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Case Report

Cytokine-Enhanced Vaccine and Suicide Non-Viral Gene Therapy in Advanced Metastatic Melanoma Patients: Two Case Reports

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ARTICLE INFO

Article history:
Received: 15 April, 2020
Accepted: 4 May, 2020
Published: 9 May, 2020

Keywords:
Metastatic melanoma
gene therapy
suicide gene
cytokine
immunotherapy
case report

ABSTRACT

The prognostic for metastatic melanoma is very poor when treated with standard cytotoxic chemotherapies and it is often refractory to check point inhibitors and/or molecular targets. In this context the development of new treatments with better efficacy and safety profiles is highly desirable. Based on our successful experience applying suicide and immune gene therapy in a veterinary clinical setting, we are proposing its translation to human patients. We are presenting here the first-in-human safety assay of this approach. We report two cases of refractory metastatic melanoma. The first-one was a 27-years-old pharyngeal mucosal melanoma patient with a primary tumor in his left tonsil. Despite transient slowing down, the disease successively progressed to radiotherapy, radical surgery, ipilimumab, nivolumab, imatinib and temozolomide. The second-one was a 72-years-old malignant melanoma patient with a primary tumor in his left hallux. Despite transient slowing down, the disease successively progressed to hallux amputation, inguinal lymphadenectomy, radiotherapy, interferon-alpha, ipilimumab, pembrolizumab and temozolomide. The proposed treatment included local intratumoral suicide gene therapy concomitant with a subcutaneous vaccine composed by allogeneic tumor extracts and liposomes with plasmids bearing IL-2 and GM-CSF genes. The treatment was safe: the only side effects were from mild to moderate and manageable: pyrexia, swelling of the injected tumor and partial hair loss (alopecia). Due to disease progression both patients were withdrawn from the study before completing the complete series of interventions. These preliminary data encourage the completion of further clinical trials to establish the possible clinical benefit of the proposed approach.

Introduction

Malignant melanoma is the most destructive and fatal variety of skin cancer with a global estimate of 288,000 new cases and 61,000 deaths in 2018 [1]. When early diagnosed at the primary site, most of cutaneous melanomas can be surgically excised and the disease definitely cured. Conversely, the prognostic for advanced or metastatic melanoma is very poor when treated with standard cytotoxic chemotherapies [2]. Due to the meager results obtained by these treatments, emergent new drugs were adopted for treatment of advanced melanoma: (i) monoclonal antibodies like ipilimumab, pembrolizumab and nivolumab and (ii) small molecules targeting different regulatory pathways like vemurafenib, trametinib, encorafenib and dabrafenib [3-5]. Unfortunately, these new treatments present a high rate of adverse effects and increase overall survival only in a limited number of patients. Other approaches, including intratumoral immunotherapies with diverse mechanisms of action such as non-oncolytic viral therapies (PV-10 and toll-like receptor 9 agonists) and oncolytic viral therapies (T-Vec, CAVATAK and HF10), are currently tested with promising results in clinical trials alone or in combination with checkpoint inhibitors [6].

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http://dx.doi.org/10.31487/j.COR.2020.05.02

Mucosal melanoma is an infrequent sort of malignant melanoma, representing only about 1.3% of melanoma cases. It is different from melanomas appearing in other places of the body and treatment options are very limited [7]. The rich lympho-vascular supply of the mucous membranes promoting early spread and metastasis of mucosal melanoma poses unresolved challenges and the urgent need for effective, safe and accessible therapies. Despite the different biology and because there are few specific studies, mucosal melanoma patients are usually treated in the same way than those bearing cutaneous melanoma, but with poorer outcomes [8].

Based on successful results obtained treating malignant mucosal melanoma in a veterinary clinical setting and aiming a translational objective, we planned a phase I clinical trial for human patients with advanced melanoma: "Suicide plus immune gene therapy for advanced melanoma" (NCT03338777) [9-11]. The main aim of this study was to evaluate the safety of combined gene therapies in melanoma patients. This treatment combines the high local cytotoxicity of the suicide *HSV thymidine kinase* gene system (*HSVtk*) associated to the prodrug ganciclovir (GCV) with the immune stimulation of interleukin-2 (hIL-2) and immune amplification of granulocyte and macrophage colony stimulating factor (hGM-CSF) in the presence of tumor antigens. The proposed scheme consists in the periodic intra/peritumoral application of plasmid DNA:cationic lipid complexes (lipoplexes) containing the HSV*tk* gene, co-administered with the prodrug GCV, and subcutaneous injections (far from melanoma lesions) of a vaccine produced with formolized allogeneic melanoma extracts combined with lipoplexes carrying the *hIL-2* and *hGM-CSF* genes (Table 1).

Table 1: Proposed treatment scheme.

