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The Role of Electrochemotherapy in the Treatment of Malignant Melanoma

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1. Introduction

About 68,130 new melanomas will be diagnosed in the United States during 2010 (38,870 men: 29,260 women) and of those 8,700 people will die of the disease (5,670 men; 3,030 women). The death rate has been dropping since the 1990s for those younger than 50, but has remained stable or is rising for older individuals. However, the incidence of melanoma has been increasing for at least 30 years, and this trend has become more pronounced in young white females and in older white men[1].

Malignant Melanoma is the seventh most common type of cancer, but it is the first cause of death from cutaneous skin cancers[2]. It has been estimated that the lifetime risk of developing malignant melanoma is 2% (1 in 50) for Caucasians, 0.1% (1 in 1,000) for those of black descent, and 0.5% (1 in 200) for Hispanics[3] .

When melanoma is detected in advanced stages, it carries a dismal prognosis, with a mean survival of about 8 months and a 5-year survival as low as 5% [4-6]. The disease spreads both by the lymphogenous and the haematogenous routes and can metastasize to virtually any organ in the body. When secondary tumours emerge, these usually follow a sequential pattern to regional lymph node basins, followed by distant sites including skin, subcutaneous tissue, lung, liver, brain, bone and other viscera[5-6]. Local recurrence, in-transit metastases and satellitosis (cutaneous metastases within 2 cm of original lesion) represent the same dissemination process[4] in the dermal lymphatics. When the patients present with cutaneous metastases, they are considered to have stage IIIB disease[7]. Cutaneous metastases occur in 2-20% of patients, depending on tumor thickness [6, 8-9] and can occur either during the early or late phase of the disease [10]. In many instances they can represent the first site of recurrence after surgical excision of the primary tumor [11] . The majority (70-80%) of recurrences are diagnosed within the first 3 years of initial diagnosis, and the median time to the presence of in-transit disease could range between 3 to 16 months[4]. The recurrences present as local or in-transit disease in 20-28%, regional disease in 26-60% and as distant metastases in 15-50% of patients. Even though local recurrence is

not yet considered stage IV disease, the prognosis is poor with 5 year survival rates of less than 50% [9,12]. The risk of developing metastases may be predicted at excision of the primary lesion and determinants include, increasing thickness of the tumour, the anatomical location, histological subtype and gender [13]. In general tumours of the trunk, non cutaneous and subungual regions behave more aggressively than those of the extremity [14-16] .

2. Treatment limitations for recurrent or in-transit melanoma

Treating extensive cutaneous/subcutaneous nodules or in-transit disease is a clinical challenge because of common unresectability and relative insensitivity to conventional systemic therapies[6]. These recurrences provide a significant psychological burden for patients whose quality of life is negatively affected by the symptoms caused by the tumor, such as pain, bleeding, ulceration, and malodorous discharge [4, 17-18]. Many patients suffering from in-transit disease also manifest with systemic disease, within 6-13 months of the onset of the local lesions. Their life expectancy is foreshortened and is determined by factors such as the burden of the loco-regional disease, the interval from initial treatment and their immune status [4].

The aim of the treatment should be the elimination of both local and systemic disease with the benefit of improved life expectancy and quality of life, while minimizing toxicities or deformities. It is important to note that metastatic cells have disseminated prior to surgical intervention, outlining the critical need for the development of adjuvant therapeutic strategies[19]. Thus the treatment of unresectable and in-transit disease is both systemic and loco/regional. The options include surgery, which is the most effective method for limited recurrent or in-transit disease, systemic chemotherapy[12,21], radiation therapy[22], cryosurgery[23]; carbon dioxide laser ablation,[12,24-26], intralesional therapies[12,21] with the Bacille Calmette-Guerin vaccine[27], TNFerade a non replicant adenovirus complex, expressing the Tumor Necrosis Factor alpha (TNF-α) gene (hTNF-α cDNA)[12,28], and Interleukin-2[29], Cell Vaccine and Immunotherapy [30-33], Interferon alpha[34-36], Regional therapies such as hyperthermic isolated limb perfusion (HILP) and Isolated limb infusion (ILP)[37], Novel Molecular Therapies with c-Kit inhibitors (Imatinib), C-RAF-inhibitors (Sorafenib) [38], and blocking B-RAF regulation of mitogen-activated protein kinase pathway (MAPK) [38]. In more recent years electroporation with anticancer drugs which is termed electrochemotherapy (ECT) has been successfully applied for local tumour control [17, 19, 39]. In general malignant melanoma is refractory to systemic treatments and survival after treatment of regional cutaneous metastases has been reported to be between 20-28% at 10 years[6,9,40]. However all current treatment modalities have individual limitations and variable response rates [4, 12, 19, 41-42].

3. Electrochemotherapy (ECT)

Definition and physiological effect:

The cell membrane represents a physical barrier to the intracellular transfer of hydrophilic drugs, macromolecules, nucleotides and peptides. The movement of polar and hydrophilic molecules across the membrane is restricted by the phospholipid bilayer[18] Studies suggest that exposure to sufficiently strong and long electrical fields, could rearrange the lipid bilayer, by reorienting the hydrophillic heads in pore-like fashion, while the hydrophobic

tails are embedded within the plasma membrane. This porosity is transient and reversible if short duration high amplitude square wave electrical pulses are optimized.[18]
Electroporation (EP) of tumour nodules allow the permeation, into the cancer cells, of poorly permeable antineoplastic drugs, such as bleomycin or cisplatin which are given either systemically or intratumorally (IT)[18, 39, 43-46]
The temporary permeability of the cell membrane caused by the electric pulses facilitates a potent localized effect and magnifies the drugs cytotoxicity by orders of magnitude [18, 39, 46-50].

