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Novel Indications for Electrochemotherapy in Dermato-Oncology

Thesis of the Dissertation

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1. INTRODUCTION

Electrochemotherapy (ECT) is a well-established method for treating cutaneous and subcutaneous tumors. It operates on the principle of reversible electroporation, serving as a physical drug-delivery system to facilitate the entry of non-permeant or poorly permeable molecules into cells. Short electric pulses locally applied induce an increase in membrane permeability, allowing the penetration of chemotherapeutic drug molecules. This technique combines electroporation with chemotherapeutic drugs, with bleomycin and cisplatin identified as the most efficient agents in conjunction with electroporation, exhibiting increased cytotoxicity by 8000 and 80-fold, respectively.

Noteworthy advantages include cell-type selectivity, sparing healthy cells while targeting rapidly dividing tumor cells, thereby enabling treatment with a wide safety margin. ECT is compatible with previously irradiated or operated areas and can be seamlessly integrated with other local or systemic therapies, such as radiation or immunotherapy, with the option for repetition if necessary. An additional favorable aspect of ECT is its vascular effect: it induces a "vascular lock" through vasoconstriction at the site of the electric pulse application. This effect negatively affects neovascularization, particularly affecting rapidly dividing endothelial cells similar to tumor cells. This feature is particularly advantageous when treating bleeding tumor nodules

ECT can be used to treat cutaneous or subcutaneous tumor nodules regardless of their histological types. In melanoma patients, who have in-transit metastases without systemic involvement, or patients with lentigo maligna (LM) or lentigo maligna melanoma (LMM) it can be used with a curative intent. An additional curative application of ECT is the treatment of patients with numerous NMSCs, particularly those located in the head and neck area. In such instances, ECT offers the potential of comparable tumor control to surgical excision and better cosmetic outcomes. In the context of unresectable, locally invasive and/or destructive tumors (either primary or secondary), ECT serves a palliative role, providing relief for the patients with large, symptomatic cutaneous tumors, such as melanoma or breast cancer metastases.

LM, LMM and acral lentiginous melanoma (ALM) are characterized by ill-defined borders, often extensive involvement, and typically manifest on the face or the soles of the feet. The difficulty in treating these melanomas lies in the need to preserve function and achieve satisfactory aesthetic outcomes while ensuring effective tumor control. Elderly patients with larger lesions may not be suitable for extensive reconstructive surgeries, particularly in

cosmetically sensitive areas of the face, as such procedures could lead to significant morbidity. This underscores the need for alternative treatment options, which currently include radiotherapy, topical imiquimod and other local ablative treatments, like ECT.

In 10-17% of patients with malignant melanoma, cutaneous metastases develop, posing a significant treatment challenge. In addition to systemic therapy, addressing these metastases is crucial, as it greatly impacts patients' quality of life. Treatment options include surgical excision, irradiation, locoregional chemotherapy and lesional therapies, such as diphencyprone, Rose Bengal (PV-10), oncolytic virotherapy (T-VEC), imiquimod and ECT. The latter has already been included in the ESMO melanoma guidelines. Interestingly, preliminary experiences suggest that ECT may elicit an immunogenic cell death, thus functioning as a form of in situ vaccination. Therefore, its combination with either ICIs or other forms of immunotherapy is of potential interest.

The most common malignancies associated with impaired immune status are NMSCs, which can be numerous, fast growing, and aggressive. After surgical resection, local recurrence and/or distant dissemination occurs often, in about 40% of cases. The treatment of multiple tumours is challenging for several reasons. The large number of tumours means that surgical treatment can only be performed in several sessions, imposing great burden on patients. Preclinical studies have demonstrated that ECT induces immunogenic cell death and that the levels of certain proinflammatory cytokines (IL-1β, IL-10, IL-6, TNF-α, IFNγ) and markers specific for macrophages and natural killer (NK) cells are elevated after treatment. Studies to date have shown that the treatment in immunocompetent mice is more effective and the tumour response is longer lasting compared to immunodeficient animals. ECT has the advantage of being able to treat several tumours at the same time, offers a high objective tumour response, a favourable side effect profile, repeatability and a favourable aesthetic outcome.

The advent of advanced systemic oncological treatments like immunotherapy and checkpoint inhibitors has led to increased patient survival. Consequently, individuals now must cope with cutaneous malignancies for an extended duration. In managing cutaneous metastases, the objective is to mitigate symptoms such as pain, odor, ulceration, suppuration, or bleeding.

2. AIMS

-Electrochemotherapy in the Treatment of Lentigo Maligna, Lentigo Maligna Melanoma, and Acral Lentiginous Melanoma: Our aim was to report the treatment outcome in three cases of elderly patients with LM, LMM, and ALM treated with ECT.

