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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

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1. Aim of the document

The aim of this document is to define the standard operating procedures (SOP) in order to safely and conveniently treat, by electrochemotherapy, patients with cutaneous and subcutaneous nodules. To this end this document provides the reader with the basis for understanding the mechanisms of the electrochemotherapy as well as its possibilities as antitumour treatment. It also has a decision chart to help the physician in choosing among the different treatment modalities reported in this SOP.

1.1. *Definition of ECT*

Electrochemotherapy is a new tumor ablation modality providing delivery into cell interiors of non-permeant drugs with intracellular targets. It is based on the local application of short and intense electric pulses that transiently permeabilize cells in tissues. To date, its main application has been the treatment of tumour nodules when the electric pulses are associated with non-permeant drugs having high intrinsic cytotoxicity. The most convenient drug is bleomycin, a currently used anticancer drug, but cytotoxicity of cisplatin is also increased in vivo by means of this original drug delivery approach.

It is important to note that the physico-chemical basis of this treatment allow to predict that it works on all the tumours types. Both the preclinical and clinical results published until now clearly support this assessment. It actually brings a new world of indications for the two drugs that until now have been proved efficient under this approach, the cisplatin and the bleomycin. The data already collected around the world have demonstrated the effectiveness of this technique which overcomes the ineffectiveness of classical chemotherapy and often allows avoidance of surgery, for example in previously irradiated areas. Moreover, it has repeatedly shown that hemorrhagic nodules stop bleeding immediately after the treatment, and the pain of painful lesions is also greatly reduced. The consequences of this treatment are simple, and taking into account the economic issues, the cost of this method is really low. This therapy should therefore be offered to the patients to improve their quality of life independently of life expectancy, to heal painful or bleeding lesions, as well as to improve the patient's cosmesis and associated social interactions.

Although ECT following the SOP here described can be performed with any electroporation system approved for clinical use and delivering 8 pulses of 100 μs at appropriate voltages, and with IT bleomycin doses different from those here recommended, we recommend that electrochemotherapy is performed with the newly developed Cliniporator™ machine that is CE certified for use on patients. This electric pulses generator has several advantages that make it a leading product on the market in this area. It generates square wave electric pulses with variable amplitude of electric pulses, and possesses two options for the frequency of the delivered electric pulses (1 or 5000 Hz). The device is computer controlled. There are several levels of the control: on the level of the machine manipulation as well as on the level of the electrical parameters that can be delivered. In addition it provides storage of the patient's characteristics as well as of the electrical parameters used for the treatment including traces of the voltage actually applied as well as the current delivered during the treatment. Moreover, the Cliniporator™ is the only device that offers the control of the pulses delivered on a screen, just after the delivery of the pulses. The operator can then receive a visual confirmation of the quality of the delivered pulses. In the case of an inadequate positioning of the electrodes, a trained user will detect the potential failure of the treatment and may repeat it immediately, without supplementary constraints for the patient (the electric pulses can be delivered again taking advantage of both the anaesthesia already operated and the dose of chemotherapeutic agent already injected). The Cliniporator™, CE marked, is produced by IGEA, an Italian spin-off company of the University of Modena that placed its experience at the disposal of the Cliniporator and ESOPE European consortia for the development, elaboration and distribution of Cliniporator™ to the medical community.

2. Principle of electrochemotherapy

Electrochemotherapy combines administration of non-permeant or poorly permeant chemotherapeutic drugs with application of electric pulses to the tumours in order to facilitate the drug delivery into the cells. Thus, enhanced drug delivery can substantially potentiate chemotherapeutic drug effectiveness, locally at the site of cell electropermeabilization by electric pulses, without affecting the tissues unexposed to electric pulses.

Based on numerous preclinical studies on electrochemotherapy using either bleomycin or cisplatin, the first clinical study on electrochemotherapy with bleomycin was performed in 1991 by Mir et al.[1] demonstrating good antitumour effectiveness on cutaneous metastases of head and neck carcinoma patients. After that initial study, several clinical studies on electrochemotherapy using bleomycin and cisplatin administered locally or systemically were initiated. Cutaneous metastases of different tumours were treated, such as head and neck squamous cell carcinoma, malignant melanoma, basal cell carcinoma, adenocarcinoma of the breast and salivary gland, hypernephroma, Kaposi sarcoma and transitional cell carcinoma of the bladder (reviewed in Gothelf et al.[2] and in Sersa et al.[3]). All together 1009 nodules in 247 cancer patients have been treated. Overall results of these studies show that electrochemotherapy is an effective treatment; objective responses were obtained in 48–100% of the treated nodules. Better response was obtained on smaller tumour nodules where the whole tumour mass could be adequately electroporated, than in bigger and thicker tumour nodules, where optimal tumour electroporation is more difficult to obtain. In some cases the treatment had to be repeated in consecutive sessions.

Electrochemotherapy is a new approach for the treatment of accessible tumour nodules, either cutaneous (this SOP) or

[1] Mir LM, Belehradek M, Domenge C, Orlowski S, Poddevin B, Belehradek J, Schwaab G, Luboinski B, Paoletti C, 1991. Electrochemotherapy, a novel antitumour treatment: first clinical trial. C R Acad Sci Paris 313:613–8.

[2] Gothelf A, Mir LM, Gehl J, 2003. Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. Cancer Treat Revs 29:371–87.

[3] Sersa G, Cemazar M, Rudolf Z, 2003. Electrochemotherapy: advantages and drawbacks in treatment of cancer patients. Cancer Ther 1:133–42.

accessible by fibroscopy, endoscopy, laparoscopy or open surgery (SOP and equipment still under development). Currently, treated lesions can be cutaneous or subcutaneous tumour nodules of any histologically proven cancer, where palliative treatment is needed. In some cases electrochemotherapy can also be organ-sparing treatment that can reduce tumour burden and predispose tumours for further treatment, like surgery. Tumour nodules must be accessible for application of electric pulses, either for single application or multiple applications in order to cover the whole tumour area for good antitumour effect. The treatment is not recommended for patients with symptomatic or rapidly progressive non-skin metastases, due to reduced expectancy and ethical implications. Furthermore, in patients that have had allergic reaction to bleomycin or cisplatin, electrochemotherapy is contraindicated to the corresponding drug. Electrochemotherapy with bleomycin is also contraindicated in patients where the cumulative dose of 400 000 IU bleomycin/m^2 (or less if recommended by the local regulatory agencies) was previously exceeded, because of possible severe pulmonary fibrosis. Still, because of its advantages (capability to improve the patient's quality of life independently of life expectancy, to heal painful or bleeding lesions, as well as to improve the patient's cosmesis and associated social interactions, to preserve the organ function, and, in case of isolated lesions, to potentially cure the patient), the anticancer centres should be equipped with the device for electrochemotherapy independently of the number of eligible cases, and offer this new technology to the patients.

