University of Missouri

# GENE SEQUENCING IDENTIFIES POTENTIAL BIOMARKERS OF EARLY FAILURE AND DEATH IN DOGS WITH OSTEOSARCOMA TREATED WITH AMPUTATION AND VACCINE-ENHANCED ACTIVATED T CELL THERAPY

Jeffrey N Bryan[1], Noe Reyes[2], Tammie Wahaus[2], Guannan Wang[3], Will Hendricks[3,4], Brian K Flesner[1], Olivia Hritzkowin[1], Carolyn J Henry[1]

[1]University of Missouri, [2]ELIAS Animal Health, [3]Vidium, [4]TGen

## BACKGROUND

Actionable biomarkers of early metastatic failure in osteosarcoma are lacking, complicating treatment planning. New technologies that facilitate rapid gene sequencing may allow for more accurate prognosis and selection of cytotoxic and immunologic therapy.

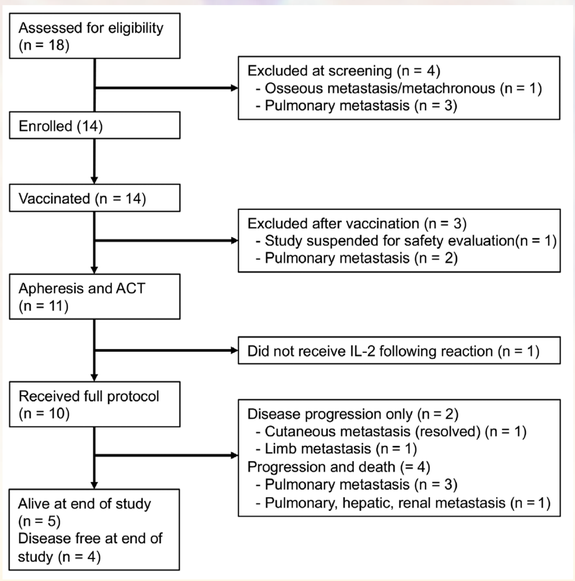
## METHODS

14 dogs underwent amputation for osteosarcoma with intent to receive autologous vaccination, activated T cell infusion, and low-dose IL-2 immunotherapy.

Targeted gene sequencing assessing copy number variation, single nucleotide variations, and internal tandem duplicates in 120 genes was performed on 9 dogs with suitable FFPE undecalcified samples after QC evaluation.

Variables (genes, mutations, signalment, ALP, tumor location) were fitted with univariate Cox models to identify factors associated with disease-free interval (DFI) and overall survival (OS). P < 0.05 was considered significant to advance the gene into future studies.

### CONSORT DIAGRAM OF TRIAL




The bioinformatics pipeline used to extract these features has been described in Chon E, et al. Genomic tumor analysis provides clinical guidance for the management of diagnostically challenging cancers in dogs. J Am Vet Med Assoc. 2023 Mar 1;261(5):668-677.

