10 examination. The fluorescence pattern is considered according to the preoperative diagnosis
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13 because liver lesions have differing fluorescence patterns on the basis of their tumour
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15
16 biology.[25] If we identify lesions with isolated fluorescence signal on fusion-fluorescence
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19 imaging that were not identified by preoperative imaging, we evaluate the lesions by
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22 intraoperative ultrasound sonography, and, if necessary, frozen section biopsies are performed
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24
25 to determine whether additional hepatectomy is required. The recurrence-free survival is
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28 analysed for each case of liver tumour, including primary liver cancer and liver metastases.
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30
31 Recurrence-free survival time is defined as the time from enrolment until first recurrence after
32
33
34 the surgical intervention. Patients without recurrence will be censored at the date of last
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36
37 confirmation of recurrence-free status. Patients lost to follow-up without a diagnosis of
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40 recurrence and those who die will be censored at the date of last confirmation of recurrence-
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43 free status.
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49 **Data collection**

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52 Three experienced surgeons will judge the intraoperative identification of hepatic
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55 segment boundaries. The entire surgical procedure, including ICG-fluorescence imaging, will
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58 be digitally recorded and analysed by an additional expert panel consisting of three highly
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4 experienced surgeons, different from those performing the surgeries, to confirm the
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7 identification of hepatic segment boundaries. The success rate of their identification is used as
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10 the end point. A flow chart of the study procedure is presented in Figure 1.
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13 Postoperative complications will be graded according to the extended Clavien-Dindo
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16 classification of surgical complications, which was published by the Japan Clinical Oncology
17
18
19 Group and more precisely described in the original criteria of the Clavien-Dindo
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21
22 classification.[26, 27]
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25 Follow-up visits will be carried out at two weeks after hospital discharge, and every
26
27
28 three months thereafter. Follow-up evaluation will be performed using routine blood tests,
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31 including liver function tests, coagulation function tests, serum tumour maker levels
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34 depending on the type of liver tumour, abdominal ultrasonography, and abdominal enhanced
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37 computed tomography.
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43 **Study timeline**

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46 Data will be collected from February 2018 to January 2020, and analysis is estimated to
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49 be completed by January 2022.
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52 Participants will be informed about the study during their preoperative visit to our
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55 hospital, and will have ample time to consider participation. Possible complications will be
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58 evaluated in the year following the surgery. The schedules of enrolment, interventions, and
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4 assessments are shown in Table 1.
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10 **Statistical analysis**

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13 The analysis populations will include the following three sets. Firstly, the full analysis
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16 set (FAS) will consist of all participants who completed the surgery with navigation by ICG-
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19 fluorescence images and have efficacy data available, excluding those who have missing
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21
22 baseline data or have had significant protocol violations (e.g., absence of informed consent,
23
24
25 enrolment outside the contract period). Secondly, the per protocol set (PPS) will consist of
26
27
28 the FAS participants completing 1 year of follow-up, excluding those with any significant
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30
31 protocol violations involving the study method, the inclusion criteria, the exclusion criteria,
32
33
34 and concomitant therapy. Lastly, the safety analysis set (SAS) will consist of the participants
35
36
37 who enrolled in this study and were given at least one dose of ICG.
38
39

40 The analysis will be performed after the data lock following completion of study drug
41
42
43 administration to all participants. For all efficacy endpoints, the FAS will be used in the
44
45
46 primary analysis, while the PPS will be used in a reference analysis. Safety will be analysed
47
48
49 using the SAS. The baseline distribution of participant characteristics and summary statistics
50
51
52 will be calculated according to group in each analysis population.
53
54

55 All statistical analyses will be performed as indicated using JMP software, version
56
57
58 13.0.0 (SAS Institute, Inc., Cary, NC, USA).
59
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4 Interim analyses will not be performed in this study.
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10 *Primary outcome*
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13 The primary objective of this study is to estimate the success rate, which is defined as the
14
15 proportion of hepatic segments identified by the ICG-fluorescence imaging system. The point
16
17 estimate of the rate and the 95% confidence interval (CI) will be calculated.
18
19
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24

25 *Secondary outcomes*
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28 The point estimate and 95% CI of the success rate of tumour detection by the ICG-
29
30 fluorescence imaging system will be calculated. For analysis of other secondary outcomes, we
31
32 will conduct a test using historical data collected at our facility as the control group. No
33
34 multiplicity adjustment will be performed in the analysis of secondary efficacy endpoints. We
35
36 will estimate the recurrence-free survival by the Kaplan Meier method. The recurrence-free
37
38 survival will also be analysed by univariate COX proportional hazard model for each clinical
39
40 variable. Multivariate Cox proportional hazard models will be adopted to analyse the risk
41
42 factors of recurrence-free survival. The following variables will be included in the
43
44 multivariate model: the success or failure of identifying liver segments using the ICG
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46 fluorescence imaging system and other variables for which the p-value is under 0.05 in the
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48 univariate analysis.
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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

