UNIVERSITY OF PENNSYLVANIA'S SCHOOL OF VETERINARY MEDICINE
DEPARTMENT OF CLINICAL STUDIES AND PATHOBIOLOGY

Combination *Lm-LLO* Immunotherapy plus Radiation Delays Tumor Progression and Prolongs Survival in Osteosarcoma

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I have the following disclosures* related to my presentation:

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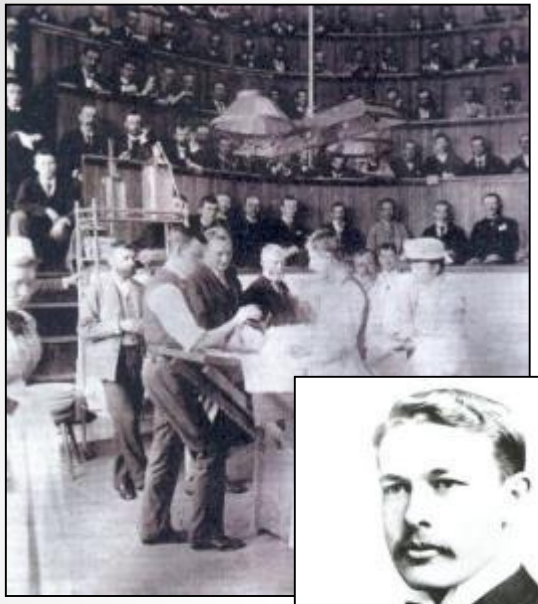
Consulting: **Advaxis Inc.**
Aratana Therapeutics

Investments: **Advaxis Inc**

I will discuss results of clinical trial for the following agents that are currently NOT approved for use in animals.

***Disclosures include spouse and immediate family where relevant.**

Osteosarcoma is an "immune responsive" tumor



ANNALS OF SURGERY

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ORIGINAL MEMOIRS.

SARCOMA OF THE LONG BONES.*

THE DIAGNOSIS, TREATMENT AND PROGNOSIS, WITH A REPORT OF SIXTY-NINE CASES.

BY WILLIAM B. COLEY, M.D.,

OF NEW YORK.

Attending Surgeon to the General Memorial Hospital; Associate Surgeon to the Hospital for Ruptured and Crippled.

Annals of Surgical Oncology, **12**(12): 1073–1083
DOI: 10.1245/ASO.2005.01.011

Improved Survival Associated With Postoperative Wound Infection in Dogs Treated With Limb-Salvage Surgery for Osteosarcoma

B. Duncan X. Lascelles, BVSc, PhD,[1] William S. Dernell, DVM, MS,[2] Maria T. Correa, MSc, PhD,[3] Mary Lafferty,[2] Chad M. Devitt, DVM, MS,[2] Charles A. Kuntz, DVM, MS,[2] Rodney C. Straw, DVM, MS,[2] and Stephen J. Withrow, DVM[2]

Veterinary Surgery
35:518–533, 2006

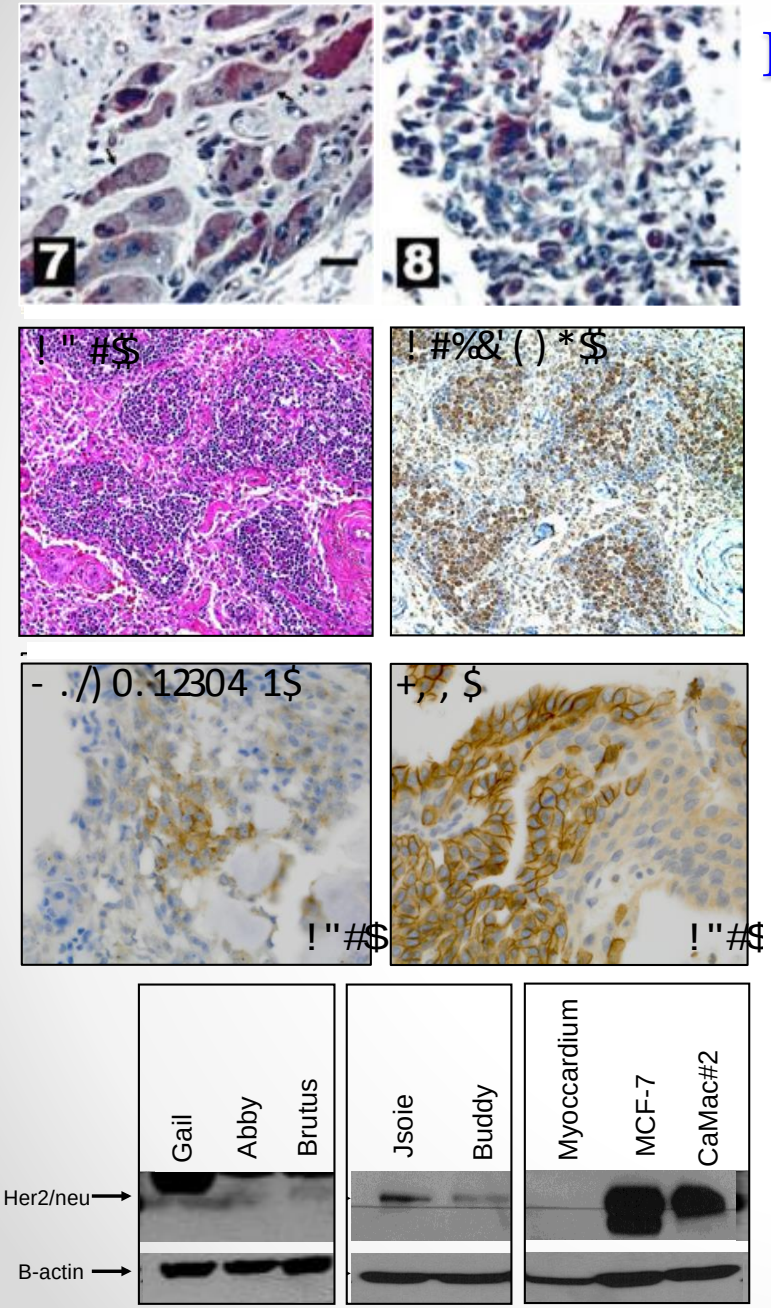
Cortical Allograft and Endoprosthesis for Limb-Sparing Surgery in Dogs with Distal Radial Osteosarcoma: A Prospective Clinical Comparison of Two Different Limb-Sparing Techniques

JULIUS M. LIPTAK, BVSc, MVCS, FACVSc, Diplomate ACVS & ECVS, WILLIAM S. DERNELL, DVM, MS, Diplomate ACVS, NICOLE EHRHART, VMD, MS, Diplomate ACVS, MARY H. LAFFERTY, CVT, GABRIELLE J. MONTEITH, BSc, and STEPHEN J. WITHROW, DVM, Diplomate ACVS & ACVIM (Oncology)

Evaluation of outcome and prognostic factors for dogs living greater than one year after diagnosis of osteosarcoma: 90 cases (1997–2008)

William T. N. Culp, VMD; Francisco Olea-Popelka, DVM, PhD; Jennifer Sefton, DVM; Charles F. Aldridge, DVM; Stephen J. Withrow, DVM; Mary H. Lafferty; Robert B. Rebhun, DVM; Michael S. Kent, DVM; Nicole Ehrhart, VMD

Flint AF, et al. Vet Pathol. 2004 May;41(3):291-6.