Agent (mg/ml)	Volume (ml)	Way	Schedule (week)	Cycle
psCMV*tk* (1.0) + GCV (12.5) depending on tumor accessible surface or volume	Min: 0.5 Max: 2.0	Intra-/peri-tumoral	1, 2, 3, 4, 5, 7, 9, 11, 13, 15	10 rounds or until limiting toxicity or progression
psCMV*hIL-2* (0.5) + psCMV*hGM-CSF* (0.5) + allogeneic tumor extract	1.0	Sub-cutaneous vaccine	1, 2, 3, 4, 5, 7, 9, 11, 13, 15	10 rounds or until limiting toxicity or progression

#Patients start with a minimum 0.5 ml (weeks 1 and 2). Starting the 3rd week, the maximal doses (according the tumor burden) are applied. Follow-up until week 30.

*Each vaccine containing 0.5 mg psCMV*hIL-2* + 0.5 mg psCMV*hGM-CSF* + 0.25 ml formolized allogenic tumor extracts.

Every plasmid was constructed using the same psCMV backbone and the corresponding gene and was amplified, purified and supplied by GenScript (Piscataway, NJ, USA) with the following specifications: ≥95% supercoiled, ≤0.005 EU/μg endotoxin, animal-free [12]. Plasmids were provided at 2.0 mg/ml in sterile phosphate-buffered saline. DMRIE/DOPE liposomes, lipoplexes, allogeneic tumor extracts and tumor vaccines were prepared as previously described [10-12]. This phase I clinical trial was approved by the Ethics Committee for Research Protocols of the Italian Hospital of Buenos Aires (Argentina) and all the patients signed the Informed Consent before being recruited. Within the frame of this study we treated two patients with very advanced metastatic melanoma, for testing the feasibility of the treatment scheme and for getting a first-in-human insight about the treatment toxicity.

Case Presentation

Patient 1

A 26-year-old man presented in March 2017 with pain sore on his left tonsil due to an unresectable, stage IV pharyngeal mucosal malignant melanoma [13].

I Previous Management

- March 2015: The patient 1 was diagnosed pharyngeal mucosal malignant melanoma in the left tonsil with cervical N3 involvement and non-mutated BRAF V600.
- June 2015: He received radiotherapy (4200 cGy) and started weekly carboplatin/paclitaxel chemotherapy.
- September 2015, radical left cervical excision evidenced 2 compromised lymph nodes (1 capsule disrupted).
- December 2015: Due to disease progression, he started with ipilimumab treatment.
- April 2016: In the absence of response, a disease spread was found at lungs, lymph nodes and bones and treatment with nivolumab started.
- Again, the disease was refractory to treatment. The surgery of a metastatic lesion at the T3 vertebral body was followed by radiotherapy.
- September 2016, the mutated c-KIT (CD 117) at exon 11 allowed treatment with imatinib that also failed in controlling the disease.
- January 2017: Because progression of bone metastases, he was treated with temozolomide but again the tumor control failed.
- April 2017: Due to the poor response to all the available treatments, the patient accepted to enter the suicide plus immune gene therapy protocol and signed the informed consent. The ^{18}FDG-PET/CT imaging previous to recruitment displayed pharyngeal hypermetabolic compromise with secondary multiple lymph node, bilateral pulmonary, hepatic and osseous, as well as glandular (right submaxillary and adrenal, thyroid and pancreas) and muscular (left leg) high metabolic activity.

II Treatment Course

- May 2017: The patient received four weekly applications of subcutaneous vaccine (in the arm) and intralesional suicide gene therapy (in the cervical mass). As specified in (Table 1), the two initial intralesional injections had 0.5 ml and the next ones 2.0 ml. The injections were applied fractionated into 0.1-0.2 ml portions and applied in different areas of the tumor. Clinical parameters such as oxygen saturation, body temperature, respiratory frequency, cardiac rhythm and blood pressure were checked every hour up to 6 h after injections. No changes in such parameters were

observed in all the treatment sessions. After the first and second sessions, the patient reported occasional temperature increase up to 38.5º C. In the third session the patient mentioned diffuse hair loss and increased sensitivity in the injection areas. In the fourth visit he reported some degree of functional dyspnea.

- June 2017: In agreement with the Oncology and Palliative Care Services, it was decided to exclude him from the protocol because of disease progression. One month later, the patient died.

Patient 2

A 72-year-old man presented in April 2017 with progressing unresectable, stage IV malignant melanoma [13].

