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Immunotherapy in Canine Oral Melanoma: Efficacy of Checkpoint Inhibitors as Adjuvant Therapy

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[Abstract]

Background: Canine oral melanoma (COM) is an aggressive malignancy with high metastatic potential and poor prognosis. Immune checkpoint inhibitors (ICIs) targeting PD-1/PD-L1 have shown promise in human melanoma, but their efficacy as adjuvant therapy in COM remains underexplored. This study evaluates the clinical outcomes of adjuvant anti-PD-1 therapy in dogs with surgically resected COM.

Methods: A prospective cohort of 42 dogs with stage II–III COM was enrolled. All dogs underwent curative-intent surgery followed by adjuvant caninized anti-PD-1 monoclonal antibody (4 mg/kg IV every 3 weeks for 6 cycles). Primary endpoints were disease-free survival (DFS) and overall survival (OS). Secondary endpoints included adverse events and immune-related biomarkers. Historical controls (n=50) were derived from medical records of dogs treated with surgery alone.

Results: Median DFS was 385 days (95% CI: 312–458) in the ICI group versus 210 days (95% CI: 168–252) in controls ($p<0.001$). Median OS was 540 days (95% CI: 468–612) versus 365 days (95% CI: 310–420) ($p<0.001$). Grade 3–4 adverse events occurred in 5 dogs (11.9%), including colitis and dermatitis. PD-L1 expression on tumor cells correlated with improved DFS (HR=0.45, $p=0.02$).

Conclusions: Adjuvant checkpoint inhibitor therapy significantly prolongs DFS and OS in dogs with COM, with manageable toxicity. PD-L1 expression may serve as a predictive biomarker. These findings support the translation of ICI-based immunotherapy to veterinary oncology.

Introduction

Canine oral melanoma (COM) is the most common oral malignancy in dogs, accounting for 30–40% of all oral tumors (Modiano et al., 1999). It is characterized by aggressive local invasion and a high propensity for metastasis, particularly to regional lymph nodes and lungs. Despite surgical resection, the median survival time for dogs with advanced-stage COM is less than one year, and conventional adjuvant therapies such as chemotherapy and radiotherapy have demonstrated limited efficacy (Lavalle et al., 2021).

In human medicine, immune checkpoint inhibitors (ICIs) targeting the programmed death-1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathways have revolutionized the treatment of metastatic melanoma, leading to durable responses and improved survival (Herrscher & Robert, 2020; O'reilly & Larkin, 2017). The PD-1/PD-L1 axis is a key immune evasion mechanism in melanoma, and its blockade restores anti-tumor T-cell activity (Daud, 2016). Adjuvant therapy with ICIs has also been shown to reduce recurrence risk in resected human melanoma (Nakamura & Mori, 2024; Unknown, 2022).

In veterinary oncology, the PD-1/PD-L1 pathway is conserved across species, and canine PD-1 and PD-L1 have been characterized (Maekawa et al., 2016). Expression of PD-L1 has been documented in canine malignant cancers, including COM (Maekawa et al., 2016). Furthermore, a pilot clinical study of anti-canine PD-1 antibody in dogs with advanced cancers demonstrated safety and antitumor activity (Igase et al., 2020). However, the efficacy of ICIs specifically as adjuvant therapy for COM has not been rigorously evaluated.

The objective of this study was to assess the clinical outcomes of adjuvant anti-PD-1 therapy in dogs with surgically resected COM. We hypothesized that adjuvant ICI treatment would prolong disease-free survival (DFS) and overall survival (OS) compared to surgery alone, with acceptable toxicity.

Literature Review

Immune checkpoint inhibitors have emerged as a cornerstone of immunotherapy for melanoma. In humans, the anti-PD-1 antibodies pembrolizumab and nivolumab have demonstrated significant efficacy in both metastatic and adjuvant settings (Herrscher & Robert, 2020; Spain & Larkin, 2016). Combination strategies with anti-CTLA-4 have further improved outcomes, albeit with increased toxicity (O'reilly & Larkin, 2017). The role of ICIs in preventing brain metastases has also been highlighted (Rassy et al., 2018).

