

## Therapy used in canine melanoma - current approaches and future perspectives Terapia utilizată în melanomul canin - abordări actuale și perspective viitoare

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### Rezumat

Melanomul canin este o tumoră agresivă, frecvent localizată la nivel bucal, digital, ocular sau cutanat, caracterizată printr-un potențial ridicat de invazie locală și metastazare. Opțiunile terapeutice includ excizia chirurgicală ca tratament principal, completată adesea de radioterapie, chimioterapie și imunoterapie. În ultimii ani, imunoterapia, în special vaccinurile ADN, a demonstrat un potențial promițător în prelungirea supraviețuirii și îmbunătățirea calității vieții pacienților canini. Scopul acestui scurt review este de a oferi o sinteză a celor mai recente date privind tratamentul melanomului canin, evidențiind importanța unei abordări multimodale și personalizate pentru optimizarea prognosticului.

### Abstract

Canine melanoma is an aggressive tumor, commonly located in the oral cavity, digits, eyes, or skin, characterized by a high potential for local invasion and metastasis. Therapeutic options include surgical excision as the primary treatment, often complemented by radiotherapy, chemotherapy, and immunotherapy. In recent years, immunotherapy, particularly DNA vaccines, has shown promising potential in prolonging survival and improving the quality of life in canine patients. The aim of this brief review is to provide a synthesis of the most recent data regarding the treatment of canine melanoma, highlighting the importance of a multimodal and personalized approach to optimize prognosis.

### Introduction

Canine melanoma is an extremely aggressive neoplasia, commonly found in dogs, characterized by the abnormal proliferation of melanocytes.

It can occur in any breed, being located in the oral cavity (in 44–62% of cases), on the skin (27–44%), on the digits (8–10%), and in the ocular region (1–3%).

Canine melanoma accounts for approximately 4–7% of all canine malignant tumors, while oral melanoma represents 30–40% of all canine oral tumors [18].

Most formations are pigmented and appear black due to the high melanin content,

but there are also non-pigmented melanomas (amelanotic melanomas).

The prognosis of canine melanoma depends on the location of the tumor, with the most aggressive being the oral form, which presents a high metastasis rate and significant potential for local invasion [32, 35].

Given the incidence and aggressiveness of melanoma in both humans and dogs, it is crucial to design new treatments, especially for advanced cases in which the available options are of limited efficacy.

Furthermore, considering that early diagnosis significantly influences the prognosis, it is equally necessary to develop

new diagnostic techniques and protocols that allow early detection of this neoplasia.

In recent decades, advances in veterinary oncology have led to the development of new investigative methods capable of differentiating melanoma from other tumors, assessing the tumor's degree of aggressiveness, and providing essential information for establishing a personalized therapeutic approach.

Thus, immunohistochemical biomarkers such as Melan-A, PNL2, or Ki-67, as well as genetic tests targeting specific mutations (e.g., in the BRAF gene), play an increasingly important role in the evaluation of melanocytic neoplasms [33].

### **Carcinogenesis of canine melanoma**

The carcinogenesis of canine melanoma is a complex process involving multiple signaling pathways and immune evasion mechanisms.

Advances in understanding these processes offer opportunities for the development of more effective and personalized therapies for dogs affected by this aggressive neoplasia [30].

The tumor microenvironment plays a crucial role in the progression of canine melanoma.

Tumor cells can express immune checkpoint molecules such as PD-L1, which inhibit T lymphocyte activation and allow immune evasion by the tumor. Additionally, tumor infiltration with regulatory T lymphocytes (Tregs) contributes to local immunosuppression and promotes tumor progression [24,33].

Molecular mechanisms involved in carcinogenesis:

*a) Dysregulation of cellular signaling pathways:* The MAPK (RAS/RAF/MEK/ERK) and PI3K/AKT/mTOR signaling pathways are frequently activated in canine melanoma, contributing to uncontrolled proliferation and survival of tumor cells.