Types of Drugs

After several studies of different cytotoxic drugs, two have been identified as the best candidates for ECT: Bleomycin and Cisplatin [17, 45]. Of importance in these studies EP or the drugs on their own do not influence the growth of tumours, yet their combination have potent tumoricidal effects[18, 51-53] (See Figure 1). An advantage of ECT is that it requires much lower doses of the cytotoxic drug for optimal effects than those usually used for systemic treatments. In addition there is little in the way of collateral damage or complications, since the the cell killing is confined to the tissues affected by the electric field. Bleomycin intercalates into the cellular chromatin causing single and double stranded breaks in DNA resulting in a mitotic cell death by pseudoapoptosis. (Figure 1 B,C & D). On the other hand, Cisplatin creates an apoptosis effect on the cell [18, 46]. This cytolytic activity is potentiated more than 1,000 fold for Bleomycin and 100 fold for Cisplatin by the addition of EP [12, 19, 48-49].

The Vascular Lock

The electric pulses produce a transient state of hypoperfusion by local reflex vasoconstriction at the arteriolar level (lasting 1-2 minutes) and a phase of interstitial edema (that resolves with membrane resealing). However the effect may last longer (12 hours to 5 days) in rapidly dividing tumor cells and is more prominent in tumours with a less mature endothelial lining and higher interstitial pressure. This phenomenon mediated by the sympathetic nervous system is termed the "vascular lock" and it has implications for timing of drug administration[18-19]. After application of the electric current, there is a retention of drugs already in the tumour but there is also an impairment of entry of drugs from the circulation. Thus when the cytotoxic drugs are administered systemically sufficient time should be allowed to achieve optimal intratumoral drug concentration prior to application of EP. ECT produces other vascular influences, which are believed to be secondary to a reduction in local angiogenic factor production, such as endothelial cell destruction and neovascular reorganization [46, 54-55]. The combined vascular influences have been successfully exploited in the treatment of bleeding melanomas [56-57]– which may be a difficult to manage problem, sometimes refractory and fatal, in patients with unresectable disease.

4. Technique of electrochemotherapy

1. The Equipment

The electric pulses may be applied to the tumors either by plate electrodes on the skin surface or by needle electrodes inserted into the lesion (figure2). The electric field distribution is determined by the geometry of the electrodes. Regardless of the type of electrode the electric field is highest around and between the electrodes [17-18]. The current pulse generators in use are the Cliniporator (IGEA, Carpi, Italy) (FIG 2) and the Medpulser™ (Inovio Biomedical Corporation, CA, USA). It appears that plate electrodes are

