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**Title: A Video Protocol of a Randomized Controlled Clinical Trial -
Electrochemotherapy of Cutaneous Metastases with Reduced Dose
Bleomycin (Bless Trial)**

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes**
If **Yes**, how far apart are the locations? 100 m

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **NO**

Current Protocol Length

Number of Steps: 16

Number of Shots: 34

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Marie Abildgaard Tolstrup:** Our research investigates whether half-dose bleomycin provides non-inferior tumor control in electrochemotherapy for cutaneous metastases.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Marie Abildgaard Tolstrup:** Cutaneous metastases lack an optimal treatment consensus; this protocol aims to minimize toxicity while maintaining oncological outcomes and quality of life.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Marie Abildgaard Tolstrup:** This protocol supports treatment de-escalation, investigates the pharmacokinetics of bleomycin, provides insight into patients' perspectives, and advances knowledge in electrochemotherapy.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. Videographer's NOTE: Extra take - C0033 file (slate as 1.4.1)

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.4. **Marie Abildgaard Tolstrup:** Future research could focus on validating reduced bleomycin dosing in larger cohorts, investigating long-term outcomes, and exploring pharmacokinetics and patient perspectives to further optimize electrochemotherapy combinations of electrochemotherapy and immunotherapy, and integrating electrochemotherapy earlier in treatment lines.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Danish Medicines Agency and the Regional Committee on Health Research Ethics at Zealand University Hospital and Herlev Hospital

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Measuring the Metastases

Demonstrator: Camilla Kjaer Lonkvist

- 2.1. To begin, identify a maximum of seven metastases in a patient [1], and mark each metastasis with a number [2]. Then, record this number in the database and combine it with the unique patient identification [3].
 - 2.1.1. WIDE: Talent identifying the metastasis on the patient's skin.
 - 2.1.2. CU: Shot of marking and giving a number the metastasis.
 - 2.1.3. Talent assigning unique identification number in the database.
- 2.2. Measure the largest diameter of each metastasis along the longest axis using a ruler [1] and measure the widest diameter perpendicular to the longest axis [2].
 - 2.2.1. CU: Shot of measuring the largest diameter of the metastasis along the longest axis using a ruler.
 - 2.2.2. CU: Shot of measuring the widest diameter perpendicular to the longest axis.
Videographer's NOTE: 2.1 and 2.2 one shot.
- ~~2.3. Also, measure the elevation from the surrounding skin surface to the highest point of the metastasis [1].~~
 - ~~2.3.1. CU: Shot of measuring the metastasis elevation from the surrounding skin surface to its highest point. Videographer's NOTE: Not filmed.~~
- 2.4. Photograph each measurement with the ruler visible [1] and record the measurements in the study form [2].
 - 2.4.1. Talent clicking picture of the measurement with the ruler visible.
Videographer's NOTE: Not filmed.
 - 2.4.2. Talent recording the measurement in the study form.

3. Collection of Biological Samples and Administration of Bleomycin

Demonstrator: Lisbet Rosenkrantz Hölmich, Mads Gustaf Jørgensen, Medical doctor, Department of Plastic Surgery, Copenhagen University Hospital Herlev and Gentofte Hospital, ~~eg~~ Marie Abildgaard Tolstrup, and Lotte Mouritsen, Nurse, Department of Anaesthesia, Copenhagen University Hospital Herlev and Gentofte Hospital
NOTE: Added Mads Gustaf Jørgensen and Lotte Mouritsen

- 3.1. Punch 3-millimeter biopsies from the registered tumors [1-TXT] and from normal skin located 2 to 3 centimeters from the tumor [2]. NOTE: One shot file C0012
 - 3.1.1. Talent punching tumor biopsy on the patient. TXT: Tumor ≥ 10 mm in diameter
 - 3.1.2. Talent punching normal skin biopsy on the patient.
- 3.2. Protect the samples from light [1] and immediately place them on ice [2]. Record the collection time [3].
 - 3.2.1. Talent placing the samples in flamingo box containing ice.
 - 3.2.2. Talent placing the flamingo box on ice. NOTE: 3.2.1 and 3.2.2 were filmed together and actions in the shot were modified.
 - 3.2.3. Talent recording the collection time. NOTE: C0022
- 3.3. Collect venous blood samples in lithium heparin tubes [1] and record the collection time [2].
 - 3.3.1. Talent collecting venous blood from patient in lithium heparin tubes.
 - 3.3.2. Talent recording the collection time. NOTE: C0022

4. Administration of Bleomycin and Post-Infusion Sampling

Demonstrator: Louise Ascanius, Nurse, Department of Oncology, Copenhagen University Hospital Herlev and Gentofte Hospital, Marie Tolstrup, Amanda Dohn, Medical doctor, Department of Anaesthesia, Copenhagen University Hospital Herlev and Gentofte Hospital, and Lisbet Rosenkrantz Hölmich NOTE: Added Amanda Dohn

