

Recommendations for improving the quality of reporting clinical electrochemotherapy studies based on qualitative systematic review

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Background. Electrochemotherapy is becoming a well-established treatment for malignancies of skin and non-skin origin and its use is widening across Europe. The technique was developed and optimized from solid experimental and clinical evidence. A consensus document is now warranted to formalize reporting results, which should strengthen evidence-based practice recommendations. This consensus should be derived from high quality clinical data collection, clinical expertise and summarizing patient feedback. The first step, which is addressed in this paper, aims to critically analyze the quality of published studies and to provide the recommendations for reporting clinical trials on electrochemotherapy.

Methods. The quality of reporting in published studies on electrochemotherapy was analyzed in order to produce procedure specific reporting recommendations. A comprehensive literature search of studies published from 2006 to 2015 was performed followed by qualitative analysis of manuscripts assessing for 47 quality criteria grouped into four major clusters: (1) trial design, (2) description of patient population, (3) description of treatment delivery and patient outcome, (4) analysis of results and their interpretation. The summary measure during literature assessment was the proportion of studies fulfilling each manuscript quality criteria.

Results. A total of 56 studies were screened, from the period 2006 to 2015, of which 33 were included in the qualitative analysis, with a total of 1215 patients. Overall, the quality of reporting was highly variable. Twenty-four reports (73%) were single-center, non-comparative studies, and only 15 (45%) were prospective in nature (only 2 of them were entered into a clinical trials registry). Electrochemotherapy technique was consistently reported, with most studies (31/33) adhering closely to published standard operating procedures. The quality of reporting the patient population was variable among the analyzed studies, with only between 45% and 100% achieving dedicated quality criteria. Reporting of treatment delivery and patient outcome was also highly variable with studies only fulfilling between 3% and 100%. Finally, reporting study results critically varied, fulfilling from 27% to 100% of the quality criteria. Based on the critical issues emerging from this analysis, recommendations and minimal requirements for reporting clinical data on electrochemotherapy were prepared and summarized into a checklist.

Conclusions. There is an increasing body of published clinical data on electrochemotherapy, but more high quality clinical data are needed. Published papers often lack accurate description of study population, treatment delivery as well as patient outcome. Our recommendations, provided in the form of a summary checklist, are intended to ameliorate data reporting in future studies on electrochemotherapy and help researchers to provide a solid evidence basis for clinical practice.

Key words: electrochemotherapy; clinical trials, recommendations

Introduction

Electrochemotherapy is becoming a well-established non-thermal ablative technique for malignancies of skin and non-skin origin.^{1,2} The medical applications of electrochemotherapy are based on the principle of electroporation, which dates back to 1982, when sequences of electric pulses were applied to deliver naked DNA molecules within mouse lymphoma cells.³ Preclinical studies carried out by several research groups, coupled with technical developments, culminated in the clinical application of electroporation during the early 1990s.⁴⁻¹³ These initial data on electroporative uptake of molecules are viewed as seminal for various biotechnological and medical applications.^{14,15} The principle of electrochemotherapy is the use of electroporation to enhance chemotherapeutic drug delivery. Two agents, bleomycin or cisplatin, can achieve a several fold increase in their intracellular availability, and consequently cytotoxicity, when the tumor tissue is exposed to reversible electroporation and transient cell membrane permeabilization, thus achieving an optimal intratumor drug distribution.^{7,16-18} Electrochemotherapy has proven effective for the treatment of different tumor histotypes, including both skin and non-skin cancers, as well as for the palliation of metastases involving cutaneous and subcutaneous tissues.¹⁹⁻²² The treatment of primary skin tumors is largely restricted to multifocal cutaneous tumors, most notably some selected cases of basal cell carcinoma, when tumor anatomical location and patient medical conditions contraindicate more aggressive treatments.²³

The publication of the European Standard Operating Procedures of Electrochemotherapy (ESOPE) in 2006 facilitated a broad acceptance of electrochemotherapy for treatment of cutaneous tumors and metastases.²⁴ Over a number of years, several clinical reports have confirmed its effectiveness. Interestingly, the vast majority of studies used the Standard Operating Procedure (SOP) as a guideline for electrochemotherapy. The availability of SOP allowed for reproducibility and improvement of results in the clinical practice. Several large follow up series confirmed the efficiency of electrochemotherapy. A recent meta-analysis of the use of electrochemotherapy in the treatment of cutaneous metastasis places it well amongst other, more established, treatment options.² Recently, electrochemotherapy has also been recognized by the National Institute for Health and Care Excellence (NICE) as an integral part of the multidisciplinary treatment for pa-

tients with skin metastases of non-skin origin and melanoma (NICE interventional procedure guidance IPG 446, <http://www.nice.org.uk/guidance/ipg446>). More recently, electrochemotherapy has been introduced into the treatment of deep-seated and endoluminal tumors.²⁵⁻²⁸ The first clinical report on visceral metastases indicates its effectiveness, and suggests a possible role of electrochemotherapy for the treatment of liver metastases, especially when located close to major blood vessels and when not manageable with surgery or other ablative techniques.²⁹

Overall, literature data from Web of Science database indicate a steady increase in number of publications and their citations under the key word "electrochemotherapy" (Figure 1A,B) and "clinical electrochemotherapy" (Figure 1C,D). Despite a steady increase in the number of published reports, a higher quality and standardization of reported studies is needed to improve and support a truly evidence based practice. In our study we only included papers published after 2006, specifically only to include reports published after the Standard Operating Procedures (SOP).²⁴

The purpose of this recommendation paper is to provide practical recommendations in order to improve the precision of reported clinical studies on electrochemotherapy (a summary checklist is provided as Supplementary file). This, in turn, we hope will stimulate the scientific community to report research using these guidelines to give comprehensive reports on areas including study design, definition of study endpoints, patient selection criteria, treatment plan and outcome assessment. The adoption of more precision in reporting will enable researchers and clinicians to perform more meaningful outcome comparisons with other ablative techniques, to clarify the direction for future research, and to produce more evidence-based practice. It is our hope that these advancements may improve patient selection, resource allocation, and ultimately patient outcome.

This report was prepared based on initiative of the Steering Committee of the COST TD 1104 Action (www.electroporation.net) and in response to a general call for increased awareness and concern for low quality reporting practice³⁰; moreover, it has been prepared by the committee within the Working group of Medical applications of electroporation, in COST action TD 1104 EP4Bio²Med, and is included in the series of publications addressing the same topic in preclinical research in electroporation as well as in the pulsed electric fields for industrial purposes.³¹

