Clinical Study Protocol

Zdravljenje primarnih jetrnih tumorjev z elektrokemoterapijo (ECT)

The Treatment of Primary Liver Tumours with Electrochemotherapy (ECT)

Acronym: PLECT

Clinical Trials number: NCT02291133

Principal investigator:	Mihajlo Đokić, MD Assist. Prof. Blaž Trotovšek, MD, PhD
Coordinators of the clinical part of the study:	Head physician Dragoje Stanisavljević, MD Assist. Valentin Sojar, MD
Study coordinators:	Prof. Gregor Serša, PhD Prof. Borut Štabuc, MD, PhD
Institution:	University Medical Centre Ljubljana
Participating institutions:	Institute of Oncology Ljubljana, (Prof. Gregor Serša, PhD) University of Ljubljana, Faculty of Electrical Engineering, Laboratory of Biocybernetics (Prof. Damijan Miklavčič, PhD)
Phone number:	5223168 (Mihajlo Đokić) 5223756 (head physician Dragoje Stanisavljević) 5879 434 (Prof. Gregor Serša, PhD)
E-mail:	mihajlo.djokic@kclj.si gsersa@onko-i.si

November 6, 2014

Table of Contents

1 PURPOSE OF THE STUDY

The purpose of this clinical study is to evaluate the safety and efficacy of bleomycin-based electrochemotherapy (ECT) in treating primary liver tumours in a phase I and II clinical study.

This will be achieved by:

- applying treatment in a controlled manner based on pre-clinical and clinical experience obtained in the ESOPE project;
- evaluating the procedures and results achieved;
- preparing standardized procedures;
- disseminating these new approaches to treatment using written and audio-visual materials, as well as by attending and organizing expert meetings.

The study is designed as an institutional study (at the University Medical Centre Ljubljana) conducted in cooperation with the Institute of Oncology Ljubljana and the Faculty of Electrical Engineering (University of Ljubljana), as well as an industrial partner, IGEA s.r.l., Carpi, Italy, which will provide technical support for the study. In phase I, 10 patients will be included in the study, and in phase II (i.e. the extension of the study), an additional 15 patients who meet the inclusion criteria will be included.

The study will be carried out within the P3-0003 "Razvoj in ovrednotenje novih terapij za zdravljenje malignih tumorjev" (Development and Evaluation of New Therapies for Treating Malignant Tumours) research programme headed by Prof. Gregor Serša, PhD.

Principal investigators:

Mihajlo Đokić, MD (surgeon)
Assist. Prof. Blaž Trotovšek, MD, PhD (surgeon)

Study coordinators:

Prof. Gregor Serša, PhD (researcher)
Prof. Borut Štabuc, PhD (gastroenterologist)

Coordinators of the clinical part of the study:

Head physician Dragoje Stanisavljević (surgeon)
Assist. Valentin Sojar, (surgeon)

Colleagues:

University Medical Centre Ljubljana

Rado Janša, PhD (gastroenterologist)
Peter Popović (radiologist)
Miha Petrič (surgeon)
Assist. Robert Juvan (surgeon)
Assist. Prof. Aleš Tomažič, PhD (surgeon)
Head physician Vojka Gorjup, PhD (cardiologist)

Institute of Oncology Ljubljana

Prof. Eldar Gadžijev, PhD (surgeon)
Assist. Ibrahim Edhemović (surgeon)
Erik Brecelj, PhD (surgeon)
Tjaša Pečnik (research nurse)

Prof. Maja Čemažar, PhD (researcher)

Faculty of Electrical Engineering, University of Ljubljana
Prof. Damijan Miklavčič, PhD (researcher)
Prof. Tomaž Jarm, PhD (researcher)
Barbara Mali, PhD (researcher)
Denis Pavliha (researcher)
Bor Kos (researcher)
Marija Marčan (researcher)

2 STARTING POINTS

The Principles of Electroporation and Electrochemotherapy (ECT)

By exposing a cell to an external electric field, a transmembrane voltage is established on the cell membrane. When the threshold value is exceeded, this voltage causes electroporation of the cell membrane, i.e. the formation of hydrophilic pores, which allow the increased passage of molecules into the cell.[1]

The principle of electrochemotherapy (ECT) is based on exposing the tumour to pulses of electroporation delivered by specific electrodes. This allows the passage of previously applied hydrophilic cytostatics, e.g. bleomycin and cisplatin, into the cells.[2] Because the intake of cytostatics is only increased in the areas exposed to the electroporation pulses, ECT is a local treatment method. In addition to the direct action of cytostatics on tumour cells, ECT has an indirect vascular-disrupting effect, because cytostatics act on the endothelial cells in the tumour vasculature.[3] The doses of cytostatics used in ECT are extremely low, resulting in minimal or no systemic toxicity.[4] Furthermore, the application of electroporation pulses reduces the blood flow in the tumour, which keeps the cytostatics in the tumour for a longer period, thus making the treatment more effective.[3]

Clinical Applications of ECT

In the early 1990s, the team at the Institute of Oncology Ljubljana headed by Prof. Serša, PhD, played an active part in pioneering clinical studies on treating cutaneous and subcutaneous tumours with ECT.[5] According to the findings of these first clinical trials, an 80% objective response of the treated tumour nodules is achieved using ECT.[6] Our team also helped draw up and publish standard surgical procedures for treating cutaneous and subcutaneous tumours using ECT. Today, ECT is a well-established method for treating malignant melanoma metastases, especially as part of palliative care,

in 15 European countries. In 2012, 2500 patients were treated with ECT and, according to several databases, ECT is now used in 135 oncology centres.

