Potential biomarkers as prognostic indicators and drug targets in canine OSA using targeted gene sequencing.

Veterinary Research Scholars Program
University of Missouri

Olivia Hritzkowin[1], Jeffrey Bryan[1], Noe Reyes[2], Tammie Wahaus[2], Guannan Wang[3]

[1]College of Veterinary Medicine, University of Missouri, Columbia, MO [2]ELIAS Animal Health [3]Vidium

Introduction

- Osteosarcoma (OSA) in companion dogs is defined by low survival rate and complicated treatment. The current standard of care provides an MST ~10 months
- OSA has a complicated genome, making targeted treatment complicated. Technologies that make rapid gene sequencing possible may allow for the discovery of biomarkers that can be used for precision therapy
- In this study, targeted genome sequencing for 120 genes with multiple mutations was performed on 9 cOSA tumors from patients treated with amputation and targeted immunotherapy
- The goal of this study was to identify novel mutations that may act as biomarkers correlating to disease free interval (DFI) and identify druggable mutations

Methods

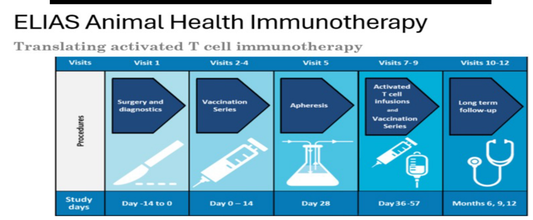

Figure 1: ELIAS Animal Health immunotherapy protocol performed following amputation of affected limb.

- Targeted gene sequencing performed on 9 treated tumors focusing on 120 specific mutations
- Careful literature review performed to created a list of previously identified mutations in cOSA
- Data analysis performed to compare known mutations to the 48 variants identified via targeted sequencing
- Potential novel biomarkers identified

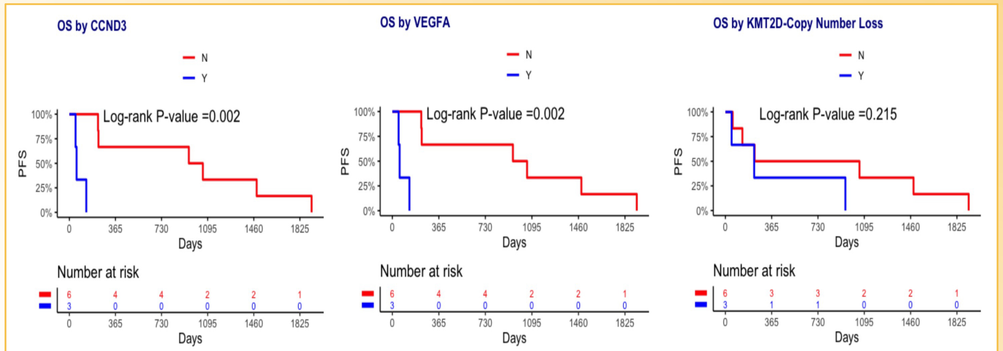

Figure 3,4,5: Kaplan-Meier Curves representing CCND3, VEGFA, and KMT2D copy number loss mutations. CCND3 and VEGFA may serve as prognostic indicators of poor DFI. KTMD2 was found in patients with varying DFI, indicating it is not useful as a prognostic indicator.