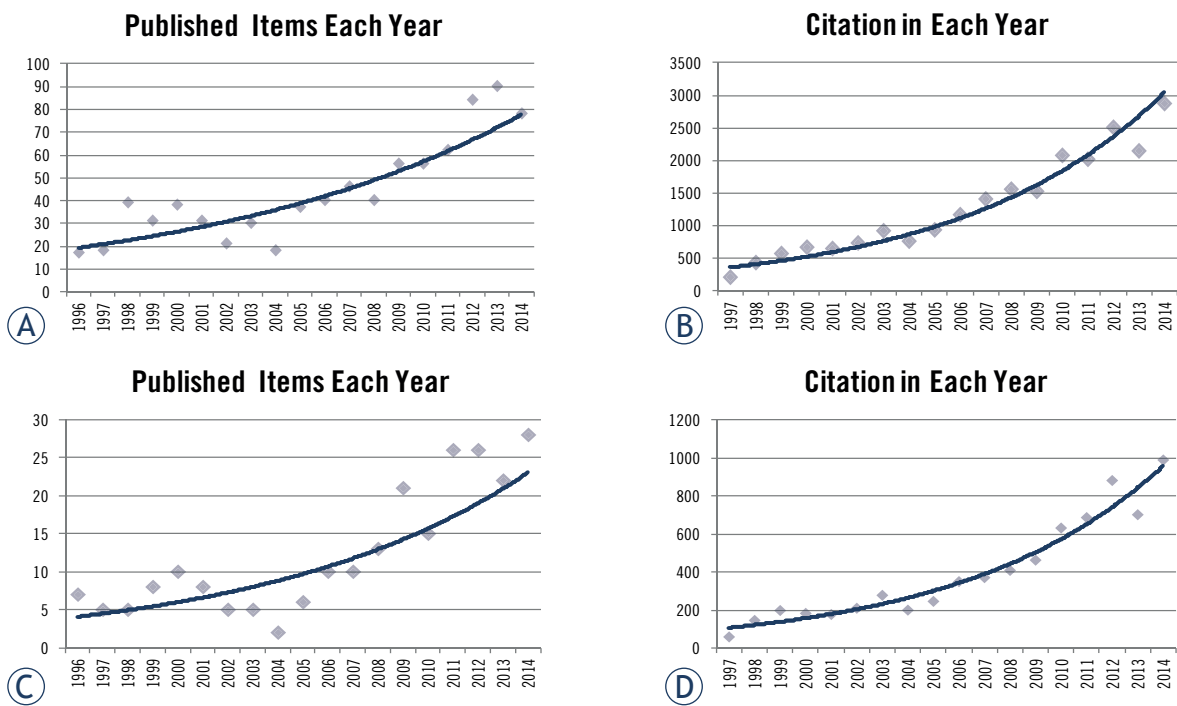


FIGURE 1. Search in Web of Science demonstrates a steady increase in number of publications under the key word “electrochemotherapy” (A,B) as well under the “electrochemotherapy, clinical” (C,D). The Meta data indicate the expanding field.

Several guidelines exist with the aim of assuring sound research practices, and improving the quality of clinical trials and, ultimately, allow for generalizable results. At a basic level, Good Clinical Practice (GCP) represents an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. At a higher level, dedicated guidelines and recommendations have been developed according to the specific type of study performed. For instance, the STROBE statement (www.strobe-statement.org) indicates a checklist for details that should be reported in observational trials; the Consolidated Standards of Reporting Trials (CONSORT) statement (www.consort-statement.org/consort-statement/) provides guidance for reporting the aim, methods, results and implications of randomized controlled trials; the PRISMA statement (www.prisma-statement.org) indicates preferred reporting for systematic reviews; finally, the REporting recommendations for tumor MARKer prognostic studies (REMARK, www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumour-marker-prognostic-studies-remark/) suggest guidelines to provide relevant information about study design, preplanned hypotheses, patient and specimen characteristics, assay methods, and statistical analyses

when evaluating tumor markers in oncology. In addition, these guidelines provide helpful suggestions on how to present data and important elements to include in discussions. Although these guidelines provide a fundamental guidance for conducting a valid clinical trial and reporting generalizable findings, nonetheless it is recognized that there is a need for specialty-specific guidelines and that these guidelines will lead to improvement in the quality of reports and to higher impact publications.^{32,33} In the field of electrochemotherapy, comprehensive meta-analyses or Cochrane style reviews of efficiency are hampered by the lack of some relevant clinical data in published reports. Therefore, we evaluated the published papers on clinical electrochemotherapy and identified possible pitfalls in data reporting. On this basis, we prepared recommendations for improving the quality of future studies and fostering further rational development of electrochemotherapy.

Systematic review and qualitative analysis of publications

Methods

The initial step was to identify and access all published trials evaluating the efficacy of electrochem-

TABLE 1. Manuscript quality criteria

Manuscript quality criteria			
Trial design	Description of Patient population	Treatment delivery and outcome assessment	Analysis of results and interpretation
1. Prospective trial	1. Setting (curative / palliative)	1. Type of anaesthesia	1. Summary of trial endpoints
2. Trial registration		2. Drug route and dosages	
3. Comparative trial	2. Demographic data (in tabular form)	3. Pulse generator	2. Predictive factors
4. Mention of trial design		4. EP parameters	
5. Multicenter study	3. No of tumors	5. Electrode description	3. Other patient outcome parameters
6. Mention of sponsor		6. Tumor safety margins indicated	
7. Trail hypothesis and sample size	4. Tumor location	7. Deviation from SOPs	4. Results interpretation
8. Informed consent	5. Tumor histotype	9. Criteria for retreatment	5. Comparison to historical controls
9. EC approval		8. Tumor coverage with EP	
10. Structured abstract		10. Total No of ECT sessions	
11. Rationale of the trial	6. Tumor size	11. ECT sessions required ^a	6. Future directions
12. A priori inclusion criteria		12. Toxicity criteria	
13. Follow-up dates	7. Visceral mts indicated	13. Response criteria	7. COI statement
14. Statistical methods		14. Evaluation of tumor control	
15. Software used	8. Concomitant treatments	15. ECT success ^b	
16. C.I., p-values		16. Keep track of patients lost to follow-up	

C.I. = confidence intervals; COI = conflict of interest statement; EC = Ethic Committee; EP = electric pulses (including number, duration and amplitude); mts = metastases; SOPs = Standard Operating Procedures.

^a Number of electrochemotherapy (ECT) sessions required for achieving response (either complete or partial) on baseline tumors

^b Decision rule for determining ECT success

otherapy in the treatment of tumors including skin cancers, cutaneous/subcutaneous metastases from other histotypes, deep-seated tumors or visceral metastases.

From October 4 to 10, 2015, we conducted a comprehensive literature assessment that included searches of Medline (EBSCO), Pubmed (NLM), Web of Science and Embase. The search terms used were “electrochemotherapy”, “electrochemotherapy” AND “clinical trial”. We limited our search to humans. Articles published from January 2006 to September 30, 2015 were retrieved. We included studies on the clinical application of electrochemotherapy regardless of study design (both prospective and retrospective) patient population, tumors histotype and anatomical location or electrochemotherapy treatment protocol. However, treatment outcome had to include tumor response and follow-up tumor control evaluation, procedural morbidity and toxicity or patient quality of life. Two of the authors (LGC and SV) and an external collaborator with experience in clinical trials indepen-

dently screened the retrieved studies based on the title, key words, and abstract to exclude non-relevant and non-English written studies. After completion of all searches, duplicates were removed and only the most recent report from follow-up series was included in order to avoid overlapping series. Both retrospective and prospective studies were included, while case reports and small series were excluded because of their intrinsic lower level of evidence (the minimum number of patients was arbitrarily set at 9). Published reviews on electrochemotherapy were similarly excluded, but their reference list was reviewed in order to identify possible additional studies. Studies whose main purpose was unrelated to electrochemotherapy efficacy and biological studies (*i.e.*, those exploring immune effects of treatment) were also excluded, unless clear and standardized description of patient outcome was retrievable from the manuscript. Studies that did not meet the inclusion criteria were discarded during the initial review. When uncertainty existed in the abstract evalu-

ation, we retrieved and assessed the full text. A third author (GS) resolved differing opinions. Full text of the included articles was independently reviewed by two of the authors using a predefined checklist quality criteria. These quality criteria were discussed and agreed among the authors in a series of operative meetings which were held during the 1st World Congress on Electroporation in Portoroz, Slovenia, between September 6 to 10 2015 and were also based on deliberations at the Recommendation paper workshop organized by COST TD1104 on 28th March 2014 in Copenhagen, Denmark. The checklist was also adapted from similar reporting standard guidelines in the field of neuro-oncology, isolated limb perfusion and in phase II cancer trials.³⁴⁻³⁶ As a result, we had a final count of 47 quality criteria that were clustered into four domains: trial design, description of patient population, treatment delivery and outcome assessment, and analysis of results and their interpretation (Table 1). The summary measure during literature assessment was the proportion of studies fulfilling each manuscript quality criteria.