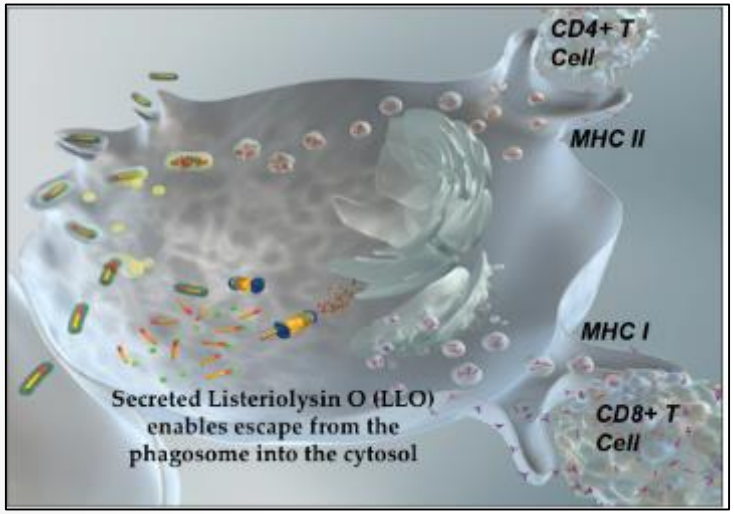


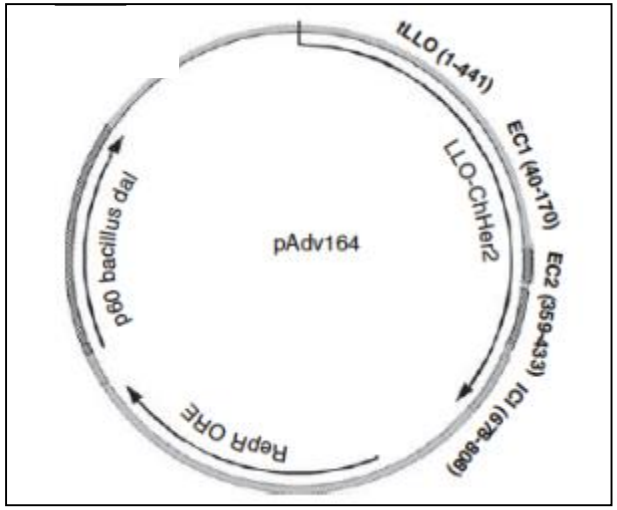
HER2/neu is a molecular target in OSA

- HER2/neu is expressed in 40-60% of pediatric and canine primary OSA and in pulmonary metastatic disease
- Supporting evidence for HER2/neu expression in tumor initiating cells
- Expression is associated with aggressive disease, increased risk of metastasis and decreased OS
- Not associated with gene amplification
- IHC indicates staining is predominantly cytoplasmic
- Trastuzumab showed minimal efficacy in a phase I clinical trial in children
- Represents a therapeutic target for T cell mediated therapies

Listeria monocytogenes

- Gram positive intracellular bacteria
  - Preferentially infects APCs
  - Induces potent innate (IL-12) and adaptive (CD4 & CD8 T cell) immune responses
  - Readily genetically modified to deliver TAA into MHC I and II pathways





Sequence identity with canine HER2

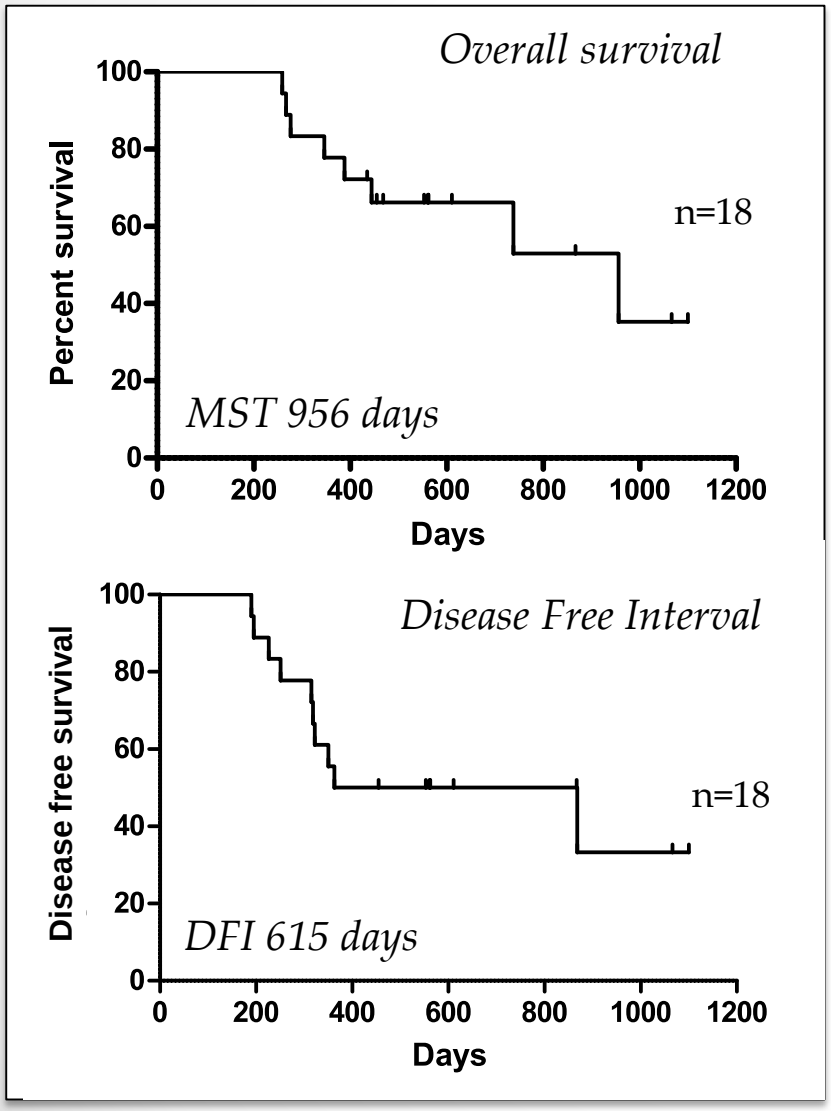
EC1 89%

EC2 93%

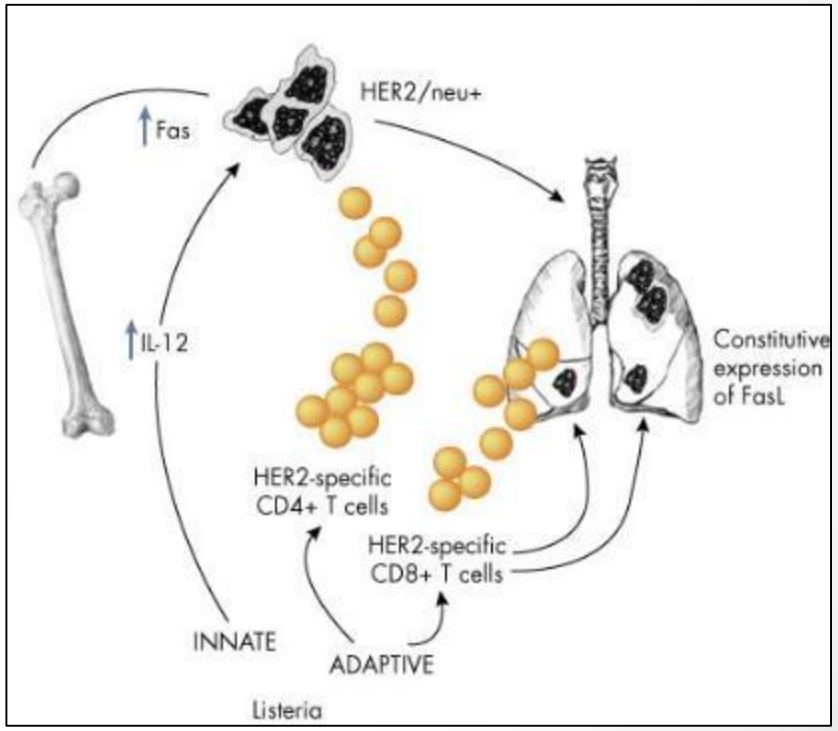
IC1 98%

- Influences the tumor microenvironment
  - Increases TIL and reduces % of Tregs and MDSC within tumors
- In mouse models:
  - Induces HER2 specific CD8+ cytotoxic T cell responses
  - Eliminates established HER2+ mammary tumors
  - Prevents HER2+ metastatic disease

