# Immunotherapy in canine melanoma

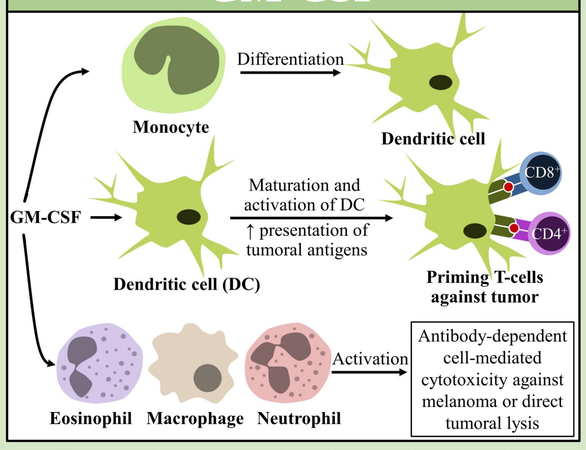
Carla Domenech Palacios

June 2017

## INTRODUCTION AND OBJECTIVES

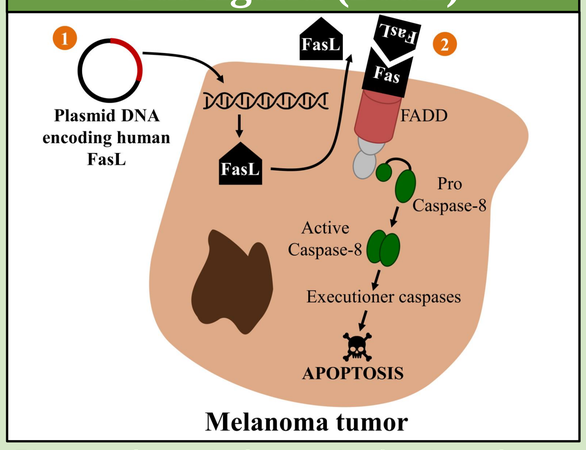
Canine malignant melanoma is a spontaneous and highly aggressive tumor of the oral cavity, digit and mucocutaneous junctions which is similar biologically to malignant melanoma in humans. Following conventional treatments (surgery, radiation therapy and/or chemotherapy), median survival times range from 4 to 12 months. Therefore, new strategies to treat canine melanoma are warranted. In the past decade, the rapidly advancing field of cancer immunology has produced several new immunotherapies for treating cancer that increase the strength of immune system against tumors. The objective of this work is the accomplishment of a bibliographic review to understand the mechanisms of action of the different immunotherapeutic strategies developed against canine malignant melanoma, and to review their effectiveness. The different immunotherapeutic strategies have been classified into four sections: innate immunotherapy, specific immunotherapy, vaccination and future prospects.

### GM-CSF



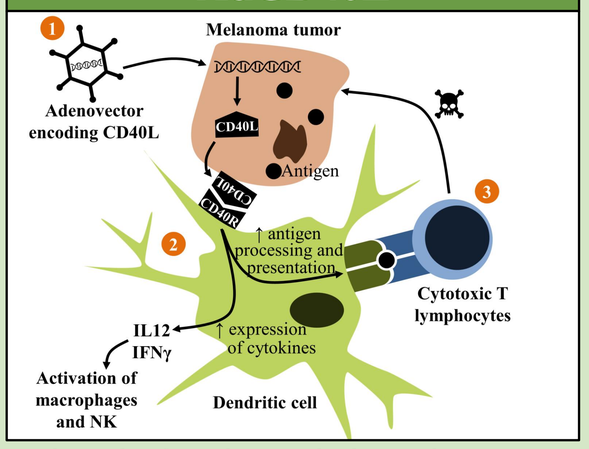

MacEwen et al. aministered to dogs with oral malignant melanoma L-MTP-PE alone or in combination with recombinant canine granulocyte macrophage colony-stimulating factor (GM-CSF). In early stages, the treatment showed a prolongation of survival time. However, there was no significant antitumor activity in advanced stages of canine oral melanoma.