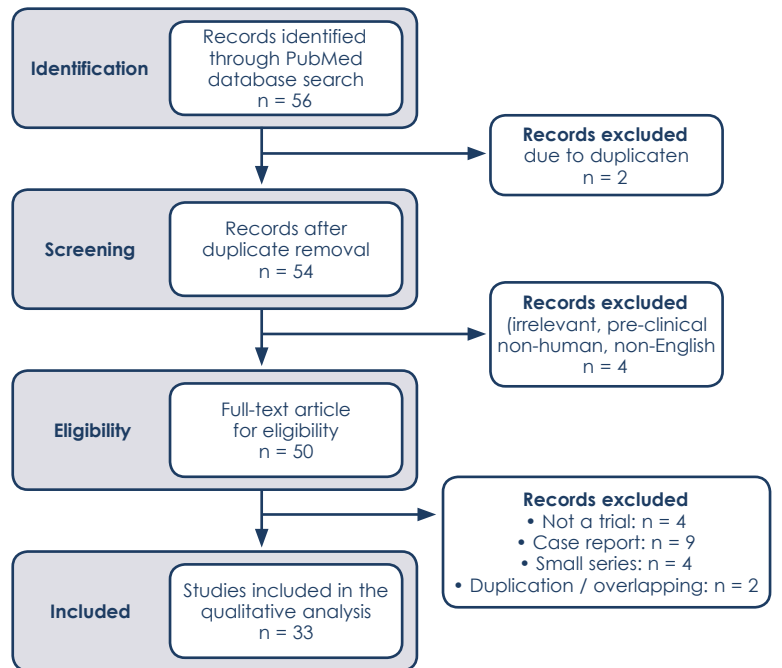


FIGURE 2. PRISMA flow diagram of identification, screening, eligibility and inclusion of studies.

Results

A total of 56 papers were initially identified. Of these, only 33 reports were finally retained in the qualitative synthesis; the reasons for exclusion of the remaining reports are listed in Figure 2.

A summary of the studies included in the final analysis is presented in Table 2.^{20-22,29,37-65} The total number of patients across all studies was 1215. Electrochemotherapy protocol was following the SOP as defined in ESOPE study in all but two cases.^{40,65}

The majority (24/33) of reports were single-center studies. There were 24 tumor-specific studies (melanoma, n=8; breast cancer, n=5; head and neck squamous cell carcinoma, n=4; Kaposi sarcoma, n=3; pancreatic cancer, n=1; colorectal cancer, n=1; soft tissue sarcomas, n=1; vaginal squamous cell cancer, n=1) and 9 studies including heterogeneous histologies. Response assessment was based on clinical evaluation in all except 3 studies on pancreatic cancer⁴¹, liver metastases from colorectal cancer²⁹, and chest wall recurrence from breast cancer⁵⁷, where response assessment was radiological (ultrasound scan, magnetic resonance imaging, computed tomography, or fluorine-18-deoxyglucose PET-CT scan). Details of the quality criteria used to assess trial design are presented in Figure 3. Less than half (15/33, 45%) of studies

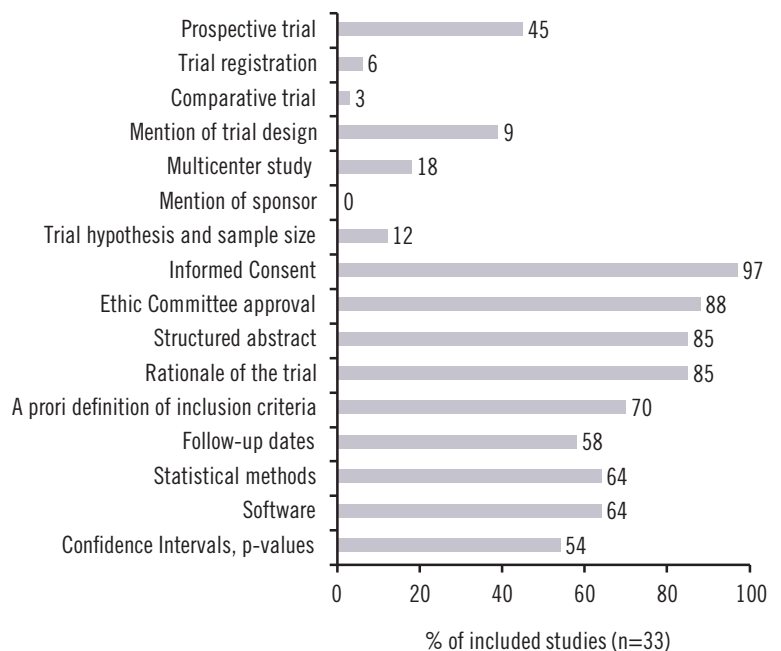


FIGURE 3. Assessment of published studies according to quality criteria concerning trial design.

were prospective and only two of them (6%) were entered into a publicly accessible clinical trials registry.^{29,57} Eighteen percent (6/33) of papers represented the report of a multicenter study. There was a single comparative trial (an internally controlled

TABLE 2. Trials identified included in the qualitative analysis

Study, year	Setting	No of pts	Tumor histotype	ECT protocol
Rotunno, 2015 ³⁷	Two-center, Italy	55	non-melanoma SC	ESOPE
Cabula, 2015 ³⁸	Multi-center, Italy	125	BC	ESOPE
Mozzillo, 2015 ³⁹	Single-center, Italy	15	melanoma	ESOPE
Landstrom, 2015 ⁴⁰	Single-center, Sweden	19	HNSCC	Other ^a
Granata, 2015 ⁴¹	Single-center, Italy	13	pancreatic cancer	ESOPE
Kreuter, 2015 ⁴²	Multi-center, Germany	56	various	ESOPE
Quaglino, 2015 ⁴³	Multi-center, Europe	121	various	ESOPE
Mir-Bonafé, 2015 ⁴⁴	Single-center, Spain	31	melanoma	ESOPE
Campana, 2014 ⁴⁵	Single-center, Italy	39	HNSCC	ESOPE
Ricotti, 2014 ⁴⁶	Single-center, Italy	30	melanoma	ESOPE
Campana, 2014 ⁴⁷	Single-center, Italy	55	BC	ESOPE
Edhemovic, 2014 ²⁹	Single-center, Slovenia	16	CRC-liver mts	ESOPE ^b
Seccia, 2014 ⁴⁸	Single-center, Italy	9	HNSCC	ESOPE
Campana, 2014 ⁵⁰	Two-center, Italy	34	STS	ESOPE
Solari, 2014 ⁵¹	Single-center, Italy	39	various	ESOPE
Di Monta, 2014 ⁵²	Single-center, Italy	19	KS	ESOPE
Caracò, 2013 ⁴⁹	Single-center, Italy	60	melanoma	ESOPE
Perrone, 2013 ⁵³	Single-center, Italy	9	V-SCC	ESOPE
Benevento, 2012 ⁵⁴	Single-center, Italy	12	BC	ESOPE
Mevio, 2012 ⁵⁵	Single-center, Italy	15	HNSCC	ESOPE
Campana, 2012 ²⁰	Single-center, Italy	35	BC	ESOPE
Latini, 2012 ⁵⁶	Single-center, Italy	18	KS	ESOPE
Matthiessen, 2012 ⁵⁷	Single-center, Denmark	12	BC	ESOPE
Gargiulo, 2012 ⁵⁸	Single-center, Italy	52	non-melanoma SC	ESOPE
Campana, 2012 ²¹	Single-center, Italy	85	melanoma	ESOPE
Curatolo, 2012 ⁵⁹	Two-center, Italy	23	KS	ESOPE
Kis, 2011 ⁶⁰	Single-center, Hungary	9	melanoma	ESOPE
Matthiessen, 2011 ²²	Two-center, Denmark-UK	52	various	ESOPE
Skarlatos I, 2011 ⁶¹	Multi-center, Greece	52	various	ESOPE
Campana, 2009 ⁶²	Single-center, Italy	52	various	ESOPE
Quaglino, 2008 ⁶³	Single-center, Italy	14	melanoma	ESOPE
Larkin, 2007 ⁶⁴	Single-center, Ireland	30	various	ESOPE
Gaudy, 2006 ⁶⁵	Single-center, France	12	melanoma	Other ^c

BC = breast cancer; ECT = electrochemotherapy; CRC-liver mts = colorectal cancer liver metastases; HNSCC = head and neck squamous cell cancer; KS = Kaposi's sarcoma; SC = skin cancer; STS = soft tissue sarcomas; V-SCC = vaginal squamous cell cancer

^a Intratumoral BLM injection (1000 IU/cm³) and tumor electroporation by means of six 1100 V/cm square wave pulses with 0.1 ms duration

^b In this trial, the ESOPE protocol was integrated by the application of variable geometry electrodes for the treatment of deep visceral metastases.

