Potential dual synergy between electrochemotherapy and sequence of immunotherapies in metastatic melanoma: A case report

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Abstract. Immune checkpoint inhibitors have changed the natural history of advanced melanoma. Despite this, a notable proportion of patients immediately relapse or develop resistance during immunotherapy, especially with the appearance of superficial metastases and consequently with a dramatic impact on clinical outcomes. Local treatment by electrochemotherapy (ECT), parallel to regional control with palliative aim, seems to release neoantigens potentially determining a significant systemic anticancer immune reactivation. The present study reported a case of a patient with metastatic melanoma receiving Pembrolizumab, electrochemotherapy and then Ipilimumab for in-transit and finally locoregional lymph nodes and distant bone metastases with experience of clinic-radiological remission. Specifically, the present patient progressed during adjuvant treatment with in-transit metastases on the scalp; he underwent two cycle of ECT obtaining partial and then unexpected and very fast nearly complete response with the Ipilimumab treatment. Concomitantly, he developed grade 4 endocrine adverse events (hypophysitis and diabetes mellitus type I) as immune-related toxicities. At 12 months from ECT the patient is in ECOG Performance Status 0 and he has resumed a regular social life. In our experience, ECT in two administrations increased and accelerated the response of Ipilimumab. The present confirmed its promising contribution in inducing a powerful immune response in order to overcome primary or acquired resistance to immune checkpoint inhibitors such as anti-programmed death antigen-1 drugs.

Introduction

Recently, novel immunotherapies directed against CTLA-4 (Cytotoxic T Lymphocyte Antigen-4) and PD-1 (Programmed Death antigen-1) afford the opportunity to effectively treat loco-regional and metastatic melanoma, ensuring significant reduction of risk for relapse, with relatively durable responses and definitely with a considerable improvement of survival (1,2).

Monotherapy with anti-PD-1 drugs, i.e. Nivolumab or Pembrolizumab, represents the standard adjuvant treatment in BRAF wild type melanoma patients (3,4).

The combination of Nivolumab and anti-CTLA4, Ipilimumab, in BRAF wild type and negative PD-L1 tumors, or anti-PD1 alone in melanoma with PD-L1 expression, are instead the most recent two options for metastatic disease (1,5).

Nevertheless, in this new therapeutic scenario, over half patients with melanoma, especially melanotic one, during or immediately after adjuvant anti-PD1 treatment recurs, or does not respond to modern immunotherapy or, moreover, relapses after a temporary response (1,3-5).

For patients with primary resistance or progressing to anti-PD-1 or even to the combination the prognosis is extremely unfavorable, with a second/following line including Ipilimumab, alkylating chemotherapy, or agents in experimental trials.

Among them, there are many patients that can develop peculiar refractory superficial metastases.

In-transit metastases represent cutaneous localization of melanoma that appear between a primary tumor and its regional lymph node basin as part of the natural lymphatic dissemination process. In presence of lymph node involvement their incidence is 20% (6).

The prognosis of patients with in-transit melanoma metastases is unfavorable with 5-year survival ranging from 12 to 37% (7).

The surgical excision of isolated lesions represents the elective treatment.

For metastases, which primarily show or develop resistance to standard therapies, electroporation associated with chemotherapy (electrochemotherapy-ECT) seems to be a new potential therapeutic option in particular in combination with immunotherapy, since it elicits a systemic immune response other than directly treat deep-seated tumors. The biological rational is represented by the ECT induction of immunogenic cancer cells death. In fact, this procedure can determine a more efficient antigen presentation of tumor-derived antigens by APCs (antigen-presenting cells), particularly CD8+

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antigen-specific T cells. Anti-PD-1 drugs revert T-cell exhaustion induced by PD-1/PD-L1 (programmed death-ligand 1) and PD-L2 (programmed death-ligand 2) engagement on CD8+ T cells. Moreover, ECT may also be beneficial in the priming phase of the antitumor immune responses, provoking long-lived tumor antigen-specific CD8+ T-cell effectors (8).