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4 surface and transection surface during parenchymal resection. We can evaluate the efficacy
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6
7 and safety of hepatectomy using ICG-fluorescence imaging systems by analysing the
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10 association between the success rate of identifying hepatic segments and clinical outcomes.
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13 This study will help to determine whether the boundaries detected by ICG-fluorescence
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16 imaging systems during hepatectomy are valid and useful.
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19 The findings obtained through this study will help to establish the utility of ICG-
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22 fluorescence imaging systems and therefore the study is expected to contribute to the
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25 improvement of prognostic outcomes in patients who undergo hepatectomy due to various
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28 causes.
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34 **Ethical approval**

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37 This study was approved by the Kobe University Clinical Research Ethical Committee.
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40 Possible protocol amendments will be sent to the Kobe University Clinical Research Ethical
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42
43 Committee.
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50 **Consideration of participants' human rights, safety, and disadvantages**

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52 The principal investigator and sub-investigators will comply with the principals of the
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54
55 protection of participants' privacy rights. Study personnel will make the utmost effort to
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57
58 protect the participants' personal information and privacy, and will not divulge any personal
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4 information learned from this study without due reasons, even outside working hours. In this
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6
7 study, a list of subject identification codes will be prepared to link the subject source data
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9
10 with the study database or study-related documents. Limited participant information, such as
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12
13 sex and date of birth, may be used to identify participants or verify the list of subject
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15
16 identification codes, within the range of all applicable laws and regulations.

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19 All effort will be taken to ensure that participants will not be personally identifiable from
20
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22 publications arising from this study.
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28 **Foreseeable disadvantages (burdens and risks)**

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30
31 The administration of ICG will be the only additional invasive intervention performed in
32
33
34 each patient. ICG administration rarely causes anaphylactic reactions (<1:10,000). Patients
35
36
37 with terminal renal insufficiency seem to be more prone to such an anaphylactic reaction. The
38
39
40 estimated mortality rate due to anaphylactic reaction is reported as <1 per 330,000.[28-31]
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43 To minimize the risk of adverse events and disadvantages that may occur in this study,
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45
46 the inclusion and exclusion criteria have been carefully discussed. All adverse events
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49 occurring in this study will be monitored to ensure that they are within the expected range. If
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52 any serious or unexpected adverse events occur, the event will be carefully examined and
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55 reviewed, and necessary countermeasures will be taken. Participation in this study may
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58 require increased hospital visits, test frequency, and blood sampling volume, compared to
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4 routine medical care. In the event of tumour progression, severe organ dysfunction, physical
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7 weakening, etc., during the preoperative treatment or during the waiting period for surgical
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10 resection, the planned surgical resection may not be possible.
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33 34 35 36 37 **AUTHOR STATEMENT**

38
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40 H. Gon, S. Komatsu, S. Murakami, M. Kido, M. Tanaka, K. Kuramitsu, D. Tsugawa, M.
41
42
43 Awazu, H. Toyama, and T. Fukumoto all made substantial contributions to the conception
44
45
46 and design of the study. H. Gon, S. Komatsu, and S. Murakami drafted the manuscript. All
47
48
49 authors provided critical review and final approval of the present manuscript.
50

51 52 53 54 55 **FUNDING STATEMENT**

56
57
58 This research received no specific grant from any funding agency in the public,
59
60

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4 commercial, or not-for-profit sectors.
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10 **DATA SHARING STATEMENT**

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13 This is a research protocol. That means the data for this study are being collected
14
15 currently. All authors have access to these data, and these data will be published as described
16
17 in the protocol, coordinated by H. Gon and S. Komatsu.
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25 **COMPETING INTERESTS STATEMENT**

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28 None declared.
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34 **FIGURE LEGENDS**

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

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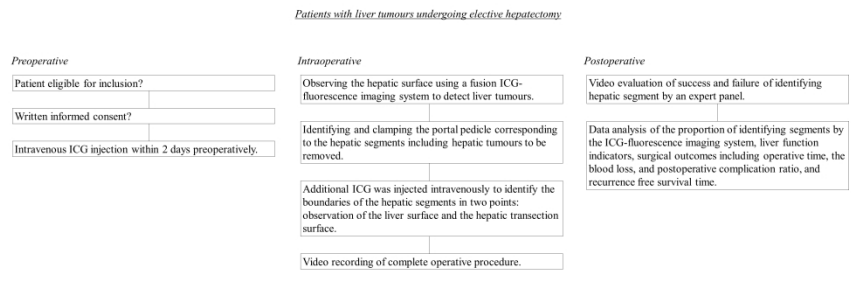


Figure 1. Flowchart of the study procedures.

