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Case Report

Electrochemotherapy in The Treatment of Melanoma and Non-Melanoma Cancer: The Synergistic Effect with Immunotherapy-A Series of Clinical Cases

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ABSTRACT

Objective: In the current oncologic landscape, where immunotherapy plays a central role in the management of both melanoma and non-melanoma skin cancers across various clinical settings, we explored the potential added value of Electrochemotherapy (ECT) as a loco-regional treatment. Can ECT, by enhancing the intracellular delivery of chemotherapeutic agents *via* reversible electroporation, offer synergistic benefits when integrated with systemic immunotherapeutic strategies? In this study we presented three cases of patients with advanced cutaneous malignancies treated with ECT in combination with Immune Checkpoint Inhibitors (ICIs), with the aim of exploring the therapeutic potential of this multimodal strategy.

Methods: Three patients diagnosed with squamous cell carcinoma or melanoma underwent one or more ECT procedures alongside systemic immunotherapy (cemiplimab or nivolumab). Clinical outcomes were assessed through radiological and clinical evaluations.

Results: All patients achieved a complete response, with sustained local and systemic disease control and good overall tolerability.

Discussions: These findings supported the hypothesis that ECT may potentiate the efficacy of immunotherapy by enhancing the immunogenicity of the tumor microenvironment.

Conclusions: The combination of ECT and immunotherapy appears to be a promising approach for patients with cutaneous malignancies, particularly in clinical settings where standard therapies are contraindicated or less effective. Further studies are warranted to optimize patient selection and treatment timing and to validate the synergistic potential of this therapeutic strategy.

Keywords: Electrochemotherapy; ECT; Immunotherapy; Melanoma; Squamous cell carcinoma of the skin; Cemiplimab; Nivolumab

INTRODUCTION

Electrochemotherapy (ECT) is a locoregional treatment that facilitates the intracellular delivery of cytotoxic agents, such as bleomycin or cisplatin, through high-intensity electrical pulses. This mechanism, known as reversible electroporation, transiently increases cell membrane permeability [1]. ECT has demonstrated clinical efficacy in both cutaneous and deep-seated malignancies, including melanoma, basal cell carcinoma and cutaneous metastases from various origins [2].

The use of ECT was associated with a 700-fold increase in bleomycin cytotoxicity compared to the drug alone. The treatment has demonstrated favorable efficacy and safety, with

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minimal side effects, also due to the localized delivery of the drug directly into cancerous tissue [3]. Additionally, the use of ECT resulted in an organ-sparing effects, preserving the functionality and patient quality of life. This appears particularly valuable in areas where traditional surgery or radiotherapy may cause disfigurement or significant morbidity [1].

Emerging research has reported a potential synergy between ECT and immunotherapy [4-7] in the treatment of skin tumors, melanoma and non-melanoma, although its role, as a choice of local-regional skin treatment, is not yet sufficiently clear.

Based on the literature and what has been observed in our clinical practice, it can be hypothesized that the ability of ECT to induce immunogenic cell death primes the immune system, creating a favorable environment for increased efficacy of immune checkpoint inhibitors or other immunotherapeutic strategies [8].

The role of the microenvironment, therefore, appears to represent a key figure in describing the synergy and mutual enhancement of the two mechanisms: immunotherapy and electrochemotherapy. In solid tumors, particularly cutaneous ones, the higher electrical conductivity compared to surrounding tissues directs the current mainly through the tumor. The cell membrane permeabilization further increases the conductivity, leading to redistribution of the electric field and enhancing the effectiveness of electrochemotherapy. Appropriately calibrated electrical pulses, transmitted through special needles, induce a reversible permeabilization, allowing the absorption of the cytotoxic drug by tumor cells, while preserving healthy tissues. Conversely, excessively high pulses result in irreversible permeabilization and localized necrosis [9].

Furthermore, ECT not only induces direct tumor cell death, but also has the effect to triggering systemic antitumor immune responses. This effect could optimize the treatment efficacy, prolong the response duration and ultimately improve the quality of life of patients with advanced or metastatic tumors.