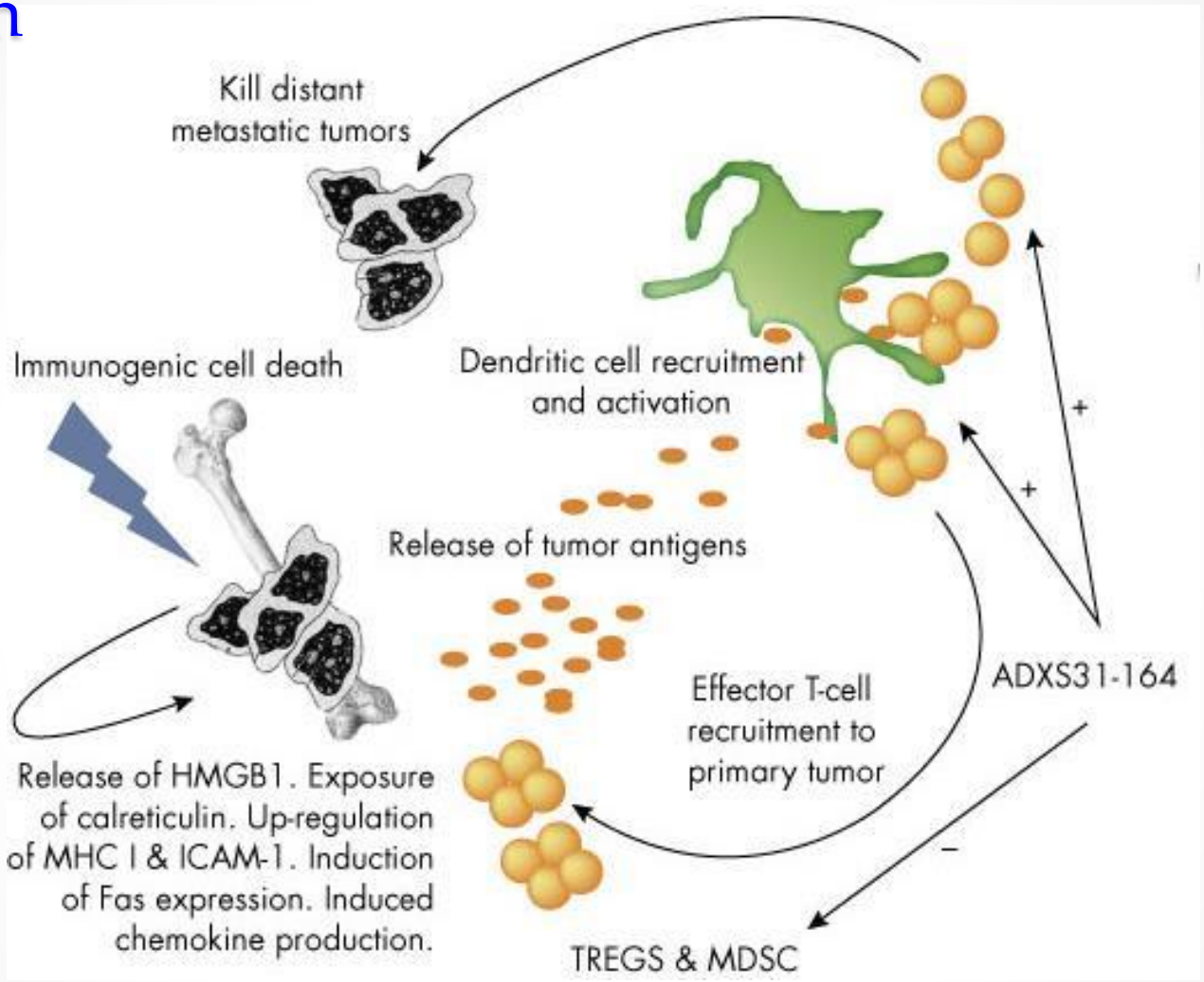
ADXS31-164 administered in the setting of minimal residual disease prevents metastatic disease and prolongs overall survival



MOA to prevent metastatic disease

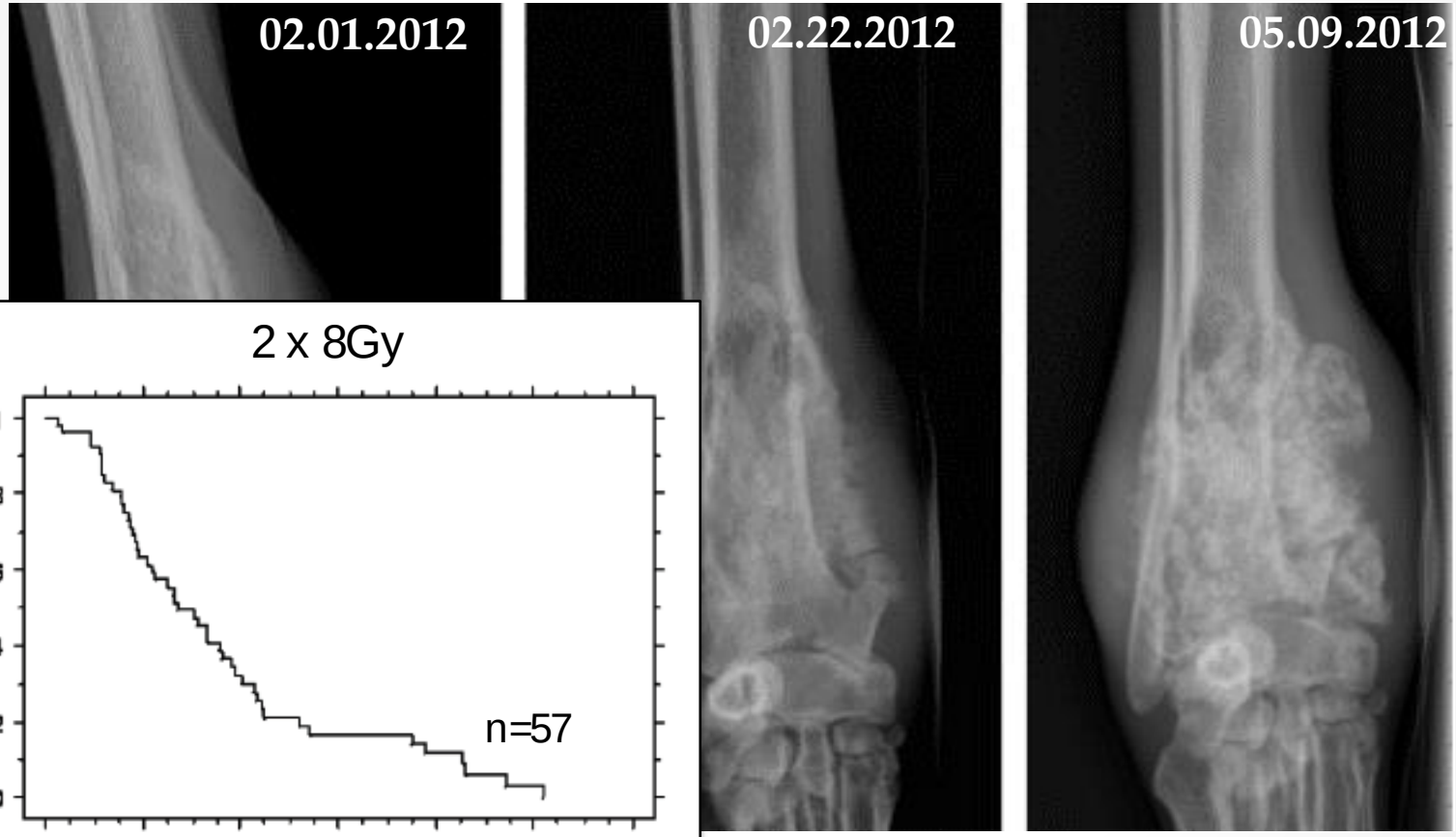


Proposed synergy between ADXS31-164 and palliative radiation



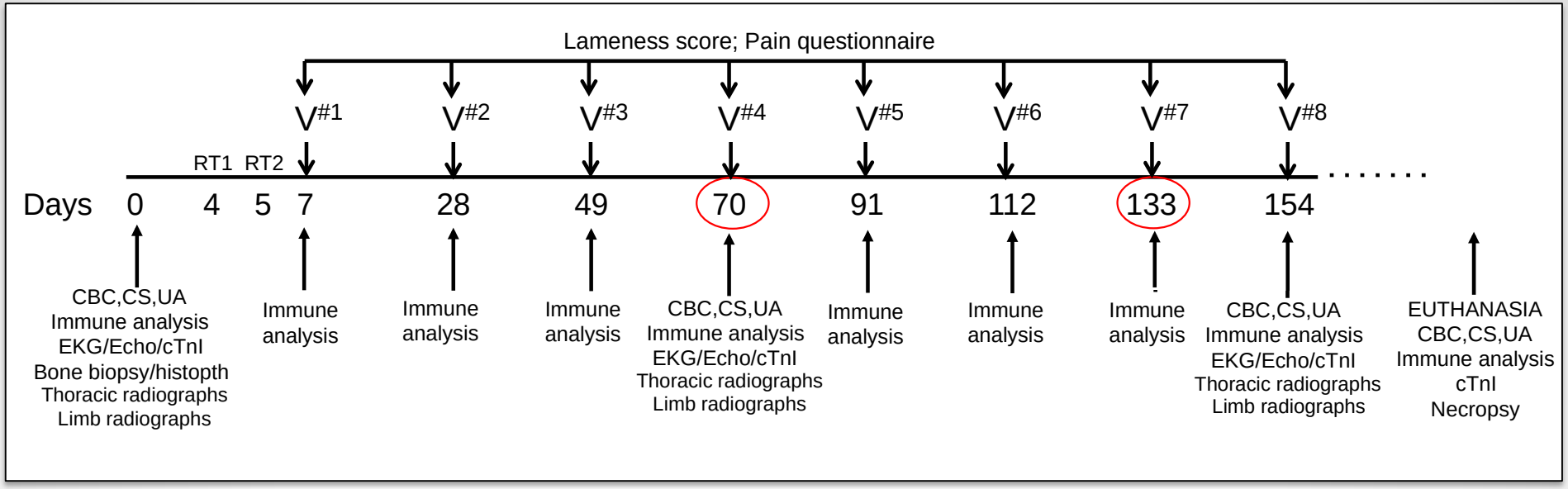
ADXS31-64 plus RT will synergize to promote anti-tumor immune responses that retard primary tumor progression and delay/prevent metastatic disease in dogs with non-resectable appendicular OSA

Characteristic Radiographic Progression of OSA following Radiation Therapy Alone



Knapp-Hoch et al. J Am Anim Hosp Assoc. 2009 Jan-Feb;45(1):24-32.