However no data from trials exist to evaluate the potential role of the combination or the correct sequence of these two therapeutic options-loco-regional and systemic treatment-but only anecdotic/retrospective cases in papers. Moreover, nowadays it is unknown the appropriate timing and sequence in terms of restoring efficacy of immunotherapy in resistance events (9-13).

Our experience on a patient with initial in-transit metastases on the scalp and then loco regional lymph node and bone metastases showed the crucial beneficial role of a peculiar sequence of electrochemotherapy and immunotherapies.

Case report

A 73-year old male patient with no significant medical history, except for rheumatic polymyalgia regressed after steroid therapy, was subjected to excision of cutaneous lesion on the scalp in November 2020. The histological exam revealed a lentigo-maligna melanoma in vertical growth phase, with the following features: Clark level IV invasion, 1.7 mm Breslow index, 10 mitoses/mm^2, TIL non brisk, absence of ulceration, regression, microsatellitosis and lymphovascular invasion, presence of perineural invasion and of melanin pigmentation.

In December 2020, the patient underwent intra-parotid sentinel lymph nodes biopsy (SLNB) and radicalization of previous exeresis. The histological skin-related report was positive for the presence of multiple melanoma foci accountable as satellite nodules of lentigo-maligna melanoma infiltrating the dermis for a maximum thickness of 1.1 mm (greater nodule). The parenchyma of salivary gland resulted free from pathological lesions. The histological examination of SLNB in the intra and periglandular tissue showed: one of these, in its subcapsular location, was positive for rare cells ascribable to metastases from melanoma. Total body CT-scan resulted in plausible no evidence of loco-regional and distant metastases (some unspecific micro-nodular to pulmonary parenchyma bilaterally, mainly to the upper lung lobes and angiomatous lesion of ~68 mm localized in the fourth hepatic segment, known in patient's history).

Furthermore, no BRAF gene mutation was identified. It ended for an onset stage IIIC (AJCC 2017) of the melanoma disease (pT2apN2cM0).

In January 2021 the patient started anti-PD1 Pembrolizumab 200 mg flat dose therapy with adjuvant purpose.

For the appearance of a suspected pigmented peri-scar skin lesion on the scalp (frontal region), the patient underwent surgery in May 2021. Histological examination confirmed the clinical suspicion of in-transit metastases.

A total body CT-scan of June 2021 showed unchanged findings and the patient continued Pembrolizumab without interruptions.

Subsequently for the rapidly progressive disease on the scalp in May-June 2021, the patient, after discussion in the multidisciplinary context and signing the informed consent to the treatment, was subjected to ECT to treat the scalp and its superficial lymphatic drainage pathways. Considering the oligo-progression of the disease (no visceral and brain melanoma localizations), the characteristics resistance of cutaneous lesions to elective treatments and the potential synergy between ECT and immunotherapy, the patient followed the same oncological treatment as first line therapy.

In particular, he underwent two ECT administrations with intravenous injection of bleomycin 30,000 UI through linear electrode and with a 6 weeks interval (June 2021-131 pulses- and August 2021-273 pulses-). He showed a partial response (persistence of disease on the left frontal-temporal region) and no significant adverse events [only pruritus G2 according to common toxicities criteria adverse events (CTCAE) version 5.0 was referred] Figs. 1-3.

In September 2021, the patient repeated a total body CT-scan with the evidence of a strange behavior of the known liver lesion (dimensional reduction from 68 to 40 mm) Fig. 4.

To better define the subsequent therapeutic process, the multidisciplinary team decided for a diagnostic investigation using 18-F-FDG PET-CT scan.

PET-CT scan carried out in October 2021 showed pathological uptake in the right iliac bone, in the right intra-parotid and left preauricular lymph nodes, and in the in-transit metastases especially at the left frontal-parietal region. There was no liver uptake. Thus, the case was discussed with radiologists: the focal lesion of liver seemed to be a fibrotic involution of the known angioma, but the appearance of lymph nodes in the neck could indicate a progression of disease. Instead, for the complex identification of intramedullary localization by CT scan, the time of onset of bone metastasis remained uncertain Fig. 5.