Mechanism of action of electrochemotherapy

ECT is performed according to standardized protocols defined by the European Standard Operating Procedures of Electrochemotherapy (ESOPE), most recently updated in 2018 [10]. These guidelines define both the criteria for the selection of patients eligible for treatment and the technical modalities for the proper performance of the procedure [11]. The ESOPE protocol involves the delivery of eight square-wave pulses, each lasting 100 microseconds, typically using voltages ranging from 100 to 1,000 volts. These settings are designed to generate an electric field in the range of 0.6 to 1.5 kV/cm, which is sufficient to induce reversible electroporation of the cell membrane [12] and the cornerstone is applying electric pulses to tumor tissue *via* electrodes. In ECT for cutaneous tumors, increasing the electrode-tissue contact surface, by compressing the tumor between parallel plate electrodes, improves electric field distribution, enhances complete response rates and reduces damage to surrounding healthy tissue, underscoring the importance of optimized electrode placement [13]. These pulses generate transient nanopores in the cell membrane's lipid bilayer, a process known as reversible electroporation. This increases the membrane's permeability, allowing hydrophilic or poorly permeable molecules, such as bleomycin or cisplatin, to penetrate the cell [1-5]. The parameters of the pulses, including their amplitude, frequency and duration, are finely tuned to optimize cell permeabilization while minimizing thermal or structural damage to surrounding tissues [14]. Electroporation facilitates the intracellular transport of cytotoxic drugs, dramatically increasing their concentration within tumor cells. For instance, the cytotoxicity of bleomycin increases up to 700-fold under electroporation conditions compared to drug administration alone. This mechanism bypasses drug resistance pathways, such as efflux pumps, which can otherwise limit the efficacy of chemotherapeutic agents in multidrug-resistant tumors [1-5].

The immunotherapy and its potential synergy with electrochemotherapy

ECT exerts cytotoxic effects and simultaneously activates the immune system through Immunogenic Cell Death (ICD). This process converts dying cancer cells into an anticancer vaccine by releasing Damage-Associated Molecular Patterns (DAMPs) such as Adenosine Triphosphate (ATP), High-Mobility Group Box 1 (HMGB1) and Calreticulin (CRT) [15]. CRT promotes phagocytosis by dendritic cells, ATP attracts and activates immune cells and HMGB1 stimulates the maturation of antigen-presenting cells, collectively triggering an adaptive immune response that may, in preclinical models, elicit an in-situ vaccination effect [16].

In this context, Interleukin-12 (IL-12) is a potent immunostimulatory cytokine that activates T and NK cells, induces Interferon-gamma (IFN-γ) production and reshapes the tumor microenvironment toward a more immunogenic profile. Gene Electro-Transfer (GET) of IL-12 plasmids enables localized and controlled cytokine release, demonstrating efficacy in preclinical studies, veterinary models and early clinical trials [17]. Combining ECT with IL-12 GET enhances tumor control, especially in poorly immunogenic tumors such as B16F10 melanoma, where ECT alone is less effective. In contrast, the addition of IL-12 provides limited benefit in more immunogenic tumors like CT26 colorectal carcinoma [18].

The first-in-human phase I trial using intra-tumoral gene electro-transfer of a novel IL-12 plasmid (phIL12) in basal cell carcinoma, aimed to boost local and systemic immune responses as an adjuvant to local therapies like ECT. The study evaluates its safety, tolerability and immunological activity, supporting the hypothesis that IL-12 GET is a promising immuno-stimulatory strategy in cutaneous tumors [19].

In a 73-year-old patient diagnosed with melanoma after disease progression treated with pembrolizumab, ECT was performed followed by ipilimumab and a rapid almost complete remission was obtained, despite severe immune-related toxicity [20].

Despite these promising findings, clinical translation faces challenges, including variability in tumor immunogenicity, optimization of gene dosing parameters and management of cytokine-related systemic toxicity.

The aim of this description of clinical cases was to implement the number of cases of treated patients, highlighting the particular connection between immunotherapy and local-regional treatment.

MATERIALS AND METHODS

Three clinical cases, involving patients diagnosed with skin cancer in different immunotherapy treatment settings, followed at the Sant' Andrea University Hospital in Rome, Italy, are presented. The patients have signed consent for data processing.

