RESEARCH PROTOCOL

Study promoter	**Castilla y Institute of Health Sciences Studies Foundation (IECSCYL)- Institute of Biomedical Research of Salamanca (IBSAL)**
EUDRA CT number	2022-000904-36
Promoter's Protocol Code	IBS-DOTIG-ECM-2202
Title	Randomised clinical trial to evaluate the dose and time of administration of indocyanine green in near-infrared fluorescein cholangiography during laparoscopic cholecystectomy.
Date	16/12/2022
Version	V 2.0

1. SUMMARY OF THE STUDY

Background. Laparoscopic cholecystectomy is one of the most commonly performed surgical procedures worldwide. One of its most serious complications is injury to the main bile duct, with an incidence of less than 1%. There are different surgical strategies that attempt to reduce this complication, with indocyanine green fluorescence cholangiography being one of the most recent to appear. This technique is becoming a great tool during laparoscopic cholecystectomy. Despite the boom in the procedure, there is currently a wide disparity in the protocols for administration of indocyanine green during the procedure.

Objectives. The main objective of the study is to analyse whether there are differences between different types of doses and administration intervals of indocyanine green to obtain quality fluorescein cholangiography during laparoscopic cholecystectomy. In addition, factors influencing the results of the technique will be sought.

Material and Methods. Open multicentre randomised clinical trial with 4 intervention groups (two doses and two administration times). The expected duration of the study will be 12 months. The main variables to be studied will be the degree of identification critical anatomical structures during laparoscopic cholecystectomy. Patients will be analysed per protocol, without intermediate analyses.

2. SIGNATURE OF APPROVAL OF THE PROTOCOL

Signature of the Promoter:

PROTOCOL TITLE: Randomised clinical trial to evaluate the dose and time of administration of indocyanine green in near-infrared fluorescein cholangiography during laparoscopic cholecystectomy: DOTIG study.

Project: IBS-DOTIG-ECM-2202

Eudract number: 2022-000904-36

Promoter's representative: Ms. María Lorenzo Santiago

Signature Date

Signature of the Coordinating Researcher:

PROTOCOL TITLE: Randomised clinical trial to evaluate the dose and time of administration of indocyanine green in near-infrared fluorescein cholangiography during laparoscopic cholecystectomy: DOTIG study.

Project: IBS-DOTIG-ECM-2202

Eudract number: 2022-000904-36

Coordinating Researcher of the Study: Prof. Luis Muñoz Bellvís.

Signature Date

3. PRINCIPAL INVESTIGATOR'S SIGNATURE HOMEPAGE

PROTOCOL TITLE: Randomised clinical trial to evaluate the dose and time of administration of indocyanine green in near-infrared fluorescein cholangiography during laparoscopic cholecystectomy.

Project: IBS-DOTIG-ECM-2202

EudraCT Number: 2022-000904-36

CONFIDENTIALITY AND DECLARATION OF CONFORMITY OF THE BPC

I have read the above clinical study protocol entitled: "Randomised clinical trial to evaluate the dose and time of administration of indocyanine green in near-infrared fluorescein cholangiography during laparoscopic cholecystectomy", and agree that it contains all the information necessary to conduct the study.
I hereby confirm that I have thoroughly read and understand this clinical trial protocol, and agree that my staff and I will conduct the trial in accordance with the protocol and comply with its requirements, including ethical and safety considerations.
I understand that if the Promoter decides to terminate or suspend the study prematurely for any reason, this decision will be communicated to me in writing. , if I decide to withdraw from the execution of the study, I will immediately communicate this decision to the Promoter.
I agree not to publish any part of the results of the study conducted under this clinical trial protocol without the prior written consent of the Sponsor.

Principal Investigator:
Hospital Centre:

_____

Signature

_____

Date

Fundación Instituto de Estudios Ciencias de la Salud de Castilla y León (IECSCYL) - Institute of Biomedical Research of Salamanca (IBSAL)
Salamanca University Hospital
Edificio Virgen de la Vega, 10.ª planta Pº San Vicente, 58-182. 37007
Salamanca

4. ACRONYMS

AA: Adverse event.

AAG: Serious adverse event.

AEMPS: Spanish Agency for Medicines and Health Products.

ASA: American Society of Anaesthesiologists.

GCP: Good Clinical Practice. CCAA:

Autonomous Communities

CRD: Data Collection Notebook.

CRDe: Electronic Data Collection Logbook.

CEIm: Committee on Ethics and Medicines Research.

CF: Fluorescent cholangiography.

IC: Informed consent. **LC**:

Laparoscopic cholecystectomy.

DSUR: Periodic Safety Reports. EC: Clinical

Trial

EMA: European Medicines Agency.

FDA: Food and Drug Administration of the United States of America. FV:

Pharmacovigilance

HC: Medical History.

HIP: Patient Information Sheet.

IBSAL: Institute of Biomedical Research of Salamanca.

ICH: International Conference on Harmonisation.

IECSCYL: Castilla y León Institute of Health Sciences Studies Foundation**. PI**: Principal

Investigator.

BMI: Body Mass Index.

LVB: Bile duct injury.

RIP: Product under investigation.

RAGI: Serious Unexpected Adverse Reactions.

VCS: Critical Security Vision.

VI: Indocyanine green.

5. Table of contents

6. General Information

6.1. Title

- Randomised clinical trial to evaluate the dose and time of administration of indocyanine green in near-infrared fluorescein cholangiography during laparoscopic cholecystectomy.
- IBS-DOTIG-ECM-2202
- Protocol Version 1.4.

6.2. Name and address of the promoter:

- Fundación Instituto de Estudios Ciencias de la Salud de Castilla y León (IECSCYL)-Instituto de Investigación Biomédica de Salamanca (IBSAL).
- Complejo Asistencial Universitario de Salamanca. Hospital Virgen de la Vega, 10th Floor. Paseo de San Vicente, 58-182. 37007 Salamanca.

6.3. Reference CEIm.

- Salamanca Health Area.

6.4. Phase of the study.

- Phase IV.

6.5. Name and title of study coordinator, address and contact details.

- Prof. Luis Muñoz Bellvís.
  - Head of the General and Digestive System Surgery Department. University Hospital of Salamanca. Salamanca, Spain. Paseo de San Vicente 182, 37007 Salamanca. Tlf: 923 29 11 00
  - Institute of Biomedical Research of Salamanca (IBSAL). University of Salamanca, Salamanca, Spain.
  - e-mailluismb@usal.es

6.6. In case of a multi-centre study, name and title of all investigators responsible for the conduct of the trial and the address and telephone numbers of trial sites.

- Dr. Jaime López Sánchez.
  - Department of General and Digestive System Surgery. University Hospital of Salamanca. Salamanca, Spain. Paseo de San Vicente 182, 37007 Salamanca. Tlf: 923 29 11 00
  - Institute of Biomedical Research of Salamanca (IBSAL). University of Salamanca, Salamanca, Spain.
  - e-mailjalopsan@hotmail.com
- Dr. Fernando Pardo Aranda
  - Department of General and Digestive System Surgery. Germans Trias i Pujol University Hospital. Badalona, Spain. Carretera de Canyet s/n, 08916 Badalona, Barcelona. Tlf: 934 651200
  - e-mailfpardoaranda@gmail.com

6.7. Name and addresses of clinical laboratories and medical or technical departments or institutions involved in the trial.

- Department of General and Digestive System Surgery. University Hospital of Salamanca. Salamanca, Spain. Paseo de San Vicente 182, 37007 Salamanca.
- Department of General and Digestive System Surgery Hospital Universitario Germans Trias i Pujol. Badalona, Spain. Carretera de Canyet s/n, 08916 Badalona, Barcelona.

7. Justification

Symptomatic cholelithiasis is a highly relevant pathology in the world population, with prevalence rates of up to 20%. Regardless of the symptom or complication causing cholelithiasis, the current standard treatment is cholecystectomy, preferably through the minimally invasive laparoscopic approach(1,2). One of the most serious complications is injury to the main bile duct (MBV). Although the incidence of this complication is less than 1% (0.3-0.7% in different series), the consequences are very important. LVB is associated with a significant increase in patient morbidity and mortality, a significant deterioration in quality of life, a major increase in healthcare costs and significant medical-legal consequences(3). Misidentification of gallbladder anatomy has been described as one of the main factors in LVB(4). This could be favoured by the great anatomical variability of this region and the inflammatory changes that occur in many patients.

