Acta Scientiae Veterinariae, 2019. 47(Suppl 1): 435.

CASE REPORT
Pub. 435

ISSN 1679-9216

Electrochemotherapy Associated with Calcium Electroporation in Metastatic Feline Cutaneous Malignant Melanoma

Denner Santos Dos Anjos[1], Cecilia Gomes Rodrigues[2], Nicilene Cardoso Silva[3], Andrigo Barboza De Nardi[1] & Carlos Eduardo Fonseca-Alves[4]

ABSTRACT

***Background*:** Calcium electroporation (CaEP) is a novel therapeutic treatment that has been studied for cancer due to its selective killing cancer cells by necrosis and danger signals. Besides that, electrochemotherapy (ECT) is an effective local treatment that involves the administration of chemotherapeutic drugs followed by delivery of electrical pulses to the tumor. The combination with ECT and CaEP has been reported in literature suggesting that additional response of immune system could have been enhanced by electroporation with calcium. This case, report on the successful treatment with CaEP combined with ECT for treatment of a regional metastasis in a feline model of malignant melanoma.

***Case*:** A 9-year-old, mixed breed cat was referred to the veterinary clinic with a 2-month history of cutaneous peripalpebral plaque lesion (0.19 cm³) and a submandibular lymph node enlargement (0.5 cm³). Incisional biopsy of the cutaneous lesion and fine-needle aspiration of submandibular lymph node confirmed a cutaneous melanoma with submandibular lymph node metastasis. Tumor staging was set in T1N1M0 according to WHO staging criteria. ECT for the primary lesion and lymph node metastasis was proposed. For the ECT, bleomycin (15,000 UI/m²) application was performed intravenous followed by electroporation (8 pulses of 100 µs at 1000 V/cm, and 1 Hz) using a needle array electrode consisted of two parallel rows with six needles in each row. At 28-day post-ECT complete remission of the primary tumor and metastatic foci was achieved. However, 120 days after ECT, recurrence was observed in submandibular and retropharyngeal lymph nodes. A second ECT approach was performed adding to bleomycin the intra lymph nodal application of calcium gluconate. The dose of calcium gluconate was diluted in an isotonic 0.9% NaCl solution resulting in a low concentration at 9 mM, injected in both metastatic lymph nodes (submandibular total volume: 1.4 ml; retropharyngeal total volume: 0.5 mL) and pulses were delivered immediately after drug administration. No systemic adverse effects were observed. Local adverse effects were considered mild as transitory edema and ulceration post procedure. One-week post-ECT+CaEP, complete remission of local metastasis occurred. However, the patient achieved five months disease free interval, and died during a surgical approach, achieving nine months of survival time.

***Discussion*:** Feline cutaneous malignant melanoma is an aggressive disease with a short survival time for the patients with mean of 4.5 months. CaEP is a novel anticancer treatment that has been study in the past years due to its selective killing cancer cells by necrosis and danger signals. The CaEP induces supraphysiological calcium influx into neoplastic cells leading to acute ATP depletion and necrosis of tumor cells. This use could be an interesting therapeutic choice for both human and veterinary medicine. In this patient, it was demonstrated a good clinical response with its use, showing temporarily tumor remission from the case presented with disease free interval of five months when compared to other report of two months. This description showed that ECT associated with CaEP improved outcome of regional melanoma lymph node metastasis in a cat. However, further investigations are needed to understand the use of CaEP in patients with metastasis as well as evaluate the use of both modalities to determine its synergistic effect.

Keywords: calcium electroporation, cutaneous melanoma, electrochemotherapy, feline, gluconate calcium.

DOI: 10.22456/1679-9216.96498

Received: 4 June 2019 Accepted: 19 September2019 Published: 30 October 2019

[1]Department of Veterinary Clinic and Surgery, São Paulo State University (UNESP), Jaboticabal, SP, Brazil. [2]Tribos and Bichos Clinic & [3]Animed Veterinary Clinic, Uberlândia, MG, Brazil. [4]Department of Veterinary Surgery and Anesthesiology, School of Veterinary Medicine and Animal Science, UNESP, Botucatu, SP. CORRESPONDENCE: D.S. Dos Anjos [denner.anjosoncology@gmail.com]. Department of Veterinary Clinic and Surgery - UNESP. Prof. Paulo Donato Castellane s/n. CEP 14884-900 Jaboticabal, SP, Brazil.