Three clinical cases, involving patients diagnosed with cutaneous cancer, in different immunotherapy treatment settings, followed at the Sant' Andrea University Hospital in Rome, Italy, are presented.

Case 1: Clinical history and findings

I.G., a male 90-year-old patient at diagnosis, ECOG Performance Status 1.

In remote pathological history: Parkison's disease, hypothyroidism and arterial hypertension. The oncological history began in November 2023, with the development of a 5 cm irregular lesion in the left peri-auricular region. The lesion was initially excised; a local recurrence was observed within a few months. Histological examination was conclusive for squamous cell carcinoma. Staging investigations were performed, including ultrasound of the neck, lymph nodes and peri-auricular region, as well as a contrast-enhanced total body CT scan. All tests were negative for distant metastatic lesions.

Following a comprehensive assessment of the patient's overall clinical status, including a cardiological evaluation, we decided for a local-regional electrochemotherapy procedure. This decision was based on the progressive enlargement of the lesion, the sudden change in color over the weeks, tending towards bright red and the hearing difficulty reported by the patient, the need for rapid and effective treatment. The patient underwent electrochemotherapy procedure on 16 April 2024 (Figure 1), without complications related. He was discharged after one night of hospital observation. Topical anti-edema drugs and antibiotics were administered and Mr. I.G. was monitored periodically with the dressings. The treatment was well tolerated. The lesion was necrotic after treatment, with crusted lesions that gradually disappeared, as expected. First-line therapy with cemiplimab at a dosage of 350 mg every 21 days was subsequently initiated, however, the patient received only two administrations [21]. Treatment was interrupted as a result of the exacerbation of hypothyroidism, which was already known in the patient's medical history. ECT procedure was repeated on 17 September 2024 as the lesion increased in size again, it presented purulent and inflammatory material (Figure 2), which we assumed is linked to the hyperactivation of the immune system against tumor cells. The lesion was carefully medicated in the following weeks. After 2 months, a total loco-regional response was evident (Figures 3 and 4). The patient continued to be monitored with clinical and instrumental tests, negative for distant recurrence. Hearing function improved and the reported quality of life, considering the age, was very good.

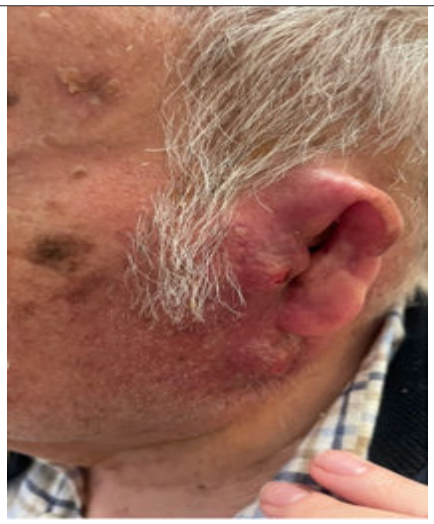

Figure 1: Lesion on the left per-auricular region, before the first elelctrochemotherapy.

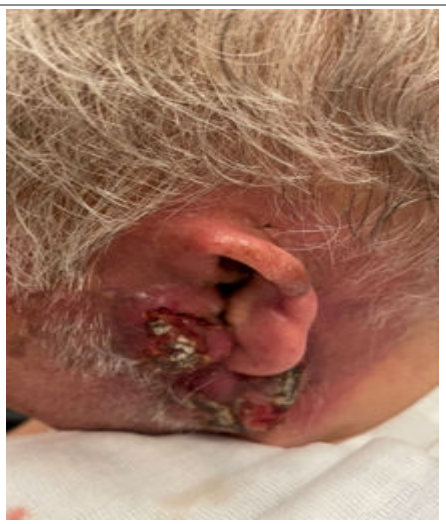

Figure 2: Lesion on the left per-auricular region, after the first elelctrochemotherapy (immune reaction).

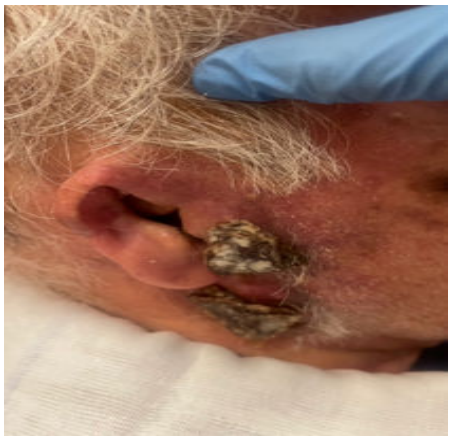

Figure 3: Lesion on the left per-auricular region, after the second elelctrochemotherapy.