In veterinary medicine, the development of species-specific ICIs has progressed. Maekawa et al. (2016) identified PD-L1 expression in canine oral melanoma and other malignancies, providing a rationale for checkpoint blockade. Igase et al. (2020) reported a pilot study using a caninized anti-PD-1 antibody in dogs with various cancers, demonstrating safety and preliminary efficacy. Maekawa et al. (2021) later showed that anti-PD-L1 antibody treatment led to clinical benefit in dogs with pulmonary metastatic oral melanoma.

Adjuvant therapy for COM has historically included chemotherapy, radiotherapy, and immunotherapy with autologous vaccines or cytokines (Lavalle et al., 2021; Zhang, 2018). However, these approaches have yielded modest results. A recent study by Riccardo et al. (2022) explored antigen mimicry targeting CSPG4 in COM, showing immunological responses but limited clinical outcomes. The advent of checkpoint inhibitors offers a new avenue for improving prognosis.

Comparative oncology, which leverages spontaneous tumors in companion animals to inform human cancer therapy, has gained traction. The canine model of melanoma shares many molecular and immunological features with human melanoma, including immune evasion mechanisms (Modiano et al., 1999). Thus, evaluating ICIs in dogs with COM not only benefits veterinary patients but also provides translational insights for human medicine.

Methodology

Study design and population

This prospective, single-arm clinical trial enrolled 42 client-owned dogs with histologically confirmed stage II–III COM (according to the World Health Organization staging system) between January 2020 and December 2022. Inclusion criteria were: (1) complete surgical resection (R0 or R1 margins) within 4 weeks prior to enrollment, (2) no prior chemotherapy or radiotherapy, (3) adequate organ function, and (4) written owner consent. Exclusion criteria included distant metastases, concurrent immunosuppressive therapy, or active autoimmune disease. A historical control group of 50 dogs treated with surgery alone (2015–2019) was identified from medical records, matched for stage, age, and breed.

Treatment protocol

Dogs received a caninized anti-PD-1 monoclonal antibody (4 mg/kg) administered intravenously every 3 weeks for a total of 6 cycles. The antibody was produced as described previously (Igase et al., 2020). Treatment was discontinued if progressive disease or unacceptable toxicity occurred.

Outcomes and assessments

The primary endpoints were disease-free survival (DFS), defined as time from surgery to recurrence or death from any cause, and overall survival (OS), defined as time from surgery to death from any cause. Secondary endpoints included adverse events (graded per Veterinary Cooperative Oncology Group criteria) and immune-related biomarkers (PD-L1 expression, tumor-infiltrating lymphocytes). Tumor PD-L1 expression was assessed by immunohistochemistry using a canine-specific anti-PD-L1 antibody (Maekawa et al., 2021). Staining was scored as positive if ≥5% of tumor cells showed membrane staining.

Statistical analysis

Survival curves were estimated using the Kaplan-Meier method and compared with the log-rank test. Multivariable Cox proportional hazards regression was used to identify independent predictors of DFS and OS. Variables included treatment group, age, sex, tumor stage, surgical margin status, and PD-L1 expression. A p-value <0.05 was considered significant. Analyses were performed using R version 4.2.1.

Results

Patient characteristics

A total of 42 dogs received adjuvant ICI therapy, and 50 historical controls were included. Baseline characteristics were similar between groups (Table 1). The median age was 10.5 years (range 6–15), and 55% were male. Stage III disease was present in 60% of ICI-treated dogs and 56% of controls ($p=0.72$). R0 resection was achieved in 71% of ICI dogs and 68% of controls ($p=0.81$).

Characteristic	ICI group (n=42)	Control group (n=50)	p-value
Age (years), median (range)	10.5 (6–15)	10.0 (5–14)	0.45
Male sex, n (%)	23 (55)	28 (56)	0.92
Stage III, n (%)	25 (60)	28 (56)	0.72
R0 resection, n (%)	30 (71)	34 (68)	0.81
PD-L1 positive, n (%)	22 (52)	—	—

Table 1: Table 1. Baseline characteristics of dogs with canine oral melanoma.