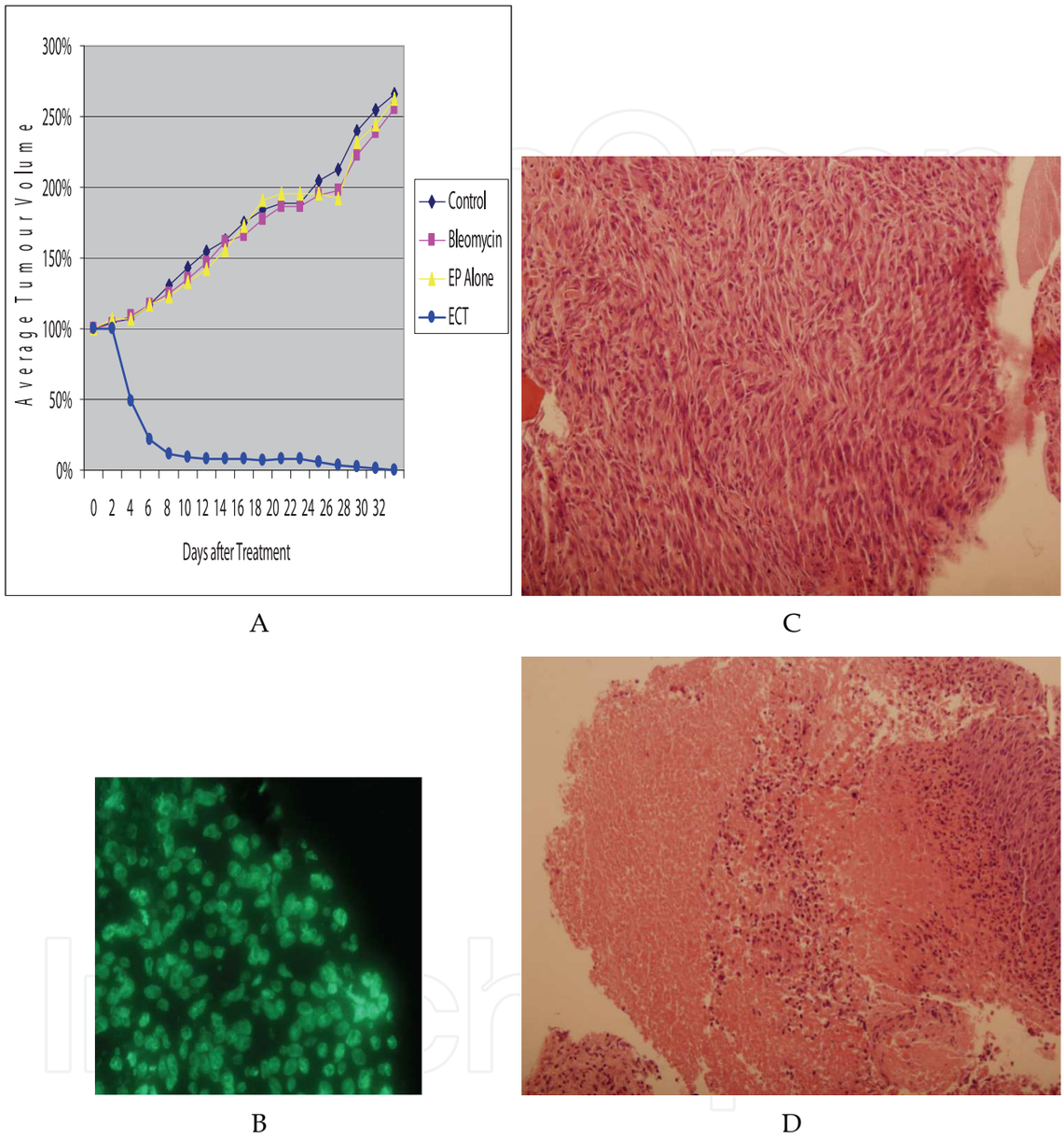

Fig. 1. A. Growth curves of experimental cancers in mice, which demonstrate that electropermeabilisation or intratumoural bleomycin alone, have no influence on tumour growth but when used together completely ablate the tumour. B) Positive Tunel stain 48 hrs post ECT indicating tumor cell death by apoptosis. C &D Tumour before showing normal cellularity and D 48 hrs post ECT showing regions of denucleation

more suitable for use in superficial skin lesions, while needle electrodes are used for deeper seated lesions, such as exophytic and thick lesions (maximum depth 3 cm)[52]. A disadvantage of plate electrodes over the needle type is the potential skin damage that may be generated by the higher impedance/resistance of the skin, especially when treating larger affected areas[19, 58]. Care must be taken to avoid inserting the needle electrodes into the healthy tissue surrounding the tumors, which may also result in local subcutaneous burns [39]. There are three types of electrodes in common usage *(FIG 2)*. Type I electrodes consist of two parallel stainless-steel plate electrodes, used for superficial lesions and do not penetrate the skin. Type II electrodes are used for smaller lesions and consist of two rows of eight needles with 4 mm distance between them, while Type III electrodes are recommended for larger lesions (>1cm), with the needles in a hexagonal configuration. The needles are inserted encircling the tumor and down to the subcutaneous tissue, slightly deeper than tumor depth [17, 19, 52, 59] . It is recommended to elevate or tent the skin at the time of delivering the electric current , if the electrodes are to be inserted in a superficial or shallow subcutaneous areas such as near the knees, tibial tuborosities, the scalp, or close to any other osseous structures[52]

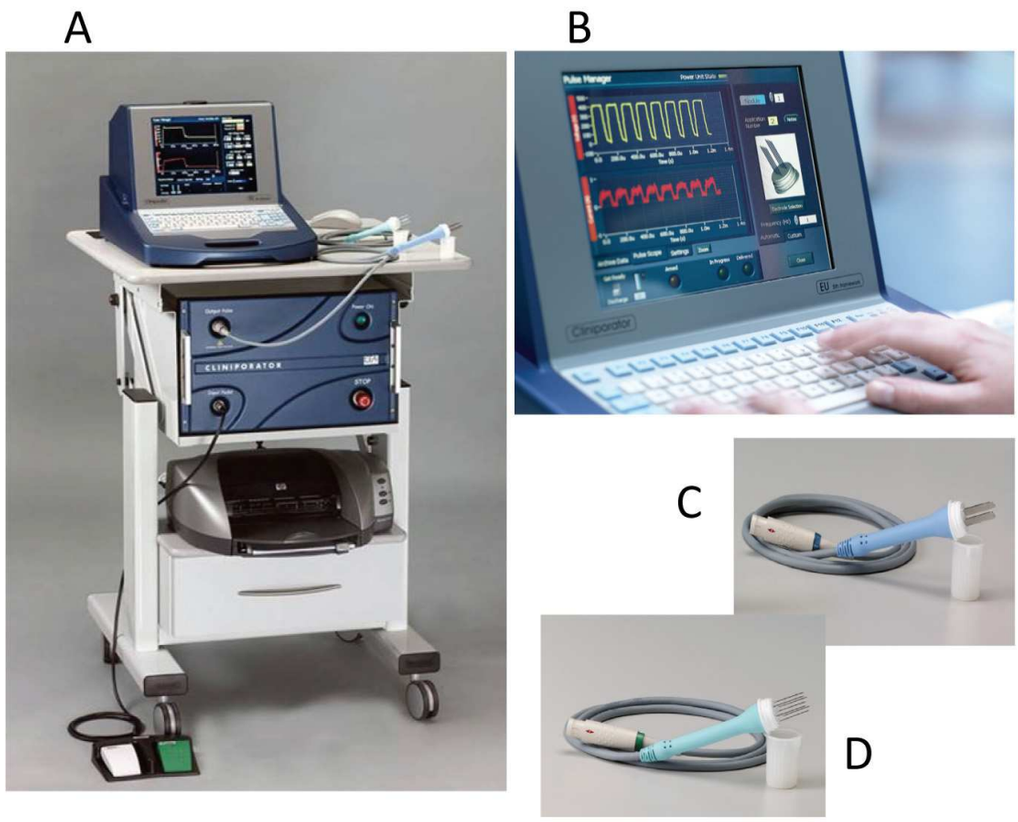

Fig. 2. (A) The ECT pulse generator. B) Note an entrainment of 8 square wave pulses. C) Electrodes type1 (plates) and (D) type 3 (Needle array).

2. Anesthesia

The procedure can be performed in the outpatient or ambulatory setting, under local anesthesia in association with conscious sedation. General anesthesia may be preferred for larger tumors, or tumors located in prior irradiated or fibrotic tissues where infiltration of local anesthetic may be painful and less likely to diffuse and thus achieve adequate pain control and for those located too close to vascular or bony structures. The dose of local anesthetic should be recorded and should not exceed the maximum allowed per body weight for Lidocaine without epinephrin (3 mg/kg) [52, 59-60]. In addition, a mixture of 0_2/air with $Fi0_2$ of 40 % is administered to the patients during conscious sedation.

