ONKOLOŠKI INŠTITUT
INSTITUTE OF ONCOLOGY
LJUBLJANA

Information for patients / Informed consent form for participating in a clinical study

Institute of Oncology Ljubljana

Title	**Electrochemotherapy Induces Changes in the Tumor Microenvironment of Cutaneous and Subcutaneous Metastases in Patients with Cutaneous Melanoma**
Sponsor	Institute of Oncology Ljubljana
Principal Investigator/ Co-Investigator	*Asst. prof. Barbara Perić, MD, PhD; Sara Milićević MD*

1. Introduction

You are invited to participate in a clinical study (hereinafter: »the study«) in which the research team will investigate changes in the tumor microenvironment of cutaneous and subcutaneous metastases in patients with cutaneous melanoma induced by electrochemotherapy.

In the last 10 years, the treatment of metastatic cutaneous melanoma has changed dramatically. The new systemic treatment with immunotherapy has led to a dramatic improvement in quality of life and overall survival. Systemic treatment means that the patient receives the drug as an infusion into a vein. Unfortunately, we know that immunotherapy is not equally successful in all patients. Recent studies have shown that the success of the treatment is not only influenced by the cellular composition of the metastasis, but also by its surroundings. This is called tumor microenvironment. Depending on the differences in the composition of this microenvironment, some metastases can be described as immunologically hot and others as immunologically cold. Immunologically hot metastases respond better to immunotherapy than immunologically cold metastases.

Studies have shown that with some interventions we can change the tumor microenvironment from being immune-cold to being immune-hot. Electrochemotherapy is one of the interventions

that might improve the efficacy of immunotherapy in cutaneous melanoma. Electrochemotherapy is an established method for the local treatment of tumors, in which only a certain tumor is treated with special electrodes, to which a weak electric current is applied. We hypothesize that electrochemotherapy stimulates the body's own immune response and enables more effective treatment. Since immunotherapy also stimulates the body's own immune response to cutaneous melanoma cells, the interaction of the two drugs could be even more successful. Recent research results support this assumption.

The primary objective is to evaluate the changes in the tumor microenvironment of cutaneous and subcutaneous melanoma metastases induced by electrochemotherapy, based on the histologic analysis of treated and untreated metastases before and after treatment. The secondary aim is to determine whether the changes in the tumor microenvironment differ depending on the chemotherapeutic agent used.

With your help, we will investigate the impact of electrochemotherapy on the tumor microenvironment of cutaneous and subcutaneous melanoma metastases. The results will help us to better understand the synergistic effects of electrochemotherapy and immunotherapy on cutaneous melanoma metastases. The combination of systemic immunotherapy and electrochemotherapy could become an important treatment method for patients with metastatic melanoma.

The details of the study are described below. **It is important that you carefully read and understand the information provided**, so that you can make an informed decision whether to participate in the study or not. Please ask questions about anything you do not understand or in case you would like to receive additional explanations. Before you decide to participate in the study, you may want to talk with your relative, friend, or general practitioner (GP).

Participation in the study is voluntary and you can refuse 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 treatment or relationship with your doctor at that institution.

If you decide to participate in the study, we will ask you to sign the informed consent form. With your signature, you confirm that:

- you have read and understood the description of the study,
- you agree to participate in the study,
- you agree with the proposed procedures and examinations,
- you allow your study-related medical data to be used for the purposes of the study and to be passed on to health authorities and institutions.

If you decide to participate, you will receive a copy of the signed consent form.

2. What is the purpose of the study?

The purpose of the study is to investigate the changes in the tumor microenvironment of cutaneous and subcutaneous melanoma metastases induced by electrochemotherapy. The research results will show us whether immunologically cold tumors can be successfully converted into immunologically hot tumors using electrochemotherapy and thus improve the effect of systemic immunotherapy.
In addition, we will determine whether the changes in the tumor microenvironment differ depending on the chemotherapeutic agent used.