### Fas ligand (FasL)



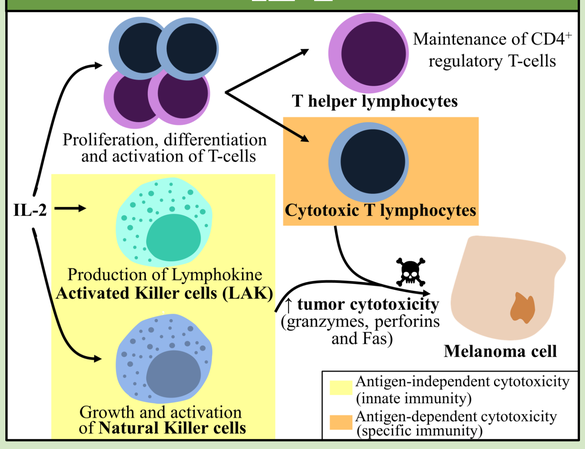

Bianco et al. examined apoptosis of canine melanoma cells lines in vitro by overexpression of FasL DNA. Overexpression of FasL induced apoptosis in each of five Fas$^+$ canine melanoma cell lines whereas Fas$^-$ cell line was resistant. Direct intratumoral administration of FasL DNA to dogs with melanoma was safe and a tumoral regression was seen in three out of five dogs.

### AdCD40L



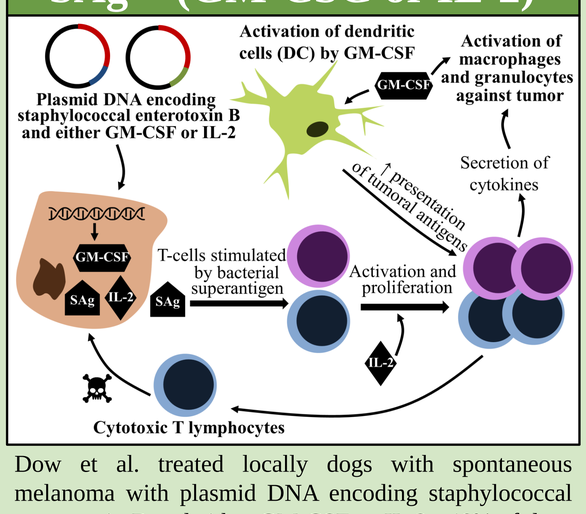

In the study of Westberg et al., dogs with spontaneous melanoma were locally infected with a recombinant adenovirus vector expressing human CD40L. 68% of the patients had a tumor regression higher than 50%, with 26% of them having a complete regression. Tumor tissue was infiltrated with T and B lymphocytes and treatment was safe.

### IL-2



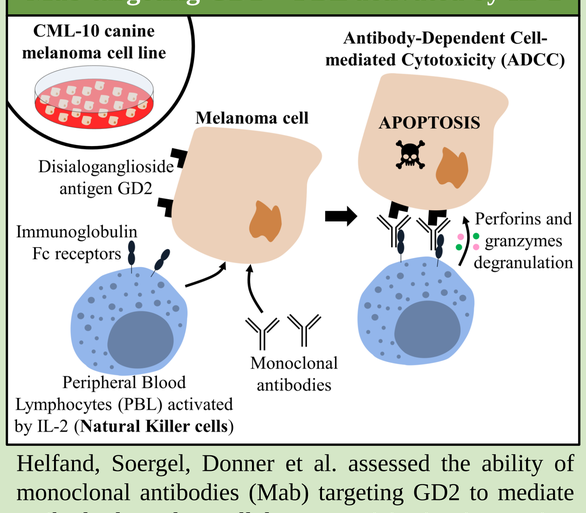

Helfand et al. examined the ability of a low dose of human recombinant IL-2 to enhance tumoricidal properties of canine peripheral blood lymphocytes (PBL) in vitro. Human recombinant IL-2 could significantly increase tumor cytotoxicity mediated by canine PBL in vitro, even when used at a concentration unlikely to induce in vivo toxicity in dogs.