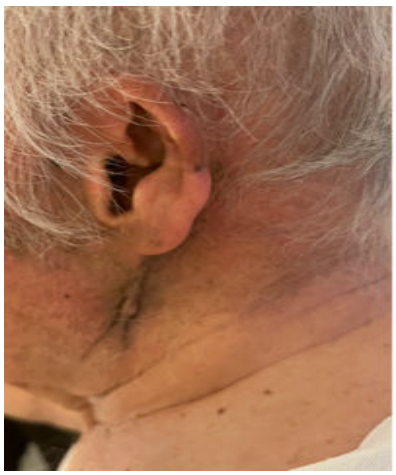

Figure 4: Complete response after the second elelctrochemotherapy.

Case 2: Clinical history and findings

M.R., a male 74-year-old patient at the onset of the disease, diagnosis of melanoma, ECOG Performance Status 0. The patient underwent surgical resection and histopathological examination revealed: PD-L1 negative; *V600E*, *V660K*, *V600D*, *V600R*, *V600M* mutations in exon 15 of the gene *BRAF* absents (wild type).

In remote pathological history: chronic ischemic heart disease, atrial fibrillation and chronic renal failure. On 6 June 2023, loco-regional ECT surgeries were carried out on a scalp lesion and on minute facial lesions, metastasis in transit (Figures 5 and 6).

In June 2023 an adjuvant medical therapy according to the nivolumab 240 mg every 14 days was started [22]. The treatment continued until September 2024, when it was interrupted due to the onset of gallstones and gallbladder empyema, for which the patient underwent cholecystectomy.

In May 2024, the patient developed cutaneous lesions on the face: frontal region and cheekbones. In light of the clinical findings, loco-regional ECT was proposed and administered. Treatment was well tolerated, the facial lesions, after an initial necrotizing phase, went towards total disappearance (Figures 7 and 8).

The last re-evaluation with total body global tomoscintigraphy with 18 fluorodeoxyglucose (PET/CT) and brain resonance (MRI) with contrast, carried out in December 2024, resulted negative for disease recurrence.

The patient's quality of life is excellent, with rare interruptions from work. He continues to be followed with clinical and instrumental tests, as per guidelines. The loco-regional treatment has allowed us to block the disease reactivation mechanism triggered.

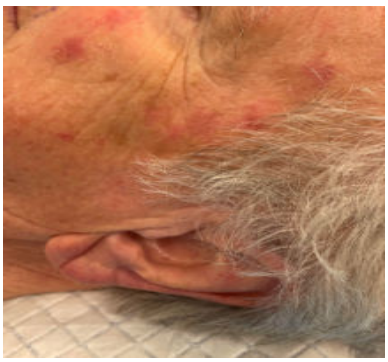

Figure 5: Left facial lesion before the last elelctrochemotherapy (metastasis in transit).

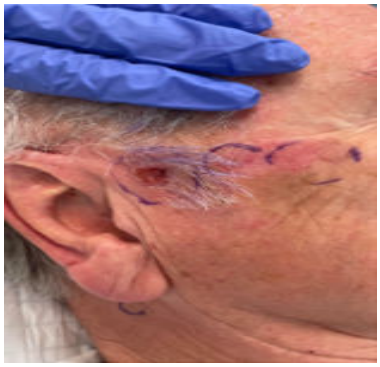

Figure 6: Right facial lesion before the last elelctrochemotherapy (metastasis in transit).

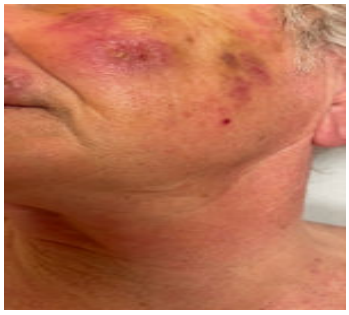

Figure 7: Left facial lesion (healing areas), after the last elelctrochemotherapy.