3. What does participation in the study entail?

In short: In the first step, a cutaneous/subcutaneous metastasis of a cutaneous melanoma is removed, followed by electrochemotherapy of the remaining metastases. The surgical oncologist then removes one or two cutaneous/subcutaneous metastases of the cutaneous melanoma on the 3rd and 10th day after the electrochemotherapy. The removed metastases are sent to the pathologist at the Institute of Oncology Ljubljana for examination. The pathologist examines the removed metastases and performs additional (immunohistochemical) analyses. A scar will remain in the place where the tumor is removed. At the same time points, we will take 5 ml of peripheral blood from your vein and store it. You will complete a quality-of-life questionnaire upon enrollment in the study, after electrochemotherapy, at 3 months, 6 months, and 12 months after electrochemotherapy, and then once a year during follow-up. The questionnaires are designed to offer various responses and filling them out takes approximately 5 minutes.

Before the procedure: After you have read the explanation, you will have the opportunity to ask questions. You can discuss the protocol of the study with the surgical oncologist. If you agree to participate in the study, the following will happen:

- complete clinical examination;
- review of records of previous treatments for cutaneous melanoma and accompanying diseases;
- photography of the site of cutaneous/subcutaneous metastases of cutaneous melanoma;
- completion of quality-of-life questionnaires (EORTC C30 and EQ-5D-5L);
- examination by an anesthesiologist if electrochemotherapy will be performed under general/regional anesthesia;
- preparation for the surgical procedure.

Procedure:
After signing the written informed consent form, we will remove one cutaneous/subcutaneous metastasis of cutaneous melanoma prior to electrochemotherapy. We will then perform electrochemotherapy on the remaining cutaneous/subcutaneous metastases.

Electrochemotherapy involves the application of a (chemotherapeutic) drug followed by the local delivery of electrical pulses to facilitate easier entry of the drug into the cell. You will not feel any pain during the procedure. The type of anesthesia will depend on the anatomical location and the number of lesions that need to be treated. The procedure can be performed under deep sedation (general anesthesia), or under local anesthesia only—the drug is injected around the metastasis, providing pain relief in the area for approximately two hours. Alternatively, the procedure can also be performed with mild sedation (intravenous sedation), along with local anesthesia.

Electrochemotherapy procedure: First, we administer a drug known as a chemotherapeutic agent (cisplatin or bleomycin). The choice of chemotherapeutic agent depends on the size and number of lesions to be treated. Cisplatin is injected directly into the cutaneous/subcutaneous metastases, while bleomycin is given as an infusion into the vein. Short electrical pulses are then delivered via electrodes to the tumor lesions and the surrounding skin. These pulses enable the cells to uptake the drug (chemotherapeutic agent) without causing any other consequences. Treatment is completed when the electrical pulses have been delivered to the entire area intended for treatment. You will remain in hospital under observation until you can be discharged home, usually on the evening of the day of the procedure or the day after the procedure.

On the third day after the electrochemotherapy treatment, we will remove one cutaneous/subcutaneous metastasis, and on the tenth day after the treatment, we will remove two cutaneous/subcutaneous metastases of cutaneous melanoma (one treated and one untreated with electrochemotherapy).

Excision of cutaneous/subcutaneous metastases: After you have signed the written informed consent form, we will inject up to 20 ml of local anesthetic near the metastasis. Once the skin area is anesthetized and sterilely prepared, we remove the cutaneous/subcutaneous metastasis of malignant melanoma with a simple elliptical excision. The metastasis is removed with a minimal margin of healthy skin. We stop the bleeding and suture the wound primarily. You will be discharged home after the procedure. You will be given instructions on wound infection management and a mild painkiller in accordance with the guidelines of the Institute of Oncology Ljubljana. The excisions of cutaneous/subcutaneous metastases are performed under local anesthesia in the operating room of the Institute of Oncology Ljubljana, as part of the day hospital. Excisions are performed by a surgical oncologist.

During this process, we will collect data about your cutaneous melanoma treatment. The database contains information about the type of cutaneous melanoma, previous treatments, location, size and number of cutaneous/subcutaneous metastases, type of anesthesia, electrochemotherapy performed, response to treatment, as well as photographic documentation and clinical evaluation of electrochemotherapy treatment.