INTRODUCTION

Calcium electroporation (CaEP) is a novel therapeutic perspective for patients with cutaneous metastasis of malignant melanoma [3] and remission of metastatic melanoma foci [4]. Its approach leads to supraphysiological calcium influx into neoplastic cells leading to acute ATP depletion killing cancer cells by necrosis and cellular danger signals (expression of ATP, calrecticulin and HMGB1) [7]. Besides this, electrochemotherapy is an effective local treatment that involves the administration of chemotherapeutic drugs followed by delivery of electrical pulses (electroporation) to the tumor.

This technique is mainly used on skin tumors, cutaneous metastasis of several tumors and head and neck cancers [9,13]. New evidences suggested on small skin metastasis that CaEP has a similar objective response rate compared to ECT with limited side effects [3]. The combination with ECT and CaEP has been reported in a dog with oral melanoma with resolution of regional metastasis 30 days after treatment, suggesting that additional response of immune system could have been enhanced by electroporation with calcium [8]. Taking into theses previous considerations, this report a description of ECT combined with CaEP for treatment of a regional metastasis in a cat with malignant melanoma.

CASE

The animal included were client-owned and written owner consent was obtained. A 9-years-old, mixed breed cat was referred to the veterinary clinic with a 2-month history of cutaneous peripalpebral plaque lesion (0.19 cm^3) and a submandibular lymph node enlargement (0.5 cm^3). Tumor volume was calculated as $ab^2\varpi/6$ ('a' is the larger diameter of the tumor nodule, and 'b' is the diameter of the tumor nodule perpendicular to 'a') [14]. Incisional biopsy of the cutaneous lesion and fine-needle aspiration (FNA) of submandibular lymph node were performed and confirmed a cutaneous melanoma with submandibular lymph node metastasis. Abdominal ultrasound, three-view thoracic radiograph, complete blood count and chemistry panel were performed and revealed no abnormalities. Tumor staging was set in T1N1M0 according to WHO staging criteria. Since the owner declined the surgical excision associated with systemic chemotherapy, ECT for the primary lesion (Figure 1A) and lymph node metastasis (Figure 1C) was proposed. Electric pulses were administered in time intervals of 8-28 min after 5 min of intravenously injecting bleomycin sulfate[1] [Cinaleo®) at 15,000 IU/m^2 in bolus 1.05 mL] under general anesthesia. The cat was premedicated with pethidine[2] [Pethidine® - 0.3 mg/kg] injected intramuscularly; anesthetic induction was performed with propofol[2] [Provive® - 3 mg/kg] and ketamine[3] [Ketamine® - 1 mg/kg] in the same syringe applied intravenously (IV) followed by endotracheal intubation; anesthesia was maintained with isoflurane[4] [Isoforine®]. The electroporation parameters consisted of eight unipolar square electric pulses with frequencies of 1 Hz and 1000 V/cm, lasting 100 μs each and delivered by six needle electrodes arranged in rows (parallel array) with distances of 0.3 mm between them using pulse generator LC BK-100 (São Paulo, SP, Brazil). All the extension of the primary tumor as well as the regional metastasis was covered with the needles. Antitumor response was performed by clinical examination according to RECIST guideline defined as measurable tumor lesions in at least one dimension with a minimum size of 10 mm caliper measurement [1]