3. The Electric Current

Permeabilization occurs in the cell when the internal transmembrane potential has surpassed the critical value between 200-300mV. The extent of the electroporative effect depends on the number and duration of the electric pulses[18,55]. Two pulse parameters have been evaluated - exponentially decaying pulses and square wave pulses. Of the two, square wave pulses are preferred as they permit independent control of the length and amplitude. The pulse parameters selected for treatment depend on the type of electrode [18, 52].

Pulse parameters

Ideally for Type I (plate) electrodes, pulse parameters of 8 square waves with amplitude of 1300 V/cm, duration of 100 μs, and frequency of 1Hz are used. The current should be delivered in two perpendicular directions.
For type II (needle) electrodes the voltage amplitude may be reduced to 1000V/cm. The pulses are administered simultaneously between the needle pairs.
For Type III, the needles are positioned hexagonally and 96 pulses are given together (12 pairs x 8 pulses) at a frequency of 5 kHz [61].
Due to the already described vascular lock phenomenon, it is recommended that the electric current is applied between 8 to 28 minutes after IV administration of the drug[62], or immediately (within 2-10 minutes) after intratumoral administration [19, 59, 63-64].

4. Drug Dosage Recommendations

Initial studies compared the routes of administration of Bleomycin suggested advantages for the intralesional over intravenous administration (77% vs. 45 % complete responses) [45] but later the prospective multi-institutional ESOPE (European Standard Operating Procedures of Electrochemotherapy) study in 2006, concluded that IV or IT Bleomycin were comparable when given to tumors of volumes less than 0.5 cm^3 [52]. For Cisplatin, the studies have shown that IT is more effective than the IV route, with CR rates of 82% vs. 48% respectively[19, 45]. In the ESOPE study local tumor control was achieved in up to 88% of tumors treated with IV Bleomycin, 73% with IT Bleomycin and 75% with IT Cisplatin[52].
If a lack of uniformity of drug distribution within the tumor is anticipated either by the presence of large and extensive disease, harder fibrotic tumor nodules, lymphedema or limb fibrosis, the IV route would be more suitable [52]. The Intratumoral route is more feasible for those less perfused nodules located in previously pretreated areas[19].
When Bleomycin is given IV the dose is 15,000 IU/m2 of body surface area in a bolus lasting 30-45 seconds. But when the IT route is chosen, both drugs are given in a dose calculation based on tumor burden. The IT dose of Bleomycin based on tumor size is calculated as follows: for tumor nodules less than 0.5 cm^3 a dose of 1000 IU/cm^3, tumor nodules between 0.5 and 1 cm^3 a dose of 500 IU/cm^3 and for tumors larger than 1 cm^3 a dose of 250IU/cm3 is

administered. The cumulative dose should not exceed 400,000 IU/m^2 due to the cumulative risk of lung fibrosis[46,52]. If the cumulative dose surpasses 60,000 IU /m2, objective respiratory function tests should be made at intervals and the drug discontinued if the respiratory diffusion capacity is abnormal[60]. The IT dose of Cisplatin is given as follow: for nodules less than 0.5 cm^3 a dose of 2mg/cm^3, for nodules between 0.5-1 cm^3, a dose of 1mg/cm^3 and for nodules larger than 1 cm^3 a dose of 0.5mg/cm^3 is given. (Table 1)

Tumor Volume ($V=ab^2\pi/6$)	Bleomycin dose (1000 IU/mL concentration)	Cisplastin dose (2mg/mL concentration)
<0.5 cm^3	1 mL/cm^3 tumor	
>0.5 < 1 cm^3	0.5 mL/ cm^3 tumor	
>1 cm^3	0.25 mL/ cm^3 tumor	

Table 1. Tumor volume and IT drug dose concentration

The rationale for reducing the intralesional dose per cm^3 when treating areas with larger tumor burdens is to reduce the risk of systemic toxicities of the absorbed drug without compromising local efficacy[19].

Prophylactic antibiotics against skin flora should be given intravenously prior to the start of the procedure, especially when lesions are ulcerated or necrotic.

The required time for the procedure is short, with a median treatment duration of 25 minutes[32]. Repeated treatments are usually well tolerated by the patients [11, 65-66] and these can usually be performed at 1-6 weekly intervals, without evident resistance [45].

5. Patient monitoring

Prior to each treatment session patients should have an electrocardiogram (ECG), blood work for evaluation of renal function, coagulation and electrolyte values. All lesions should be photographed and the tumor burden documented. Cellular debris may be released from the tumour during electroporation and these may interfere with the clearance of cytotoxic drugs by the kidney. Thus, when using IV Bleomycin, the serum creatinine should be maintained at less than 150mol/L to ensure proper renal clearance. Physiological monitoring includes visual display of O2 saturation, pulse rate, blood pressure, continuous ECG tracing and respiratory parameters. Acetominophen or an anti-histaminic medication may be given to prevent the mild febrile reaction that may occur in the early post procedural period when Bleomycin is administered [46, 59].

Technical pearls

1. Test the device and the electrodes prior to beginning the procedure
2. Prep and drape following sterile technique principles
3. Keep in mind correct timing of drug administration and application of the electric current
4. Protect osseous surfaces by tenting the skin
5. Communicate with awake patients and assisting staff, prior to delivery of electric current
6. Check electroporator traces and waves to confirm proper current delivery
7. Do not overlap treating fields with normal tissue
8. Dress wounds according to presenting symptoms