^c Intratumoral BLM injection (concentration, 4 mg/mL; dose, 1 mg/cm³ of tumor volume was followed, after 10 minutes, by the application of electric pulses (six 100 µsec-long pulses, 4 pulses/sec, electric field >600V/cm

study with inpatient randomization of melanoma metastases to intralesional bleomycin versus intralesional bleomycin followed by electric pulses⁶⁵; a formal sample size calculation or analysis of “intent-to-treat” population was found in only 4/33 (12%) studies.^{20,50,57,65}

Details of the quality criteria used to assess the description of patient population are presented in Figure 4. Treated tumors were described in detail in most reports: number of tumors, 94%; tumor location, 100%; tumor histotype, 100%; tumor size, 91%. On the other hand, additional clinical information was less frequently reported: study setting -palliative/curative-, 54%; presence of visceral metastases, 54%; concomitant oncologic treatments, 45%.

Details of the manuscript quality criteria used to assess the description of treatment delivery and response assessment are presented in Figure 5. Treatment details were accurately described in most reports: type of anaesthesia, 32/33 (97%); drugs, 33/33 (100%); pulse generator, 33/33 (100%); electrode types, 31/33 (93%); electric pulse parameters, 32/33 (97%). The criteria for response assessment were clearly stated in 29/33 (88%) of studies, while toxicity criteria were indicated in only 14/33 (42%) of papers.

Details of the quality criteria used to assess the analysis of results and their interpretation are presented in Figure 6. The majority of reports included a critical analysis: interpretation of results, 33/33 (100%); comparison to historical control, 25/33 (76%); indication of possible future directions, 33/33 (100%); conflict of interest statement, 27/33 (82%). On the contrary, only a minority of them fulfilled other specific quality criteria: summary of primary and secondary endpoints, 13/33 (39%); indication of predictive factors, 9/33 (27%); additional patient outcome parameters, 9/33 (27%).

Based on the results of this analysis, the consensus between authors was to recommend some minimal requirements for reporting clinical data in future studies.

Recommendations and minimal requirements for reporting clinical trial results on electrochemotherapy

Trial design

Any consolidation of the evidence base of electrochemotherapy requires that reports adhere strictly to research reporting standards and are the result of well-designed clinical trials. Much of these

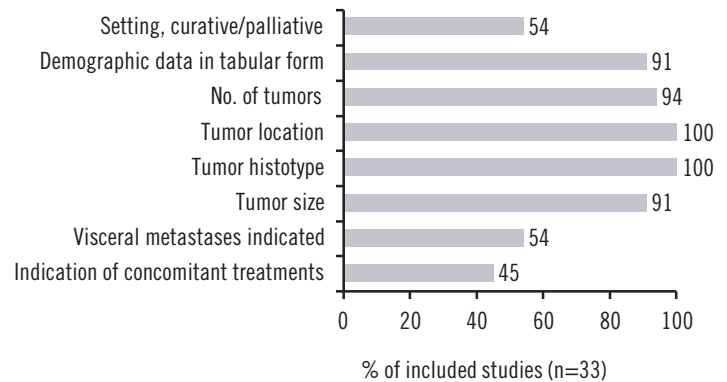


FIGURE 4. Assessment of published studies according to quality criteria concerning description of patient population.

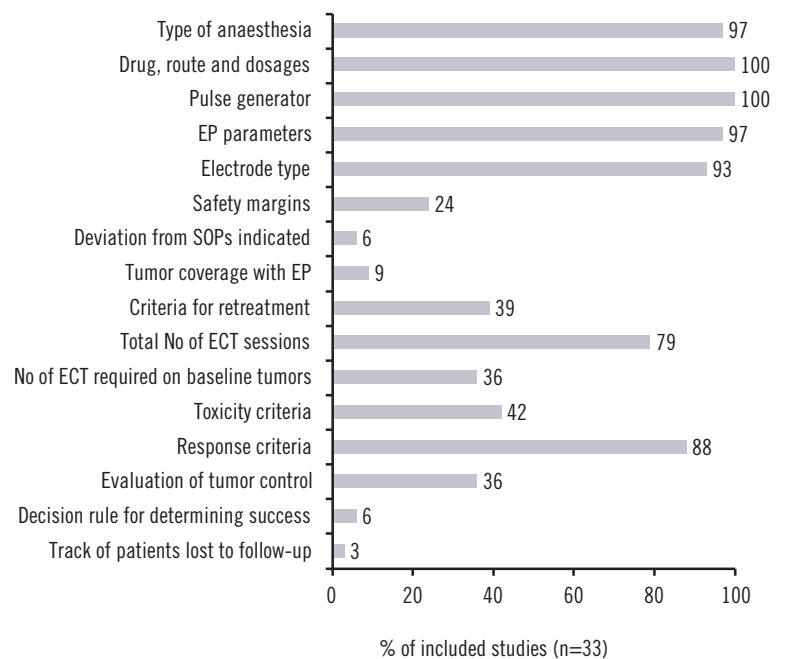


FIGURE 5. Assessment of published studies according to quality criteria concerning treatment delivery and outcome assessment.

ECT = electrochemotherapy; EP = electric pulses.

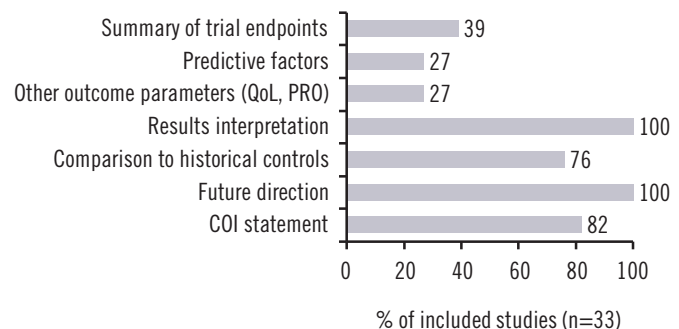


FIGURE 6. Assessment of published studies according to quality criteria concerning analysis of results and interpretation.

COI = conflict of interest statement; PRO = patient reported outcomes; QoL = quality of life.

topics are covered by STROBE (STrengthening the Reporting of Observational studies in Epidemiology, <http://www.strobe-statement.org/>) checklist and CONSORT (CONsolidated Standards of Reporting Trials, <http://www.consort-statement.org/checklists/view/32-consort/66-title>) guidelines which should be adhered to as much as possible when reporting observational studies and randomized controlled trials, respectively. Incorporation of these electrochemotherapy guidelines will further improve the quality of the reports. So far, only phase I-II single-arm trials have been reported, with the exception of a single small-sized study, which included an intra-patient randomization of tumors to direct bleomycin injection or bleomycin injection followed by electroporation.⁶⁵ It is likely that improving the evidence base will involve conducting properly designed, prospective comparative - possibly randomized - clinical trials in order to perform accurate analyses of the advantages of electrochemotherapy against other ablative procedures or alternative local treatments. Of utmost importance, future trials should aim to be prospective and preferably multicentric, with clearly defined endpoints and inclusion criteria. It is also advisable that all trials should be registered at publicly accessible clinical trials registries, (e.g., clinicaltrials.gov, [ISRCTN registry at http://www.isrctn.com](http://www.isrctn.com), [WHO registry at www.apps.who.int/trialsearch](http://www.apps.who.int/trialsearch), or similar) and approved by institutional review boards or respective national bodies. Finally, according to the current requirements of most scientific journals – which refer to the recommendation of the International Committee of the Medical Journal Editors (ICMJE, <http://www.icmje.org/>), manuscripts should conform to well-defined general principles and include, for example, a statement about patient informed consent, modalities of study conduct, as well as authors conflicts of interest.