No clinical trials were active for our patient, thus he started a second line therapy with anti-CTLA4 Ipilimumab. After the first administration the patient had immediately benefit with the resolution of feeling of encumbrance of lymph node in parotid region and with the remission of the known nodules of the scalp. Nevertheless after four canonical doses of Ipilimumab ended in February 2022, the patient developed acute hypophysitis (grade 4-G4 according to CTCAE version 5.0). This peculiar immune-related adverse event was characterized by asthenia, hyporexia with consequent dehydration and hospital admission. He performed RMN of the sella turcica with reduction of physiological contrast uptake in the neurohypophysis. Thus a replacement steroid therapy was started after the load dose because of the corticotropic axis failure, with immediate advantage.

Then, he was hospitalized again for evidence of significant asymptomatic hyperglycemia (G4 CTCAE 5.0). Thus, diabetes mellitus type I was diagnosed and the patient undertook insulin therapy.

PET-CT scan performed in March 2022 showed a complete response of disease Fig. 6.

During Pembrolizumab treatment we observed the onset of lymphopenia; instead eosinophilia, progressive lymphocyte increase to normal values and lastly neutropenia appeared firstly during the ECT and then with Ipilimumab.

Although these adverse effects, the patient had a very fast and considerable improvement on its quality of life especially for the remission of strongly pronounced skin-lesions on the

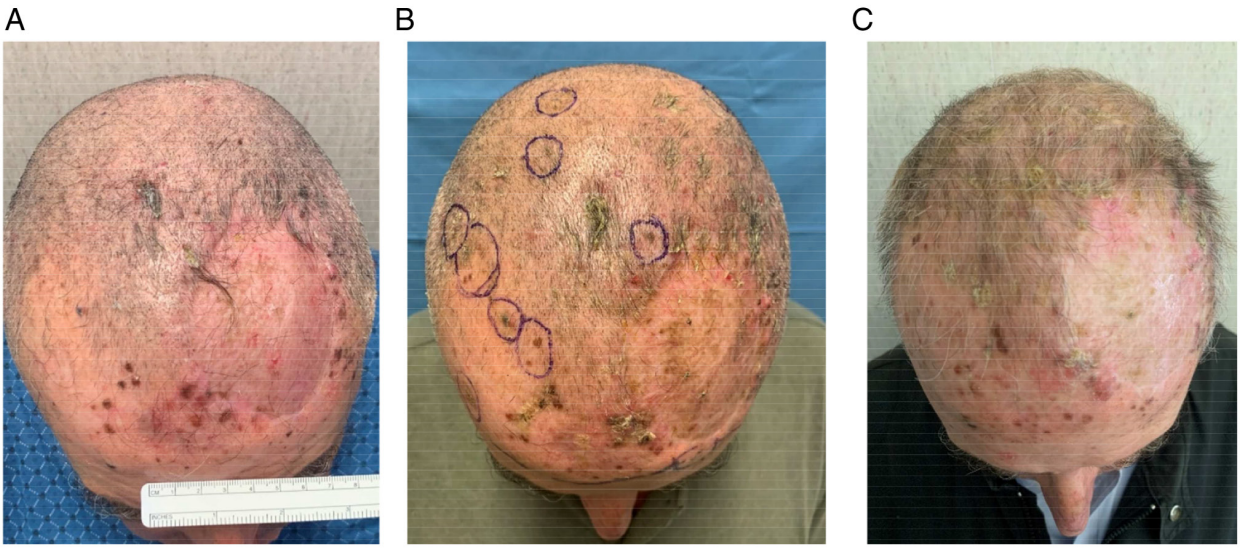

Figure 1. Frontal view of the patient's scalp before and after the ECT. (A) June 2021 baseline. (B) August 2021 after first ECT administration. The circles indicate refractory metastasis. (C) October 2021 after second ECT administration. ECT, electrochemotherapy.