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	<u>#1</u>	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	<u>#2a</u>	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	<u>#2b</u>	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	<u>#3</u>	Date and version identifier	4
Funding	<u>#4</u>	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	<u>#5a</u>	Names, affiliations, and roles of protocol contributors	21,22
Roles and responsibilities: sponsor contact information	<u>#5b</u>	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	<u>#5c</u>	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	<u>#5d</u>	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

1	Background and	<u>#6a</u>	Description of research question and justification for undertaking	6-8
2	rationale		the trial, including summary of relevant studies (published and	
3			unpublished) examining benefits and harms for each intervention	
4				
5				
6	Background and	<u>#6b</u>	Explanation for choice of comparators	N/A
7	rationale: choice of			
8	comparators			
9				
10				
11	Objectives	<u>#7</u>	Specific objectives or hypotheses	8
12				
13				
14	Trial design	<u>#8</u>	Description of trial design including type of trial (eg, parallel	8
15			group, crossover, factorial, single group), allocation ratio, and	
16			framework (eg, superiority, equivalence, non-inferiority,	
17			exploratory)	
18				
19				
20				
21	Study setting	<u>#9</u>	Description of study settings (eg, community clinic, academic	8
22			hospital) and list of countries where data will be collected.	
23			Reference to where list of study sites can be obtained	
24				
25				
26	Eligibility criteria	<u>#10</u>	Inclusion and exclusion criteria for participants. If applicable,	8,9
27			eligibility criteria for study centres and individuals who will	
28			perform the interventions (eg, surgeons, psychotherapists)	
29				
30				
31	Interventions:	<u>#11a</u>	Interventions for each group with sufficient detail to allow	9
32	description		replication, including how and when they will be administered	
33				
34				
35	Interventions:	<u>#11b</u>	Criteria for discontinuing or modifying allocated interventions for	16
36	modifications		a given trial participant (eg, drug dose change in response to	
37			harms, participant request, or improving / worsening disease)	
38				
39				
40	Interventions:	<u>#11c</u>	Strategies to improve adherence to intervention protocols, and any	N/A
41	adherence		procedures for monitoring adherence (eg, drug tablet return;	
42			laboratory tests)	
43				
44				
45	Interventions:	<u>#11d</u>	Relevant concomitant care and interventions that are permitted or	N/A
46	concomitant care		prohibited during the trial	
47				
48				
49	Outcomes	<u>#12</u>	Primary, secondary, and other outcomes, including the specific	10,11
50			measurement variable (eg, systolic blood pressure), analysis metric	
51			(eg, change from baseline, final value, time to event), method of	
52			aggregation (eg, median, proportion), and time point for each	
53			outcome. Explanation of the clinical relevance of chosen efficacy	
54			and harm outcomes is strongly recommended	
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1	Participant timeline	<u>#13</u>	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
2				
3				
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6	Sample size	<u>#14</u>	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
7				
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11	Recruitment	<u>#15</u>	Strategies for achieving adequate participant enrolment to reach target sample size	10
12				
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14				
15	Allocation: sequence generation	<u>#16a</u>	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
16				
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25	Allocation concealment mechanism	<u>#16b</u>	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
26				
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32	Allocation: implementation	<u>#16c</u>	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
33				
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36	Blinding (masking)	<u>#17a</u>	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
37				
38				
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41	Blinding (masking): emergency unblinding	<u>#17b</u>	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
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46	Data collection plan	<u>#18a</u>	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
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1	Data collection plan:	<u>#18b</u>	Plans to promote participant retention and complete follow-up,	N/A
2	retention		including list of any outcome data to be collected for participants	
3			who discontinue or deviate from intervention protocols	
4				
5				
6	Data management	<u>#19</u>	Plans for data entry, coding, security, and storage, including any	16,17
7			related processes to promote data quality (eg, double data entry;	
8			range checks for data values). Reference to where details of data	
9			management procedures can be found, if not in the protocol	
10				
11				
12				
13	Statistics: outcomes	<u>#20a</u>	Statistical methods for analysing primary and secondary outcomes.	13-15
14			Reference to where other details of the statistical analysis plan can	
15			be found, if not in the protocol	
16				
17				
18	Statistics: additional	<u>#20b</u>	Methods for any additional analyses (eg, subgroup and adjusted	14,15
19	analyses		analyses)	
20				
21				
22	Statistics: analysis	<u>#20c</u>	Definition of analysis population relating to protocol non-	N/A
23	population and		adherence (eg, as randomised analysis), and any statistical	
24	missing data		methods to handle missing data (eg, multiple imputation)	
25				
26				
27	Data monitoring:	<u>#21a</u>	Composition of data monitoring committee (DMC); summary of	15
28	formal committee		its role and reporting structure; statement of whether it is	
29			independent from the sponsor and competing interests; and	
30			reference to where further details about its charter can be found, if	
31			not in the protocol. Alternatively, an explanation of why a DMC is	
32			not needed	
33				
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37	Data monitoring:	<u>#21b</u>	Description of any interim analyses and stopping guidelines,	14
38	interim analysis		including who will have access to these interim results and make	
39			the final decision to terminate the trial	
40				
41				
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43	Harms	<u>#22</u>	Plans for collecting, assessing, reporting, and managing solicited	15
44			and spontaneously reported adverse events and other unintended	
45			effects of trial interventions or trial conduct	
46				
47				
48	Auditing	<u>#23</u>	Frequency and procedures for auditing trial conduct, if any, and	N/A
49			whether the process will be independent from investigators and the	
50			sponsor	
51				
52				
53	Research ethics	<u>#24</u>	Plans for seeking research ethics committee / institutional review	16
54	approval		board (REC / IRB) approval	
55				
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57	Protocol amendments	<u>#25</u>	Plans for communicating important protocol modifications (eg,	N/A
58			changes to eligibility criteria, outcomes, analyses) to relevant	
59				
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1		parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
2			
3			
4	Consent or assent	<u>#26a</u> Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12,13
5			
6			
7			
8	Consent or assent: ancillary studies	<u>#26b</u> Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
9			
10			
11	Confidentiality	<u>#27</u> 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
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17	Declaration of interests	<u>#28</u> Financial and other competing interests for principal investigators for the overall trial and each study site	22
18			
19			
20			
21	Data access	<u>#29</u> Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
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26	Ancillary and post trial care	<u>#30</u> Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
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30	Dissemination policy: trial results	<u>#31a</u> 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
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38	Dissemination policy: authorship	<u>#31b</u> Authorship eligibility guidelines and any intended use of professional writers	N/A
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42	Dissemination policy: reproducible research	<u>#31c</u> Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
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46	Informed consent materials	<u>#32</u> Model consent form and other related documentation given to participants and authorised surrogates	N/A
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50	Biological specimens	<u>#33</u> 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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Manuscripts

