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# Electrochemotherapy – An easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: Results of ESOPE (European Standard Operating Procedures of Electrochemotherapy) study

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ABSTRACT

*Purpose:* To evaluate and confirm efficacy and safety of electrochemotherapy with bleomycin or cisplatin on cutaneous and subcutaneous tumour nodules of patients with malignant melanoma and other malignancies in a multicenter study.
*Patients and methods:* This was a two year long prospective non-randomised study on 41 patients evaluable for response to treatment and 61 evaluable for toxicity. Four cancer centers enrolled patients with progressive cutaneous and subcutaneous metastases of any histologically proven cancer. The skin lesions were treated by electrochemotherapy, using application of electric pulses to the tumours for increased bleomycin or cisplatin delivery into tumour cells. The treatment was performed using intravenous or intratumoural drug injection, followed by application of electric pulses generated by a Cliniporator™ using plate or needle electrodes. Tumour response to electrochemotherapy as well as possible side-effects with respect to the treatment approach, tumour histology and location of the tumour nodules and electrode type were evaluated.
*Results:* An objective response rate of 85% (73.7% complete response rate) was achieved on the electrochemotherapy treated tumour nodules, regardless of tumour histology, and drug used or route of its administration. At 150 days after the treatment (median follow up was 133 days and range 60–380 days) local tumour control rate for electrochemotherapy was 88% with bleomycin given intravenously, 73% with bleomycin given intratumourally and 75% with cisplatin given intratumourally, demonstrating that all three approaches were

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similarly effective in local tumour treatment. Furthermore, electrochemotherapy was equally effective regardless of the tumour type and size of the nodules treated. Side-effects of electrochemotherapy were minor and acceptable, as reported by the patients.
*Conclusion*: We demonstrated that electrochemotherapy is an easy, highly effective, safe and cost-effective approach for the treatment of cutaneous and subcutaneous tumour nodules of different malignancies. Electrochemotherapy can provide immediate clinical benefit in patients with advanced cutaneous and subcutaneous metastases.
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## 1. Introduction

Electrochemotherapy is an efficient local tumour ablation modality that has already proven its effectiveness clinically in the treatment of various types of tumours. It uses electroporation, a physical drug delivery system, to deliver non-permeant or poorly permeant chemotherapeutic agents into the cells.[1] Among several clinically approved drugs that have been tested in preclinical studies, bleomycin and cisplatin have been demonstrated to be the most suitable drugs for clinical use of electrochemotherapy.[2–5] Exposure of cells to electric pulses increases cytotoxicity of bleomycin (~8000 fold) and cisplatin (~80 fold).[2,3] *In vivo* application of electric pulses to the tumours significantly potentiates antitumour effectiveness of bleomycin or cisplatin, given either intravenously[3,6] or intratumourally.[7,8] The treatment results in complete responses of the tumours with drug doses that by themselves have minimal or no antitumour activity and induce no side-effects. Application of electric pulses to the tumours can be performed either by plate electrodes that are placed on the skin above the tumour or by needle electrodes that are inserted into it. Like the drugs themselves, the application of the electric pulses alone, delivered by either type of electrodes, has minimal or no effect on tumour growth.[3,6,9]

Several clinical phase I and II studies, aiming at evaluating safety, efficacy and dosage, demonstrated clinical applicability of electrochemotherapy using bleomycin (reviewed in 10) or cisplatin (reviewed in 11). Cutaneous and subcutaneous tumour nodules were treated in patients with progressive disease of different malignancies: sarcomas, carcinomas, but predominantly malignant melanoma. Electrochemotherapy with bleomycin resulted in 85% objective responses, with high percentage (56%) of long lasting complete responses.[12] Electrochemotherapy with cisplatin resulted in 77% long lasting complete responses of tumour nodules treated by electrochemotherapy, compared to 19% for those that were treated with cisplatin only.[13] Besides these first clinical reports, several others followed with similar results. All together, in all published clinical studies, 1009 tumour nodules in 247 patients were treated, demonstrating that electrochemotherapy is a feasible and effective treatment for local tumour control, with few side-effects.[10,11,14,15] Efficacy of electrochemotherapy was demonstrated in palliative treatment of cutaneous and subcutaneous tumour nodules, thus suggesting that it may provide meaningful clinical benefit in this important field of cancer treatment.

In the above mentioned studies different protocols for electrochemotherapy were performed with different doses of chemotherapeutics, different pulse parameters and different electric pulse generators in conjunction with different electrode types. A European project (ESOPE, European Standard Operating Procedures of Electrochemotherapy) was therefore launched with the aim to prepare standard operating procedures (SOP) for electrochemotherapy, based on the experience of the leading European cancer centers on electrochemotherapy. The ESOPE project also anticipated the use of a new, CE approved, medical grade, electric pulses generator Cliniporator™. The Cliniporator™ allows monitoring and storage of current and voltage parameters as well as pulse parameter setting, for immediate control and later retrieval and analysis. Furthermore, also a high pulse frequency can be delivered, enabling use of more complex electrodes.

The four participating cancer centers prepared protocols for treatment and for pain control based on their previous experience on electrochemotherapy along with the list of electrochemotherapy indications. Here we report the results of this trial. Cutaneous and subcutaneous tumour nodules in patients with progressive disease were treated by electrochemotherapy using bleomycin (given either intravenously or intratumourally) or cisplatin (given intratumourally). The goals of this trial were to: determine the objective response rate and complete response rate of the treated tumour nodules after single treatment; investigate the efficacy of each of the two drugs used in electrochemotherapy and the influence of the route of bleomycin administration; determine the treatment response according to the tumour type, size, its location, type of electrodes and electrical parameters, and cancer centre where the treatment was performed, evaluate the toxicity and document the safety of the treatment.

## 2. Patients and methods

### 2.1. *Patients*

Between 31 March 2003 and 20 April 2005 62 patients were eligible according to the inclusion criteria and were included in the study; five patients died within 60 days, two did not return for treatment evaluation, and one withdrew informed consent. In addition 13 patients were excluded from the evaluation of response due to less than 60 days follow up. While 62 patients were evaluable for toxicity and 42 patients were evaluable for response, respectively, 61 and 41 patients are reported in relation to late withdrawal of informed consent by one patient.

The patients were recruited from the four cancer centers participating in this study: Institut Gustave-Roussy (IGR),

Villejuif, France, Institute of Oncology Ljubljana (OI), Ljubljana, Slovenia, University of Copenhagen at Herlev Hospital (HH), Herlev, Denmark and Cork Cancer Research Center Bio-Sciences Institute and Mercy University Hospital, National University of Ireland (CCRC), Cork, Ireland.