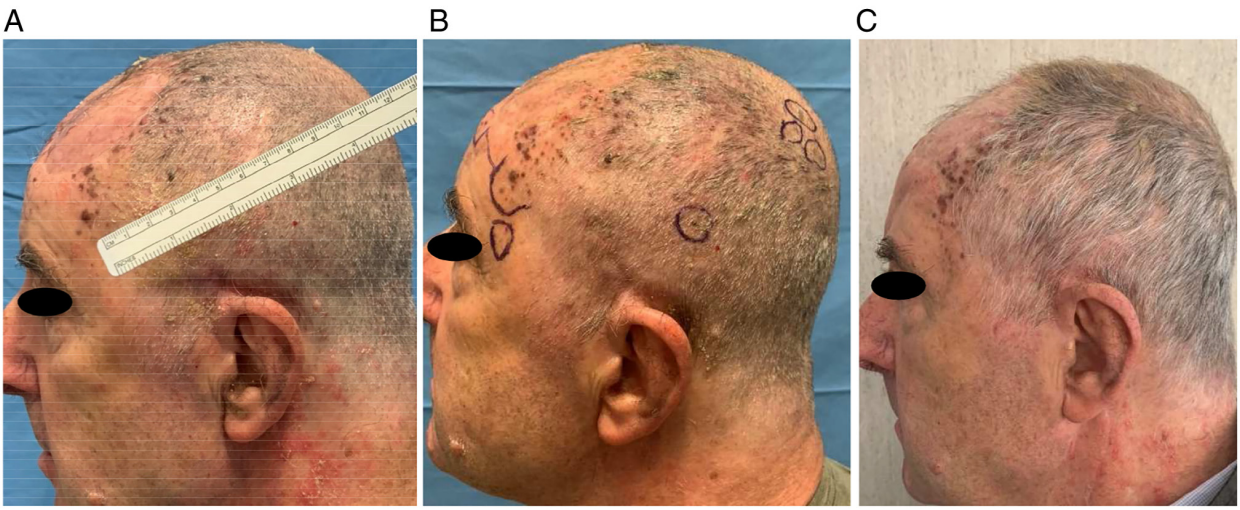

Figure 2. Left profile of the patient before and after the ECT. (A) June 2021 baseline. (B) August 2021 after first ECT administration. (C) October 2021 after second ECT administration. ECT, electrochemotherapy.

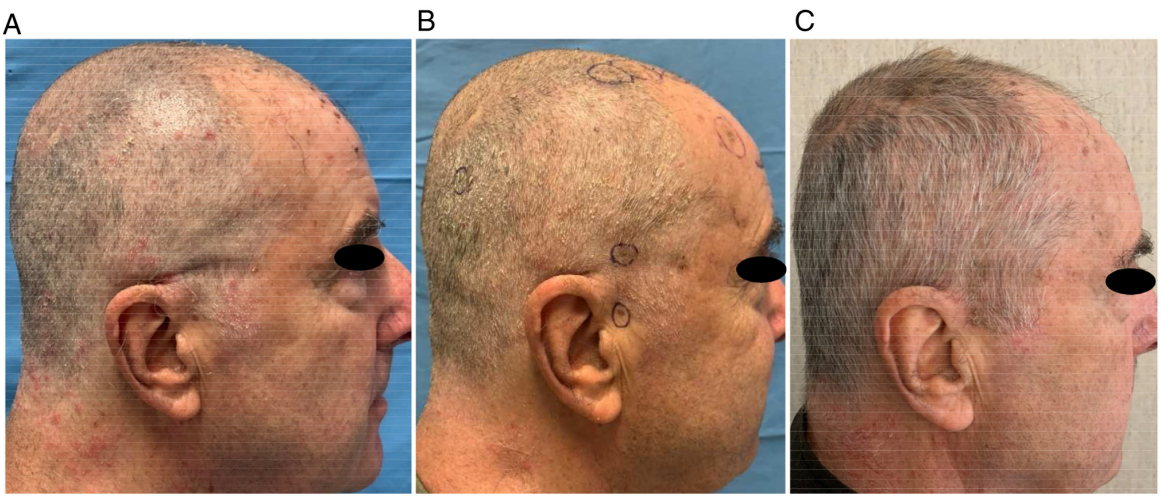

Figure 3. Right profile of the patient before and after the ECT. (A) June 2021 baseline. (B) August 2021 after first ECT administration. (C) October 2021 after second ECT administration. ECT, electrochemotherapy.