-Combination of Pembrolizumab with Electrochemotherapy in Cutaneous Metastases from Melanoma: A Comparative Retrospective Study from the InspECT and Slovenian Cancer Registry: The aim of this study was to evaluate the local response and tumor control on cutaneous metastases in melanoma patients who received pembrolizumab and ECT. Moreover, we assessed toxicity, systemic disease progression, and survival.

-Electrochemotherapy for Non-Melanoma Skin Tumors in Immunosuppressed Patients – a Prospective Cohort Analysis: The aim of this study was to evaluate the efficacy, toxicity and impacts on quality of life of ECT for NMSCs in immunosuppressed patients compared to non-immunosuppressed patients.

- Quality of Life Changes After Electrochemotherapy: This study aims to assess the impact of ECT on the QoL of patients, addressing a gap in the current literature.

3. MATERIALS AND METHODS

- Three elderly patients were treated with ECT for extensive, difficult-to-treat tumor lesions (LM, LMM and ALM) according to the ESOPE guidelines

- Data of patients with histologically proven stage IIIC–IV melanoma with measurable cutaneous metastases suitable for ECT application were collected (patient demographics, disease stage, location, number and size of skin metastases, previous treatments, ECT parameters, and toxicity). The study cohort (n = 130) included three subgroups according to the received treatment: pembrolizumab plus ECT (n = 45), pembrolizumab alone (n = 44), and ECT alone (n = 41). The data of the pembrolizumab–ECT and ECT groups were retrieved from the InspECT database, while those of the pembrolizumab cohort were collected from the Clinical Registry of Skin Melanoma (CRRS). The median interval between the initiation of immunotherapy and ECT was 2.4 months (range, from−3 to 41 months). The median time between diagnosis and ECT in the pembrolizumab-ECT group was 2.8 years (0.6–10.4), and, in the ECT group, it was 2.0 years (0.3–22.3) (p = 0.180). Only two patients received the first ECT treatment before the start of immunotherapy, at 89 and 5 days. ECT was performed

according to the ESOPE. Local response was evaluated at 6 months after ECT, adapting the Response Evaluation Criteria in Solid Tumors (RECIST version 1.0). Systemic response was assessed at 6 months after ECT in the pembrolizumab–ECT group and 6 months after immunotherapy initiation in the pembrolizumab group according to the immune-related response criteria (irRC) using PET-CT, CT, MR, and bone scintigraphy. Statistical analysis was conducted with NCSS software (Hintze, J. (2013).

- Fourty-four patients underwent ECT at the Department of Dermatology and Allergology in Szeged, Hungary between 2016 and 2024. All patients had a histopathologically verified diagnosis of keratinocyte skin cancer. The study population consisted of two distinct groups: one comprising immunosuppressed patients and the other consisting of non-immunosuppressed individuals. ECT treatment was performed according to the ESOPE guidelines. After treatment, patients were followed regularly (every 3 months) – prospective data collection was carried out. During visits, tumor response, side effects, QoL questionnaires were recorded, as well as photographic documentation, and the appearance of novel tumors was also documented. Tumor response was evaluated according the RECIST 1.0 criteria. Adverse events were assessed using the Common Terminology Criteria for Adverse Events (CTCAE 4.0). QoL was assessed using the EQ-5D (Euro Quality of Life - 5 dimensions) questionnaire, which was completed by the patients themselves. R 4.2.3. software was used for the statistical analysis.

- A prospective, observational, single-center study was carried out to evaluate the impact of ECT on the patients' QoL. Data collection was carried out on patient demographics, medical history, nodule sizes and localization, details of treatment and QoL. Patients with primary or secondary cutaenous tumors were included in the study. At the baseline visit before the treatment (1 day before or on the day of the treatment), data were collected about patients' demographics, primary tumor, disease stage, previous local and systemic treatments. QoL and pain questionnaires were also completed before the treatment. The follow-up visit was scheduled in 1-3 months, where the patients were asked to fill out the questionnaires. The median follow-up time was 47 days (28-91). IBM SPSS software (Statistical Packages for Social Sciences) 29.0.0.0 (version 241) software was used for the statistical analysis.

4. RESULTS

- The first two patients showed a complete, the third patient showed a partial response to the treatment. All three patients experienced only transient, local, low grade side effects including mild erythema, edema and crusting.