Patients were to have a life expectancy $\geqslant$3 months, measurable cutaneous or subcutaneous tumour nodules suitable for application of electric pulses but not bigger than 3 cm in diameter, a treatment free interval of at least 2 weeks from previously applied therapy, Karnofsky performance status greater than 70% or WHO $\leqslant$ 2, and adequate haematological and renal function. Patients must have been offered standard treatment according to the policies of the country of residence. Electrochemotherapy was considered either in the case of progression of the disease despite use of standard treatments or when patients did not wish to receive standard treatment. The following exclusion criteria were applied: symptomatic and/or rapidly progressive visceral disease, allergic reactions to bleomycin or cisplatin observed in a previous treatment or if cumulative bleomycin dose of 250000 IU/m$^2$ was exceeded, peripheral neuropathy $\geqslant$ grade 2, abnormal haemostasis, chronic renal dysfunction, clinically manifested arrhythmia or with pacemaker, epilepsy and pregnancy or lactation. Institutional or national review board approval had been given for this study to each of the participating cancer centers. All patients gave their written, informed consent to participate in the study.

### 2.2. *Study design*

This study was a prospective non-randomised study, conducted in accordance with protocol for electrochemotherapy agreed among participating centers, based on their previous experience. Briefly, the patients were treated by electrochemotherapy using either bleomycin or cisplatin in low doses followed by application of electric pulses to the tumours by the CE labelled electric pulse generator Cliniporator™ (IGEA S.r.l., Carpi, Italy), in order to potentiate cytotoxicity of the chemotherapeutics (Fig. 1). In the case that the patient had more than seven nodules, all nodules were treated for compassionate reason (or at least all those treatable during the session time). However a maximum of seven nodules were identified at the first visit and only these were considered for the study and analysis purposes.

Bleomycin (either Blenamax, Pharmachemie B.V., The Netherlands, Bleomycin, Asta Medica, Sweden, Bléomycine, Roger Bellon, France, or Bleomycin Sulphate, Mayne Pharma Plc, Wawickshire, UK) was given either intravenously (15000 IU/m$^2$, in a bolus lasting 30–45 s) or intratumourally, at a dose dependent on the size of the tumour nodules. On the bases of previous personal clinical experience Authors selected for intratumoural administration of bleomycin the dose of 1000 IU/cm$^3$ of tumour for tumors smaller than 0.5 cm$^3$. For larger tumors the concern was that maintaining the same concentration per cm$^3$ of tumour would deliver locally a high bleomycin dose. Thus the bleomycin/cm$^3$ of tumor was scaled as it

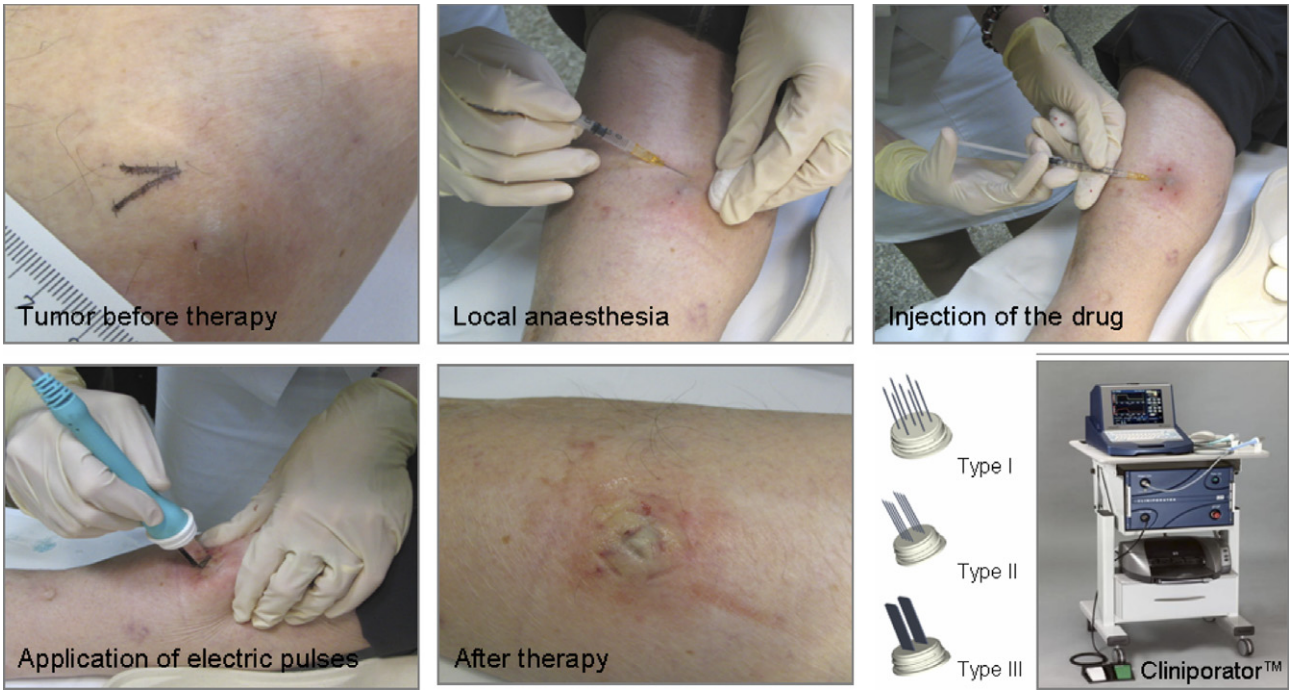


**Fig. 1 – Electrochemotherapy procedure for local drug administration (in the case of intravenous drug administration, electric pulses are delivered to the tumour 8–28 min later). After anaesthesia tumour nodule is injected with cisplatin or bleomycin. Electric pulses, generated by the Cliniporator™, are delivered to the tumour nodule 1–3 min later by electrodes, plate or needle type. If the tumour nodule is larger than the gap between the electrodes, electric pulses are delivered in multiple applications so that the whole tumour volume is electroporated.**

follows: bleomycin solution, at a concentration of 1000 IU/ml, was injected intratumourally at the dose of 250 IU/cm$^3$ of tumour for tumour nodules bigger than 1 cm$^3$, at the dose of 500 IU/cm$^3$ of tumour for nodules bigger than 0.5 cm$^3$ and smaller than 1 cm$^3$, and at the dose of 1000 IU/cm$^3$ of tumour for nodules smaller than 0.5 cm$^3$. Cisplatin (Cysplatyl, Aventis, France), dissolved at a concentration of 2 mg/ml was given intratumourally only, since previous reports did not prove high efficacy of electrochemotherapy using intravenous injection of cisplatin.[16] The injection volume was again dependent on the size of the tumour nodules. Nodules bigger than 1 cm$^3$ were treated with 0.5 mg/cm$^3$ of tumour, nodules bigger than 0.5 cm$^3$ and smaller than 1 cm$^3$ were treated with 1 mg/cm$^3$ of tumour and nodules smaller than 0.5 cm$^3$ with 2 mg/cm$^3$ of tumour. All these drug doses had neither systemic nor local side-effects.[11,13]