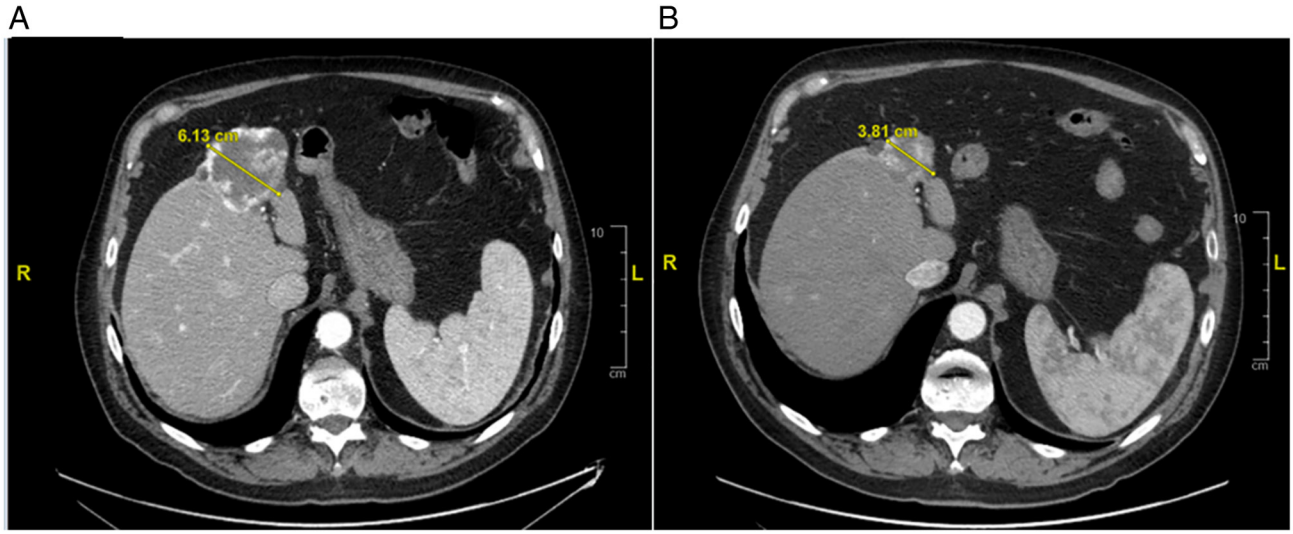

Figure 4. Evidence of liver angioma reduction (with yellow line) during immunotherapy on CT scan. (A) Abdomen CT scan of June 2021. (B) Abdomen CT scan of September 2021. CT, computerized tomography.