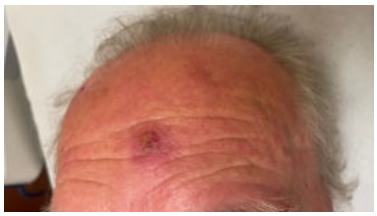

Figure 8: Left facial lesion (healing areas), after the last elelctrochemotherapy.

Case 3: Clinical history and findings

P.P., a male, 81-year-old patient at diagnosis, ECOG Performance Status 1. In remote pathological history, in 2019

cardiac defibrillator implant. Diagnosis of invasive squamous cell carcinoma of the scalp. The patient's oncological history began in May 2022 with the appearance of a suspicious 4 cm scalp lesion. A biopsy was performed at that site and histopathological examination revealed an invasive, moderately differentiated (G2) squamous cell carcinoma of the skin. Considering the disease biological and molecular characteristics, staging, the patient's clinical condition and excluded distant metastases, the patient underwent an ECT procedure targeting the scalp lesion on July 2022 (Figures 9 and 10). The patient was scheduled for clinical and radiological reassessment. On February 2023 the patient underwent surgery to remove the skin lesion on a new onset scalp with a widening of the margins of DM 0.4 cm, histological examination: squamous carcinoma invasive. He was again sent for clinical and instrumental follow-up checks. On April 2024, exeresis of left lateral cervical lymphadenopathy was carried out DM 2.2 cm, description macroscopic: lymph node site of metastatic neoplastic repeat of squamous carcinoma (p40+, ck7-) keratinizing. TC Total body in August 2023 described a new lymphadenopathy in the left lateral cervical area with a diameter of 27 mm and other lymph-nodes smaller in size, site of disease recurrence. Taking into account the biological characteristics and staging, there was an indication for first-line treatment according to the cemiplimab 350 mg every 21 days, which the patient began on September 2024 [21].

The second session of ECT was carried out on November 2024 (cranial theca and surrounding area to the nasal region), without complications (Figures 11 and 12). A complete loco-regional response was obtained, but the extent, also in terms of size, of the ulcerated and bleeding lesion treated, at the level of the scalp, makes it a case of notable response.

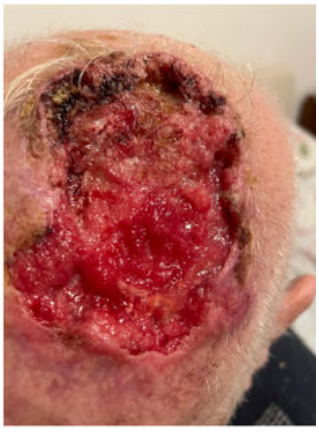

Figure 9: Lesion scalp before the first elelctrochemotherapy procedure.

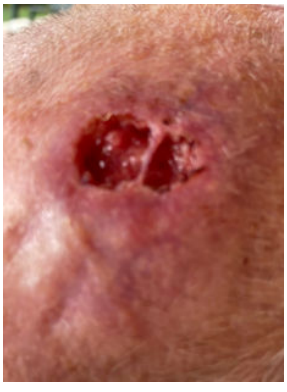

Figure 10: Lesion scalp before the first elelctrochemotherapy procedure.

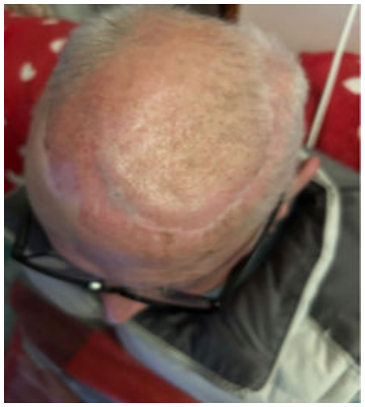

Figure 11: Complete response after elelctrochemotherapy.