Electric pulses were applied to the tumour nodules in a time window between 8 and 28 min after the intravenous injection of bleomycin,[17] or immediately (within 2 min) after the intratumoural injection of either bleomycin or cisplatin, according to experience in previous clinical studies. In general, electric pulses were applied by plate electrodes (Type I) to superficial tumour nodules and by needle electrodes to deeper seated tumours (subcutaneous nodules of maximum depth 3 cm). However, needle electrodes were also used for the treatment of large superficial (even exophytic) and thick nodules. The needle electrodes were of two types, needle row (Type II) for small tumour nodules and hexagonal centred configuration (Type III) for the large nodules (Fig. 1). Electrical parameters were: for Type I electrodes, eight electric pulses of 1300 V/cm amplitude over distance ratio and 100 μs duration, delivered at either 1 or 5000 Hz repetition frequency; for Type II electrodes, eight electric pulses of 1000 V/cm amplitude over distance ratio and 100 μs duration, delivered at either 1 or 5000 Hz repetition frequency; and for Type III electrodes, 96 electric pulses (eight pulses per pair of needles) of 1000 V/cm amplitude over distance ratio and 100 μs duration, delivered at 5000 Hz repetition frequency. The amplitude was determined as to provide sufficiently high electric field everywhere in the tumour.

Local or general anaesthesia of the patients was performed for alleviation of the pain associated with drug injection and application of electric pulses to the tumours. Local anaesthesia was performed around the area of the treated tumour nodules by injection of lidocaine (2%) with epinephrine (0.5%), taking into account not to exceed the maximal dose of anaesthetic for the patient, specifically in patients with multiple tumour nodules treated. General anaesthesia was performed when tumour nodules were too numerous, too large or too painful to be anesthetised by local anaesthesia, with propofol for general sedation and an opiate like remifentanil for analgesia. Inhaled anaesthetics (halogenated and nitrous oxide) were avoided. Patients breathed a mixture of $O_2$/air with $FiO_2$ limited <40%. The choice of the approach for the general anaesthesia was up to each participating cancer centre.

The treatment was performed either on an out-patient basis, or during a one-day hospitalisation of the patient. In one centre (IGR) all patients treated were kept in the hospital for 24 h. The time from the beginning to the end of the treatment was short, median time 25 min (range 6–60 min). Following electrochemotherapy the patients treated on an out-basis were released from the hospital within an hour in the case of local anaesthesia or after, e.g. 3 h in the case of general anaesthesia.

### 2.3. *Patient monitoring*

During the pre-treatment period the following evaluations and procedures were performed: medical history of chronic, non-malignant and malignant disease, physical examination, baseline laboratory tests, ECG and imaging studies. Following the treatment patients were regularly physically examined. Before and after the treatment tumour nodules were measured with calliper in two perpendicular diameters, and photo documented. The tumour volume was calculated by the formula $V = ab^2\pi/6$ ('$a$' was the larger diameter of the tumour nodule and '$b$' the diameter of the tumour nodule perpendicular to '$a$'), and used for drug dosage calculation when bleomycin or cisplatin were injected intratumourally. For evaluation of treatment response the tumour size was calculated by the formula $A = ab$, in accordance with WHO response criteria.[18] All patient data and parameters of the treatment procedure were stored in a centralised electronic database storing the electronic Case Record Forms (CRF).[19] The electronic CRF included measurements and photographs of the treated tumour nodules before and after the treatment, data on quality of life and economics, and report on side-effects such as pain level during and after the procedure (evaluated by means of visual analogue scale, VAS). Muscle contractions at the time of pulse delivery were recorded in the CRF. All adverse events were reported immediately.

### 2.4. *Response criteria and data analysis*

During the first month after the treatment, the patients were seen at two weeks intervals, and thereafter monthly. Antitumour efficacy was evaluated based on criteria of WHO Handbook for Reporting Results of Cancer Treatment[18]; complete response (CR) was determined when the tumour nodule was not palpable; partial response (PR) was defined as a decrease of more than 50% in the products of the largest perpendicular diameters of the measurable lesions. A <50% reduction and up to 25% in the increase in the above measurements was defined as no change (NC). For all response definitions minimum 4-week duration was required for qualifying for each type of response. Progressive disease (PD) was defined by an increase of more than 25%. In cases where it was not possible to measure tumour nodule because nodules were ulcerated or covered with a crust, they were rated as non-evaluable. Response of the treated nodules to the treatment of each participating centre underwent an internal review by the other three centers and had to be confirmed by at least two out of three centers.

### 2.5. *Statistics*

Statistical analysis was performed using the SPSS 11.0 software (Statistical Packages for Social Sciences, Chicago, USA). The differences in the distribution of objective response of

the nodules in the different analyzed groups were tested by contingency tables and Chi square test. Local tumour control was estimated as a function of time by the Kaplan–Meier product limit method, and the difference between curves was analyzed using log-rank test. Data from patients which were lost to follow up were used as censored data. Statistical significance was tested at the 5% level.

## 3. Results

### 3.1. *Patient population*

Characteristics of the 61 patients treated at the four cancer centers from 31 March 2003 to 20 April 2005 are detailed in Table 1. 61 patients were evaluable for treatment toxicity, and 41 completed clinical response evaluation at day 60 or more, with 171 tumour nodules. For evaluation purposes the metastatic tumour nodules were segregated into malignant melanoma tumour nodules (98 tumour nodules, 57%) and non-melanoma (i.e. carcinoma and sarcoma) tumour nodules (73 tumour nodules, 43%). The median observation time of the treated nodules was 133 days with a range from 60 to 380 days (Table 1).