To date, there have been many recommendations to reduce the incidence of this complication, perhaps the most widely implemented being the critical safety view (CSV) published by Strasberg in 1995(5). Other techniques, such as the B-SAFE rule, are in the process of implementation(6). A recent approach using artificial intelligence and *machine learning* has been applied in the field of safe laparoscopic cholecystectomy (LC)(7). Among invasive methods, intraoperative cholangiography (IOC) has classically been the most widely used system for the prevention and early detection of LVB, as well for the diagnosis of choledocholithiasis(8). However, the disadvantages of this technique are the use of ionising radiation, the learning curve, the need prior surgical dissection and consequently the possibility of iatrogenic injury, as well as the need for infrastructure, organisation and coordination that is not widely available(9). Furthermore, it has not been shown to decrease the incidence of LVB.

Near-infrared (NIR) fluorescence cholangiography (FC) is a relatively recent technique(10) that applies the fluorescent property and biliary-only elimination of indocyanine green (IV) to map the extrahepatic biliary tree(3). VI has previously been described in the fields of cardiology, ophthalmology and currently in hepatology for the study of hepatic functional reserve(11). CF-VI allows minimally invasive, real-time visualisation of the anatomy of the extrahepatic bile duct. It is a fast method with a minimal learning curve. In addition, it does not require prior surgical dissection and costs less than CIO. However, it is currently not widely available and requires a dedicated imaging infrastructure(9,12). Recently, CF-VI has been shown to be superior to traditional white light CL in reducing LVB(13), and a 2021 meta-analysis estimates that this technique could significantly reduce LVB during CL(14). However, it has not yet been shown to be significantly superior to CIO(9,12). Furthermore, different authors show disparate rates

anatomical visualisation depending on the characteristics of the patient, the pathological process, the technique used, the instruments, etc. (3,15).

Currently, there are major differences in LV administration protocols during FC in LC. The precise dose and timing of administration are key to achieve adequate visualisation of critical vascular and biliary structures and to reduce the fluorescence emitted by the liver parenchyma, which could hinder correct anatomical visualisation(15). Regarding dosage, there are a multitude of VI administration protocols, using single doses or doses adjusted for the patient's body weight. Some authors advocate administration of the IV 24 hours before the procedure in order to avoid hepatic fluorescence(16). However, in the context of major ambulatory surgery, outpatient surgery or short-stay surgery(17), we believe that this practice is not logistically feasible at present. Other groups administer LV with a variable range of time interval(15). Recent preliminary results from the European Fluorescence Image Surgery Registry show the great disparity of preoperative LV administration protocols(18). Therefore, there is a need for protocolisation of drug administration through the results of randomised clinical studies.

The investigational medicinal product is Verdye. Verdye (*Diagnostic Green* GMBH, Aschheim-Dornach, Germany) contains the active substance VI sodium as a powder for solution for injection. This product is approved by the US Food and Drug Administration (FDA), the European Medicines Agency (EMA) and the Spanish Agency for Medicines and Medical Devices (AEMPS) as of 30/8/2017. It is a medical device for hospital use and authorised diagnostic centres. VI is a water-soluble agent with a peak spectral absorption of 800 nm. Once administered intravenously, it has an affinity for plasma proteins (β-apolipoprotein B in 95% of cases), remaining constant in the intravascular space. The IV does not undergo any metabolisation process in the body. The uptake of the drug from plasma is exclusively hepatic, with biliary excretion within 30 minutes to 2 hours after intravenous injection. The fluorescent characteristics of the product appear after stimulation with near-infrared light beams (700-900 nm). It is indicated for cardiac, circulatory and microcirculatory diagnostics, liver function diagnostics and ophthalmic angiography.

Risks from the administration of the medicinal product are rare. Severe allergic reactions have been reported in one in 10,000 patients, causing symptoms such as: constriction of the throat, pruritus, blotchy skin, urticaria, coronary artery spasm, facial oedema, shortness of breath, chest tightness or pain, tachycardia, low blood pressure, dyspnoea, heart failure or cardiac arrest, restlessness, nausea, sensation of heat, flushing. The possibility of severe allergic reaction is higher in patients with severe renal insufficiency.

The route of administration of the IV will be intravenous, as specified in its data sheet. The drug administration protocols will be divided according to the dosage and time interval until surgery, as detailed below:

- **Dosage**. 2 groups: fixed bolus dose of 2.5 mg and bolus dose adjusted for body weight (0.05 mg/kg total body weight).

- **Time of administration.** On admission (>3 hours prior to surgery) and immediately preoperatively (15-30 minutes prior to surgery).

There is no treatment period as it is considered a diagnostic method. This period shall include intraoperative time.

The investigator shall ensure that this study is conducted in accordance with the principles of the 1964 Declaration of Helsinki and its subsequent revisions, GCP as well as

The study will be conducted in accordance with the ICH guidelines and in full compliance with ethical and deontological standards. Individuals participating in this study will be qualified by education, training and experience to perform their respective tasks.

The study population in this CEA is patients with an indication for elective, scheduled or early CL. The inclusion criteria are detailed below:

- Over 18 years of age.
- Autonomy, self-sufficiency and independence.
- Indication of CL programmed:
  - Symptomatic cholelithiasis: history of biliary colic, acute calculous cholecystitis, choledocholithiasis, acute ascending cholangitis of calculous origin or acute calculous pancreatitis.
  - Gallbladder polyps with indication for laparoscopic surgery.
  - Vesicular adenomyomatosis with indication for laparoscopic surgery.
- Indication for early LC (<72 hours of admission for acute calculous cholecystitis/acute calculous cholecystitis/complicated biliary colic).
- Indication of deferred emergency CL.
- Understanding information.
- Signing the informed consent form.

The exclusion criteria are detailed below:

- Under 18 years of age.
- Incapacity.
- Pregnancy or breastfeeding.
- Chronic kidney disease (Stage> IIIb).
- Adverse reactions or allergies prior to IV.
- Previous adverse reactions or allergies to VI excipients.
- Confirmed adverse reactions or allergies to iodinated contrast agents.
- Functional thyroid pathology (hyperthyroidism, thyroiditis, toxic multinodular goitre, functioning thyroid adenoma).
- Urgent non-deferrable/emergent gallbladder surgery.
- Initial surgery by laparotomy.
- Previous suspicion of gallbladder carcinoma.
- Inability to understand the information needed to participate in the study.
- Refusal of inclusion in the study protocol.

8. Aim and Purpose of the Trial

The objectives of this CE are detailed :

- **Main objective.** To analyse whether there are differences between different doses (single dose and weight-adjusted dose) and different VI administration intervals to achieve ideal visualisation of biliary structures during CF in LC.

- **Secondary objectives.**
  - 1. To analyse the influence of body mass index on FC outcomes during CL.
  - 2. To analyse the influence of the type of biliary pathology requiring surgery on the results of FC during LC.
  - 3. To analyse the influence of the type of surgery (elective/early/ delayed surgery) on the outcome of FC during LC.
  - 4. To analyse the influence of previous inflammatory changes on the results of FC during LC.
  - 5. To analyse the influence of previous gallbladder or bile duct instrumentation on the results of FC during LC.
  - 6. To analyse the influence of different laparoscopic imaging systems on the results of FC during LC.
  - 7. To analyse the rate of intraoperative complications related to CF during LC.
  - 8. To analyse the rate of postoperative complications related to CF during LC.
  - 9. To analyse the impact of FC during LC on general surgeons and/or surgeons specialised in hepatobiliopancreatic pathology and their subjective assessment of the procedure.
  - 10. To assess the impact of a history of previous liver disease on fluorescein cholangiography.