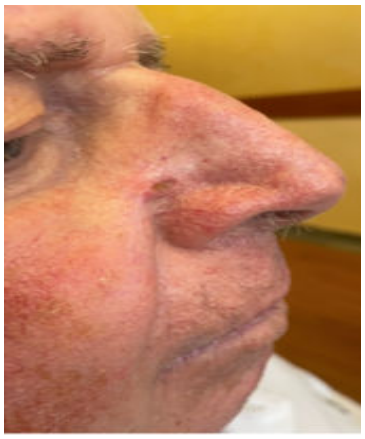

Figure 12: Complete response after elelctrochemotherapy.

DISCUSSION

ECT has emerged as a valuable loco-regional therapeutic option capable of inducing long-lasting local responses, particularly in patients with cutaneous malignancies where systemic therapies alone may be insufficient or contraindicated. Beyond its established cytotoxic effect, ECT is increasingly recognized for its immunomodulatory potential. The ability of ECT to induce immunogenic cell death may serve as an in-situ vaccination strategy, promoting the release of tumor-associated antigens and activating innate and adaptive immune responses. This mechanism provides a strong biological rationale for combining ECT with Immune Checkpoint Inhibitors (ICIs) in selected patients.

Our research group have already reported that Electrochemotherapy is a valid loco-regional treatment with a long-lasting long-term response [23].

This case series report includes three patients, two with advanced cutaneous Squamous Cell Carcinoma (cSCC) and one with melanoma in the adjuvant setting, treated with ECT integrated into systemic immunotherapy regimens. All patients achieved a complete clinical and radiological response. Importantly, the treatments were well tolerated, with minimal adverse effects and rapid functional recovery. These results translated into preserved or even improved quality of life, a key factor in treatment selection, particularly for elderly or comorbid patients.

The therapeutic synergy observed between ECT and mmunotherapy found supports the hypothesis that ECT can activate the immune system and enhance systemic antitumor responses. This is consistent with recent findings reported in the literature, such as in the recent study by [18]. In their work, the authors systematically reviewed and analyzed data from 10 clinical trials, highlighting the growing clinical relevance of this combinatorial strategy in different tumor types. Although available clinical data remain limited, this review, together with other retrospective studies in melanoma and breast cancer, suggests that the combination of ECT and immunotherapy may lead to superior outcomes compared to loco-regional treatment or immunotherapy alone.

However, despite these promising results, significant questions remain unanswered, also considering the small number of cases described in the literature. Larger prospective studies and randomized controlled trials are needed to confirm the efficacy and durability of this therapeutic synergy. Specifically, future research should focus on optimal patient selection, timing of ECT concerning systemic therapy and identification of predictive biomarkers of response. Furthermore, understanding the tumor microenvironment's role and how ECT modulates immune infiltration may help tailor treatment to maximize benefit.

Another relevant clinical consideration is the high tolerability profile of ECT, which makes it especially suitable for frail populations and patients with comorbidities, as seen in our cases. Moreover, the rapid resolution of symptoms and recovery of function, such as improved hearing and maintenance of occupational activities, highlight the added value of ECT in preserving quality of life alongside disease control.

Future research should focus on integrating predictive biomarkers and combining ECT+IL-12 with immune check point inhibitors to maximize systemic tumor control. Combining locoregional therapies such as ECT with systemic immunomodulatory strategies represents an attractive avenue to improve therapeutic efficacy, prolong treatment response and improve quality of life in patients with advanced or metastatic cancers.

In summary, our experience suggests that the combination of electrochemotherapy and immunotherapy represents a promising and well-tolerated strategy for the management of advanced skin cancers. While further studies are warranted to define its place in clinical guidelines, the growing body of evidence, including our own, supports a wider use of ECT as a key component of integrated oncologic care.

CONCLUSION

This case series confirms the potential of the combination of ECT with immune checkpoint inhibitors as a promising and well-tolerated therapeutic strategy for patients with advanced skin cancer, in line with the other results already reported in the literature. All patients achieved complete clinical and radiological responses, with good overall tolerability and durable disease control, both locally and systemically. These results reinforce the growing evidence that ECT has a synergistic role with immunotherapy, enabling the modification of the microenvironment and triggering mechanisms that determine apparently better responses, compared to monotherapy. Future research should focus on clarifying the underlying synergistic mechanisms to fully exploit the therapeutic potential of this multimodal approach.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

CONSENT TO DATA PROCESSING AND PUBLICATION OF IMAGES

Consent was adequately provided by patients.

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