**Table 1 – Patient's and tumour nodule characteristics**

| Characteristics | All patients | | Patients evaluable for clinical response | |
|---|---|---|---|---|
| | Patients (*N* = 61) | Nodules (*N* = 290) | Patients (*N* = 41) | Nodules[a] (*N* = 171) |
| *Age, years* | | | | |
| Range | 22–91 | | 37–91 | |
| Median | 66 | | 66 | |
| *Sex* | | | | |
| Male | 20 | | 11 | |
| Female | 41 | | 30 | |
| *Years from diagnosis* | | | | |
| Range | 0–27 | | 0–27 | |
| Median | 4.0 | | 5.0 | |
| *Patients treated at:* | | | | |
| IGR | 16 | 92 | 11 | 52 |
| OI | 24 | 85 | 16 | 64 |
| CCRC | 13 | 69 | 10 | 37 |
| HH | 8 | 44 | 4 | 18 |
| *Cancer type* | | | | |
| Malignant melanoma | 32 | 190 | 20 | 98 |
| Carcinoma | 27 | 91 | 19 | 64 |
| Sarcoma | 2 | 9 | 2 | 9 |
| *Location of metastasis* | | | | |
| Head and neck | 9 | 23 | 5 | 13 |
| Trunk | 27 | 130 | 19 | 81 |
| Limbs | 25 | 137 | 17 | 77 |
| *Performance status* | | | | |
| 0 | 35 | | 24 | |
| 1 | 14 | | 11 | |
| 2 | 12 | | 6 | |

a 85 of them in pre-irradiated areas.

## 4. Treatment response

### 4.1. *Overall treatment response*

Overall treatment results on 41 patients that completed evaluation of the response demonstrated good antitumour effectiveness. After electrochemotherapy treatment, a response was achieved in 145 of the treated nodules (84.8%) at the end of follow up of each patient, with a few PR (11.1%), CR being the prevalent response (73.7%). Negative response was observed in low percentage of the treated nodules (15.2%), being either NC (10.5%) or PD (4.7%) (Fig. 2).

The ESOPE protocol was designed in a way to evaluate treatment response for more than 60 days after the treatment. Due to this limitation some patients (13 patients with 67 nodules) were excluded from the study because of a follow up shorter than 60 days. Antitumour effects of the electrochemotherapy were evident in 11 of these nodules (5CR and 6PR), 26 could not be evaluated (NA) because of formation of a crust over the treated nodules and 30 did not respond (16NC and 14PD) within the short follow up. If these patients would also be included in the evaluation of the overall treatment response, including the NA as a negative treatment response, then OR rate would still be 65.5%.

Electrochemotherapy was performed with bleomycin injected intravenously or intratumourally and with cisplatin injected intratumourally. According to the drug used, at 150 days after the treatment, local tumour control probability (CR) for electrochemotherapy was 88.2% ($n = 86$) with bleomycin given intravenously, 73.1% ($n = 41$) with bleomycin given intratumourally, and 75.4% ($n = 44$) with cisplatin given intratumourally (Fig. 3). No statistical difference was observed between the three approaches ($p = 0.09$).

In addition, results of OR rate of the tumours showed no statistical difference between OR rate after systemic bleomycin administration (89.5%) and local administration of either bleomycin or cisplatin (80.0%, $p = 0.08$). Furthermore, no difference in responsiveness (OR) to electrochemotherapy between bleomycin and cisplatin when injected intratumourally was observed (80.5% and 79.5%, respectively; $p = 0.91$) (Fig. 3).

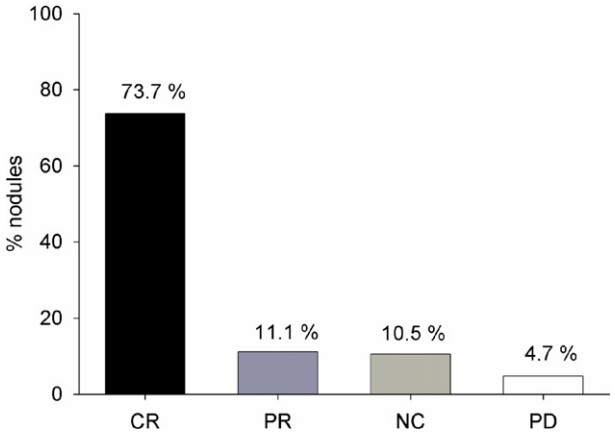


**Fig. 2 – Therapeutic efficacy of electrochemotherapy on cutaneous and subcutaneous tumour nodules of different histology.**

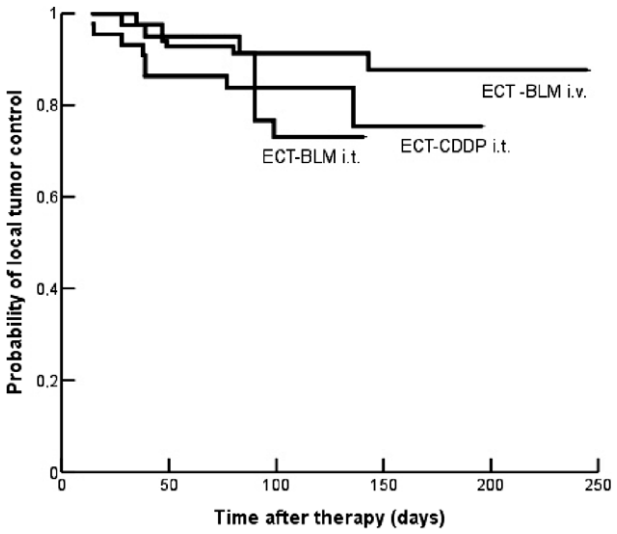


**Fig. 3 – Local tumour control curves for electrochemotherapy with bleomycin given intravenously (ECT–BLM i.v.; 86 nodules) or intratumourally (ECT–BLM i.t.; 41 nodules), and cisplatin given intratumourally (ECT–CDDP i.t.; 44 nodules). Local tumour control rate was estimated using the Kaplan–Meier product limit method, and the difference between the curves was analyzed by means of a log-rank test.**

### 4.2. *Treatment response according to the tumour type*

In the study, malignant melanoma nodules ($n = 98$) and non-melanoma tumour nodules ($n = 73$) were treated. Among the latter there were breast cancer ($n = 58$), colon cancer ($n = 1$), squamous cell carcinoma of the skin ($n = 3$), squamous cell carcinoma of cervix ($n = 2$) and Kaposi and leiomyosarcoma ($n = 9$) tumour nodules (Figs. 4–6). Although not significant ($p = 0.07$) there was a trend toward higher antitumour activity in non-melanoma nodules (OR 90.4% versus 80.6%) supported also by a higher CR rate (83.6% versus 66.3%, $p = 0.018$) (Figs. 4–6).

