Combination therapy with APAVAC® immunotherapy and low dose cyclophosphamide as adjuvant treatment for feline aggressive mammary carcinomas – A Pilot study

Petrucci, G.[1,2], Martins, J.[3], Lobo, L.[1,4,5], Queiroga, F.[2], Prada, J.[6], Pires, I.[6], Frayssinet, P.[7], Henriques, J.[3]

[1] Hospital Veterinário do Porto, Porto, Portugal
[2] CITAB, Centre of Technologies Agro-environmental and Biologics, University of Trás-os-montes and Alto Douro, Vila Real, Portugal
[3] Hospital Veterinário de Berna, Lisboa, Portugal
[4] CECA, Centro de Estudos em Ciência Animal, Universidade do Porto, Porto, Portugal
[5] Lusófona University, Lisboa, Portugal
[6] CECAV, Animal and Veterinary Research Centre, University of Trás-os-montes and Alto Douro, Vila Real, Portugal
[7] Urodelia, Bioengineering Department, Toulouse, France

INTRODUCTION

Feline mammary carcinomas are a very aggressive disease with high rates of metastasis after radical surgery[1]. Adjuvant chemotherapy has been suggested but reports are few and with heterogeneous results[2]. The purpose of this preliminary evaluation is to assess the efficacy of a chemoimmunotherapy (tumor microenvironment-oriented therapy) combining APAVAC® (Heat-Shock-Protein96 based vaccine) and metronomic cyclophosphamide.

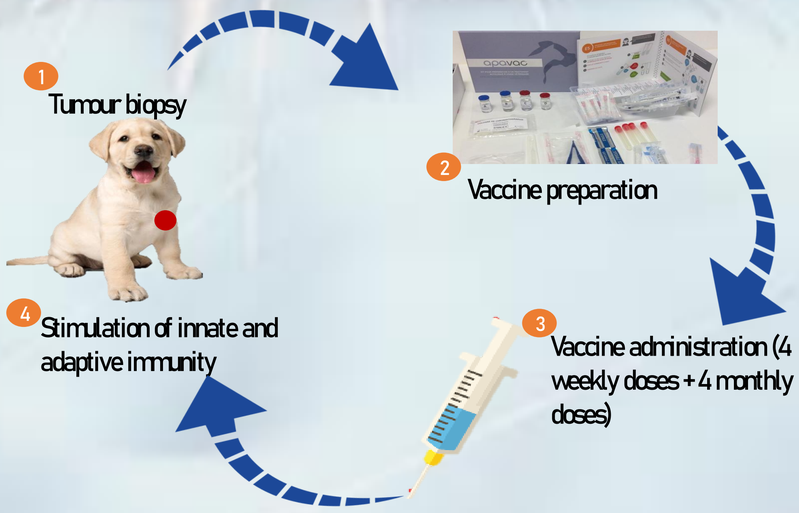

Figure 1: Preparation of autologous tumour cell vaccine APAVAC®

MATERIAL AND METHODS

Eleven female cats with mammary Carcinoma were enrolled after full staging procedures. All cats were submitted to radical mastectomy and tumour classification was performed (WHO classification scheme). For vaccine preparation, HSP96 were isolated and purified from the tumour. Oral cyclophosphamide (15mg/m² SID) + meloxicam (0,05mg/Kg SID) and vaccine administration were used as adjuvant treatment. Time to relapse (TTR), time to progression (TTP), tumour specific survival (TSS) and treatment toxicity were assessed and compared with previous reported literature.

Stage	Histologic classification	Grade	Linfnode metastasis	Mitosis*
I (n=1) III (n=10)	Tubulo-papillary (n=5)	1 (n=1)	Yes (n=7) No (n=4)	>15 (n=9) 0-7 (n=2)
	Solid carcinoma (n=4)	2 (n=3)		
	Ductal carcinoma (n=1)	3 (n=7)		
	Anaplastic carcinoma (n=1)			

Figure 2: Characterization of the study sample.
*Number of mitoses per field area (400x field)

RESULTS

Most of the cats (82%) were stage III. Five cats (45%) were diagnosed with mammary carcinoma for the first time, the remaining with recurrent disease. Side effects were observed in 82% of patients (grade 1 and 2 VCOG-CTOE). Only two cats died before completing the immunotherapy protocol. The median TTR and TTP were 297 and 377 days respectively. 64% of the cats are still alive.

Category	Adverse Event	Absolute Frequency	Grade
Renal/Genitourinary	Cystitis	2	1 and 2
Blood/Bone marrow	Neutropenia	3	1 and 2
	Anaemia	1	2
Administration site conditions	Injection site reaction	2	1
Gastrointestinal	Vomiting	2	1 and 2
	Anorexia	1	2

Figure 3: Classification of adverse events observed during chemoimmunotherapy, based on VCOG-CTCAE (2011).

