jove | Cancer Research

DATE PUBLISHED June 9, 2026
DOI 10.3791/70098
URL jove.com/t/70098

A Video Protocol of a Randomized Controlled Clinical Trial - Electrochemotherapy of Cutaneous Metastases with Reduced Dose Bleomycin (BLESS Trial)

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Citation

Tolstrup, M.A., Kjaer Lonkvist, C., Emilie Rosted, E., Abdulmohsen M Alrasheed, T., Armstrong Bastrup, F., Mojsoska, B., Plesnik, H., Kosjek, T., Rosenkrantz Hölmich, L., Krezdorn, N., Gehl, J. A Video Protocol of a Randomized Controlled Clinical Trial - Electrochemotherapy of Cutaneous Metastases with Reduced Dose Bleomycin (BLESS Trial). *J. Vis. Exp.* (232), e70098, doi:10.3791/70098. (2026).

Abstract

Cutaneous metastases in patients with incurable cancer represent a significant problem as they often cause pain, discomfort, and emotional distress that affect everyday life. Finding treatment options that are both effective and gentle is essential. ECT offers one such possibility. Here, short, high-voltage electrical pulses are applied directly to the tumor, briefly opening tumor cells, allowing chemotherapy to enter more effectively and kill cancer cells. Traditionally, patients receive 15.000 IU/m2 of bleomycin intravenously, but emerging evidence suggests that a lower dose may be just as effective while causing fewer side effects. This protocol describes an ongoing double-blinded, randomized clinical trial that tests whether ECT with half the standard bleomycin dose is non-inferior to the conventional regimen for tumor control in patients with cutaneous metastases. The article outlines randomization and blinding procedures, pretreatment evaluation, bleomycin preparation and administration, electrode placement, pulse delivery, and response evaluation using the modified Response Evaluation Criteria in Solid Tumors (mRECIST) criteria. In addition to clinical response at three months, the protocol includes pharmacokinetic blood sampling and qualitative interviews with the patients to enable a comprehensive evaluation of treatment impact. Baseline tumor characteristics from the first 15 enrolled patients and an example of how mRECIST is applied are presented. Critical steps to ensure methodological rigor are discussed, including standardized tumor measurements, consistent electrode positioning, and predefined management of confluent or poorly demarcated tumors. By visually outlining the procedural workflow and key methodological considerations, this article provides a reproducible framework for dose optimization in ECT. It supports future implementation of reduced-dose regimens in clinical oncology practice.

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Introduction

Cutaneous metastases represent a distressing clinical manifestation of advanced malignancy, most commonly observed in patients with breast cancer, malignant melanoma, and lung cancer, although they may arise from a wide range of primary tumors[1]. Between 0.7% to 9% of patients within these diagnostic groups develop cutaneous metastases, a condition generally associated with poor prognosis and substantial disease burden[1]. These patients are often heavily pretreated and clinically fragile, making therapeutic options limited. In addition to reflecting advanced disease, cutaneous metastases significantly impair quality of life due to pain, odor, ulcerations, and psychological distress from their visible and progressive nature[2,3].

Local treatment modalities, therefore, play an important role in symptom control and local disease management. In addition to surgery and radiotherapy, ECT has emerged as an established local treatment option for cutaneous and subcutaneous metastases. ECT combines the administration of chemotherapy – most commonly bleomycin – with the delivery of short high-voltage electric pulses that transiently increase cell membrane permeability, thereby enhancing intracellular drug uptake and cytotoxicity[4]. In the broader literature, ECT is increasingly recognized as a valuable component of multidisciplinary cancer care, with European guidelines recommending it as a treatment option for cutaneous metastases of various origins[5,6]. Given its localized nature, high response rates[7], and quality-of-life benefits, ECT may be particularly appropriate for patients with limited treatment options and recurrent tumors.