Electric pulses can be delivered by three different types of electrodes that were developed along with the Cliniporator™ generator. Type I electrodes are plate electrodes with different gap between the plates. They are aimed at treating small and superficial tumour nodules. Needle electrodes are suitable for treatment of thicker and deeper-seated tumour nodules. There are two types of needle electrodes with either two parallel arrays of needles (Type II electrodes) with a 4 mm gap between them for treatment of small nodules, or an hexagonal array of electrodes (Type III electrodes) for bigger (>1 cm in diameter) nodules. This variety of different electrodes was developed in order to encompass the varying cutaneous tumour nodules encountered that may be treated by electrochemotherapy.

Either bleomycin or cisplatin can be used in the treatment. Good antitumour effectiveness has been obtained by either of the drugs. Clinical data obtained so far have proved antitumour effectiveness of bleomycin and cisplatin when given intratumourally, however intravenous injection is recommended for bleomycin only (for large tumours). Since the drug treatment can be performed either intratumourally or intravenously it gives numerous possibilities for the varying treatment modality. Solitary or multiple nodules can be treated, and correspondingly using local or systemic anaesthesia, as described in the various operating modalities of this SOP.

2.1. *Important references of general interest*

- Gothelf, A et al. 2003. Electrochemotherapy: Results of cancer treatment using enhanced delivery of bleomycin by electroporation. *Cancer Treatment Reviews* **29**:371–87.
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- www.cliniporator.com.

3. Patient selection

This part covers the criteria that must be checked during the pre-inclusion visit for the treatment by Electrochemotherapy (ECT) of patients with cutaneous and subcutaneous nodules. It will therefore allow determination of whether the patient will benefit from this treatment.

3.1. *History*

A full history should be taken from the patient and special attention should be paid to the following:

The presence of a pacemaker precludes treatment on the anterior chest wall.
If the patient is taking anticoagulants then check INR (see Haematology).
If any previous difficulties with local anaesthesia (lidocaine) or general anaesthetic this will determine treatment method.
If the patient has sensitivity to bleomycin or a history of pulmonary fibrosis then this will preclude the administration of intravenous bleomycin.

3.2. *ECG*

ECG before treatment depends on the location of the nodules i.e. it is less important if the nodules are on the limbs and more important if on the trunk. It also depends on the patient's pre-morbid condition i.e. cardiac history. It is highly recommended in the case of manifest cardiac arrhythmia or previous cardiac event.

3.3. *Haematology*

a. An INR >1.5 precludes invasive treatment (i.e. needle insertion).
b. If Platelets <70 000/mm^3 then haematological opinion regarding the risk of bleeding versus the benefit of the therapy. The clinician can then make an informed decision.

3.4. *Biochemistry*

If using intravenous bleomycin, creatinine should be <150 μmol/l to ensure adequate renal clearance.

3.5. *Size and number of nodules*

Assess size of largest nodule and number of nodules. These determine the branch of the treatment protocol.

3.6. *Feasibility of local anaesthetic*

This is determined by the clinician (using the depth of the nodule and its proximity to large vessels as well as its mobility with respect to deep tissues. This will ensure that injection and diffusion of the anaesthetic around the tumour will be correct). This determines the branch of the treatment protocol.

3.7. *Determination of body surface area or of total tumour volume*

3.7.1. *Intravenous route*

If intravenous bleomycin administration is the therapeutic option chosen, measure patient height and weight in order to calculate the surface area (NB: in the electrochemotherapy modality treatments, only bleomycin can be administered intravenously). Doses are as follows: Intravenous bleomycin is injected at a dose of 15 000 IU/m^2.

3.7.2. *Intratumoural route*

If administering intratumoural bleomycin or cisplatin, measure the lesions to foresee the volumes and concentrations of these agents that will have to be prepared or ordered at the pharmacy.

Intratumoural bleomycin concentration is 1000 IU/ml and doses are as follows:

Tumour volume ($V = ab^2\pi/6$)	<0.5 cm^3	0.5 $cm^3 \ll 1$ cm^3	>1 cm^3
Bleomycin dose, concentration 1000 IU/ml	1 ml (1000 IU)/cm^3 of tumour	0.5 ml (500 IU)/cm^3 of tumour	0.25 ml (250 IU)/cm^3 of tumour

Cisplatin is applied intratumourally only, concentration used is 2 mg/ml and doses are as follows:

Tumour volume ($V = ab^2\pi/6$)	<0.5 cm^3	0.5 $cm^3 \ll 1$ cm^3	>1 cm^3
Cisplatin dose, concentration 2 mg/ml	1 ml (2 mg)/cm^3 of tumour	0.5 ml (1 mg)/cm^3 of tumour	0.25 ml (0.5 mg)/cm^3 of tumour

In both cases, determine the number of nodules to be treated.

3.8. *In the case of patient inclusion within a clinical trial of ECT (if patient is actually eligible), obtain patient's written consent*

If available, photographic documentation would be useful to document the size and location of the nodules in order to monitor treatment efficacy.

3.9. *Treatment option*

(Go to decision chart, section IV of this SOP).

3.10. *Feasibility of the chosen therapeutic option*

Firstly general rules for use of cisplatin or bleomycin:

Bleomycin is the sole option for intravenous administration route.
Both bleomycin and cisplatin can be used in intra-lesional injections.

3.10.1. *Bleomycin*

If the therapeutic option chosen requires bleomycin, verify:

- Absence of known allergic reaction to bleomycin Yes? No?
- Limit of previous bleomycin delivery of 400 000 IUg/m^2 not reached Yes? No?
- Absence of pulmonary fibrosis of whatever origin Yes? No?
- Creatinine level <150 μmol/l. Yes? No?

Only if all answers are yes can you use bleomycin.
If at least one answer is no, check whether you can replace bleomycin with cisplatin

3.10.2. *Cisplatin*

If the therapeutic option chosen uses cisplatin, verify:

- Absence of known allergic reaction to cisplatin Yes/No?

If answer is yes, check whether you can replace cisplatin by bleomycin.

