Leids Universitair Medisch Centrum

CLINICAL STUDY PROTOCOL

FOCUS GREEN

Fluorescence-guided resection Of Colorectal liver metastases Using SGM-101 and Indocyanine GREEN.

Short Title: SGM-101 and ICG in Colorectal Liver Metastasis
Acronym: Focus Green
Version: 3.1
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Ethics Committee number: -
CTIS: 2023-504615-33-00

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Date	04-08-2023
Coordinating investigators	Mats I. Warmerdam, MD, PhD-candidate Site: LUMC Telephone: 071-52 98420 Email: M.I.Warmerdam@lumc.nl Tom H. Dijkhuis, Msc, PhD-candidate Site: LUMC Telephone: 071-52 98420 Email: T.H.Dijkhuis@lumc.nl Ana Matoshi, Msc, PhD-candidate Site: LUMC Telephone: 071-52 98420 Email: a.matoshi@lumc.nl
Principal investigator(s)	Alexander L. Vahrmeijer, MD, PhD Site: LUMC Telephone: 071-52 99976 Email: A.L.Vahrmeijer@lumc.nl J. Sven D. Mieog, MD, PhD Site: LUMC Telephone: 071-5299143 Email: J.S.D.Mieog@lumc.nl
Sponsor (in Dutch: verrichter/opdrachtgever)	Leiden University Medical Center (LUMC) Albinusdreef 2 2333 ZA Leiden, the Netherlands
Independent expert	Dr. F.A. Holman, MD, PhD Site: LUMC

	Telephone: 071-5298420 E-mail: f.a.holman@lumc.nl
Subsidising party	Surgimab 1025 Avenue Henri Becquerel 34000 Montpellier FRANCE Françoise Cailler, Ph.D. Telephone: + 33 467 612 417 e-mail: fcailler@surgimab.com
Pharmacy	Linda van der Hulst Telephone: +3171 52 64285 E-mail: trials@lumc.nl

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor or legal representative: Department of Surgery Leiden University Medical Center Head of Department: Prof. Dr. J.F. Hamming		
Principal Investigator Leiden University Medical Center *Dr. A.L. Vahrmeijer*		

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
IMD	**Investigation with Medical Device**
IMDD	**Investigational Medical Device Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: 25-30% of patients with colorectal cancer develop colorectal liver metastases (CRLM). Cornerstone for optimal survival is achieving radical surgical resections of all metastases . To asisst the surgeon in achieving this, the use of intra-operative ICG for fluorescent tumour delineation has widely been adopted as standard of care. Multiple international trials have demonstrated that the use of ICG increases the rate of radical resections and result in the detection of additional malignant lesions invisible to the naked eye. However, the rate of false positives is still high and although it has increased the number of radical resections, even in a minimal invasive cohort the unintended R1 rate is still as much as 8%. Therefore, we are in need of an additional real-time intra-operative tool to detect R1 resections, especially in patients with a priori high risk for R1. To illustrate, in a large shared database of the Erasmus University Medical Center we found that patients that either received neoadjuvant chemotherapy, underwent a resection for >3 CRLM or patients that had a locally recurrent liver metastasis were independently associated with high R1 rates, ranging between 23-29%. Therefore, we propose the addition of SGM-101, a tumour targeted (carcinoembryonic antigen, CEA) NIR-fluorescence probe to ICG in patients scheduled for a resection with high risk of R1, ultimately to reduce the R1 ratio. This is the first trial testing the feasibility of working simultaneously with the two fluorescent dyes. If feasibility is met in this trial, this is a step-up towards a powered trial with primary objective to reduce the rate of R1 resections.

An additional exploratory objective of this study is to investigate the feasibility of SGM-101's potential to isolate circulating tumour cells (CTCs) and tumour-derived extracellular vesicles (EVs) as biomarkers for CRC. The load of CTCs and EVs in the circulation is strongly associated with poor clinical outcomes. Studies have shown that they contain information about the molecular profile of the tumour. The administration of SGM-101 may enable the detection of CEA positive CTCs and EVs.

Primary objective: To assess the feasibility of simultaneous use of ICG and SGM-101 for intraoperative imaging of colorectal liver metastases.

Study design: This is an open-label, single dose, exploratory study in the Leiden University Medical Center (LUMC).

Study population: In total 10 patients will be included who are scheduled for resection of colorectal liver metastases and meet at least one of the following criteria:

1. Scheduled for resection of >3 CRLM or,
2. Completed neo-adjuvant chemotherapy, of which the last course was completed within 3 months before surgery or,
3. Scheduled for surgery because of a locally recurrent liver metastasis.

Main study endpoints:

The main endpoint is feasibility of the simultaneous use of ICG and SGM-101. It is deemed feasible when it meets all of the following criteria:

1. A positive score on practical workability measured with survey A *'practical workability during surgery'*, defined as an average score of at least neutral.

2. A positive score on the patient's experience measured with survey B *'patient experience'*, defined as an average score of at least neutral.
3. SGM-101: at least 80% sensitivity, measured as follows:
   - For *capsular* lesions, that are visible in white light are counted positive if TBR ≥ 1.5 *in vivo*.
   - For *subcapsular* lesions that are not visible in white light will be counted as positive if:
     A: TBR ≥ 1.5 *in vivo,* OR
     B: TBR ≥ 1.5 *ex vivo* on whole specimen or on bread loafs
   All the above measurements are performed with the Quest Open Camera System
4. No cross-interference of fluorescent signal of both dyes within the two different excitation and emission channels. Cross-Interference is deemed insignificant if at macroscopic bread loaf imaging with the Quest spectrum System:
   A: At the 800nm channel the rim signal (ICG, 800nm) to tumour (possibly due to SGM-101, 700nm) ratio (STR) ≥ 1.5
   B: At the 700nm channel the tumour (SGM, 700nm) to rim signal (ICG, 800nm) ratio (TRR) ≥ 1.5 in SGM-101 positive tumours (defined in bullet point 3).

Feasibility is reached when we get a positive result on all four items.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: NIR fluorescence imaging using ICG results in improved detection of CRLM, although improvements could still be made. By using a tumour-specific fluorophore, SGM-101, which could be visualized on another wavelength channel than ICG, we aim to improve the intraoperative detection of CRLM and the rate of R0 resections. The latter is directly correlated with an improved overall survival. A potential advantage of SGM-101 compared to ICG is the fact that SGM-101 is tumour specific and is able to visualize the primary tumour and other metastases as well. Furthermore, the use of ICG only has a high false positive rate. By using the tumor specific fluorescence probe SGM-101, this rate could decrease. Moreover, in patients pretreated with chemotherapy it is found that the accumulation of ICG around the metastases is significant lower compared to patients who did not receive preoperative chemotherapy. This leads to a lower detectability of these metastases. The addition of SGM-101 is therefore believed to decrease the false negative rate, increase the detectability of CRLM and increase the R0-rate. As ICG is currently standard of care during oncologic liver surgery, participation in this study only requires an additional visit 4 days before surgery for SGM-101 administration. SGM-101 has already shown great potential in phase I/II studies and in over around 300 patients so far it proved to be safe and well tolerated.

1 INTRODUCTION AND RATIONALE

Colorectal liver metastases

Surgical resection is an important curative treatment option for liver metastases of colorectal origin (CRLM) (1). Irradical resection margins (<1 mm) are one of the main factors associated with lower overall survival. Although the percentage of R0 resections has improved over the past decades, recent literature reports unintended microscopic incomplete (R1) margins in up to 14-27% of cases (2, 3). To aid the surgeon in discriminating between tumour tissue and normal tissue, real-time intra-operative imaging techniques have been developed.

Intraoperative near infrared fluorescence imaging with ICG

Intraoperative near infrared (NIR) fluorescence imaging using indocyanine green (ICG) can assist the surgeon in improved visualization and resection of CRLM. ICG is a non-targeted fluorophore, with an excitation-emission spectrum +-800nm and should be administered 1-14 days before colorectal liver surgery. After infusion, it binds to albumin and is rapidly cleared from the blood by the liver. However, it was coincidentally found that ICG get entrapped around the CRLM. This results in the typical 'rim-shaped' fluorescent pattern surrounding the tumor (see figure 1a). Previous studies have extensively described the advantages of ICG in CRLM such as the identification of additional malignant lesions that would not have been found without the aid of fluorescence (4). In the recent multicentre MIMIC-trial it was shown that the R1 resection rate decreased from 12.6% to 7.6% in minimal invasive resections for CRLM (under submission). Also it resulted in a change in surgical management in 28% of patients (5). In the same study it was shown that in nearly 10% of patients additional malignant lesions were found due to ICG, Therefore, the use of ICG during liver resections is currently standard-of-care in the LUMC.

Drawbacks ICG and remaining challenges

Although ICG led to improved intra-operative tumour detection and increased the rate of radical resections, several drawbacks for this non-specific dye exist:
Firstly ICG is non-targeted dye and therefore lacks tumor specificity. This results in substantial false positive rates, ranging from 2-40% (5-9).
Secondly Interpretation of ICG fluorescence is more challenging when patients are pretreated with neoadjuvant chemotherapy. Analysis of our own data, shows a significant lower fluorescent signal to background ratio (SBR) for patients that received neoadjuvant chemotherapy, 2.41, than patients that did not receive neoadjuvant treatment, 6.33, see figure 1a/b. Accordingly, current Asia-Pacific consensus guidelines also state that signal interpretation can be more difficult and ICG administration should be altered in this patient group to prevent high false positive rates (9). Therefore they suggest to avoid ICG administration one day before surgery.
Lastly, although the aforementioned MIMIC-trial has proven ICG aids to increase the rate of radical resections in minimal invasive colorectal liver surgery, an R1 rate of 12% remained, of which nearly 8% unintended and 4% forced due to anatomical limits. This cohort consisted exclusively of minimal invasive surgeries and there was no specific selection of patients with high risk for positive resection margins, suggesting that this cohort comprised of less advanced oncological resections. From a large up-to-date database shared by the Erasmus University medical Center we found that high R1-rates were found in patients that received

neoadjuvant chemotherapy (22.6%), patients that underwent a resection for >3 CRLM (25.5%) and patients that had at least one local recurrent liver metastasis (29.1%). Using multivariate logistic regression all three variables are independently positively correlated with R1, with respective significant ORs of 1.8, 1.71 and 1.85.

To potentially overcome the drawbacks of ICG and to overcome the high R1-rate in neoadjuvant treated patients, patients undergoing a resection for >3 CRLM and patients with at least one locally recurrent liver metastasis we propose a first feasibility trial simultaneously combining two fluorescent probes: ICG and SGM-101.

SGM-101– a tumour-targeting fluorescent probe

In the past years, the concept of specifically targeting tumour cells was developed and led to the conjugation of agents with affinity for a specific target, such as an overexpressed cancer cell membrane protein, to a fluorophore. For instance, for detection of colorectal cancer, SGM-101 was developed by Surgimab (Montpellier, France) showing promising results. SGM-101 is a tumour-targeting agent consisting of a NIR-emitting moiety (700nm) covalently bound to the monoclonal antibody that targets carcinoembryonic antigen (CEA), a membrane protein that is overexpressed >90% of colorectal cancers (10-13). To illustrate its potential, in the phase 2 cohort, consisting of patients with locally advanced rectal cancer or locally recurrent rectal cancer, it was found that in 19% of patients, SGM-101 led to more complete tumour tissue removal by highlighting tumour tissue that was not detected by the naked eye, and thereby increased the R0 rate (12). Furthermore, in a very small cohort performed in the LUMC, it was shown that SGM-101 was perfectly able to visualize all 14 CRLM in 8 patients (14). Currently several clinical studies (Phase II and III) are enrolling patients with colorectal cancer for evaluation of SGM-101. The multicenter national phase II trial on locally advanced rectal cancer and recurrent rectal cancer (L19-069, NL69838.056.19), still open for inclusion aims to reduce the R1 rate, similar as in the proposed study. The international multicentric phase 3 trial in colorectal cancer is currently in progress in 10 clinical centers and aims for FDA approval (P19.004 and EudraCT 2018-000151-40). In over >300 patients so far, SGM-101 has proven to be well tolerated and safe. In contrast to ICG, SGM-101 will fluoresce the entire tumor instead of a rim surrounding the tumor (see figure 2).

Double dye NIR imaging

To our knowledge, only one trial worldwide has been conducted that tested the simultaneous use of two fluorescent probes (15). In this trial they combined the use of ICG and the porphyrin precursor 5-aminolevulinic acid (5-ALA) for the detection of colorectal liver metastases. They showed that the addition of 5-ALA to ICG resulted in higher specificity.
A requisite to work with two dyes is that they emit different wavelengths. In the proposed study ICG emits 800nm and SGM-101 700nm. In addition a camera system is required that is able to capture both wavelengths. The Quest Spectrum system is a CE-marked fluorescence camera developed to capture these two wavelengths. During intra-operative NIR-fluorescence imaging only one channel at the time can be projected. It is possible to switch between these two channels limitlessly.
ICG will be infused 7 days before surgery and SGM-101 4 days before surgery.

In this trial the feasibility of the use of two simultaneous fluorescent dyes, ICG and SGM-101, with two different wavelengths will be tested and evaluated. If feasibility is met, this is a step-up towards a powered trial with primary objective to reduce the rate of R1 resections.