4. What do you need to do?

Before the planned surgical procedure, you must inform the surgical oncologist about any prior treatments and medications that you take regularly. After the procedure, follow the instructions of the surgical oncologist and medical staff regarding wound care, pain management, suture removal, and follow-up appointments.

5. What happens to the tissue and blood samples taken?

The removed metastases will be sent to a pathologist who will confirm your diagnosis and perform additional analyses. The remaining tissue from the removed metastases will be stored at the Department of Pathology, Institute of Oncology Ljubljana.

It is important to emphasize that the blood samples taken for translational examinations are not the same as the samples for regular routine examinations used to assess your health. Your blood samples will be stored in the laboratory of the Institute of Oncology Ljubljana.

The biological samples will be anonymized and labeled with a specific number assigned to you. The staff involved in the research will ensure your anonymity. Your sample number cannot be used to trace your identity. At the discretion of the research coordinators these samples may later be used for new research purposes. The purpose of collecting biological material is to identify the biological characteristics of tumors, which could help predict the most appropriate treatment for patients.

The Institute of Oncology Ljubljana guarantees that your biological samples will not be used or misused for commercial purposes.

6. Is participation in the study mandatory?

Participation in the study is voluntary. You can refuse to participate. Despite verbal and written consent, you can withdraw your participation in the study at any time and without giving reasons. This will not affect your further treatment or the relationship with your doctor and other employees of the Institute of Oncology Ljubljana.
If you wish to withdraw your participation in the study, please inform your surgical oncologist. Any reservations or concerns can be communicated to your surgical oncologist at any time.

7. What are the potential benefits of the study for me?

We cannot guarantee that you will benefit from participating in the study. Your participation in the study will contribute to the advancement of medicine and the better treatment of patients with cutaneous melanoma in the future. We expect that the results of the study will help us to plan a combined treatment with electrochemotherapy and systemic immunotherapy, which could

significantly contribute to the improvement of relapse-free survival and overall survival of patients with cutaneous melanoma.

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

Electrochemotherapy is used as a standard treatment for the treatment of cutaneous and subcutaneous metastases in patients with cutaneous melanoma. Therefore, the potential risks or adverse effects associated with participation in the study are comparable to those of patients who do not participate in the study. Additional risks are associated with two procedures under local anesthesia, the excisions of four cutaneous or subcutaneous metastases, and venous blood sampling.

As with any drug or treatment, adverse effects may occur during treatment, so we cannot guarantee that you will not experience adverse effects during the study. Adverse events may be mild, moderate, or severe. It is important that you inform your surgical oncologist of any symptoms or adverse effects.

<u>Risks and adverse effects associated with electrochemotherapy:</u>
When cisplatin is injected directly into tumor nodules, the area around the injection may become inflamed for a few days. Bleomycin has been reported to cause a number of adverse effects: fever on the day of injection, loss of appetite, fatigue or nausea, and loss of appetite. Bleomycin is not used in patients with acute lung infection or severely impared lung function. If you have previously been treated with bleomycin, you should inform your surgical oncologist.

The device used to deliver the electrical pulses is called Cliniporator. It has been certified by European electrical safety bodies and is compliant with current security rules for electrical devices. Side effects and possible complications associated with the administration of pulses include:

- Involuntary muscle contraction at the time of the electrical pulse, which stops at the end of the pulse; this is usually unpleasant, but painless.
- Skin burns; sometimes observed when patients have been treated with plate electrodes, but not when needle electrodes have been used.
- Hyperpigmentation of the skin.
- Minimal skin or mucosal sloughing.