The first recorded clinical case of treating a deep-seated tumour with ECT involved treating melanoma metastases in thigh muscles.[7] Experience in treating other types of soft tissue tumours with ECT is good but limited to a few clinical cases and studies involving a small number of patients.[8–11] In addition to treating deep-seated soft tissue tumours, ECT is used to treat deep-seated tumours in the internal organs. Brain and liver metastases have already been shown to be effective targets of ECT.[12–14] The results of a clinical study on electrochemotherapy used to treat liver metastases of stage I and II colorectal cancer prove the safety and efficacy of this approach. Preliminary results have already been published for the case of one patient, where the treatment procedure is described in detail.[14] Conclusions on safety and efficacy are in the process of publication for the first 17 patients treated with electrochemotherapy. No serious side effects attributable to ECT were observed in the study, and the efficacy results suggest an 80% response from the treated metastases to ECT, which is comparable to that of skin metastases.

Treatment of Primary Malignant Liver Tumours

Primary malignant liver tumours (carcinomas) represent a larger group of tumours that originate from various liver cells. Among such tumours, hepatocellular carcinoma (90%) and intrahepatic cholangiocarcinoma (8.5%) are the most common, while other primary liver carcinomas are significantly rarer. Liver tumours are the sixth most common cancer in the world and represent the third most common cause of death from cancer. The incidence of liver tumours is increasing worldwide, including in Slovenia, where the incidence is 9.3 in men and 4.6 in women per 100,000 people per year. The current treatment strategy is based on the resection of the affected liver parenchyma or radiofrequency ablation – these being the two methods of definitive treatment or bridging therapy to possible liver transplantation. Depending on the stage of the disease, transarterial chemoembolization (TACE), hepatic intra-arterial injection of lipiodol and systemic therapy with sorafenib are also considered palliative treatments. Radical liver resection or liver transplantation are the only promising treatment methods with a 5-year survival rate that can be greater than 50%. Radiofrequency ablation (RFA) can be used as a definitive treatment method (it can be repeated) or as bridging therapy to possible life-saving liver transplantation. RFA and percutaneous ethanol-lipiodol injection are suitable for smaller tumours and for tumours in specific locations (the proximity of blood vessels, subcapsular location). RFA is also offered to patients with individual smaller metastases as a

local form of treatment. Tumours that grow into the inferior vena cava or into the area of the large hepatic and portal veins, or on which RFA has little or no effect due to cooling, are particularly problematic. Therefore, new and more effective approaches to treating liver metastases are being sought for such patients. Distant cancer is usually treated with systemic chemotherapy or biological drugs, which are only partially successful while producing high systemic toxicity.

At the University Medical Centre Ljubljana, in cooperation with the Institute of Oncology Ljubljana, a phase I (10 patients) / II clinical study will be designed to assess the safety and efficacy of treatment for primary liver tumours using ECT. We aim to include an additional fifteen (15) patients within phase II using this protocol. The study aims to prove that treating primary liver tumours using ECT is a safe method even in hard-to-reach locations. Using imaging, we will also assess the response to therapy and the asymptomatic interval.

Planning ECT Treatment

The ECT treatment of deep-seated tumours in internal organs requires planning based on diagnostic radiology imaging. The electrode type is selected based on the precise location and measured size of the tumour nodules; for tumours located up to 3 cm (the lower edge of the metastasis) below the surface of the liver, which are more easily accessible to ECT, short-needle electrodes with a fixed hexagonal geometric arrangement are appropriate, while for hard-to-reach and deep-seated metastases, long-needle electrodes with arbitrary geometric arrangement have recently been made available. In line with technological advances and the development of new types of electrodes, experts at the Italian company IGEA designed a new electric pulse generator called the Cliniporator VITAE for treating larger and deep-seated tumours. The generator meets the essential requirements for consumer safety, health and environmental protection as defined by the EU guidelines or regulations, as it has the CE mark.

The distribution of the electrical field within the tissue is an important predictor for the success of ECT tumour treatment, as the change in the transmembrane potential of the cell membrane, which is a prerequisite for successful electroporation, is directly proportional to the strength of the electric field.[19] Numerical modelling is currently the only effective method for predicting the electric field distribution in tissue, as its structure is, by definition, inhomogeneous, nonlinear and in some cases anisotropic. When using long-needle electrodes with an arbitrary geometric arrangement, it is necessary to determine the depth of electrode insertion into the tumour nodule, as well as the

voltage, length and frequency of the supplied electroporation pulses based on numerical modelling in order to achieve the optimal coverage of the tumour nodules with the electric field.[7]

Electroporation Pulse Synchronization Using Electrocardiogram (ECG)

In ECT treatment, the electric current reaches the area around the target due to the high electrical conductivity of the tissue. This increases the likelihood of possible interactions between the electroporation pulses and the heart function when treating deep-seated tumours in internal organs due to the anatomical proximity of the heart. In our clinical study, we will use a synchronization procedure built into the electroporation pulse generator (Cliniporator VITAE) to avoid cardiac dysfunction caused by ECT. So far, this procedure has proven to be effective in preventing cardiac pacing during the 'ventricular sensitivity' period.[14] Nevertheless, further research is urgently needed to verify the effectiveness of the existing synchronization procedure and to determine any short- or long-term effects of electroporation pulses on the heart function.