### SAg + (GM-CSG or IL-2)



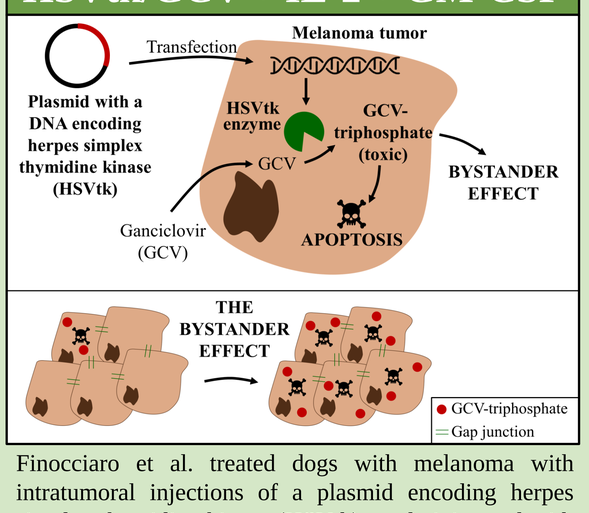

Dow et al. treated locally dogs with spontaneous melanoma with plasmid DNA encoding staphylococcal enterotoxin B and either GM-CSF or IL-2. 46% of dogs had complete or partial response. Tumor tissue was infiltrated with macrophages and T lymphocytes. There were increased levels of cytotoxic CD8$^+$ lymphocytes in the bloodstream. Increased survival time was observed and treatment was safe.

### Mab targeting GD2 + PBL activated by IL-2



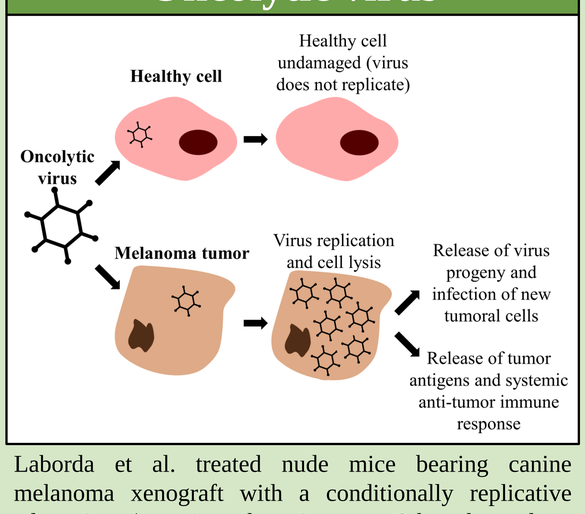

Helfand, Soergel, Donner et al. assessed the ability of monoclonal antibodies (Mab) targeting GD2 to mediate antibody-dependent cellular cytotoxicity in vitro against a canine melanoma cell line (CML-10). Monoclonal antibodies potentiated lysis of the canine melanoma cell line by canine peripheral blood lymphocyte (PBL) stimulated with IL-2.

### HSVtk/GCV + IL-2 + GM-CSF



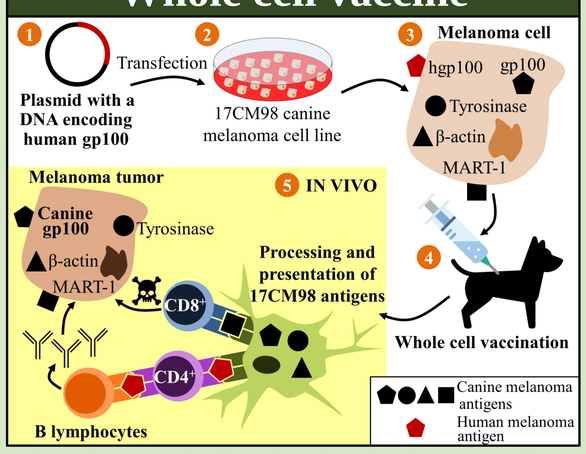

Finocciaro et al. treated dogs with melanoma with intratumoral injections of a plasmid encoding herpes simplex thymidine kinase (HSVtk) coadministrated with ganciclovir (GCV), and irradiated xenogeneic cells secreting GM-CSF and IL-2. 16% of cases had complete response and 31% had partial response, with an average of 80% of tumor regression. Treatment was safe and significantly improved median survival time.