Key elements of trial design:

- Explanation of the rationale of the study
- Description of trial design and sponsorship
- Indication of trial endpoints
- Indication of inclusion and exclusion criteria
- Trial approval and registration
- Informed consent statement

Description of patient population

Electrochemotherapy was initially used with palliative intent. First trials demonstrated remark-

able efficiency in the treatment of skin metastases from malignant melanoma.^{21,60,63} Subsequently, electrochemotherapy was also evaluated for the treatment of other tumor histotypes (e.g., non-melanoma skin cancers and cutaneous metastases from other tumor histotypes) with equally high success.^{20,37-38,47,57-58} Reports of small series indicate also its possible usefulness in the treatment of primary basal cell carcinomas²³ and a clinical trial is currently ongoing comparing the effectiveness of electrochemotherapy to standard surgical resection and is due to report 5 year follow up data next year (EudraCT Number: 2010-019260-37). A particular advantage of electrochemotherapy is that it is a reliable alternative treatment option for patients who have exhausted more conventional oncological treatments or are judged unfit for or refuse repetitive surgical interventions.⁴⁷ Therefore, future reports need to include detailed description of patient's demographic and clinical data including detailed description of previous treatments. A detailed description of tumor location, histotype as well as number and size of the electrochemotherapy target and non-target lesions is paramount. Authors should also specify whether targeted lesions had previously received irradiation or not, whether visceral metastases are present and whether the treatment is intended as palliative or curative. Additionally, since electrochemotherapy is finding its place among other oncologic treatments, and will be increasingly used also in combination with them, an accurate record of concomitant treatments is also advisable.⁶⁶

Key elements of patient population:

- Patient demographic data (in tabular form)
- Setting - palliative or curative
- Tumor histology
- Disease stage (lymph node or visceral metastases)
- Description of target lesions treated with electrochemotherapy (anatomical location, number and size)
- Previous local treatments
- Concomitant oncological treatments
- Adjuvant and / or following oncological treatments

Treatment information

The treatment is applied by performing a procedure conjugating the administration of a drug and local application of electric pulses. In one "session"

or “cycle” a single or several tumor nodules can be treated. Since the procedures can be repeated on the same and also on newly emerged tumor nodules, patient treatment may require one or more sessions of electrochemotherapy. Therefore, reports should clearly indicate how many sessions (or cycles) were needed for the treatment of baseline tumors and, overall, for patient management. If retreatment is necessary, the indication should be clear, detailing previous response and disease status in target and non-target tumors. In order to ensure the maximum efficacy, electrochemotherapy needs two key elements: the presence of a cytotoxic agent within tumor tissue and the adequate coverage of tumor with electric pulses above the threshold of reversible membrane electroporation.⁶⁷ The results of the ESOPE study and the adoption of SOP that were prepared within the ESOPE project (QLK-2002-02003) were of great importance for the development of electrochemotherapy.^{68,24} In fact, they provided practical guidelines and standardization of the procedure. The clinical data evaluation demonstrated that the use of guidelines and a standardized protocol enabled to reach the same level of effectiveness also in the centers without previous experience with electrochemotherapy.¹⁷ The ESOPE study provided evidence for electrochemotherapy in the treatment of skin metastases of different histotypes.⁶⁸ It included use of bleomycin or cisplatin as chemotherapeutics, different routes of administration (intravenous or intratumoral) and the use of either local or general anaesthesia. The pulse parameters (number, sequence and amplitudes) for different electrodes were however well defined within the Cliniporator project.⁴⁵ A specific electric pulse generator has been consistently used with different electrodes, according to the size, depth and anatomical location of treated tumors.

As confidence with the procedure has developed, treatment indications have also widened. The first studies were based on patients with tumors less than 3 cm, however lesions greater than 3 cm are now routinely treated^{57,62,69}, representing a natural development of the field based on success with smaller tumors for which the ESOPE guidelines were prepared. As such, there is a need to adapt and revise the SOP and this is already underway. Furthermore, new producers of electric pulse generators are coming to the market, and new electrodes with different design for different treatment settings are emerging. All these changes will make the clinical data evaluation even more challenging. First of all, to address this topic, future reports will

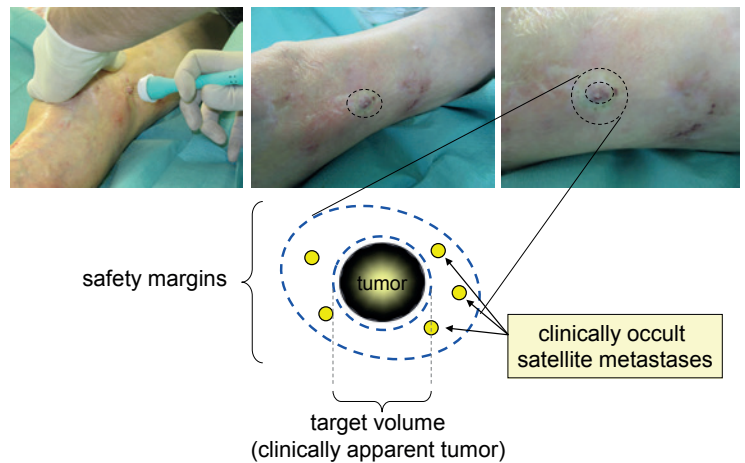


FIGURE 7. Importance of covering whole tumor area along with safety margins. Reporting of the type of electrode applied is essential.

need to state the type of anesthesia used (local or general; drugs and doses), the chemotherapeutic agent, drug concentration and dose used, which both depend on the route of administration. The duration of bolus injection, as well as time interval between the drug administration and application of electric pulses, should be specified. The type of electric pulse generator as well as the type of electrodes and their manufacturers should be reported. Additional information should include if the pulse generator is under software control and the specification of the version of that software. If new types of electrodes are used, a detailed description of the design and the sequence and amplitude of pulses is needed. It must be clearly stated whether applied electrodes are needle or plate, the distance between the electrodes, their shape and size, the amplitude of applied electric pulses, their duration, number and repetition frequency. Furthermore, the total number of pulse deliveries, as well as the time interval required for electrode applications after drug injection, should be specified. Additionally, the report of adequate or inadequate coverage of the tumor as well as the way the pulses are applied (e.g., from the margins to the tumor centre or if the pulses were applied in 4+4 (perpendicular) configuration each time) would also be advisable, when possible (Figure 7).⁷⁰

Electrochemotherapy has a high therapeutic index, therefore after successful treatment minimal damage is observed on normal surrounding tissues. During treatment, it is also possible for the treating physician to include a safety margin around the target tumor, depending on tumor size, biologic aggressiveness and propensity for

developing satellite lesions such as in the case of malignant melanoma or soft tissue sarcomas. In order to improve reporting, the information about the safety margins and their extent should also be reported. In addition, electrochemotherapy can be repeated several times (however there is a ceiling for the total lifetime dose of bleomycin), according to tumor response and disease behavior.^{62,63} This fundamental aspect is not covered by the currently available SOP. For providing a more informative report, data regarding repetitive treatments should be included, along with the description on what basis the retreatment was performed and at what time interval.

Key elements on treatment information:

- Indication of electroporation protocol (adherence to SOP or other)
- Type of anesthesia
- Drug (producer)
- Drug details (dose, concentration and route of administration)
- Time interval between drug administration and application of electric pulses
- Technical details of the electric pulse generator, including type, manufacturer and version of software, if applicable
- Information about the electrodes used, for respective tumor(s)
- Number of electric pulses application per tumor
- Inclusion of a report on electrical parameters (n, T, U, I, f)*
- Adequacy of tumor treatment (treatment application success rate)
- Extent of the safety margins treated
- Number of treatment sessions (with interval between sessions)

* Legend: n = number; T = duration of pulses; U = voltage amplitude applied; I = measured current; f = pulse repetition frequency

Outcome assessment

The early studies on electrochemotherapy antitumor activity have carefully evaluated the response of treated tumors. Response assessment was initially performed by the bidimensional WHO criteria.⁷¹ According to these criteria, baseline and post-treatment tumor size is determined by bidimensional measurements *e.g.* the sum of the two longest diameters in the perpendicular dimensions. The tumor response to treatment is divided into four cat-

egories (complete response, partial response, stable disease, progressive disease, according to the change from baseline tumor measurement).