Overview of Pilot Study Timeline



Median Duration of Pain Relief with RT alone = 70 days

Median Survival Time with RT alone = 136 days

Ref: *Knapp-Hoch et al. J Am Anim Hosp Assoc. 2009 Jan-Feb;45(1):24-32.*

Inclusion/Exclusion criteria

- Confirmed diagnosis of OSA by bone biopsy and histopathology
- No evidence of metastatic disease
- Systemically healthy with no evidence of cardiac disease
- Treatment naïve (other than pain medications)

Patient Signalment and Tumor Characteristics

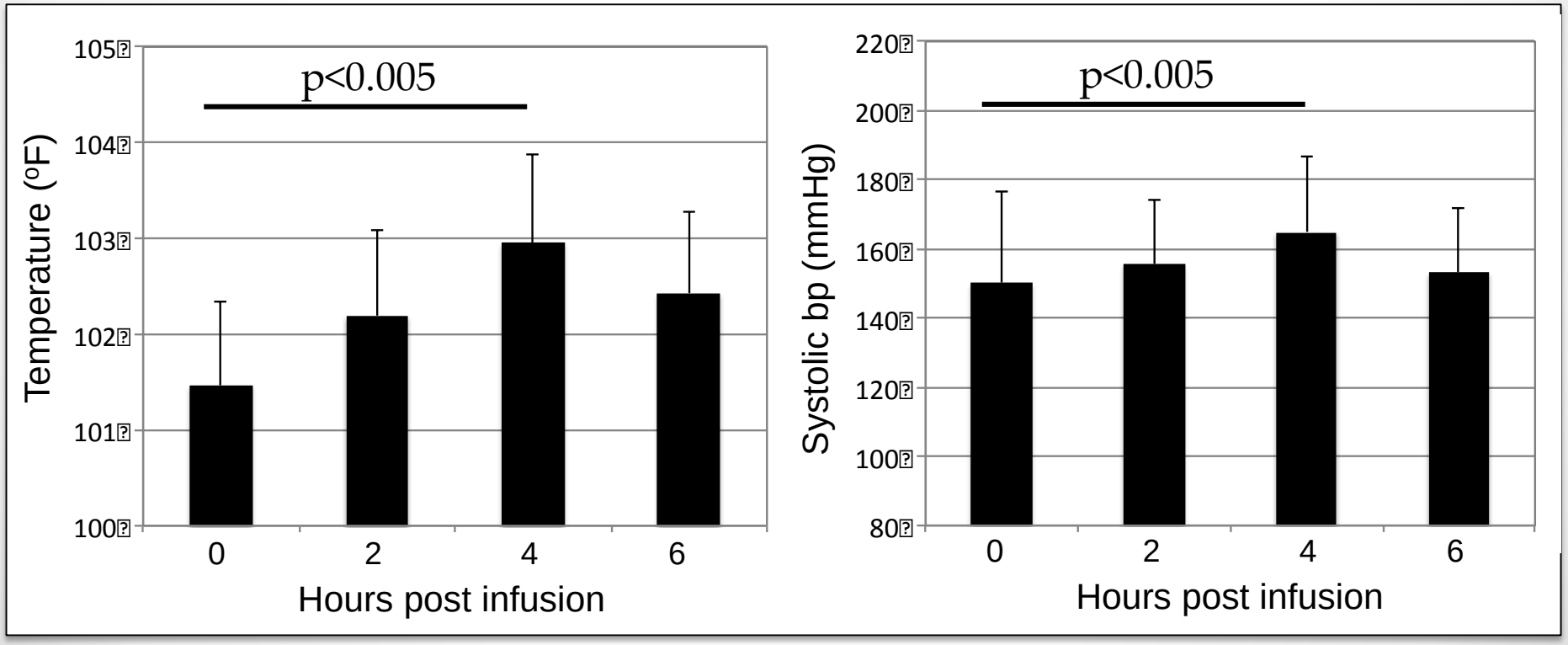
AGE	BREED	SEX	TUMOR LOCATION	SUBTYPE	HER2/Neu expression	Number of vaccines administered to date	Concurrent treatments	Time to progression (days)	Overall survival (days)
Vaccine Group									
9	Italian Spinone	MC	Proximal humerus	Osteoblastic	Pending	8	T,G,NSAIDs	238 B	285
6	Great Pyrenees	FS	Proximal humerus	Osteoblastic	1	8 (+2)	T,G, Pamidronate		479+
9	Irish Setter	FS	Distal radius	Osteoblastic	6	2	T,G	62 L	62
8	Golden Retriever	MC	Distal tibia	Osteoblastic	Pending	8 (+2)	None	243 L	378+
7	GSD	MC	Distal femur	Osteoblastic	2	8 (+1)	None		354+
7	Great Dane	MC	Distal radius	Osteoblastic	6	5	T,G,A,NSAIDs	113 (PF)	187*
7	Greyhound	MC	Proximal humerus	Fibroblastic	3	1	None	57 (PF)	57*
9	Mixbreed	FS	Proximal humerus	Osteoblastic	Pending	7	None	204 (PF)	322+
9	Mixbreed	MC	Distal tibia	Osteoblastic	Pending	8 (+2)	T,G		269+
6	Great Pyrenees	FS	Distal radius	Osteoblastic	Pending	2	None	90 (PF)	115*
7	Greyhound	FS	Proximal humerus	Osteoblastic	Pending	3	None		116+
9	Saluki	M	Distal radius	Osteoblastic	Pending	2	None		66+

T Tramadol
G Gabapentin
NSAID Non Steroidal Anti-Inflammatory Drug

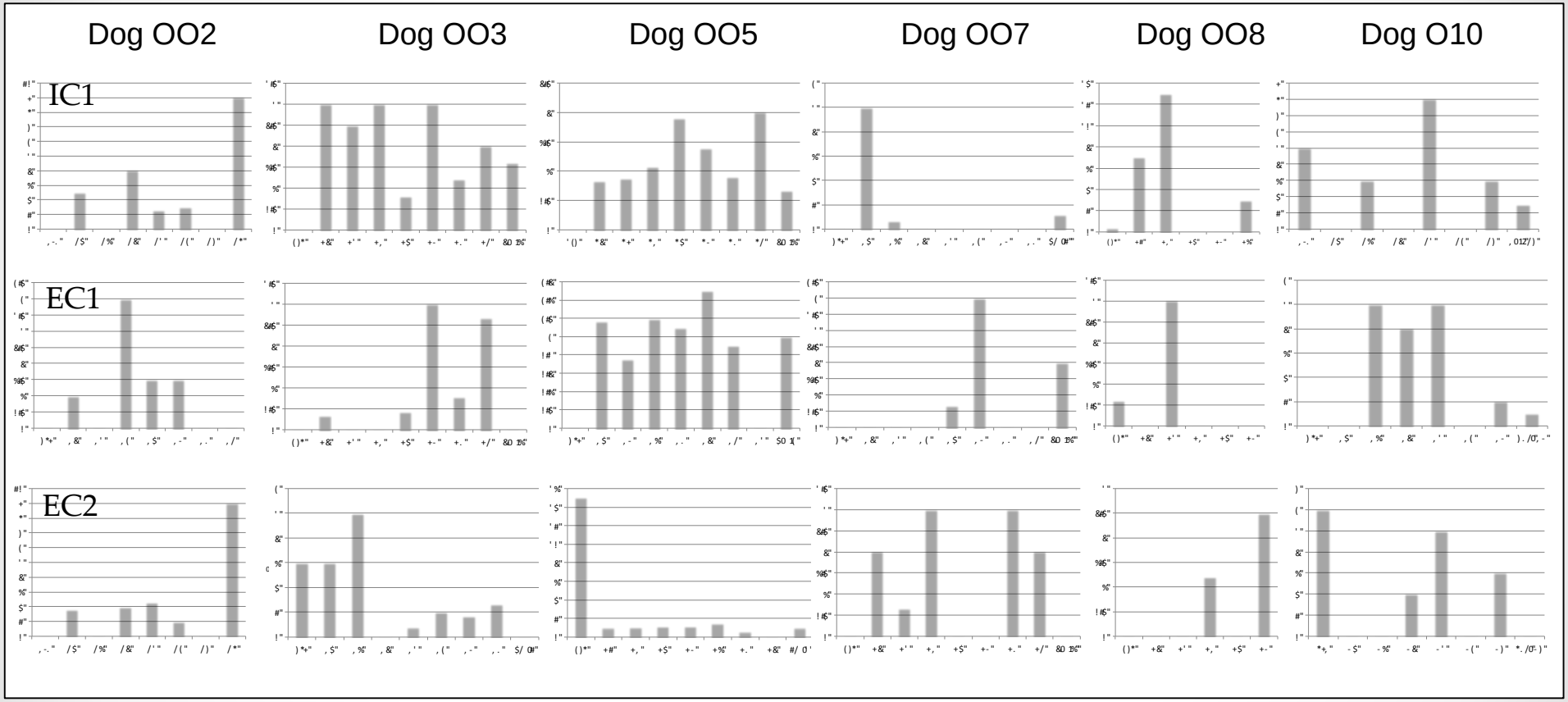
B Bone Metastases
L Lung Metastases
PF Pathologic Fracture