## RESULTS—DFI

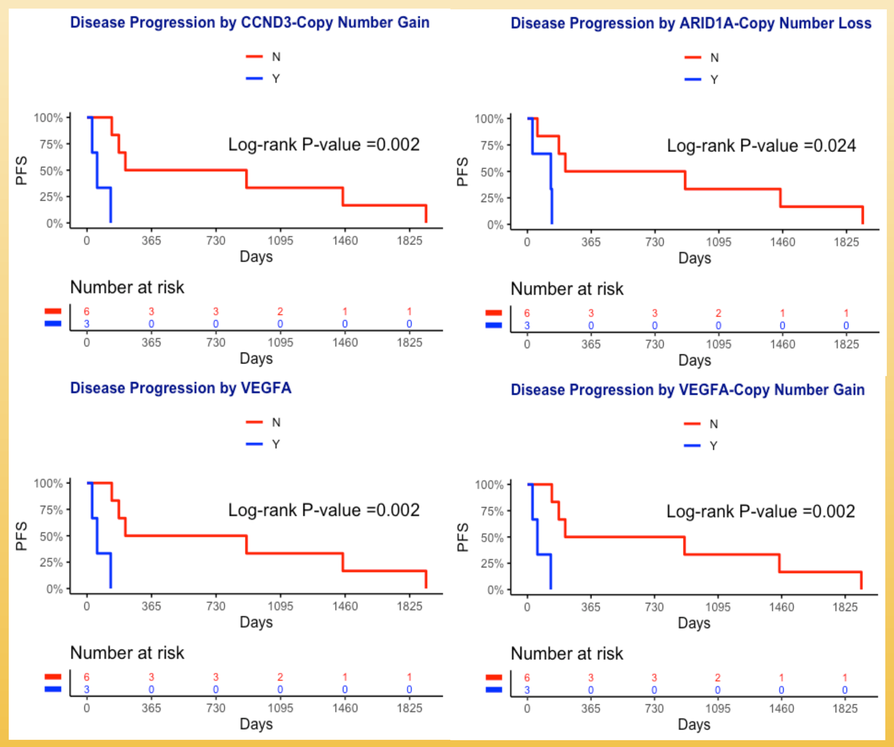



Figure 1: Kaplan-Meier curves of univariate analysis of dogs assessing presence or absence of CCND3 copy number gain, ARID1A copy number gain, and VEGFA mutation (SNV) and copy number gain on the DFI of dogs treated with amputation and immunotherapy. Dogs with CCND3 mutation, VEGFA mutation, or ARID1A mutation relapsed earlier than dogs without those features (median 483d vs. 57d).

| Mutation | Cell Color |
|---|---|
| CNV- copy number gain | |
| CNV- copy number loss | |
| SNV- missense | |
| SNV- loss of function | |
| ITD- loss of function | |

| Patient # → / Mutated Gene ↓ | 1 545d | 2 483d | 3 540d | 4 546d | 5 230d | 6 227d | 7 134d | 8 57d | 9 49d |
|---|---|---|---|---|---|---|---|---|---|
| TP53 | | | | | | | | | |
| CDKN2A/B | | | | | | | | | |
| FANCG * | | | | | | | | | |
| ATM * | x2 | | | | | | | | |
| SETD2 | | | | | | | | | |
| MEN1 * | | | | | | | | | |
| ARID1A | | | | x2 | | | | | |
| MYC | | | | | | | | | |
| KMT2D | | | | | | | | | |
| CCNE1 * | | | | | | | | | |
| BAP1 | | | | | | | | | |
| SMARCA4 | | | | | | | | | |
| STK11 | | | | | | | | | |
| ATRX | | | | | x2 | | | | |
| APC | | | | x3 | | | | | |
| RB1 | | | | | | | | | |
| PTEN | | | | | | | | | |
| MSH3 * | | | | | | | | | |
| BRCA2 | | | x2 | | | | | | |
| PDGFRA | | | | | | | | | |
| KIT * | | | | | | | | | |
| KDR * | | | | | | | | | |
| NF1 | | | | | | | | | |
| BRCA1 * | | | | | | | | | |
| CCND3 * | | | | | | | | | |
| VEGFA * | | | | | | | | | |
| CDK12 * | | | | | | | | | |
| ATR * | | | | | | | | | |
| PIK3CA | | | | | | | | | |
| AKT1 * | | | | | | | | | |
| MSH2 * | | | | | | | | | |
| MSH6 * | | | | | | | | | |
| FANCL * | | | | | | | | | |
| NRAS * | | | | | | | | | |
| FANCA * | | | | | | | | | |
| MYCN * | | | | | | | | | |
| FLCN * | | | | | | | | | |
| PALB2 * | | | | | | | | | |
| NOTCH1 * | | | | | | | | | |
| FGF3 * | | | | | | | | | |
| CCND1 | | | | | | | | | |
| IKZF1 * | | | | | | | | | |
| POLE * | | | | | | | | | |
| FBXW7 * | | | | | | | | | |
| VHL * | | | | | | | | | |
| REL * | | | | | | | | | |
| RUNX1 * | | | | | | | | | |
| TSC2 * | | | | | | | | | |

Table 1: Identified mutations in each of the nine samples analyzed including copy number gains and losses, missense mutations, single nucleotide variants leading to loss of function, and internal tandem duplicates leading to loss of function. Survival is included in days in each column heading. Some of the longest-lived dogs were alive at the end of study but not censored in these analyses.

## RESULTS

48 genes were variant. 25 genes with 26 mutations were present in more than two dogs differentiating DFI. Nine features segregated dogs with a P<0.25.

Mutation of VEGFA (P=0.002) and copy number gain of CCND3 and VEGFA (P=0.002) separated the 6 longest from the 3 shortest remissions (median 483d vs. 57d). Copy number loss of ARID1A followed (P=0.024).

A second set of genes with 26 mutations were present in more than two dogs differentiating OS. Eight features segregated dogs with a P<0.25.

Mutation of VEGFA (P=0.002) and copy number gain of CCND3 and VEGFA (P=0.002) separated the 6 longest lived from the 3 shortest lived dogs (median 540d vs. 57d).

Rb1 mutation and copy number loss segregated the 5 longest lived from the 4 shortest lived dogs (median 540d vs. 96d; P=0.048).

TP53 was mutated in 89% of the tumors.