In vitro evaluation of novel applications for SGM-101

Primary tumors shed CTCs in the blood from which a minority has the possibility to become metastatic lesions. Research in breast cancer patients has shown that CTC detection provides earlier detection of the disease status than conventional imaging methods. The detection of CTCs has been the focus of several studies in the recent years. It has been shown that CTCs express several biomarkers such as CEA, EpCAM, and CD44v6 making these cells interesting targets for the diagnosis and monitoring of cancer patients (16-18).

Most cell types, and in particular neoplastic cells, shed vesicles called extracellular vesicles (EVs) which consist of lipids, nucleic acids, and proteins (such as CEA). These molecules give specific information about their parental cancer cells and tumor microenvironment, which can be used to characterize the tumor. This allows for diagnosis, disease monitoring, and patient stratification in various types of cancers such as CRC (19-22).

The low amount in which CTCs and EVs can be found in the blood has made the isolation and characterization challenging and prevented them from entering the clinical route. An exploratory aim of this study is to determine the clinical utility of CTCs and EVs as biomarkers by detecting and quantifying the fluorescent signal released from SGM-101 labeled CTCs and EVs shed by the tumor in the blood. The blood will be collected from the peripheral and portal veins. Since the tumor is drained in the portal vein the blood samples will be enriched with CTCs and EVs presenting a unique opportunity to analyze a higher amount of CTCs and EVs providing material for quantification and profiling using methods such as flow cytometry and cryo-Electron microscopy. The information will be correlated with the clinical parameters of the patients. The analysis of the samples collected from the portal vein will reveal key information to selectively target relevant CTCs and EVs for the peripheral blood which could enable monitoring of the patients after surgery.

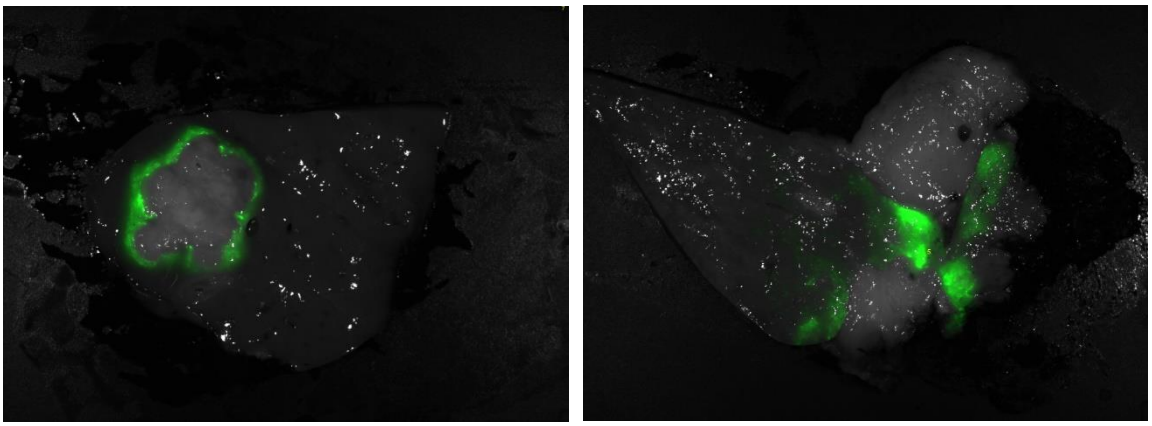

Figure 1: Illustrations of the fluorescent rim around a CRLM in a bread loaf specimen.
Left: typical rim-shaped pattern around a CRLM that was not pre-treated with neoadjuvant chemotherapy.
Right: Neoadjuvant treated patient resulting in a less intense and distinct rim.

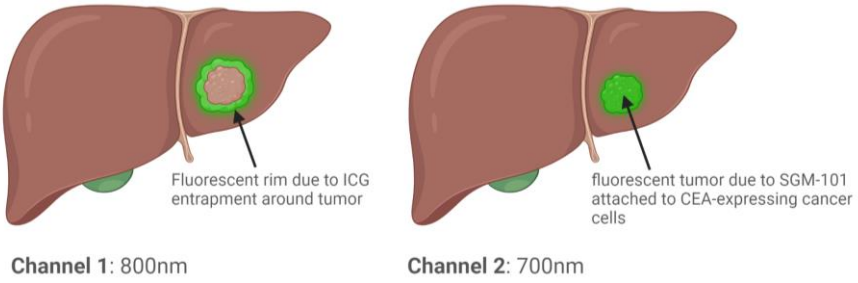

Figure 2: illustration of characteristic fluorescence pattern of ICG and SGM-101. non-specific ICG is entrapped around the tumor creating the typical fluorescent rim (left), while SGM-101 is localized within the tumor (right).
Channel 1: 800nm, only absorbs and projects fluorescent wavelength of ICG.
Channel 2: 700nm, only absorbs and projects fluorescent wavelength of SGM-101

2 OBJECTIVES

Primary objective:
To assess the feasibility of simultaneous use of ICG and SGM-101 for intraoperative imaging of colorectal liver metastases.

If feasibility is met, this is a step-up towards a powered trial which primary objective will be to increase the amount of R0-resections in colorectal liver metastases by the use of ICG and SGM-101.

Secondary objectives:
1. To assess the accuracy of ICG, SGM-101 or both to demonstrate an irradical resection per lesion and per patient level.
2. To assess the *in vivo* and *ex vivo* concordance rate of ICG, SGM-101 or both to histopathological status of the resected lesions (benign/malignant), ultimately to diminish the amount of false positive resected lesions
3. To determine the *in vivo* and *ex vivo* signal-to-background ratios (SBR) for SGM-101 and ICG;
4. To assess the number of intraoperative changes in surgical plan based on fluorescence (ICG, SGM-101 or both) and correlate this to histopathological status of the resected tissue. These changes comprise of
    a. Performing an extra resection because of
        i. An additional fluorescent lesion
        ii. Suspected fluorescent irradicality
    b. Preservation of tissue
5. To determine the *surgeon's satisfaction and judged potency* (Survey C) on working with SGM-101 in addition to ICG
6. To determine the correlation between fluorescent intensity and tissue CEA expression
7. To determine the effect of tumour depth on fluorescent signal for both ICG and SGM-101

Exploratory objective(s):

1. To evaluate the feasibility of minimally invasive diagnostic approach for the early detection of colorectal cancer through circulating tumor cells and extracellular vesicles
2. To evaluate the feasibility of using CTCs and EVs in the blood of CRC patients as biomarkers for early detection of recurrence/metastasis
3. To evaluate the feasibility of using CTCs and EVs from peripheral blood for disease monitoring and early cancer detection.

3 STUDY DESIGN

3.1 Overall study design and plan

This is an open-label, single dose, exploratory study in patients undergoing surgery for CRLM in the Leiden University Medical Center (LUMC). For ICG and SGM-101, the optimal doses (0.5mg/kg and 10 mg, respectively) and injection times (7 days and 4 days before surgery, respectively) will be used.

The total duration of the study for a subject is approximately 6-8 months. Starting from visit 1 where informed consent will be performed until the results of the routine CT/MRI +-6 months after surgery.
The study procedures coincide as much as possible with clinical procedures to minimize discomfort for the patient. Only visit 3 is fully additional.

It will consist of the following visits (see table 1):

- Visit 1: Screening at baseline (a few weeks before the planned surgery) where the clinical eligibility of the patient will be assessed by the surgeon and verbal and written informed consent will be obtained. When the patient meets all of the inclusion and does not meet any of the exclusion criteria the patient can be included in the study.
- Visit 2: Administration of ICG, 7 (+/- 1) days before surgery. This is standard of care for liver surgery in the LUMC, patients will be administered a single (i.v.) dose of 0.5mg/kg
- Visit 3: Administration of SGM-101: 4 (+/-1) days prior to surgery the patient will be admitted to the hospital. Before any study related procedures written informed consent will be obtained. Administration of SGM-101 will take 30 minutes. Post-injection, patients will stay at the hospital for at least 2 hours for observation. They will complete survey B ‘ patient experience’.
- Visit 4: Day of surgery: The duration of the admission is based on the clinical post-operative course of the individual patient;
- Visit 5: Day of discharge from the surgical department.
- Visit 6: The follow-up will coincide with the first outpatient routine post-surgical follow-up approximately 4 weeks (+-2 weeks), after surgery. If there is no visit in that period, then the study coordinator will consult the patient by telephone to ask for (S)AEs.
- Visit 7: The results of the routine CT/MRI-scan performed 6 months (+-2 months) after surgery will be assessed for follow-up on non-suspicious nodules that were not resected and to note for recurrences. This is no extra visit for the patient.

Table 1: visits and assessment schedule

STUDY ASSESSMENT	SCREENING	ICG ADMINISTRATION (standard-of-care)	SGM-101 ADMINISTRATION (extra infusion/visit)	HOSPITALIZATION PERIOD			OUTPATIENT CLINIC [3]	END OF STUDY
	W-4 (+/- 3W)	D-7 (+/- 1 day)	D-4 (+/- 1 day)	D0 (Day of surgery)	D5 (+-2 days)	Day of discharge	W4 (+/- 2W)	M6 (+/- 2M)
Inclusion and exclusion criteria	X							
Informed consent	X							
Demography	X							
Medical history	X							
General symptoms	X	X	X[1]	X	X	X	X	
Concomitant medication	X	X	X	X	X	X	X	
Physical examination			X[1]					
Vital Signs (Pulse Rate, BP, RR, BT)			X[1]	X	X	X		
ECG			X[1]	C				
Blood sample peripheral (CEA, hematology, chemistry,			X					
Blood sample peripheral (CTCs & EVs)			X	X	X			
Blood sample portal (CTCs & EVs)				X				
Urine Pregnancy Test			X[2]					
2x EDTA blood sample			X	X	X			
SGM-101 administration			X					
ICG administration		X						
(S)AE			X	X	X	X	X	
End of study								X
Routine liver CT/MRI follow-up								X

BP = Blood Pressure, BT = Body Temperature, HR = Heart Rate, RR = Respiratory Rate, (S)AE = (Severe) Adverse Event
1: Pre-dosing and at discharge (2 hours after study drug administration)
2: Pre-dosing; only for female patients with child-bearing potential
3: Allowed to be performed by telecommunication or to retrieve from EPD
C = continuously

4 STUDY POPULATION

4.1 Population (base)

Potential patients will be selected during multidisciplinary team meetings regarding liver metastases.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

1. Diagnosed with liver metastases of colorectal origin for which surgical resection is proposed and meet at least one of the following criteria:
    a. Scheduled for surgical resection of >3 CRLM or;
    b. completed neo-adjuvant therapy, of which the last course was completed within 3 months before surgery or;
    c. Scheduled for surgery because of a locally recurrent liver metastasis.
2. ≥18 years old.
3. Willing and capable to give informed consent before study specific procedures

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Patients with contraindications for SGM-101
    a. History of any anaphylactic shock;
    b. Patients pregnant or breastfeeding (pregnancy should be ruled out by a pregnancy test within two weeks prior to administration of the conjugate);
    c. Known positive test for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAG) or hepatitis C virus (HCV) antibody or patients with untreated serious infections;
    d. Previous administration of SGM-101
2. Patients with contraindications for Indocyanine green:
    a. Allergy for shells and/or clamps
    b. Hyperthyroidism
    c. Known allergy for ICG
3. Any condition that the investigator considers to be potentially jeopardizing the patient's well-being or the study objectives

4.4 Lifestyle restrictions

Patients must comply with general restrictions concerning surgical treatment. Patients who could become pregnant must agree to use an acceptable form of birth control from the time of study entry until 90 days after the withdrawal of the investigational product.

4.5 Sample size calculation

This is an exploratory study; therefore, the sample size is not based on statistical considerations. A total of 10 patients will be included to test for feasibility. If the combination of SGM-101 and ICG is deemed feasible, this is a step-up towards a powered trial

5 TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Intervention: According to standard-of-care an infusion of 0.5mg/kg ICG will be given 7 days (+-1) before surgery. 4 days (+-1) before surgery an extra visit is planned to administer a single intravenous injection of 10mg of SGM-101.
During surgery the Quest Spectrum camera system v2/3.0 will be used to visualize the lesions and look for additional lesions. After resection ex-vivo assessment of NIR-fluorescence of all resected tissue, on gross-macroscopy, bread loafs and microscopic slides.
Investigational drug: Intravenous single injection of the CEA-targeted NIR fluorophore SGM-101. This targeted 700nm fluorophore is developed by the SurgiMab, Montpellier, France.
Imaging: Intraluminal imaging will be performed with the CE-marked near-infrared (NIR) fluorescence imaging systems from Quest Medical Imaging: Quest Spectrum imaging platform v2/3.0. With this NIR-imaging system real-time *in-vivo* fluorescent signals from the administered fluorophore can be evaluated. The Quest Spectrum platform will also be used for evaluation of ex-vivo fluorescence of resected tissue on the back table and pathology department, which shall be performed during and after every procedure.

5.2 Use of co-intervention (if applicable)

5.2.1 Concomitant medications

As the trial will be performed during routine care and no drug-drug interactions with SGM-101 are known, concomitant medication can be used as in routine care. The use of other fluorescent contrast agent, other than SGM-101 and ICG, is not allowed in this study.

For possible interactions of ICG, a standard-of-care drug in the LUMC, we refer to the SMPC.