This protocol evaluating reduced-dose bleomycin is particularly relevant for patients with multiple cutaneous metastases who require effective local control but may be vulnerable to systemic toxicity due to prior treatments, comorbidities, or advanced disease status. In such patients, optimizing the balance between efficacy and safety is critical. Questions regarding optimal drug dosing—such as whether bleomycin doses can be reduced without compromising efficacy—remain an important area of ongoing research[8,9,10,11], as reducing systemic exposure could further improve safety for vulnerable patients.

This protocol describes a randomized, double-blind clinical trial investigating whether a half-dose of bleomycin is non-inferior to the standard dose for the treatment of cutaneous metastases using ECT. The study design is shown in **Figure 1**. In addition, the protocol outlines how the pharmacokinetics of bleomycin in tumors, normal skin, and patient blood samples will be assessed, as well as how qualitative interviews will be conducted to explore patients' experiences of living with cutaneous metastases and undergoing ECT treatment. The protocol aims to include approximately 110 tumors from around 55 patients with non-curable cancer and has been described in detail previously[12]. Outcomes for the BLESS trial are presented in **Supplementary Table 1**.

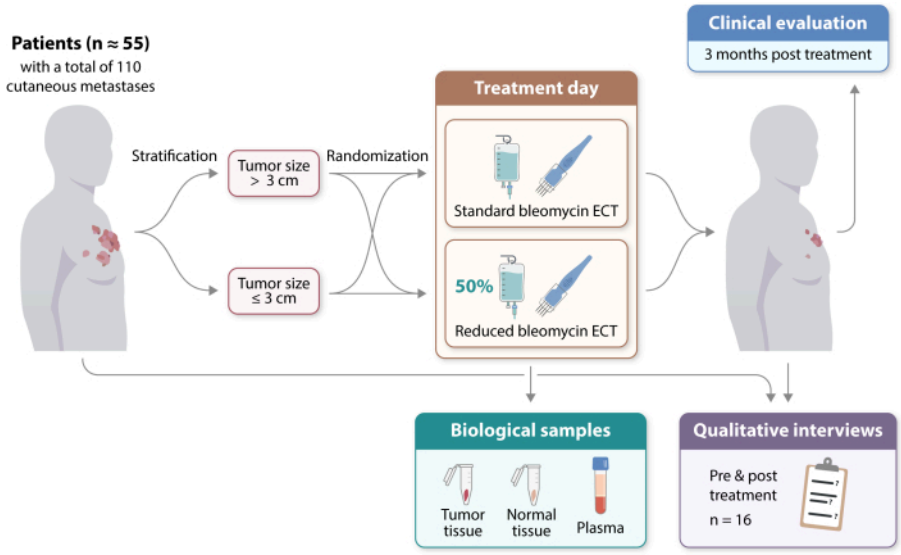

Figure 1: Study design of the BLESS trial. This figure outlines the design of the BLESS trial, a randomized controlled study. Around 55 palliative patients, representing roughly 110 tumors, will be enrolled. After stratification by tumor size, patients are randomly allocated to receive either the full bleomycin dose or a half dose. The main outcome measure is tumor response three months after treatment. Sixteen patients will also take part in qualitative interviews conducted before and approximately three months after therapy. On the treatment day, blood and tissue samples are collected for pharmacokinetic analysis; as previously published by Tolstrup et al. (2025)[12]. Please click here to view a larger version of this figure.

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Protocol

The trial has received approval from both the Danish Medicines Agency and the Regional Committee on Health Research Ethics (CTIS no. 2024-513360-25-00). It is conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice (GCP) and is overseen by the GCP Unit in Copenhagen. Eligible participants are patients who would otherwise qualify for ECT, thereby ensuring that study participation does not introduce additional treatment-related risks. Written informed consent is mandatory, and all participants are covered by the Danish Patient Compensation Scheme. The patients who participate in this video will be carefully informed and will sign written informed consent.