At 28-day post-ECT complete remission of the primary tumor and metastatic foci was achieved (Figure 1B and D). CR of lymph node was considered based on its reduction < 10 mm (at baseline examination 0.5 cm^3) and also negative FNA. The subject was followed-up monthly by clinical examination (measurement of lymph node was classified as non-measurable due to < 10 mm in its axis) and 120 days after ECT, submandibular and retropharyngeal lymph nodes enlargement was noted 0.4 cm^3 and 2.77 cm^3, respectively. FNA of both lymph nodes confirmed tumor recurrence metastasis (Figure 1E). Then, a second ECT approach was performed as mentioned above, however, based on the previous human literature [3,4,7] it was proposed adding to bleomycin the calcium gluconate[5] [Calcium gluconate® 10%]. The dose of calcium gluconate was diluted in an isotonic 0.9% NaCl solution resulting in a low concentration at 9 mM, injected in both metastatic lymph nodes (submandibular total volume: 1.4 mL; retropharyngeal total volume: 0.5 mL) and pulses were delivered immediately after drug administration.

One-week post-second ECT and CaEP, PR was observed in retropharyngeal lymph node (1.31 cm^3) and CR in submandibular lymph node (non-measurable). No systemic adverse effects were observed. Local adverse effects were considered mild as transitory edema

and ulceration post-ECT and CaEP that resolved in 21 days after use of cefovexin[6] [Convenia® - 0.1 mg/kg SC single dose] plus non-steroidal anti-inflammatory Piroxicam[7] [Anflene® 0.3 mg/kg once daily for 7 days]. The patient achieved CR at 28-day post-second ECT+CaEP observed by non-measurable lymph nodes and negative FNA with scar and fibrous tissue locally (Figure 1F). Clinical outcome can be observed in Table 1. Five months disease free interval was achieved (after second ECT and CaEP), however, at this moment, recurrence was observed in the retropharyngeal lymph node confirmed by FNA (9 months after first session). The owner declined a new ECT session, and submitted the patient to surgical excision, but during surgery a cardio-respiratory arrest occurred, and patient died presenting overall survival of 9 months. The owner declined a post-mortem examination.

DISCUSSION

Feline cutaneous malignant melanoma is an aggressive disease with a short survival time for the patients (mean 4.5 months) [15]. On the other hand, canine cutaneous melanoma seems to be less aggressive and metastatic [11] than feline cutaneous melanoma [12,15] prognosis for feline cutaneous melanoma seems to be poor, as is seen in a metastatic human melanomas and cats could be an interesting spontaneous model for human cutaneous metastatic melanomas. According to the previous literature, the mean survival time after surgical removal of cutaneous metastatic melanoma in cats is 4.5 months [15], resembling human cutaneous melanoma.

Due the poor prognosis in this case, the owner rejected surgical treatment and accepted a the ECT as a more conservative therapeutic approach. Recently, it was observed in canine oral malignant melanoma that ECT generates an increase of cytotoxic T-cells alongside a decrease of regulatory T-cells post-treatment, indicating that activation of immune system occurs delaying metastatic lesions [10]. CaEP is a novel anticancer treatment that has been study in the past years due to its selective killing cancer cells by necrosis and danger signals (expression of ATP, calrecticulin and HMGB1) [7]. The CaEP induces supraphysiological calcium influx into neoplastic cells leading to acute ATP depletion and necrosis of tumor cells [7]. However, since this is singular case report, we were not able to correlate immune system alteration with the CaEP.

The use of CaEP could be an interesting therapeutic choice for both human and veterinary medicine. Previous *in vitro* studies showed the effect of CaEP and bleomycin electroporation on three different cancer cell spheroids (breast, cancer and colon cancer) and normal dermal fibroblast spheroids, showing spheroid size reduction in all three cancers cells but not in normal cell spheroids [5]. These interesting results indicated that normal cells seem less sensitive to CaEP [5]. The first randomized clinical trial in humans with cutaneous metastases treated with CaEP or ECT using bleomycin, showed similar results between both techniques [8].

Table 1. Clinical outcome after first and second electrochemotherapy treatments.