No drug-to-drug interaction is expected to occur between ICG and SGM-101 because ICG is administered 6-8 days before surgery while SGM-101 is administered 3-5 days before surgery. In practice this means that at least 24 hours have elapsed between ICG and SGM-101 administration. This will most unlikely result to a drug-drug interaction in the circulation as ICG well be completely removed from the circulation, given its very short half-life (23). More importantly ICG will also be entirely cleared from the liver (tissue) as the biliary excretion is reported by Meijer et al to be almost complete in 18 hrs. It is indeed expected that ICG will only be present around the tumor, based on our previous experiments in which it was shown that the fluorescence of the liver is more or less absent except for a rim-like structure around the malignant tissue. More importantly, a recently performed study in nude mice showed that the combination of SGM-101 (3 days before surgery) and ICG (1 day before surgery) did not mention problems (24). Obviously, in this trial the administration is in reverse order: In this trial ICG is administered firstly and subsequently SGM-101. Nevertheless, the cited study could well be used for this trial. If 3 days of SGM-101 exposure of the liver followed by ICG for a part of the liver (eg a ligated part that was first exposed to SGM-101) is without problems, this can be interpreted as absence of a relevant interaction between ICG and SGM-101. We are aware that SGM-101 will accumulate preferentially in the hepatic tumor, but that is not synonymous with non-exposure to SGM-101 of the other parts of the liver.
Both papers suggest that the circulatory and tissue concentrations of ICG are likely to be low. Finally, both compounds are pharmacologically inactive as evidence that a relevant drug-drug-interaction is unlikely.

5.2.2 Lifestyle restrictions

Patients must comply with general restrictions concerning surgical treatment including strenuous physical activity (e.g., heavy lifting, weight or fitness training) at least 24 hours prior to the day of screening and planned endoscopy, followed by standard of care clinical observation until hospital discharge.

5.2.3 Contraception requirements

All women of childbearing potential and all males must practice effective contraception during the study and be willing and able to continue contraception for at least 30 days after the injection of study treatment.
Women of childbearing potential are defined as all women physiologically capable of becoming pregnant, unless they meet one of the following conditions:

- Postmenopausal: 12 months of natural (spontaneous) amenorrhea or 6 weeks after surgical bilateral oophorectomy with or without hysterectomy;
- Posthysterectomy.

For the purposes of the study, effective contraception is defined as follows:

- Females: Using 1 or more of the following acceptable methods of contraception: surgical sterilization (e.g., bilateral tubal ligation), intrauterine contraception/device, hormonal contraception, or any 2 barrier methods (a combination of male or female condom with spermicide; diaphragm, sponge, cervical cap).
- Males: Effective male contraception includes a vasectomy with negative semen analysis at follow up, or the use of condoms with spermicide.

Abstinence can be considered an acceptable method of contraception at the discretion of the investigator. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post ovulation methods) and withdrawal are not considered acceptable methods of contraception.

5.3 Escape medication (if applicable)

Not applicable.

6 INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational products

The injectable conjugate SGM-101 is composed of a chimeric monoclonal antibody specific of CEA, bound to a fluorochrome. The study drug, SGM-101, will be administered to the patients in a time span of 30 minutes as detailed in the schedule of assessments. There will be no placebo.

The injectable dye ICG is not an experimental product and is already widely adopted in routine care, therefore it is not described in this section.

6.2 Summary of findings from non-clinical studies

Please find all the relevant information in the IB and IMPD. Find below a brief summary.

6.2.1 Non-clinical pharmacology

Non-clinical pharmacology studies were undertaken to characterize SGM-101's *in vitro* efficacy in binding the CEA antigen as well as its potential cytotoxicity, and to assess its *in vivo* efficacy in targeting CEA-expressing tumors, allowing their visualization and surgical removal in a number of different animal models. These studies have demonstrated SGM-101's efficacy in binding, in a highly specific manner, human CEA *in vitro* as well as in a variety of *in vivo* human CEA expressing mouse models epitope of CEA. The binding efficacy of SGM-101 to human CEA-expressing cells was demonstrated *in vivo* in terms of specificity, sensitivity and targeting pattern using four different human tumor xenograft mouse models.

SGM-101 allowed *in vivo* identification of tumors overexpressing CEA in all models tested, regardless of the location of tumors and their origin (colorectal, pancreas and breast), including subcutaneously, in the cecum, pancreas or breast in orthotopic tumor models, in the peritoneal cavity in peritoneal carcinomatosis mouse models, and in the liver in metastatic models, confirming the targeting ability of the anti-CEA antibody. Subcutaneous gastric and breast tumors overexpressing CEA were also clearly fluorescent after SGM-101 injection.
In addition, a high-level signal-to-noise ratio allows the detection of very small nodules invisible to the naked eye, even for an experienced investigator.
The injection of SGM-101 was well tolerated in all mouse models during the *in vivo* pharmacology studies.

Moreover, GLP-compliant safety pharmacology studies demonstrated that intravenous SGM-101 administration does not lead to any adverse effects neither on the central and peripheral nervous systems in Wistar rats, nor on the cardiovascular and respiratory functions in Beagle dogs at dose levels of up to 40 mg/kg and 20 mg/kg, respectively.

6.2.2 Non-clinical pharmacokinetics and metabolism

The pharmacokinetics, metabolism and biodistribution of SGM-101 were assessed in a total of 5 studies performed in mice and *in vitro* in human plasma. The stability of the covalent amide bond between the SGM-Ch511 antibody and the BM-104 fluorochrome in the

conjugate was confirmed *in vitro* in human plasma at 37°C, with no significant amounts of BM-104 released after up to 96 hours of incubation.

Pharmacokinetic studies were conducted in transgenic mice expressing human CEA and in the corresponding wild-type strain with [^{125}I]-SGM-101. No apparent differences were observed, and this agrees with reports that CEA expression in this transgenic model is exclusively apical, with the antigen not being accessible to circulating SGM-101. Normal Swiss mice treated with 30 or 60 µg of [^{125}I]-SGM-101 displayed similar pharmacokinetic profiles with AUC values increasing with dose but less than proportionally.

Biodistribution studies were also performed in an intraperitoneal human colorectal cancer model overexpressing CEA (in immunosuppressed NMRI mice) to evaluate the ability of the SGM-101 conjugate to target CEA-expressing tumors. Administration of [^{125}I]-SGM-101 resulted in 10-15 % of the injected dose (per gram of tissue) located at the tumor 24 hours after treatment. Normal tissue labelling was very low and rapidly decreasing. In these animals, as well as in normal Swiss mice, total remaining radioactivity levels in the animal accounted for less than 10% of the administered dose 10 days after treatment. Similar tissue distribution was observed in the transgenic human CEA-expressing mice.

Toxicokinetic evaluation was performed within the main GLP-compliant toxicology study in Wistar rats. SGM-101 plasma levels in rats treated intravenously at 5, 20 or 40 mg/kg/day increased with dose in an almost proportional manner and exposure to SGM-101 was slightly higher in males than in females at the highest dose level tested.

6.2.3 Non-clinical toxicology and safety pharmacology

As none of the animal species commonly used in toxicology studies express CEA and transgenic mice expressing CEA have not been sufficiently characterized, toxicity was assessed in Wistar rats after intravenous administration, consistent with the intended clinical use.

The evaluation of treatment toxicity over 28 days and after three administrations by slow continuous infusion (4 hours) of SGM-101 to rats at doses of up to 40 mg/kg per day (which corresponds to 85-fold the intended maximum clinical dose) led to the conclusion that this dose level was the NOAEL as no signs of toxicity were observed.

The toxicology evaluation performed within the scope of the safety pharmacology study evaluating the effects of SGM-101 on the cardiac and respiratory functions of Beagle dogs revealed no significant adverse effects, besides transient, spontaneously resolving signs of an anaphylactoid or anaphylactic type of reaction at the high dose level 20 mg/kg (approximately 45-fold the maximum anticipated clinical dose-level). Based on these observations, the NOAEL of SGM-101 was considered to be 5 mg/kg in the dog.

A tissue cross-reactivity study of SGM-101 conducted on a panel of 42 human frozen tissues and blood smears resulted only in the staining of tissues that have been described in the literature to express CEA, confirming on-target only binding of SGM-101.

The toxicity and genotoxicity of the BM-104 fluorochrome alone were also evaluated, in Wistar rats and in an Ames test, in order to cover its potential release from the SGM-101 conjugate *in vivo*. The highest tested dose of 3.4 mg/kg (corresponding to 140-fold the theoretical maximal clinical dose assuming 100% release) was considered the NOAEL. Furthermore, BM-104 was found not to be mutagenic in the Ames test.

Phototoxicity of SGM-101 and BM-104 was evaluated in a neutral red uptake assay in which both were considered non phototoxic.

6.3 Summary of findings from clinical studies

Please find all the relevant information in the IB and IMPD. Find below a brief summary.

6.3.1 Clinical pharmacology

A first-in-human trial that was performed in France (SGM-CLIN01) in advanced cancer patients presenting peritoneal carcinomatosis originating from digestive malignancies, and a second trial (SGM-CLIN02), in the Netherlands, for the delineation of pancreatic and colorectal tumors. Clinical pharmacokinetics and metabolism.

During surgery a fluorescent signal could be detected at the location of tumors in a number of cases. This signal could be distinguished from the background signal in most of them, but the TBR appears to vary depending on tumor heterogeneity and type of background. Pathology results show good concordance between fluorescent signal and tumor tissue.

For more information, see the IB version 13.

6.3.2 Clinical toxicology and safety pharmacology

In the SGM-CLIN01 trial SGM-101 was injected to 18 patients. All doses, including the maximal dose of 15 mg, were well tolerated; no dose-limiting toxicity (DLT) has been recorded during the dose escalation phase and none of the adverse events observed have been considered related to the IMP.

SGM-101 has been administered up to 12,5 mg in 14 patients with pancreatic cancer, and up to 15 mg in 61 patients with primary, recurrent and metastatic colorectal cancer in the SGM-CLIN02 trial. Neither drug-related adverse events nor DLTs have been reported. The (serious) adverse events occurring during the study were either related to the disease or the surgical procedure.

Recently SGM-101 was administered to 13 patients in the SGM-CLIN05 trial. Also in this trial there were no AEs and SAEs related to the product. Additionally it has been administered to >100 patients in the ongoing SGM-CLIN04 trial. In this trial only 2 SAEs occurred that might have been related to the IMP, due to action taken on fluorescence. No Suspected Unexpected Serious Adverse Reaction (SUSAR) was reported and no clinically important emerging efficacy or safety findings have been obtained from this clinical trial. In the ongoing international multicenter SGM-CLIN03 trial, >150 patients have received the IMP so far. No AEs or SAEs

have been reported so far that are related to the IMP. In all these trials SGM-101 has already shown (and is showing in the current trials) great potential and proven to be safe and effective. The big majority of (serious) adverse events occurring during the study were either related to the disease or the surgical procedure.

Of the treated patients, many additional malignant lesions have been found and resected that would otherwise not have been detected by the surgeon in white light.

6.4 Summary of known and potential risks and benefits

Nonclinical pharmacology studies have demonstrated SGM-101's efficacy in binding, in a highly specific manner to human CEA. Safety pharmacology studies demonstrated that intravenous SGM-101 administration did not lead to any adverse effects at doses up to 20-40mg/kg. Toxicity associated with SGM-101 use should therefore be minimal. More details are described in the risk analysis (Chapter 13).

6.5 Description and justification of route of administration and dosage

Description of route of administration:

The study drug will be administered using a Infusion Pump (Fresenius, Agilia VP MC (Version FR/EU/non-US) via an intravenous line (Volumat IV Set 0.2um Filter Fresenius, VL ON70 / M46444600S), giving a single dose of 10mg (100ml) injection in 30 minutes. The line will be flushed with 50 mL in 20 minutes normal saline after administration.

Justification of dosage-selection:

In previous clinical studies, SGM-101 was injected to patients with colorectal cancer between 5-15 mg. It was shown that intravenous administration of SGM-101 is safe and provides successful detection of primary, recurrent and metastasized CRC (10, 12). In the phase II study for colorectal tumours, SGM-101 showed the best tumor to background ratios with 10 mg, 4 days (+/- 1 day) prior to surgery. Therefore, we will administer a single dose of 10 mg SGM-101 intravenously in this study and no dose-adjustments will be made during the course of this study.

6.6 Dosages, dosage modifications and method of administration

6.6.1 Dosage and method of administration

The study drug (SGM-101) will be intravenously administered as a single dose of 10mg (100ml) 3-5 days before scheduled surgery. SGM-101 administration will take place on the surgical ward (day admission ward) under medical supervision and performed by the coordinating investigator. The administration of SGM101, 10mg (100ml) injection takes 30 minutes via an intravenous line. After infusion of SGM-101 the line will be flushed with 50 mL in 20 minutes normal saline after administration. Vital signs will be checked at t=predosing and t=120min after finishing the single dose administration of 10mg (100ml) SGM-101.

6.6.2 Dose-ranging

Not applicable, no dose-ranging or adjustments will be made during the course of this study.

6.6.3 Early-escalation rule

Not applicable, single dosing cohort.

6.6.4 Dose-interval optimization

This study is designed as a single dose 3-5 days before intervention, the dosing interval is based on previous experience with SGM-101 (IMP) in studies performed in colorectal cancer

(see section 6.5). The exact moment for administration between 3-5 days before surgical intervention is mainly based on scheduling considerations of the intervention.

6.6.5 Stopping criteria

Administration will be stopped in case of an unacceptable tolerability profile based on the nature, frequency, and intensity of observed AEs judged jointly by the principal investigator, SurgiMab and the Leiden University Medical Center Leiden (LUMC), the Netherlands.