\+ Alive
* Euthanasia due to pathologic fracture

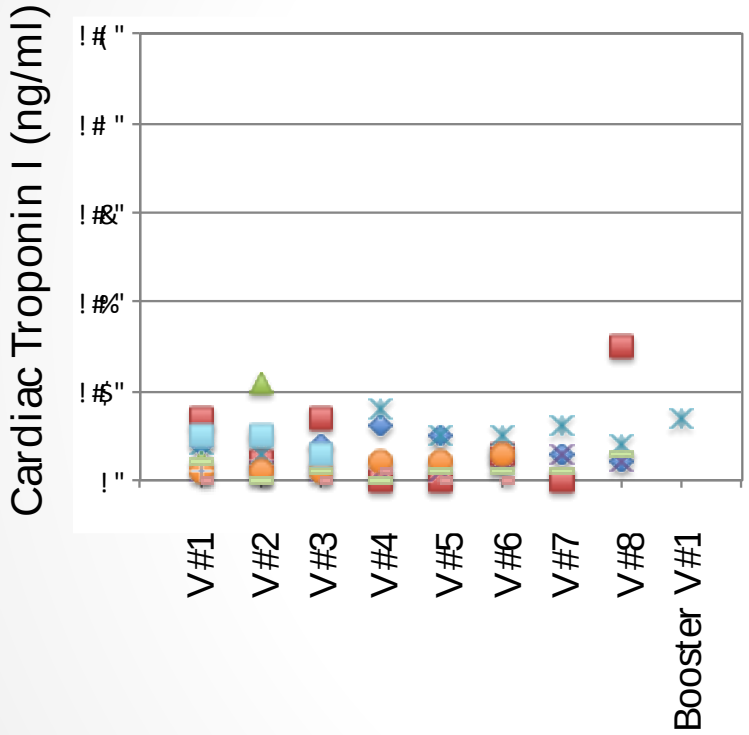
Mild, transient increases in temperature and systolic blood pressure following ADXS31-164

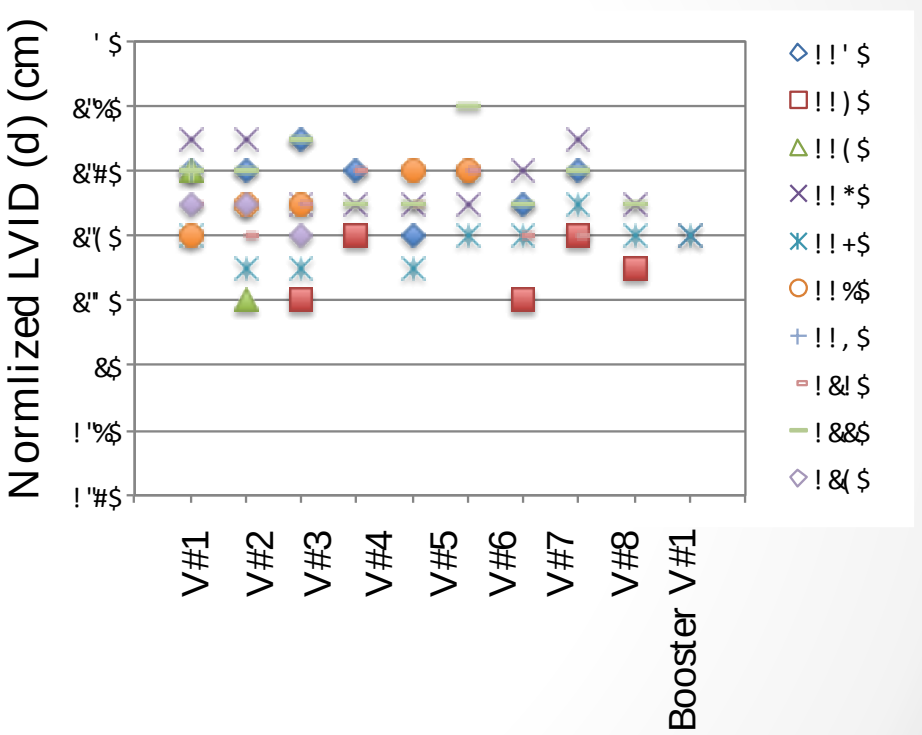


ADXS31-164 breaks tolerance to HER2/neu and induces antigen-specific IFN-γ production



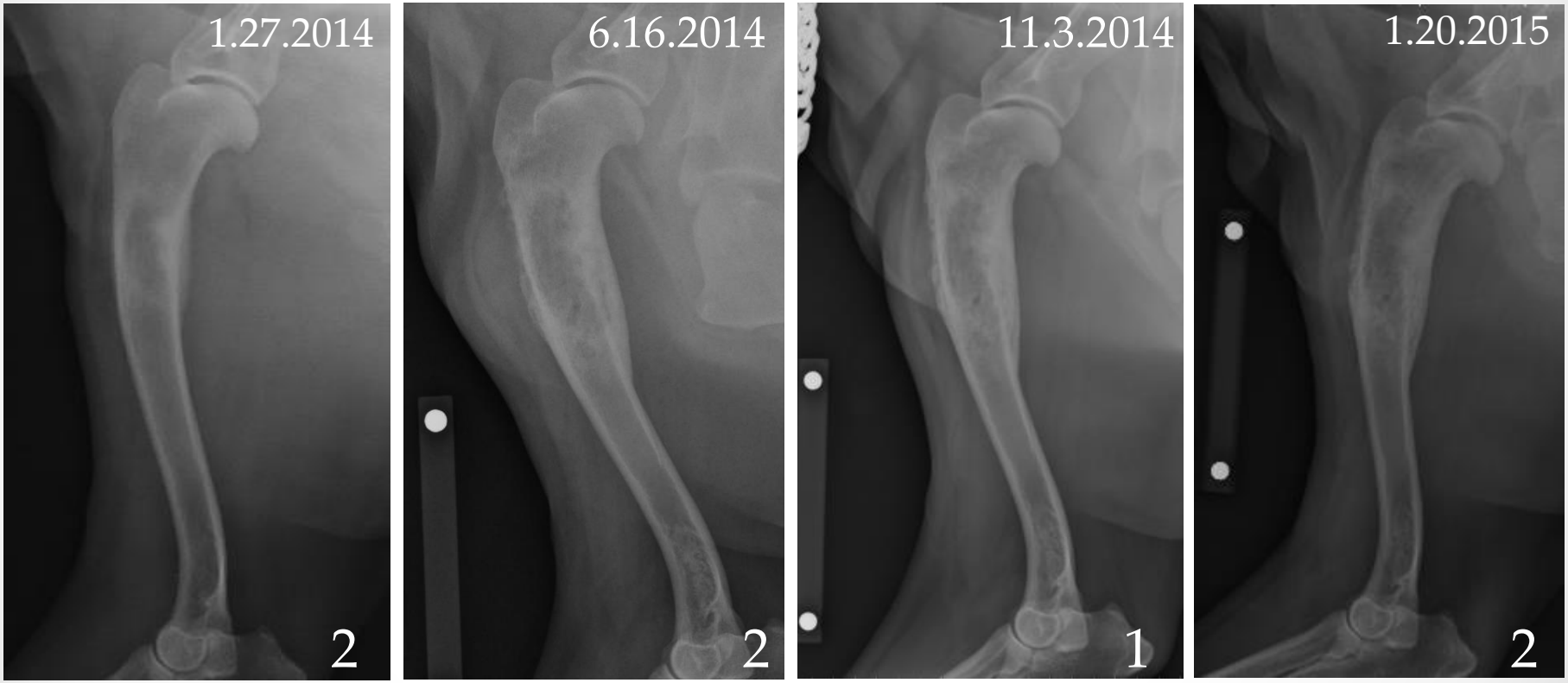
No evidence of cardiotoxicity with repeat doses of ADXS31-164