	Clinical measurement/Adverse effects				
1st session ECT	D0	D7	D14	D21	D28
Primary tumor (peripalpebral plaque)	0.19 cm^3	Local edema, ulceration, and crust	Ulceration	Crust	CR observed by scar tissue with palpebral retraction
Submandibular LN	0.5 cm^3 (FNA +)	Local edema, and crust	Crust (resolution of edema)	LN non-measurable (<10 mm)	non-measurable (<10 mm) and FNA -
2nd session ECT (120 days after 1st session ECT)					
Submandibular LN	0.4 cm^3	non-measurable (<10 mm)	Local ulceration, edema and crusts	Crust	Scar tissue (FNA -)
Retropharyngeal LN	2.77 cm^3	1.31 cm^3; no local AE	Local ulceration, edema and crusts	Crust (non-measurable)	Scar tissue (FNA -)

FNA: Fine needle aspiration; LN: Lymph node; ECT: Electrochemotherapy; AE: Adverse effects.

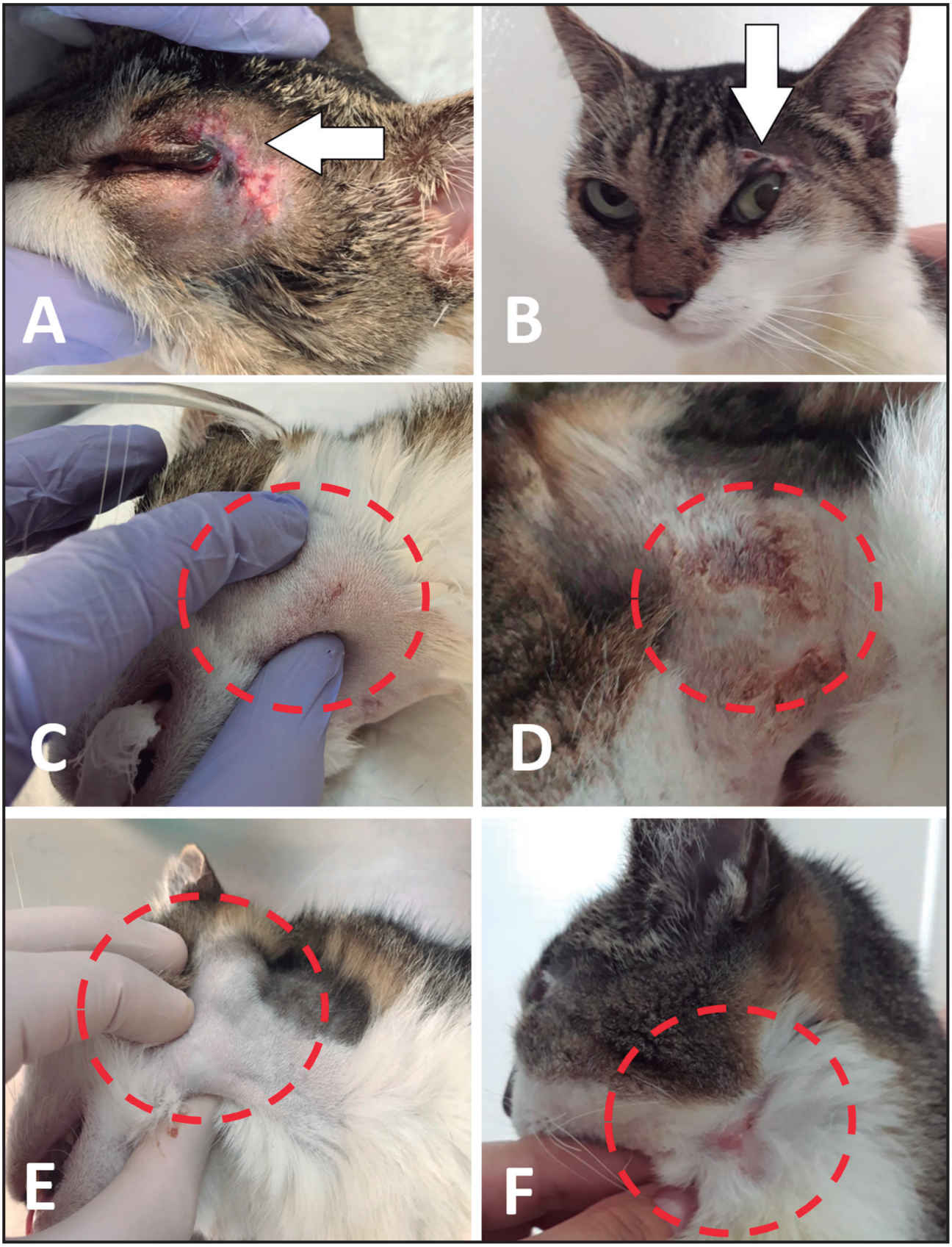

Figure 1. Metastatic cutaneous malignant melanoma in a cat. A- primary cutaneous peripalpebral plaque (arrow) diagnosed as a malignant melanoma by biopsy. B- Complete remission of the peripalpebral lesion (arrow), 28 days after electrochemotherapy. C- Submandibular lymph node enlargement (red dotted circle), diagnosed as a metastatic melanoma site by fine needle biopsy. D- Submandibular lymph node remission and crust formation (red dotted circle), 28 days after electrochemotherapy (ECT). E- Lymph node metastasis relapse (redo dotted circle) after four months of the previous ECT with bleomycin. Complete remission of the lymph node metastasis (red dotted circle), 28 days after ECT plus CaEP.

However, seems that tumor cells are more sensitive to bleomycin than normal ones [2]. Thus, need to be conducted to better understand the complex mechanism behind the CaEP [3-7]

A previous study compared the use of bleomycin with calcium compounds as calcium chloride and calcium glubionate [5]. These authors demonstrated decreased cell viability in three cancer cell lines independent of calcium compound [5], indicating the use of different calcium compounds associated with CaEP. Based on this fact, it was use calcium gluconate due the readiness way to be acquired. It was demonstrated