### 4.3. *Treatment response according to the tumour size and location*

Electrochemotherapy treated tumour nodules, regardless of the drug and route of administration used, were segregated into three categories according to their size (small <0.1 $cm^3$, medium >0.1 and <0.5 $cm^3$, and large >0.5 $cm^3$) in order to evaluate whether the size of the treated nodules affects treatment outcome. No statistical difference between the treatment responses to electrochemotherapy according to the tumour size was found ($p = 0.59$). OR rate was 81.0% on small tumour nodules, 87.7% on medium, and 85.7% on large tumour nodules, indicating that OR rate is independent of the size of the treated nodules. However, when considering the administration route, a statistically significant difference was found between systemic and local drug administration, namely systemic injection of bleomycin (32 nodules, 93.8% OR) resulted in better antitumour effectiveness than the local injection of cisplatin or bleomycin (24 nodules, 75% OR) if the nodules where bigger than 0.5 $cm^3$ ($p = 0.047$).

Tumour nodules were located on different parts of patient's body: head and neck region, trunk or limbs. Electrochemotherapy was the most effective in tumour nodules located on the trunk (OR = 92.6%) while on limbs OR rate was 79.2% and in head and neck region 69.2%. The difference in OR rate according to the tumour nodules' location was statistically significant ($p = 0.01$). Moreover, on the limb nodules, a significant ($p = 0.006$) difference was found in favour of electrochemotherapy efficacy after systemic versus local drug injection.

It has to be stressed that 49.7% of the treated nodules were in previously irradiated areas: the OR rate of these nodules

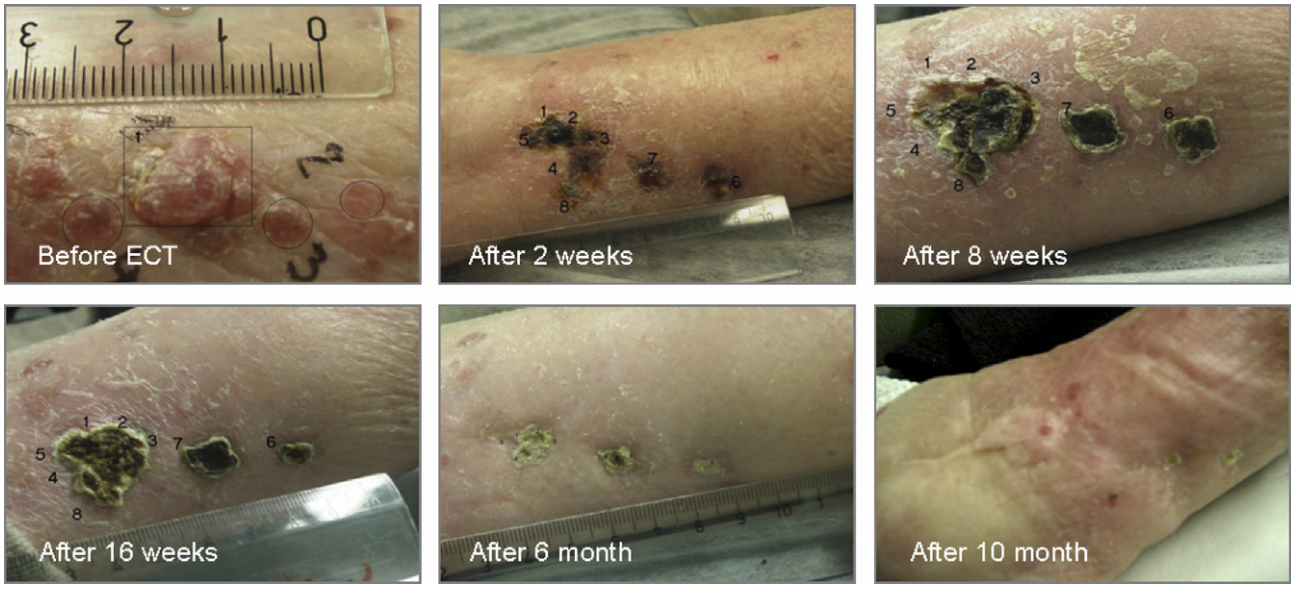


**Fig. 4 – Response of multiple cutaneous in transit metastases of malignant melanoma. Tumour nodules were treated by electrochemotherapy with intratumoural drug injection of cisplatin in local anaesthesia. Electric pulses were applied using plate electrodes. Two weeks after the treatment the scab over the treatment area was visible, which thereafter fell off, displaying good antitumor effect with minimal scaring of the treated area.**

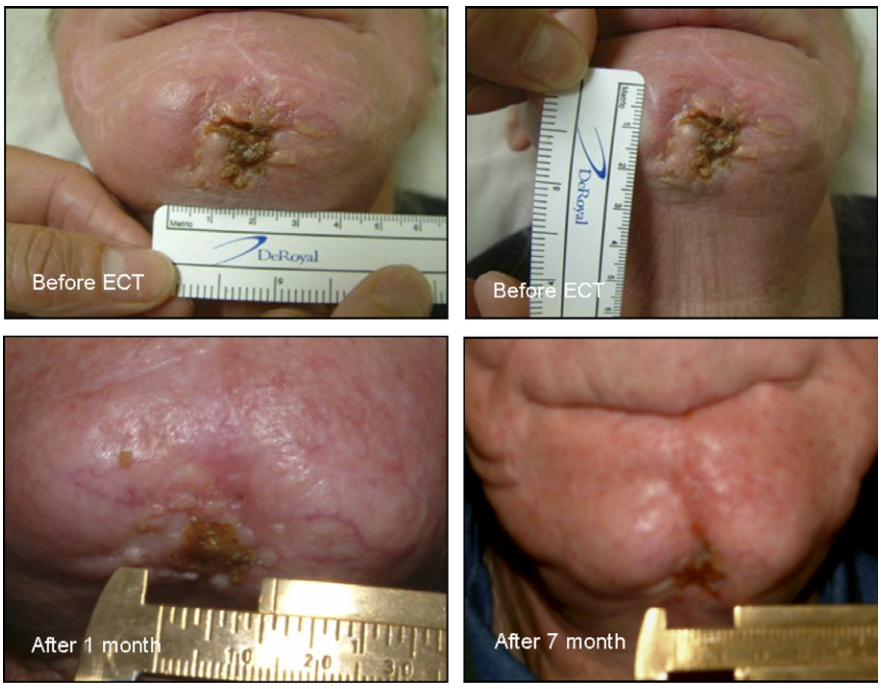


**Fig. 5 – Electrochemotherapy of a local recurrence of squamous cell carcinoma. The upper images display a local recurrence of squamous cell carcinoma of the lower lip, the primary was treated with surgical excision and flap reconstruction. The recurrence was resistant to systemic chemotherapy and local radiotherapy. The patient underwent two treatments with electrochemotherapy, 6 months apart. The lower left image was taken 1 month after the initial treatment and the lower right image 1 month post the second. There is no evidence of tumour present in the remaining chronic ulcer.**