Indeed, most past studies were focused on tumor response and on patient early outcomes. Nevertheless, a number of reports indicate that the disease locally relapsed or progressed elsewhere, but only few reports indicated the value of electrochemotherapy in the local management of patient symptoms. Hence, the clinical benefit for patients, especially in the palliative setting, where preservation of quality of life (QoL) and evaluation of patient reported outcome (PRO) are crucial, should be based on dedicated assessments and described.

The new RECIST (Response Evaluation Criteria in Solid Tumors) version 1.1 criteria, with some adaptations, have proven a suitable tool for response assessment of superficial tumors^{21,72}, whereas for the setting of treatment of deep-seated tumors (*i.e.*, electrochemotherapy application on liver metastases) the modified RECIST criteria represent the most appropriate and standardized method for the evaluation of tumor response.⁷³ In general, for standard electrochemotherapy on superficial tumors, the RECIST 1.1 criteria, which are based on one-dimensional measurements, seem even more practical and offer highly concordant response assessment compared with the bidimensional WHO criteria.⁷⁴

So far, most of the published papers do not report on any serious treatment related adverse event after electrochemotherapy. Nevertheless, the process surrounding the determination, recording and reporting of adverse events remains moderately challenging especially for the clinician who may not be involved in drug or device-related research. Nevertheless, it is important to understand the basic definitions of adverse events reporting in order to ensure that the proper information is collected in clinical protocols. Moreover, a comprehensive patient observation and a detailed report of all types and grades of toxicities are essential for providing a comprehensive report of treatment outcome, not only in the early, but also in the long-term follow-up. In this way, only large cohorts of patients will enable in-deep view of long-term toxicity and more detailed analyses of treatment-related adverse events according to different patients subgroup, as demonstrated by a recently published report on electrochemotherapy-related pain.⁴³ For this purpose, Common Terminology Criteria for Adverse Events (CTCAE v4.0) is widely accepted throughout the oncology community as the standard classification and severity grading scale for

adverse events. Unfortunately, most of the studies conducted so far do not report consistently on this crucial aspect.

Key elements of treatment outcome assessment:

- Time of response assessment
- Standardized response evaluation criteria (e.g. WHO, RECIST 1.1, mRECIST)
- Time to local and systemic disease progression
- Standardized toxicity criteria (e.g. CTCAE v4.0)
- Quality of Life (QoL), patient reported outcomes (PRO)
- Track of patients lost to follow-up

Analysis and interpretation of the results

A clear summary of the trial endpoints is essential. In fact, the field is moving beyond simply reporting on tumor control, as treatment now includes, in some instances, also primary tumors. Here it is important to report and discuss other parameters, such as time to local/systemic progression and, if possible, also the patient survival time and QoL as well. Such data will increase the evidence level of electrochemotherapy effectiveness, and consolidate a role for electrochemotherapy outside the palliative setting and into a confirmed primary treatment modality.

It has been clearly demonstrated that tumor size is the most reliable predictive factor for response in patients who underwent electrochemotherapy.^{21,22,69} In future, detailed reports including data on previous local therapies (e.g., radiation) as well as on local (within electrochemotherapy field) tissue status (e.g., presence of lymphedema or fibrosis) and concomitant/adjunct oncologic treatments would allow for the identification of other reliable predictive indicators for response.

Key elements for analysis and interpretation of the results:

- Summary of trial endpoints
- Additional patient outcome parameters (e.g., QoL, PRO)
- Predictive factors
- Results interpretation
- Future research directions

Conclusions

Electrochemotherapy represents an effective treatment option for an increasing number of cancer patients with superficial tumors. Nevertheless, to further improve its evidence basis, it will be crucial to raise the quality of future reports.

In this study, we have highlighted some relevant aspects of clinical data reporting, with the aim of improving the quality of future studies in the field of electrochemotherapy. Although a large amount of data are published so far, clinical research needs to adopt detailed and accurate reporting as well as moving from small, non-comparative series to well-designed, possibly randomized, clinical trials. Despite the encouraging results indicated, the vast majority of included reports are case series from single institutions. Although there was a wide consensus to use previously published SOP for the treatment protocol, these studies often present a variety of designs and reporting methods, thus limiting the understanding of patient selection, treatment effect, toxicity and overall patient outcome. Of note, published studies often lack sufficient procedural as well as patient data. These shortcomings represent a major hurdle to performing systematic reviews or meta-analysis, which may provide a more robust evaluation of treatment effectiveness and, ultimately, encourage wider acceptance of electrochemotherapy in the clinical practice.

Our study has some limitations. We identified a set of manuscript quality criteria from available literature and we have expanded this list by including additional, procedure-specific criteria that were discussed and agreed among the authors. The list of 47 quality criteria that were used for reviewing published reports represents an arbitrary selection of criteria performed by a relatively small number of authors. There is potential for selection bias in the inclusion of papers for analysis, as the initial screen was based on broad, non-selective inclusion criteria. However, we feel that these were widely inclusive and fitting in order to develop the proposed recommendations. Nevertheless, we believe that our suggestions largely cover the most crucial aspects, which are required to improve the quality of clinical practice and future research: trial design and conduction, definition of study endpoints, patient selection, treatment delivery, patient management and follow-up, standardization of outcome assessment. Our recommendations are open to a broader discussion with the community users of electrochemotherapy and, possibly, to further improvements in line with other interventional

oncology procedures.^{75,76} Electrochemotherapy requires standardization of terminology and reporting criteria to facilitate effective communication among researchers and appropriate comparison between different treatment technologies. As such, investigators involved in this field should be familiar with these recommendations and use them for future study design and conduction, treatment application as well as data reporting. We envision that the adoption of these recommendations will further improve the quality of future studies and allow more meaningful comparisons of outcome data of patients treated with electrochemotherapy (Supplementary file).