a good clinical response with its use, showing temporarily tumor remission from the case presented (disease free-interval of five months) when compared to other report (two months) [8]. A previous case report recorded the use of ECT and CaEP in canine melanoma, observing similar results [8], as well as observed in another study using combination of ECT and calcium electroporation, suggesting that this approach could be used as a strategy to enhance immune system response and improve survival in patients with disseminated malignant melanoma [4].

This description showed that ECT associated with CaEP improved outcome of regional melanoma lymph node metastasis in a cat. Further investigations are needed to understand the use of CaEP in patients with metastasis as well as evaluate the use of both modalities to determine its synergistic effect.

MANUFACTURERS

[1]Meizler Biopharma. Barueri, SP, Brazil.
[2]União Química - Farmacêutica Nacional S/A. São Paulo, SP, Brazil.
[3]Vetnil-Indústria e Comércio de Produtos Veterinários Ltda. Louveira, SP, Brazil.
[4]Cristália Produtos Químicos Farmacêuticos Ltda. Itapira, SP, Brazil.
[5]Laboratório Halex Istar. Goiânia, GO, Brazil.
[6]Laboratório Zoetis. São Paulo, SP, Brazil.
[7]Laboratório Teuto. Anápolis, GO, Brazil.

***Declaration of interest*.** The authors report no conflicts of interest.

REFERENCES

1 **Eisenhauer E.A., Therasse P., Bogaerts J., Schwartz L.H., Sargent D., Ford R., Dancey J., Arbuck S., Gwyther S., Mooney M., Rubinstein L., Shankar L., Dodd L., Kaplan R., Lacombe D. & Verweij. 2009.** New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). *European Journal of Cancer*. 45: 228-247.

2 **Calvet C.Y. & Mir L.M. 2016.** The promising alliance of anti-cancer electrochemotherapy with immunotherapy. *Cancer Metastasis Review*. 35: 165-177.

3 **Falk H., Matthiessen L.W., Wooler G. & Gehl J. 2018.** Calcium electroporation for treatment of cutaneous metastases; a randomized double blinded phase II study, comparing the effect of calcium electroporation with electrochemotherapy. *Acta Oncology*. 12: 42.