6.7 Study drug packaging and labelling

The SGM-101 active substance will be present as a sterile solution for injection of the SGM-Ch511-BM104 antibody-fluorochrome conjugate at the nominal concentration of 5.0 mg/mL in 10 mM KH_2PO_4, 10 mM Na_3citrate, 300 mM arginine, 0.02% Tween-20, pH 6.0. The SGM-101 finished product is a sterile solution for injection of the same composition. It will be supplied as 4R type I amber glass vials sealed with FluroTec® elastomeric stoppers and aluminium flip-off seals, containing an extractable volume of 2.0 mL and stored at +2 to +8°C.

The SGM-101 active substance and investigational medicinal product (IMP) are manufactured and controlled in compliance with good manufacturing practice (GMP) by Polymun Immunobiologische Forschung (Klosterneuburg, Austria).

The labels fulfil GMP Annex 13 requirements for labelling. Label text will be translated into local language. The label will include the following information:

Vials labels:

Onderzoek: Focus Green
SGM-101, ampul met 2,3 mL (2ml extraheerbaar)
11,5mg (10 mg extraheerbaar)
Steriele oplossing voor i.v. injectie

Partijnummer: SGM1010522-A
Patiëntnr:_____ Unitnr:_____
Bewaren tussen +2°C en +8°C Vervaldatum: 30/11/2026

Sponsor name: Leiden University Medical Center

Uitsluitend voor gebruik in een klinish onderzoek

Onderzoek: Focus Green
SGM-101, Ampul met 2,3 mL (2ml extraheerbaar)
11,5mg (10 mg extraheerbaar)

Steriele oplossing voor I.V. injectie
Bewaren tussen +2°C en +8°C
Partijnummer: SGM1010522-A
Patiëntnr:_____ Unitnr:_____
Vervaldatum: 11/2026
Naam van de onderzoeker: A.L. VAHRMEIJER, MD, PhD
Volgens de protocolinstructies gebruiken
Adres van de sponsor: Leiden University Medical Center - Albinusdreef 2 - 2333 ZA Leiden - The Netherlands - 071-52 99976
IMP leverancier : SurgiMab SAS - 10 Parc Club du Millenaire – 1025 Avenue Henri Becquerel - 34000 Montpellier – FRANKRIJK - +33 467798382
Uitsluitend voor gebruik in een klinisch onderzoek

The dispensing of the study drug will be performed by the pharmacy. Study drug will be dispensed for each subject. Study drug packaging will be overseen by the Leiden University Medical Center Pharmacy and bearing a label with the identification required by local law, the protocol number, drug identification, and dosage.

6.8 Study drug preparation

Study drug will be made ready for use by the LUMC pharmacy staff according to the pharmacy manual.

For the preparation of a 10mg dose, 2 mL of SGM-101 will be transferred into a 100 mL pouch of 0.9% NaCl. The pouch will be gently shaken to ensure proper dilution of the SGM-101. The diluted solution will then be injected to the patient in a 30 minutes infusion, through a perfusion tubing system containing a 0.22µm filter. The pouch will be protected from light when possible by using an aluminium foil in order to reduce fluorescence bleaching.

When all the solution has been injected, an additional 50 mL of saline solution will be injected to account for the dead volume of the filter and tubes. The IMP injection and NaCl flush will be registered in the "IMP Injection Log" filed in the pharmacy files.
After drug preparation, SGM-101 is stable for 16 hours at +5°C ± 3°C, and for 1 hour at room temperature.

6.9 Drug accountability

Drug accountability will be maintained by the Leiden University Medical Center Pharmacy and assessed by maintaining adequate study drug dispensing records.
The investigator is responsible for ensuring that dosing is administered in compliance with the protocol. Delegation of this task must be clearly documented and approved by the investigator. All study drug administrations will occur under medical supervision.

The IMP will be sent from Polymun depots (Austria) to Leiden University Medical Centre's pharmacy.

Upon arrival at the pharmacy, the investigational product will be checked for damage and verified for proper identity, quantity, integrity of seals and temperature conditions. Any deviation or product complaints will be reported upon discovery.

At all times, the number of supplied, used and remaining treatment doses should match. The investigator (or designee or pharmacist) will be responsible for IMP accountability,

reconciliation, and record maintenance. In accordance with all applicable regulatory requirements, the investigator or designated site staff or pharmacist must maintain the following inventory records:

- Receipt of IMP boxes at the LUMC's pharmacy;
- IMP administered to each patient (including vial numbers);
- Log of any discarded/unused/expired IMP;
- Monitoring of the storage conditions.

Any unused or expired IMP, as well as empty vials or vials with remaining volume, must be destroyed on site using locally approved safety procedures and documentation.

All used IMP will be kept in their original packaging and stored at ambient temperature in a dedicated room with restricted access until check of inventory count performed by the monitor.

6.10 Treatment assignment

Patients will be assigned in a consecutive order starting with the lowest number. Replacement patients will be numbered +100, e.g. subject 5 will have subject 105 as replacement, and subject 105 will have subject 205 as replacement, etc.

7 INVESTIGATIONAL MEDICAL DEVICE – NEAR-INFRARED FLUORESCENCE IMAGING

7.1 Near-infrared fluorescence imaging system

Near-infrared fluorescence will be assessed with dedicated NIR-fluorescence imaging systems in-vivo as well as ex-vivo as described in the Methods > Study Procedures (chapter 8.3). These CE-marked systems will be used to provide the ground truth for NIR-imaging. Dedicated NIR-fluorescence imaging systems are produced for *in-vivo* (open-, and laparoscopic procedures) imaging, for example by Quest Medical Imaging, the Quest Spectrum platform v2/3.0. This CE-marked system is regularly used during image-guided perfusion assessment and the detection of liver metastases. The use of the system in this study is not different compared to these indications. During image-guided perfusion assessment with for example Indocyanine Green (ICG), the NIR fluorescence imaging system (i.e. Quest) detects the fluorescence signal from the fluorophore (i.e. ICG) bound to albumin that circulates in the bloodstream within the NIR spectrum of 700 – 900 nm. The detection of the fluorescence signal during tumor-imaging is similar to perfusion assessment (without any modifications to the NIR fluorescence imaging system), but during tumor-imaging the fluorophore is bound to an antibody (e.g. SGM-101, a fluorochrome-labeled anti-CEA monoclonal antibody) or peptide that binds to the tumor. In our previous studies, we have demonstrated the applicability of the Quest Medical Imaging system in detecting fluorescent CEA expressed tumors with SGM-101. (10, 25, 26) The Central Committee on Research Involving Human Subjects (CCMO) will be notified through the review procedure according to the Medical Device Regulation considering off-label (outside intended CE-mark, art 82.) use of the Quest Spectrum imaging platform. Quest Medical Imaging is aware of the approval and the use of the system for tumor imaging in the context of the study design. In the supplement D2. Investigational Medical Device Dossier (IMDD), the manufacturer has provided all relevant safety and testing information for the Quest Spectrum imaging system. The manufacturer as well as the principal investigator declare no intent of performing conformity assessment purposes for this imaging devices using this study, since the aim of this study is feasibility assessment and is designed as exploratory pilot study (phase II), conform article 82. of the Medical Device Regulation.

7.1.1 Imaging systems used for in-vivo imaging

Quest Spectrum NIR-imaging system V2/3.0 (Olympus Europe / Quest Photonic Devices, Hamburg, Germany)

Name of device	**Manufacturer**	**Model**	**CE conform 93/42/EEG of EU/2017/745**	**Conform intended use as described in the instruction manual?**
Quest Spectrum imaging platform (v2/3.0)	Olympus Europe, Hamburg, Germany / Quest Innovations	Quest Spectrum imaging platform v2 / v3.0	Yes, conform 93/42/EEG	No, art. 82 MDR

	Photonic Devices, Middenmeer, the Netherlands			

7.1.2 Imaging systems used for *ex-vivo* imaging

- Backtable imaging: Quest OPEN camera - NIR fluorescence system (Olympus Europe / Quest Photonic Devices, Hamburg, Germany)
- Gross macroscopy/fresh-frozen biopsy: PEARL imaging system (LI-COR, Biosciences, Lincoln, NE, U.S.A.).
- Bread loafs/Casettes: PEARL imaging system (LI-COR, Biosciences, Lincoln, NE, U.S.A.).
- Microscopy: Odyssey CLx scanner (LI-COR, Biosciences, Lincoln, NE, U.S.A.).

7.2 Summary of findings from non-clinical studies

Summary of findings from non-clinical studies including references is described in the IMDD of the camera system (Quest Medical Imaging).

Camera system	**Filename**	**Pages**
Quest Spectrum Imaging platform v2/3.0	D2. Investigational Medical Device Dossier (IMDD)_Quest_Medical_Spectrum_Imaging_ Platform_2_and_3.0_Final_07102021	6-10, 16-18

7.3 Summary of findings from clinical studies

Summary of findings from non-clinical studies including references is described in the IMDD of the camera system (Quest Medical Imaging).

Camera system	**Filename**	**Pages**
Quest Spectrum Imaging platform v2/3.0	D2. Investigational Medical Device Dossier (IMDD)_Quest_Medical_Spectrum_Imaging_ Platform_2_and_3.0_Final_07102021	6-10, 16-18

7.4 Summary of known and potential risks and benefits

Summary and structured benefit-risk analysis and risk-management is described in the IMDD of the camera system (Quest Medical Imaging). A structured risk analysis including all study details is given in chapter 13 (IMP) and 14 (IMD):

Camera system	**Filename**	**Pages**

Quest Spectrum Imaging platform v2/3.0	D2. Investigational Medical Device Dossier (IMDD)_Quest_Medical_Spectrum_Imaging_ Platform_2_and_3.0_Final_07102021	13-16

8 METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The simultaneous use of ICG and SGM-101 is deemed feasible when it meets all of the following criteria:

1. A positive score on practical workability measured with survey A *'practical workability during surgery' (see section 8.1.4)*, defined as an average score of at least neutral. This survey will be handed to the surgeon at the end of every surgery.
2. A positive score on the patient's experience measured with survey B *'patient experience' (see section 8.1.4)*, defined as an average score of at least neutral. This survey will be handed to the patient at the end of the last infusion (SGM-101).
3. SGM-101: at least 80% sensitivity, measured as follows:
   - C*apsular* lesions, that are visible in white light are only counted positive if TBR ≥ 1.5 *in vivo*.
   - S*ubcapsular* lesions that are not visible in white light will be counted as positive if:
     A: TBR ≥ 1.5 *in vivo,* OR
     B: TBR ≥ 1.5 *ex vivo* on whole specimen OR
     C: TBR ≥ 1.5 *ex vivo* on bread loafs

   All the above measurements are performed with the Quest Open Camera System
4. No cross-interference of fluorescent signal of both dyes within the two different excitation and emission channels. Cross-Interference is deemed insignificant if at macroscopic bread loaf imaging with the Quest spectrum System:
   A: At the 800nm channel the rim signal (ICG, 800nm) to tumour (possibly due to SGM-101, 700nm) ratio (STR) ≥ 1.5 in ICG positive tumours.
   B: At the 700nm channel the tumour (SGM, 700nm) to rim signal (ICG, 800nm) ratio (TRR) ≥ 1.5 in SGM-101 positive tumours (defined in bullet point 3).

Feasibility reached when we get a positive result on all four items.

8.1.2 Secondary study parameters/endpoints (if applicable)

1. The accuracy of ICG, SGM-101 or both to demonstrate an irradical resection. The accuracy comprises of the rate of true positives, true negatives, false positives and false negative with accompanied sensitivity, specificity, positive predictive value and negative predictive value. An irradical resection may be demonstrated by:
   a. A fluorescent hotspot in the wound bed of either ICG, SGM-101 or both. If this results in a reresection of the hotspot in the wound bed this is correlated to the pathology outcome. When the surgeon was not able to perform a reresection of the wound bed then the assumed corresponding site at the ex-vivo specimen will be marked to correlate it to pathology. A margin of <1mm is classified as R1. Additionally, when the surgeon performs a reresection based on white light only (SGM-101 and ICG negative), this will be noted and correlated to pathology.
   b. A fluorescent hotspot on the ex-vivo specimen of either ICG, SGM-101 or both. This hotspot will be marked with a stitch and correlated to pathology. Likewise, the possible reresection will be correlated to pathology.