Risks and complications associated with general anesthesia:

Complication	**How often does it occur?**	**How prominent can it be?**	**Duration**
Pneumonia	Rarely	Mild to severe	One week
Cardiovascular complications	Very rarely	Mild to severe	Low risk, very rarely present
Pulmonary embolism	Rarely	Mild to severe	Low risk, may appear within the first month after the general anesthesia, rare after more time has passed

Risks and complications associated with local anesthesia:

Complication	**How often does it occur?**	**How prominent can it be?**	**Duration**
Infection	Sometimes	Moderate	A few weeks
Bleeding	Rarely	Mild to moderate	Usually right after the procedure
Pain at the procedure site	Often	Moderate	A few days
Seroma or hematoma	Rarely	Moderate	Up to 6 weeks
Allergic reaction	Rarely	Mild to severe	Right after the procedure

Risks and complications associated with venous blood sampling:

Complication	**How often does it occur?**	**How prominent can it be?**	**Duration**
Sensitivity, pain, bruising at the blood sampling site	Sometimes	Mild	One week
Infection, vertigo, loss of consciousness	Rarely	Mild	During the procedure

Most risks and complications disappear within a short time after the procedure or when the wound has completely healed. On rare occasions, serious complications may occur. In addition, complications that are not expected by researchers or have not yet been described may appear. It is important that you inform your surgical oncologist of any unusual symptoms. The surgical oncologist will advise you on the most appropriate treatment.

9. Can I receive other medications and treatments during and after participation in the study?

Absolutely. During your participation in the study, you can continue to take all your usual medications and continue your treatment for cutaneous melanoma or other diseases. However, it is important that you inform your surgical oncologist about your treatment and medications. You should also tell them about any over-the-counter medications, vitamins, or herbal supplements you are taking, as well as any acupuncture or alternative treatments. All important information will be recorded. Your surgical oncologist and the research team will carefully monitor your treatment and use of medications that may affect your health during the study.

10. Termination of participation

You can withdraw from the study at any time. This will not affect your current or future medical care. To withdraw from the study, please contact your surgical oncologist.

11. Unexpected termination of the study

The study may be terminated unexpectedly for various reasons:

- It turns out that one of the surgical procedures is not effective or causes unacceptable complications.
- The number of patients included in the study is insufficient for more significant conclusions.

The principal investigator and co-investigator will decide whether to terminate the study. Your surgical oncologist will inform you about the termination of the study in a timely manner.

12. What will happen to the data?

By signing the consent form, you agree that the surgical oncologist and the research team may collect and use your personal data. We will collect the following information for research purposes: name, identification number, date of birth, gender, health status information at entry into the study (diagnosis, medical history, status, discharge report), information about previous treatments for cutaneous melanoma, information about the electrochemotherapy performed (date of procedure, number of lesions treated, number of electrical pulses per lesion, type of electrode used, duration of electrical pulse and chemotherapy application, etc.), information about the chemotherapeutic agent used (pharmacological name, dosage, strength, etc.), information about health services provided during the study period (including any complications), clinical data on treatment success (results of imaging studies and measurements, clinical assessment of treatment effectiveness), professional assessment of treatment success, data on histological and immunohistochemical analysis (including histological images), photographic documentation of cutaneous/subcutaneous metastases before and after electrochemotherapy, completed questionnaires, and other data listed in the protocol. When collecting and using this data, we will comply with Regulation (EU)

2016/679 of the European Parliament and of the Council of April 27, 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data; we will also comply with the Personal Data Protection Act (ZVOP-2). All data is confidential and pseudonymized. The data is encrypted, and you cannot be traced back through it. All data collected will be stored securely, and only research staff will handle it. The data will only be used for the purposes of this study and will only be disclosed with your consent or if required by law.

Your health and cutaneous melanoma data will be stored at the Institute of Oncology Ljubljana. By signing the consent form, you also allow the staff to receive health data and tumor tissue from other institutions if they are relevant to the work being done. Please note that your personal data will be stored in accordance with the principles of lawfulness, purpose limitation, and data minimization, pursuant to Article 5 of GDPR 2016/679, for as long as necessary to achieve the purpose for which they are collected and processed, provided that you give your voluntarily consent on the form at the end of this notice. The retention period of the data is limited to 20 years, after which they will be deleted.

In accordance with Articles 9 and 10 of Regulation (EU) GDPR No. 2016/679, we may provide the data controller with data classified as "special categories of personal data," which are data containing information about an individual's physical and mental health or "data concerning health." The processing of these types of personal data by the data controller is only permitted with your voluntary, explicit, and written consent, which you give on the form at the end of this notice.