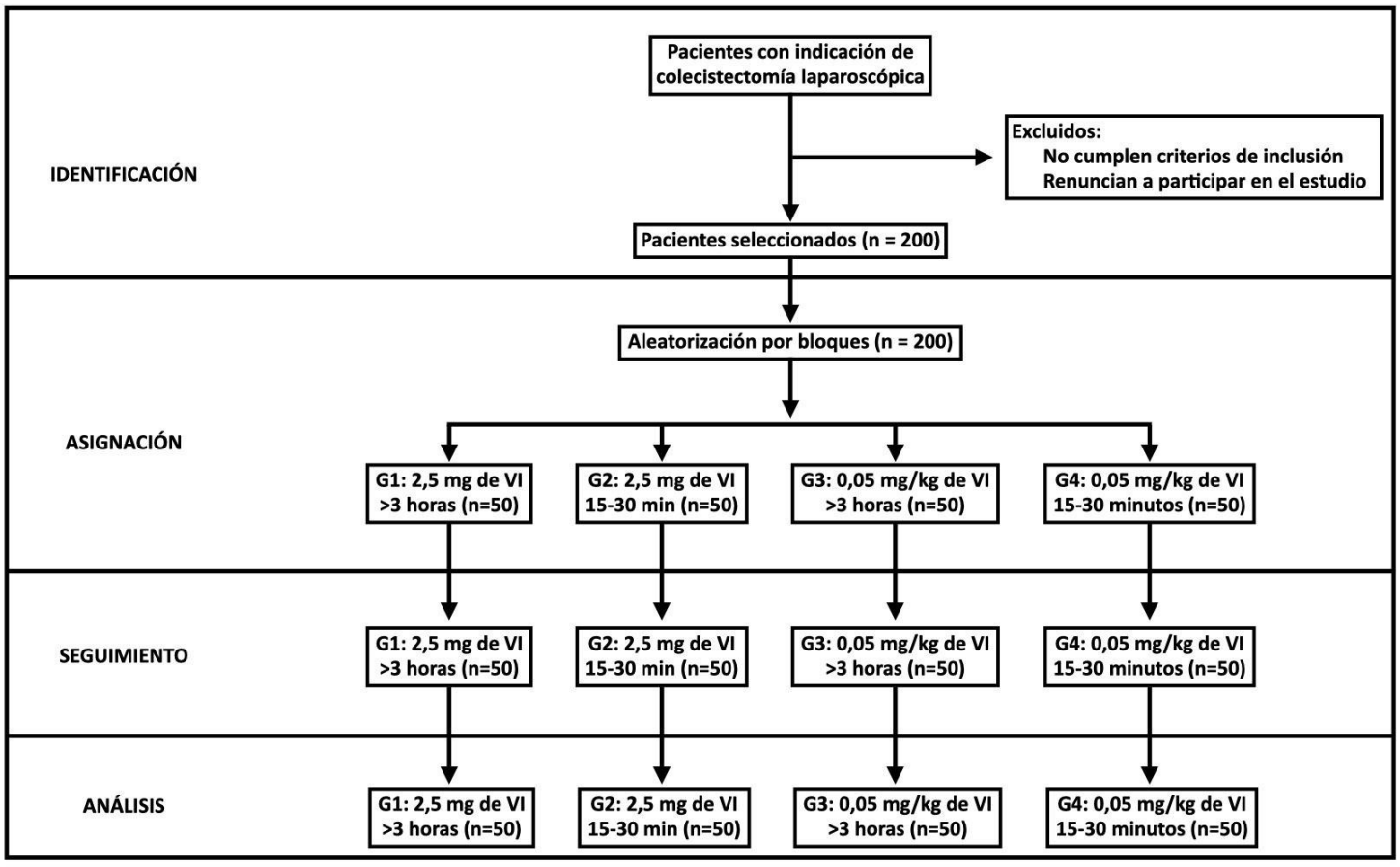
9. Trial Design

For this JU, the following key variables to be assessed in the trial are determined. Primary variables:

- Identification of biliary structures prior to dissection of the hepatocystic triangle.
  - Identification of cystic duct (yes/no).
  - Identification of common bile duct (yes/no).
  - Identification of the junction of the cystic duct with the common bile duct (yes/no).
  - Identification of the junction of the cystic duct with the gall bladder (yes/no).
  - Identification of the common hepatic duct (yes/no).
  - Identification of anatomical variables (yes/no. Specify).
- Identification of biliary structures after dissection of the hepatocystic triangle.
  - Identification of cystic duct (yes/no).
  - Identification of common bile duct (yes/no).
  - Identification of the junction of the cystic duct with the common bile duct (yes/no).
  - Identification of the junction of the cystic duct with the gall bladder (yes/no).
  - Identification of the common hepatic duct (yes/no).
  - Identification of anatomical variables (yes/no. Specify).
- Degree of identification of biliary structures prior to dissection of the hepatocystic triangle (1=poor, 2=sufficient, 3=sufficient, 4=good, 5=excellent).

- Degree of identification of biliary structures after dissection of the hepatocystic triangle (1=poor, 2=sufficient, 3=sufficient, 4=good, 5=excellent).
- Degree to which fluorescence cholangiography was perceived as useful for surgery (0=not useful, 1=moderately useful, 2=very useful).
- Degree to which the fluorescence of the liver background (contrast between liver and ducts) was perceived as disturbed (0=absence of disturbance, 1=slightly disturbed, 2=disturbed visualisation, but cystic-choledochal junction was clearly visible before dissection, 3=disturbed visualisation and cystic-choledochal junction was only visible after dissection, 4=very disturbed: it was impossible to visualise the biliary structures correctly).

The design of this CE is reflected in the following flowchart.



G1: Grupo 1, G2: Grupo 2, G3: Grupo 3, G4: Grupo 4, VI: Verde de indocianina

10. Research Product Characteristics (RPP)

Verdye 25 mg contains VI sodium powder for solution for injection. VI is a water-soluble agent with a peak spectral absorption of 800 nm. It is approved for diagnostic use only and is indicated for the measurement of the excretory function of the liver.

liver. This medicine is included in the University Hospital of Salamanca and routinely used in the General and Digestive System Surgery Service.

Diagnostic use of this medicinal product should be performed under surveillance. The total daily dose should be less than 5 mg/kg body weight. The medicinal product should not be diluted with saline solutions and be stored at a temperature below 30°C. Administration should be intravenous and handled under sterile conditions. A previous tolerance test with Verdye in which no problems have been encountered should not be considered an absolute guarantee of safety. Patients with end-stage renal failure have a higher rate of adverse effects. Two cases of death from anaphylactic reaction following administration of IV during cardiac catheterisation have been reported.

The delivery of the IMP will be carried out through the Hospital Pharmacy Service. Each vial of medicine will be for individual use, discarding the remaining active ingredient according to the protocol for handling pharmacological products. A document will be sent to the Hospital Pharmacy Service detailing the vials needed for the study in each centre (estimating 10 additional vials per centre). These vials will be stored in the operating room storerooms in a place outside the usual product use and with a "only use for DOTIG study" label. Once the study is completed, unused VI vials will be returned to the Hospital Pharmacy Service. A drug traceability document will be signed to ensure proper delivery and storage of the drugs.

VI administration protocols will be prespecified depending on the randomised treatment group. The product will be prescribed by the medical staff once the dosage and administration interval is known.

11. Selection and Withdrawal of Subjects

11.1. Criteria for inclusion of subjects

- Over 18 years of age.
- Autonomy, self-sufficiency and independence.
- Indication of CL programmed:
  - Symptomatic cholelithiasis: history of biliary colic, acute calculous cholecystitis, choledocholithiasis, acute ascending cholangitis of calculous origin or acute calculous pancreatitis.
  - Gallbladder polyps with indication for laparoscopic surgery.
  - Vesicular adenomyomatosis with indication for laparoscopic surgery.
- Indication for early LC (<72 hours of admission for acute calculous cholecystitis/acute calculous cholecystitis/complicated biliary colic).
- Indication of deferred emergency CL.
- Understanding information.
- Signing the informed consent form.

11.2. Exclusion criteria for subjects.

- Under 18 years of age.
- Incapacity.
- Pregnancy or breastfeeding.

- Chronic kidney disease (Stage> IIIb).
- Adverse reactions or allergies prior to IV.
- Previous adverse reactions or allergies to VI excipients.
- Confirmed adverse reactions or allergies to iodinated contrast agents.
- Functional thyroid pathology (hyperthyroidism, thyroiditis, toxic multinodular goitre, functioning thyroid adenoma).
- Urgent non-deferrable/emergent gallbladder surgery.
- Initial surgery by laparotomy.
- Previous suspicion of gallbladder carcinoma.
- Inability to understand the information needed to participate in the study.
- Refusal of inclusion in the study protocol.

11.3. Termination and interruption criteria

- **Study termination criteria**. Completion of the 30-day follow-up period of the last patient included in the study.
- **Patient completion criteria**. Completion of the follow-up 30 days after the intervention.
- **Patient discontinuation criteria**. At least one of the following must be met:
  - Serious adverse events related to drug administration.
  - Development of contraindications or study exclusion criteria within the inclusion period and prior to surgery.
  - Voluntary drop-out of previously included patients.

12. Informed Consent (IC) and Patient Information Sheet (PIS)

The investigator will explain to each patient (witness or legal representative) the nature of the study/trial and the purposes, procedures, estimated duration, possible risks and benefits of their participation in the study/trial and possible inconveniences that this may cause them. All patients will be informed of the voluntary nature of their participation and that they may withdraw at any time, without affecting their future medical care or their relationship with the physician responsible for their treatment.

The patient shall be given sufficient time to read and understand the explanations included in the patient information sheet before giving informed consent (Annex 3). No patient shall be included in the study/trial before informed consent has been obtained.

Each patient will be assigned to an intervention group which will involve the administration of a dose at a predetermined time. All patients will receive the study product as there is no placebo group. The reason for the existence of 4 comparative groups is to study the efficacy of different dosage and interval of administration of the IV. Participation in the study

does not involve a different surgical procedure or an increase in the number of scheduled visits.