RT+ ADXS31-164 delays the radiographic progression of primary OSA

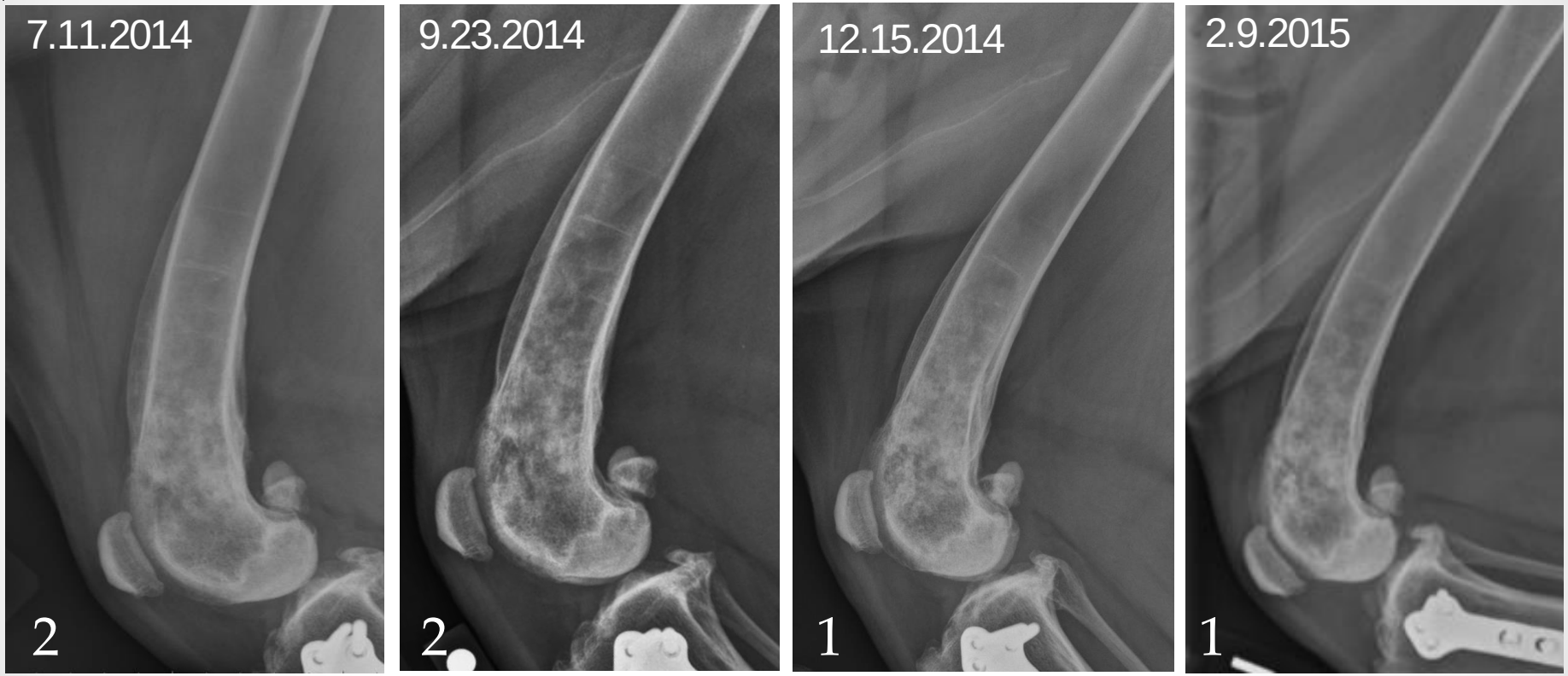
Dog 003



LAMENESS SCORE:
0=clinically sound, 1=barely detectable lameness, 2=mild lameness, 3=moderate lameness, 4=severe lameness, 5=non weight bearing

RT+ ADXS31-164 delays the radiographic progression of primary OSA

Dog 007



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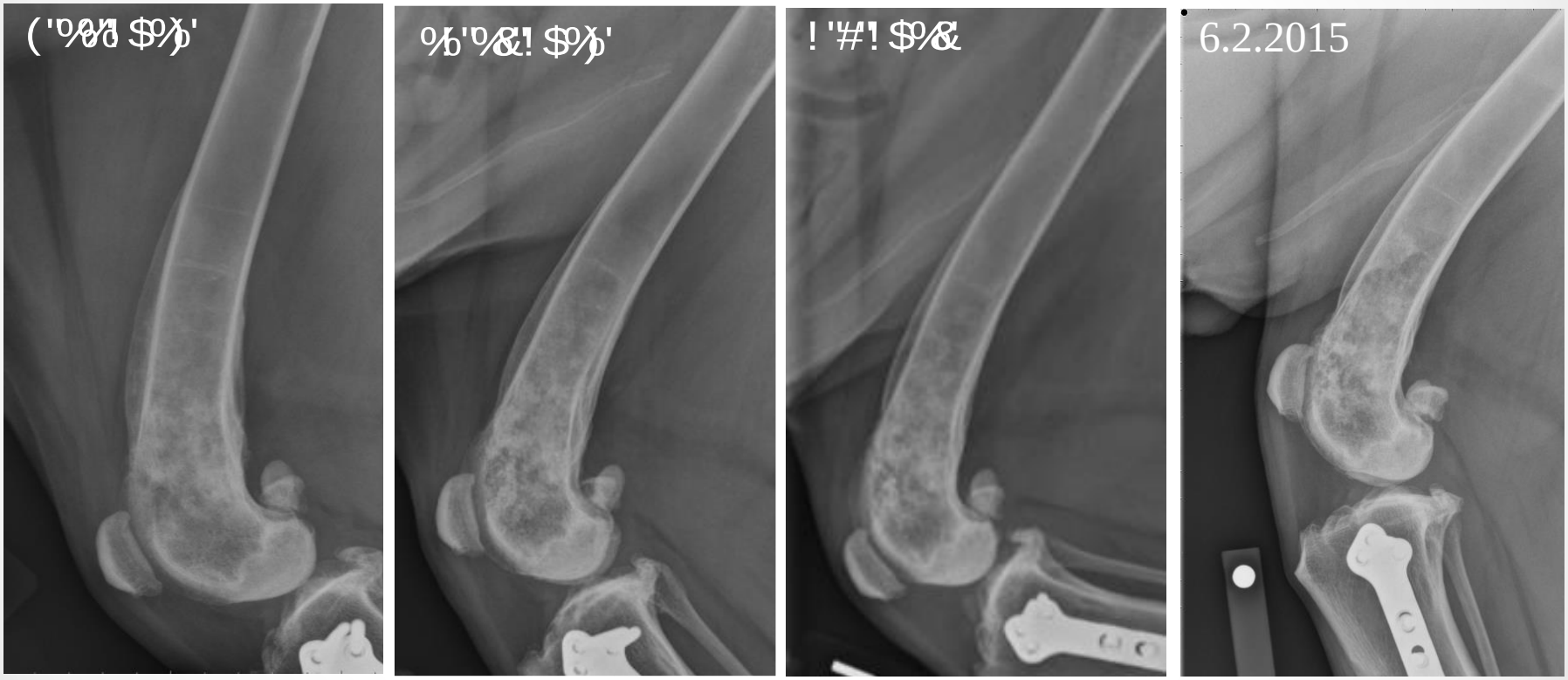
RT+ ADXS31-164 delays the radiographic progression of primary OSA