5. ECT development and clinical applications in melanoma

Neumann in 1982[67] published the first paper regarding EP as a method to transfer genes into mammalian and bacterial cells. There were other *in vitro* [65, 68] and *in vivo* studies in early and late 1980's using EP in combination with drugs[69], but the first clinical trials demonstrating its effectiveness over Bleomycin alone for the treatment of a diversity of cutaneous tumors of the head and neck region, were published in early 90's by Mir et al[47] and Belehradek et al.[70] from the Institute Gustave Roussy, in Villejuif France. These first 8 patients were treated for squamous cell carcinoma tumors and a complete response (CR) was observed in 57% of the lesions. These early publications encouraged other investigators to expand the principles of this technique to other tumor types including basal cell carcinomas, Kaposi sarcoma, and melanoma metastases [6, 62, 71-74]. By the mid 90's, Rudolf et al.[71] and Heller et al. [73] published the results of an initial small group (5) of melanoma patients that underwent treatment with ECT and IV Bleomycin. They reported overall response rates (OR) in 92% and 50% of 24 and 10 metastatic nodules respectively[19]. That same year, Glass et al. [75] from the University of South Florida in Tampa, USA, reported on the first study using ECT with IT Bleomycin in melanoma metastases obtaining OR rates of 92% (78% CR and 14 % PR) in 20 metastatic lesion – results similar to those obtained by the IV route in the previous studies. Later in 1998, the same group of investigators published the results of a bigger cohort of patients with a variety of cutaneous and subcutaneous malignant nodules. Twelve of 34 patients had 84 metastatic melanoma nodules with documented OR rates up to 99% (89% complete response (CR) and 10 % partial response (PR))[76]. That same year, the combined data produced by five institutions in USA, France and Slovenia was published by Mir et al.[39] In this study, twenty patients with metastatic melanoma lesions showed responses in 131 (92%) of 142 lesions, with CR of 53% and PR of 39%. A major finding of this study was that the results were comparable among the institutions even though their treatment protocols and the route used for administering the Bleomycin were not standardized. Additional small studies [56, 77-79] using ECT with IT Bleomycin for melanoma lesions continued to show good responses, with OR rates of 71 to 100% (CR 23%-100% and PR 0%-62%). Rols et al.[80] in 2000 continued to demonstrate OR's of 93% using ECT and IV Bleomycin in the treatment of 54 metastatic melanoma nodules.

Sersa et al.[81-82] introduced Cisplatin as a therapeutic option in 1998. In their studies they reported CR rates of 100% for 2 patients with 13 lesions treated with IT Cisplatin, but low CR rates of 11 % in 9 patients with 27 lesions treated using the IV route. Additional studies were published using this drug by the IT route only [61, 82-83] with OR rates ranging from 81% to 100% (CR 0%-70 % and PR 6%-100%).

The main issue with most of the studies of the 1990's and early 2000's was the utilization of a variety of treatment protocols with different pulse parameters and pulse generators in combination with different electrode types and drug administration routes [6, 39, 44, 47, 52, 56, 58, 63, 70, 72-73, 75-78, 81-88]. But in 2006 the results of a pivotal prospective non-randomized multicenter ESOPE study [52] were published, thus providing recommendations for a standardized protocol for the procedure. The study included 102 patients, 61 evaluated for response and 41 for toxicity respectively. The protocol allowed for administration of Bleomycin either IV or IT, or with Cisplatin IT, and included several histological types of lesions. Ninety-eight lesions from 20 melanoma patients were evaluated - The OR rates were 81% with a CR of 66%. The results confirmed the effectiveness of ECT in the treatment of lesions of different histology, demonstrating an 85% objective response rate and a CR rate of 74% for all lesions.

These results were independent of the drug used or the route of administration chosen. Additionally subsequent studies [11, 49, 66, 89-92] of ECT evaluating its effect in the treatment of melanoma and other skin cancers continue to demonstrate the efficacy of the treatment, with response rates comparable to the earlier studies, ranging from 46-100%.
Repeated treatments are feasible as demonstrated by Campana et al.[90] and Quaglino et al.[11] producing additional clinical responses in patients who had initial non or partial responses or who presented with new recurrent lesions. In the Campana[90] study 34 patients out of 52 were diagnosed with unresectable melanoma, but the response rate for the entire cohort of patients treated with either IT or IV Bleomycin improved significantly from a CR of 50%, up to 83 % after the third ECT treatments.
There are approximately 60 institutions in Europe and in the United States that continue to investigate and offer ECT as a palliative treatment for a variety of unresectable tumors, including melanoma; and in an occasional report it has been used as an alternative curative therapy [93].

6. ECT in the treatment of advanced melanoma

In patients with unresectable recurrent or in-transit melanoma disease who are not candidates for standard surgical or medical treatment, ECT is now an important therapeutic option[18,43,49,75,84,94]. These cases include those with unresectable disease due to the extensive number of nodules or lesions located in compromising anatomic areas, such as those around joints, nerves, distal leg and in previously operated fields. Encouraging results with long term remissions have been documented [17, 45, 78, 93, 95-96]. (Figure 3)