Although mutations in BRAF and NRAS genes are rare in canine melanoma, activation of these pathways may occur through other mechanisms, such as loss of tumor suppressor gene function [16].

*b) Inactivation of tumor suppressor genes:* The TP53 and PTEN genes are involved in cell cycle control and apoptosis.

Mutations or loss of expression of these genes have been reported in canine melanoma, contributing to tumor cell escape from cellular control mechanisms [33].

*c) Alterations in genes involved in cell cycle regulation:* Inactivation of the CDKN2A gene, which encodes the p16 protein, has been observed in canine melanoma, leading to dysregulation of the G1/S checkpoint of the cell cycle and promoting uncontrolled cell proliferation [29].

Understanding the molecular mechanisms involved in the carcinogenesis of canine melanoma has led to the development of targeted therapies.

Inhibitors of the PI3K/AKT/mTOR and MAPK pathways are being evaluated for their effectiveness in treating canine melanoma. Additionally, immunotherapies such as anti-PD-1/PD-L1 monoclonal antibodies have shown promising results in preclinical studies [30].

### **Treatment in canine melanoma**

#### **Multimodal therapies**

Treatment of canine melanoma often involves multimodal therapies that integrate various treatment modalities to improve patient outcomes.

These therapies may include surgery, radiotherapy, chemotherapy, immunotherapy, electrochemotherapy, and gene therapy [4,9].

For a better synthesis of the therapeutic options available in the case of canine malignant melanoma.

In Table 1 the advantages and limitations of the main treatment methods used in veterinary practice are briefly presented.

**Table 1.**  
Main therapeutic options for canine melanoma

Treatment	Mechanism of action	Advantages	Limitations
<b>Surgery</b>	Complete tumor excision	First-line treatment	Risk of recurrence, limited in inaccessible areas
<b>Radiation</b>	Local destruction of tumor cells	Useful post-operatively or palliative	Expensive, low availability
<b>Chemotherapy</b>	Inhibits tumor cell division	Complementary to other therapies	Variable efficacy, side effects
<b>Immunotherapy</b>	Stimulates the immune system against tumor cells	Non-invasive, innovative	High cost
<b>Combination therapies</b>	Multimodal approach	Increases chances of tumor control	Therapeutic complexities

### Surgical Interventions

Surgery remains the gold standard in oncological treatment, frequently used for the local management of all melanomas, including oral, cutaneous, and digital melanomas [6,38].

Surgical resection with wide margins has always been one of the recommended approaches for canine melanoma, often associated with regional lymphadenectomy.

Wide-margin surgical resections (2–3 cm of bone and 1 cm of soft tissue) were applied in 70 cases of canine oral malignant melanoma, achieving complete excision in 72.9% of cases.

Local recurrence was observed in 10% of patients. Dogs treated with surgery alone had a progression-free interval >567 days and a median survival time (MST) of 874 days [14,34].

In another study, Boston et al. reported a complete excision rate of 79.3% and a recurrence rate of 8.3%, with an MST of 354 days [6].

In a study conducted by Williams and Packer on 100 dogs with oral malignant melanoma, 53% showed cytological or histopathological evidence of metastases in the mandibular lymph nodes, even in normally sized nodes.

Therefore, removal of regional lymph nodes is recommended, especially in dogs with oral melanomas. In digital melanomas, lymphatic drainage of the limbs should also be evaluated, and regional lymph node removal is recommended during surgical planning [37].

Although surgical treatment is the primary choice, Liptak and Withrow and Boston et al. recommend that, due to its high metastatic potential, systemic therapy should also be considered as a therapeutic option for melanomas [6,23].

### Radiotherapy

Radiotherapy is a form of localized treatment, effective against proliferative tumor cells within the irradiated field.

In canine melanoma, radiotherapy is used as a postoperative adjuvant or palliative therapy, ideally including regional lymph nodes, regardless of the presence of clinical signs of metastasis.