1. Randomization and blinding

1. Generate the allocation sequence independently of the study team before patient enrollment begins.
2. Use a web-based randomization service.
3. Perform block randomization with a block size of ten within each stratification group: one with patients' largest tumor ≤ 3 cm, and the other with patients' largest tumor > 3 cm.
4. Store one set of randomization envelopes in the hospital pharmacy, where the bleomycin is produced.

   NOTE: This allows the unblinded pharmacy personnel to prepare the study medicine according to the randomization code.
5. Store a second set of sealed randomization envelopes in a secure location with controlled access. Allow access to these envelopes only in the event of an emergency unblinding.

2. Participants' inclusion and pretreatment assessment

1. Screen patients according to the prespecified inclusion and exclusion criteria[12]. Detailed inclusion and exclusion criteria are presented in **Supplementary Table 2**.
2. Obtain informed consent.
3. Confirm eligibility before assigning a randomization number.
4. Measure all visible cutaneous metastases at baseline.
5. Stratify patients into two groups according to their largest cutaneous metastasis (≤ 3 cm or > 3 cm).
6. Assign each patient a pre-generated randomization number corresponding to both the stratification group and treatment allocation (full-dose or half-dose bleomycin).
7. Register a maximum of seven metastases per patient for response evaluation (baseline registration).

   NOTE**:** If a patient has more than seven metastases, treat all metastases; however, follow up to seven metastases for response assessment.
8. Assign each registered metastasis a unique identification number and measure it in three dimensions using rule-based measurements. Perform photographic documentation.
   1. Length: Measure the largest diameter of the metastasis using a ruler aligned with the longest axis. Include the measuring device in the photograph.
   2. Width: Measure the widest diameter perpendicular to the longest axis. Document with a photograph including the measuring device.
   3. Height: Measure the elevation from the surrounding skin surface to the highest point of the metastasis.
9. Record all measurements in a study form.

3. Treatment with electrochemotherapy

1. Preparation of bleomycin must be done in the hospital pharmacy.
   1. Reconstitute the bleomycin powder with sterile water for injection to obtain a stock solution of 3,000 IU/mL, according to the manufacturer's instructions.
   2. Dilute the stock solution with 0.9% sodium chloride to obtain a final infusion volume of 100 mL.

      NOTE: Maintain a total infusion volume of 100 mL for all patients, regardless of whether the full or half dose of bleomycin is prepared.
   3. Ensure the infusion bag is labeled only with the patient ID to maintain blinding.

      CAUTION: Bleomycin is a cytotoxic chemotherapeutic agent. Handle with appropriate personal protective equipment and prepare in a certified biological safety cabinet, following institutional cytotoxic drug handling guidelines. Dispose of contaminated materials as cytotoxic waste[13].
2. Induction of anesthesia
   1. Induce general anesthesia according to institutional standards. **NOTE:** Local anesthesia may be used if clinically indicated.
   2. If the treatment area involves the head and neck, use a spiral tube when appropriate.
   3. Initiate bleomycin infusion when the fraction of inspired oxygen (FiO_2) is below 30% and oxygen saturation is clinically acceptable.

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NOTE: An association between oxygen exposure and bleomycin-induced pulmonary toxicity has been reported; therefore, a low FiO_2 is recommended[14].

3. Bleomycin infusion
    1. Administer bleomycin intravenously over 4–5 min. Record the exact time of the infusion start and completion.
    2. Wait 8 min after completion of the infusion before starting the electroporation.
4. Electroporation
    1. Select needle electrodes (linear or hexagonal) according to tumor size. Use hexagonal electrodes for tumors > 3 cm and linear electrodes for tumors ≤ than 3 cm[4].
    2. Connect the needle electrode to the external pulse generator.
    3. Apply electric pulses to the tumor using the external pulse generator (**Figure 2**).