Dog 007



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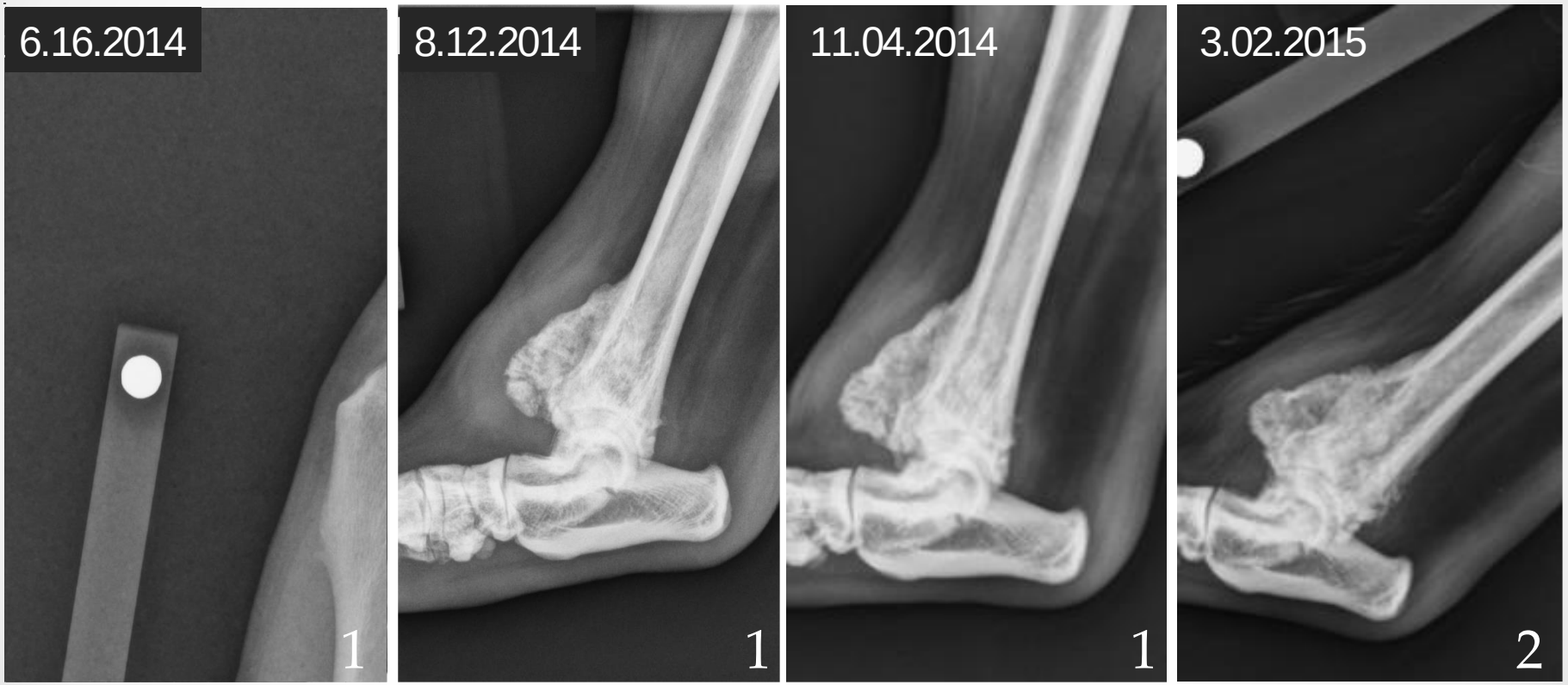
RT+ ADXS31-164 delays the radiographic progression of primary OSA

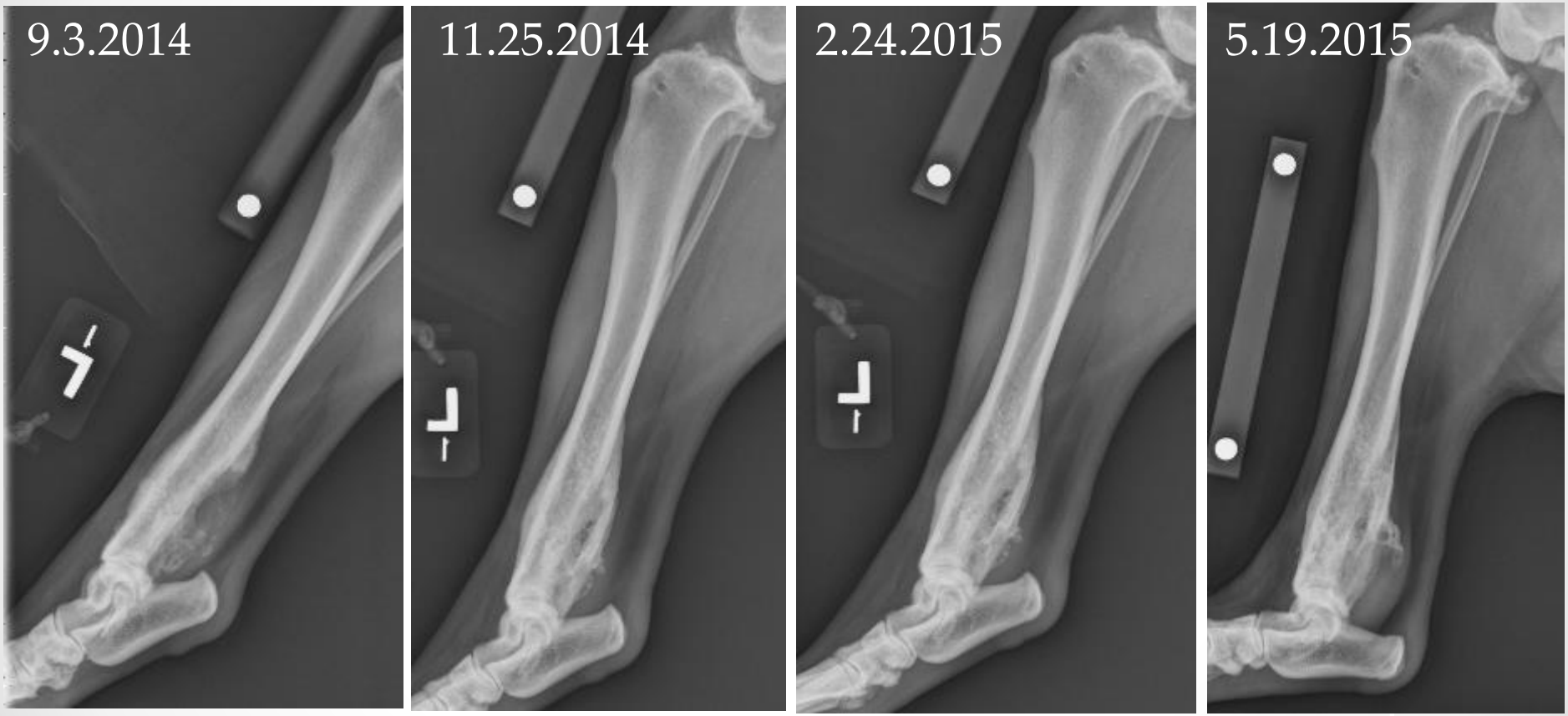
Dog 005



LAMENESS SCORE:
0=clinically sound, 1=barely detectable lameness, 2=mild lameness, 3=moderate lameness, 4=severe lameness, 5=non weight bearing

Radiographic evidence of bone remodeling and "healing" of primary OSA lesion following RT+ ADXS31-164

Dog 011



- Increase boney deposition and apparent cortical bone replacement
- Decrease in lysis of distal tibia
- Smoothening of periosteal reaction on cranial aspect of tibia

