LM-LLO–HER2/NEU IMMUNOTHERAPY COMBINED WITH RADIATION SAFELY DELAYS TUMOR PROGRESSION AND PROLONGS OVERALL SURVIVAL IN A PHASE I CLINICAL STUDY IN CANINE OSTEOSARCOMA

LB-113

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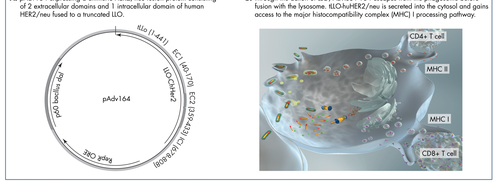
INTRODUCTION

- Osteosarcoma (OSA) is an aggressive, highly malignant pediatric mesenchymal bone tumor.
- Despite chemotherapy, radiotherapy (RT), and radical surgery, metastatic disease is common and results in 30% mortality within 5 years.[1]
- No improvements to survival have been seen over the past 15 years, so novel treatments are required.[2]
- Human epidermal growth factor receptor 2 (HER2)/neu is expressed in ~40% of pediatric primary OSA and in OSA tumor-initiating cells, suggesting that immune targeting of HER2/neu might delay or eliminate metastatic disease.
- ADXS31-164 is a highly attenuated recombinant *Listeria monocytogenes* (*Lm*) expressing a chimeric human HER2/neu protein fused to immune-activating, truncated listeriolysin O (LLO) (**Figure 1**).

Figure 1. ADXS31-164 recombinant HER2/neu plasmid and mechanism of action



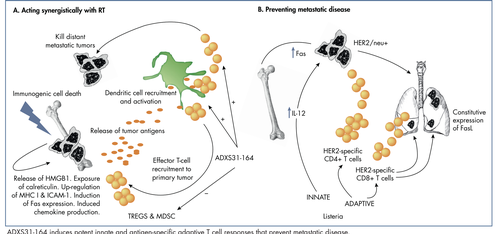
CD, cluster of differentiation; HER2, human epidermal growth factor receptor 2; LLO, listeriolysin O.

- Canine OSA is a clinically relevant large animal OSA model in which to study novel therapies, as it shares many clinical, biologic, and genetic features of human OSA.[2,3]
- A previous phase I study in the setting of minimal residual disease showed that ADXS31-164 treatment delays time to metastases and prolongs overall survival (OS) in dogs with OSA.[4]

HYPOTHESIS

- RT plus ADXS31-164 will synergize to promote anti-tumor immune responses that retard primary tumor progression (**Figure 2A**) and delay/prevent metastatic disease (**Figure 2B**) in dogs with spontaneous appendicular OSA that do not undergo primary tumor removal (amputation).

Figure 2. Potential mechanisms of action of ADXS31-164



ADXS31-164 induces potent innate and antigen-specific adaptive T cell responses that prevent metastatic disease.
RT induces immunogenic tumor cell death and acts in concert with ADXS31-164–induced HER2-specific T cells, and its inhibitory effect on Tregs and MDSC to augment anti-tumor immune responses at both the primary and metastatic sites. Strong induction of IL-12 upregulates Fas on circulating metastatic OSA cells, leading to their elimination as they pass through the lung that constitutively expresses FasL.
CD, cluster of differentiation; HER2, human epidermal growth factor receptor 2; hMGB1, high-mobility group protein B1; ICAM-1, intercellular adhesion molecule 1; MDSC, myeloid-derived suppressor cells; MHC I, major histocompatibility complex I; RT, radiotherapy; TREGS, regulatory T cells.

METHODS AND STUDY DESIGN

TRIAL POPULATION

- Systemically healthy, pet dogs with histopathologically confirmed, treatment-naive, HER2+ appendicular OSA, and no evidence of cardiac or metastatic disease.

STUDY DESIGN (Figure 3)

- RT: 16 Gy of RT in 2 fractions on consecutive days.
- ADXS31-164: given intravenously once every 3 weeks for 8 doses following RT.

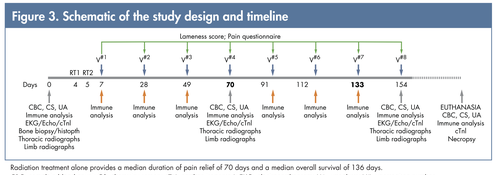
ENDPOINTS

- Primary: Time to Progression.
- Secondary: Safety, Assessment of Anti-Tumor Immunity and OS.