3.10.3. *Pacemaker*

If the patient has a pacemaker, verify:

- Any nodule located on the anterior chest wall Yes/No?

If Yes:

- If the only nodules of the patient are located in the anterior chest wall, patient is not eligible for treatment by ECT
- If there are nodules located elsewhere, only these nodules will be eligible for treatment by ECT.

3.10.4. *Coagulation abnormalities*

If the patient has coagulation problems, verify if Type II or type III electrode are supposed to be used.

If answer is yes, treatment protocol must be modified to use Type I electrodes and also:

- If the nodules of the patient are all deep (more than 1 cm below the skin) nodules, patient is not eligible for treatment by ECT.
- If there are nodules less deeply located, only these nodules will be eligible for treatment by ECT.

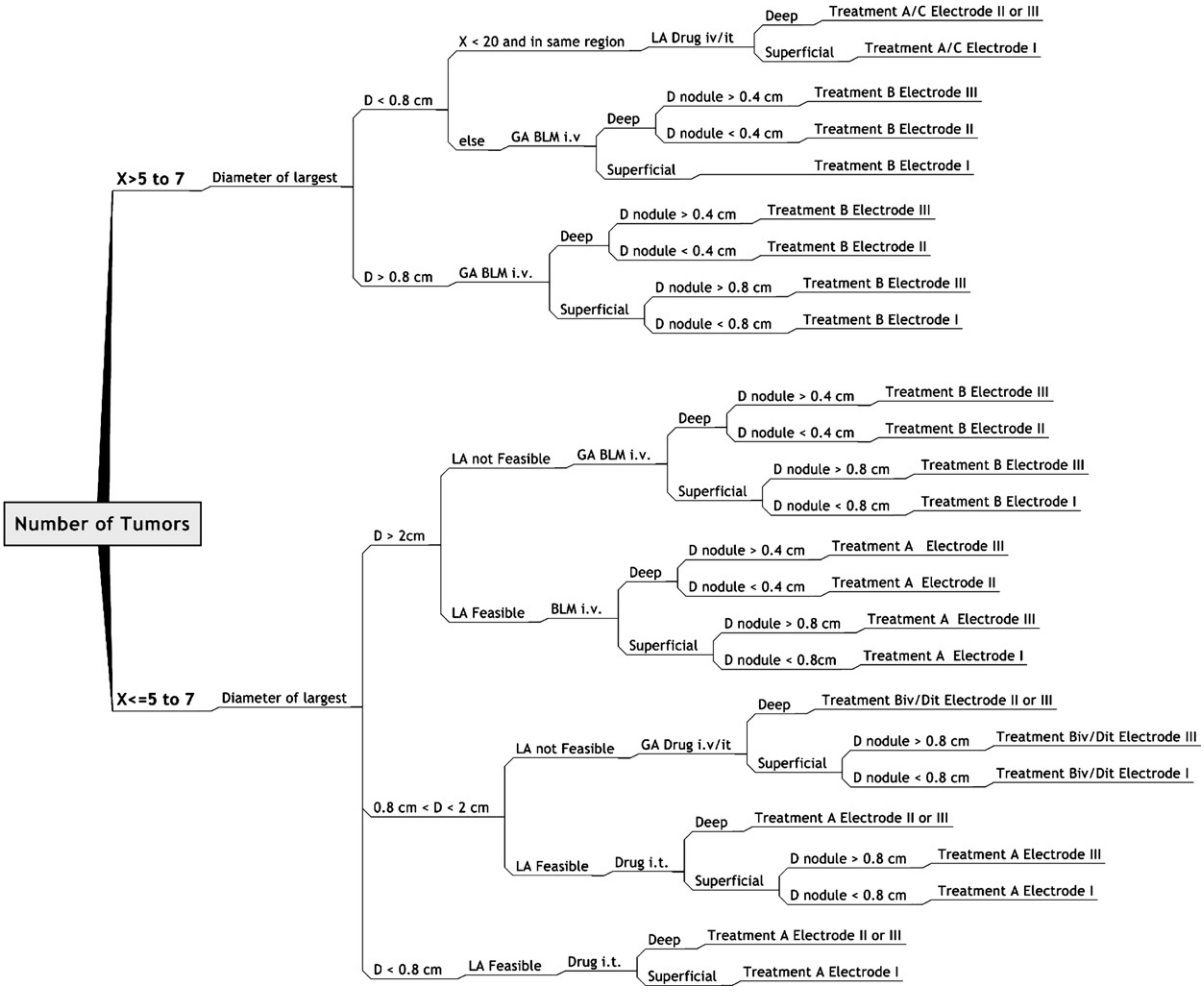
If nodules are superficial, but very thick (thickness greater than 1 cm), one single treatment with Type I electrodes will probably not be sufficient; a second treatment can therefore be foreseen, as well as eventual superficial necrosis in case of repetitive treatments of this (these) nodules.

4. Choice of the treatment modality

This section provides physicians using electrochemotherapy with the information necessary to select drugs (BLM = bleomycin, and Drug = either bleomycin or cisplatin), the delivery routes (it = intratumoural, and iv = intravenous), the type of anaesthesia (LA = local anaesthesia, and GS = general sedation) and the type of electrodes to use. Electrode I, II, III stand respectively for the electrodes of type I, II III described in the section "Principles of Electrochemotherapy". Electrodes are one time use for a particular patient and for a single session: they can be used to treat thus several nodules of the same patient in a given session. It may happen, in the case of the treatment of a large number of nodules or of lesions of very different sizes, that more than one electrode type have to be used for a particular patient in the same session.

For the Cliniporator™ machine, type I electrodes are coded P-xx-yz (where P means plates, xx is the length (in mm) of the plates, y is the distance between the plates and z standing for G or B, respectively for the Green or Blue handle and cord to connect the electrodes to the Cliniporator™), type II electrodes are coded N-xx-yB (where N means needles and B refers to the Blue Handle cord to connect the electrodes to the Cliniporator™), and type III electrodes are coded N-xx-HG (where xx is the length of the needles (18, 20 or 30 mm), H represents the Hexagonal electrodes geometry and G the green handle).

This section consists thus in a decision tree that takes in consideration the number of skin nodules (X), the largest diameter of the largest nodule, the localization of the nodules and their individual diameter (D nod). The various branches of the decision tree result in just four treatment



modalities (A, B, C and D) described in the following section, each of them with 3 options corresponding to the 3 types of electrodes developed for the treatment of cutaneous or subcutaneous nodules.