13. Data Collection Notebook -CRD-.

The data collected will be recorded in a CRD, the veracity of which will be validated by the designated monitors. Each patient will be identified in the CRD by means of an alphanumeric code that identifies the patient (and the centre if it is a Multicentre) and in correlative order. The data protection provisions of Organic Law 3/2018 on Personal Data Protection and guarantee of digital rights will be respected at all times.

The data will be obtained from the personal interview with the patient and access to the patient's clinical history. If the patient does not attend any of the scheduled visits, the investigator will contact the patient by telephone to confirm that the absence is not due to a conscious abandonment of follow-up. If the patient is not contacted, the researcher will access the local or regional digital medical record to find out if there have been any events related to the study that have been assessed in another health centre.

The procedures for correctly completing the data collection notebook will be through the specific sessions of the General and Digestive System Surgery Department, by means of the presentation and explanation of the notebook. In addition, any doubts and problems that may arise will be resolved.

The specific requirements will be determined by the electronic data collection record keeper:

1. The principal investigators (PIs) will be the only individuals who can sign the registry. These individuals have all functions with the exception of the monitor function and the electronic data collection logbook manager (CRDe).

2. Sub-researchers and study coordinators may include data in the study, but may not sign the registry. You can resolve *queries*.

3. The monitor will be able to review the data and generate *queries*.

4. The data manager has the power to block-unblock the electronic data collection logbook. In addition, he/she is responsible for data management. He/she has access to all the centres included in the study.

Randomisation will be carried out using algorithms proposed by the CRD. Block randomisation will be carried out.

All patient data will be included for the first 45 days after the patient's surgery.

14. Medical History -HC- and source documents

The electronic medical record will be available through the Jimena computer software (version III and version IV) of the Castilla y León Health System (SACYL) and the SAP software of the Catalan Health System. In addition, those paper documents that must be kept in the medical record and cannot be computerised (informed consent for surgery, informed consent for clinical trials, surgical protocol, hospital discharge report), will be collected in the paper medical record of each patient, and stored in the Clinical Documentation Service of the University Hospital of Salamanca and the University Hospital of Salamanca.

Germans Trias i Pujol University of Badalona. Paper documents can be scanned and stored in the digital repository of both servers.

Definitions:

- Source data: All information in original documents and certified true copies of original records of clinical results, observations, or other activities of a clinical trial necessary for the reconstruction and evaluation of the trial. Source data shall be included in the source documents (original records or certified copies).
- Source documents: original documents, data and records (e.g., patient records, medical or consultation records, laboratory notes, memoranda, registers, data recorded on automated instruments, copies or transcripts certified after verification, microfiche, photographic negatives, microfilm or magnetic media, X-rays, subject files, and pharmacy records in the laboratories and in the medico-technical departments involved in the clinical trial).

Patients must have given written consent for their medical records to be read by personnel authorised by the sponsor and regulatory authorities. This information is included in the informed consent.

The following data specific to this CB shall be collected in the HC and shall also be collected in the CRDe:

- **Pre-operative period:**
  - Date of birth (dd-mm-yyyy), age (years), sex (male/female), weight (kg), height (cm), body mass index-BMI (kg/m2), anaesthetic-surgical risk classification (American Association of Anaesthesiologists-ASA classification), previous abdominal surgeries (biliary tract, liver, pancreas, intestinal, others), previous endoscopic or radiological explorations of the biliary tract (endoscopic retrograde cholangiopancreatography (ERCP) or endoscopic retrograde cholangiopancreatography (ERCP)), liver, pancreas, intestinal, others), previous endoscopic or radiological explorations of the biliary tract (endoscopic retrograde cholangiopancreatography (ERCP) or transparihepatic cholangiography (TPHC)), previous biliary prosthesis placements (yes/no). History of acute cholecystitis (yes/no).
    /previous acute cholangitis (yes/no). History of previous percutaneous gallbladder drainage (no/radiological percutaneous cholecystectomy or endoscopic transduodenal/transgastric). Previous liver disease (no/fatty liver steatosis/hepatitis/cirrhosis).
  - Indication for surgery [Symptomatic cholelithiasis (biliary colic, acute cholecystitis, choledocholithiasis, acute ascending cholangitis, acute lithiasic pancreatitis), gallbladder polyps, gallbladder adenomyomatosis].

- **Perioperative period:**
  - Date of entry (dd-mm-yyyy).
  - Time of entry (hour:minutes)
  - Date of surgery (dd-mm-yyyy).
  - Time of surgery (hour:minutes).

- Type of surgery (elective/early/ delayed emergency).
- Grade of cholecystitis in case of early surgery / delayed emergency (18th Tokyo Classification).
- Intervention group (group 1/group 2/group 3/group 4).
- Characteristics of the procedure. IV dosage (fixed dose/weight-adjusted dose). IV administration interval (>3 hours preoperatively, 15-30 minutes preoperatively). Immediate adverse reactions (yes/no. Specify). Number of trocars (3/4). Imaging system used (Olympus/Karl-Storz/Stryker). Optics used (0º/30º). Gall bladder puncture/emptying (yes/no). Bile culture (yes/no). Result of bile culture if any (specify).
- Conversion to laparotomy (yes/no), intraoperative complications (yes/no), specification of intraoperative complications (cystic artery injury, right hepatic artery injury, liver injury with/without bleeding, gallbladder opening (intraperitoneal biliary contamination: yes/no, lithiasis outflow into peritoneal cavity: yes/no), hollow viscus perforation, trocar bleeding, other). Operating time (minutes).

- **Postoperative period:**
  - Hospital stay (days), postoperative complications (intra-abdominal haematoma, bilioma, abscess, paralytic ileus, surgical site infection-ISQ, trocar evisceration, trocar eventration, others), postoperative complications according to Clavien-Dindo classification,bile duct injury (Strasberg classification), time of diagnosis bile duct injury (intraoperative / postoperative), treatment of bile duct injury (immediate surgical, radiological, endoscopic or delayed surgical). Mortality during admission (yes/no), 30-day mortality (yes/no), causes of mortality (specify). Adverse drug reactions within 30 days (yes/no. Specify).

All preoperative data will be collected for patients withdrawn from the study, as well the type of complication developed and any adverse events during the first 30 days of the study (if any). Subjects will not be replaced in the study. The follow-up period will be identical for subjects who do not undergo the study intervention.

15. Monitoring

Required data to be accurately recorded in CRDs and agree with source data. Revised according to the Monitoring Manual.

- Patient identification.
- Date and time of surgery.
- Dose and time of VI administration.
- Target variables (set out above).

After each monitoring, the monitor will be able to generate a discrepancy document with the inconsistencies and errors detected in the study. These problems can be communicated through the CDRe or by e-mail.

16. Researcher and collaborators

The principal investigator shall maintain a register of all persons involved in the trial (medical, nursing, and other personnel). He/she shall confirm that appropriate training relevant to the trial is given to all such personnel and that any new information relevant to the conduct of the trial is conveyed to the personnel involved.

17. Facilities

- **Hospitalisation ward**. This is where the patient is admitted prior to surgery. This is where all mandatory documentation is checked and the patient is prepared for surgery.
- **Pre-operative conditioning room**. A surgical checklist and a new preparation of the patient will be performed.
- **Operating theatres**. In addition to the equipment necessary for any laparoscopic surgical activity, which is no different from the study activity, these operating theatres will be equipped with the necessary equipment to perform CF-VI (optics, light cable and compatible image processing system).
- **Postoperative recovery room**. Controlled by the Anaesthesiology and Resuscitation Service.
- **Outpatient clinics**. Place where the postoperative control will be carried out after the patient is discharged.
- **Hospital Pharmacy Service**. Responsible for dispensing the medicine.
- **Operating theatre storeroom**. This is where the necessary equipment and drugs are stored, including the active ingredient of the study.