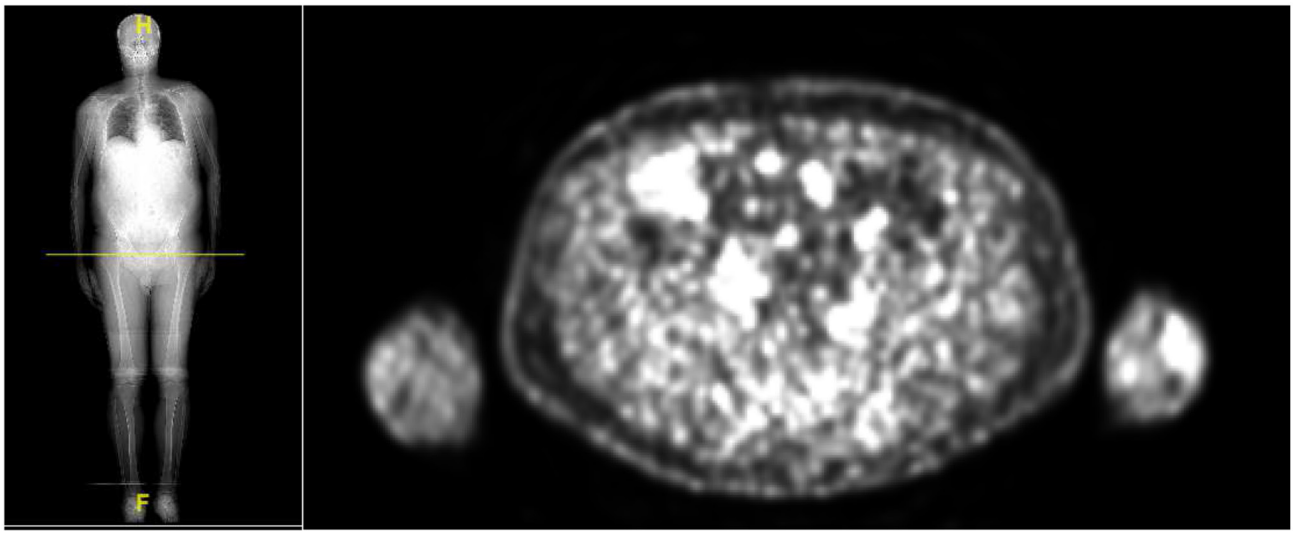

Figure 5. Right iliac bone metastasis on PET-CT scan of October 2021. PET-CT, positron emission tomography-computerized tomography.

scalp. Until now, after 12 months from the first ECT and 6 months from the starting of anti-CTLA4 immunotherapy, the patient is in excellent clinical condition and complete response of disease still maintains Fig. 7.

Discussion

In this manuscript we reported a case report of metastatic melanotic melanoma patient receiving Pembrolizumab, electrochemotherapy and then Ipilimumab for cutaneous and finally loco-regional lymph nodes and distant bone metastases with experience of dramatic clinical-radiological benefit.

Specifically our patient progressed during adjuvant treatment with in-transit melanotic metastases on the scalp. It is reported that melanin pigment and melanogenesis have a crucial function in the progression of melanotic melanoma determining resistance to immunotherapy, probably by glycolysis and hypoxia-inducible factor 1-alpha (HIF-1a) activation and their immunosuppressive effects. In fact a negative correlation between tumor pigmentation and diseases outcome was shown (14).

Therefore, after the discussion in the Skin Cancer Multidisciplinary Group of our University Hospital, to overcome the melanotic metastasis' resistance to Pembrolizumab, the patient underwent electrochemotherapy obtaining partial and then nearly complete response with the Ipilimumab treatment.

The electrochemotherapy was safe; the patient only reported pruritus in the scalp, treated with antihistamine medications. During the treatment with Ipilimumab the patient developed hypophysitis with corticotropic axis failure and type 1 diabetes mellitus: thus an insulin therapy and cortisone acetate was started.

ECT combines the inducted electroporation of cancer cells with the concurrent infusion of bleomycin as cytotoxic chemotherapy, more often than cisplatin. A pulsed electrical