### Oncolytic virus



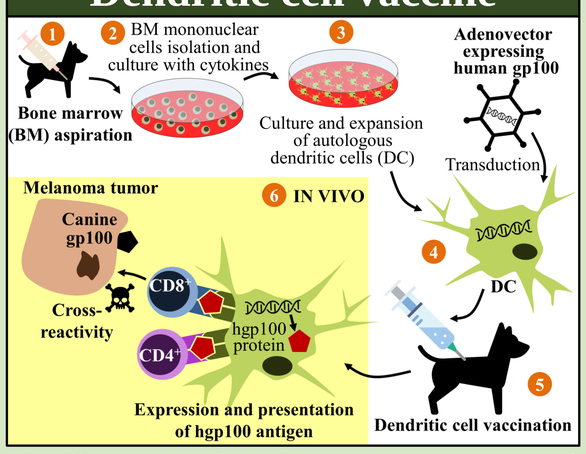

Laborda et al. treated nude mice bearing canine melanoma xenograft with a conditionally replicative adenovirus (a canine adenovirus type 2-based oncolytic virus). Treatment resulted in inhibition of tumor growth and prolonged survival of mice. Local administration of the same adenovirus in six tumor bearing dogs led to two partial responses. There was no direct virus associated adverse effects.

### Whole cell vaccine



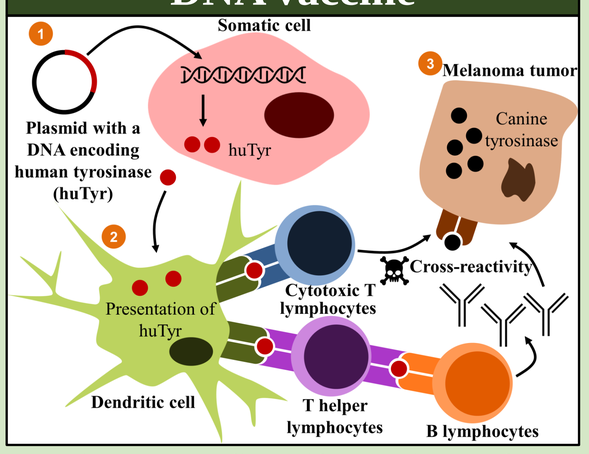

Alexander et al. describe the development of an allogeneic whole-cell tumor vaccine (17CM98 canine melanoma cell line) transfected with xenogeneic human gp100. The overall response rate was 17% (3% complete response and 14% partial response). Dogs with evidence of tumor control had a median survival significantly longer (337 days). Treatment was well tolerated.

### Dendritic cell vaccine



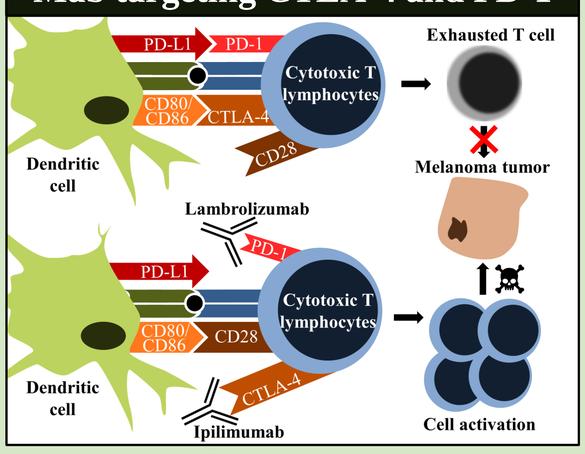

Gyorffy et al. performed an ex vivo expansion of dendritic cells (DC) derived from bone marrow of three dogs with spontaneous melanoma. DC were transduced with the xenoantigen hgp100 and were used as a vaccine. A dog demonstrated antigen-specific cytotoxic T lymphocyte (CD8+) activity in peripheral blood lymphocytes. This dog had no signs of recurrent disease 48 months after initial vaccine injection.