- 4.1. Administer bleomycin intravenously over 4 to 5 minutes according to the study protocol [1] and record the start and completion timings [2].
 - 4.1.1. Talent administering bleomycin intravenously to the patient.
 - 4.1.2. Talent recording the start and completion timings.
- 4.2. Punch 3-millimeter biopsies from the same tumors at 2, 4, 6, and 8 minutes intervals after infusion [1] and from normal skin located 2 to 3 centimeters from the tumor 8 minutes after infusion [2]. Record the collection time [3].
 - 4.2.1. Talent punching tumor biopsy on the patient.
 - 4.2.2. Talent punching normal skin biopsy on the patient.
 - 4.2.3. Talent recording the collection time. NOTE: C0022
- 4.3. Collect venous blood samples, using a separate IV (i-v) line at 5, 10, 20, 30, and 40 minutes intervals in lithium heparin tubes [1]. Record the collection time [2].
 - 4.3.1. Talent collecting venous blood from patient in lithium heparin tubes.
 - 4.3.2. Talent recording the collection time. NOTE: C0022

~~4.4. Protect the samples from light [1] and centrifuge at a relative centrifugal force of 2000 for 10 minutes at 20 degrees Celsius [2]. Transfer the plasma into 2-milliliter tubes [3] and store at minus 80 degrees Celsius freezer until further analysis [4].~~

~~4.4.1. Talent placing samples in the flamingo box.~~

~~4.4.2. Talent placing the samples into the centrifuge.~~

~~4.4.3. Talent transferring the plasma into 2 milliliter tubes.~~

~~4.4.4. Talent storing the samples in a -80 °C freezer.~~ NOTE: The author's requested to move this between 5.3 and 5.4.

5. Electroporation and Treatment Assessment

Demonstrator: Lisbet Rosenkrantz Hölmich and Marie Abildgaard Tolstrup, Camilla Lønkvist, and Millen Tesfaldet, Head nurse, Department of Oncology, Copenhagen University Hospital Herlev and Gentofte Hospital NOTE: Added Camilla Lønkvist, and Millen Tesfaldet

5.1. Select the needle electrodes according to the tumor size [1-TXT]. Following the safety guidelines, connect it to the external pulse generator [2]. NOTE: One shot C0015 (wrong audio slate, referred as 4.1.2) extra shot C0021

5.1.1. Talent holding the needle electrode. TXT: Hexagonal electrode: >3cm tumor; Linear electrode: ≤ 3 cm tumor

5.1.2. Talent connecting needle electrode to the external pulse generator.

5.2. After 8 minutes of infusion, apply electric pulses to the tumor, ensuring complete coverage with a 1-centimeter safety margin [1-TXT].

5.2.1. CU: Shot of applying electric pulse to the tumor.

TXT: Pulse delivery: 8 square-wave pulses of 0.1 ms at 1 kV/cm and 1 kHz

5.3. Record treatment time, pulse duration, and delivered current for each treated tumor [1-TXT].

5.3.1. Talent documenting the parameters. TXT: Assess tumor response after 3 months NOTE: The removed step 5.4 is provided concisely as on-screen text.

4.4 Protect the samples from light [1] and centrifuge at a relative centrifugal force of 2000 for 10 minutes at 20 degrees Celsius [2]. Transfer the plasma into 2-milliliter tubes [3] and store at minus 80 degrees Celsius freezer until further analysis [4].

4.4.1 Talent placing samples in the flamingo box.

4.4.2 Talent placing the samples into the centrifuge. NOTE: C0029 as extra and C0018

4.4.3 Talent transferring the plasma into 2 milliliter tubes.

4.4.4 Talent storing the samples in a -80 °C freezer. NOTE: This was placed here as per author's request.

~~5.4 After 3 months of treatment, measure and document the registered tumors as previously demonstrated, including the entire area if covered by crust or scab [1]. Assess tumor response using mRECIST (*M-RECIST*) criteria [2] and record it [3].~~ **NOTE:**
Not filmed and the VO is added in concise format in 5.3.

~~5.4.1 Talent measuring the tumor with ruler.~~

~~5.4.2 TEXT ON A PLAIN BACKGROUND:~~

~~Complete Response: Disappearance of lesion~~

~~Partial Response: $\geq 30\%$ reduction~~

~~Progressive Disease: $\geq 20\%$ increase~~

~~Stable Disease: Neither PR nor PD~~

~~5.4.3 Talent recording the tumor response.~~

5.5 Conduct qualitative interview through telephone or in person with willing patient using a predefined semi-structured interview guide before and after [1].