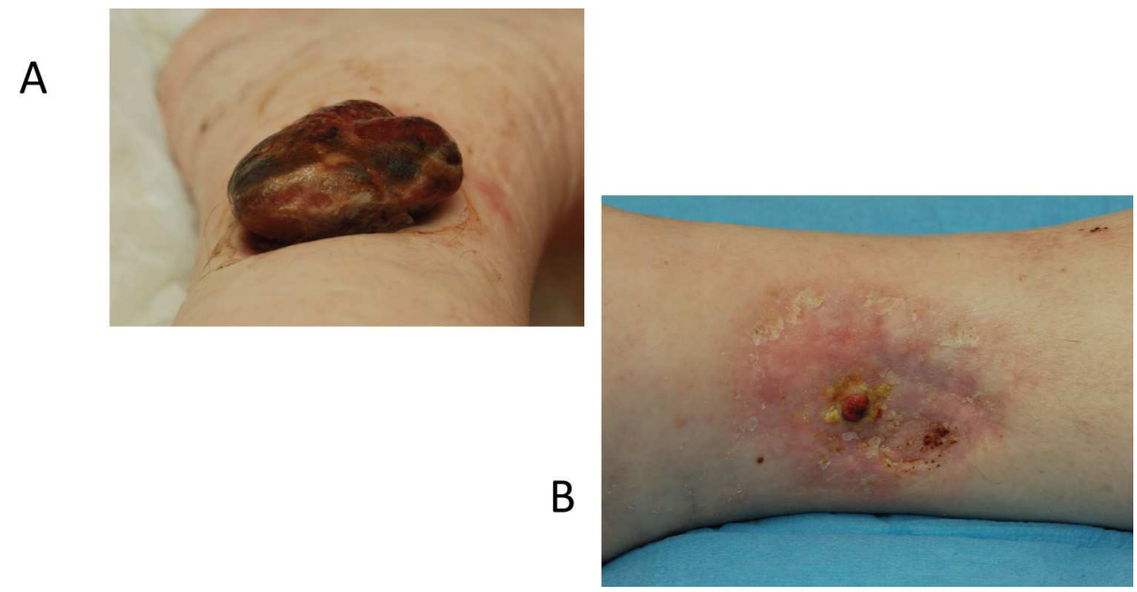

Fig. 3. A) An Exophytic type recurrent malignant melanoma after isolated limb perfusion.
B) Six months following treatment by ECT .

Author	No. Patients	Nodules	Drug/route	CRR %	ORR (PR+CR)%
Rudolf 1995[135]	2	24	Bleomycin IV	92	92
Heller 1996[137]	3	10	Bleomycin IV	30	50
Glass 1996[140]	5	23	Bleomycin IT	78	96
Heller 1998[141]	12	84	Bleomycin IT	75	99
Kubota 1998[144]	1	8	Bleomycin IT	100	100
Mir 1998[27]	20	142	Bleomycin IV	53	92
Sersa 1998[146]	2	13	Cisplastin IT	100	100
Sersa 2000[147]	9	27	Cisplastin IV	11	48
Sersa 2000[153]	10	82	Cisplastin IT	80	87
Rols 2000[145]	4	55	Bleomycin IV	9	93
Gehl 2000[128]	1	9	Bleomycin IT	100	100
Rodriguez 2001[143]	2	13	Bleomycin IT	23	85
Sersa 2003[149]	14	211	Cisplastin IT	70	81
Byrne 2005[144]	21	52	Bleomycin IT	63	71
Snoj 2005[148]	1	1	Cisplastin IT	0	100
Marty 2006[124]	20	98	Bleomycin IT/IV* larger 3 cm	66	81
Gaudy 2006[159]	12 *(7 per protocol)*	30 23	Bleomycin IT	36 74	46 82
Snoj 2006[165]	1	16	Cisplastin IT	100	100
Larkin 2007[121]	4 *(2 pts LTF)*	56	Bleomycin IT or IV for nodules > 3 cm	0	50
Snoj 2007[164]	1 *(RETC x4)*	224	Bleomycin IV	100	100
Tauceri 2007[157]	3	ns	Bleomycin IT	ns	ns
Qualigno 2008[12]	14 *(7 pts RETC)*	160 73	Bleomycin IV	50 58	93 93
Campana 2009[158]	34 MM *(52 cohort 27 RETC)*	373 608 257	Bleomycin IT/IV	ns 50 66	ns 74 90

IV: Intravenous, IT: Intratumoral, CRR: Complete Response Rate, ORR: Overall Response Rate, PR: Partial Response, CR: Complete Response
RETC: Repeated Electrochemotherapy, LTF: Lost to follow up, ns: No specified, MM: Malignant melanoma
*: Patients with different histological types

Table 2. Summary of most relevant studies using ECT for unresectable or In-Transit Melanoma

Its effectiveness has been proven when providing palliative treatment for hemorrhagic and painful tumor nodules [56-57, 79, 97-98]. This is a benefit believed to be secondary to the "vascular lock" phenomenon The vasoconstriction at the arteriolar level produces an imediate and dramatic reduction of perfusion of the malignant lesions, thus controlling the bleeding [18, 45, 98]. ECT can also be useful as a neoadjuvant treatment for cytoreduction and organ sparing treatment. Its benefits has been reported in patients with perineal melanoma treated with Cisplatin [85] and for a sphincter saving procedure in anal melanoma [83].

ECT can also be suitable for and more tolerable by those patients with a prohibitive surgical risk due to significant comorbidities[52] because the length of the procedure is relatively short [52] and patients are able to tolerate multiple sessions. In some anatomical locations ECT could provide good and sometimes better cosmetic results than surgery [93].

ECT in combination with cytokine therapy or gene coding immunotherapies has advanced to clinical trials in advanced melanoma. In a phase II study, patients treated with injections of low dose perilesional IL-2 and ECT with Bleomycin, the cytotoxic T lymphocytes response against the known melanoma antigens initially decreased after treatment to reappear when IL-2 was stopped. The tumor-specific peripheral T cells could be detected later in the lesions. The authors theorized that cell death produced by ECT may have attracted and primed denditric cells with the tumor antigens, which later migrated to the draining lymph node basin, and elicited a T cell response against those antigens expressed by the melanoma[100].

The first Human phase I trial of in vivo DNA electroporation of recurrent malignant melanoma was published in the USA in 2009[101]. EP with dose escalation of the interleukin-12 plasmid (in vivo DNA EP) was used in the treatment of 24 patients with stage III B/C or IV disease. It resulted in significant necrosis of melanoma cells and regression of the majority of treated lesions. In addition, clinical regression of untreated lesions suggested the induction of systemic anti tumor immune responses. The treatment was found to be safe with no significant reported toxicities . While additional studies in larger cohorts of patients are still necessary, to obtain reproducibility of these results, these data show a new method of inducing potent tumour specific immune responses individual to the patient.