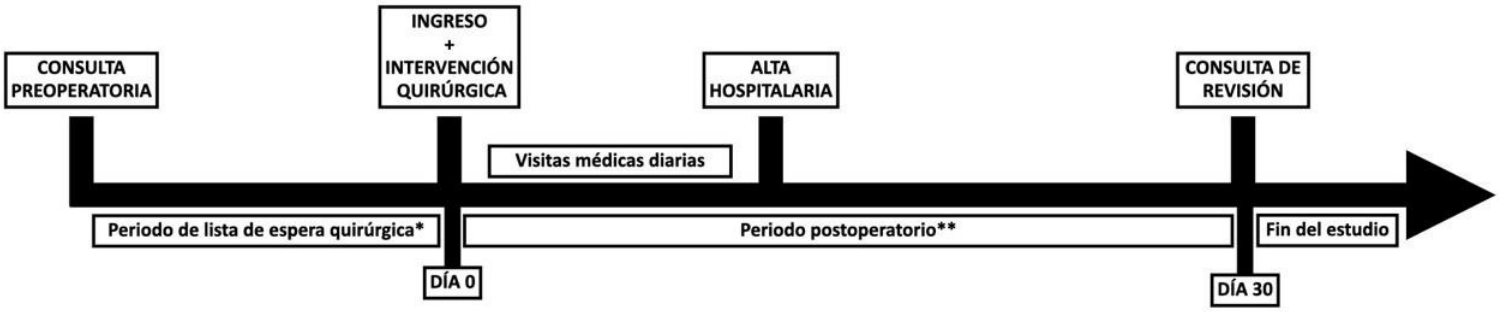
18. Treatment of Subjects

Subjects included in this CE will be treated with VI [Verdye (Diagnostic Green GMBH, Aschheim-Dornach, Germany)]. The following patient groups are established in the CE.

- **Group 1**. Fixed dose 2.5 mg with IV administration more than 3 hours before surgery.
- **Group 2**. Fixed dose 2.5 mg with immediate pre-operative VI administration (15-30 minutes before surgery).
- **Group 3.** Weight-adjusted dose (0.05 mg/kg total body weight) with administration of the LV more than 3 hours before surgery.
- **Group 4.** Weight-adjusted dose (0.05 mg/kg total body weight) with immediate pre-operative administration of the IV (15-30 minutes prior to surgery).

Verdye contains the active substance VI sodium powder for solution for injection. Each ampoule contains 25 mg of active substance. The dissolution medium for the product should be water for injectable preparations. The solution for injection should be inspected before administration and discarded in case of turbidity. The drug solution shall be prepared with 10 ml of water for injectable solutions, obtaining a ratio of 2,5 mg/ml. Once the solution is prepared according to the above protocol, it shall be injected directly through a peripheral venous line of the patient's upper limb. The medicinal product should be stored at a temperature below 30°C, keeping its original outer packaging in order to protect the medicinal product from light. Once the solution for injection has been prepared, it should be protected from light and used immediately.

The expected duration of subject participation will be 31 days, including preoperative, intraoperative, immediate postoperative (<24 hours), early postoperative (<7 days) and late postoperative (<30 days) periods. The patient recruitment period will be 12 months. The end of the clinical trial will take place from day 30 after discharge of the last patient recruited, with the aim of analysing the 30-day morbidity and mortality of the study patients.



* El periodo de lista de espera quirúrgica podría variar entre pacientes según necesidades asistenciales.
** El periodo postoperatorio normal tras colecistectomía laparoscópica programada es de 24-48 horas. No obstante, puede ampliarse debido a complicaciones y/o necesidades.

The frequency of patient visits will be as follows:

1- **Consultation prior to admission**. The objective of the study and the entire study protocol will be explained, along with the delivery of the HIP and the IC.

2- **Visit prior to surgery.** This visit will corroborate the correct understanding of the information, the presence of the correctly signed ICs and the resolution of any doubts or problems.

3- **In-hospital post-operative visits**. These will be carried out daily in the morning shift (8-15 hours). No different from postoperative visits of other operated patients.

4- **Postoperative control visit**. This will take place 30 days after the surgical intervention through the General and Digestive System Surgery Outpatient Department.

There is no treatment period as it is considered a diagnostic method. This period shall include intraoperative time.

A computerised block randomisation will be performed through CDRe. Participants will be assigned to each of the 4 treatment groups by non-stratified 1:1:1:1:1 randomisation.

As it is an intervention without a control group, blinding will not be performed in the trial. The

end-of-study criterion will be completion of the follow-up period at the end of the trial.
30 days from the last patient included in the study.

19. Effectiveness Assessment

The following efficacy parameters shall be used:

- Degree identification of biliary structures prior to dissection of the hepatocystic triangle (1=poor, 2=sufficient, 3=sufficient, 4=good, 5=excellent).
- Degree of identification of biliary structures after dissection of the hepatocystic triangle (1=poor, 2=sufficient, 3=sufficient, 4=good, 5=excellent).
- Degree to which FC was perceived as useful for surgery (0=not useful, 1=moderately useful, 2=very useful).
- Degree to which the fluorescence of the liver background (contrast between liver and ducts) was perceived as disturbed (0=absence of disturbance, 1=slightly disturbed, 2=disturbed visualisation, but cystic-choledochal junction was clearly visible before dissection, 3=disturbed visualisation and cystic-choledochal junction was only visible after dissection, 4=very disturbed: it was impossible to visualise the biliary structures correctly.

20. Security Assessment

20.1. Definitions

- **Adverse Event (AE):** An adverse event is any undesirable medical reaction experienced by the patient at any time during the course of the study, whether or not it is considered to be related to the study treatment. This definition includes new disease onset and exacerbation of pre-existing conditions other than the indication under study.

- **Adverse Reaction (AR):** An AR is any unintended, harmful reaction to an investigational medicinal product, regardless of the dose administered.

- **Serious Adverse Event (SAE) and Serious Adverse Reaction (SAR)**
  Serious AEs or ARs are considered to be those which, at any dose, may result in **death, threaten the life of the subject, require hospitalisation of the patient or prolong an existing hospitalisation, cause permanent or major disability or incapacity, or result in a congenital anomaly or malformation**. **Suspected AE or AR that is medically significant**, even if it does not meet the above criteria, including major medical events that require intervention to prevent one of the consequences described above, **are also considered serious**. In addition, all suspected transmission of an infectious agent via a medicinal product shall be reported as serious.

  The concept of "threatening the life of the subject" in the definition refers to the fact that, in the opinion of the investigator, the patient at the time of the AA or AR is at real risk of death; it does not refer to the fact that the AA/AR hypothetically could have resulted in death had it been more severe.

  The term "requiring hospitalisation" shall exclude both planned hospitalisations for scheduled treatments and hospitalisations that have been planned or are anticipated prior to the start of the study in relation to a pre-existing medical condition.

- **Unexpected Adverse Reaction (UAR)**
  Any AR whose nature, intensity or consequences do not correspond to the reference safety information.

- **Serious Unexpected Adverse Reaction (SUAR)**
  RAG (previously defined), the nature, severity or consequences of which do not correspond to the reference safety information.

20.2. Causality Criteria (see section 20.7)

• **Related AA:** The temporal relationship of the AA with the study medication indicates a possible causal relationship and cannot be explained by factors such as the patient's clinical status, therapeutic interventions.

• **Unrelated AA:** The temporal relationship of the AA to the study medication indicates an unlikely causal relationship, or other factors (medication or concomitant conditions), other therapeutic interventions provide a satisfactory explanation for the AA.

20.3. Security reference information

In this study, the reference safety information (RSI) will be the information available in the corresponding product label of the medicinal product under study: Verdye.

20.4. Safety variables and parameters

The safety assessment of the drugs under study will be carried out by means of clinical examination (physical, analytical and/or radiological) and collecting serious adverse effects and those that may be directly related to the medication under study.

The key safety parameter will be the bile duct injury rate, which is currently at incidences of 0.3%-0.7%.

20.5. Information on Adverse Events

All adverse events shall be recorded in the data collection notebook. For each adverse event, the dates of onset and resolution, intensity, duration, and its impact on study treatment (e.g. discontinuation) shall be recorded. The severity of the adverse event and its relationship to the study should be assessed according to specific guidelines. Any action taken or outcome achieved (e.g. hospitalisation, withdrawal of treatment, etc.) should also be noted.

The investigator or a co-worker shall question and/or examine the subject for the occurrence of adverse events.

Adverse events will be collected once the subject has been randomised. If a subject experiences an adverse event after signing the informed consent document (inclusion), but prior to treatment assignment (recruitment), the event will NOT be recorded unless the investigator considers it to be causally related to a procedure in the protocol.

During the course of the study, site staff will again note any changes in the disorder and nature of possible adverse events.

20.6. Assessment of the intensity of adverse events and their relation to treatment

The following definitions shall be used to assess the intensity of adverse events:

- Mild: sign, symptom or event is known but easily tolerated.

- Moderate: there is sufficient discomfort to interfere with normal activity and may warrant intervention.

- Severe: is disabling and results in inability to perform usual activities or significantly affects the clinical condition and warrants intervention.

- Life-threatening: immediate risk of death.

- Death

20.7. Assessment of the imputability of adverse events and serious adverse events with study treatment: Imputability criteria.

The sponsor will classify adverse events, based on their causal relationship with the drug, according to the Algorithm of Karch and Lasagna (1977), as follows:

- **Definitive:** there is a reasonable time sequence between the administration of the drug and the appearance of the AE. The event coincides with the ARs described for the drug, improves with drug withdrawal, reappears after re-administration and cannot be explained by alternative causes.