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4 **Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence**
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7 **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

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4 prognostic outcome improvements in patients undergoing hepatectomy for various causes.
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7 **Ethics and dissemination:** The protocol has been approved by the Kobe University Clinical
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10 Research Ethical Committee. The findings of this study will be disseminated widely through
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12
13 peer-reviewed publications and conference presentations.
14
15

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

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4 which albumin is the principal carrier - following intravenous injection. Also, ICG is excreted
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7 in bile in an unconjugated form and is not cleared by extrahepatic mechanisms. Furthermore,
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10 single or repeated intravenous injections or infusions rarely cause unfavourable adverse
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13 effects. Taking advantage of these characteristics and the development of concomitant
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16 fluorescence imaging techniques, ICG-fluorescence imaging systems are widely used for
17
18
19 detecting sentinel lymph nodes and arterial blood flow, and their effectiveness has been
20
21
22 recognized.[11, 12] Moreover, the potential utility of this approach to identify liver tumours
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24
25 and hepatic segment boundaries, as well as to detect the bile duct tree intraoperatively, has
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27
28 recently been demonstrated.[7, 13-19]

31 The ICG-fluorescence imaging system was initially introduced for use during open
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33
34 hepatectomy. Similar fluorescence imaging systems have been recently developed for use
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37 during laparoscopic hepatobiliary surgery. Several reports have demonstrated the efficacy of
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40 such systems during laparoscopic cholecystectomy and hepatectomy.[20] However, whether
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43 the hepatic boundaries visualised by ICG-fluorescence imaging systems are clinically precise
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46 and useful has not been adequately assessed. For example, there may be minor deviations due
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49 to the confluence of communicating vessel branches between hepatic segments; the injected
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52 ICG likely passes through the hepatic segments and the tumour to be removed. Evidence
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55 regarding the efficacy of ICG-fluorescence imaging systems is not fully established, and
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58 further investigation is required.
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4 The purpose of this study is to evaluate the efficacy of the ICG-fluorescence imaging
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7 system during hepatectomy for patients with liver tumours by analysing the rate of detection
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10 of hepatic boundaries and tumours. In addition, we assess the precision of the detected hepatic
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13 boundaries by evaluating the postoperative clinical data.
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19 **METHODS AND ANALYSIS**

21 **Study design**

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25 This prospective study is a single-arm, exploratory clinical trial. Patients with liver
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28 tumours will undergo hepatectomy using the ICG-fluorescence imaging system. This study
29
30
31 will be performed at Kobe University.
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37 **Target population**

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40 From 2018 to 2020, patients with liver tumours treated at Kobe University will be
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42
43 enrolled. The inclusion criteria are as follows: male or female patients with liver tumours,
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46 aged 20 years and older, scheduled for elective hepatectomy, have preserved liver function,
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48
49 able to understand the nature of the study procedures, and willing to participate and give
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52 voluntary written consent. Liver functional reserve will be assessed by serum biochemical
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55 data (albumin level, total bilirubin level, and prothrombin time) and ICG retention for 15
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58 minutes (ICG-R15). The patients will be categorized according to the severity of liver disease
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4 based on Child-Pugh stages and the liver damage classification, defined by the Liver Cancer
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6
7 Study Group of Japan.[21, 22] Preserved liver function is defined as ICG-R15 <15% and a
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10 Child-Pugh classification of A or B.