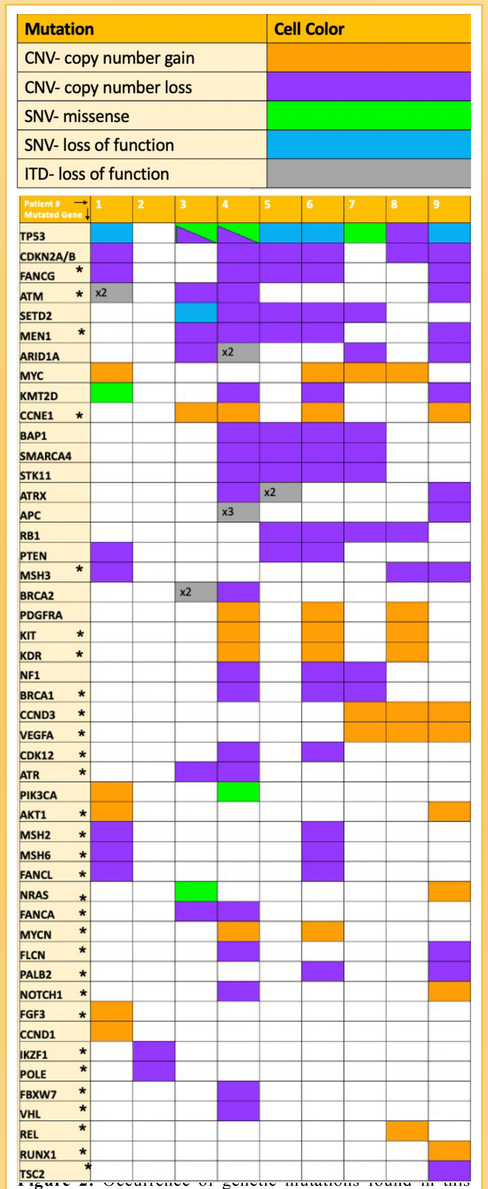

Figure 2: Occurrence of genetic mutations found in this study. Asterix* placed next to novel mutations.

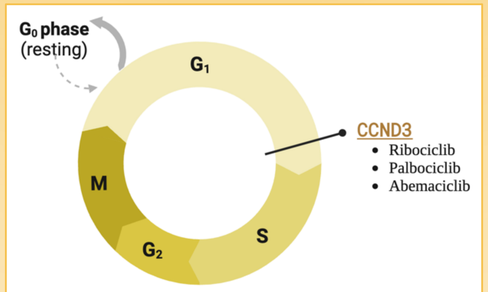

Figure 6: Cell cycle diagram with indication of actionable location of CCND3. Listed are pharmaceuticals to block mutational effect.

Results

- MST of patients in this study was 677 days
- CCND3 and VEGFA copy number gain found in the 3 shortest surviving patients (Fig. 2)
- TP53 mutation found in 89% of patients and is the most common mutation (Fig. 2)
- ARID1A mutation is currently correlated with higher degree of metastasis, 75% of patients with ARID1A mutation succumbed to illness related to pulmonary metastasis
- Copy number loss was the most prevalent mutation (Fig.2)
- Significant increased DFI is attributed to immunotherapy protocol and not genetic predisposition
- Quantity of mutation was not found to correlate with DFI (Fig. 3,4,5)
- Histopathologic diagnosis was not found to correlate with quantity of mutations or prognosis of disease (Fig. 7)
- Several novel genes were in the same gene family as those previously identified
- Novel mutations occurred at a lower rate than mutations already identified (Fig. 2)

Conclusions

- Identification of unique mutations contributes to the growing knowledge of the canine OSA genome.
- CCND3, VEGFA, and ARID1A may be prognostic biomarkers
- CCND3 presence indicates the use of cell cycle drugs during cOSA treatment (Fig. 6)
- Chromothripsis is indicated (Fig. 2)
- Novel mutations found via targeted sequencing suggests further studies

Acknowledgements

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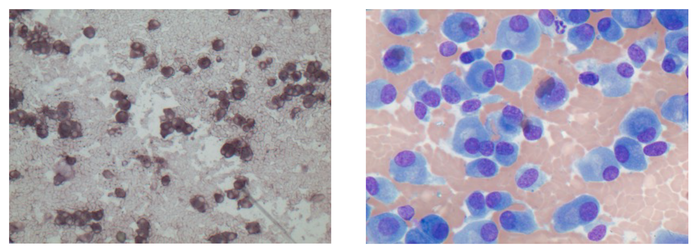

Figure 7: ALP stain cytology (Left) and cytology (Right) of canine OSA. Histopathologic diagnosis was found to not be a prognostic indicator or indicative of mutational burden.