- **Probable:** there is a reasonable time sequence between the administration of the drug and the occurrence of the AE. The event coincides with the ARs described for the drug, improves after discontinuation of treatment and cannot be explained by other alternatives.

- **Possible:** there is a reasonable time sequence between drug administration and the occurrence of AA. The event coincides with the ARs described for the drug but can be explained by alternative causes.

- **Conditional or Improbable:** there is a reasonable time sequence between the administration of the drug and the occurrence of the AE. The event does not coincide with the ARs described for the drug and can be explained by alternative causes.

- **Unrelated:** there is no reasonable time sequence between the administration of the drug and the occurrence of the AE. The event does not coincide with the ARs described for the drug and can be explained by alternative causes.

For the purposes of expedited reporting, the definite, probable and possible categories of the Karch and Lasagna (1977) algorithm shall be considered as related and the conditional or improbable category of the Karch and Lasagna (1977) algorithm shall be considered as unrelated.

The determination of the possible relationship to the study treatment is the responsibility of the principal investigator of the research site or his/her designee and, once notified, will be reviewed by the study sponsor and/or his/her designee.

20.8. Monitoring of adverse events

Subjects with adverse events will be monitored by appropriate clinical assessments and laboratory tests as directed by the investigator. All adverse events should be tracked until their satisfactory resolution or their

stabilisation. All actions taken and monitoring results should be recorded in the appropriate section of the data collection notebook. If abnormalities in laboratory values are clinically significant and result in clinical symptoms or meet the criteria for a serious adverse event, the diagnosis shall be reported as a serious adverse event.

20.9. AAG notification, expedited notification and notification of pregnancy

Serious Adverse Event Notification

All serious adverse events (as defined below), whether or not they are considered treatment-related or expected, should be reported to the Pharmacovigilance department by the principal investigator or a collaborator within **24 hours** (one working day) of becoming aware of them, using the appropriate reporting form. Serious adverse events occurring at any time from the patient's randomisation into the study until the subject completes or leaves the study must be reported. A subject is considered complete: upon completion of the last visit or contact (e.g., telephone contact with the investigator or a collaborator) as indicated in the protocol evaluation schedule or after the last dose of study medication, whichever is later. Withdrawal is defined as the date on which a subject and/or the investigator determines that the subject can no longer meet the study requirements at any subsequent visits and assessments. AAG follow-ups will follow the same form indicating follow-up and should be submitted within 7 days, (noting that it is the "follow-up" form). The follow-up report will indicate whether the event has resolved or is still active, whether and how it has been treated, and whether the patient's participation in the trial has continued or ended. The trial site will retain the form.

The notifying investigator should complete and sign the AAG notification form (Annex 4) for the trial and send it by fax or e-mail to:

PhD Patricia Rodríguez Fortúnez

Head of Pharmacovigilance

Telephone: 615510900

E-mailpatriciarodriguezfortunez@yahoo.es

The PV staff shall review the form received and, if appropriate, request additional information from the investigator. The investigator shall provide information to the sponsor or to the person assuming the tasks delegated by the sponsor (PV Manager) whenever requested to do so, and in any case when the investigator's initial assessment of severity or causality changes.

The reporting procedure described above shall be followed for communicating follow-up information.

The original copy of the AAG Forms (either initial or follow-up) shall be retained at the trial site, along with all other trial documentation.

The PV manager shall keep a detailed record of all AAGs or of special interest reported to him/her by the investigators.

In the event that a medication error occurs or the investigational medicinal product is used outside the protocol during the conduct of the trial, the investigator shall notify the VF manager within 24 hours of becoming aware of it. The reporting circuit and form will be the same as for AAGs.

Serious adverse events are those AEs that meet the severity criteria described in the definitions section. Clinically significant events that not life-threatening, life-threatening or require hospitalisation may be considered as serious adverse drug experiences when, based on sound medical judgement, they are likely to endanger the subject or require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events are allergic bronchospasm requiring intensive, home or emergency department treatment, blood dyscrasias or seizures not resulting in hospitalisation, or development of dependence or substance abuse.

Laboratory test abnormalities that are considered clinically relevant also need to be reported, unless otherwise stated in this section of the protocol.

Expedited notification of RAGIs

The sponsor or the person delegated by the sponsor is responsible for notifying the EMA/AEMPS and the Autonomous Regions where the trial is conducted of all RAGI collected in the study, following the procedure indicated in the legislation in force.

The maximum period for notification of an individual case of suspected RAGI shall be 15 calendar days from the time the sponsor becomes aware of the suspected RAGI. Where the suspected RAGI has resulted in the death or endangerment of the patient, the sponsor shall submit the information within 7 calendar days of becoming aware of the suspected . He/she shall complete the information, if possible, within 8 days.

This information should include an assessment of the significance and implication of the findings, including relevant previous experience with the same or similar medicinal products.

Likewise, the competent body of each of the Autonomous Communities where the trial is carried out should be notified of suspicions of RAGI occurring in the centres.

health services in your Community. In both cases, the RAGI notification form shall be used for this purpose.

Expedited notification of other relevant safety information

The sponsor or its delegate should report as soon as possible and no later than 15 days after becoming aware of it any information that could change the risk/benefit balance of the investigational product (e.g. an increase in the rate of occurrence of expected AGRs, AGIRs occurring after completion of a clinical trial, new events related to the conduct of the trial or the development of the investigational product, any recommendations of the data monitoring committee relevant to subject safety, etc.).

Pregnancy Notification

In the event of any pregnancy occurring during the conduct of the trial, the investigator shall notify the sponsor or the person assuming the tasks delegated by the within 24 hours of knowledge of the pregnancy. The pregnancy will also be followed up to document the outcome of the pregnancy and the health status of the newborn. If the outcome of the pregnancy meets GABA criteria or if the newborn presents a serious event, the procedures described for GABA notification will be followed. Notification shall be made using the specific pregnancy notification form (Annex 5) which shall be sent by e-mail to the same contact person who will receive the HCA notifications.

The original copy of the Pregnancy Form will be kept at the trial site, along with the rest of the study documentation.

20.10. Blind opening and unmasking

Not applicable as the study is open-ended and therefore there is no blind.

20.11. Periodic security report

The annual safety reports, which will include the RAGIs and AAGs collected in the study from the time the patient is randomised, will be sent by the sponsor or the person delegated by the sponsor to the AEMPS (Clinical Trials Area of the Subdirectorate General for Medicines for Human Use), to the corresponding Autonomous Regions and to the CEIm, within the deadlines established in the legislation in force.

The submission of the annual safety report and other safety reports by the developer shall in any case comply with the criteria and procedure specified in Articles 43, 45 and 53 of Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 and in accordance with RD1090/20015.

20.12. Report to researchers

The sponsor will submit safety information that could impact the safety of the patients enrolled in the trial to the investigators as early as possible. In addition, the investigator will be informed throughout the study of any safety issues, including protocol modifications due to safety concerns.

21. Statistics

For the calculation of the sample size we estimated an average annual number of laparoscopic cholecystectomies of 300 cases. Assuming a minimum recruitment rate of 50% in the study, a duration of 12 months and possible losses of patients, we estimate a sample size of 200 patients.

Since the main objective is to analyse whether there are differences between the doses and administration times of the 4 treatment groups in which categorical variables will be measured, the effect of the chosen sample size (n= 200) on the statistical contrasts in this type of variables is w=0.25 with a power.95 and a significance error of the null hypothesis α=0.05.

The procedure used to account for missing, unused and erroneous data will be the "*Pairwise Delection*" method. The confidence intervals of the statistics will be 95% and the contrasts will be performed at a significance level of 5%. All analyses will be performed with the free software R (version 4.1.2+, R Core Team, Vienna, https://www.R-project.org/) and the RStudio interface (version 1.4.1103+).

The study will have no planned intermediate analyses as no partial analysis of results is envisaged. Once all data have been collected, verified and validated, a statistical analysis of the data will be performed according to the specifications described in the protocol. If this analysis leads to the conclusion that further analysis is necessary, this will be reported in the final analysis of results. All treated and evaluable subjects shall be analysed. Different statistical tools will be used depending on the nature of the variables collected in the study, accompanying the results with their corresponding graphs.

Lilliefors-Kolmogorov-Smirnov and Shapiro-Wilks tests shall be performed to study the normality of the distribution of quantitative variables, and to their distribution they shall be represented by the mean (standard deviation) or the median (median deviation) and may be accompanied by their corresponding confidence intervals. The relationship between such variables shall be measured with Pearson's or Spearman's correlation coefficients.