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13 The exclusion criteria are as follows: has liver or renal insufficiency, or known ICG
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16 hypersensitivity, pregnant or breastfeeding, or unable to understand the nature of the study
17
18
19 procedure.

20 21 22 23 24 25 **Intervention**

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28 ICG is injected intravenously at a dose of 0.5 mg/kg body weight within 2 days
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30
31 preoperatively. Intraoperatively, we will initially observe the hepatic surface using a fusion
32
33
34 ICG-fluorescence imaging system (PINPOINT; Stryker, Kalamazoo, MI, US) to detect liver
35
36
37 tumours. Among several methods for identifying liver segments with fluorescence imaging,
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39
40 we will use the negative staining technique to identify the liver segments in this study.[23]

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43 After identifying and clamping the portal pedicle corresponding to the hepatic segments to be
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46 removed, additional ICG is injected intravenously at a dose of 0.5 mg/kg body weight to
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48
49 identify the boundaries of the hepatic segments.[24] Hepatectomy is performed based on the
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52 demarcation between fluorescing and non-fluorescing areas, which are assumed to be the
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54
55 boundaries of the hepatic segments. The demarcation will also be checked as continuously as
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58 possible during parenchymal resection. Parenchymal resection will be performed using an
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4 ultrasonic surgical aspirator (CUSA; Cavitron Lasersonic Corp., Stamford, CT, USA), and a
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6
7 bipolar clamp coagulation system (ERBE, Tübingen, Germany). The fusion ICG-fluorescence
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10 images will only be used for the hepatectomy. The Pringle manoeuvre will be performed and
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12
13 a drainage tube will be routinely inserted around the cut surface of the liver parenchyma.
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19 **Sample size calculation**

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22 The purpose of the primary analysis of this study is to estimate the success rate, which is
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24 defined as the proportion of hepatic segments identified by the ICG-fluorescence imaging
25
26 system during hepatectomy. In order to judge the procedure as useful, a success rate of at least
27
28 80% is thought to be required. When the expected success rate is 90% and the two-sided 95%
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30 confidence interval width is 0.12, the required number of participants is 98. To allow for an
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32 approximately 10% dropout, the target sample size of this study has been set to 110.
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43 **Outcome measures**

44 *Primary endpoint*

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48 The primary endpoint is the success and failure of identifying hepatic segments using the
49
50 ICG-fluorescence imaging system. We will evaluate the identification of hepatic segments at
51
52 two points: observation of the liver surface and the hepatic transection surface. We assume
53
54 that identification is successful when fulfilling the following two criteria:
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4 (1) Hepatic surface
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7 Identification of hepatic segments by the ICG-fluorescence imaging system is considered
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9
10 successful when the demarcation between fluorescing and non-fluorescing areas is consistent
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12
13 with the ischemic demarcation area observed by clamping the portal pedicle.
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16 (2) Hepatic transection surface
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19 Hepatic parenchymal resection is performed based on the demarcation between
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21
22 fluorescing and non-fluorescing areas, which are assumed to be the boundaries of the hepatic
23
24
25 segments. We divide the time taken to perform parenchymal resection into three equal
26
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28 intervals by reviewing the recorded videos after surgery, and the identification of hepatic
29
30
31 segment boundaries is evaluated at each interval. Identification of hepatic segments is
32
33
34 considered successful when we can identify the hepatic segments in more than 80% of the
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37 transected area during parenchymal resection at more than two intervals.
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43 *Secondary endpoints*
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46 The secondary endpoints are the success and failure of identifying liver tumours by the
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49 ICG-fluorescence imaging system, liver function indicators (alanine transaminase, albumin,
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52 total bilirubin, international normalized ratio of prothrombin time, platelet count), the
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55 operative time, the blood loss, the rate of postoperative complications, and recurrence-free
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58 survival. Successful identification of liver tumours is determined when any isolated
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4 fluorescence signals are detected, also considering liver tumours diagnosed by other
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7 modalities, including preoperative imaging and IOUS, and finally confirmed by pathological
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10 examination. The fluorescence pattern is considered according to the preoperative diagnosis
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13 because liver lesions have differing fluorescence patterns on the basis of their tumour
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15
16 biology.[25] If we identify lesions with isolated fluorescence signal on fusion-fluorescence
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19 imaging that were not identified by preoperative imaging, we evaluate the lesions by
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22 intraoperative ultrasound sonography, and, if necessary, frozen section biopsies are performed
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24
25 to determine whether additional hepatectomy is required. The recurrence-free survival is
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28 analysed for each case of liver tumour, including primary liver cancer and liver metastases.
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30
31 Recurrence-free survival time is defined as the time from enrolment until first recurrence after
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33
34 the surgical intervention. Patients without recurrence will be censored at the date of last
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37 confirmation of recurrence-free status. Patients lost to follow-up without a diagnosis of
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40 recurrence and those who die will be censored at the date of last confirmation of recurrence-
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43 free status.
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49 **Data collection**