2. Concordance rate of ICG, SGM-101, ICG and SGM-101 combined (both fluorescent) or ICG and SGM-101 combined (for which only one is fluorescent), to histopathological result (lesion benign vs malignant). The concordance rate comprises of the rate of true positives, true negatives, false positives and false negative with accompanied sensitivity, specificity, positive predictive value and negative predictive value.
    a. In-vivo: An in-vivo SBR (ICG)/TBR (SGM-101) of ≥1,5 is determined as positive for both SGM-101 as ICG.
    b. ex-vivo: this will be performed with the Quest open imaging system. An ex-vivo SBR/TBR of ≥1,5 is noted as positive for both SGM-101 as ICG.
    c. Ex-vivo bread loafs: This will be performed with both the ex-vivo Quest open imaging system as with the Pearl imaging system. A TBR/SBR of ≥1,5 is noted as positive for both SGM-101 as ICG.
3. For every lesion a TBR (SGM-101) /SBR (ICG) will be calculated in vivo (Quest open camera), ex vivo whole specimen (Quest open camera, Pearl system) and ex vivo bread loafs (Quest open camera, Pearl system).
4. Modification of operative plan due to imaging (e.g. extension of resection margins, additional resection, preservation of tissue) and the correlation to histopathology.
5. Survey C ‘surgeon’s satisfaction and judged potency’ questionnaire (see section 8.1.4) will be handed to all the surgeons at the end of the trial that have at minimum operated 3 times within this trial. Outcomes will be analysed.
6. To assess the correlation between lesion CEA expression, performed using immunohistochemistry staining, to bread loaf TBR (SGM-101) measured with the Pearl system. Hereby also correlating the effect of:
    a. Immunohistochemical score for CEA to bread loaf TBR. The amount of CEA expression is determined by immunohistochemistry and quantified using the immunoreactive score (IRS).
    b. Percentage of tumour comprising of vital tumour cells to bread loaf TBR
7. Effect of tumour depth on fluorescent status of ICG/SGM-101 will be calculated in three ways
    a. Distance between tumour to liver surface assessed by pre-operative CT or MRI
    b. Distance between tumour to liver surface as assessed by post-operative pathology
    c. Distance between tumour to closest resection margin as assessed by post-operative pathology

8.1.3 Exploratory endpoints

1. To evaluate the feasibility of minimally invasive diagnostic approach for the early detection of colorectal cancer through circulating tumor cells and extracellular vesicles.
2. To evaluate the feasibility of using CTCs and EVs in the blood of CRC patients as biomarkers for early detection of recurrence/metastasis.
3. To evaluate the feasibility of using CTCs and EVs from peripheral blood for disease monitoring and early cancer detection.

8.1.4 Quantification of fluorescent ratio's

Tumor-to-background ratio (TBR), SGM-101

For SGM-101 the *tumor-to-background ratio (TBR)* will be calculated. The TBR is calculated in a standardized manner. First, the tumor and background are delineated in QuPath. If the tumour is not visible in white light it is not possible to delineate it. In that circumstance, the signal instead of the tumor will be delineated. Strictly this would result in a *signal-to-background ratio (SBR)*, however for keeping it organized, this will also be called a 'TBR'. In MeVisLab, the delineated annotations of the tumor and the background are used to compute the mean fluorescence intensities (MFI). The background that was first delineated by the researcher will be processed by a code that selects the area 2cm around the tumour delineation. From this background surface the *background fluorescence* is calculated. Lastly, the TBR is calculated by dividing the tumour fluorescence by the background fluorescence.

Signal-to-background ratio (SBR), ICG

For ICG the *signal-to-background ratio (SBR)* will be calculated. The SBR is defined as the ratio of MFI from the rim around the tumour divided by the background. The rim is defined as the area from the edge of the tumor up to 5 mm away from the tumor. First, the tumor and background are delineated in QuPath. In MeVisLab, the delineated annotations of the tumor and the background are used to compute the mean fluorescence intensities of the RIM and background (MFI). The rim will be computed in MeVisLab by generating a halo of 5 mm around the tumor. The background fluorescence is defined as the MFI of all 'healthy tissue' at a minimum distance from 5 mm from the tumor to 2cm around the tumor. At last, the SBR is calculated by dividing the signal fluorescence by the background fluorescence.

Signal-to-tumor ratio (STR), assessment of cross interference in the 800nm channel.

To assess for unwanted cross interference of SGM-101 in the 800nm channel (see main endpoint), the STR will be calculated; the MFI of the rim (caused by ICG) divided by MFI of the tumor (unwanted cross interference of SGM-101). The rim is defined as the area from the edge of the tumor up to 5 mm away from the tumor. First, the tumor and background are delineated in QuPath. In MeVisLab, the delineated annotations of the tumor and the background are used to compute the mean fluorescence intensities of the RIM and the tumor. At last, the STR is calculated by dividing the signal fluorescence by the background fluorescence.

Tumor-to-rim ratio (TRR), assessment of cross interference cross interference in the 700nm channel.

To assess for unwanted cross interference of ICG in the 700nm channel, the TRR will be calculated; the MFI of the tumor (SGM-101) divided by the MFI of the rim (unwanted cross interference of ICG). The rim is defined as the area from the edge of the tumor up to 5 mm away from the tumor. First, the tumor and background are delineated in QuPath. In MeVisLab, the delineated annotations of the tumor and the background are

used to compute the mean fluorescence intensities of the RIM and the tumor. At last, the TRR is calculated by dividing the tumor fluorescence by the rim fluorescence.

8.1.5 Surveys

Survey A: practical workability Surgeon (Dutch)

Deze vragenlijst heeft betrekking op de toevoeging van SGM-101 boven op de standard of care ICG.

1. Het gebruik van twee fluorescerende stoffen op 2 verschillende kanalen was goed werkbaar
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

2. De tijd die ik spendeerde aan deze techniek was acceptabel
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

3. Ik heb geen discomfort ervaren in het werken met 2 fluorescerende stoffen
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

4. De toevoeging van SGM-101 had een positieve invloed op de operatie (bijvoorbeeld: lokalisatie, snijrand beoordeling, discrimineren maligne/benigne, ‘surgical confidence’)
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

The practical workability is deemed positive when the average of scores of all questions for all patients is at least neutral. This will be calculated as follows. The answer “definitely not agree” will be awarded -2 points; “not agree” -1 point; “neutral” 0 points, “agree”, 1

point; definitely agree 2 points. To be deemed positive, the average of all surveys should be at least 0 points.

Survey A: practical workability Surgeon (English)

This questionnaire relates to the addition of SGM-101 on top of the standard of care ICG.

1. Using two fluorescent dyes on 2 different channels was workable.

   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

2. The time I spent on this technique was acceptable.

   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

3. I experienced no discomfort in working with 2 fluorescent dyes.

   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

4. The addition of SGM-101 had a positive impact on the surgery (e.g., localization, margin assessment, discriminating malignant/benignant, 'surgical confidence').

   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

Survey B patient experience (Dutch)

Deze vragenlijst heeft betrekking op het extra studiemiddel dat u 4 dagen van te voren toegediend heeft gekregen (SGM-101). Het middel dat u 7 dagen voor de operatie krijgt, wordt volgens de standaard zorg gegeven in het LUMC (ICG).

1. Ik vond het geen probleem om een keer extra naar het ziekenhuis te komen voor het toedienen van de studiemedicatie .
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

2. Ik heb het extra infuus niet als een te zware belasting ervaren.
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

3. Als ik de keuze nog een keer had kunnen maken dan zou ik weer meedoen aan deze studie.
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

The patient experience is deemed positive when the average of scores of all questions for all patients is at least neutral. This will be calculated as follows. The answer “definitely not agree” will be awarded -2 points; “not agree”, -1 point; “neutral” 0 points, “agree”, 1 point; definitely agree, 2 points. To be deemed positive, the average of all patients should be at least 0 points.

Survey B patient experience (English)

This questionnaire relates to the additional study drug which was administered to you 4 days prior to surgery (SGM-101). The drug that was given to you 7 days prior to surgery , is a stand-of-care drug in the LUMC (ICG).

1. I did not mind coming to the hospital one extra time to get the study drug adminstered
   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

2. I did not experience the extra infusion to be too much of a burden
   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

3. If I was able to make the choice again, I would participate in this study again.
   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

Survey C: Satisfaction and potency (Dutch)

1. Ik denk dat het systematisch gebruik van SGM-101 bovenop ICG een positief effect gaat hebben op de chirurgische lokale controle van tumorweefsel bij patiënten die geopereerd worden aan colorectale levermetastasen en die voldoen aan 1 van de volgende criteria:
   1. Neo-adjuvant voorbehandeld met chemotherapie OF
   2. Resectie van >3 metastasen OF
   3. Resectie van een randrecidief
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

2. Door de toevoeging van SGM-101 aan ICG voel ik mij zekerder in het verkrijgen van radicale marges.
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

3. Door de toevoeging van SGM-101 aan ICG voel ik mij zekerder in het detecteren van alle laesies.
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

4. Door de toevoeging van SGM-101 aan ICG voel ik mij zekerder in het discrimineren tussen goed- en kwaadaardig weefsel.
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

5. Ik wil in de toekomst vaker met beide stoffen opereren bij patiënten met colorectale levermetastasen.
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

6. Een gepowerde studie die het nut van SGM-101 (bijvoorbeeld verhogen %R0 resecties, discrimineren maligne/benigne) bovenop ICG onderzoekt bij patiënten met colorectale levermetastasen lijkt mij zinvol.
   Totaal oneens
   Oneens
   Neutraal
   Eens
   Totaal eens

Survey C: Satisfaction and potency (English)

1. I think that the systematic use of SGM-101 on top of ICG will have a positive effect on surgical local control of tumor tissue in patients undergoing surgery for colorectal liver metastases who meet 1 of the following criteria:
   1. Neo-adjuvant pretreated with chemotherapy OR
   2. Resection of >3 metastases OR
   3. Resection of a peripheral recurrence
   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

2. With the addition of SGM-101 to ICG, I feel more confident in obtaining radical margins.
   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

3. With the addition of SGM-101 to ICG, I feel more confident in detecting all lesions
   Totally disagree.
   Disagree
   Neutral
   Agree
   Totally agree

4. With the addition of SGM-101 to ICG, I feel more confident in discriminating between benign and malignant tissue.
   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

5. In the future I would like to use both fluorescent dyes more often in patients with colorectal liver metastases.
   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

6. A powered trial examining the utility of SGM-101 (e.g., increase %R0 resections, discriminate malignant/benignant) on top of ICG in patients with colorectal liver metastases seems to me to be useful.
   Totally disagree
   Disagree
   Neutral
   Agree
   Totally agree

8.1.6 Other study parameters (if applicable)

Important baseline criteria will be documented. This includes age, gender, weight, height, BMI, cancer history, general medical history, concomitant medication and substance abuse. Radiology findings will be documented. Information about the procedure and pathology will be collected.

8.2 Randomisation, blinding and treatment allocation

Randomization: not applicable

Treatment allocation: In total 10 patients will be included in this study. According to standard-of-care an infusion of 0.5mg/kg ICG will be given 7 days (+-1) before surgery. 4 days (+-1) before surgery an extra visit is planned to administer a single intravenous injection of SGM-101.

During surgery the Quest Spectrum camera system v2/3.0 will be used to visualize the lesions and look for additional lesions.

Blinding: No blinding will be performed

8.3 Study procedures

This is an open-label, exploratory study in patients undergoing surgery for CRLM. For ICG and SGM-101, the optimal doses (0.5mg/kg and 10 mg, respectively) and injection times (7 days and 4 days before surgery, respectively) will be used.

8.3.1 Visits

The total duration of the study for a subject is approximately 6-8 months. Starting from visit 1 where informed consent will be performed until the standard-of-care outpatient follow-up visit at approximately 6 month (+-2) postoperatively.
The study procedures coincide as much as possible with clinical procedures to minimize discomfort for the patient. Only visit 3 is fully additional.

It will consist of the following visits (see also table 1):

Visit 1: Screening
Screening at baseline (a few weeks before the planned surgery) where the clinical eligibility of the patient will be assessed by the surgeon and verbal and written informed consent will be obtained. When the patient meets all of the inclusion and does not meet any of the exclusion criteria the patient can be included in the study.

Visit 2: Administration of ICG, day -7 before surgery (+/-1))
As part of standard-of-care for liver surgery in the LUMC, patients will be administered a single (i.v.) dose 0.5mg/kg ICG approximately 7 (+/-1) days prior to surgery. This infusion is standard-of-care in the LUMC.

Visit 3: Administration of SGM-101, day -4 before surgery (+/- 1)
Four days (+-1day) prior to surgery patients will be admitted to the LUMC. Before dosing, a clinical exam, vital signs and ECG will be obtained. A single dose of 10 mg of SGM-101 will be administered intravenously. Administration of SGM-101 will take 30 minutes. The line will be flushed with 50 mL in 20 minutes normal saline after administration. A physical examination, ECG and vital signs will be obtained before discharge (2 hours after administration). Appropriate measures will be taken if abnormalities (compared to baseline) are found. Adverse events will be recorded throughout the treatment and observation period. Patients are allowed to eat and take medication.
After this infusion the patient is asked to complete Survey B: patient experience (see section 8.1.4). The patient experience is deemed positive when the average of scores of all questions for all patients is at least neutral. This will be calculated as follows. The answer “definitely not agree” will be awarded -2 points; “not agree”, -1 point; “neutral” 0 points, “agree”, 1 point; definitely agree, 2 points. To be deemed positive, the average of all patients should be at least 0 points.

Visit 4: Admission and surgery (day 0)
The scheduled liver resection(s) will be carried out according to standardized peri-operative planning. Besides clinical assessment, white-light visual inspection (WLI) and palpation, intraoperative ultrasound (IOUS) and ICG NIR-fluorescence imaging, SGM-101 fluorescence imaging will be used to screen and inspect the target lesion(s), the entire liver and four-abdominal quadrants as study procedure. Recordings will be made of the surgical field with the NIR fluorescence camera system for post-surgery image-analysis and TBR, SBR, STR and TRR.

The surgery consist of the following steps:

1. After the patient is intubated and surgical site incised, as part of standard surgical procedure, white light inspection (WLI) of the surgical field, four-abdominal quadrants,

peritoneum and adjacent structures will be carried out. The number and location of suspect CRLM, and possible other distant metastases detected under normal light and/or by palpation will be described and recorded on the case report form (CRF). Following WLI, NIR ICG (800nm, channel 2) fluorescence will be used to visualize the CRLM. The number and location of suspect CRLM detected using the imaging system with ICG fluorescence will be recorded on the CRF. Images will be captured with ICG from all lesions (channel 1). This step is all according to standard-of-care surgical procedures.