I Previous Management

- March 2015: After 3 years presenting melanonychia in the left hallux, with persistent inguinal lymphadenopathy, fine needle aspiration biopsy of the lymph node indicated the presence of melanoma metastasis. One month later and after hallux amputation, the diagnosis of stage III nodular melanoma was confirmed with Breslow 4.77 mm, Clark level IV, peripheral and deep margins compromised, mitotic rate≥ 10, Ki-67 proliferative index of 90% and non-mutated BRAF V600.
- April 2015: Inguinal lymphadenectomy was performed yielding 3 positive lymph nodes with massive metastases (all capsules disrupted). This was followed by radiotherapy (5000 cGy) of the perinodal area.
- June 2015: He started with systemic interferon-α2b.
- January 2016: Despite the appearance of vitiligo in his left foot indicating an immune reaction against melanotic cells, a subcutaneous metastatic mass was detected at the hypogastrium and the patient was subjected to ipilimumab treatment.
- July 2016: Due to the lack of response, a series of pembrolizumab cycles was performed until being stopped because of pyrexia and intestinal infection.
- November 2016: Following a proximal humeral pathological fracture due to bone metastasis, a successful shoulder prosthetic replacement after tumor excision was done. Subsequently he received 3 cycles of temozolomide.
- May 2017: Since all the treatments were unable of getting disease control, the patient accepted to enter the suicide plus immune gene therapy protocol and signed the informed consent. The ^{18}FDG-PET/CT imaging previous to recruitment displayed multiple lymph node, bone, liver and right adrenal metabolically active areas compatible with widespread metastases.

II Treatment Course

- June 2017: The patient received five weekly applications of subcutaneous vaccine (in the leg) and intralesional suicide gene therapy (in the hypogastric mass). As specified in (Table 1), the two initial intralesional injections had 0.5 ml and the next ones 2.0 ml. The injections were applied fractionated into 0.1-0.2 ml portions and applied in different areas of the tumor. Clinical parameters such as oxygen saturation, body temperature, respiratory frequency, cardiac rhythm and blood pressure were checked every hour up to 6 h after injections. No changes in such parameters were observed in all the treatment sessions. From the first to the fourth session, the patient reported occasional temperature increase up to 38.5º C after treatment. After the fourth session the patient reported diffused hair loss.
- July 2017: The patient received the treatment two more times every other week as described in (Table 1). Neither changes in clinical parameters were observed in all the treatment sessions nor pyrexia reported after treatment. After seven treatment applications, CT analysis confirmed disease progression in axillary lymph nodes, lungs, liver and peritoneum. Furthermore, a painful lumbar spine metastasis progressed, significantly decreasing the quality of life. In agreement with the Oncology and Palliative Care Services, the patient was withdrawn from the protocol. Three months later, the patient died.

Discussion

We report here two cases of advanced melanoma treated with non-viral suicide gene therapy and cytokine enhanced vaccines: one mucosal and other one cutaneous. Taken into account the advanced status of the disease that in both cases was refractory to standard and check point immunotherapies, the patients entered the study with an ECOG performance status of 2. In this situation the adverse events caused by the treatment could be partially hidden by the initial conditions. Anyway, we did not register any acute reaction, and the side effects that could be attributed to the treatment were from mild to moderate and completely manageable: pyrexia, swelling of the injected tumor and partial hair loss (alopecia).

Immune-related alopecia was already described in a small proportion of melanoma patients (about 1%) receiving immune checkpoints inhibitors [14-16]. This side effect could appear delayed after some months from the last immunotherapy cycle. This kind of induced alopecia displays specific perifollicular lymphocytic infiltrates that affect the functionality of hair follicles with the consequent hair loss. Even though both patients were subjected to checkpoint inhibition immunotherapy cycles before entering the study, the timing of the alopecia onset appearing few days after our treatment, strongly suggests that it was probably related to an additional immune stimulation triggered by the subcutaneous vaccine. This vaccine has not only many melanoma specific antigens but can also express immune stimulating cytokines [12].

Alopecia was also described among the cutaneous adverse effects of imatinib that can appear some months after stopping the treatment [17]. The patient 1 had been treated with this tyrosine kinase inhibitor after the immune check point inhibitors. Therefore, we cannot discard its contribution on this side effect. In this exploratory study we found that the proposed treatments scheme is feasible in melanoma patients and appears to be safe with very mild side effects. Nevertheless, the two early patients described in this work entered the study with a very advanced stage of the disease precluding the detection of any possible positive effect of the treatment. Therefore, we consider that the proposed treatment would be more appropriately conducted in a neoadjuvant setting, especially before check point inhibitory immunotherapy, as it is currently being assayed for conditionally replicating oncolytic virotherapies [18].

The successful results obtained in previous veterinary settings together with the lack of significant undesirable side effects, encourage the completion of further clinical trials to establish the possible clinical benefit and safety profile of the proposed approach.

Conflicts of Interest

None.

Acknowledgement

The authors are grateful to the patients and their families for their cooperation and participation in this study. The authors thank M. Guadalupe Pallotta, José M. Lastiri and Sergio R. Specterman (Sección Oncología Clínica-HIBA); Waldo H. Belloso (Sección Farmacología Clínica-HIBA) and Liliana B. Giménez (Área Diagnóstico/Patológico, IOAHR-UBA) for helpful discussions and support in the early stages of this study.

Funding

This work was supported by grants from ANPCYT/FONCYT (PIDC2012-0063) L.M.E.F. and G.C.G. are investigators of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina), V.A.S Chief of the Sección Farmacología Clínica-HIBA and H.L.H.H. research fellow of ANPCYT/FONCYT.

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