        NOTE: The external pulse generator must deliver eight square-wave pulses of 0.1 ms at a field strength of 1 kV/cm and a frequency of 1 kHz.
    4. Reposition the electrode as necessary to ensure complete coverage of the tumor and a 1 cm safety margin.
    5. Repeat the pulse application for each tumor requiring treatment.

        CAUTION: Follow all safety guidelines for the external pulse generator for handling and delivering electric pulses.
    6. Record treatment time, pulse duration, and delivered current for each treated tumor.

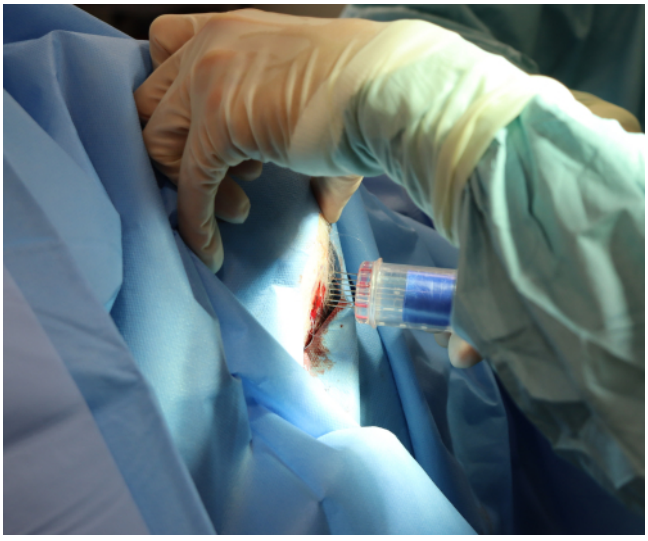

Figure 2: Intraoperative electrochemotherapy procedure for a scalp tumor. The image shows a treatment session performed in the operating theater with the patient under general anesthesia. The tumor is located on the scalp. Following sterile preparation and draping of the surgical field, and the intravenous administration of bleomycin, the treating physician inserts a sterile hexagonal electrode perpendicularly into the tumor tissue. The electrode is connected to the pulse generator, and electric pulses are delivered according to the predefined treatment parameters described in the protocol. To ensure complete tumor coverage, the electrode is repositioned sequentially until the entire tumor area, including a 10 mm margin, has been treated. Please click here to view a larger version of this figure.

4. Collection of biological samples

1. Blood samples
    1. Collect venous blood samples six times during the procedure: Before bleomycin infusion, and at 5, 10, 20, 30, and 40 min after bleomycin infusion.
    2. Use a separate peripheral intravenous catheter for blood sampling.
    3. Collect samples in lithium heparin tubes.

        CAUTION: This is critical for subsequent analysis of the concentration of bleomycin in the samples[15].
    4. Protect the samples from direct light and store at room temperature (20 °C) until centrifugation.

        NOTE: Bleomycin is light sensitive[15].
    5. Record the exact time of each collection.

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6. After final collection, centrifuge samples at 2,000 *x g* for 10 min at 20 °C. Transfer plasma to 2 mL tubes and store at -80 °C until further analysis.

2. Tumor biopsies
    1. Collect 3 mm punch biopsies from tumor tissue five times during the procedure: Before bleomycin infusion, and at 2, 4, 6, and 8 min after bleomycin infusion (**Figure 3**).

        NOTE: Obtain all biopsies from the same tumor (≥10 mm diameter) for consistency.
    2. Immediately place samples on ice and protect from light.
    3. Record exact collection times.
    4. Store biopsies in a -80 °C freezer.
3. Normal skin biopsies
    1. Collect 3 mm punch biopsies from normal skin located 2–3 cm from the tumor before bleomycin infusion and 8 min after bleomycin infusion.
    2. Place all samples on ice and protect from light until referral to freezer.
    3. Store biopsies in a -80 °C freezer.

Figure 3: Operating room setup. The image shows the operating theater during the procedure. In the foreground, a tumor biopsy is handled before electroporation. In the background, the electroporator and its display screen are visible. Simultaneously, the treating physician is preparing to start electroporation. Please click here to view a larger version of this figure.