2. The camera will be changed from channel 1 (ICG, 800nm) to channel 2 (700nm, SGM-101). NIR SGM-101 fluorescence inspection of the surgical field, the CRLM, four-abdominal quadrants and adjacent structures will be performed. From every CRLM an image will be captures. In the eCRF the findings and possible alterations in plan are noted.

3. Resections will be performed:
    a. All lesions that are suspect on standard of care assessment will be resected (WL, ICG, IOUS, pre-operative imaging), independent of fluorescent status of SGM-101.
    b. When a lesion is not suspect/visible with standard of care but SGM-101 positive then an excision biopsy will be performed.
    c. A lesion that is not suspect in WL, ICG and SGM-101 will not be resected. During the standard follow-up of 6 months this lesion will be monitored and issued as malignant when it turns out to be malignant at the follow-up.

    Surgeons are allowed to use both fluorescent channels at all moments during surgery when warranted.

4. Once the resections are done, intra-operative margin assessment by fluorescence will be performed in both channels. This is done:
    a. by imaging of the wound bed(s).
    b. by imaging of the resected specimen(s).

    Margins are suspect to be positive (i.e. irradical resection R1) when:

    - A hotspot of SGM-101 and/or ICG is detected in the wound bed. If surgically safe and feasible, a re-resection will be performed. In case of uncertainty the surgeon can take a fresh frozen section. By help of a stitch the expected corresponding irradical area on the specimen will be marked and will be correlated to pathology.
    - A hotspot of SGM-101 and/or ICG is found on the ex-vivo specimen (by imaging with the Quest). If surgically safe and feasible, a re-resection of the corresponding area in-vivo will be performed. By help of a stitch the hotspot on the specimen will be marked and will be correlated to pathology.

    In case NIR fluorescence imaging shows additional extent of the lesion in the surgical plane (positive margins, incomplete tumor removal, irradical resection) which is not visible during initial clinical assessment and WLI, IOUS inspection and ICG imaging, the surgeon could decide to resect this additional tissue or take a biopsy when it is safe and surgically feasible. All perioperative changes in surgical plan or postsurgical

treatment will be recorded on the CRF. Snapshots and recordings of NIR fluorescence imaging will be made for TBR analysis.

5. NIR fluorescence imaging at the pathology
    a. Snapshots and recordings of NIR fluorescence imaging will be captured of the entire specimen(s), with the Quest open camera system and the Pearl system
    b. Snapshots and recordings of NIR fluorescence imaging will be captured of the bread loafs, with the Quest open camera system and the Pearl system

6. After the surgery the surgeon is asked to complete Survey A: practical workability (see section 8.1.4). The surgical practical workability is deemed positive when the average of scores of all questions for all surgeons is at least neutral. This will be calculated as follows. The answer “definitely not agree” will be awarded -2 points; “not agree”, -1 point; “neutral” 0 points, “agree”, 1 point; definitely agree, 2 points. To be deemed positive, the average of all answers should be at least 0 points.

As briefly described in chapter 8.1.3 TBRs/SBRs/STRs/TRRs will be calculated to quantify fluorescent signal in tumour, rim and background area. Fluorescence quantified value of tumour, rim and background region will be divided from each other to obtain the desired ratio’s. The programs QuPath and MeVisLab will be used for this analysis. They will be performed in-vivo, ex-vivo and on bread loafs.

All intraoperative changes in surgical plan will be recorded. A distinction will be made whether these changes were based on fluorescence (either SGM-101 or ICG) or white light, or both.

Visit 5: Day of discharge from the surgical department.
(Severe) adverse events will be recorded.

Visit 6: 4 weeks after surgery (+-2 weeks)
The first follow-up will coincide with the first outpatient routine post-surgical follow-up approximately 4 weeks (+-2 weeks), after surgery. (Severe) adverse events will be recorded throughout the follow-up. If there is no outpatient post-surgical follow up the study coordinator will plan this visit to assess for possible new (S)AEs. This may be done by telecommunication.

Visit 7 and end of study: 6 months after surgery (+- 2 months)
The results of the routine CT/MRI-scan performed 6 months after surgery will be assessed for follow-up on non-suspicious nodules that were not resected. Also, liver recurrences will be registered. This is not an extra visit.

When all 10 surgeries have been performed, the surgeons that have operated three times or more within the scope of this trial will be handed *survey C: Surgical satisfaction and potency* (see section 8.1.4).

According to standard care, hematoxylin and eosin staining is performed on microscopic slides of tumor and all lymph nodes. In addition to this, immune-histochemistry staining of CEA shall be performed on 4 µm sectioned formalin-fixed paraffin-embedded tumor tissue to demonstrate and confirm CEA-expression. The amount of CEA quantified using the

immunoreactive score (IRS). Also, the percentage of vital tumour cells within the tumour will be assessed and correlated to fluorescent signal. The unstained slides of all resected tumor/lymph nodes shall be imaged using the Odyssey CLx scanner (LI-COR Biosciences, NE, USA). Concordance of fluorescence and histopathology will be assessed after the pathologist has finished the regular histopathological examination. Histopathology will be the gold standard. All previously mentioned pathology assessments are performed by a dedicated gastro-entero-oncological pathologist.

8.3.2 Collection of peripheral and portal blood (circulating tumour cells and extracellular vesicles procedures)

- Portal blood (± 5 ml) from the portal vein
- Peripheral venous blood: day of SGM-101 administration, day of the surgery and day 5 (+-2 days) post surgery. For each day 2 EDTA-coated tubes are needed (15-20 ml of blood in total per day)

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.5 Replacement of individual subjects after withdrawal

Subjects withdrawing for reasons other than adverse events or any other tolerability issues with the treatment will be replaced.

8.6 Follow-up of subjects withdrawn from treatment

Should a subject decide to withdraw from the study, all efforts should be made to complete and report the observations, particularly the follow-up examinations, as thoroughly as possible.

8.7 Premature termination of the study

Before trial medication is administered, changes in the subject health status since the previous visit have to be checked. The investigator must temporally interrupt or permanently discontinue the study drug if continued administration of the study drug is believed to be contrary to the best interests of the subject. The interruption or premature discontinuation of study drug might be triggered by an Adverse Event (AE) or for administrative reasons in particular withdrawal of the subject's consent. The reason for study drug interruption or premature discontinuation must be documented.

9 SAFETY REPORTING

9.1 Definitions of adverse events

An Adverse Event (AE) is any untoward medical occurrence in a subject who is participating in a clinical study performed. The AE does not necessarily have to follow the administration of a study drug, or to have a causal relationship with the study drug. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory or vital sign finding), symptom, or disease temporally associated with the study participation, whether or not it is related to the study drug.

9.1.1 Intensity of adverse events

The intensity of clinical AEs is graded three-point scale as defined below:

- Mild: discomfort noticed but no disruption of normal daily activity;
- Moderate: discomfort sufficient to reduce or affect normal daily activity;
- Severe: inability to work or perform daily activity.

9.1.2 Grading of adverse event

- Grade 1: Mild
- Grade 2: Moderate
- Grade 3: Severe: inability to work or perform daily activity
- Grade 4: Life-threatening
- Grade 5: Fatal

9.1.3 Relationship to study drug

For each AE the relationship to drug as judged by the investigator:

- Related
- Probable;
- Possible;
- Unlikely;
- Unrelated.

9.1.4 Chronicity of adverse events

The chronicity of the AE will be classified by the investigator on a three-item scale as defined below:

- Single occasion: single event with limited duration;
- Intermittent: several episodes of an event, each of limited duration;
- Persistent: event which remained indefinitely.

9.1.5 Action

Eventual actions taken will be recorded.

9.1.6 Serious adverse events

A Serious Adverse Event (SAE) is defined by the International Conference on Harmonization (ICH) guidelines as any AE fulfilling at least one of the following criteria:

- Is fatal
- Is life-threatening
- Is disabling
- Requires or prolongs in-patient hospitalization
- Causes congenital anomaly

will be described as a SAE. Important medical events that may not be immediately life threatening or result in death or hospitalization may be considered a SAE when, based on appropriate medical judgement, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above.

9.1.7 Suspected unexpected serious adverse reactions (SUSARs)

A SUSAR (Suspected Unexpected Serious Adverse Reaction) is a SAE that is unexpected, (nature or severity of which is not consistent with the applicable product information (e.g., investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorised product)) and suspected (a reasonable possibility of causal relationship with investigational drug, regardless of the administered dose).

9.1.8 Reporting of serious adverse events and SUSARs

SAEs and SUSAR's will be reported according to the following procedure. The investigator, on behalf of the Sponsor, will report the SAEs through the web portal ToetsingOnline (see https://toetsingonline.nl/) to the accredited EC that approved the protocol, within 24 hours of first knowledge for SAEs followed by a period of maximum of 8 days to complete the initial preliminary report. All SUSARS must be reported to the EC that approved the study, CA and the Dutch Medicines Evaluation Board (College ter Beoordeling van Geneesmiddelen) within a period of maximum 7 days.

The Sponsor will report expedited the following SUSARs to the EC:

- SUSARs that have arisen in the clinical trial that was assessed by the EC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the EC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.
The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.
In addition, all SUSARs and SAEs must be reported to SurgiMab within 72h of first knowledge of the event. The investigator must fill in the form SGM-ASF56 and send it by e-mail to the SurgiMab's Clinical Project Leader, Bérénice FRAMERY, at bframery@surgimab.com.
The Sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.
The expedited reporting will occur not later than 15 days after the Sponsor has first knowledge of the adverse reactions. For fatal or life-threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.
All adverse and serious adverse reactions will be reported through the end of the study (i.e. up to two weeks after administration of the study drug).

9.1.9 Follow-up of adverse events

All AEs will be followed until they have abated, returned to baseline status or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

9.2 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the investigator will inform the subjects and the EC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the EC, except insofar as suspension would jeopardize the subjects' health. The investigator will ensure that all subjects are kept informed.

9.3 Annual safety report or development safety update report

In addition to the expedited reporting of SUSARs, the investigator on behalf of the sponsor will submit, once a year throughout the clinical trial, a safety report to the EC, CA, MEB and CAs of the concerned Member States.
This safety report consists of:

- A list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- A report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

The investigator will report all AEs and SAEs that occurred during this study and send a list to SurgiMab once a year.

9.4 Pregnancy

9.4.1 Teratogenicity

It is not known whether a single dose of SGM-101 can cause foetal harm when administered to a pregnant woman or whether it can affect reproductive capacity. There have been no studies in pregnant women. Study drug should only be administered to women of childbearing potential when a pregnancy test is negative or the clinical condition of the patient rules pregnancy out. A negative pregnancy test must be documented on the CRF prior to the first dose of study drug in patients of child bearing potential. The investigator must counsel the patient and discuss the risks of continuing with the pregnancy and the possible effects on the foetus. Monitoring of the subject should continue until the outcome of the pregnancy is known.

9.4.2 Reporting of pregnancy

Irrespective of the treatment received by the subject, any pregnancy occurring during study drug administration or 28 days after study drug withdrawal, must be reported within 24 hours of the investigator's knowledge of the event to the sponsor.

10 STATISTICAL ANALYSIS

All statistical analysis will be performed using the Statistical Package for Social Sciences (SPSS; IBM Corporation, Armonk, NY, USA). Continuous demographic variables (e.g. age, height, weight, BMI) will be summarized by descriptive statistics (n, mean, SD, median, Min, Max). Qualitative demographic characteristics will be summarized by counts and percentages. For concordance between fluorescence signals (both ICG and SGM-101) and pathology, the signal-to-background ratio, the tumour-to-background ratio, sensitivity, specificity, positive predicting value, negative predicting value and diagnostic accuracy will be calculated.

10.1 Primary study parameter(s)

See section 8.3 for the calculations and methods for the primary endpoints. For survey A and B the average score will be calculated. Calculation of the sensitivity of SGM-101 will be assessed by the percentage of true positive lesions that were fluorescent (TBR≥ 1.5).
No statistical tests will be performed for these analyses.

10.2 Secondary study parameters

This is a single arm non-randomized exploring feasibility study. Therefore the analyses are solely descriptive. For instance, concordance between fluorescence (y/n) and malignancy at histopathology (y/n) will be expressed by sensitivity, specificity, positive predictive value and negative predictive value for ICG, SGM-101 or both; description of the amount of R1/R2 resections and; rate of surgeries in which a modification of operative plan occurred due to imaging (e.g. extension of resection margins, or additional resection), in and -ex-vivo TBRs/SBRs. With simple linear regression the correlation between bread loaf TBR/SBR and tissue CEA expression or percentage of vital tumour cells or lesion depth will be performed

10.3 Other study parameters

Exploratory data-driven analyses can be performed with the caveat that any statistical inference will not have any confirmatory value. Deviations from the original statistical plan will be documented in the clinical study report.