The distributions of the categorical variables will be studied by means of frequencies and percentages. To represent and measure the dependence between them, contingency tables will be used with Pearson's Chi-square test, Fisher's exact test, McNemar's test

or Cochran's Q. A category comparison study could also be done using odds ratios between categories of clinical interest. To study the influence that some categorical variables might have on quantitative variables, ANOVA tests could be performed.

Finally, all the statistical information obtained on the relevance of each variable in the study could be used to apply classification tools to the individuals in the study (decision trees, logistic regression, ROC curves).

22. Direct Access to Source Data/Documents

The Promoter will enter into appropriate arrangements to appoint a monitor to oversee this study and to liaise regularly with the site, including arranging site visits. The investigator agrees to allow the monitor direct access to all relevant documents and to give his/her time and that of his/her staff to the monitor to discuss the results and any issues of concern.

23. Quality Control and Quality Assurance

In order to ensure the quality of the data, the promoter:

• Provide quality instruction and training to the research staff prior to the start of the study. Training topics include, but are not limited : Good Clinical Practice (GCP) certificates, Adverse Event (AE) reporting, study details and procedures, study documentation, informed consent, and patient recruitment.

• Conduct regular monitoring in accordance with the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP). Data will be assessed for compliance with the protocol and for accuracy in relation to the source documents. Monitors will verify that the clinical trial is conducted, that data are generated, documented and reported in compliance with the protocol, good clinical practice and any applicable local regulatory requirements.

Direct access to the data should be granted to the PIs, monitors, members of the evaluating IRB/IEC and AEMPS, as well as to the authorities as necessary, to allow for study-related monitoring and surveillance, audits and inspections. At each site, they may review the trial records and compare them directly with the source documents, may discuss the conduct of the trial with the investigator, and may verify that the facilities are maintained in an acceptable manner. Audit reports will be treated confidentially.

24. Ethical and legal aspects.

The trial will be conducted in accordance with the recommendations for clinical trials and drug evaluation in man, as contained in the 1964 Declaration of Helsinki, as revised in Tokyo, Venice, Hong-Kong, South Africa, Edinburgh, Washington, Tokyo and Seoul, Fortaleza (2013) and in the AEPMS Instructions for the conduct of clinical trials in Spain (Version 9 of 27 July 2018). The investigator will conduct the study in compliance with the ethical principles of the Declaration of Helsinki.

The study/trial must adhere to the protocol, which ensures compliance with Good Clinical Practice, as described in the ICH Tripartite Harmonised Guidelines for Good Clinical Practice.

This study/trial will be submitted for evaluation by a Medicines Research Ethics Committee and notified to the AEMPS.

25. Data Management and Archiving of Records

The confidentiality of individual patient data will be respected at all times. The original data will be kept in the relevant department and will only be accessible to the study/trial investigators or to inspectors in case of inspection the Spanish Health Authorities.

Patients in the study/trial will be identified by a patient code. The investigator will inform patients that data collected during the study/trial will be stored and analysed electronically, in compliance with Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of individuals with regard to the processing of personal data and on the free movement of such data and with Organic Law 3/2018 of 5 December on the Protection of Personal Data).

The researcher is the only person who can and should know the origin of the data obtained and who can associate it with the patient.

The personal data (full name, address, place of work, VAT number) of the investigators will be recorded in a computer file, for the sole purpose of facilitating the organisational and logistical aspects necessary for the conduct of the study/trial.

In compliance with the above regulations, the data file will be treated confidentially and researchers may exercise their rights of access, rectification, cancellation and opposition with respect to the personal data recorded, if they so request by writing to the Data Protection Officer of the promoter at protecciondedatos@ibsal.es

26. Financing and Insurance

The trial sponsor is responsible for ensuring that an insurance or financial guarantee is in place to cover damages incurred as a result of the clinical trial.

This clinical trial is considered a low-intervention level clinical trial accordance with RD 1090/2015 and European Regulation No. 536/2014 due to the following conditions:

1. The medicinal product (indocyanine green) studied in the present project is used in accordance with the authorised label.
2. In the present trial, no additional procedures are performed with respect to those that would have been performed on participants in the context of routine clinical practice, both in Spain and in the European Union and other international settings.

Therefore, and in accordance with chapter III, article 9 of Royal Decree 1090/2015: "Damage to the study subject that may result as a consequence of a low-intervention clinical trial will not need to be covered by an insurance contract or financial guarantee, if they are covered by the individual or collective professional liability insurance or equivalent financial guarantee of the healthcare centre where the clinical trial is carried out"; no *ad hoc* insurance has been taken out for this clinical trial.

27. Other test procedures. Management Delivery of other test materials

No "extra" materials are required for the realisation of this CE. All materials are available at participating CE centres.

Details of the documentation to be submitted to the Protocol are set out in Annex 2.

28. Audits and inspections

Health authorities or IRB/IECs may visit the site to conduct audits or inspections with verification of the original data. The objective of this audit is to systematically and independently evaluate all trial-related activities and documentation to determine whether these activities have been performed and whether the data have been analysed and reported in accordance with the protocol, GCP Standards, ICH Guidelines, and legal requirements.

29. Changes to the protocol

If an amendment to the trial protocol is necessary, the amendment or a new version of the trial protocol (amended protocol) should be notified to the IRB/IEC for approval by the IRB/IEC and, if applicable, also by the health authorities, prior to implementation. Local requirements should be met.

If an amendment to the protocol requires a change in the informed consent for a particular site, the IRB/IEC must be notified. Approval by the IRB/IEC is required prior to use of the new version of the consent form. The sponsor will distribute the amendments and new versions of the protocol to each principal investigator, who in turn is responsible for distributing these documents to the site staff. The distribution of these documents to the health authorities will follow the applicable regulations.

30. Difficulties and limitations of the study

Some of the difficulties could be found in the period of patient selection and inclusion in the study, due to the presence of numerous exclusion criteria. Other difficulties arise during the intervention, due to alleged problems arising from the use of fluorescent technology or laparoscopic surgical equipment. The subjectivity inherent in the visual assessment of the target variables and the presence of the surgeon factor is another limitation of the study. Finally, four groups with specific doses and administration periods are being evaluated, which could lead to the establishment of multiple different combinations, although methodologically impossible to carry out with an adequate number of cases.

31. Periodic Security Reports (DSUR)

Following the standards of good clinical practice and current regulations, the Pharmacovigilance Officer will prepare a safety report once a year. This report will contain the safety assessment of the drug according to all AAGs received by the Node's Pharmacovigilance Officer.

The Pharmacovigilance Officer will send the DSUR to the Project Manager for review, indicating the need for a response within 15 days and the deadline.

The Pharmacovigilance Officer will then send the DSUR to the Sponsor for approval, indicating the need for a response within 15 days and the deadline.

All modifications suggested by the GP or sponsor will be carried out by the Pharmacovigilance Officer.

The latest version of the DSUR will be signed by the PV manager, then by the GP (as reviewer) and finally by the developer giving his approval.

The annual safety report (DSUR) shall be sent by the developer to:

- AEMPS: via ECM portal using "E iv) Annual Safety Report (DSUR) [Only AEMPS]"
- CEIm del Área de Salud de Salamanca: via ECM portal using "E ii) Trial progress ".
- Contact points for clinical trials in the Autonomous Communities via email (https://).www.aemps.gob.es/investigacionClinica/ptoContacto.do)

The Project Manager will send a copy of the acknowledgement of receipt from the AEMPS to the Pharmacovigilance Officer.

Follow-up and completion reports

The Sponsor, in accordance with the current regulations and instructions regulating clinical trials by the AEMPS, will send the relevant follow-up reports.

Three months after the completion of patient enrolment, a statistical report including all results of the study shall be drawn up. This report shall be made available to the participating investigator. In addition, copies of the report shall be sent to the IRB that evaluated the study and to the AEMPS.

Any report obtained from this study will be treated as confidential, at least until it has been reviewed and analysed by the person responsible for the study. The data set may be used for general publications, which should always refer to the study. The investigator agrees not to provide information related to the study or to provide access to the study data to third parties.

32. Publication Policy

Regardless of the results of the study, the sponsor agrees to present them to the medical community through scientific publications, conferences or other means.

Any formal presentation or publication of the results of the study shall be considered a joint publication by the investigator(s) and the sponsor.

33. References

1. Ansaloni L, Pisano M, Coccolini F, Peitzmann AB, Fingerhut A, Catena F, et al. 2016 WSES guidelines on acute calculous cholecystitis. World J Emerg Surg 2016;11:25.

2. Tazuma S, Unno M, Igarashi Y, Inui K, Uchiyama K, Kai M, et al. Evidence-based clinical

practice guidelines for cholelithiasis 2016. J Gastroenterol 2017;52(3):276-300.