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References

- Mali B, Jarm T, Snoj M, Sersa G, Miklavcic D. Antitumor effectiveness of electrochemotherapy: A systematic review and meta-analysis. *EJSO* 2013; **39**: 4-16.
- Spratt DE, Spratt EAG, Wu SH, DeRosa A, Lee NY, Lacouture ME, et al. Efficacy of skin-directed therapy for cutaneous metastases from advanced cancer: A meta-analysis. *J Clin Oncol* 2014; **32**: 3144-55.
- Neumann E, Schaefer-Ridder M, Wang Y, Hofschneider PH. Gene transfer into mouse lymphoma cells by electroporation in high electric fields. *EMBO J* 1982; **1**: 841-5.
- Glass LF, Pepine ML, Fenske NA, Jaroszeski M, Reintgen DS, Heller R. Bleomycin-mediated electrochemotherapy of metastatic melanoma. *Arch Dermatol* 1996; **132**: 1353-7.
- Heller R, Jaroszeski MJ, Glass LF, Messina JL, Rapaport DP, DeConti RC, et al. Phase I/II trial for the treatment of cutaneous and subcutaneous tumors using electrochemotherapy. *Cancer* 1996; **77**: 964-71.
- Mir LM, Belehradec M, Domenge C, Orlowski S, Poddevin B, Belehradec J Jr, et al. Electrochemotherapy, a new antitumor treatment: first clinical trial. *C R Acad Sci III* 1991; **313**: 613-18.
- Mir LM, Orlowski S, Belehradec J Jr, Paoletti C. Electrochemotherapy potentiation of antitumor effect of bleomycin by local electric pulses. *Eur J Cancer* 1991; **27**: 68-72.
- Giraud P, Bachaud JM, Teissie J, Rols MP. Effects of electrochemotherapy on cutaneous metastases of human malignant melanoma. *Int J Rad Oncol Biol Phys* 1996; **36**: 1285.
- Rols MP, Bachaud JM, Giraud P, Chevreau C, Roche H, Teissie J. Electrochemotherapy of cutaneous metastases in malignant melanoma. *Melanoma Res* 2000; **10**: 468-74.
- Mir LM, Glass LF, Sersa G, Teissie J, Domenge C, Miklavcic D, et al. Effective treatment of cutaneous and subcutaneous malignant tumours by electrochemotherapy. *Brit J Cancer* 1998; **77**: 2336-42.
- Glass LF, Fenske NA, Jaroszeski M, Perrott R, Harvey DT, Reintgen DS, et al. Bleomycin-mediated electrochemotherapy of basal cell carcinoma. *J Am Acad Dermatol* 1996; **34**: 82-6.
- Heller R, Jaroszeski MJ, Reintgen DS, Puleo CA, DeConti RC, Gilbert RA, et al. Treatment of cutaneous and subcutaneous tumors with electrochemotherapy using intralesional bleomycin. *Cancer* 1998; **83**: 148-57.
- Belehradec M, Domenge C, Luboinski B, Orlowski S, Belehradec J Jr, Mir LM. Electrochemotherapy, a new antitumor treatment. First clinical phase I-II trial. *Cancer* 1993; **72**: 3694-700.
- Eisenstein M. A shock to the system. *Nat Meth* 2006; **3**: 66.
- Kotnik T, Frey W, Sack M, Haberl Meglič S, Peterka M, Miklavcic D. Electroporation-based applications in biotechnology. *Trends Biotechnol* 2015; **33**: 480-8.
- Sersa G, Miklavcic D, Cemazar M, Rudolf Z, Pucihar G, Snoj M. Electrochemotherapy in treatment of tumours. *EJSO* 2008; **34**: 232-40.
- Miklavcic D, Mali B, Kos B, Heller R, Sersa G. Electrochemotherapy: from the drawing board into medical practice. *BioMedical Engineering Online* 2014; **13**: 29.
- Bureau MF, Gehl J, Deleuze V, Mir LM, Scherman D. Importance of association between permeabilization and electrophoretic forces for intramuscular DNA electrotransfer. *Biochim Biophys Acta* 2000; **1474**: 353-9.
- Quaglino P, Mortera C, Osella-Abate S, Barberis M, Illengo M, Rissone M, et al. Electrochemotherapy with intravenous bleomycin in the local treatment of skin melanoma metastases. *Ann Surg Oncol* 2008; **15**: 2215-22.
- Campana LG, Valpione S, Falci C, Mocellin S, Basso M, Corti L, et al. The activity and safety of electrochemotherapy in persistent chest wall recurrence from breast cancer after mastectomy: a phase-II study. *Breast Cancer Res Treat* 2012; **134**: 1169-78.
- Campana LG, Valpione S, Mocellin S, Sundararajan R, Granziera E, Sartore L, et al. Electrochemotherapy for disseminated superficial metastases from malignant melanoma. *Brit J Surg* 2012; **99**: 821-30.
- Matthiessen LW, Chalmers RL, Sainsbury DC, Veeramani S, Kessell G, Humphreys AC, et al. Management of cutaneous metastases using electrochemotherapy. *Acta Oncol* 2011; **50**: 621-9.
- Salwa SP, Bourke MG, Forde PF, O'Shaughnessy M, O'Sullivan ST, Kelly EJ, et al. Electrochemotherapy for the treatment of ocular basal cell carcinoma; a novel adjunct in the disease management. *J Plast Reconstr Aesthet Surg* 2014; **67**: 403-6.
- Mir LM GJ, Sersa G, Collins CG, Garbay JR, Billard V, et al. Standard operating procedures of the electrochemotherapy: Instructions for the use of bleomycin or cisplatin administered either systemically or locally and electric pulses delivered by the Cliniporator by means of invasive or non-invasive electrodes. *EJC Suppl* 2006; **4**: 14-25.
- Miklavcic D, Snoj M, Zupanic A, Kos B, Cemazar M, Kropivnik M, et al. Towards treatment planning and treatment of deep-seated solid tumors by electrochemotherapy. *BioMedical Engineering Online* 2010; **9**: 10.
- Edhemovic I, Gadzije EM, Breclj E, Miklavcic D, Kos B, Zupanic A, et al. Electrochemotherapy: a new technological approach in treatment of metastases in the liver. *Tecnol Cancer Res Treat* 2011; **10**: 475-85.
- Miklavcic D, Sersa G, Breclj E, Gehl J, Soden D, Bianchi G, et al. Electrochemotherapy: technological advancements for efficient electroporation-based treatment of internal tumors. *Med Biol Eng Comput* 2012; **50**: 1213-25.
- Soden D, Larkin J, Collins C, Piggott J, Morrissey A, Norman A, et al. The development of novel flexible electrode arrays for the electrochemotherapy of solid tumour tissue. (Potential for endoscopic treatment of inaccessible cancers). *Conf Proc IEEE Eng Med Biol Soc* 2004; **5**: 3547-50.
- Edhemovic I, Breclj E, Gasljevic G, Marolt Music M, Gorjup V, Mali B, et al. Intraoperative electrochemotherapy of colorectal liver metastases. *J Surg Oncol* 2014; **110**: 320-7.
- Journals unite for reproducibility. *Nature* 2014; **515**: 7.
- Miklavcic D. Network for development of electroporation-based technologies and treatments: COST TD1104. *J Membrane Biol* 2012; **245**: 591-8.
- Khan AA, Clover AJ. New guidelines for reporting observational studies and their implications for plastic surgery (STROBE). *J Plast Reconstr Aesthet Surg* 2009; **62**: 155-6.