5. Primary endpoint assessment

1. Evaluate tumor response three months after treatment.
2. Perform tumor-level assessment using ruler-based measurements and photographic documentation, as described in section 2.7.

    NOTE: If the tumor surface is covered by a crust or scab, include the entire area in the measurements.
3. Assess all tumors registered at baseline (up to seven tumors per patient).
4. Apply the mRECIST criteria[16].
    1. Complete response (CR): full disappearance of the lesion.
    2. Partial response (PR): reduction of at least 30% in the largest diameter.
    3. Progressive disease (PD): an increase of at least 20% in the largest diameter.
    4. Stable disease (SD): changes not meeting the criteria for PR or PD.

6. Qualitative interviews

1. At inclusion, ask patients whether they wish to participate in qualitative interviews regarding their experiences with and expectations regarding cutaneous metastases and ECT. If included, proceed with the following.
2. Conduct one semi-structured interview before treatment. Use a predefined semi-structured interview guide.

    NOTE: The interviews may take place by telephone, at the patient's home, or at the treatment center and will last approximately 1 h.
3. Conduct the second interview as described above, three months after treatment.

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7. Analysis of the biological samples

1. Prepare and analyze biological samples for bleomycin concentration according to previously established protocols[12,15,17].
2. Quantify bleomycin using ultra-high-performance liquid chromatography (UHPLC) coupled (**Figure 4**) with quadrupole mass spectrometry[15].