NB: If it is estimated, that there will be less that 0.5 cm between the tip of the needles and periosteum, consider using general anaesthesia rather than local anaesthesia.

5. Operating modality A: Local anaesthesia – intratumoural chemotherapy

Room: Room with couch
Personnel: Doctor and assistant (e.g. nurse)
Assistance in case of emergency: No extra precautions necessary
Required equipment:

- Pillows, and cover
- Gloves
- Lidocaine 2%, with epinephrine (0.5%)
- Syringe, size 5 ml
- Hypodermic needles, app. 25 G
- Gauze pads
- Ruler for measuring tumour volume
- Drug for injection
- Skin disinfectant pads
- Clock, pen and paper
- Cliniporator and selection of electrodes

5.1. *Anaesthesia*

Lay a rectangular infiltration of local anaesthetic (Lidocaine, 2%, with epinephrine 0.5%) around the area to be treated, by injecting along 4 lines so that the nodule is 'fenced in' by local anaesthetic. Be sure that an even amount of anaesthetic is injected along all 4 sides of the rectangle, so that pain transmission is completely blocked. If more than one nodule is to be treated, it is necessary to consider the total amount of lidocaine: for example, in a 70 kg patient the maximal dose without adrenaline is 210 mg (21 ml of 1% lidocaine or 10.5 ml of 2% lidocaine) and with adrenaline 420 mg (42 ml of 1% or 21 ml of 2%). This will set the maximum of nodules treatable by one session with local anaesthesia, not surpassing 3 mg/kg of lidocaine without adrenaline or 6 mg/kg for lidocaine with adrenaline.

If it is anticipated that there will be less than 0.5 cm from the tip of the electrodes to periost, local anaesthesia should be reconsidered, since it may be difficult to palliate the patient sufficiently. Another potential restriction to local anaesthesia is the localization of the nodules in previously irradiated areas: experience has shown that is seems more difficult to apply local anaesthesia in these previously irradiated areas and if the option "local anaesthesia" is confirmed, it is recommended to put more anaesthetic and allow more time for lidocaine diffusion.

Small cutaneous tumours may be anaesthetized by local infiltration of lidocaine just below the tumour, however, still covering the area of electroporation. This may increase the number of small nodules that can be treated with local anaesthetic in one session.

Premedication with sedatives does not seem necessary.

5.2. *Injection of chemotherapy*

Use the chemotherapy agent chosen at the time of the preinclusion visit, according to the guidelines described in the corresponding modality treatment.

Measure the tumour(s) to be treated, in the longest diameter (a) and the diameter perpendicular to that (b).

From the formula $V = ab^2\pi/6$, calculate the dose to be injected in each nodule, see below.

Intratumoural treatment can be performed either with cisplatin or bleomycin, according to the instructions below.

5.2.1. *Cisplatin*

- Cisplatin (CDDP) (e.g. Platamine, Pharmacia&Upjohn S.p.a., Milan, Italy, or other national suppliers) 10 mg, powder for injection:
- Intratumoural injection
  - dissolve to 2 mg/ml with sterile water
  - protect from direct light
- Injected dose should be 0.25 ml (0.5 mg) per cm^3 of tumour tissue for tumours larger than 1 cm^3, and 0.5 ml (1 mg) per cm^3 of tumour tissue for tumours smaller than 1 cm^3 but larger than 0.5 cm^3. For tumours smaller than 0.5 cm^3 injected dose should be 1 ml (2 mg) per cm^3, as a somewhat larger loss to surrounding tissues would be anticipated (see table below)

Calculated tumour volume ($V = ab^2\pi/6$)	<0.5 cm^3	0.5–1 cm^3	>1 cm^3
Cisplatin dose (concentration: z2 mg/ml)	1 ml/cm^3 tumour tissue	0.5 ml/cm^3 tumour tissue	0.25 ml/cm^3 tumour tissue

5.2.2. *Bleomycin*

- Bleomycin (e.g. Bleomycin, Asta Medica AB, Täby, Sweden or Laboratoires Roger Bellon, France, or other national suppliers). Bleomycin is distributed in vials each containing 15 000 IU of bleomycin, determined by its activity. Previously, the term units has been used, where 15 000 IU = 15 units. Some investigators report bleomycin in mg, and 15 000 IU is approximately equal to 8–9 mg or 15 mg of bleomycin depending on the activity of the drug and on the manufacturer. This may be defined by the institute pharmacy.
- The recommended concentration of injection solution is 1000 IU/ml, dissolve in sterile water.
- Injected dose should be 0.25 ml (250 IU) per cm^3 of tumour tissue for tumours larger than 1 cm^3, and 0.5 ml (500 IU) per cm^3 of tumour tissue for tumours smaller than 1 cm^3 but larger than 0.5 cm^3. For tumours smaller than 0.5 cm^3 injected dose should be 1 ml (1000 IU) per cm^3 (see table below)

Calculated tumour volume ($V = ab^2\pi/6$)	<0.5 cm^3	0.5–1 cm^3	>1 cm^3
Bleomycin dose (concentration: 1000 IU/ml)	1 ml/cm^3 tumour tissue	0.5 ml/cm^3 tumour tissue	0.25 ml/cm^3 tumour tissue

5.3. *Choice of electrodes, pulse parameters, frequency*

Choose the appropriate electrode. If the tumour is less than 1 cm, consider using either plate or parallel array electrodes. In case the tumour is more than 1 cm, consider using the hexagonal array electrodes. Plates or needles depending on the location of the small nodule, respectively superficial or more deeply located.

Enter the electrode code sequence (see description in section IV of this SOP) into the Cliniporator™. Insert electrode in appropriate handle (see colour code, B or G). Verify electrode cap insertion in the handle.

Treating at a frequency of 5 kHz will reduce the number of contractions to one – however this contraction will be more forceful than if a frequency of 1 Hz is used. The use of the 5 kHz frequency is mandatory for the electrodes of type III (hexagonal geometry).

5.4. *Pulsing procedure*

Make a test run with the Cliniporator™ and electrodes.

Please note, that if any of the lesions to be treated are near large vessels, ultrasound guided application of needles may be needed.

Position patient comfortably lying down. Use pillows to support head, leg or arm so that a relaxed position is obtained. Offer blanket in case patient feels cold.

Disinfect the area to be treated. Sterile cover will not be needed.