- Local response was significantly higher among patients who received ECT—alone or with pembrolizumab—when compared to pembrolizumab alone. The analysis of patients with local progression and time to local progression favored the pembrolizumab–ECT group over the pembrolizumab group. The systemic ORR was similar between groups. The number of patients who had systemic progression was similar, but the time to systemic progression, as well as the systemic control of the disease, was significantly longer in the pembrolizumab–ECT group than in the pembrolizumab group. The patients in the pembrolizumab–ECT group had better local PFS, systemic PFS, and OS when compared to the pembrolizumab group. No serious adverse events were reported. We observed the side effects associated with pembrolizumab and ECT separately, as well as during the combined treatment. In the case of PD-1 inhibitors, immune-related side effects were observed, while for ECT, local toxicity was noted, including ulceration, suppuration, odor, and hyperpigmentation. In patients who received both treatments, the side effects did not accumulate.

- NMSCs were treated located in the head and neck region, trunk and upper limbs, predominantly on skin surfaces exposed to light. Treated lesions included actinic keratoses (AK), basal cell carcinomas (BCC), and squamous cell carcinomas (SCC). None of the patients had known lymph node or internal organ metastasis at the time of treatment.
In the immunosuppressed group, a total of 156 tumours, 82 target lesions were treated. In the control group, we treated 183 tumours, with 157 target lesions. The average number of lesions treated per patient was 11 in the first group, 6 in the second group. The mean of the largest diameter of the tumours was 35.8 mm (12-160 mm) in the first group, 27.6 mm (9-105 mm) in the second group. Fisher's exact test was used to compare tumour response rate in the two groups. Three months after treatment, tumour lesions in the non-immunosuppressed group showed a significantly higher chance to respond to ECT treatment ($p=0.001$, odds ratio=3.18). Six months after ECT statistically significant difference was not observed in tumour response between the two groups ($p>0.05$). ECT treatment was repeated in 6 out of the 14 immunosuppressed patients. Among them, 3 patients showed PR in the treated area and/or experienced local recurrence, therefore, ECT was repeated 5, 11 and 12 months after the first session. In addition, surgical excision, PDT treatment and cryotherapy were used to treat the

novel tumours, which appeared in some cases outside the treated area. One patient developed histologically confirmed lymph node metastasis of SCC, and we opted for radiotherapy for the primary tumour region and the metastasis. The patient with the perineal verrucous carcinoma received PD-1 inhibitor immunotherapy after ECT, due to PR after ECT treatment. No systemic toxicity was observed. Local side effects included odour, suppuration, hyperpigmentation, ulceration, and transient pain well controlled with analgesics. At 3 and 6 months after ECT, no significant difference was noted in toxicity between the two groups (Fisher's exact test, $p>0.05$). The patients had an ECOG performance status 0 or 1, therefore no problems with mobility, self-care, or performing usual activities were reported. Regarding the EQ-VAS and pain VAS scores, there was no significant difference between the two groups (Fisher's exact test, $p>0.05$).

- Our study included 62 patients (42 male and 20 female) who underwent ECT between 2015.10.22. and 2022.06.21. The median age was 70 years (12-91 years). All patients had an ECOG status of 0 or 1. The three subtypes according to histology were the following: 14 patients (23%) had metastases of malignant melanoma (MM), 40 (56%) had keratinocyte cancer (KC: basal cell carcinoma (BCC) or squamous cell carcinoma (SCC)) and 13 patients (21%) had other cutaneous tumors (metastases of breast ductal adenocarcinoma, Kaposi's sarcoma, angiosarcoma, epitheloid sarcoma, malignant Schwannoma). 39 patients (47%) had tumor nodules smaller than 3 centimeters ($T<3$), and 14 patients (28%) had previously received radiotherapy (RT1). The number of tumors treated per patient ranged from 1 to 33 (median: 6). Before the ECT session, the mean EQ-VAS reported by our patients was 72.39 ± 13.201, which increased to 73.55 ± 15.696 three months later. Although the increase was not statistically significant ($p=0.438$), this is still a favorable outcome considering the treatment was carried out with a palliative intention in most of the cases and we could preserve the patients' overall health state. There was no statistically significant difference in the changes in EQ-VAS values between the subgroups divided by histological types. Patients with previously irradiated tumor nodules had a significantly lower EQ-VAS score after the treatment ($p=0.047$), while the other group had a statistically not significant increase ($p=0.240$). Tumor size also had an effect on changes in the EQ-VAS. Those patients, who had smaller tumors, their EQ-VAS was significantly higher after the treatment ($p=0.035$). Patients with bigger tumor nodules had a slight decrease in this value. The pain value of the patients decreased from 1.89 ± 2.765 to 1.68 ± 2.715 months after ECT ($p=0.287$). There was no difference observed according to tumor histology or previous irradiation. Patients with

smaller tumor nodules had a significantly lower level of pain after the treatment (p=0.029). As for those patients, who had at least one tumor bigger than 3 cm, a minimal increase was observed in the pain scores. The average value was 0.8044 ± 0.2297 before ECT and 0.8447 ± 0.2208 after, so the increase is not statistically significant (p=0.132). There were no statistically significant differences between the EQ-5D index in patients with different histological tumor types. Patients who had previously received radiotherapy presented with a significant increase in the EQ-5D index values (p=0.012). When assessing the index values of those with smaller diameter tumors, they had a significant increase (p=0.012), those with larger lesions, they had a nonsignificant decrease.