Preliminary results suggest that ECT does not seriously affect cardiac function though there are statistically significant changes in some parameters of heart rate variability during and after therapy relative to the pre-therapy parameter values.

The analysis of ECG signals recorded during ECT applied to liver tumours shows short-term effects on the cardiac function, even though the delivery of the electroporation pulses was synchronized with the ECG. The effects were expressed as a temporary shortening of the corrected QT interval and an increase in short-term heart rate variability. The analysis of the ECG signals recorded before and after ECT was applied to liver tumours indicates that ECT has longer-term effects on cardiac function, which are expressed as increased heart rate or a shortened RR interval and as reduced long-term heart rate variability parameters (low-frequency component). We find that these changes are most likely at least partly attributable to the effects of analgesics and other medications received by patients in intensive care, as well as to postoperative pain, and not to the effects of the electrochemotherapy itself, although these results have yet to be confirmed. These results could be confirmed by capturing ECG signals before, during and after abdominal surgery, which would include procedures similar to those performed during the ECT of liver tumours. The only difference should be that ECT is not performed during these surgical procedures. The ECG signals captured in this way would serve as a control and would allow these assumptions to be confirmed.

1. Kotnik T, Pucihar G, Miklavcic D. Induced transmembrane voltage and its correlation with electroporation-mediated molecular transport. *J Membr Biol* 2010; 236(1): 3–13.

2. Sersa G, Stabuc B, Cemazar M, Jancar B, Miklavcic D, Rudolf Z. Electrochemotherapy with cisplatin: potentiation of local cisplatin antitumour effectiveness by application of electric pulses in cancer patients. *Eur J Cancer* 1998; 34(8): 1213–8.

3. Sersa G, Jarm T, Kotnik T, Coer A, Podkrajsek M, Sentjurc M *et al.* Vascular disrupting action of electroporation and electrochemotherapy with bleomycin in murine sarcoma. *Br J Cancer* 2008; 98(2): 388–98.

4. Marty M, Sersa G, Garbay J, Gehl J, Collins C, Snoj M *et al.* Electrochemotherapy – An easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: Results of ESOPE (European Standard Operating Procedures of Electrochemotherapy) study. *Ejc Supplements* 2006; 4(11): 3–13.

5. Rudolf Z, Štabuc R, Čemažar M, Miklavčič D, Vodovnik L, Serša G. Electrochemotherapy with bleomycin. The first clinical experience in malignant melanoma patients. *Radiol Oncol* 1995; 29: 229–35.

6. Sersa G, Miklavcic D. Electrochemotherapy of tumours. *J Vis Exp* 2008; (22).

7. Miklavcic D, Snoj M, Zupanic A, Kos B, Cemazar M, Kropivnik M *et al.* Towards treatment planning and treatment of deep-seated solid tumours by electrochemotherapy. *Biomed Eng Online* 2010; 9: 10.

8. Shimizu T, Nikaido T, Gomyo H, Yoshimura Y, Horiuchi A, Isobe K *et al.* Electrochemotherapy for digital chondrosarcoma. *J Orthop Sci* 2003; 8(2): 248–51.

9. de Bree R, Tijink BM, van Groeningen CJ, Leemans CR. Electroporation therapy in soft tissue sarcoma: a potentially effective novel treatment. *Sarcoma* 2006; 2006: 85234.

10. Curatolo P, Quaglino P, Marenco F, Mancini M, Nardò T, Mortera C *et al.* Electrochemotherapy in the treatment of Kaposi sarcoma cutaneous lesions: a two-center prospective phase II trial. *Ann Surg Oncol* 2012; 19(1): 192–8.

11. Campana LG, Mocellin S, Basso M, Puccetti O, De Salvo GL, Chiarion-Sileni V *et al.* Bleomycin-based electrochemotherapy: clinical outcome from a single institution's experience with 52 patients. *Ann Surg Oncol* 2009; 16(1): 191–9.

12. Soden DM, Larkin JO, Collins CG, Tangney M, Aarons S, Piggott J *et al.* Successful application of targeted electrochemotherapy using novel flexible electrodes and low dose bleomycin to solid tumours. *Cancer Lett* 2006; 232(2): 300–10.

13. Agerholm-Larsen B, Iversen HK, Ibsen P, Moller JM, Mahmood F, Jensen KS *et al.* Preclinical validation of electrochemotherapy as an effective treatment for brain tumours. *Cancer Res* 2011; 71(11): 3753–62.

14. Edhemovic I, Gadzijev EM, Brecelj E, Miklavcic D, Kos B, Zupanic A *et al.* Electrochemotherapy: a new technological approach in treatment of metastases in the liver. *Technol Cancer Res Treat* 2011; 10(5): 475–85.

15. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer* 2010; 46(4): 765–81.

16. Gatta G, Capocaccia R, Sant M, Bell CM, Coebergh JW, Damhuis RA *et al.* Understanding variations in survival for colorectal cancer in Europe: a EUROCARE high resolution study. *Gut* 2000; 47(4): 533–8.

17. Briux J, Sherman M. Management of Hepatocellular Carcinoma: an update. Hepatology 2011; 53(3): 1020–2

18. Miklavcic D, Beravs K, Semrov D, Cemazar M, Demsar F, Sersa G. The importance of electric field distribution for effective in vivo electroporation of tissues. *Biophys J* 1998; 74(5): 2152–8.