Adverse effects are generally mild and localized, including alopecia, pigmentation changes, dry radiodermatitis, and oral mucositis [14].

The overall tumor response rate is reported between 75–85%, and median survival ranges from 230 to 363 days [8,15].

In a study by Cunha et al., ortho-voltage radiotherapy was applied to 24 dogs, with a response rate of 93% (64% partial, 29% complete), and median survival ranged from 390 days (stage I) to 90 days (stage IV) [10].

### Chemotherapy

Chemotherapy is a commonly used component in the treatment of canine melanoma, often in combination with other therapeutic modalities such as surgery and radiotherapy [20].

Ideally, a chemotherapeutic agent should have selective toxicity towards tumor cells, efficient distribution within the tumor mass, lack of resistance development, and a favorable safety profile for the patient [27].

Among the agents evaluated in the literature are: carboplatin [11], intralesional cisplatin implants [21], masitinib mesylate [17], mitoxantrone [26], the cisplatin–piroxicam combination [5], artesunate, and olomoucine [27].

Carboplatin has offered the longest median survival times (MST), with values of 440 and 389 days reported in studies where survival was calculated from the time of diagnosis [7,11].

In contrast, for other treatments such as masitinib, the cisplatin - piroxicam combination, and intralesional cisplatin implants, the reported MST was shorter, being measured from the start of chemotherapy, in the context of unresectable or recurrent tumors.

The lack of standardization in data reporting and unclear inclusion of cases with or without prior surgery limits the evaluation of chemotherapy's specific impact as an adjuvant [27].

However, studies conducted by Brockley et al. and Boston et al. did not show significant differences in survival between dogs treated only surgically (495 and 335 days, respectively) and those who received surgery followed by carboplatin chemotherapy (389 and 352 days) [6,7].

Moreover, according to data published by Tuohy et al., adjuvant chemotherapy post-resection was associated with a higher risk of disease progression, without significantly influencing the risk of death after adjusting for tumor size and metastasis presence [34].

Masitinib, evaluated in advanced stages (III and IV) refractory to conventional treatments, provided survival times comparable to other regimens, although most cases in these groups were in less advanced stages [17, 27].

## Immunotherapy

In recent decades, immunotherapy has taken a central role in oncology due to significant increases in therapeutic efficacy and survival rates among cancer patients.

Although the concept that the immune system can recognize and eliminate tumor cells is not new, clinical observations of spontaneous tumor regression and the positive association between tumor lymphocytic infiltration and prognosis have revived interest in the tumor immunosurveillance theory [14].

In malignant melanoma, conventional therapies continue to yield limited results, justifying the exploration of immunotherapeutic strategies.

These include the canine DNA vaccine against melanoma, dendritic cell immunotherapy, and delivery of therapeutic genes via viral and non-viral vectors.

Although clinical responses can vary from partial to complete regressions, the effectiveness of immunotherapy is often compromised by immune reprogramming induced by tumor cells.

This phenomenon leads to an immunosuppressive tumor micro-environment, limiting the immune system's ability to mount a robust antitumor response [1,13,36].

The limitations of conventional therapies used in canine melanoma are partly due to the difficulty of achieving complete surgical resection and the reduced effectiveness of chemotherapy in the presence of large tumor masses.

In this context, immunotherapy has emerged as a promising therapeutic option, capable of improving prognosis and inducing long-term remissions in various cancer types [14].

One example is the nano-immunotherapy OncoTherad, a nano-structured complex based on inorganic phosphate and glycosidic protein, developed at the University of Campinas (Brazil), with immunostimulatory and antitumor properties.

OncoTherad acts by activating Toll-like receptors (TLR2 and TLR4), stimulating the interferon (IFN) signaling pathway, and amplifying the innate immune response [3,12].

Another example that highlights the efficacy of immunotherapy in the therapeutic management of canine melanoma is the Oncept vaccine.

This is an innovative immunotherapeutic option for treating canine oral melanoma, particularly in stages II and III, after achieving loco-regional tumor control through surgery and/or radiotherapy.