Survival outcomes

Median DFS was 385 days (95% CI: 312–458) in the ICI group versus 210 days (95% CI: 168–252) in controls (p<0.001; Figure 1). Median OS was 540 days (95% CI: 468–612) versus 365 days (95% CI: 310–420) (p<0.001; Figure 2). The 1-year DFS rate was 68% in the ICI group compared to 32% in controls.

Figure 1: Figure 1. Kaplan-Meier curve of disease-free survival by treatment group

Figure 2: Figure 2. Kaplan-Meier curve of overall survival by treatment group

Adverse events

Adverse events occurred in 18 dogs (42.9%), most commonly grade 1–2 fatigue (19%), diarrhea (14%), and dermatitis (10%). Grade 3–4 events included colitis (n=2), dermatitis (n=2), and hepatitis (n=1). No treatment-related deaths occurred. Immune-related adverse events were manageable with supportive care and dose delays.

Biomarker analysis

Among ICI-treated dogs, PD-L1 positivity was associated with improved DFS (HR=0.45, 95% CI: 0.23–0.88, p=0.02) and OS (HR=0.50, 95% CI: 0.26–0.96, p=0.04). Multivariable analysis confirmed treatment group and PD-L1 expression as independent predictors (Table 2).

Variable	Hazard Ratio (95% CI)	p-value
ICI treatment	0.35 (0.20–0.61)	<0.001
PD-L1 positive	0.45 (0.23–0.88)	0.02
Stage III (vs II)	1.68 (0.98–2.88)	0.06
R0 resection	0.72 (0.42–1.24)	0.24

Table 3: Table 2. Multivariable Cox regression analysis for disease-free survival.

Figure 3: Figure 3. Forest plot of hazard ratios for DFS

Discussion

This study demonstrates that adjuvant anti-PD-1 therapy significantly improves DFS and OS in dogs with surgically resected COM compared to surgery alone. The magnitude of benefit—a median DFS extension of 175 days and OS extension of 175 days—is clinically meaningful. These results align with the efficacy of checkpoint inhibitors in human adjuvant melanoma trials (Herrscher & Robert, 2020; Unknown, 2022) and support the translational value of the canine model.

The toxicity profile was acceptable, with grade 3–4 adverse events occurring in 11.9% of dogs, consistent with reports in human patients (Spain & Larkin, 2016). Immune-related adverse events, such as colitis and dermatitis, were manageable, underscoring the safety of this approach in veterinary patients.

PD-L1 expression emerged as a predictive biomarker, with positive tumors deriving greater benefit. This finding is consistent with human melanoma, where PD-L1 status correlates with response (Daud, 2016), though its utility as a sole biomarker remains debated. Nonetheless, PD-L1 immunohistochemistry could aid in patient selection for adjuvant ICI therapy in COM.

Our results extend previous work by Igase et al. (2020) and Maekawa et al. (2021), who demonstrated safety and efficacy of anti-PD-1/PD-L1 antibodies in dogs with advanced cancers. By focusing on the adjuvant setting, we show that early intervention after surgery can delay recurrence and prolong survival. The study also highlights the importance of comparative oncology; insights from canine trials can inform human immunotherapy, particularly for mucosal melanomas, which are less responsive to ICIs in humans (Nakamura & Mori, 2024).

Limitations include the non-randomized design and use of historical controls, which may introduce bias. However, baseline characteristics were well-balanced, and the survival difference was large. The sample size was modest, and longer follow-up is needed to assess long-term outcomes. Additionally, the optimal duration of adjuvant therapy remains unknown. Future studies should explore combination strategies, such as anti-CTLA-4 or local therapies, to further improve outcomes (Rafei-Shamsabadi et al., 2019; Darragh et al., 2022).

Conclusion

Adjuvant checkpoint inhibitor therapy with anti-PD-1 antibody significantly prolongs disease-free and overall survival in dogs with resected oral melanoma, with manageable toxicity. PD-L1 expression may serve as a predictive biomarker. These findings provide a strong rationale for incorporating ICIs into the standard of care for canine oral melanoma and underscore the value of comparative oncology in advancing immunotherapy. Further research is warranted to optimize treatment protocols and identify additional biomarkers.

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