We assume that the results will be presented and published several times. Your data will be used in publications or presentations in a way that you cannot be identified. Often, data from research is presented as a whole, without highlighting individual participants. This prevents your identification. If we wish to present a specific case from the research, we will only use the assigned number and initials of the name.

The information about your participation will be stored in your file at the Institute of Oncology Ljubljana.

In accordance with the applicable Slovenian laws on access to data, you have the right to access your data collected as part of the study. You can also request correction of any incorrect data. If you wish to access your data, please contact the contact person listed below.

The existence of automated decision-making, including profiling

“Profiling” means any form of automated processing of personal data consisting of the use of personal data to evaluate certain personal aspects relating to an individual, particularly to analyze or predict aspects concerning that individual's performance at work, economic situation, health, personal preferences, interests, reliability, behavior, location, or movements.

The data controller does not use any form of automated decision-making, including 'profiling,' as defined in the first and fourth paragraphs of Article 22 of Regulation (EU) No. 2016/679, for this specific processing of personal data.

13. Complaints and compensations

If you suffer any injuries as a result of your participation in the study, please inform your surgical oncologist as soon as possible. They will arrange for appropriate treatment.

In the event of injuries or complications resulting from participation in the study, you have the right to legal proceedings in accordance with the applicable legislation of the Republic of Slovenia. By signing the consent form, you do not waive these rights.

In the event of injury or complications related to the study treatment, you are entitled to compensation in accordance with the general rules of civil law. By participating in the study, you do not waive any of your rights.

14. Who conducts the study?

The research is conducted by physicians from the Department of Surgical Oncology together with researchers from the Department of Experimental Oncology at the Institute of Oncology Ljubljana. The employees involved in the research do not receive any additional remuneration for their work.

15. Who approved the study?

The study was reviewed and approved by the National Medical Ethics Committee of the Republic of Slovenia (July 18 2023; No. 0120-297/2023/3).

16. Additional information and contact details

According to the General Data Protection Regulation (GDPR 2016/679), you have following rights:

- to request confirmation of the existence or non-existence of personal data;
- to obtain information about the purpose of the processing and the type of personal data, recipients or types of recipients to whom the personal data have been or will be disclosed, and the duration of the retention period;
- to obtain data corrections and data deletion;
- to obtain all information about your treatment, about your well-being and limitations of the treatment;
- to receive data in a portable form;
- to object to data processing at any time;
- to oppose the process of automated decision-making in relation to individuals;
- to ask the data controller for access to personal data, correction, deletion, limitation or opposition to data processing, in addition to the right to data portability;
- to withdraw your consent at any time, without cost, regardless of the lawfulness of the processing your personal data;
- to exercise your rights by contacting the or data controller or processor.

This consent and the provision of personal data is not mandatory (it is voluntary), but without it, participation in the research is not possible. Refusal of consent has no influence on the regular treatment of your disease. Refusal to consent to the processing of certain personal data has no consequences for you.

If you believe that there has been a breach of your personal data, please contact the Data Protection Officer of the Institute of Oncology Ljubljana (Taja Džambasović, LLB, Zaloska cesta 2, 1000 Ljubljana, email: tdzambasovic@onko-i.si, phone: +386(0)1 5879 058), or the Information Commissioner (Slovenian National Supervisory Body for Personal Data Protection, Dunajska cesta 22, 1000 Ljubljana, email: gp.ip@ip-rs.si, phone: +386(0)1 2309 730).

You can contact the patients' rights advocate if you need advice, assistance or representation (by authorization) in exercising your rights under the Patients' Rights Act. The patients' rights advocate can provide you with basic information, offer professional assistance, and give concrete guidance in exercising your rights in the field of health care, health insurance, and the provision of health services. The counseling, assistance, and advocacy provided by the patients' rights advocate is free and confidential. Patients' rights advocates are listed at https://www.gov.si/teme/pacientove-pravice/.