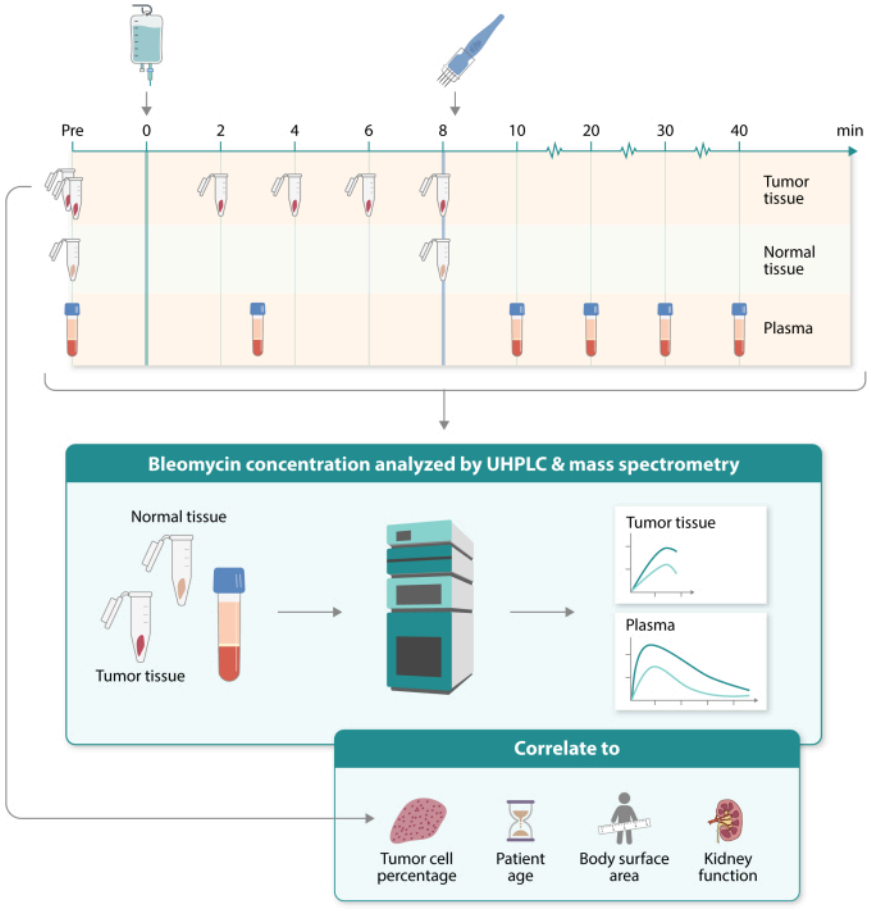

Figure 4: Timeline and Analytical Workflow for Biological Sample Collection and Bleomycin Quantification. The figure presents the timeline for biological sample collection during the treatment day. Plasma and tissue samples will be analyzed using Ultra-High-Performance Liquid Chromatography (UHPLC) coupled with mass spectrometry. Measured bleomycin concentrations will be correlated with tumor cell fraction, patient age, body surface area, and renal function parameters; as previously published by Tolstrup et al. (2025)[12]. Please click here to view a larger version of this figure.

Results

As this protocol describes an ongoing randomized, double-blind clinical trial, no definitive results on tumor response by bleomycin dose are currently available. Instead, this section summarizes preliminary observations from the first 15 enrolled patients: 13 women and 2 men. Among these patients, two were diagnosed with malignant melanoma, three with lung cancer, and ten with breast cancer. Baseline tumor characteristics are presented in Table 1. An example of tumor measurements and response evaluation according to the mRECIST criteria is presented in **Figure 5**.

	N=52	%
Primary tumor origin		
Breast cancer	38	73

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Malignant melanoma	10	19
Lung cancer	4	8
Size of tumors (length)		
< 10 mm	7	13
10 – 19 mm	22	42
20 – 29 mm	10	19
30 – 59 mm	8	15
60 – 99 mm	2	4
> 100 mm	3	6
Previously irradiated		
Yes/no	13/39	25
Previous ECT		
Yes/no	5/47	10
Site of tumor		
Head/neck	4	8
Chest	22	42
Abdomen	9	17
Back	9	17
Upper limps	7	13
Lower limps	1	2
Bleeding		
Yes/no	13/39	25
Oozing		
Yes/no	10/42	19

Table 1: Tumor characteristics for the first 52 tumors included in the BLESS trial. The table presents key tumor characteristics, including primary tumor origin, tumor size, and previous electroporation status. The number and corresponding percentage of tumors within each category are shown.

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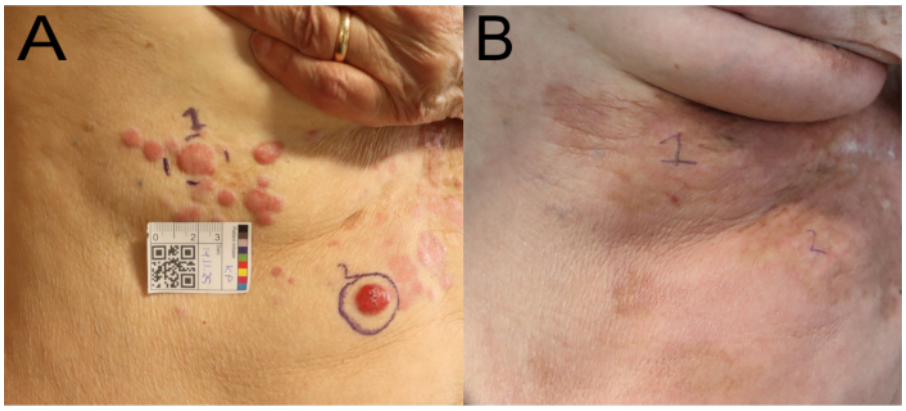

Figure 5: Representative clinical response assessment using mRECIST criteria. (A) Baseline clinical presentation of a 77-year-old woman with breast cancer and multiple cutaneous metastases located at the anterior thoracic wall and abdomen. The image shows several cutaneous metastases beneath the right breast. Two tumors in this area were registered at baseline for response evaluation according to mRECIST criteria. Tumor 1 measurements: largest diameter 20 mm; perpendicular diameter 15 mm; thickness 2 mm. Tumor 2 measurements: largest diameter 20 mm; perpendicular diameter 14 mm; thickness 4 mm. **(B)** Clinical appearance at follow-up after treatment. No measurable tumors are detectable in the previously treated areas. Post-treatment skin changes, including thickening and hyperpigmentation, are visible within the treated field. According to mRECIST criteria, the patient achieved a complete response for the registered tumors. Please click here to view a larger version of this figure.