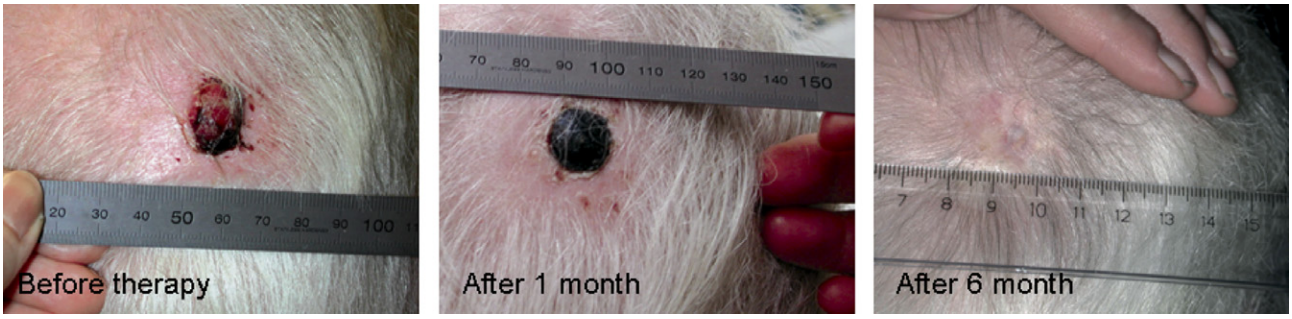


**Fig. 6 – Course of treatment for patient with malignant melanoma metastases, treated with intravenous bleomycin and type III (hexagonal) electrodes under general anaesthesia. One of eight treated metastases is shown. Before treatment the metastasis was ulcerated and caused haemorrhage, pain and discomfort. One month after treatment the lesion was covered by a crust. Around the crust, needle marks in normal tissue are visible, because the tumour was covered including a margin of normal tissue. Whereas the tumour area became necrotic, the normal tissue was very little affected, indicating a therapeutic window. Six months after treatment the treated nodule was in CR. The crust fell off after 10 weeks, revealing normal skin that had healed underneath the nodule (from Gehl, Ugeskrift for Laeger, 2005, with permission).**

(OR = 88.2%) was the same as the OR rate of the nodules that were not in previously irradiated areas (OR = 81.4%).

### 4.4. *Treatment response per patient*

In order to evaluate treatment effectiveness in individual patients, the percentage of patients with all treated nodules in OR rate was calculated. We also calculated the number of patients with at least 75% and with at least 50% of nodules in OR. One third of the patients (34%) had single nodules, whereas the rest had two to more than five tumour nodules. Regardless whether they had treatment of single or multiple nodules, all considered nodules were in OR in 26 patients (63.4%), in three other patients 75% of nodules were in OR, and in another two patients 50% of nodules were in OR.

### 4.5. *Treatment response according to the cancer centre*

Overall treatment response rate to electrochemotherapy was evaluated with respect to centers where recruitment and

treatment was performed. No statistical difference in response to treatment was observed between the four cancer centers ($p = 0.09$). The lowest OR rate observed was 77.8% and the highest 94.2%, indicating that in all centers the treatment was performed with similar positive treatment outcome.

### 4.6. *Tumour response according to the type of electrodes and electrical parameters used for treatment*

Electrochemotherapy was performed with three types of electrodes; type I plate electrodes for superficial tumour nodules and type II and III needle electrodes for deeper seated tumour nodules. Statistical analysis of the treatment response according to the type of electrodes used for treatment did not show differences in responsiveness of tumour nodules to electrochemotherapy ($p = 0.18$). OR rate was 88.2% with plate electrodes, 72.0% with needle electrodes type II and 88.6% with hexagonal needle electrodes type III. The electric pulses generator Cliniporator™ has two options: electric pulses can be delivered at a repetition frequency of either 1 or 5000 Hz. Evaluating the treatment response from all three types of electrodes together, the 5000 Hz repetition frequency of the applied electric pulses resulted in statistically significantly better ($p = 0.05$) antitumour effect than the 1 Hz repetition frequency (87.2% and 73.3% of OR, respectively). Since the hexagonal type III needle electrodes can be used only with the 5000 Hz electric pulse frequency, while type I and type II electrodes operate with both frequencies, further analysis was made to compare the treatment effectiveness of type I and type II electrodes only, using either 1 or 5000 Hz. Even though almost the same difference was found (85.4% of OR at 5000 Hz versus 73.3% of OR at 1 Hz), the difference was not statistically significant ($p = 0.11$), because 126 nodules were treated using electrodes of type I or II.

Analysis of electric current delivered to the tumours during application of electric pulses demonstrated that significantly ($p = 0.01$) higher OR rate (100%) was achieved for hexagonal needle electrodes (type III) when electric current exceeded the value of 1.5 A, compared to 73.3% OR rate with currents below 1.5 A. With plate electrodes the current never fell below 2 A and no association between current level and nodule response was observed.

### 4.7. *Toxicity evaluation*

The analysis of toxicity was performed on 61 patients that were eligible according to the inclusion criteria and were included and treated in the study.

Due to the low dosages of the chemotherapeutics used, limited side-effects related to bleomycin or cisplatin treatment were recorded during the study.

Local or general anaesthesia was used for alleviation of the symptoms associated with application of the electric pulses. In the first few patients (7 pts, 12% cases), EMLA cream (lidocaine 2.5% and prilocaine 2.5%, AstraZeneca) was used for local anaesthesia, but was shown not to be fully efficacious and was not used thereafter. In the rest of the patients local anaesthesia (30 pts, 49% cases) by injection of lidocaine with epinephrine and general anaesthesia (24 pts, 39% cases) were used.