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52 Three experienced surgeons will judge the intraoperative identification of hepatic
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55 segment boundaries. The entire surgical procedure, including ICG-fluorescence imaging, will
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58 be digitally recorded and analysed by an additional expert panel consisting of three highly
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4 experienced surgeons, different from those performing the surgeries, to confirm the
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7 identification of hepatic segment boundaries. When we perform an open hepatectomy, the
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10 video will be captured by another surgeon using the scope of a fusion ICG-fluorescence
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13 imaging system. When we perform a laparoscopic hepatectomy, the ICG-fluorescence images
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16 can be accessed through the laparoscope. The success rate of their identification is used as the
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19 end point. A flow chart of the study procedure is presented in Figure 1.
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21

22 Postoperative complications will be graded according to the extended Clavien-Dindo
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25 classification of surgical complications, which was published by the Japan Clinical Oncology
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28 Group and more precisely described in the original criteria of the Clavien-Dindo
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31 classification.[26, 27]
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34 Follow-up visits will be carried out at two weeks after hospital discharge, and every
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36
37 three months thereafter. Follow-up evaluation will be performed using routine blood tests,
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40 including liver function tests, coagulation function tests, serum tumour maker levels
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43 depending on the type of liver tumour, abdominal ultrasonography, and abdominal enhanced
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46 computed tomography.
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52 **Study timeline**

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54 Data will be collected from February 2018 to January 2020, and analysis is estimated to
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57 be completed by January 2022.
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4 Participants will be informed about the study during their preoperative visit to our
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7 hospital, and will have ample time to consider participation. Possible complications will be
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10 evaluated in the year following the surgery. The schedules of enrolment, interventions, and
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13 assessments are shown in Table 1.
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19 **Statistical analysis**

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22 The analysis populations will include the following three sets. Firstly, the full analysis
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25 set (FAS) will consist of all participants who completed the surgery with navigation by ICG-
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28 fluorescence images and have efficacy data available, excluding those who have missing
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31 baseline data or have had significant protocol violations (e.g., absence of informed consent,
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34 enrolment outside the contract period). Secondly, the per protocol set (PPS) will consist of
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36
37 the FAS participants completing 1 year of follow-up, excluding those with any significant
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40 protocol violations involving the study method, the inclusion criteria, the exclusion criteria,
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42
43 and concomitant therapy. Lastly, the safety analysis set (SAS) will consist of the participants
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45
46 who enrolled in this study and were given at least one dose of ICG.
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49 The analysis will be performed after the data lock following completion of study drug
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52 administration to all participants. For all efficacy endpoints, the FAS will be used in the
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54
55 primary analysis, while the PPS will be used in a reference analysis. Safety will be analysed
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58 using the SAS. The baseline distribution of participant characteristics and summary statistics
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4 will be calculated according to group in each analysis population.
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7 All statistical analyses will be performed as indicated using JMP software, version
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10 13.0.0 (SAS Institute, Inc., Cary, NC, USA).
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13 Interim analyses will not be performed in this study.
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19 *Primary outcome*
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22 The primary objective of this study is to estimate the success rate, which is defined as the
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24 proportion of hepatic segments identified by the ICG-fluorescence imaging system. The point
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26 estimate of the rate and the 95% confidence interval (CI) will be calculated.
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34 *Secondary outcomes*
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37 The point estimate and 95% CI of the success rate of tumour detection by the ICG-
38
39 fluorescence imaging system will be calculated. For analysis of other secondary outcomes, we
40
41 will conduct a test using historical data collected at our facility as the control group. No
42
43 multiplicity adjustment will be performed in the analysis of secondary efficacy endpoints. We
44
45 will estimate the recurrence-free survival by the Kaplan Meier method. The recurrence-free
46
47 survival will also be analysed by univariate COX proportional hazard model for each clinical
48
49 variable. Multivariate Cox proportional hazard models will be adopted to analyse the risk
50
51 factors of recurrence-free survival. The following variables will be included in the
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4 multivariate model: the success or failure of identifying liver segments using the ICG
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7 fluorescence imaging system and other variables for which the p-value is under 0.05 in the
8
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10 univariate analysis.
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16 *Safety analysis*

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18 The safety endpoint of this study is the frequency of adverse events. A table will be
19
20 prepared to summarize the endpoint. For estimation of the rates of adverse events, a two-sided
21
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25 95% CI will be calculated.
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31 **Data monitoring**

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34 Monitoring will be performed in order to periodically check whether the study is being
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37 conducted safely in accordance with the protocol and whether the data are properly collected.
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40 The following items are reviewed every six months: informed consent, obtained and signed;
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42
43 participant retention; study implementation system; study safety and data; and study progress.
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49 **Patient and Public involvement**

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52 There was no patient and/or public involvement in planning of this study.
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58 **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