Supplementary Table 1: Endpoints of the BLESS trial. Overview of the clinical and analytical endpoints evaluated in the BLESS trial, including treatment response outcomes and related objective definitions. The table summarizes the assessment methods, evaluation time points, and outcome measures used to determine clinical response and other study endpoints during follow-up; as previously published by Tolstrup et al. (2025)[12].Please click here to download this file.

Supplementary Table 2: Inclusion and exclusion criteria of the BLESS trial. Eligibility criteria used for patient selection in the BLESS trial. The table outlines the conditions required for study participation, including demographic, clinical, and safety considerations, as well as factors leading to exclusion from the trial; as previously published by Tolstrup et al. (2025)[12].Please click here to download this file.

Discussion

A detailed description of the protocol has previously been published[12]. This protocol provides a visual and practical overview of the double-blinded, randomized clinical trial, including qualitative interviews and a description of the pharmacokinetic analyses. By illustrating the procedural workflow and critical methodological steps, the video complements the written protocol and facilitates reproducibility. The study offers a structured framework for evaluating both the mechanisms and clinical application of ECT[12].

Several steps in the protocol are critical to ensure valid evaluation of ECT administered with two different doses of bleomycin. One key step is standardized tumor measurement at baseline and at the three-month follow-up, when treatment response is assessed. The tumors in this heterogeneous patient population vary considerably in size, morphology, and anatomical location, which may complicate consistent measurement. Purely demarcated tumors require predefined consensus-based assessment strategies. For example, when multiple small tumors coalesce, the study team agreed to measure the confluent area as a single tumor. To ensure consistency and minimize interobserver variability, all ambiguous cases must be discussed within the study team. Furthermore, only a limited number of trained investigators perform tumor measurements, and whenever possible, the same investigator conducts both baseline and follow-up assessments for each patient.

Another critical procedural component is electrode placement, including electrode length selection and repositioning during treatment. In this protocol, electrode placement is performed by trained plastic surgeons who have received standardized instructions in both study protocol and electroporation techniques before participation. Although this approach promotes procedural consistency, some degree of individual clinical judgement remains unavoidable, particularly regarding needle depth and positioning. Reproducibility could potentially be further strengthened by limiting the number of treating surgeons to one or two dedicated operators.

Protocol deviations most commonly occur during intra-procedural blood sampling for pharmacokinetic analyses. Many of the included patients are heavily pretreated and may have compromised venous access due to prior systemic therapy and comorbidities. Consequently, complete blood sampling at all predefined time points is not always feasible. These deviations are documented and considered in the pharmacokinetic analyses.

Compared to existing ECT protocols[4], this protocol provides a structured and holistic approach to evaluating ECT with reduced-dose bleomycin. Beyond measuring tumor response, it incorporates patient-centered outcomes and pharmacokinetic insights, thereby strengthening the evidence base for optimizing ECT in clinical oncology. If non-inferior tumor control can be demonstrated, dose reduction may expand the therapeutic window, particularly for elderly or multimorbid patients, by reducing the risk of toxicity without compromising treatment efficacy.

Disclosures

None of the authors declare any conflict of interest.

Acknowledgments

This work was supported by The Danish Cancer Society (Grant number R325-A18717). The study also received funding from Region Zealand Health Science Research Foundation (grant number R46-A2242), the NEYE foundation, the Agnethe Løvgreen Foundation, and the Dagmar Marshall Foundation.

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