10.4 Interim analysis (if applicable)

No interim analysis will be performed

11 ETHICAL CONSIDERATIONS

11.1 Regulation statement

The investigator will ensure that this study is conducted in full compliance with the protocol, the principles of the Declaration of Helsinki, version 64, 2013; the ICH GCP guidelines (http://www.ich.org/products/guidelines.html), and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

Patients planned for surgical intervention in the LUMC and eligible for inclusion according to the inclusion and exclusion criteria will be preselected during the weekly multidisciplinary liver team meetings. With a minimum of 2 week before surgery, patients will be recruited from the LUMC. During the consecutive or next outpatient clinic appointment with their clinician (surgeon) or specialized nurse-practitioner will inform the patient about the study and provides them with the patient information forms (PIF, in Dutch: patient informatie formulier). The coordinating investigator will plan a (telephone) appointment 5 days after the patient has received the information, to answer questions and ask for the final decision to participate in the study. If patients indicate they need longer, a new appointment will be made. It is the responsibility of the investigator to obtain written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives and potential hazards of the study. The investigator must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time for any reason.

The informed consent and subject information will primarily be provided in Dutch, if patients have insufficient proficiency in the Dutch language, the information can be provided in English. All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care.

Documented informed consent must be obtained for all patients included in the study before they are registered or randomized in the study. This must be done in accordance with the national and local regulatory requirements. For European Union member states, the informed consent procedure must conform to the ICH guidelines on Good Clinical Practice. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative".

11.3 Benefits and risks assessment, group relatedness

NIR fluorescence imaging using ICG results in improved detection of CRLM, although improvements could still be made. By using a tumour-specific fluorophore, SGM-101, which could be visualized on another wavelength channel, we aim to improve the intraoperative detection of CRLM and thus the rate of R0 resections. This is directly correlated with an improved overall survival. As ICG currently standard of care during oncologic liver surgery, participation in this study only requires an additional visit 4 days

before surgery. SGM-101 has already shown great potential in phase I/II studies and proven to be safe and effective. No DLT have occurred. An extensive risk analysis is described in Chapter 13.

The potential benefits and risks of intra-operative NIR-Fluorescence imaging during curative-intent surgical resection of CRLM using SGM-101:

Potential benefits:

- Potential improved intra-operative visualization of local tumor status and extent, and surgical plane, potentially resulting in:
    - Lesion removal with greater precision; higher chance of achieving an R0 resection.

Patients participating in this study will undergo intraoperative NIR-fluorescence imaging after injection of a single dose of ICG, which is standard-of-care in the LUMC, and SGM-101. NIR-Fluorescence imaging is a clinical technology that requires administration of a fluorescence-imaging agent that can be excited at near-infrared (NIR) wavelengths of ~700-800 nm. Upon illuminating tissue surfaces with penetrating NIR light to excite the imaging agent within the tissues, the generated fluorescence is collected to form a two-dimensional (2D) image demarking the tissue deposition of the imaging agent. Intraoperative NIR-fluorescence imaging is an additional intra-operative tool for surgeons which could provide them with real-time visual enhancement and guidance during open and minimally invasive (laparoscopic/robotic) interventions. NIR-fluorescence imaging with SGM-101 is used in conjunction with and as addition to standard white-light visual inspection (WLI), IOUS inspection and NIR-fluorescence ICG inspection and will not substitute standardized perioperative clinical. The potential benefits of NIR-Fluorescence imaging with SGM-101 on top of ICG during resection of CRLM need to be sought within the additional visual information it generates for the surgeon. The scheduled resection will be carried out according to standardized principles, besides white-light visual inspection (WLI), palpation, IOUS and NIR-fluorescence ICG imaging, NIR-fluorescence SGM-101 imaging will be used to inspect the target lesion(s), infiltration, radicality and to screen for new (liver) lesions as described in section 8.3.1. In the recent SGM-101 phase 2 trial, comprising of patients with locally advanced rectal cancer or locally recurrent rectal cancer, it was found that in 19% of patients, SGM-101 led to more complete tumour tissue removal by highlighting tumour tissue that was not detected by the naked eye, and thereby reduced the R0 rate (12). It is therefore that we suspect that the addition of SGM-101 to ICG in patients with CRLM has the potential to increase the R0 rate. Therefore, in the case NIR fluorescence imaging shows: 1) additional extent of the lesion in the surgical plane, or 2) a fluorescent hotspot in the wound bed suggesting incomplete tumor removal or 3) a fluorescent hotspot on the back table – suggesting limited marges or incomplete tumor removal- the surgeon could decide to resect this additional tissue when it is safe and surgically feasible. All lesions that are suspect on standard of care assessment will be resected (WL, ICG, IOUS, pre-operative imaging), independent of fluorescent status of SGM-101. When a lesion is not suspect/visible with standard of care but SGM-101 positive then an excision biopsy will be performed. A lesion that is not suspect in WL, ICG and SGM-101 will not be resected. During the standard follow-up of 6 months this lesion will be monitored and issued as malignant when it turns out to be malignant at the follow-up. Lastly, in case of doubt, the surgeon is able to perform a small

fresh frozen biopsy (FFS) and send this to pathology for real-time analysis. Depending on the pathological result on this FFS the surgeon can decide how to judge the fluorescence. Post-operative, additionally to the standard histopathological analyses, the pathologist will evaluate the additional (FLI+, WLI-) biopsies/tissue that have been resected separately for presence of malignant tissue. In case, pathological examination shows that the additionally (FLI+, WLI-) resected tissue is 'true positive' i.e. tumor+, this could result in an (oncological) benefit for the patient. In case pathological examination shows that the additionally (FLI+, WLI-) resected tissue is 'false positive', there will be no (oncological) benefit. The additional resected tissue is minimal compared to the magnitude of the planned surgery, the likelihood of harm caused by potential tissue removal based on a false positive signal could be graded minimal. Therefore, the potential harm for the patient is estimated to be negligible compared to the potential benefit of a more complete removal of the CRLM.

This study drug (SGM-101) and study design have been used previously in colorectal and pancreatic cancer patients (phase II), without significant drug-related events, as well as no significant changes in vital signs, ECG or laboratory analysis were observed.
Although, when administrating an investigational product, it is possible that unknown side effects or (hyper)sensitivity reactions occur. Based on experience with other fluorescent tracers, such reactions are generally mild and transient in nature. The risk of damage in this study related to administration of this compound is considered negligible.
Currently several clinical studies (Phase II and III) are enrolling patients with colorectal cancer for evaluation of SGM-101. The multicenter national phase II trial on locally advanced rectal cancer and recurrent rectal cancer (L19-069, NL69838.056.19), still open for inclusion aims to reduce the R1 rate, similar as in the proposed study. The international multicentric phase 3 trial in colorectal cancer is currently in progress in 10 clinical centers and aims for FDA approval (P19.004 and EudraCT 2018-000151-40). In over >300 patients so far, SGM-101 has proven to be well tolerated and safe.
All study drug administrations will be done in the clinic under medical supervision. The patients receiving any study drug will remain in the clinic after the administration of the study drug. Thus, the patients can be closely monitored for any adverse signs during the treatment. Therefore, providing the protocol is adhered to, careful observation and medical management will minimize any associated risk in this study.
Issues of possible concern with the use of the SGM-101 and accompanying imaging system are:

- Adverse reactions to SGM-101.
- Presence and functioning of a Near-Infrared- Fluoresence camerasystem in the operating room;
- Nonspecificity of localization;
- Phototoxicity from the light source;
- Fading of the chromophore (photobleaching);
- Inability to excite SGM-101 or to record emission;

As proven with extensive knowledge of the Leiden University Medical Center, the presence of a camera system in the operating room is not novel and should create little problem with maintaining a sterile field. The Quest Spectrum Platform Camera will be used initially prior to surgical excision to record the localization of tumors and post-excision to document the status. As such, it needs not be intrusive during the procedure.

Standard hospital procedures to ensure sterilization or masking of the equipment will be employed.

There is limited potential for phototoxicity from any light source. The degree of risk is related to the power of the beam and the extent of exposure. The power of the beam in this study is low and potential phototoxicity is negligible. There have been no adverse events with this system or similar systems.

While SGM-101 appears to specifically localize both colorectal and pancreas carcinomas, there is a possibility that a minority of patients will have non-CEA expressing (CEA-negative) tumors and will therefore not benefit from use of this CEA-targeted agent. Although, based on various studies evaluating biomarker expression using immunohistochemical analyses show that CEA expression is expressed in more than 90% of colorectal tumors. More importantly, this expression is not influenced by neoadjuvant therapy, as described by Boogerd et al (27).

Like all chromophores, excitation by appropriate wavelength of light will result in molecular activation in which a different wavelength of light is emitted. This is an active process which changes the excitability of the molecule leading to photobleaching. Since white light contains all wavelengths of light, extended room light exposure could also lead to bleaching. During administration of SGM-101, the infusion bag will be wrapped in aluminium foil. In addition, exposure to excitation light (laser and LED light) will be limited to necessary exposure to properly perform the study-related handlings and surgery. Previous research with SGM-101 has shown that limited exposure to excitation light does not result in decreased fluorescence signal and is therefore negligible.

The risk that the imaging system will not excite SGM-101 or record an image after emission is minimal. An external source of SGM-101 can be used to check that the system is working. In the event of such failure, the surgeon continues as he/she would without the system.

For a detailed structured risk assessment see Section 13-14.

11.4 Study funding

This study is an investigator initiated clinical trial, the study is funded by the Leiden University Medical Center. SurgiMab S.A.S. will be a subsidy provider by providing the drug product (IMP), SGM-101. Financial details are provided in the separate contract(s) between LUMC and SurgiMab.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance (Onderlinge Waarborgmaatschappij Centramed B.A., Voorburg.) which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1) € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2) € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the Research;
3) € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

12 ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Data will be recorded on paper data collection forms and will be entered after quality control in a CASTOR EDC database for subsequent tabulation and statistical analysis. The data will be handled confidentially and will be encoded to ensure patients privacy. A Subject Screening and Enrolment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion. All data from paper source will be entered into the CASTOR EDC. Results of clinical laboratory analyses will be sent electronically to LUMC and loaded into the database. Images and videos will be analysed and stored at the surgical oncology department of LUMC. After the database has been declared complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement between the investigator, sponsor and the statistician.

Patient clinical history (e.g. patient history, substance abuse, etc.), baseline characteristics (e.g. age, length, body weight, BMI, etc.), operation characteristics (e.g. indication, procedure, etc.), intraoperative findings (including primary and secondary endpoints), as well as hospital course and postoperative follow-up evaluation will be prospectively recorded and computerized in the online electronic data capture system Castor (https://data.castoredc.com/#studies).

All patient data will be encoded to ensure the patient's privacy. The key of the sequential code to the patient data/material is safeguarded by the participating centre itself in the screening log. Patients will be assigned in a consecutive order starting with the lowest number. Replacement patients will be numbered +100, e.g. subject 5 will have subject 105 as replacement, and subject 105 will have subject 205 as replacement, etc. Only authorized staff members (see signature log) will have access to the database. Data (including video recordings) will be kept for fifteen years after the end of the study.

12.2 Storage of tissue and associated data

When biobank informed consent is given by the participant, leftover material of the study will be stored in the Biobank CRC. The material and associated data will be used for various future scientific studies into the onset, diagnosis, and treatment of colorectal cancer (CRC). The Biobank CRC is subject to the LUMC Regulations Biobank (LUMC Beheerreglement Biobank), where the LUMC Biobank Facility acts as administrator. Tissue will be stored indefinitely at the department of Pathology of the LUMC and will be registered in the Biobank Information Management System. The decision-making regarding the availability of body material and/or personal data to internal and external applicants is described in detail in the LUMC Beheerreglement Biobank.

12.3 Monitoring and Quality Assurance

Some people can access all the data at the research location, including data without a code. This is necessary to check whether the study is being conducted in a good and reliable manner. Persons who have access to the data for review are: the project leaders, the committee that monitors the safety of the study, a monitor working for the LUMC, national and international supervisory authorities, for example, the Healthcare and Youth Inspectorate (IGJ) and Food and Drug Administration (FDA). They will keep the data

confidential. If the included patients have any questions or complaints about the processing of the data, one can contact the Data Protection Officer of the LUMC or the Dutch Data Protection Authority. Monitoring visits and contacts will occur at regular intervals, according to a frequency defined in the study-specific monitoring plan. A close-out visit will be performed after study closure. Monitoring will be executed by (internal) monitors of the LUMC according to the monitor plan.

12.4 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.5 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.6 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final

study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.7 Public disclosure and publication policy

In accordance with standard editorial and ethical practice, the results of the study will be published in an open access peer-reviewed scientific journal.

13 STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

13.1.1 Level of knowledge about mechanism of action

SGM-101 is a CEA-specific chimeric antibody conjugated with a NIR emitting fluorochrome. Nonclinical pharmacology studies have demonstrated SGM-101's efficacy in binding, in a highly specific manner to human CEA. CEA has no known natural ligand and the binding of anti-CEA antibodies to their target does not trigger the activation of cell signalling pathways. Safety pharmacology studies demonstrated that intravenous SGM-101 administration did not lead to any adverse effects at doses up to 20-40mg/kg. Toxicity associated with SGM-101 use should therefore be minimal.

13.1.2 Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

The radiolabeled anti-CEA antibody CEA-Scan (arcitumomab) from Immunomedics GmbH was authorized in the European Union in 1996 for the assessment of recurrent or metastatic colorectal carcinoma in patients with colorectal cancer. The product has since been removed from the market for commercial reasons but over its 9 years of use, no adverse effects have been reported.

To date, the use of SGM-101 has been successfully implemented in several trials.