3 PATIENT SELECTION

The suitability of patients for inclusion in the study will be assessed using inclusion and exclusion criteria.

3.1 Inclusion Criteria

1. Patients with primary liver tumours; hepatocellular carcinoma, intrahepatic cholangiocarcinoma and other primary tumours, up to 7 cm in size, that are difficult to resect due to their position (likelihood of insufficient healthy liver after resection) or inaccessible for treatment with standard ablation techniques because they are located on large hepatic vascular structures (portal veins, hepatic veins or their presence in the inferior vena cava or its immediate vicinity, subcapsular location of many smaller tumours), but are otherwise operable.
2. Patients in which imaging has conformed disease progression after prior therapy with TACE, RFA or percutaneous alcohol injection and who are not candidates for potentially curative treatment, but have a satisfactory performance status and a Child-Pugh score of less than 8.

   Patients in groups 1 and 2 are patients for whom the standard treatment options (systemic and/or surgical) have been exhausted. ECT will be offered to these patients as the only therapeutic option. If a patient has a large number of tumours, the tumours that are both unresectable and unsuitable for RFA will be treated with ECT, while any other metastases will be resected or treated with RFA.
3. Patients with smaller tumours that are not suitable for RFA or percutaneous alcohol injection due to their location (used as bridging therapy to liver transplantation)
4. Patients with tumours that are larger than 4 cm in hard-to-reach locations (unsuitable for surgical resection) and unsuitable for other treatment methods.

   Patients in groups 3 and 4 are potentially curable using standard treatment. The added ECT used on these patients will not affect the standard treatment recommended in the current guidelines.
5. The patient is offered ECT treatment even if the patient refuses standard treatment options.
6. A primary liver tumour confirmed histologically and/or by a multidisciplinary team for liver disease based on radiological and laboratory diagnostics.
7. Age above 18 years.
8. Life expectancy over 3 months.
9. Performance status (PS) ≥ 70 according to Karnofsky PS or < 2 according to WHO recommendations.
10. Depending on the type of active substance, 2-5 weeks have passed since the last treatment, if any.
11. The patient must understand the treatment process and any side effects.
12. The patient must be able to give consent to participate in a clinical trial (‘informed consent’).
13. Prior to inclusion in the study, the patient should be treated by a multidisciplinary team for gastrointestinal tumours.

3.2 Exclusion Criteria

1. Any previously confirmed form of cancer other than surgically treated non-invasive uterine cancer or basal cell carcinoma treated with surgery or radiotherapy.
2. Proven visceral and/or bone metastases or diffuse metastases.
3. Life-threatening infection and/or heart failure and/or liver failure and/or other life-threatening systemic conditions.
4. Clinically significant ascites.
5. Significantly reduced lung function.
6. Age under 18 years.
7. Major coagulation system disorders (coagulation system disorders that do not respond to standard therapy, such as vitamin K replacement or fresh frozen plasma).
8. Received cumulative dose of bleomycin ≥ 250 mg/m^2.
9. Allergic reactions to previous treatment with bleomycin.
10. Chronic decrease affecting renal function (creatinine > 150 µmol/L).
11. Epilepsy.
12. Heart rhythm disorders.
13. Implanted pacemaker or defibrillator.
14. Pregnancy.
15. Patients who are unable to understand the purpose of the study or do not agree to participate in the study.

3.3 Examinations and Tests Before Treatment

Two weeks before inclusion in the study, the following will be performed:

- clinical examination, anamnesis
- radiological imaging techniques to discover liver metastases:
  - dynamic contrast-enhanced ultrasound (DCE-US)
  - computed tomography (CT) perfusion
  - magnetic resonance imaging
- Systemic disease evaluation
  - X-ray or CT of the lungs
  - other examinations depending on the clinical picture (bone scintigraphy, PET/CT)

One week before inclusion in the study, the following will be performed:

- laboratory tests:
  - haematological tests with a complete blood count
  - coagulation tests

- biochemical tests: electrolytes, creatinine, transaminases
- immunohistochemical examinations: tumour markers

- ECG

4 TREATMENT PLAN

Where there are liver tumours (up to 3 cm in maximum diameter) with the lower edge located up to 3 cm below the liver capsule, ECT using short-needle electrodes with a hexagonal geometric arrangement will be performed in line with the standard surgical procedures for treating cutaneous and subcutaneous tumours/metastases with ECT.

Where there are larger liver tumours (up to 5 cm) located near the vena cava or along large hepatic or portal veins, ECT using long-needle electrodes with an arbitrary geometric arrangement will be performed. Using numerical modelling, we will prepare a treatment plan to determine the optimal position of the electrodes and the required electric current voltage for the optimal electroporation of the target tissue. The preparation of a treatment plan using numerical modelling will take place in two basic steps (see below) based on radiological images captured up to 2 weeks before the procedure.

1. Decomposing medical images and constructing a model:
   - importing medical radiology images into an appropriate program,
   - determining important areas,
   - pretreating and decomposing images,
   - constructing a 3D model.
2. Planning treatment:
   - The 3D model will be supplemented with electrodes supplying electroporation pulses, as well as with corresponding tissue properties and boundary conditions based on the calculation using the finite element method and the calculation processed by automatic algorithms for determining the criterion function,
   - the possibility of statistical and probability functions to determine the probability of electroporation will be examined, the effect probability being a function of the electric field strength applied.