3. Pesce A, Piccolo G, Lecchi F, Fabbri N, Diana M, Feo CV. Fluorescent cholangiography: An up-to-date overview twelve years after the first clinical application. World J Gastroenterol 2021;27(36):5989-6003.

4. Nuzzo G, Giuliante F, Giovannini I, Ardito F, D'Acapito F, Vellone M, et al. Bile duct injury during laparoscopic cholecystectomy: results of an Italian national survey on 56591 cholecystectomies. Arch Surg. 2005;140:986-92.

5. Strasberg SM, Hertl M, Soper N. An analysis of the problem of biliary injury during laparoscopic cholecystectomy. J Am Coll Surg 1995;180(1):101-25.

6. Gupta V, Jain G. Safe laparoscopic cholecystectomy: Adoption of universal culture of safety in cholecystectomy. World J Gastrointest Surg 2019;11(2):62-84.

7. Mascagni P, Vardazaryan A, Alapatt D, Urade T, Emre T, Fiorillo C, et al. Artificial Intelligence for Surgical Safety: Automatic Assessment of the Critical View of Safety in Laparoscopic Cholecystectomy Using Deep Learning. Ann Surg 2020 [Epub ahead of print].

8. Flum DR, Dellinger EP, Cheadle A, Chan L, Koepsell T. Intraoperative Cholangiography and Risk of Common Bile Duct Injury During Cholecystectomy. JAMA 2003;289(13):1639.

9. Vlek SL, van Dam DA, Rubinstein SM, de Lange-de Klerk ESM, Schoonmade LJ, Tuynman JB, et al. Biliary tract visualization using near-infrared imaging with indocyanine green during laparoscopic cholecystectomy: results of a systematic review. Surg Endosc 2017;31(7):2731-42.

10. Ishizawa T, Bandai Y, Ijichi M, Kaneko J, Hasegawa K, Kokudo N. Fluorescent cholangiography illuminating the biliary tree during laparoscopic cholecystectomy. Br J Surg 2010;97(9):1369-77.

11. Reinhart MB, Huntington CR, Blair LJ, Heniford BT, Augenstein VA. Indocyanine Green: Historical Context, Current Applications, and Future Considerations. Surg Innov 2016;23(2):166-75.

12. Lim SH, Tan HTA, Shelat VG. Comparison of indocyanine green dye fluorescent cholangiography with intra-operative cholangiography in laparoscopic cholecystectomy: a meta-analysis. Surg Endosc 2021;35(4):1511-20.

13. Dip F, LoMenzo E, Sarotto L, Phillips E, Todeschini H, Nahmod M, et al. Randomized Trial of Near-infrared Incisionless Fluorescent Cholangiography. Ann Surg 2019;270(6):992-9.

14. Dip F, Lo Menzo E, White KP, Rosenthal RJ. Does near-infrared fluorescent cholangiography with indocyanine green reduce bile duct injuries and conversions to open surgery during laparoscopic or robotic cholecystectomy? - A meta-analysis. Surgery 2021;169(4):859-67.

15. van den Bos J, Wieringa FP, Bouvy ND, Stassen LPS. Optimizing the image of fluorescence cholangiography using ICG: a systematic review and ex vivo experiments. Surg Endosc 2018;32(12):4820-32.

16. Verbeek FPR, Schaafsma BE, Tummers QRJG, van der Vorst JR, van der Made WJ, Baeten CIM, et al. Optimization of near-infrared fluorescence cholangiography for open and laparoscopic surgery. Surg Endosc 2014;28(4):1076-82.

17. Widjaja SP, Fischer H, Brunner AR, Honigmann P, Metzger J. Acceptance of Ambulatory Laparoscopic Cholecystectomy in Central Switzerland. World J Surg 2017;41(11):2731-4.

18. Agnus V, Pesce A, Boni L, Van Den Bos J, Morales-Conde S, Paganini AM, et al. Fluorescence-based cholangiography: preliminary results from the IHU-IRCAD-EAES EURO-FIGS registry. Surg Endosc 2020;34(9):3888-96.

19. Dindo D, Demartines N, Clavien P-A. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 2004;240(2):205-13.

20. Yokoe M, Hata J, Takada T, Strasberg SM, Asbun HJ, Wakabayashi G, et al. Tokyo Guidelines 2018: diagnostic criteria and severity grading of acute cholecystitis (with videos). J Hepatobiliary Pancreat Sci 2018 J;25(1):41-54.

ANNEXES

Annex 1: Classifications

Classification of postoperative complications (Clavien-Dindo classification)(19)**:**

- **Grade I.** Any deviation from the normal postoperative course not requiring pharmacological, endoscopic, radiological or surgical treatment. The following are allowed: antiemetics, antipyretics, analgesics, diuretics, electrolytes and physiotherapy. Includes drainage of the surgical wound infection on the hospital ward.
- **Grade II.** Use of other medication not included in Grade I (blood transfusion or parenteral nutrition).
- **Grade III.** Complications requiring surgical, radiological or endoscopic intervention:
  - **Grade IIIA.** Interventions without general anaesthesia.
  - **Grade IIIB.** Interventions under general anaesthesia.
- **Grade IV.** Severe complications requiring admission to the Intensive Care Unit.
  - **Grade IVA.** Organ failure, including dialysis.
  - **Grade IVB.** Multi-organ failure.
- **Grade V.** Death of the patient.

LVB classification (Strasberg)(5)**:**

- **Type A.** Injury from small hepatic ducts draining from the hepatic bed or cystic duct.
- **Type B.** Aberrant right hepatic duct obstruction.
- **Type C.** Transverse section, without ligation of the aberrant right hepatic duct.
- **Type D.** Lateral lesion of the main bile duct.
- **Type E.** Lesions of the main hepatic duct.
- **E1.** Lesion more than 2 cm from the confluence of the hepatic ducts.
- **E2** Lesion less than 2 cm from the confluence of the hepatic ducts.
- **E3.** Hilar lesion with preservation of the confluence of the hepatic ducts.
- **E4.** Destruction of the biliary confluence.
- **E5.** Aberrant right hepatic duct lesion.

Grade of severity of acute cholecystitis (Tokyo Guidelines 2018)(20)**:**

- **Grade I (mild).** Acute cholecystitis in a healthy patient, without organ dysfunction, only mild inflammatory changes in the gallbladder.
- **Grade II (moderate).** Acute cholecystitis accompanied by the following conditions:
  - Leukocytosis >18,000 mm3.
  - Palpable mass in the right upper quadrant of the abdomen.
  - Duration of the picture of more than 72 hours.
  - Marked local inflammation (biliary peritonitis, perivesicular abscess, liver abscess, gangrenous cholecystitis, emphysematous cholecystitis).
- **Grade III (severe). Cholecystitis associated with dysfunction of the following organs or systems:**
  - Cardiovascular dysfunction (hypotension requiring treatment with dopamine >5 ug/kg/min or any dose of dobutamine).
  - Neurological dysfunction (decreased level of consciousness).
  - Respiratory dysfunction (PaO2/FiO2 <300).
  - Renal dysfunction (oliguria, creatinine >2 mg/dl).
  - Liver dysfunction (PT-INR >1.5).
  - Haematological dysfunction (platelets <100,000/mm3).

Annex 2: Documentation upon delivery of the Protocol:

1. **Essential documents and other documents provided**
   - **1.1.** Researcher's file revised and complete in all its sections
   - **1.2.** Place for the maintenance of the Researcher's Archive. Detail
   - **1.3.** The submission of the Researcher's File assumes that all documentation is complete. Highlight, if appropriate, specially submitted or annotated documents. Examples:
     - **1.3.1.** Contract(s) and agreement of the centre's management
     - **1.3.2.** Authorisation by the competent health authority, e.g. AEMPS.
     - **1.3.3.** Favourable opinion of the ECIC
2. **Essential documents and other collected documents (if applicable)**
   - **2.1.** Signing of the visit log (with the Investigation Team and/or Pharmacy as appropriate)
   - **2.2.** Signed CVs of researchers, collaborators and pharmacy personnel
   - **2.3.** Laboratory ranges
   - **2.4.** Laboratory and equipment certificates. Accreditations
   - **2.5.** Signed Declaration of Conflict of Interest forms (if applicable) /"". Financial Disclosure".
   - **2.6.** Signed receipt of delivery of Researcher's File and other trial material
   - **2.7.** Signed receipt of delivery of medication
   - **2.8.** Page for registering signatures, functions and delegation of tasks
   - **2.9.** Training record (if applicable) specific to the project - on GCP, visit attendance, procedures for completing CRD, etc.

Annex 3 (attached separately): Patient Information Sheet - Informed Consent.

Annex 4 (attached separately): AAG Notification Form. Annex 5

(attached separately): Pregnancy Notification Form.