ANALYSES

- Radiographs taken at baseline, week 10, week 22 and every 2 months thereafter to assess the effect of treatment on primary tumor progression and development of metastases.
- Systemic and cardiac toxicity, lameness, and quality of life (QoL; using a validated owner questionnaire) were assessed at each treatment, and then every 2 months.
- Peripheral blood mononuclear cells (PBMCs) were collected every 3 weeks to assess HER2/neu-specific T-cell responses.
  - Interferon-gamma (IFN-γ) responses were determined using the Enzyme-Linked ImmunoSpot (ELISpot) assay

Figure 3. Schematic of the study design and timeline



Radiation treatment alone provides a median duration of pain relief of 70 days and a median overall survival of 136 days.
CBC, complete blood count; CS, chemistry screen; cTnI, cardiac troponin I; EKG, electrocardiogram; UA, urinalysis; V#, vaccination number.

RESULTS

- A total of 10 dogs were enrolled in the study. The signalment and tumor characteristics are shown in **Table 1**.

Table 1. Patient signalment and tumor characteristics

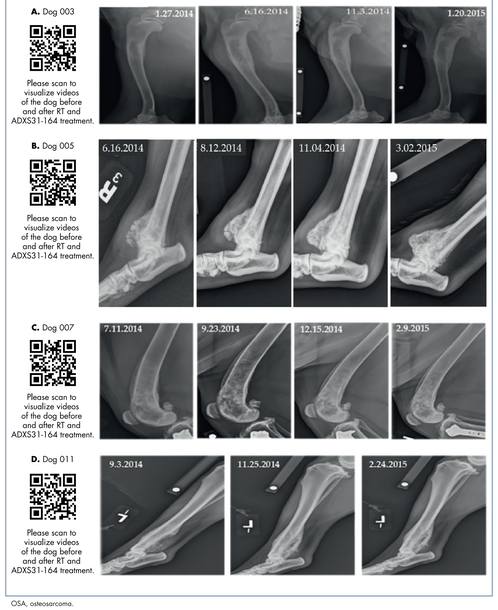
Age	Breed	Sex	Tumor location	Subtype	Number of vaccines administered	Concurrent treatments	Time to progression (days)	Overall survival (days)	Median survival (days)
Vaccine group									
9	Italian Spinone	MC	Proximal humerus	Osteoblastic	8	T, G, NSAIDs	238 B	285	262
6	Great Pyrenees	FS	Proximal humerus	Osteoblastic	8	T, G, Pamidronate		411+	
7	Greyhound	MC	Proximal humerus	Fibroblastic	1	None	57 (PF)	57*	
9	Mixbreed	FS	Proximal humerus	Osteoblastic	7	None	204 (PF)	243+	
8	Golden Retriever	MC	Distal tibia	Osteoblastic	8	None	243 L	310+	251.5
9	Mixbreed	MC	Distal tibia	Osteoblastic	8	T, G		201+	
7	GSD	MC	Distal femur	Osteoblastic	8	None		286+	286
7	Great Dane	MC	Distal radius	Osteoblastic	5	T, G, A, NSAIDs	113 (PF)	187*	111
9	Irish Setter	FS	Distal radius	Osteoblastic	2	T, G	62 L	62	
6	Great Pyrenees	FS	Distal radius	Osteoblastic	3	T, G, NSAIDs	90 (PF)	115*	

B, bone metastases; G, gabapentin; L, lung metastases; NSAID, non-steroidal anti-inflammatory drug; PF, pathologic fracture; T, tramadol.
+Alive.
*Euthanasia due to pathologic fracture.

EFFICACY

- To date, 7 of 10 dogs, and 5 of 10 dogs showed minimal primary tumor progression by radiographic evaluation at 10 weeks and 22 weeks, respectively.
- Stable clinical disease was observed in 5 dogs (please scan QR codes in Figure 4 to visualize videographic assessment of dogs before and after treatment).
- The radiographic progression of primary OSA was delayed by the combination of RT and ADXS31-164 (**Figures 4A, B, and C**).
  - In 1 dog, combination therapy led to radiographic evidence of bone remodeling and apparent healing of the primary OSA lesion (**Figure 4D**)
  - Of the 10 dogs, 4 developed pulmonary metastatic disease; this appeared to resolve in 1 dog with continued treatment

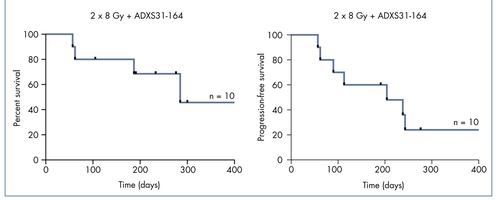
Figure 4. Delayed progression of primary OSA and prevention of metastatic disease



OSA, osteosarcoma.