Pain level was evaluated by visual analogue scale (VAS), where 0 was no pain and 100 mm the worst imaginable pain. In patients treated under local anaesthesia patient's pain level was evaluated immediately after the treatment and 2 days later. After general anaesthesia, patient's pain level was evaluated only 2 days after the treatment. In patients with local anaesthesia median level of pain was 35 (range 0–100) immediately after the treatment. After 2 days there was very little pain left, and VAS dropped to a median level of 20 (range 0–70) in patients who had local anaesthesia and to a median level of 10 (range 0–45) in patients who had general anaesthesia, indicating that patients treated with general anaesthesia reported significantly lower VAS than the patients treated in local anaesthesia ($p = 0.05$). The pain was limited predominantly to the treated tumour and surrounding tissue. According to these data the control of the pain level during the electrochemotherapy was good and acceptable for the patients.

Muscle contractions associated with application of electric pulses were evaluated by the treating physician on four levels and in more than 78% of patients ($n = 48$) no or low level muscle contractions were observed. The least muscle contraction was observed when hexagonal centred electrodes (Type III) were used and the strongest muscle contractions were observed with plate electrodes (Type I).

Probably the best indicator that electrochemotherapy is not too stressful or painful procedure is that among the interviewed patients the majority (57 pts, 93%) of them, would be willing to accept the treatment next time if it would be indicated.

In the study five serious adverse events, in four patients, were declared, but none was found not to be associated with the electrochemotherapy. Three of the adverse events were the death of the patient because of pulmonary metastases or disease progression unrelated to the electrochemotherapy. The two other serious adverse events, hypoxia and thoracic pain, on the same patient, spontaneously disappeared in one hour and were unrelated to the treatment.

## 5. Discussion

The ESOPE study demonstrates that electrochemotherapy is an easy, highly effective and safe treatment approach for cutaneous and subcutaneous tumour nodules of various malignancies. It confirmed efficacy of electrochemotherapy using bleomycin or cisplatin, in a multicenter non-randomised trial based on procedures prepared by European leading cancer centres in electrochemotherapy. Electrochemotherapy can provide immediate clinical benefit in patients with advanced cutaneous and subcutaneous metastases.

Until now clinical studies on electrochemotherapy were conducted according to the protocols of individual groups. A lack of homogeneity was apparent between those protocols predominantly in the use of electric pulse generators and electrodes, in the methods for pain control, and in drug dosage and route of drug administration. The harmonisation of the procedures for electrochemotherapy enabled homogenous data analysis in this study.

Several clinical studies on electrochemotherapy were published, demonstrating efficacy of electrochemotherapy in local tumour control of various malignancies. The treatment response of previous studies is comparable to the overall treatment response of this study. OR rate in previously published studies was 85% for bleomycin and 77% for cisplatin (reported in this issue by G. Sersa), which is the same level of local tumour control as in this study, where we obtained an overall OR rate 85%. Specifically, OR rate of electrochemotherapy with systemically administered bleomycin was 89.5% whereas by electrochemotherapy with local cisplatin or bleomycin administration 80% OR rate was obtained. No difference in OR rate between electrochemotherapy with cisplatin or bleomycin local administration was observed. The effectiveness of electrochemotherapy in local tumour control is still very good (65.5%), when, because of short follow-up of some patients, the non-evaluable nodules with crusts are taken into account as being negative treatment outcome.

There was no difference in the success rate among the four different oncological centres participating in the study; demonstrating that knowledge and practice of the treatment can be gained in short time considered that the CCRC centre that had no previous experience with electrochemotherapy.

The patients, with progressive and metastatic disease involving cutaneous and subcutaneous sites were treated by electrochemotherapy in palliative intent. The vast majority of the patients had previously received all available standard treatments, and were only thereafter included into this study. Effective local tumour control was achieved also in areas heavily pre-treated either by surgery or radiotherapy. For example in this study 85 tumour nodules out of 171 nodules (49.7%) were in previously irradiated areas. Indeed, local recurrence in previously irradiated skin is considerable challenge in the management of, e.g. melanoma, head- and neck cancer and breast cancer. Electrochemotherapy was very effective and is a valuable supplement to the treatment options available to these patients.

Furthermore, it can be also emphasised that electrochemotherapy is very acceptable for elderly patients, where surgery is contraindicated. In our study 16 pts over 75 years, comprising 39% of all treated patients, were treated with significant benefit to them, without side-effects.

Electrochemotherapy has been reported to be effective on all histological types of tumours.[10,11] In this study melanoma and non-melanoma tumours were equally responsive to electrochemotherapy ($p = 0.07$). Its efficacy is based on its physico-chemical approach: (a) drug delivery system by electric pulse application, useful in the electropermeabilisation of all kind of cells, and (b) use of drugs that have low or no membrane permeability, that are highly cytotoxic once in the cell and that exert their cytotoxic effects through a direct interaction with the cell's DNA. In fact, when therapy is conducted properly, having sufficient amount of the drug within the tumour, and having electroporated the whole tumour mass, electrochemotherapy is effective independently of histological types of tumours. Furthermore, it was demonstrated that in the majority of patients (75.6%) at least 50% of the treated nodules were in OR, and 63.4% of the patients treated by electrochemotherapy achieved meaningful local tumour control of all treated nodules.

In this study, electrochemotherapy with bleomycin given systemically or locally and electrochemotherapy with cisplatin given locally were compared. The cumulative results clearly demonstrated that all three treatment approaches were effective, resulting in 88.2%, 73.1% and 75.4% tumour control probability (CR), respectively, without statistically significant difference in their antitumour effectiveness. Electrochemotherapy with bleomycin given intravenously shows the tendency to be the most effective with higher CR rate over local administration of either bleomycin or cisplatin. Specifically, for the tumour nodules smaller than 0.5 $cm^3$ electrochemotherapy with bleomycin given systemically or locally were equally effective. Furthermore, in these small tumours, electrochemotherapy with bleomycin given intratumorally was equally effective compared to previously published data on electrochemotherapy with intratumoural administration of bleomycin, although the doses of bleomycin used in this study were lower compared to previously published data.[20] For larger tumours (more than 0.5 $cm^3$) electrochemotherapy with bleomycin given intratumourally was less effective than electrochemotherapy with bleomycin given intravenously as well as than electrochemotherapy with bleomycin given intratumourally reported in other studies.[20] This could be explained by the lower dose of bleomycin per $cm^3$ of tumour. Nevertheless in the Authors' opinion the higher efficacy of electrochemotherapy with intravenous injection of bleomycin for large tumours could be explained by the more uniform distribution of the drug inside the tumour. Moreover, the intravenous route is technically more convenient for the treatment of multiple tumour nodules. Intravenous drug injection is recommended also in tumour nodules with hard consistency where intratumoural drug injection would be difficult and in large nodule treatment where it was significantly more efficient. Whereas intratumoural drug injection, either of bleomycin or cisplatin would be more convenient for less perfused tumour nodules, predominantly in heavily pre-treated areas, like skin flaps and pre-irradiated areas. Equal tumour responsiveness to electrochemotherapy was found either with bleomycin or cisplatin both given intratumourally. The choice of the drug and its route of administration should be based on the tumour size, and the number of tumour nodules to be treated and is described in details in the SOP (reported in this issue by L.M. Mir et al.).