4 **Falk H., Lambaa S., Johannesen H.H., Wooler G., Venzo A. & Gehl J. 2017.** Electrochemotherapy and calcium electroporation inducing a systemic immune response with local and distant remission of tumors in a patient with malignant melanoma - a case report. *Acta Oncology*. 56(8): 1126-1131.

5 **Frandsen S.K., Gissel H., Hojman P., Eriksen J. & Gehl J. 2014.** Calcium electroporation in three cell lines; a comparison of bleomycin and calcium, calcium compounds, and pulsing conditions. *Biochimica Biophysica Acta*. 1840(3): 1204-1208.

6 **Frandsen S.K., Hansen H.F. & Gehl J. 2016.** New Drugs for Electrochemotherapy with Emphasis on Calcium Electroporation. In: Miklavčič D. (Ed). *Handbook of Electroporation*. Basel: Springer International Publishing, pp.1-13.

7 **Frandsen S.K., Gissel H., Hojman P., Tramm T., Eriksen J. & Gehl J. 2012.** Direct therapeutic applications of calcium electroporation to effectively induce tumor necrosis. *Cancer Research*. 72(6): 1336-1341.

8 **Kulbacka J., Paczuska J., Rembialkowska N., Saczko J., Kielbowicz Z., Kinda W., Liszka B., Kotulska M., Kos B., Miklavcic D., Tozon N. & Cemazar M. 2017.** Electrochemotherapy combined with standard and CO2 laser surgeries in canine oral melanoma. *Slovenian Veterinary Research*. 54: 181-186.

9 **Marty M., Sersa G., Garbay J.R., Gehl J., Collins C.G., Snoj M., Billard V., Geertsen P.F., Larkin J.O., Miklavcic D., Pavlovic I., Paulin-Kosir S.M., Cemazar M., Morsli N., Soden D.M., Rudolf Z., Robert C., O'Sullivan G.C. & Mir L.M. 2006.** Electrochemotherapy - An easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: results of ESOPE (European Standard Operating Procedures of Electrochemotherapy) study. *European Journal Cancer*. 4(11): 3-13.

10 **Milevoj N., Tratar U.L., Nemec A., Brozic A., Znidar K., Sersa G., Cemazar M. & Tozon N. 2019.** A combination of electrochemotherapy, gene electrotransfer of plasmid encoding canine IL-12 and cytoreductive surgery in the treatment of canine oral malignant melanoma. *Research Veterinary Science*. 122: 40-49.

11 **Nishiya A.T., Massoco C.O., Felizzola C.R., Perlmann E., Batschinski K., Tedardi M.V., Garcia J.S., Mendonça P.P., Teixeira T.F. & Zaidan Dagli M.L. 2016.** Comparative Aspects of Canine Melanoma. *Veterinary Science*. 19: E7.

12 **Patnaik A.K. & Mooney S. 1988.** Feline melanoma: a comparative study of ocular, oral, and dermal neoplasms. *Veterinary Pathology*. 25: 105-112.

13 **Plaschke C.C., Gehl J., Johannesen H.H., Fischer B.M., Kjaer A., Lomholt A.F. & Wessel I. 2019.** Calcium electroporation for recurrent head and neck cancer: a clinical phase I study. *Laryngoscope Investigative Otolaryngology*. 4(1): 49-56.

14 **Tozon N., Lampreht T.U., Znidar K., Sersa G., Teissie J. & Cemazar M. 2016.** Operating procedures of the electrochemotherapy for treatment of tumor in dogs and cats. *Journal of Visualized Experiments*. 116: 54760**.**

15 **van der Linde-Sipman J.S., de Wit M.M.L., van Garderen E., Molenbeek R.F., van der Velde-Zimmermann D. & de Weger R.A. 1997.** Cutaneous malignant melanomas in 57 cats: identification of (amelanotic) signet-ring and balloon cell types and verification of their origin by immunohistochemistry, electron microscopy, and *in situ* hybridization. *Veterinary Pathology*. 34: 31-38.