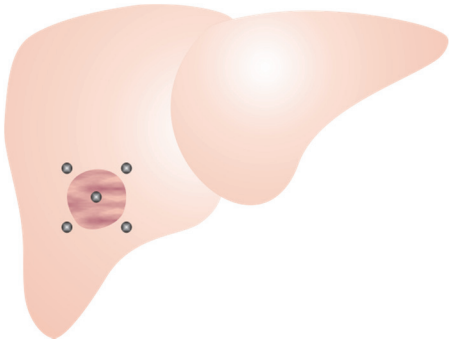

Figure 1: Schematic representation of electrode placement in the middle and the periphery of a metastasis in healthy liver tissue.

5 TREATMENT: ELECTROCHEMOTHERAPY (ECT)

Patients meeting the inclusion criteria with primary liver tumours for which surgery is not possible due to the proximity of the main hepatic veins, for whom the success of other ablation techniques cannot be predicted, will undergo ECT.

5.1 Chemotherapeutic Agents

- The cytostatic used will be bleomycin (BLM) manufactured by Heinrich MackNachf. GmbH & Co. KG, Illertissen, Germany.
- The BLM will be dissolved in saline solution and administered intravenously with a bolus injection at a dose of 15 mg/m^2.
- 8-28 min after BLM application, electroporation pulses will be delivered to the metastases.

5.2 Electroporation

5.2.1 Electric Pulse Generator

- We will use the Cliniporator VITAE electric pulse generator manufactured by IGEA, Carpi, Italy, designed to treat larger and deep-seated tumours, such as liver metastases.
- It has the CE mark and is authorized for use in a clinical setting.

5.2.2 Electrodes

Two types of electrodes will be used:

a) Short-needle electrodes with a fixed hexagonal geometric arrangement:

- for metastases with their lower edge located up to 3 cm below the liver capsule;
- ECT will be performed in line with the standard surgical procedures for cutaneous and subcutaneous tumours/metastases;

b) Long-needle electrodes with an arbitrary geometric arrangement:

- for deeper-seated tumours and tumours located ≥ 3 cm below the liver capsule;
- to ensure the safety margin, the required number of electrodes (presumably from 4 to 6) will be inserted into the tumour and its surrounding area using ultrasound guidance in line with the treatment plan prepared based on numerical modelling before the procedure.

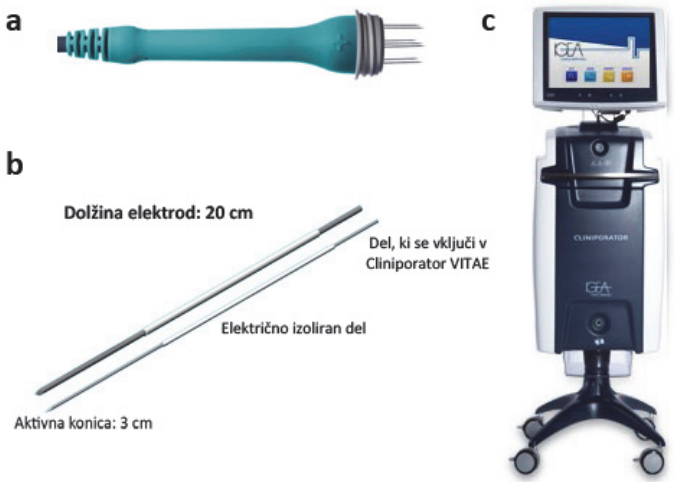

Figure 2: **Short-needle electrodes with a fixed hexagonal geometric arrangement (a), long-needle electrodes with an arbitrary geometric arrangement (b) and Cliniporator VITAE (c).**

5.2.3 Electroporation Pulses

- Between each pair of electrodes, 8 rectangular electroporation pulses (length: 100 µs) will be supplied to the tumours in two sequences, each containing 4 electroporation pulses of reversed polarity and a repeating frequency of 1000 Hz.
- The delivery of individual pulse sequences will be synchronized with the occurrence of successive heartbeats.
- When using long-needle electrodes with an arbitrary geometric arrangement, the voltage of the electroporation pulses for each electrode pair will be determined on the basis of

numerical modelling, and when using short-needle electrodes with a fixed hexagonal geometric arrangement, a voltage of 730 V will be supplied.

- If excessive electric current (> 50 A) makes it impossible to supply a sufficiently high voltage, a larger number of lower-voltage electroporation pulses will be supplied.

5.3 The Synchronization of Electroporation Pulses with ECG and Holter ECG Recording

- To ensure synchronization, the Cliniporator VITAE will be connected to an AccuSync 42 external unit, which produces trigger pulses based on the measured ECG signal to deliver electroporation pulses on the R wave of each heartbeat.
- For interoperative patient monitoring, patients will be connected to AccuSync via three ECG electrodes independently of ECG capture.
- On the selected ECG lead (standard leads I, II, III; the most suitable lead is chosen depending on the intensity of the R wave), AccuSync detects the R wave of individual heartbeats in the early phase of the increasing R wave slope and produces a trigger pulse.
- The trigger pulse is brought to the Cliniporator VITAE, which uses its built-in synchronization algorithm for the further verification of trigger pulse suitability and, meeting a series of conditions, produces electroporation pulses with a 50 ms delay behind the trigger pulse to avoid ventricular sensitivity (T wave range).
- The ECG signal and trigger pulses will be captured and stored for later analysis of the synchronization algorithm function and of any immediate effects of the treatment on heart function.