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4 The principal investigator and sub-investigators will comply with the principals of the
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7 protection of participants' privacy rights. Study personnel will make the utmost effort to
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10 protect the participants' personal information and privacy, and will not divulge any personal
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13 information learned from this study without due reasons, even outside working hours. In this
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15
16 study, a list of subject identification codes will be prepared to link the subject source data
17
18
19 with the study database or study-related documents. Limited participant information, such as
20
21
22 sex and date of birth, may be used to identify participants or verify the list of subject
23
24
25 identification codes, within the range of all applicable laws and regulations.
26
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28 All effort will be taken to ensure that participants will not be personally identifiable from
29
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31 publications arising from this study.
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37 **Foreseeable disadvantages (burdens and risks)**

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40 The administration of ICG will be the only additional invasive intervention performed in
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42
43 each patient. ICG administration rarely causes anaphylactic reactions (<1:10,000). Patients
44
45
46 with terminal renal insufficiency seem to be more prone to such an anaphylactic reaction. The
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48
49 estimated mortality rate due to anaphylactic reaction is reported as <1 per 330,000.[28-31]
50
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52 To minimize the risk of adverse events and disadvantages that may occur in this study,
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54
55 the inclusion and exclusion criteria have been carefully discussed. All adverse events
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58 occurring in this study will be monitored to ensure that they are within the expected range. If
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4 any serious or unexpected adverse events occur, the event will be carefully examined and
5
6
7 reviewed, and necessary countermeasures will be taken. Participation in this study may
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9
10 require increased hospital visits, test frequency, and blood sampling volume, compared to
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12
13 routine medical care. In the event of tumour progression, severe organ dysfunction, physical
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16 weakening, etc., during the preoperative treatment or during the waiting period for surgical
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19 resection, the planned surgical resection may not be possible.
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46 **AUTHOR STATEMENT**

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49 H. Gon, S. Komatsu, S. Murakami, M. Kido, M. Tanaka, K. Kuramitsu, D. Tsugawa, M.
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52 Awazu, H. Toyama, and T. Fukumoto all made substantial contributions to the conception
53
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55 and design of the study. H. Gon, S. Komatsu, and S. Murakami drafted the manuscript. All
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58 authors provided critical review and final approval of the present manuscript.
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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.

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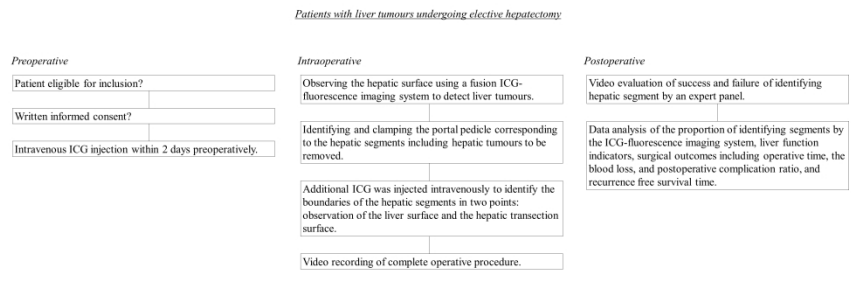


Figure 1. Flowchart of the study procedures.

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	<u>#1</u>	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	<u>#2a</u>	Trial identifier and registry name. If not yet registered, name of intended registry	4
Trial registration: data set	<u>#2b</u>	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	<u>#3</u>	Date and version identifier	4
Funding	<u>#4</u>	Sources and types of financial, material, and other support	22
Roles and responsibilities: contributorship	<u>#5a</u>	Names, affiliations, and roles of protocol contributors	21,22
Roles and responsibilities: sponsor contact information	<u>#5b</u>	Name and contact information for the trial sponsor	N/A
Roles and responsibilities: sponsor and funder	<u>#5c</u>	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	<u>#5d</u>	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