5. DISCUSSION

- The management of LM, LMM and ALM poses significant challenge due to their ill-defined margins, large sizes, and frequent localization in cosmetically and functionally sensitive areas. Our cases highlight ECT as a promising alternative treatment modality for these tumors, supported by histological results from post-ECT biopsies. ECT's ability to activate the immune response through non-thermal tumor ablation is particularly beneficial for immunogenic tumors like melanoma. It can be used alone, combined with other treatments, or repeated if necessary, and has shown efficacy even in previously irradiated tumors. Notably, the combination of ECT with topical imiquimod appears to enhance therapeutic outcomes. This aligns with the American Academy of Dermatology (AAD) guidelines, which recommend imiquimod as an adjuvant therapy after surgery, noting that combination therapies outperform surgery alone. Additionally, ECT offers favorable cosmetic outcomes by preserving healthy tissue, making it less invasive and improving quality of life, especially for visible tumors. However, ECT has limitations. This case series involves a small patient cohort, and longer follow-up is needed to assess long-term outcomes. Further studies with larger groups and extended observation are essential to optimize protocols and expand its clinical use.

- In this exploratory analysis, skin-directed treatment with ECT in melanoma patients treated with pembrolizumab proved to be a safe and effective therapy to improve tumor response and local control on cutaneous metastases. Interestingly, our data also show improved disease PFS and OS in the pembrolizumab–ECT group compared to pem-

brolizumab alone. There were no significant differences in terms of local toxicity among the three groups, indicating that pembrolizumab does not add to ECT local side-effects. Based on the significant improvement of local control, we believe that ECT should be considered as a safe and effective adjunct to the standard treatment of patients with persistent or nonresponsive cutaneous metastases. Additionally, we observed a longer systemic PFS and OS in the combined treatment group. Although our type of study cannot provide any causative correlation, the better outcome of patients in the combined treatment group suggests the involvement of a systemic immune response.

-Our prospective cohort analysis shows that ECT seems to be as effective and safe in immunosuppressed patients as in non-immunosuppressed patients for NMSCs. Treated tumours per patient and the largest tumour diameter were higher in the immunosuppressed group, which correlates with the findings of the above mentioned studies from the literature. Three months after ECT, immunocompetent patients showed a significantly better tumor response. However, by the 6-month follow-up, there was no statistically significant difference in response between the two groups. The antitumor effect of ECT is based on three major mechanisms: it induces mitotic cell death by causing double-strand DNA breaks, leads to transient hypoperfusion (the vascular effect), and triggers immunogenic cell death9. In immunosuppressed patients, the number and/or function of both innate and adaptive immune cells are diminished, which might explain the delayed effect of ECT observed at the later 6-month follow-up.

-Our study is the first one focusing on reporting quality of life outcomes of patients with various histological types of tumors. When evaluating the 3 levels of the EQ-5D questionnaire, we noticed a decrease in the proportion of patients who reported some problems with respect to pain/discomfort and anxiety/depression (Table 2). Overall, we observed a slight, but not statistically significant improvement in EQ-VAS, Pain-VAS and EQ-5D-index values after ECT. In the subgroup analysis, we found that patients who previously underwent radiotherapy had a significant decrease in EQ-VAS scores and a significant increase in EQ-5D index. This somewhat controversial result shows the importance of using multiple QoL questionnaires – one’s overall health state might decrease due to other factors (such as comorbidities or age), and at the same time, they might experience improvement in the five dimensions: mobility, self-care, performing usual activities, pain/discomfort and anxiety/depression. EQ-VAS, Pain-VAS and EQ-5D index both

increased significantly after ECT in those who had tumor nodules smaller than 3 cm. This result shows that this particular subgroup might benefit the most from ECT regarding QoL.

6. CONCLUSIONS

Our data demonstrates the efficacy and potential of ECT as a valuable treatment modality in dermatolo-oncology. These findings support the role of ECT as both a palliative and curative approach, broadening its clinical applicability and reinforcing its integration into oncologic treatment strategies.