## RESULTS—SURVIVAL

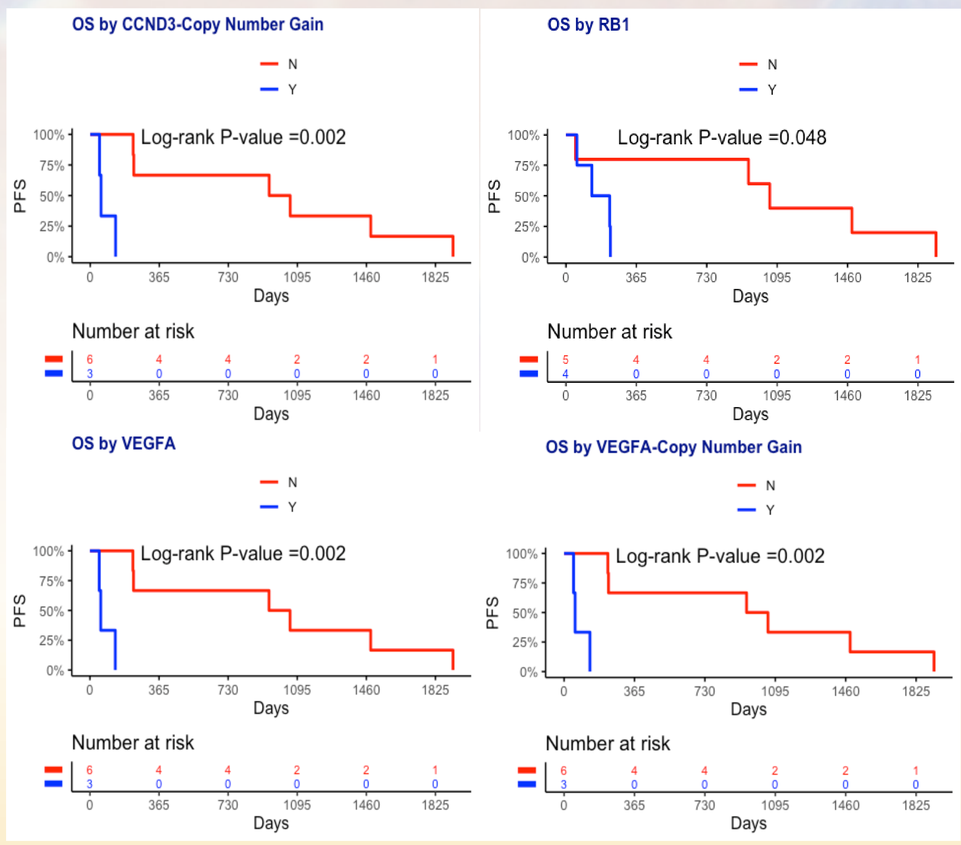



Figure 2: Kaplan-Meier curves of univariate analysis of dogs assessing presence or absence of CCND3 copy number gain, RB1 mutation, and VEGFA mutation (SNV) and copy number gain on the overall survival (OS) of dogs treated with amputation and immunotherapy. Dogs with CCND3 mutation, VEGFA mutation, or ARID1A mutation survived shorter than dogs without (median 540d vs. 57d).

## DISCUSSION

Identifying gene mutations common to short and long term survival with osteosarcoma could yield biomarkers of aggressive disease, with some mutations potentially druggable. CCND3 (Cyclin D3) amplification has been reported previously to be a prognostic biomarker in a Vidium publication evaluating multiple tumor types in which the amplification was identified including osteosarcoma and hemangiosarcoma. Bortezomib may target dysregulated cyclin function. Palbocilib, ribociclib, and abemociclib target dysregulated cyclin-dependent kinases (CDK4 and 6) in human patients with early data generated in dogs for palbociclib. CCND3 has been reported to be mutated and amplified in human osteosarcoma. VEGFA overexpression has been associated with poorer outcomes in many cancers in people, including osteosarcoma. The VEGF pathway can be targeted by anti-vascular drugs including toceranib. ARID1A mutation has been described less commonly in human osteosarcoma. Its effect on malignant cells may be targeted with olaparib, dasatinib, fruquinitinib, and tazmetostat. Olaparib and dasatinib have some reported use in dogs with cancers, and tazmetostat has shown effects on canine cancer cells *in vitro*. The results of this pilot investigation support further prospective study in dogs with osteosarcoma with the goal of improving therapy.

Comparative Oncology Radiobiology and Epigenetics Laboratory
University of Missouri

## ACKNOWLEDGEMENTS

Funding for this study provided by ELIAS Animal Health

ELIAS ANIMAL HEALTH