Inject the drug into the tumour, and note the time. For small tumours, choose to inject either directly into the nodule or to tunnel the hypodermic needle under the skin to the nodule before injection. For larger tumours, several injections are needed. Make a pattern of injections using parallel insertions points. (If bleomycin is used make a calculation of the total amount of drug required for the entire session, if this exceeds or equals 15000 IU/m^2 give this dose intravenously instead.)

Within 10 min of drug injection, electric pulses must be applied. Take the gauze pad in one hand, and the electrode in the other. The gauze pad can enable you to grasp the skin around the lesion to be treated, to lift the lesion up from the underlying musculature. In this way, muscle contractions and associated discomfort can be considerably reduced.

Inform patient when the first pulse is to be delivered.

Several pulse applications may be needed to cover the tumour volume. Overlapping fields should be avoided in normal tissue (including skin overlying subcutaneous tumours), but is acceptable in the tumour.

The electric field drops off very quickly outside the area of the electrodes, so the area to be treated by pulses must be encompassed within the electrodes – if necessary by running more than one application. Therefore, not only must the appropriate electrode be chosen, but the pattern of applications necessary to cover the tumour volume must be decided on as well.

After treatment, a loose dry dressing can be applied, or the lesion can be left without dressing.

Patients can be retreated. Even though bleomycin doses are low, it is recalled that the cumulative bleomycin dose must not exceed 400000 IU/m^2 due to risk of lung fibrosis. Pulmonary function tests should be considered if cumulative dose of bleomycin exceeds 60000 IU/m^2. If the diffusion capacity is abnormal, bleomycin must be discontinued.

To avoid potential pain at the end of the procedure (some localizations can be uncomfortable), paracetamol can be given in a prophylactic way, but can also be offered to patients after the procedure.

5.5. *Follow-up*

See patient again at 4 weeks post-treatment or earlier if required. At that time, treatment efficacy can be determined in most cases. For larger lesions, more healing time may be necessary. Re-treatment can be considered upon the evaluation at 4 weeks post-treatment, but also later.

A healing time of up to 10 weeks is possible for lesions over 1.5 cm. For smaller lesions, healing time is in the order of 4–8 weeks.

6. Operating modality B: Local anaesthesia – intravenous chemotherapy

Room: Room with couch.
Personnel: Doctor and assistant (e.g. nurse)
Assistance in case of emergency: No extra precautions necessary.
Required Equipment:

- Couch, pillows, and cover
- Gloves
- Lidocaine, 2%, with epinephrine 0.5%
- Syringe, size 5 ml
- Hypodermic needles, app. 25 G
- Gauze pads
- Ruler for measuring tumour volume
- Equipment for iv infusion
- Bleomycin, 15000 IU/m^2
- Skin disinfectant pads
- Clock, pen and paper
- Cliniporator and selection of electrodes

6.1. *Infusion of chemotherapy*

If, at your institute, premedication with antihistaminic and/or anti-inflammatory and anti-allergic drugs (e.g., Methylprednisolonum, Clemastinum, etc.) is the rule when patients are treated with bleomycin, follow the usual domestic procedures. The doses of bleomycin are low and there is not a mandatory need for such premedication.

Infuse bleomycin, 15000 IU/m^2 (e.g. Bleomycin, Asta Medica AB, Täby, Sweden, or other national suppliers). Bleomycin is distributed in vials each containing 15000 IU of bleomycin, determined by its activity. Previously, the term units has been used, where 15000 IU = 15 units. Some investigators report bleomycin in mg, and 15000 IU is approximately equal to 8–9 mg of bleomycin, or 15 mg, depending on the activity of the drug and the manufacturer. This may be defined by the institute pharmacy.

Infuse as bolus in not less than 30 s but in not more than 1 min.

After 8 min, allowing the drug to diffuse into tissues, proceed with administration of local anaesthetic.

6.2. *Anaesthesia*

Lay a rectangular infiltration of local anaesthetic around the area to be treated, by injecting along 4 lines so that the nodule is 'fenced in' by local anaesthetic. Be sure that an even amount of anaesthetic is injected along all 4 sides of the rectangle, so that pain transmission is completely blocked. If more than one nodule is to be treated, it is necessary to consider the total amount of lidocaine: for example, in a 70 kg patient the maximal dose without adrenaline is 210 mg (21 ml of 1% lidocaine or 10.5 ml of 2% lidocaine) and with adrenaline 420 mg (42 ml of 1% or 21 ml of 2%). This will set the maximum of nodules treatable by one session with local anaesthesia, not surpassing 3 mg/kg of lidocaine without adrenaline or 6 mg/kg for lidocaine with adrenaline.

If it is anticipated that there will be less than 0.5 cm from the tip of the electrodes to periost, local anaesthesia should be reconsidered, since it may be difficult to palliate the patient sufficiently. Another potential restriction to local anaesthesia is the localization of the nodules in previously irradiated areas: experience has shown that is seems more difficult to apply local anaesthesia in these previously irradiated areas and if the option "local anaesthesia" is confirmed, it is recommended to put more anaesthetic and allow more time for lidocaine diffusion.

Small cutaneous tumours may be anaesthetized by local infiltration of lidocaine just below the tumour, however, still covering the area of electroporation. This may increase the number of small nodules that can be treated with local anaesthetic in one session.

Premedication with sedatives does not seem necessary.

6.3. *Choice of electrodes, pulse parameters, frequency*

Choose the appropriate electrode. If the tumour is less than 1 cm, consider using either plate or parallel array electrodes. In case the tumour is more than 1 cm, consider using the hexagonal array electrodes.

Enter the electrode code sequence (see description in section IV of this SOP) into the Cliniporator^{TM}. Insert electrode in appropriate handle (see colour code, B or G). Verify electrode cap insertion in the handle.

Treating at a frequency of 5 kHz will reduce the number of contractions to one – however this contraction will be more forceful than if a frequency of 1 Hz is used. The use of the 5 kHz frequency is mandatory for the electrodes of type III (hexagonal geometry).

6.4. *Pulsing procedure*

Make a test run with the Cliniporator^{TM} and electrodes.

Please note, that if any of the lesions to be treated are near large vessels or nerves, ultrasound guided application of needles may be needed.

Position patient comfortably lying down. Use pillows to support head, leg or arm so that a relaxed position is obtained. Offer blankets in case patient feels cold.

Disinfect the area to be treated. Sterile cover will not be needed.

Take the gauze pad in one hand, and the electrode in the right. The gauze pad can enable you to grasp the skin around the lesion to be treated, to lift the lesion up from the underlying musculature. In this way, muscle contractions and associated discomfort can be considerably reduced.