There are no studies comparing ECT with other surgical modalities, however when taking into consideration the learning curve of other complex regional treatment modalities, the techniques of ECT are considered to be "user friendly" and easy to teach and learn. This technique can also be highly advantageous and useful in countries or hospitals where other modalities or resources are limited.[45]

7. Advantages of ECT

1. Excellent local tumor control rates (80-90%)
2. Minimal risk of damage to healthy surrounding tissue
3. Lower chemotherapy doses needed, minimizing the toxicity profile of the drug
4. There is no protein denaturation, which may elicit an undesireable immune response against self antigens
5. High safety profile without severe side effects
6. Good cost/benefit ratio profile: ambulatory setting, lower cost for drugs, and minimal equipment needed.
7. Treatments are well tolerated by patients
8. Improvement in the perceived quality of life

8. Patient selection and limitations of the procedure

The contraindications of the ECT could be divided into drug and procedure related.

A. Drug related contraindications[60]:

1. Known allergy to the drug to be administered
2. Interstitial lung fibrosis, if Bleomycin is going to be used
3. Kidney failure or limited renal function
4. When the cumulative dose of Bleomycin has reached >400,000 UI/m^2

B. Procedure related contraindications[43]:

1. For safety reasons ECT should not be used in patients with implanted electric devices such as pacemakers
2. In patients who may carry a higher risk of bleeding such as those on anticoagulants or with increased INR and platelets count < 70,000.

9. Limitations

With the current available electrodes, ECT has limitations when treating deep seated tumors[43]. Tumors larger than 3 cm^2 appear to have lower response rates (CR 73 %) to ECT [11,49,90] as compared to nodules smaller than 1cm^2 (CR 98%). These findings were not affected by either the cutaneous or subcutaneous location of the nodules [11].

When tumor nodules are located in irradiated or fibrotic tissues the needle electrode penetration may be problematic with a suboptimal delivery of the electrical current or drugs [90]. Nonetheless, if optimal needle penetration is achieved, ECT is equally effective in irradiated as in non-irradiated tissue[52]. If extensive disease (more than 15 lesions) is present repeated sessions may be necessary. In aggressive disease – while undergoing ECT new cutaneous nodules may emerge but palliative retreatment is worthwhile eventhough the systemic disease progresses rapidly[60].

This treatment modality has not been studied in randomized trials with other treatment techniques, such as ablative and perfusion or infusion procedures, or radiation therapy. More studies with longer follow up are still needed to evaluate disease free survival and to compare ECT to surgical excision, not only in the palliative setting but as a curative alternative in those patients unsuitable or unfit for a surgical procedure[19].

10. Toxicity and side effects

ECT has a low toxicity profile with limited side effects as compared to other regional therapies such as HILP or ILP [41]. However one of the limitations of fully assessing the toxicities of the treatment is the inconsistency of the large majority of the published studies documenting complications.

The systemic dose of Bleomycin is one twentieth of that used in the majority of chemotherapeutic regimens and thus the systemic side effects appear to be limited to nausea [18, 90]. There has been two reported cases of post procedure lypothymia[90].

The most common local side effects reported by the majority of patients are pain (75%) and erythema limited to the tumor and surrounding treated tissue [11, 45, 52, 66]. Most of the patients considered those symptoms tolerable as documented by the ESOPE study [52]. The

erythematous reaction usually recedes within a few days[11]. Local tumor necrosis has been reported in 42% of cases[66]. Delayed wound healing that may take several weeks or months to resolve, and epidermal erosions, and hematomas have been reported as rare events[66,73,75]. The injection site reactions appear to be low (Type I and II) in the Wiebendirk toxicity scale[90].

Transient muscle spasms myoclonus, secondary to muscle stimulation by the electrical pulses, have been reported in 25% with lower intensity contractions in up to 78% of patients[52,66]. Some authors advocate the administration of diazepam to alleviate these particular symptoms[73,75]. Interestingly the majority of patients are willing to continue treatment if indicated, since the side effects are tolerable[19].

11. What the future holds

Equipment evolution

Bioengineering developments and evolution of the technique continue to expand the applications of ECT as an alternative treatment for tumors that are inaccessible to current electrical probes. A redeveloped electroporator generator provides more flexibility to deliver the electric current at different phases of the cardiac cycle and the facility to connect several electrode probes around the tumor to deliver the electric pulses in synchrony. The other development is related to the type of electrodes. Longer array electrodes are now available to treat larger and deeper seated tumors, which in the past was a limitation of the procedure. These longer electrodes are insulated proximally to prevent short circuiting of current and to protect the normal tissues that are transgressed en-route to the tumor. These new devices have recently been applied clinically[19, 102-103]. Kos et al. [103] have proposed an algorithmic computer optimized analysis to treat deeper tumors, to minimize errors and to maximize treatment benefits.

Another novelty is the creation of finger applicators which allows the application of electrodes into lesions located in difficult to reach anatomic locations, such as the inside of the oral cavity. These finger electrodes have been already tested in the treatment of melanoma of the oral mucosa, and head and neck regions, with complete tumor regression [90].

New endoluminal electrodes to reach internal lesions within the gastrointestinal tract have been used in animal models, as well as tumors transplanted into rabbit liver and murine models [87, 104-106]. These studies are encouraging and have demonstrated both *in vitro* and *in vivo* (human solid tumor masses in nude mouse models), that the use of flexible electrodes is safe, feasible and reproducible.

There is still the need to create harder needle probes for use in those difficult to treat subcutaneous and cutaneous tumors. These needles must be capable of penetrating into hard fibrotic tissue, while minimizing the bleeding risks and maintaining an adequate electrical distance between the probes.

Nanopulses

Higher amplitude electric pulses or "nanopulses" are currently being evaluated. The use of shorter pulse durations in the nanosecond range are believed to create smaller pores that allow ions but not large molecules to penetrate the membrane. These higher electric fields increase the possibility of producing non resealable pores, thus producing the effect of irreversible electroporation, and consequently allowing the cells to lose their cytoplasm with concomitant cell death. This principle is being used for tumor ablation, palliation or both [43,

[107-109]. However in the treatment of melanoma, the advantages of these higher electric fields are not immediately evident.