6 FOLLOW-UP AFTER TREATMENT

Follow-up will be carried out at three levels. First, defining the early and late effects of treating primary liver tumours using ECT; second, evaluating the short- and/or long-term effects of electroporation pulses, BLM or ECT as a whole on the heart function; and third, recording any adverse effects of ECT.

6.1 Effects of Treating Primary Liver Tumours Using ECT

- Early treatment effects will be assessed using DCE-US or CT based on changes in tumour blood flow after ECT compared to the pre-ECT blood flow.

- Late effects will be assessed based on modified RECIST criteria, taking into account the change in the size and density of the treated tumour nodules before and after ECT. These will be assessed based on CT perfusion imaging; the volume of the metastases will be calculated using the formula $V = \frac{a \times b^2 \times 6}{\pi}$, where a is the shorter and b the longer metastasis diameter.

Based on the assessment of the late ECT effects, the response to treatment will be evaluated in line with the WHO classification:

- Complete response (CR): complete disappearance of the tumour nodule
- Partial response (PR): reduction of the tumour nodule size by more than 50%
- No change (NC): decrease in tumour nodule size by less than 50% or an increase in the tumour nodule size by less than 25%
- Progressive disease (PD): growth of the tumour nodule by more than 25%

- The direct effect will be assessed by histological analysis of those metastases that are surgically removed.
- EORTC Quality of Life questionnaire according to the QLQ C30 standards

EXAMINATION/TEST	**Screening period until D −14**	**D −1**	**D7**	**D30**	**D60**	**D90**	**D120**
clinical examination, anamnesis	**X**	**X**	**X**	**X**	**X**	**X**	**X**
ECG	**X**	**X**					
DCE-US	**X**			**X**	**X**		**X**
CT perfusion / MRI	**X**			**X**			**X**
Blood count	**X**	**X**	**X**				
Coagulation tests	**X**	**X**	**X**				
Biochemical blood analysis	**X**	**X**	**X**	**X**	**X**		**X**
EORTC QLQ C30 questionnaire	**X**		**X**	**X**			

***D = day**

6.2 The Effects of Electroporation Pulses, BLM or ECT as a Whole on Cardiac Function

- To study the short-term effect, we will use the ECG signal captured during surgery, which will cover the period before, during and immediately after ECT, including BLM injection and electroporation pulses.
- To study possible late and/or long-term effects, the ECG signal will be recorded using a standard Holter system in 24-hour periods immediately before and after surgery. 5-10 patients will be included.

  ECG recordings will also be performed on 10-15 patients who will undergo abdominal surgery without ECT at the Department of Abdominal Surgery, University Medical Centre Ljubljana.

6.3 Recording ECT Adverse Effects will be Performed According to the Criteria of the National Cancer Institute (NCI)

An adverse effect is an undesirable and unexpected sign, symptom or disease, regardless of the cause, that develops or worsens during the study and that includes pathological clinical observations and deviations in the values of laboratory-measured parameters. The latter are only considered a side effect if they require additional treatment or result in the premature withdrawal of the patient from the study.

We will record any side effects that occur during the study and up to 30 days after the end of treatment. If a serious adverse reaction occurs more than 30 days after the end of treatment, the principal investigator will decide whether the complication is study-related or not.

We will record the start and end time of any adverse effect and the cause-and-effect relationship between the adverse effect and the ECT, cancer or other diseases, as well as assessing the severity of the adverse effect and describing the actions that will need to be taken.

The causal relationship between the adverse effect and the ECT will be assessed using the following criteria:

- Not related to the study.
- Probably not related to the study.
- Possibly related to the study.
- Probably related to the study.
- Certainly related to the study.

The severity of the side effects will be assessed using the following grades:

- Grade 1 – mild
- Grade 2 – moderate
- Grade 3 – a difficult complication requiring medical care
- Grade 4 – a life-threatening condition requiring immediate medical assistance
- Grade 5 – death

If a serious adverse effect is recorded during the study, the principal investigator will notify the responsible person at the National Medical Ethics Committee of the Republic of Slovenia in writing within 24 hours. A serious adverse effect is a complication that results in the following:

- death,
- a life-threatening condition requiring immediate medical assistance,
- hospitalization or extended hospitalization,
- the prolonged inability of the patient to perform any task that they were able to perform before the onset of the complication,
- any event or condition that endangers the life or health of the patient and that, according to a medical assessment, could lead to any of the serious complications referred to above.

7 DATA PROCESSING

The results of the proposed clinical trial will be analysed descriptively by describing the antitumour effect, any adverse effects and the patients' quality of life after treatment of the primary liver tumours.

8 STATISTICAL ANALSIS

All data will be entered into a Microsoft Access 2010 database, which will be used for all calculations except for statistical analysis, which will be performed with GraphPad Software (La Jolla, CA, USA). The log-rank (Mantel-Cox) test will be performed on the Kaplan-Meier estimates. A chi-squared test will be used for the statistical comparison of response according to tumor location. A two-tailed P value less than 0.05 will be considered to be statistically significant.

Consent for Participating in a Clinical Study

Treatment of Primary Liver Tumours with Electrochemotherapy

What do I need to know about the clinical study?