Inform patient when the first pulse is to be delivered.

After each pulse, the physician, the nurse or the assistant must look at the traces on the screen to have a quick visual check of the quality of the pulses delivered; go back to the recorded files in case of doubt about efficiency of the treatment.

Several pulse applications may be needed to cover the tumour volume. Overlapping fields should be avoided in normal tissue (including skin overlying subcutaneous tumours), but is acceptable in the tumour.

The electric field drops off very quickly outside the area of the electrodes, so the area to be treated by pulses must be encompassed within the electrodes – if necessary by running more than one application. Therefore, not only must the appropriate electrode be chosen, but the pattern of applications necessary to cover the tumour volume must be decided on as well.

The treatment should be finished at 28 min after the end of the infusion, in order to ensure that the tumours treated are subjected to an adequate concentration of bleomycin in the tissues. In the case there are more tumours to be treated, a second treatment round can be performed some days after the first one (see below, patients retreated). Pulses can start at 8 min, and thus the operator has 20 min to proceed to the treatment. This period can be extended eventually to 25 min, but probably not more than 30 min. So it could be said that if treatment is not totally finished within the optimal time, at 28 min after the injection, for still 5 min it is possible to treat other nodules but just to finish the areas already anaesthetized.

Some patients experience chills and fever a few hours after intravenous bleomycin administration. As paracetamol may reduce these symptoms, patients can be advised to take this drug either prior to or just after treatment.

After treatment, a loose dry dressing can be applied, or the lesion can be left without dressing.

Patients can be retreated. There should be at least one week between two intravenous bleomycin administrations. Usual precautions regarding the cumulative dose of bleomycin should be taken. Indeed, the cumulative bleomycin dose must not exceed 400 000 IU/m^2 due to risk of lung fibrosis. Pulmonary function tests should be considered if cumulative dose of bleomycin exceeds 60 000 IU/m^2. If the diffusion capacity is abnormal, bleomycin must be discontinued.

To avoid potential pain at the end of the procedure (some localizations may be uncomfortable), paracetamol can be given in a prophylactic way but it can also just be offered to patients after the procedure.

6.5. *Follow-up*

See patient again at 4 weeks post-treatment or earlier if required. At that time, treatment efficacy can be determined

in most cases. For larger lesions, more healing time may be necessary. Re-treatment can be considered upon the evaluation at 4 weeks post-treatment, but also later.

A healing time of up to 10 weeks is admissible for lesions over 1.5 cm. For smaller lesions, healing time is in the order of 4–8 weeks.

7. Operating modality C: General sedation – intratumoural chemotherapy

Room: Any room where practice of general anaesthesia is allowed, according to each country regulation, including a bed, couch or operating table, and all mandatory ventilation support and monitoring required for an operating theatre

Personnel:

- A certified anaesthesiologist for sedation,
- An operator assisted by a nurse for the procedure,
- A trained operator, or a trained nurse, or a trained technician, able to take care of the Cliniporator™ installation and of the preparation of the data recording.

Equipment Required:

For anaesthesia/sedation:

- Oxygen and air supply in order to limit FiO_2 to less than 40%
- Face mask connected to a soft 2 L balloon for assisted ventilation
- Airway canula
- Infusion line: 20 G catheter, standard tubing, ringer lactate 500 ml
- Vital monitoring: non-invasive blood pressure, ECG, end tidal CO_2, pulse oximeter
- Double syringe pump
- Remifentanil (1 mg, diluted into 25–50 ml)
- Propofol (10%): 50 ml
- Available: atropine, ephedrine and naloxone.

For the procedure

- Cliniporator™ with a selection of sterile one use electrodes (type I, II, and III) and the sterilized connection cables (sterilization according to the manufacturer recommendations).
- Chemotherapy prepared in aseptic conditions
- Sterile gloves and jacket
- Sterile Gauze pads
- Ruler for measuring tumour volume
- Digital camera to record pictures of the nodules before and after the procedure (optional)
- Skin disinfectant pads
- Clock, pen and paper.

7.1. *Anaesthesia*

General sedation may be decided, following the decision tree, when nodules are too numerous, too big or too painful to be anaesthetized by local anaesthesia, in the immediate vicinity of periost, or when they are inside a previously irradiated area (experience has shown that it seems more difficult to apply local anaesthesia in previously irradiated areas, see operating modalities A and B). The general sedation procedure regularly followed at your institute can be used. This operating modality describes in detail general sedation using reversible drugs. Indeed, as each pulse is very brief (<100 μs), the whole procedure is brief (<30 min.) and since residual pain is moderate, reversible drugs are desirable, like propofol for sedation and an opiate like remifentanil for analgesia. Volatile agents and nitrous agents should be avoided. Airway control should be ensured before bleomycin infusion. Premedication with sedatives is not necessary with general anaesthesia. The main risk is respiratory depression due to excessive sedation + analgesia; it may require transient assisted ventilation for a few minutes.

Analgesia is started at least 3 min before intratumoural injection: remifentanil 0.5 μg/kg bolus then 0.1–0.15 μg/kg/min then adjusted to the response to the first pulses or target controlled infusion (target 2 to 4 ng/ml). It may also be replaced by alfentanil boluses 250–750 μg.

Sedation is then started: propofol 0.5 mg/kg then 2–4 mg/kg/h or target controlled infusion (target 1 to 2 μg/ml).

Both are stopped at the last pulse. Post-operative pain is usually very low to moderate. Post-operative analgesia can be provided by intravenous paracetamol (1 g) and tramadol (100 mg), or paracetamol per rectum, as soon as the ECT started. It may be renewed every 6 hours until the 24th hour. Alternatively, instead of giving the paracetamol in a prophylactic way, it can be just offered to the patient after the procedure. No systematic prevention of nausea and vomiting is given but nausea or vomiting episodes can occur in a few sensitive patients. They spontaneously resume. If they appear they can be treated with standard regimens used by the institution.

7.2. *Injection of chemotherapy*

Use the chemotherapy agent chosen at the time of the pre-inclusion visit, according to the guidelines described in the corresponding modality treatment. Particular care for proper intratumoural injection must be taken to ensure treatment efficacy, particularly when very small nodules have to be treated.

Measure the tumour(s) to be treated, in the longest diameter (a) and the diameter perpendicular to that (b).

From the formula $V = ab^2\pi/6$ calculate the dose to be injected in each nodule, see below.

Intratumoural treatment can be performed either with cisplatin or bleomycin, according to the instructions below.