In the SGM-CLIN01 trial, SGM-101 was injected to 18 patients. All doses, including the maximal dose of 15 mg, were well tolerated; no dose-limiting toxicity (DLT) has been recorded during the dose escalation phase and none of the adverse events observed have been considered related to the IMP. Furthermore, SGM-101 has been administered up to 10 mg in 14 patients with pancreatic cancer, and up to 15 mg in 56 patients with primary, recurrent and metastatic colorectal cancer in the SGM-CLIN02 trial. No AEs and SAEs related to the product have occurred. Recently it was administered to 13 patients in the SGM-CLIN05 trial. Also in this trial there were no AEs and SAEs related to the product. Additionally it has been administered to >100 patients in the ongoing SGM-CLIN04 trial. In this trial only 2 SAEs occurred that might have been related to the IMP, due to action taken on fluorescence. No Suspected Unexpected Serious Adverse Reaction (SUSAR) was reported and no clinically important emerging efficacy or safety findings have been obtained from this clinical trial. In the ongoing international multicenter SGM-CLIN03 trial, >150 patients have received the IMP so far. No AEs or SAEs have been reported so far that are related to the IMP. In all these trials SGM-101 has already shown (and is showing in the current trials) great potential and proven to be safe and effective. The big majority of (serious) adverse events occurring during the study were either related to the disease or the surgical procedure.

Of the treated patients, many additional malignant lesions have been found and resected that would otherwise not have been detected by the surgeon in white light.

13.1.3 Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

In vitro characterization of SGM-101 allowed to confirm that the antibody recognizes the same epitope of CEA, GOLD 2, as the original 511 murine antibody it is derived from. The binding efficacy of SGM-101 to human CEA expressing cells was demonstrated in vivo in terms of specificity, sensitivity and targeting scope using four different xenografted or transgenic mouse models. It could be concluded that SGM-101 has selectivity for CEA and negligible affinity to receptors other than CEA.

13.1.4 Selectivity of the mechanism to target tissue in animals and/or human beings

The Carcinoembryonic antigen (CEA) is a 200 kDa glycoprotein expressed in a number of tumours of epithelial origin, such as colorectal carcinoma, pancreas carcinoma, lung adenocarcinoma, mucinous ovarian carcinoma, endometrial adenocarcinoma and gastric carcinoma. CEA was initially described as an oncofetal antigen, namely, a molecule expressed during fetal development, absent in healthy adults and re-expressed in adults with certain types of cancer. It has however since then been shown that it also has a limited expression in normal adult tissue. As mentioned in section 11.1.3, SGM-101 has selectivity for CEA and negligible affinity to receptors other than CEA.

13.1.5 Study population

The study population will consist of patients diagnosed with liver metastases of colorectal origin for which surgical resection is proposed and meet at least one of the following criteria:

a. Scheduled for surgical resection of >3 CRLM or;
b. completed neo-adjuvant therapy, of which the last course was completed within 3 months before surgery or;
c. Scheduled for surgery because of a locally recurrent liver metastasis.

Females of childbearing potential may participate in the trial, but only when they agree to use a highly effective form of contraception in the cases where the child bearing potential is retained post-surgery.

13.1.6 Pharmacokinetic considerations

The starting dose of SGM-101 and the time to perform surgery after dosing are based upon our experiences with the phase I and phase II study. The assumption that with these doses and the time between dosing and surgery sufficient amounts of SGM-101 will have reached the site of action and is supported by the nonclinical data currently available.

13.1.7 Interaction with other products

As the trial will be performed during routine care and no drug-drug interactions with SGM-101 are known, concomitant medication is allowed to be used as in routine care. However

use of other fluorescent agents than ICG are not allowed, as these agents possibly excite and emit light in the same near-Infrared range as SGM-101 and could interfere with the image acquisition of SGM-101.

13.1.8 Predictability of effect

Participation in this study could result in the intraoperative detection of more lesions with the potential of being malignant than would have been detected with standard practices. Resections will be performed according through the following criteria:

1. All lesions that are suspect on standard of care assessment will be resected (WL, ICG, IOUS, pre-operative imaging), independent of fluorescent status of SGM-101.
2. When a lesion is not suspect/visible with standard of care but SGM-101 positive then a small excision biopsy will be performed to correlate it to pathology assessment.
3. A lesion that is not suspect with standard of care assessment and SGM-101 will not be resected. During the standard follow-up of 6 months this lesion will be monitored and issued as malignant when it turns out to be malignant at the follow-up.

Furthermore we expect more complete resections with the help of both ICG and SGM-101. As earlier mentioned, in the recent multicentre MIMIC-trial - comprising of a large cohort of patients that received ICG because of CRLM - it was shown that the R1 resection rate decreased from 12.6% to 7.6% in minimal invasive resections for CRLM (under submission). Also it resulted in a change in surgical management in 28% of patients (5). In the same study it was shown that in nearly 10% of patients additional malignant lesions were found due to ICG, Therefore, the use of ICG during liver resections is currently standard-of-care in the LUMC. Similar for SGM-101, in the phase 2 cohort, comprising of patients with locally advanced colorectal cancer or recurrent colorectal cancer, it was found that in 19% of patients, SGM-101 led to more complete tumour tissue removal by highlighting tumour tissue that was not detected by the naked eye, and thereby reduced the R0 rate (12). Therefore, also in this trial, when NIR fluorescence imaging shows additional extent of the lesion in the surgical plane (positive margins, incomplete tumor removel, irradical resection) which is not visible during initial clinical assessment and WLI, IOUS inspection and ICG imaging, the surgeon could decide to resect this additional tissue when it is surgically feasible. If additional (suspect) tumor tissue will be resected, this concerns a minimal amount. In concrete terms, one or a few millimeters of tissue of the surgical margins. Given the magnitude of the planned surgery, the likelihood of harm caused by potential tissue removal based on a false positive signal could be graded nihil. Therefore, the potential harm related to intraoperative NIR-fluorescence imaging is estimated to be minimal compared to the potential benefit of a more accurate assessment of local tumor status and the potential of a more complete (radical) resection of the tumor. Based on experience from previous performed feasibility studies within colorectal liver metastases surgery the predictability of effect is adequate (14).

13.1.9 Can effects be managed?

The risks of participation for the patients in the trial include adverse (hypersensitivity) reactions. These risks are deemed minimal. Nevertheless, precautionary measures

(supervised administration by qualified staff and availability of medical treatment to treat hypersensitivity reactions) are in place and these effects are generally well manageable.

13.2 Synthesis

SGM-101 has no known biological actions other than binding to the CEA receptor and this translated into an unremarkable toxicology profile in animals. This warrants investigation of SGM-101 in humans in a dedicated hospital/clinical research unit setting.

14 STRUCTURED RISK ANALYSIS (Part II – IMD)

Camera system	Filename
Quest Spectrum Imaging platform v2/3.0	D2. Investigational Medical Device Dossier (IMDD)_Quest_Medical_Spectrum_Imaging_ Platform_2_and_3.0_Final_07102021

14.1 Potential issues of concern

14.1.1 Level of knowledge about mechanism of action

The level of knowledge about the mechanism of action for NIR-fluorescence imaging is deemed high. Clinical translation of NIR-fluorescence guided surgery in several malignancies has been the topic of interest within our research group in the last 15 years, which is shown by the number of published clinical trials evaluating NIR-fluorescence imaging using various fluorescent agents in combination with the NIR-fluorescence imaging systems on the market. Furthermore, these systems have been thoroughly tested before their market introduction and CE-certification. Although we focus on tumor-targeted imaging, the mechanism of action of these systems, namely: visualizing the emission of NIR-light of the fluorophore bound to the target tissue, using a NIR-camera and computer to digitally visualize the signal location and it's intensity. Our experience with these (section 7.1.1) systems are based on the various preclinical and clinical studies that have been carried out for evaluating *in-vivo* fluorescence of study subjects, for detailed information and study references we refer to the provided IMPD of the Quest Spectrum Imaging Platform.

14.1.2 Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Not applicable.

14.1.3 Selectivity of the mechanism to target tissue in animals and/or human beings

The selectivity of the mechanism of action is based on the selectivity of the used fluorescent agent as described in section 6 and 7, since the NIR-fluorescent imaging system only visualizes the fluorescence of the bound fluorescent agent. Based on preclinical and clinical trials we demonstrated the high selectivity of SGM-101 for tumors overexpressing CEA (10, 12, 13, 28-31).

In vitro stability studies in human plasma at 37°C have confirmed the stability of the amide bonds linking the BM-104 fluorochrome to the antibody.

In vivo studies conducted in human CEA-expressing mouse models have shown similar pharmacokinetic profiles as in wild-type strains; CEA expression in this transgenic model is exclusively apical, with the antigen not being accessible to circulating SGM-101. Tissue distribution was also comparable in transgenic human CEA-expressing mice and in wild-type animals. SGM-101 was shown to be rapidly eliminated in immunosuppressed mice bearing human intraperitoneal tumors overexpressing CEA and in normal Swiss mice, with remaining SGM-
101 levels of less than 10 % of the injected dose, 10 days after injection. Toxicokinetic evaluation was performed within the main GLP-compliant toxicology study in Wistar rats.

SGM-101 plasma levels quantified in rats treated intravenously at 5, 20 or 40 mg/kg/day increased with dose in an almost proportional manner and exposure to SGM-101 was slightly higher in males than in females at the highest dose level tested.

Preliminary analysis of the pharmacokinetic data from the first-in-man clinical trial described on page 61 indicates that SGM-101 maximal serum concentrations are usually reached at or close to the end of the infusion and decrease progressively thereafter, with a mean t1/2 of approximately 30 h regardless of the dose level (range of values between 16.9 and 36.3 h). From 5 to 12.5 mg, exposure tends to increase with dose but appears to remain unchanged between 12.5 and 15 mg.

14.1.4 Study population

See section 13.1.5

14.1.5 Pharmacokinetic considerations

The administered dose of SGM-101 and the time to perform surgery after dosing are based upon our experiences with the phase I and phase II study. The assumption that with this dose and the time between dosing and surgery sufficient amounts of SGM-101 will have reached the site of action and is supported by the nonclinical data currently available.

14.1.6 Interaction with other products

As the trial will be performed during routine care and no drug-drug interactions with SGM-101 are known, concomitant medication is allowed to be used as in routine care

14.1.7 Predictability of effect

See 13.1.8

14.1.8 Can effects be managed?

The proposed investigational intraoperative procedure is a similar procedure as standard hepatic resections, with the addition of a tumor-targeted NIR fluorescence tracer (SGM-101) to reduce the number of false-negative lesions and incomplete (R1) resections based on standard-of-care assessment (WL, ICG, IOUS, pre-operative imaging). Given the magnitude of the planned surgery, the likelihood of harm caused by potential tissue removal based on a false positive signal could be graded nihil and is estimated to be negligible compared to the potential benefit of a more complete and accurate removal of the tumor. There is no additional risk of using the intraoperative camera system above usual use of other equipment in the operation room, all personnel using the equipment are trained and updated for using these systems. A dedicated team of nurses, researchers, and surgeons in the LUMC, with over 10 years of experience in the successful completion of clinical trials evaluating fluorescence-guided surgery- especially SGM-101 - in various tumor types are engaged during this project. Based on experience from performed studies in colorectal and liver surgery the effect can be managed, and furthermore management of (undesired) effects are comparable to hepatic cancer surgery without SGM-101 NIR-fluorescence imaging.

14.2 Synthesis

NIR-fluorescence imaging using the dedicated systems from Quest Medical Imaging have shown to be capable of safe visualization of NIR-fluorescence signal in patients with various solid tumors, including secondary liver tumors using tumor-targeted moieties. The additional risk of using these intraoperative camera systems for NIR-fluorescence imaging is deemed negligible. SGM-101 NIR-fluorescence imaging is an addition on the standard practice of clinical assessment with white-light visual inspection, palpation IOUS and ICG NIR-fluorescence imaging. Safety and the clinical assessment will always be leading in the decision of responsible surgeon(s) to deviate from the initial plan. Concrete, for every (newly identified) lesion, resections will be performed according to the following criteria:

1. All lesions that are suspect on standard of care assessment will be resected (WL, ICG, IOUS, pre-operative imaging), independent of fluorescent status of SGM-101.
2. When a (newly identified) lesion is not suspect/visible with standard of care but SGM-101 positive then a small excision biopsy will be performed.
3. A lesion that is not suspect with standard of care assessment and SGM-101 will not be resected. During the standard follow-up of 6 months this lesion will be monitored and issued as malignant when it turns out to be malignant at the follow-up.

For suspected (rest-) tumor tissue in the surgical bed (i.e. suspect positive surgical margins, R1), surgeon will perform a small reresection or take a biopsy of the fluorescent hotspot to correlate this to pathology. final decision to perform a reresection, will be based on clinical (re)assessment with this additional information. In which the deviation of the resection, e.g. resection of additional tumor-suspect (liver) tissue is always carefully weighed and only performed if considered safe and surgically feasible.
Given the magnitude of the planned surgery, the likelihood of harm caused by potential tissue removal based on a false positive signal could be graded nihil. Therefore, the potential harm related to intraoperative NIR-fluorescence imaging is estimated to be minimal compared to the potential benefit of a more accurate assessment of local tumor status and the potential of a more complete (radical) resection of the tumor. In all the aforementioned trials with SGM-101 it was shown that SGM-101 was safe and effective, benefiting the surgical resections by resulting in tumor removal that would otherwise not have been removed (10, 12-14, 30).

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