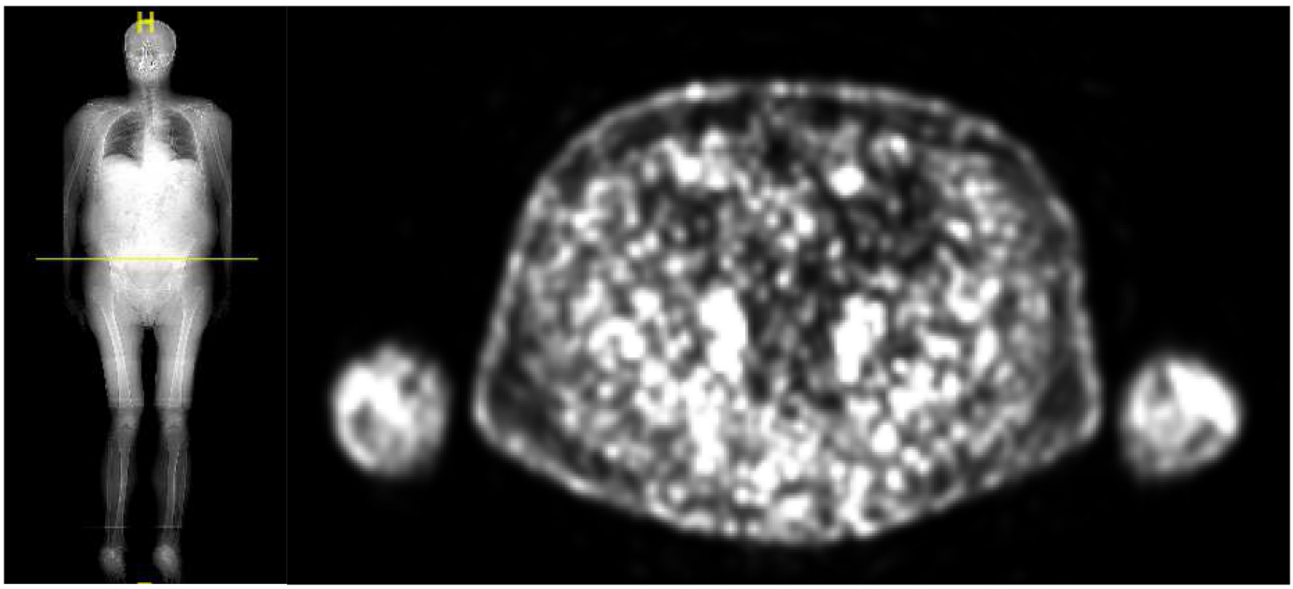

Figure 6. Disappearance of pathological uptake of right iliac bone on PET-CT scan of February 2022. PET-CT, positron emission tomography-computerized tomography.

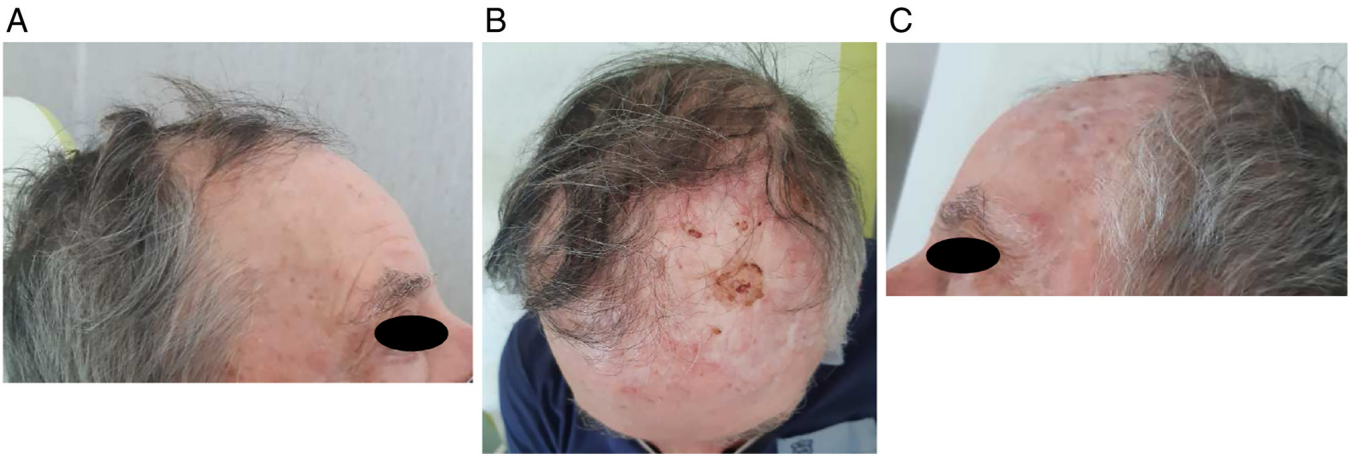

Figure 7. Clinical remission of disease on the scalp. Presence of vitiligo on the (A) right profile, (B) frontal view and (C) left profile.

current enhances the cell permeability expounding the event of revocable electroporation. ECT also determines anti-vascular effect resulting in increased tumor cell hypoxia. Therefore, locally bleomycin is more active with negligible systemic adverse events (15,16).