The advantage of electrochemotherapy is also that it is easy and quick to perform (median time of treatment ~25 min). It has to be emphasised that the good antitumour effects reported in this study were obtained with only a single treatment. The treated tumour nodules are well controlled, and after complete regression of the treated nodules good cosmetic effect is obtained. After the treatment, no specific dressing of the treated area is needed, and the first regular check up of the treatment is required one or two months after the treatment. However, if the tumours recur after the treatment, re-treatment is possible with equal treatment effectiveness, as reported in previous studies.[12,13] For example, re-treatment can be needed if during the first treatment the electric field was not covering the whole tumour or if the tumour was large or too thick.

Electric pulses for electroporation of the tissue are delivered by different sets of electrodes. In this study plate and

needle electrodes were used. Plate electrodes (type I)[17] and row needle (type II)[21] electrodes were already used in other studies while hexagonal centred electrodes (type III)[22] were used here for the first time in humans. The plate and row needle electrodes were limited to the treatment of superficial and small tumour nodules (<2 cm diameter), whereas hexagonal centred electrodes allowed to treat the bigger (2–3 cm diameter), thicker and deeper seated tumour nodules. No difference was obtained in OR rate of the treated tumour nodules with respect to the electrodes used. The data were analyzed according to the size of the tumour nodule treated, and demonstrate that there is no difference in treatment response according to the size of the tumour nodule. The only difference was that in tumours bigger than 0.5 $cm^3$ systemic bleomycin administration was more efficient than local drug administration, probably because of better diffusion of the drug in the tumour.

Electric pulses were generated by electric pulse generator Cliniporator™ with on line recording and storage of the electric pulses parameters delivered to the tumour. The analysis of the electrical parameters demonstrated that for efficient electroporation of tumour nodules electric current that has to be delivered by hexagonal electrodes has to exceed 1.5 A. In the case that the current was below this level, the treatment was less effective. Since the amplitude of the current mostly depends on the depth of needle electrodes inserted into the tissue, this observation suggests that the loss of efficacy could be due to a too low penetration into the tissue. As the device allows immediate feed back information about the electric current that was delivered to the treated area, repositioning or deeper insertion of the electrodes can be done and re-treatment with the same or other type of electrodes can be performed if measured current is too low. This information should result in more effective treatment and likely in higher OR rate.

For the first time electric pulse delivery at 5000 Hz was used in this study. We demonstrate that 1 and 5000 Hz frequencies have equal antitumour effect in electrochemotherapy and that there is no difference in the level of pain. Using a high frequency pulse delivery has several advantages. Since each treatment consists of a sequence of 8 electric pulses, and each electric pulse induces muscle contraction, increase in frequency of electric pulses reduces eight muscle contractions to one.[23,24] The use of 5000 Hz frequency has also the advantages in shortening the treatment time, especially in the treatment of multiple nodules, and in the case of the type I electrodes, in preventing electrode displacement during the treatment. Indeed, since muscle contraction occurs after the delivery of the electric pulses, consecutive muscle contractions that occur at 1 Hz frequency can influence type I electrode positioning. Finally, hexagonal electrodes operate only at 5000 Hz, so that the treatment of large tumour area is completed in less than 1 s, instead of 1 min 35 s that would be necessary at 1 Hz.

One of the goals of the study was to define the procedure to control the pain that the patient experiences during pulse delivery. Three different procedures have been tested. The use of EMLA cream proved to be insufficient and was abandoned after a few patients. Local anaesthesia by injection of lidocaine with epinephrine was effective; however care is needed that the whole area surrounding the tumour is well injected. General anaesthesia is to be preferred when very large or multiple nodules need to be treated. All types of electrodes can be used with either local or general anaesthesia. All treated patients were asked whether they would accept treatment with electrochemotherapy again, if needed. The vast majority of the patients (93%) were willing to accept the treatment again if it would be offered, which clearly indicates that electrochemotherapy is well tolerated and acceptable by the treated patients.

Local tumour growth of single or multiple nodules of progressive diseases is currently approached by local tumour surgery, isolated limb perfusion, isolated limb infusion or irradiation.[25–27] We used electrochemotherapy after failure of those local treatments. Taking into consideration the high CR rate (73.7%) after single electrochemotherapy session, its safety and relative simplicity of application, one might consider it to be a valid and valuable option in the treatment of metastatic cutaneous and subcutaneous nodules. Advantages of electrochemotherapy over isolated limb perfusion and over isolated limb infusion are its relative simplicity, the short duration of the treatment, the contained cost and the immediate return to daily life by the patients. Furthermore electrochemotherapy can be repeated on regrowing tumour nodules or newly emerging tumour nodules, with equal antitumor effectiveness in each treatment session.[12,13] On the other hand isolated limb perfusion can also treat clinically undetectable disease.[25,26]

Finally, several studies suggest other indications where electrochemotherapy can be used as a first line treatment, such as organ sparing effect of electrochemotherapy in the treatment of non-operable primary[28] or recurrent melanoma,[29] for alleviation of pain or reduction of tumour bleeding,[30] as well as a neoadjuvant treatment before conventional treatment.[28] Based on these treatment indications electrochemotherapy brings clinical benefit in various subsets of patients with advanced cancer. Further developments may broaden electrochemotherapy indications to treat less advanced diseases. These developments also include the combination of electrochemotherapy with other cytotoxic or biological treatments or technical development of the electrodes used for the electric pulse application.

The cost of the treatment is acceptable and the technology required relatively inexpensive, which makes it ideal in terms of budget constraints by all health systems, and specifically for small hospitals, or developing countries. No specific skill is required, one day training at an experienced centre is sufficient to let the physician feel confident with the treatment.

Electrochemotherapy is simple, highly effective and safe. Tumour treatment is performed in single session, for localised disease, as palliative treatment of tumour nodules of various histology. Because of short duration of the treatment and lack of side-effects, it can be performed on an out-patient basis.

Finally this study lead to the validation of standard operating procedures that are the necessary prerequisite for physician training, dissemination of the technology and its use in daily clinical practice.

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