You are invited to participate in a clinical study (hereinafter: 'the study') in which the research team will evaluate the efficacy and potential toxicity of treating primary liver tumours with electrochemotherapy. The study is led by Mihajlo Đokić, MD, and coordinated by Prof. Gregor Serša, PhD. The head physician Dragoje Stanisavljević, MD, is coordinating the clinical part of the study. Other health professionals employed at the University Medical Centre Ljubljana and the Institute of Oncology Ljubljana, as well as researchers from the Faculty of Electrical Engineering, University of Ljubljana, are also involved in the study.

It is important that you carefully read and understand this document before signing the consent. The document sets out the purpose, procedures, benefits, risks, adverse effects and precautions related to the study. If you are not completely honest with your doctor about your medical condition, participating in the study may be detrimental to you. ***If this document contains any terms or expressions that you do not understand, please ask your doctor or a healthcare professional participating in the study to explain them to you before signing.***

Participation in the study is voluntary and you can decline to participate. Furthermore, you are free to withdraw from the study at any time and without giving a reason – this will not affect your further care or your relationship with your doctor at that institution.

Details of the study are described below. It is important that you understand the information provided, as this is the only way you can make an informed decision on whether or not to participate. If you decide to participate, you will receive a copy of the signed consent.

What is the purpose of the study?

The purpose of the study is to determine the efficacy of bleomycin-based electrochemotherapy and potential toxicity during surgery on primary liver tumours.

Why was I asked to participate in the study?

We suggest the treatment of primary liver tumours using electrochemotherapy because you have been identified as having one of the following conditions:

a) Disease progression following TACE therapy.
b) Primary liver tumours that are not suitable for other forms of treatment due to their location or that are not accessible to other conventional ablation techniques.
c) Progression of the primary disease for which other treatment is either risky or expected to be less effective.

d) An advanced form of the disease, which the BCLC staging system classifies as symptomatic treatment, but the patient's WHO performance status is good (Child-Pugh < 8, > 4; minor changes in the parenchyma).

In this case, electrochemotherapy treatment will not affect the course of the disease; your treatment would be in line with the standard protocol, completely uninterrupted and unchanged. By analysing the electrochemotherapy-treated metastases, we would gain new information and knowledge that would help future patients with the same disease.

How long will the cooperation last?

Your participation in the study, including the screening period, will last approximately **4 months**.

How will the treatment take place?

You will receive treatment while under general anaesthesia. The treatment involves an intravenous injection of the chemotherapeutic agent bleomycin and, after approximately 8 minutes, the application of electrical pulses via sterile stainless-steel electrodes inserted into the metastases.

Appointments and procedures during the study

Prior to inclusion in the study, your case will be reviewed by a by a multidisciplinary team for liver disease, who will assess whether it makes sense for you to take part in the study.

During the study, you will have at least **7 appointments** over a period of **4 months**.

The treatment consists of the following:

→ **1st appointment: screening (up to 2 weeks before treatment)**

If you agree to participate in the study, your doctor will examine you to see if you are a viable candidate. You and your doctor will also discuss any previous illnesses and treatments.

The examination will consist of the following.

- General medical examination: measuring the electrical activity of the heart (ECG).
- Venous blood collection for haematological and biochemical examinations and coagulation tests.
- Radiological examinations: you will be referred for computed tomography with perfusion (CT perfusion) or dynamic contrast-enhanced ultrasonography (DCE-US).
- You will complete a quality-of-life questionnaire.

→ **2nd appointment (1 day before treatment)**

- General medical examination: measuring the electrical activity of the heart (ECG).
- Venous blood collection for haematological and biochemical examinations and coagulation tests.

→ **3rd appointment (1 week after treatment)**

- General medical examination.
- Venous blood collection for haematological and biochemical examinations and coagulation tests.
- You will complete a quality-of-life questionnaire.

→ **4th appointment (1 month after treatment)**

- General medical examination.
- Venous blood collection for biochemical analysis.
- Radiological examinations: you will be referred for CT perfusion and/or DCE-US.
- You will complete a quality-of-life questionnaire.

→ **5th appointment (2 months after treatment)**

- General medical examination.
- Venous blood collection for biochemical analysis.
- Radiological examinations: you will be referred for DCE-US.

→ **6th appointment (3 months after treatment)**

- General medical examination.
- Radiological examinations: you will be referred for CT perfusion.

→ **7th appointment (4 months after treatment)**

- General medical examination.
- Venous blood collection for biochemical analysis.
- Radiological examinations: you will be referred for CT perfusion and DCE-US.

What are the potential benefits of the study for me?

Electrochemotherapy is used as a standard method to treat cutaneous tumours. Our experience gained from having treated liver metastases using electrochemotherapy also shows the possible effectiveness of electrochemotherapy in treating primary liver tumours.

We cannot guarantee that the treatment will be effective in your case, but we hope that we will at least manage to reduce the size of the metastases.

What are the potential risks or adverse effects associated with the study?

As with any medicine or treatment, adverse effects may occur during treatment, so we cannot guarantee that you will not experience any adverse effects during the study.

It is important that you inform the doctor monitoring you during the study about any symptoms or a new medicine prescribed by another doctor.

What are my responsibilities if I decide to participate in the study?

You will need to sign a consent form for participating in the study.

You will need to visit your medical institution at least 7 times and undergo the treatments and procedures described in this document.

You will not be allowed to participate in another study while participating in this one.

Before taking any medicine that you have not taken before, you will need to consult with the doctor monitoring you during the study.