- The addition of ADXS31-164 to RT prolonged survival (**Figure 5A**).
  - The median survival time (MST) was 285 days, compared with the historical MST of 136 days with RT alone[5]
- Median time to progression was 243 days (**Figure 5B**).
  - In total, 5 of 10 dogs have died; 3 were euthanized due to pathologic fracture and 2 were euthanized due to metastatic disease

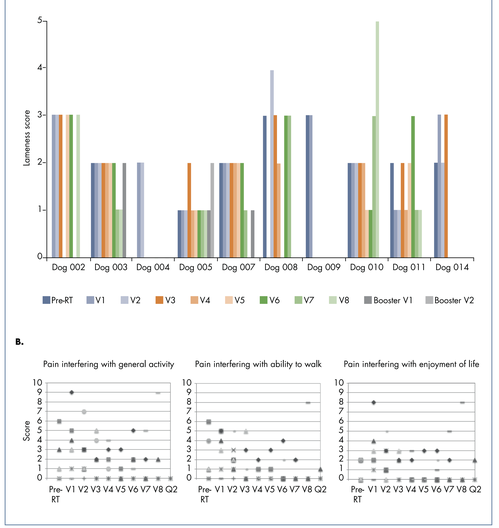
Figure 5. Survival (A) and progression-free survival (B) curves



SAFETY AND QOL

- Repeat ADXS31-164 administration was well tolerated with no systemic or cardiac toxicity.
- Lameness and other QoL parameters showed uniform stability or improvement over the entire study period (**Figures 6A and B**).

Figure 6. Lameness (A) and QoL (B) parameters

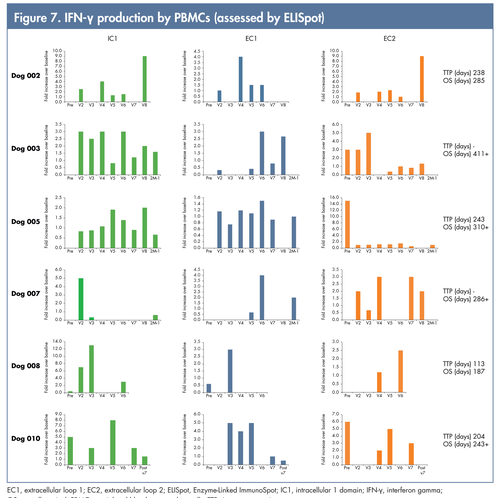


QoL was assessed through the brief validated pain score which is completed by pet owners and evaluates 11 parameters. Pain interfering with general activity, ability to walk and enjoyment of life are shown here in Fig. 6B. Each symbol represents an individual dog.
QoL, quality of life; RT, radiotherapy; V, vaccination.

IMMUNE TOLERANCE

- 5 out of 6 dogs evaluated to date developed IFN-gamma responses against the intracellular 1 (IC1) domain of HER2/neu within 3 weeks of vaccination (**Figure 7**), suggesting ADXS31-164 breaks peripheral tolerance to HER2/neu.

Figure 7. IFN-γ production by PBMCs (assessed by ELISpot)



EC1, extracellular loop 1; EC2, extracellular loop 2; ELISpot, Enzyme-Linked ImmunoSpot; IC1, intracellular 1 domain; IFN-γ, interferon gamma; OS, overall survival; PBMCs, peripheral blood mononuclear cells; TTP, time to progression.

CONCLUSIONS

- Combination RT + ADXS31-164 delays primary tumor progression and prolongs OS.
- Combination RT + ADXS31-164 maintains limb function and improves QoL.
- Repeat administration of ADXS31-164 is safe and well tolerated, with no cardiac toxicity.
- These findings may have important translational relevance for human patients with OSA and other HER2/neu+ cancers.

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DISCLOSURES

The authors are fully responsible for all content and editorial decisions for this poster.
Drs. Yvonne Paterson and Nicola Mason are consultants and shareholders in Advaxis Inc.