ECT combination with Gene transfer, immunology and nanomolecules

Several studies suggest that the immune system is also involved in the mechanisms of response to ECT treatment and that this could be exploited for systemic disease control.
In a murine model [114], ECT followed by CpG oligonucleotide injection locally, produced an enhancement of the complete regression responses of tumors from 43 to 100 % , while also triggering systemic antitumor phenomenon with specific immune memory. Activation of dendritic cells released from the tumors are believed to be involved in this response with a recruitment of CD11c and CD11b receptors and an increase of TLR9 expression .
EP in combination with gene transfer is termed "Gene Electrotransfer ". It uses gene coding plasmids in order to transfer intracellularly a combination of genes, to either knock down the expression of a particular gene, or to stimulate temporary patterns of gene expression). The technique allows for avoidance of the biohazard issues intrinsic to viral vectors [101, 115-116].
Scientists at the Cork Cancer Research Centar in Ireland have investigated in an *in vivo* murine model, the application of EP and local gene therapy for malignant tumors. A plasmid coding for two immunogenes, granulocyte-macrophage colony-stimulating factor (GM-CSF) and the B7-1 co-stimulatory immune molecule were delivered by EP. This resulted in the complete regression of the majority (60%) of non immunogenic tumors, while eliciting a tumour specific systemic response that hindered the metastatic growth in the liver in a 100 % of the mice. This was a durable potent response with tumor specificity. When the remaining non responders non responders tumors were excised, an improved survival was observed when compared to the control groups, thus suggesting that the use of neoadjuvant electroporation and gene therapy given at an appropriate time interval prior to tumour excision or ablation could prevent the surfacing of metastatic disease [117-119]. Regulatory T cell depletion at the time of electrogenetherapy stimulated improved complete response rates from 60 to near 100% suggesting a potential for improving efficacy of electroporation based immunogene therapy.
ECT with injection of TNF-α intra or peritumorally and suboptimal doses of Bleomycin in mice might have a positive immunomodulatory effect, and possibly adds a systemic component to the localized ECT treatment[120] . It has been noted that EP with INF-α used to treat Mycosis Fungoides (subcutaneous lymphoma) lesions produced a 100 % CR [121] The cytotoxic action of INF-α was attributed to its increased tumoral concentration and the prolonged time of action produced by the EP. ECT either with INF-α or TNF-α might permit the usage of less toxic doses, while enhancing clinical local and systemic immunological response rates and minimizing systemic side effects[19].

ECT, radiotherapy and activation of bioreductive drugs

The "vascular lock" principle of EP has the potential to be used to "activate" bioreductive drugs such as Tirapazamine against neoplastic cells [43], due to its vascular disrupting effect of tumor blood supply. In addition ECT has been shown to have a synergistic effect with radiotherapy in preclinical investigations, opening the possibility to use it as a radiosensitising tool for the palliation of subcutaneous lesion[43, 122].
The continued development of improved diagnostic methods will allow for earlier diagnosis of metastases, and possibly open the opportunities for *in situ* neoadjuvant treatment of

higher risk malignant melanomas by means of immunogene or cytokine therapies, hence establishing tumour specific responses which could prevent recurrence and eradicate disseminated micometastases.

12. Summary

ECT with Bleomycin or Cisplatin is an effective treatment in the palliative management of unresectable recurrent cutaneous or subcutaneous melanoma metastases or in-transit disease, with OR rates of approximately 80-90%. ECT should now be considered as part of the armamentarium for treatment of loco regional advanced melanoma. The technology of ECT continues to evolve allowing for the treatment of metastatic lesions in other organs or anatomic regions. The principles of EP are already being applied in the clinical setting for the delivery of targeted therapies such as gene transfer and immunotherapy. These therapies along with ECT have the potential not only for local, but for distant treatment of tumors such as those of malignant melanoma, by stimulating a self-driven immune response to achieve systemic control of the disease

However studies evaluating long term follow up results of ECT in melanoma are still needed prior to considering it as an option for curative intent. ECT has been proven to be an excellent palliative option in treatment of recurrent unresectable or in-transit disease.

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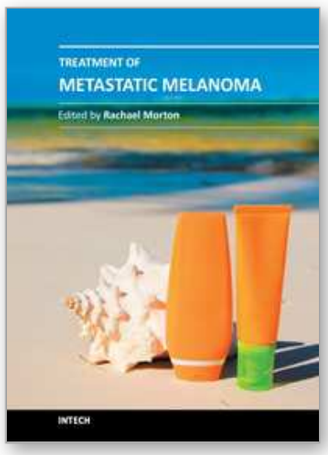

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Surgery continues to be the mainstay treatment for melanoma localized to the primary tumor and/or lymph nodes. Results from randomized controlled trials indicate that sentinel node biopsy for the treatment of cutaneous melanoma of intermediate thickness has a beneficial effect on recurrence rates, and adjuvant radiotherapy to regional lymph node fields following surgical resection reduces loco-regional recurrence in patients at high risk of relapse. Isolated limb perfusion, electrochemotherapy, and photodynamic therapy continue to be evaluated for treatment of stage IV disease. However, the greatest excitement in new treatment has been with targeted therapies for genetic mutations. In particular, the promising results of partial and complete tumor response in stage IV disease from early phase trials of the B-RAF kinase inhibitors. This book provides a contemporary insight into the therapeutic treatment options for patients with metastatic melanoma and is relevant to clinicians and researchers worldwide. In addition, an update on current clinical trials for melanoma treatment has been included, and two chapters have been reserved to discuss the treatment of oral and uveal melanoma.

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