7.2.1. *Cisplatin*

- Cisplatin (CDDP) (e.g. Platamine, Pharmacia& Upjohn S.p.a., Milan, Italy, or other national suppliers) 10 mg, powder for injection:
- Intratumoural injection
  - dissolve to 2 mg/ml with sterile water
  - protect from direct light

- Injected dose should be 0.25 ml (0.5 mg) per cm^3 of tumour tissue for tumours larger than 1 cm^3, and 0.5 ml (1 mg) per cm^3 of tumour tissue for tumours smaller than 1 cm^3 but larger than 0.5 cm^3. For tumours smaller than 0.5 cm^3 injected dose should be 1 ml (2 mg) per cm^3, as a somewhat larger loss to surrounding tissues would be anticipated (see table below)

Calculated tumour volume ($V = ab^2\pi/6$)	<0.5 cm^3	0.5–1 cm^3	>1 cm^3
Cisplatin dose (concentration: 2 mg/ml)	1 ml/cm^3 tumour tissue	0.5 ml/cm^3 tumour tissue	0.25 ml/cm^3 tumour tissue

7.2.2. Bleomycin

- Bleomycin (e.g. Bleomycin, Asta Medica AB, Täby, Sweden or other national suppliers). Bleomycin is distributed in vials each containing 15 000 IU of bleomycin, determined by its activity. Previously, the term units has been used, where 15 000 IU = 15 units. Some investigators report bleomycin in mg, and 15 000 IU is approximately equal to 8–9 mg of bleomycin, or 15 mg, depending on the activity of the drug and the manufacturer.
- The recommended concentration of injection solution is 1000 IU/ml, dissolve in sterile water.
- Injected dose should be 0.25 ml (250 IU) per cm^3 of tumour tissue for tumours larger than 1 cm^3, and 0.5 ml (500 IU) per cm^3 of tumour tissue for tumours smaller than 1 cm^3but larger than 0.5 cm^3. For tumours smaller than 0.5 cm^3 injected dose should be 1 ml (1000 IU) per cm^3 (see table below)

Calculated tumour volume ($V = ab^2\pi/6$)	<0.5 cm^3	0.5–1 cm^3	>1 cm^3
Bleomycin dose (concentration: 1000 IU/ml)	1 ml/cm^3 tumour tissue	0.5 ml/cm^3 tumour tissue	0.25 ml/cm^3 tumour tissue

7.3. *Choice of electrodes, pulse parameters, frequency*

Choose the appropriate electrode. If the tumour is less than 1 cm, consider using either plate or parallel array electrodes. In case the tumour is more than 1 cm, consider using the hexagonal array electrodes. Plates or needles depending on the location of the small nodule, respectively superficial or more deeply located.

Enter the electrode code sequence (see description in section IV of this SOP) into the Cliniporator™. Insert electrode in appropriate handle (see colour code, B or G). Verify electrode cap insertion in the handle.

Treating at a frequency of 5 kHz will reduce the number of contractions to one – however this contraction will be more forceful than if a frequency of 1 Hz is used. The use of the 5 kHz frequency is mandatory for the electrodes of type III (hexagonal geometry).

7.4. *Pulsing procedure*

Make a test run with the Cliniporator™ and electrodes.

Please note, that if any of the lesions to be treated are near large vessels (or nerves), ultrasound guided application of needles may be needed.

Position patient comfortably lying down. Use pillows to support head, leg or arm so that a relaxed position is obtained. Offer blanket in case patient feels cold.

Disinfect the area to be treated. Sterile cover will not be needed.

Inject the drug into the tumour, and note the time. For small tumours, choose to inject either directly into the nodule or to tunnel the hypodermic needle under the skin to the nodule before injection. For larger tumours, several injections are needed. Make a pattern of injections using parallel insertions points. (If bleomycin is used make a calculation of the total amount of drug required for the entire session, if this exceeds or equals 15 000 IU/m^2 give this dose intravenously instead.)

Within 10 min of drug injection, electric pulses must be applied. Take the gauze pad in one hand, and the electrode in the other. The gauze pad can enable you to grasp the skin around the lesion to be treated, to lift the lesion up from the underlying musculature. In this way, muscle contractions can be considerably reduced.

After each pulse, the physician, the nurse or the assistant must look at the traces on the screen to have a quick visual check of the quality of the pulses delivered; go back to the recorded files in case of doubt about efficiency of the treatment.

Several pulse applications may be needed to cover the tumour volume. Overlapping fields should be avoided in normal tissue (including skin overlying subcutaneous tumours), but is acceptable in the tumour.

The electric field drops off very quickly outside the area of the electrodes, so the area to be treated by pulses must be encompassed within the electrodes – if necessary by running more than one application. Therefore, not only must the appropriate electrode be chosen, but the pattern of applications necessary to cover the tumour volume must be decided on as well.

After treatment, a loose dry dressing can be applied, or the lesion can be left without dressing.

Patients can be retreated. Even though the bleomycin doses are low, it is recalled that the cumulative bleomycin dose must not exceed 400 000 IU/m^2 due to risk of lung fibrosis. Pulmonary function tests should be considered if cumulative dose of bleomycin exceeds 60 000 IU/m^2. If the diffusion capacity is abnormal, bleomycin must be discontinued.

7.5. *Follow-up*

See patient again at 4 weeks post-treatment or earlier if required. At that time, treatment efficacy can be determined in most cases. For larger lesions, more healing

time may be necessary. Re-treatment may be considered upon the evaluation at 4 weeks post-treatment, but also later.

A healing time of up to 10 weeks is possible for lesions over 1.5 cm. For smaller lesions, healing time is in the order of 4–8 weeks.

8. Operating modality D: General sedation – intravenous chemotherapy

Room: Any room where practice of anaesthesia is allowed, according to each country regulation, including a bed, couch or operating table, and all mandatory ventilation support and monitoring required for an operating theatre

Personnel:

- A certified anaesthesiologist for sedation.
- An operator assisted by a nurse for the procedure.
- A trained operator, or a trained nurse, or a trained technician, able to take care of the Cliniporator^{TM} installation and of the preparation of the data recording.