33. Al-Benna S, Clover J. The role of the journal impact factor: choosing the optimal source of peer-reviewed plastic surgery information. *Plast Reconstr Surg* 2007; **119**: 755-6.
34. Chang SM, Reynolds SL, Butowski N, Lamborn KR, Buckner JC, Kaplan RS, et al. GNOSSIS: guidelines for neuro-oncology: standards for investigational studies-reporting of phase 1 and phase 2 clinical trials. *Neuro Oncol* 2005; **7**: 425-34.
35. Trablusi NH, Patakfalvi L, Nassif MO, Turcotte RE, Nichols A, Meguerditchian AN. Hyperthermic isolated limb perfusion for extremity soft tissue sarcomas: systematic review of clinical efficacy and quality assessment of reported trials. *J Surg Oncol* 2012; **106**: 921-8.
36. Mariani L, Marubini E. Content and quality of currently published phase II cancer trials. *J Clin Oncol* 2000; **18**: 429-36.
37. Rotunno R, Marengo F, Ribero S, Calvieri S, Amerio P, Curatolo P, et al. Electrochemotherapy in non-melanoma head and neck skin cancers: a three centers experience and literature review. *G Ital Dermatol Venereol* 2015; in press
38. Cabula C, Campana LG, Grilz G, Galuppo S, Bussone R, De Meo L, et al. Electrochemotherapy in the treatment of cutaneous metastases from breast cancer: A multicenter cohort analysis. *Ann Surg Oncol* 2015; **22** (Suppl 3): 442-50.
39. Mozzillo N, Simeone E, Benedetto L, Curvietto M, Giannarelli D, Gentilcore G, et al. Assessing a novel immuno-oncology-based combination therapy: Ipilimumab plus electrochemotherapy. *Oncoimmunology* 2015; **4**(6): e1008842.
40. Landstrom FJ, Reizenstein J, Adamsson GB, Beckerath M, Moller C. Long-term follow-up in patients treated with curative electrochemotherapy for cancer in the oral cavity and oropharynx. *Acta Otolaryngol* 2015; **135**: 1070-8.
41. Granata V, Fusco R, Piccirillo M, Palaia R, Petrillo A, Lastoria S, et al. Electrochemotherapy in locally advanced pancreatic cancer: Preliminary results. *Int J Surg* 2015; **18**: 230-6.
42. Kreuter A, van Eijk T, Lehmann P, Fischer M, Horn T, Assaf C, et al. Electrochemotherapy in advanced skin tumors and cutaneous metastases - a retrospective multicenter analysis. *J Dtsch Dermatol Ges* 2015; **13**: 308-15.
43. Quaglino P, Matthiessen LW, Curatolo P, Muir T, Bertino G, Kunte C, et al. Predicting patients at risk for pain associated with electrochemotherapy. *Acta Oncologica* 2015; **54**: 298-306.
44. Mir-Bonafe JM, Vilalta A, Alarcon I, Carrera C, Puig S, Malveyh J, et al. Electrochemotherapy in the treatment of melanoma skin metastases: a report on 31 cases. *Actas Dermosifiliogr* 2015; **106**: 285-91.
45. Campana LG, Mali B, Sersa G, Valpione S, Giorgi CA, Strojan P, et al. Electrochemotherapy in non-melanoma head and neck cancers: a retrospective analysis of the treated cases. *Brit J Oral Maxillofac Surg* 2014; **52**: 957-64.
46. Ricotti F, Giuliodori K, Cataldi I, Campanati A, Ganzetti G, Ricotti G, et al. Electrochemotherapy: an effective local treatment of cutaneous and subcutaneous melanoma metastases. *Dermatol Ther* 2014; **27**: 148-52.
47. Campana LG, Galuppo S, Valpione S, Brunello A, Ghiotto C, Ongaro A, et al. Bleomycin electrochemotherapy in elderly metastatic breast cancer patients: clinical outcome and management considerations. *J Cancer Res Clin Oncol* 2014; **140**: 1557-65.
48. Seccia V, Muscatello L, Dallan I, Bajraktari A, Briganti T, Ursino S, et al. Electrochemotherapy and its controversial results in patients with head and neck cancer. *Anticancer Res* 2014; **34**: 967-72.
49. Caraco C, Mozzillo N, Marone U, Simeone E, Benedetto L, Di Monta G, et al. Long-lasting response to electrochemotherapy in melanoma patients with cutaneous metastasis. *BMC Cancer* 2013; **13**: 564.
50. Campana LG, Bianchi G, Mocellin S, Valpione S, Campanacci L, Brunello A, et al. Electrochemotherapy treatment of locally advanced and metastatic soft tissue sarcomas: Results of a non-comparative phase II study. *World J Surg* 2014; **38**: 813-22.
51. Solari N, Spagnolo F, Ponte E, Quaglia A, Lillini R, Battista M, et al. Electrochemotherapy for the management of cutaneous and subcutaneous metastasis: a series of 39 patients treated with palliative intent. *J Surg Oncol* 2014; **109**: 270-4.
52. Di Monta G, Caraco C, Benedetto L, La Padula S, Marone U, Tornesello ML, et al. Electrochemotherapy as "new standard of care" treatment for cutaneous Kaposi's sarcoma. *EJSO* 2014; **40**: 61-6.
53. Perrone AM, Galuppi A, Cima S, Pozzati F, Arcelli A, Cortesi A, et al. Electrochemotherapy can be used as palliative treatment in patients with repeated loco-regional recurrence of squamous vulvar cancer: a preliminary study. *Gynecol Oncol* 2013; **130**: 550-3.
54. Benevento R, Santoriello A, Perna G, Canonico S. Electrochemotherapy of cutaneous metastases from breast cancer in elderly patients: a preliminary report. *BMC Surg* 2012; **12** (Suppl 1): S6.
55. Mevio N, Bertino G, Occhini A, Scelsi D, Tagliabue M, Mura F, et al. Electrochemotherapy for the treatment of recurrent head and neck cancers: preliminary results. *Tumori* 2012; **98**: 308-13.
56. Latini A, Bonadies A, Trento E, Bultrini S, Cota C, Solivetti FM, et al. Effective treatment of Kaposi's sarcoma by electrochemotherapy and intravenous bleomycin administration. *Dermatol Ther* 2012; **25**: 214-8.
57. Matthiessen LW, Johannesen HH, Hendel HW, Moss T, Kamy C, Gehl J. Electrochemotherapy for large cutaneous recurrence of breast cancer: a phase II clinical trial. *Acta Oncol* 2012; **51**: 713-21.
58. Gargiulo M, Papa A, Capasso P, Moio M, Cubicciotti E, Parascandolo S. Electrochemotherapy for non-melanoma head and neck cancers: clinical outcomes in 25 patients. *Ann Surg* 2012; **255**: 1158-64.
59. Curatolo P, Quaglino P, Marengo F, Mancini M, Nardo 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**: 192-8.
60. Kis E, Olah J, Ocsai H, Baltas E, Gyulai R, Kemeny L, et al. Electrochemotherapy of cutaneous metastases of melanoma—a case series study and systematic review of the evidence. *Dermatol Surg* 2011; **37**: 816-24.
61. Skarlatos I, Kyrgias G, Mosa E, Provatopoulou X, Spyrou M, Theodorou K, et al. Electrochemotherapy in cancer patients: first clinical trial in Greece. *In Vivo* 2011; **25**: 265-74.
62. 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**: 191-9.
63. Quaglino P, Mortera C, Osella-Abate S, Barberis M, Illengo M, Rissone M, et al. Electrochemotherapy with intravenous bleomycin in the local treatment of skin melanoma metastases. *Ann Surg Oncol* 2008; **15**: 2215-22.
64. Larkin JO, Collins CG, Aarons S, Tangney M, Whelan M, O'Reilly S, et al. Electrochemotherapy: aspects of preclinical development and early clinical experience. *Ann Surg* 2007; **245**: 469-79.
65. Gaudy C, Richard MA, Folchetti G, Bonerandi JJ, Grob JJ. Randomized controlled study of electrochemotherapy in the local treatment of skin metastases of melanoma. *J J Cutan Med Surg* 2006; **10**: 115-21.
66. Valpione S, Campana LG, Pigozzo J, Chiarion-Sileni V. Consolidation electrochemotherapy with bleomycin in metastatic melanoma during treatment with dabrafenib. *Radiol Oncol* 2015; **49**: 71-4.
67. Miklavcic D, Corovic S, Pucihar G, Pavselj N. Importance of tumour coverage by sufficiently high local electric field for effective electrochemotherapy. *EJC Suppl* 2006; **4**: 45-51.
68. Marty M SG, Garbay JR, Gehlc J, Collinsd CG, Snoj M et al. Electrochemotherapy – An easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: Results of ESOP (European Standard Operating Procedures of Electrochemotherapy) study. *EJC Suppl* 2006; **4**: 3-13.
69. Mali B, Miklavcic D, Campana LG, Cemazar M, Sersa G, Snoj M, et al. Tumor size and effectiveness of electrochemotherapy. *Radiol Oncol* 2013; **47**: 32-41.
70. Sersa G, Cemazar M, Semrov D, Miklavcic D. Changing electrode orientation improves the efficacy of electrochemotherapy of solid tumors in mice. *Bioelectrochem Bioener* 1996; **39**: 61-6.
71. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981; **47**: 207-14.
72. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; **45**: 228-47.
73. Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Disease* 2010; **30**: 52-60.
74. Choi JH, Ahn MJ, Rhim HC, Kim JW, Lee GH, Lee YY, et al. Comparison of WHO and RECIST criteria for response in metastatic colorectal carcinoma. *Cancer Res Treat* 2005; **37**: 290-3.
75. Goldberg SN, Grassi CJ, Cardella JF, Charboneau JW, Dodd GD, Dupuy DE, et al. Image-guided tumor ablation: Standardization of terminology and reporting criteria. *J Vasc Interv Radiol* 2009; **20**: S377-S90.
76. Callstrom MR, York JD, Gaba RC, Gemmete JJ, Gervais DA, Millward SF, et al. Research reporting standards for image-guided ablation of bone and soft tissue tumors. *J Vasc Interv Radiol* 2009; **20**: 1527-40.