### DNA vaccine



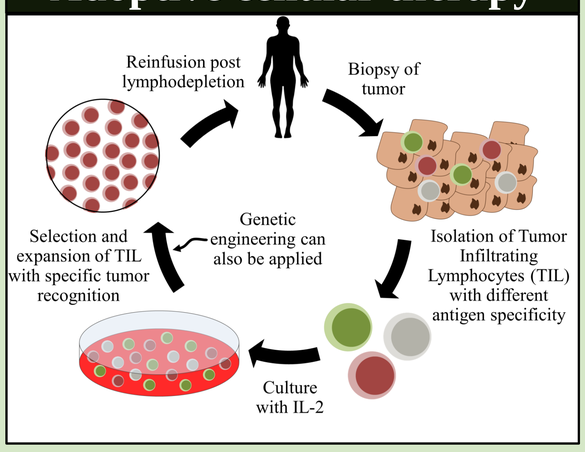

Two important studies with opposite conclusions examined the efficacy of a vaccine containing plasmid DNA encoding human tyrosinase. In the Grosenbaugh et al. paper, the use of the vaccine significantly increased survival times. The melanoma specific median survival time was not reached. In the Ottnod et al. study, the vaccine did not improve the progression free survival, disease-free interval or median survival time.

### Mab targeting CTLA-4 and PD-1




This picture, modified from Regan et al., shows the mechanism of action of monoclonal antibodies against CTLA-4 (ipilimumab) and PD-1 (lambrolizumab). This immunotherapies have not yet been studied in canine malignant melanoma.

### Adoptive cellular therapy




This picture shows the procedure of adoptive cell therapy. This immunotherapy has not yet been studied in canine malignant melanoma.

## CONCLUSION

The field of tumor immunotherapy is growing rapidly. Melanoma vaccination is having an impressive progress, with new approaches incorporating new antigen targets and delivery technologies. Recent immunotherapeutic advances against melanoma have been made in human medicine, including anti-CTLA-4 and anti-PD-1 antibodies and adoptive T-cell transfer. Hopefully, in a future, veterinary oncologists will have access to these innovative and effective immunotherapies.

Further research is needed to:

- Understand why some immunotherapies are only effective in some patients
- Improve the immunotherapeutic modalities (maximize efficacy, minimize toxicity and avoid resistance mechanisms)
- Detect reliable biomarkers (e.g. PD-L1) to increase the proportion of patients responding to immunotherapy
- Determinate the most effective immunotherapeutic combinations (potential synergistic activity)

**REFERENCES**

Alexander et al. 2006. Cancer Immunol. Immunother. 55:433–42.
Bianco et al. 2003. Gene Ther. 10:726–36.
Dow et al. 1998. J. Clin. Invest. 99:2616–24.
Finocchiaro et al 2008. Cancer Gene Ther. 15:267–276.
Grosenbaugh et al. 2011. Am. J. Vet. Res. 72:1631–1638.
Gyorffy et al. 2005. J. Vet. Intern. Med. 19:56–63.
Helfand et al. 1994b. Cancer Biother. 9:237–44.
Helfand, Soergel, Donner et al. 1994a. J. Immunother. 16:188–97.
Laborda et al. 2014. Mol. Ther. 22:986–98.
MacEwen et al. 1999. Clin. Cancer Res. 5:4249–58.
Ottnod et al. 2013. Vet. Comp. Oncol. 11:219–229.
Regan et al. 2016. Vet. J. 207:20–8.
Westberg et al. 2013. J. Immunother. 36:350–8.

FACULTAT DE VETERINÀRIA

UAB
Universitat Autònoma de Barcelona