1	Background and	<u>#6a</u>	Description of research question and justification for undertaking	6-8
2	rationale		the trial, including summary of relevant studies (published and	
3			unpublished) examining benefits and harms for each intervention	
4				
5				
6	Background and	<u>#6b</u>	Explanation for choice of comparators	N/A
7	rationale: choice of			
8	comparators			
9				
10				
11	Objectives	<u>#7</u>	Specific objectives or hypotheses	8
12				
13				
14	Trial design	<u>#8</u>	Description of trial design including type of trial (eg, parallel	8
15			group, crossover, factorial, single group), allocation ratio, and	
16			framework (eg, superiority, equivalence, non-inferiority,	
17			exploratory)	
18				
19				
20				
21	Study setting	<u>#9</u>	Description of study settings (eg, community clinic, academic	8
22			hospital) and list of countries where data will be collected.	
23			Reference to where list of study sites can be obtained	
24				
25				
26	Eligibility criteria	<u>#10</u>	Inclusion and exclusion criteria for participants. If applicable,	8,9
27			eligibility criteria for study centres and individuals who will	
28			perform the interventions (eg, surgeons, psychotherapists)	
29				
30				
31	Interventions:	<u>#11a</u>	Interventions for each group with sufficient detail to allow	9
32	description		replication, including how and when they will be administered	
33				
34				
35	Interventions:	<u>#11b</u>	Criteria for discontinuing or modifying allocated interventions for	16
36	modifications		a given trial participant (eg, drug dose change in response to	
37			harms, participant request, or improving / worsening disease)	
38				
39				
40	Interventions:	<u>#11c</u>	Strategies to improve adherence to intervention protocols, and any	N/A
41	adherence		procedures for monitoring adherence (eg, drug tablet return;	
42			laboratory tests)	
43				
44				
45	Interventions:	<u>#11d</u>	Relevant concomitant care and interventions that are permitted or	N/A
46	concomitant care		prohibited during the trial	
47				
48				
49	Outcomes	<u>#12</u>	Primary, secondary, and other outcomes, including the specific	10,11
50			measurement variable (eg, systolic blood pressure), analysis metric	
51			(eg, change from baseline, final value, time to event), method of	
52			aggregation (eg, median, proportion), and time point for each	
53			outcome. Explanation of the clinical relevance of chosen efficacy	
54			and harm outcomes is strongly recommended	
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1	Participant timeline	<u>#13</u>	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
2				
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6	Sample size	<u>#14</u>	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
7				
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11	Recruitment	<u>#15</u>	Strategies for achieving adequate participant enrolment to reach target sample size	10
12				
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15	Allocation: sequence generation	<u>#16a</u>	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
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25	Allocation concealment mechanism	<u>#16b</u>	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
26				
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32	Allocation: implementation	<u>#16c</u>	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
33				
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35				
36	Blinding (masking)	<u>#17a</u>	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
37				
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39				
40				
41	Blinding (masking): emergency unblinding	<u>#17b</u>	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
42				
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46	Data collection plan	<u>#18a</u>	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
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1	Data collection plan:	<u>#18b</u>	Plans to promote participant retention and complete follow-up,	N/A
2	retention		including list of any outcome data to be collected for participants	
3			who discontinue or deviate from intervention protocols	
4				
5				
6	Data management	<u>#19</u>	Plans for data entry, coding, security, and storage, including any	16,17
7			related processes to promote data quality (eg, double data entry;	
8			range checks for data values). Reference to where details of data	
9			management procedures can be found, if not in the protocol	
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13	Statistics: outcomes	<u>#20a</u>	Statistical methods for analysing primary and secondary outcomes.	13-15
14			Reference to where other details of the statistical analysis plan can	
15			be found, if not in the protocol	
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17				
18	Statistics: additional	<u>#20b</u>	Methods for any additional analyses (eg, subgroup and adjusted	14,15
19	analyses		analyses)	
20				
21				
22	Statistics: analysis	<u>#20c</u>	Definition of analysis population relating to protocol non-	N/A
23	population and		adherence (eg, as randomised analysis), and any statistical	
24	missing data		methods to handle missing data (eg, multiple imputation)	
25				
26				
27	Data monitoring:	<u>#21a</u>	Composition of data monitoring committee (DMC); summary of	15
28	formal committee		its role and reporting structure; statement of whether it is	
29			independent from the sponsor and competing interests; and	
30			reference to where further details about its charter can be found, if	
31			not in the protocol. Alternatively, an explanation of why a DMC is	
32			not needed	
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36				
37	Data monitoring:	<u>#21b</u>	Description of any interim analyses and stopping guidelines,	14
38	interim analysis		including who will have access to these interim results and make	
39			the final decision to terminate the trial	
40				
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43	Harms	<u>#22</u>	Plans for collecting, assessing, reporting, and managing solicited	15
44			and spontaneously reported adverse events and other unintended	
45			effects of trial interventions or trial conduct	
46				
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48	Auditing	<u>#23</u>	Frequency and procedures for auditing trial conduct, if any, and	N/A
49			whether the process will be independent from investigators and the	
50			sponsor	
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53	Research ethics	<u>#24</u>	Plans for seeking research ethics committee / institutional review	16
54	approval		board (REC / IRB) approval	
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57	Protocol amendments	<u>#25</u>	Plans for communicating important protocol modifications (eg,	N/A
58			changes to eligibility criteria, outcomes, analyses) to relevant	
59				
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1		parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
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3			
4	Consent or assent	<u>#26a</u> Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12,13
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8	Consent or assent: ancillary studies	<u>#26b</u> Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
9			
10			
11	Confidentiality	<u>#27</u> 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
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17	Declaration of interests	<u>#28</u> Financial and other competing interests for principal investigators for the overall trial and each study site	22
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21	Data access	<u>#29</u> Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	N/A
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26	Ancillary and post trial care	<u>#30</u> Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
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30	Dissemination policy: trial results	<u>#31a</u> 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
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38	Dissemination policy: authorship	<u>#31b</u> Authorship eligibility guidelines and any intended use of professional writers	N/A
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42	Dissemination policy: reproducible research	<u>#31c</u> Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
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46	Informed consent materials	<u>#32</u> Model consent form and other related documentation given to participants and authorised surrogates	N/A
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50	Biological specimens	<u>#33</u> 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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