You will need to report any changes in your general well-being to the doctor monitoring you during the study.

What are my options if I choose not to participate in the study?

If you do not wish to participate in this study, you will continue to be treated by your doctor. Your health care and treatment will not be compromised. You are free to withdraw from the study at any time. Doing so will not affect your further medical care.

What if new information becomes available during the study?

Sometimes new information about the treatment in question comes up during the study. In this case, your doctor will inform you and you can discuss whether you wish to continue participating in the study. If you decide to withdraw from the study, your doctor will offer other treatment options. If you decide to continue participating in the study, you will be asked to confirm your continued participation in the study by signing an updated consent form.

How will my privacy be protected?

In this study, your personal information and information about your medical condition will be documented anonymously (without your name, using only the number that you will be assigned upon entering the study).

In accordance with Slovenian legislation, all data collected during the study will be kept strictly confidential. The results of the study, including photographs of tumour lesions, will be available for scientific evaluation purposes and will be published in health publications in an anonymized form.

Voluntary participation and termination of participation:

Participation in the study is voluntary. You can choose to withdraw from the study at any time. Doing so will not affect your current or future medical care. To withdraw from the study, please contact Mihajlo Đokić, MD, on +386 1 5223168.

Please note that if you withdraw from the study, your doctor will still be able to use the information gathered while you were part of the study.

Your participation in the study may be terminated without your consent if you fail to follow the instructions and protocol. Further, we may exclude you from the study without your consent if the treatment being studied is detrimental to you and has adverse effects.

Will I need to pay to participate in the study?

No, the treatment is free of charge.

Who can I contact if I have a question a question about any detrimental effect or reaction associated with the study?

You have the right to ask questions about the study and to have them answered.

If you have any questions about participating in the study or if you think that you have been harmed in the study or that you have a reaction to the treatment being tested, please contact:

Mihajlo Đokić, MD, by phone: +386 1 5223168 or by email: mihajlo.djokic@kclj.si.

Who can I contact to report a breach of data protection policy:

If you believe that there has been a breach of your personal data, please contact the principal investigator:

Mihajlo Đokić, MD, by phone: +386 1 5223168 or by email: mihajlo.djokic@kclj.si.

Who can I contact if I have a question about my patient rights?

The study and study protocol will be reviewed and approved by the National Medical Ethics Committee of the Republic of Slovenia. If you have questions about your patient rights, you can contact (including anonymously): the office of the Patients' Rights Representative Duša Hlade Zore, who works for the Ministry of Health of the Republic of Slovenia, on the premises of the Ljubljana Institute of Public Health (*Zavod za zdravstveno varstvo Ljubljana*), Zaloška 29, 1000 Ljubljana, telephone number: +386 1 5423285, e-mail: dusa.zore@zzv-lj.

Patient Consent for Participating in the Study

- I, the undersigned, hereby agree to participate in the study "Treatment of Primary liver Tumours with Electrochemotherapy". I understand that my refusing to participate in the study will not affect my health care.
- I have read the description of the study. The study and the starting points have been explained to me in language that I understand. I am aware that participation is voluntary. I am sufficiently familiar with the purpose, methods, risks and benefits of the study.
- I understand that the doctor in charge of the study will inform me about any new findings that have arisen during the study that may affect my consent.
- I allow my study-related medical information to be used and passed on to health authorities and institutions. I consent to the use and disclosure of my personal health information as described in this document.
- I understand that I may decline to participate in the study. I further understand that I am free to withdraw from the study at any time and without giving a reason and that this will not affect my further care or the relationship with my doctor.
- I declare that I have read this document, have had the opportunity to ask as many questions as I wanted, and have received satisfactory answers thereto. I understand that I have not waived any legal rights that I have as a participant in the study.
- I am aware that I will receive a copy of the signed voluntary consent.
- I allow samples of metastases to be stored for histological analyses and photographic records to be published for scientific purposes.

  ❒ Yes ❒ No

I have read the whole document. I have asked all the questions I had. I agree to participate in this study.

Name of the patient/subject in block letters

Signature of the patient/subject | Date (*DD/MM/YYYY*)

Name of the person obtaining the consent in block letters

Signature of the person obtaining the consent | Date (*DD/MM/YYYY*)

Name of the person responsible for the study in block letters

Signature of the person responsible for the study | *Date (DD/MM/YYYY)*

INFORMED CONSENT STATEMENT

for the Study "Treatment of Primary Liver Tumours with Electrochemotherapy"

I, the undersigned _____, born on _____,

have been informed about the method and purpose of the above study. I understand the course of my treatment as part of the study.

I understand that participation in the study is voluntary and that I may decline to participate. I have been informed that the results of the study will be used for research purposes and contribute to knowledge about the safety and effectiveness of electrochemotherapy in treating primary liver tumours. I am aware that the course of the study has been reviewed and approved by the *National Medical Ethics Committee of the Republic of Slovenia*. I allow my tissue sample analysis results to be used in the study and for publications in the scientific literature; my personal data is confidential.

By signing, I confirm that I agree with the therapy and give my free consent.

Date: _____ Participant's signature: _____

I, the undersigned _____, hereby certify that I have explained the course, purpose, risks and potential benefits of the study to the participant in an understandable way.

Date: _____ Signature of the principal investigator: _____


univerzitetni
klinični center ljubljana
University Medical Centre Ljubljana