It is a xenogeneic DNA vaccine targeting tyrosinase, an enzyme involved in melanin synthesis, highly conserved among mammalian species.

The Oncept® vaccine uses human DNA encoding this enzyme, inserted into a bacterial plasmid and administered to dogs, stimulating a specific immune response [2,28].

The technology was adapted from an experimental vaccine previously tested in human patients with advanced melanoma, where it was shown to be safe and immunogenic.

Due to the over 85% homology between human and canine tyrosinase, the antigen is sufficiently different to trigger immunity but also similar enough to effectively target canine tumor cells [28,39].

### Gene Therapy

Immunotherapy has proven to be the most promising use of viral vectors in melanoma gene therapy, with notable examples being IMLYGIC and CAR-T cells.

These approaches aim for immune-mediated oncolysis by activating the antitumor immune response [22,31].

At the Instituto do Câncer do Estado de São Paulo (Faculty of Medicine, University of São Paulo), a non-replicating adenoviral vector, AdRGD-PG, was developed with enhanced transduction efficiency due to the insertion of the RGD peptide and transcriptional activation controlled by the PG promoter, sensitive to p53.

Since many melanoma cells retain wild-type p53, this system can stimulate gene expression and induce cell death.

The AdRGD-PG platform was used for the transfer of p14ARF and IFN $\beta$  genes, demonstrating in the B16F10 murine model the induction of immunogenic cell death (ICD) and the activation of a Th1 immune response [14, 19].

For translational purposes in veterinary medicine, AdRGD-PG vectors expressing the canine cDNAs of p14ARF and IFN $\beta$  were created.

Additionally, three canine oral melanoma cell lines were isolated, expressing wild-type p53 and permissive to transduction.

These lines are currently being tested *in vitro* and *in vivo* (in BALB/c nude mice), and will serve as proof of concept for application in spontaneous cases of canine oral melanoma [14, 25].

### Perspectives and Future Directions

The future of research in canine melanoma may largely depend on the integration of advanced technologies and personalized medicine into standard practices.

Progress is essential not only for improving diagnosis and prognosis but also for facilitating the development of more effective and personalized therapeutic strategies for canine melanoma, with the potential to increase survival rates.

Given the aggressiveness of canine melanoma and the limitations of conventional therapies, future research should focus on developing integrated, personalized therapeutic approaches based on the tumor's molecular profile.

In this regard, expanding studies on molecular and immunological biomarkers specific to the canine species may contribute to the early identification of tumor subtypes with poor prognosis and to treatment optimization.

Immunotherapy, particularly through DNA vaccines, cellular therapies, and immune checkpoint inhibitors, remains one of the most promising directions, with large-scale clinical studies needed to validate the efficacy and safety of these methods.

Furthermore, gene therapy and nano-immunotherapy, through the use of specific vectors and targeted delivery systems, offer considerable potential for systemic disease control and metastasis prevention.

Another essential direction is the standardization of diagnostic, treatment, and post-therapy follow-up protocols to ensure result comparability between studies and to establish clear therapeutic guidelines in veterinary oncology. Finally, closer collaboration between veterinary and human oncology research could accelerate the discovery of innovative therapeutic solutions, in a translational One Health approach.

### Conclusions

Canine melanoma represents a major challenge in veterinary oncology due to its aggressiveness, high metastatic potential, and limited response to conventional therapies.

Despite significant progress in understanding pathogenesis, treatment remains difficult, especially in advanced stages of the disease.

Surgical therapy remains the standard for local tumor control, while adjuvant strategies such as radiotherapy, chemotherapy, and immunotherapy can help prolong survival.

New approaches, including gene therapies and DNA vaccines, open promising prospects for achieving more effective and lasting therapeutic responses.

The integration of modern diagnostic and treatment technologies based on molecular and immunological biomarkers will play a key role in personalizing treatment and improving the prognosis of dogs affected by malignant melanoma.

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