Equipment required:

For anaesthesia/sedation:

- Oxygen and air supply in order to limit FiO_2 to less than 40%
- Face mask connected to a soft 2L balloon for assisted ventilation
- Airway canula
- Infusion line: 20 G catheter, standard tubing, ringer lactate 500 ml
- Vital monitoring: non-invasive blood pressure, ECG, end tidal CO_2, pulse oximeter
- Double syringe pump
- Remifentanil (1 mg, diluted into 25 to 50 ml)
- Propofol (10%): 50 ml
- Available: atropine, ephedrine and naloxone

For the procedure

- Cliniporator^{TM} with a selection of sterile one use electrodes (type I, II, and III) and a connection cable sterilized between uses according to the manufacturer recommendations
- Chemotherapy prepared in aseptic conditions
- Sterile gloves and jacket
- 4 sterile sheets to limit the operating zone (optional)
- Gauze pads
- Ruler for measuring tumour volume
- Digital camera to record pictures of the nodules before and after the procedure
- Skin disinfectant pads
- Clock, pen and paper

8.1. *Anaesthesia*

General sedation may be decided when nodules are too numerous, too big or too painful to be anaesthetized by local anaesthesia, in the immediate vicinity of periosteum, or when they are inside a previously irradiated area (experience has shown that is seems more difficult to apply local anaesthesia in previously irradiated areas, see operating modalities A and B). The general sedation procedures regularly followed at your institute can be used. This operating modality describes in detail general sedation using reversible drugs. Indeed, as each pulse is very brief (<100 μs), the whole procedure brief (<30 min) and since residual pain is moderate, reversible drugs are desirable, like propofol for sedation and remifentanil for analgesia. Volatile agents and nitrous agents should be avoided. Airway control should be ensured before bleomycin infusion. Premedication with sedatives is not necessary with general anaesthesia. The main risk is respiratory depression due to excessive sedation + analgesia; it may require transient assisted ventilation for a few minutes.

8.2. *Injection of chemotherapy*

If, at your institute, premedication with antihistaminic and/or anti-inflammatory and anti-allergic drugs (e.g., Methylprednisolonum, Clemastinum, etc.) is the rule when patients are treated with bleomycin, follow the usual domestic procedures. The doses of bleomycin are low and there is not a mandatory need for such premedication.

The full dose of chemotherapy bleomycin at 15 mg/m^2, that is 15 000 IU/m^2, is administered intravenously in not less than 30 s but in not more than 1 min. After 8 min, a time necessary to allow the drug to diffuse into tissues, pulses may start. They can be delivered, optimally for outcome treatment, during 20 min, i.e. until minute 28th after the end of the bleomycin injection. However, in case of many/large nodules treated for patient palliation, it is possible to continue the treatment for a few more minutes.

Analgesia is started at least 3 min before the first pulses: remifentanil 0.5 μg/kg bolus then 0.1–0.15 μg/kg/min then adjusted to the response to the first pulses or target controlled infusion (target 2 to 4 ng/ml). It may also be replaced by alfentanil boluses 250–750 μg.

Sedation is then started: propofol 0.5 mg/kg then 2–4 mg/kg/h or target controlled infusion (target 1 to 2 μg/ml).

Both are stopped at the last pulse. Post-operative pain is usually very low to moderate. Post-operative analgesia can be provided by intravenous paracetamol (1 g) and tramadol (100 mg), or paracetamol per rectum, as soon as the ECT started. It may be renewed every 6 hours until the 24th hour. Alternatively, instead of giving the paracetamol in a prophylactic way, it can be just offered to the patient after the procedure. No systematic prevention of nausea and vomiting is given but nausea or vomiting episodes can occur in some patients. They spontaneously resume. If they appear they can be treated with standard regimens used by the institution.

8.3. *Choice of electrodes, pulse parameters, frequency*

Choose the appropriate electrode. If tumour is less than 1 cm, consider using either plate or parallel array electrodes. In case the tumour is more than 1 cm, consider using the hexagonal array electrodes. Plates or needles depending on the location of the small nodule, respectively superficial or more deeply located.

Enter the electrode code sequence (see description in section IV of this SOP) into the Cliniporator™. Insert electrode in appropriate handle (see colour code, B or G). Verify electrode cap insertion in the handle.

Treating at a frequency of 5 kHz will reduce the number of contractions to one – however this contraction will be more forceful than if a frequency of 1 Hz is used. The use of the 5 kHz frequency is mandatory for the electrodes of type III (hexagonal geometry).

8.4. *Pulsing procedure*

Make a test run with the Cliniporator™ and electrodes.

Please note, that if any of the lesions to be treated are near large vessels or nerves, ultrasound guided application of needles may be needed.

Have the patient positioned comfortably lying down. Use pillows to support head, leg or arm so that a relaxed position is obtained.

Disinfect the area to be treated. Sterile cover will not be needed.

Take the gauze pad in one hand, and the electrode in the right. The gauze pad can enable you to grasp the skin around the lesion to be treated, to lift the lesion up from the underlying musculature. In this way, muscle contractions can be considerably reduced.

After each pulse, the physician, the nurse or the assistant must look at the traces on the screen to have a quick visual check of the quality of the pulses delivered; go back to the recorded files in case of doubt about efficiency of the treatment.

Several pulse applications may be needed to cover the tumour volume. Overlapping fields should be avoided in normal tissue (including skin overlying subcutaneous tumours), but is acceptable in the tumour.

The electric field drops off very quickly outside the area of the electrodes, so the area to be treated by pulses must be encompassed within the electrodes – if necessary by running more than one application. Therefore, not only must the appropriate electrode be chosen, but the pattern of applications necessary to cover the tumour volume must be decided on as well.

The treatment should be finished at 28 min after the end of the infusion, in order to ensure that the tumours treated are subjected to an adequate concentration of bleomycin in the tissues. A second treatment round can be performed, in case there are more tumours to be treated.

After treatment, a loose dry dressing can be applied, or the lesion can be left without dressing.

Patients can be retreated. However, bleomycin should maximally be given once a week. Usual precautions regarding the cumulative dose of bleomycin should be taken. Indeed, the cumulative bleomycin dose must not exceed 400 000 IU/m^2 due to risk of lung fibrosis. Pulmonary function tests should be considered if cumulative dose of bleomycin exceeds 60 000 IU/m^2. If the diffusion capacity is abnormal, bleomycin must be discontinued.

8.5. *Follow-up*

See patient again at 4 weeks post-treatment or earlier if required. At that time, treatment efficacy can be determined in most cases. For larger lesions, more healing time may be necessary. Re-treatment can be considered upon the evaluation at 4 weeks post-treatment, but also later.

A healing time of up to 10 weeks is admissible for lesions over 1.5 cm. For smaller lesions, healing time is in the order of 4–8 weeks.

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