If you would like any additional information regarding the study or have experienced complications which you would like to report to the surgical oncologist, please contact:

Contact persons:

Name	*Barbara Perić*	*Sara Milićević*
Job title	*Surgical Oncologist*	*General Surgery Resident*
Phone	*+386(0)15879915*	*+386(0)15879915*
Email	*bperic@onko-i.si*	*smilicevic@onko-i.si*

Informed consent for participating in a clinical study

Title	**Electrochemotherapy Induces Changes in the Tumor Microenvironment of Cutaneous and Subcutaneous Metastases in Patients with Cutaneous Melanoma**
Sponsor	Institute of Oncology Ljubljana
Principal Investigator/ Co-Investigator	*Asst. prof. Barbara Perić, MD, PhD; Sara Milićević, MD*

Patient statement

- I have read and understand the whole document. I declare that I have asked all the questions I had and I have received satisfactory answers. I had enough time to decide to participate in the study.
- I am aware that participation in the study is voluntary. I further understand that I am free to withdraw from the study at any time and without giving a reason. This will not affect my further care or relationship with my doctor.
- I declare that I will answer all the questions.
- I allow the samples of metastases to be stored and analyzed for the purpose of evaluating the changes in the tumor microenvironment of cutaneous/subcutaneous melanoma metastases induced by electrochemotherapy. I allow the processing of my personal data, including my health data. I allow my biological material to be used in future studies at the Institute of Oncology Ljubljana if the research protocols will be reviewed and approved by the Medical Ethics Committee. I allow the use and transfer of biological material for research purposes within the countries of the European Union or other countries, including the United States.
- I understand that in case of withdrawal of participation in the study, I will continue with regular follow-ups.
- I allow researchers to use the Cancer Registry and similar databases to track my health.
- I am aware that I will receive a copy of the signed voluntary consent.
- I allow my study-related medical information to be used for research purposes even if I decide to withdraw from the study.

☐ **I GIVE MY CONSENT** / ☐ **I DO NOT GIVE MY CONSENT** to the processing of my personal data, including those considered to be a special type of data (health data).

☐ **I ALLOW** / ☐ **I DO NOT ALLOW** the collection of peripheral venous blood samples for further translational studies and permit the use of my biological material in the future studies, provided that the research protocolsare reviewed and approved by the Medical Ethics Committee.

☐ **I AGREE** / ☐ **I DO NOT AGREE** to participate in the study.

Name of the patient (block letters) _____

Signature _____ Date _____

Signature Witness* (block letters) _____

Signature _____ Date _____

* The witness must be over 18 years old and should not be a researcher or a member of the research team. If a translator is present, he cannot be the Signature Witness.

Doctor's statement

I confirm that I explained the purpose and course of the study and its possible complications to the patient in a comprehensible manner.

Name of the doctor responsible for the study (block letters) _____

Signature _____ Date _____

Withdrawal of participation in the study

Title	**Electrochemotherapy Induces Changes in the Tumor Microenvironment of Cutaneous and Subcutaneous Metastases in Patients with Cutaneous Metastases**
Sponsor	Institute of Oncology Ljubljana
Principal Investigator/ Co-Investigator	*Asst. prof. Barbara Perić, MD, PhD; Sara Milićević MD*

Patient Statement

I want to withdraw my participation in the study named *Electrochemotherapy Induces Changes in the Tumor Microenvironment of Cutaneous and Subcutaneous Metastases in Patients with Cutaneous Metastases.* This will not affect my further care or relationship with my doctor.

I withdraw my participation in the study. However, I allow the members of the research group access to my medical records and collection of data about my health. ☐

I withdraw my participation in the study and I do not allow the members of the research group access to my medical records and collection of data about my health. ☐

Name of the patient (block letters) _____

Signature _____ Date _____

In case the reasons for withdrawal of participation in the study are stated verbally, the doctor responsible for the study states them in the box below

Doctor's statement

I confirm that I explained to the patient the procedure for withdrawing participation in the study in a comprehensible manner.

Name of the doctor responsible for the study (block letters) _____

Signature _____ Date _____