5.5.1 Talent interviewing the patient with an interview guide.

Results

6 Results

6.4 The registered tumors [1] achieved a complete response after bleomycin treatment, with no visible tumors in the treated area [2-TXT].

6.4.1 LAB MEDIA: Figure 5A. *Video editor: Circle and highlight the areas labeled 1 and 2.*

6.4.2 LAB MEDIA: Figure 5B. *Video editor: Circle and highlight the areas labeled 1 and 2.* **TXT: Representative image; not from the patient shown in the video**

Pronunciation guides:

1. Metastases
Pronunciation link: <https://www.merriam-webster.com/dictionary/metastases>
IPA: /mə'tæstə'si:z/
Phonetic Spelling: muh·tas·tuh·seez
2. Metastasis
Pronunciation link: <https://www.merriam-webster.com/dictionary/metastasis>
IPA: /mə'tæstə'si:s/
Phonetic Spelling: muh·tas·tuh·suhs
3. Diameter
Pronunciation link: <https://www.merriam-webster.com/dictionary/diameter>
IPA: /daɪ'æmətər/
Phonetic Spelling: dye·am·uh·ter
4. Perpendicular
Pronunciation link: <https://www.merriam-webster.com/dictionary/perpendicular>
IPA: /,pɜ:pən'dɪkjələr/
Phonetic Spelling: pur·puhn·dik·yuh·ler
5. Elevation
Pronunciation link: <https://www.merriam-webster.com/dictionary/elevation>

IPA: /ˌɛləˈveɪʃən/

Phonetic Spelling: eh·luh·vay·shuhn

6. Biological

Pronunciation link: <https://www.merriam-webster.com/dictionary/biological>

IPA: /ˌbaɪəˈlɑːdʒɪkəl/

Phonetic Spelling: bye·uh·laa·juh·kuhl

7. Bleomycin

Pronunciation link: <https://www.merriam-webster.com/medical/bleomycin>

IPA: /ˌbliːoʊˈmaɪsɪn/

Phonetic Spelling: blee·oh·my·sin

8. Biopsies

Pronunciation link: <https://www.merriam-webster.com/dictionary/biopsy>

IPA: /ˌbaɪˌɑːpsɪz/

Phonetic Spelling: bye·op·seez

9. Venous

Pronunciation link: <https://www.merriam-webster.com/dictionary/venous>

IPA: /ˈviːnəs/

Phonetic Spelling: vee·nuhs

10. Heparin

Pronunciation link: <https://www.merriam-webster.com/medical/heparin>

IPA: /ˈhepərɪn/

Phonetic Spelling: hep·uh·rin

11. Intravenously

Pronunciation link: <https://www.merriam-webster.com/dictionary/intravenously>

IPA: /ˌɪntrəˈviːnəsli/

Phonetic Spelling: in·truh·vee·nuhs·lee

12. Infusion

Pronunciation link: <https://www.merriam-webster.com/dictionary/infusion>

IPA: /ɪnˈfjuːʒən/

Phonetic Spelling: in·fyoo·zuhn

13. Centrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>

IPA: /ˈsentrɪˌfjuːdʒ/

Phonetic Spelling: sen·truh·fyooj

14. Centrifugal

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugal>

IPA: /sɛnˈtrɪfjʊɡəl/

Phonetic Spelling: sen·trif·yuh·guhl

15. Plasma

Pronunciation link: <https://www.merriam-webster.com/dictionary/plasma>

IPA: /ˈplæzmə/

Phonetic Spelling: Plaz·muh

16. Electroporation

Pronunciation link: <https://www.merriam-webster.com/medical/electroporation>

IPA: /ɪˌlɛktroʊpəˈreɪʃən/

Phonetic Spelling: ih·lek·troh·puh·ray·shuhn

17. Electrodes

Pronunciation link: <https://www.merriam-webster.com/dictionary/electrode>

IPA: /ɪˈlɛkˌtroʊdɪz/

Phonetic Spelling: ih·lek·trohdz

18. Hexagonal

Pronunciation link: <https://www.merriam-webster.com/dictionary/hexagonal>

IPA: /hɛkˈsæɡənəl/

Phonetic Spelling: hek·sag·uh·nuhl

19. Qualitative

Pronunciation link: <https://www.merriam-webster.com/dictionary/qualitative>

IPA: /ˈkwɑːləˌtɛtɪv/

Phonetic Spelling: kwah·luh·tay·tiv

20. Criteria

Pronunciation link: <https://www.merriam-webster.com/dictionary/criteria>

IPA: /kraɪˈtɪriə/

Phonetic Spelling: kry·teer·ee·uh