	Median	Confidence Interval 95%	
		Lower limit	Upper limit
Time to Relapse (TTR)	297,0	205,65	388,34
Time to progression (TTP)	377	254,99	499,01
Tumour specific survival (TSS)*	443	307,02	580,52

*Results for mean

Figure 4: Median and confidence interval 95% of the end-points TTR. TTP and TSS.

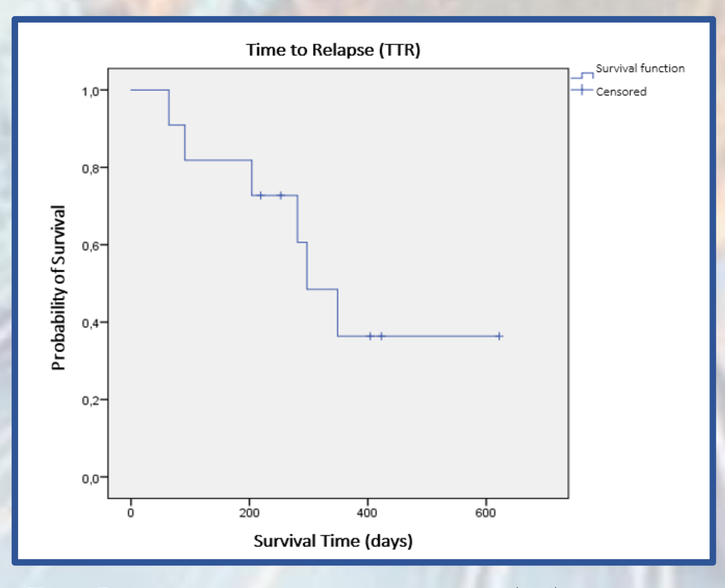

Figure 5: Kaplan-Meier curve of time to relapse (TTR).

CONCLUSIONS

To the authors knowledge this is the first description of the application of chemoimmunotherapy in feline mammary carcinomas and the first prospective study evaluating adjuvant treatment since 1984[8]. This therapy targets, microenvironment, angiogenesis and stimulation of anti-tumor immune system. We considerate that this treatment may be a valid option for aggressive feline mammary carcinomas, with low toxicity and similar disease-free intervals compared with previous reports. Further studies with a large number of patients are needed to evaluate is true efficacy.

	References	Adjuvant therapy	DFI (days)	ST (days)
Stage III	[3]Ito et al. (1996)	Cyclophosphamide/vincristine	-	270
	[4]Novosad et al. (2006)	Doxorrubicine	416	442
	[5]Borrego et al. (2009)	Doxorrubicine + cyclophosphamide	324	460
	[6]Seixas et al. (2011)	None	-	180
	[7]Cunha et al. (2015)	Mitoxantrone	360	640

Figure 6: Review of studies with and survival evaluation of cats in stage 3. (DFI, disease free-interval; ST, survival time).

References:
(1) Gimenez, F.; Hecht, S.; Craig, L. E.; Legendre, A. M., Early detection, aggressive therapy: optimizing the management of feline mammary masses. J Feline Med Surg 2010, 12 (3), 214-24
(2) Morris, J. Mammary Tumors in the Cat, Size matters, so early intervention saves lives, Journal of feline medicine and surgery (2013) 15, 391-400
(3) Ito, T.; Kadosawa, T.; Mochizuki, M.; Matsunaga, S.; Nishimura, R.; Sasaki, N., Prognosis of malignant mammary tumor in 53 cats. J Vet Med Sci 1996, 58 (8), 723-6.
(4) ovosad, C. A.; Bergman, P. J.; O'Brien, M. G.; McKnight, J. A.; Charney, S. C.; Selting, K. A.; Graham, J. C.; Correa, S. S.; Rosenberg, M. P.; Gieger, T. L., Retrospective evaluation of adjunctive doxorubicin for the treatment of feline mammary gland adenocarcinoma: 67 cases. Journal of the American Animal Hospital Association 2006, 42 (2), 110-120
(5) Borrego, J. F.; Cartagena, J. C.; Engel, J., Treatment of feline mammary tumours using chemotherapy, surgery and a COX-2 inhibitor drug (meloxicam): a retrospective study of 23 cases (2002-2007)*. Veterinary and comparative oncology 2009, 7 (4), 213-21
(6) Seixas, F.; Palmeira, C.; Pires, M. A.; Bento, M. J.; Lopes, C., Grade is an independente prognostic factor for feline mammary carcinomas: a clinicopathological and survival analysis. Veterinary journal 2011, 187 (1), 65-71
(7) Cunha, S. C., Corgozinho, K. B., Souza, H. J., Silva, K.V, Ferreira, A. M. Adjuvant chemotherapy with mitoxantrone for cats with mammary carcinomas treated with radical mastectomy. Journal of Feline Medicine and Surgery 2015, 17 (12) 1000-1004
(8) MacEwen, E. G.; Hayes, A. A.; Mooney, S.; Patnaik, A. K.; Harvey, H. J.; Passe, S.; Hardy, W.D., Jr., Evaluation of effect of levamisole on feline mammary cancer. Journal of biological response modifiers 1984, 3 (5), 541-6