In addition to determine cell death, ECT can generate a local and systemic immune reaction as a result of releasing tumor associated antigens (TAA) from electroporated cancer cells.

TAA are neoantigens able to evoke an immune response primarily mediated by cytotoxic-T lymphocytes (CTL). Furthermore, TAA seem to be captured by local dendritic cells and then presented to tumor-specific CTL in draining lymph nodes (17-20).

Some reports showed the efficacy of ECT in the treatment of in-transit or subcutaneous metastases from cutaneous melanoma. An objective response rate from 60 to 90% was achieved in the palliative management of unresectable recurrent cutaneous disease. This response is usually long-lasting; moreover ECT is an easy, rapid and effective procedure which can be repeated (21-23). However, a long-term complete response of these peculiar localizations of metastasis seems to be difficult to obtain due to spread of cancer cells into lymphatic vessels (24-27).

In a similar way as ECT, a systemic and unexpected response to a local treatment derives from the radiotherapy of lesions, due to the release of TAA, named abscopal effect. It has been also demonstrated in patients affected by metastatic melanoma: distant lesions have showed a clinical-radiological response.

Thus, it has been hypothesized that the association of ECT with anti-PD1/anti-CTLA4 could represent an effective strategy to induce an immunological durable and synergic response against the cancer (11,28-32).

The role of hypoxia in this combination treatment has virtually been examined, particularly in melanotic melanoma.

The response to radiation/ECT joint with immune checkpoint inhibition seems to be dependent on the hypoxia level (33) that could be measured to prescribe appropriate dose of radiotherapy and the number of ECT administrations.

Trials utilizing concomitant stereotactic body radiotherapy/ and immunotherapy are still ongoing or recently completed without results about their synergistic impact and the best schedule for the two treatments (NCT02659540 with preliminary results¸ NCT04581382, NCT03850691, NCT03297463, NCT02406183, NCT04017897). Only one trial-NCT03448666-, still ongoing, aims to evaluate the activity of ECT and Pembrolizumab in patients with superficial or superficial and visceral metastases. ECT is administrated after the first cycle of immunotherapy. Starting with ECT could determine intralesional necrosis reducing loco-regional immunotherapy efficacy; instead, ECT administration after the first dispensation demonstrated a synergistic effect in retrospective series (11), probably due to the aforementioned mechanism of neo-antigens release. However, immunotherapy alone could induce an optimal local and systemic performance without the use of ECT. Thus, the immunotherapy-ECT sequence can be considered in oligo-progressing disease, for instance.

Considering that Ipilimumab shows uncommon, not immediate but long lasting action: usually the complete response was achieved after some months from the start (34). According to our experience, ECT leads to a local response and probably to an acceleration of the systemic one. Thus, we hypothesize that ECT in two administrations could increase and accelerate the efficacy of Ipilimumab.

Finally, randomized clinical trials and translational researches are needed to shed the light on the improved combination/sequence/number of administrations of ECT and systemic immunotherapy in order to offer the most appropriate therapies, especially for patients with superficial (and also visceral) metastases.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FM and FDF performed the patient treatments (immunotherapy and electrochemotherapy respectively) and they were major contributors in writing the paper and contributed to design and conception. They verified efficacy, monitored patient and collected images and medical data. RB and PB formulated and supervised treatment plans. FM and FDF confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The patient consented to the collection of data and images for the aim of research and for publication in written form.

Patient consent for publication

Written consent was obtained from the patient. He authorized us to publish his disease history and his images.

Competing interests

FM was a consultant/advisory board member for BMS and MSD. The rest of the authors declare that they have no competing interests.

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