Stable clinical disease following RT+ADXS31-164

386-003

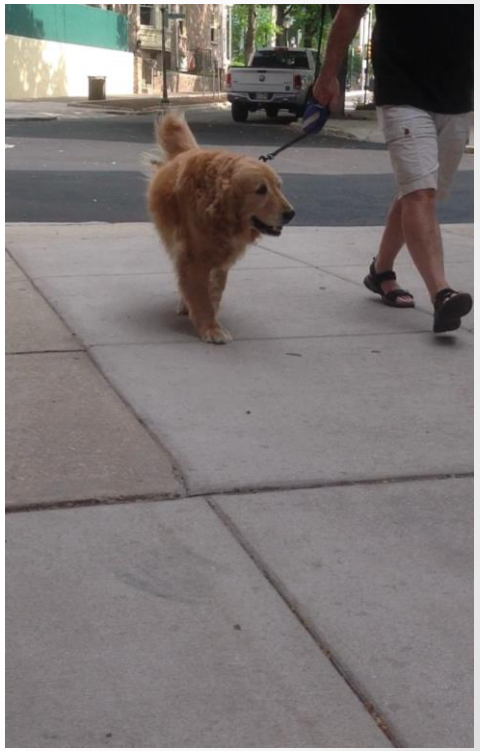
2.26.2014

11.03.2014

386-005

6.12.2014

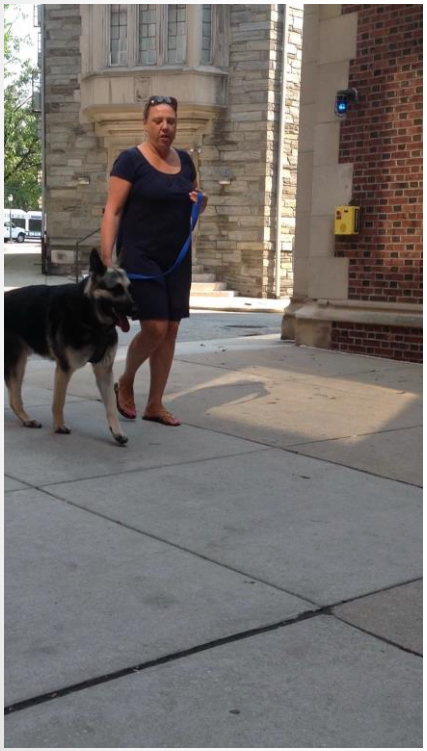
5.12.2015



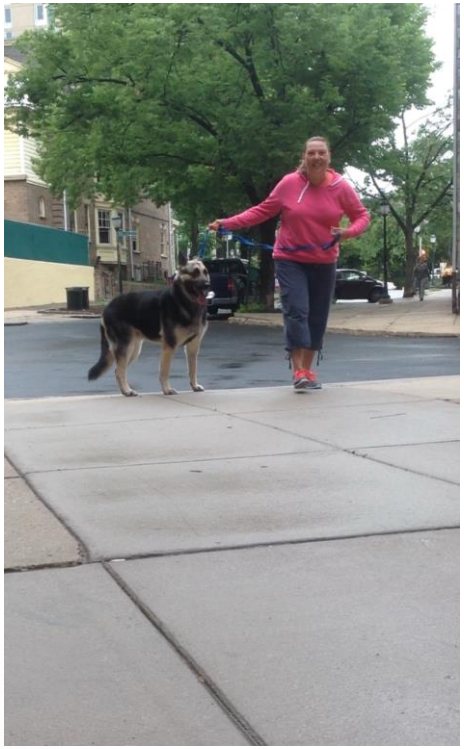
Stable clinical disease following RT+ADXS31-164

386-007

7.11.2014

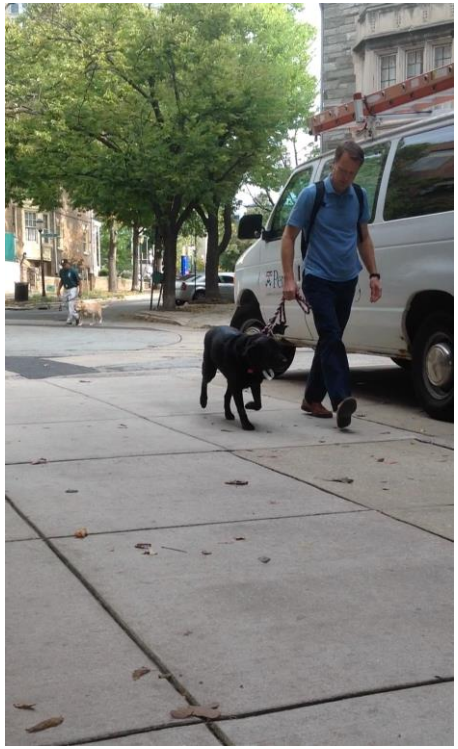


6.02.2015



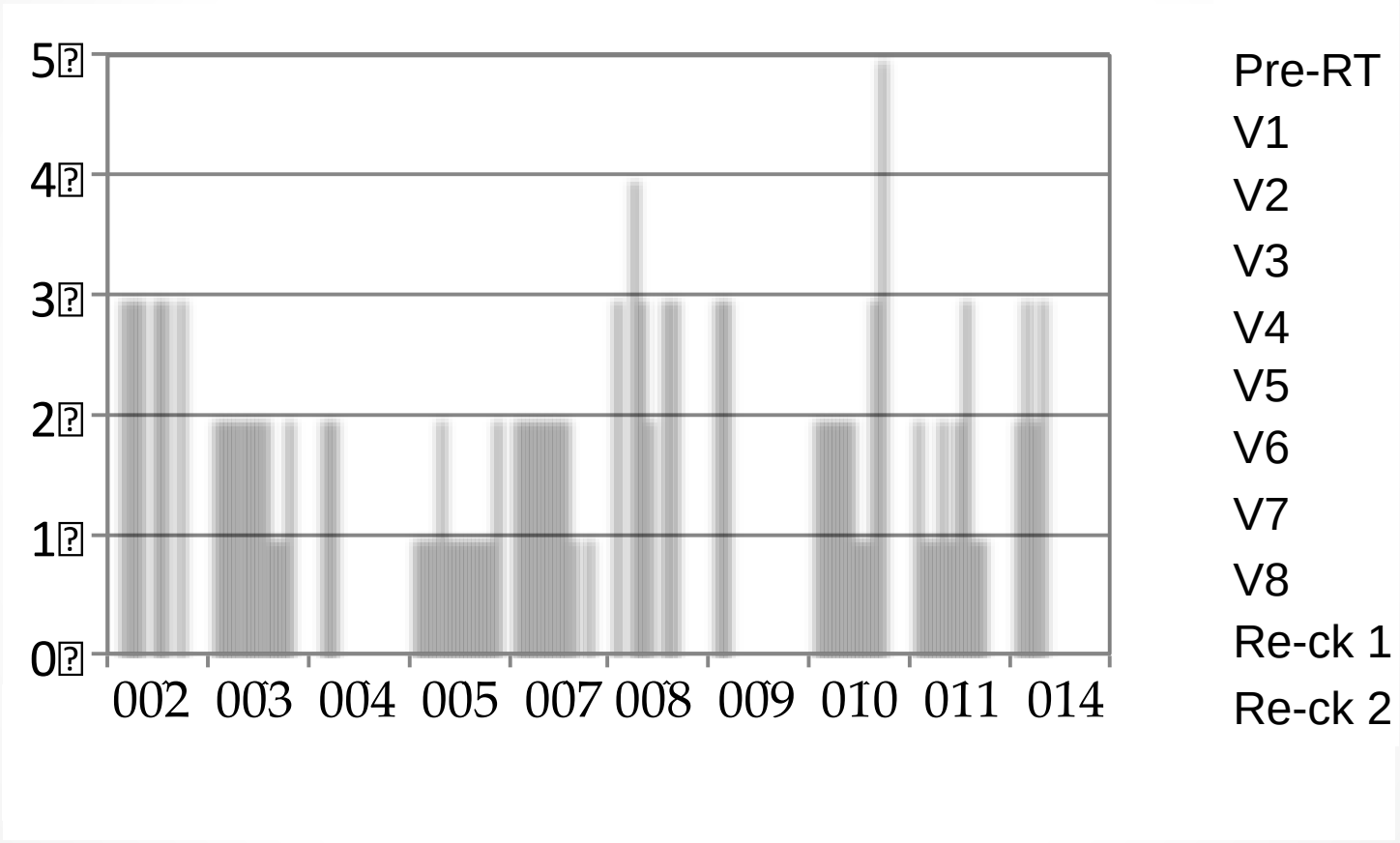
386-011

9.03.2014



05.19.2015

Lameness scores



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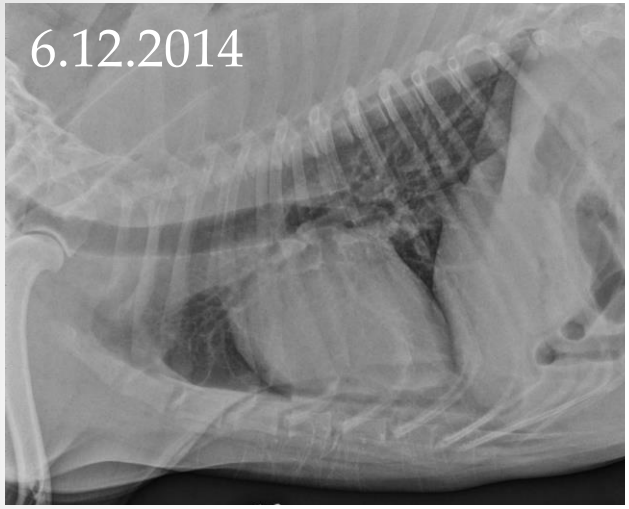
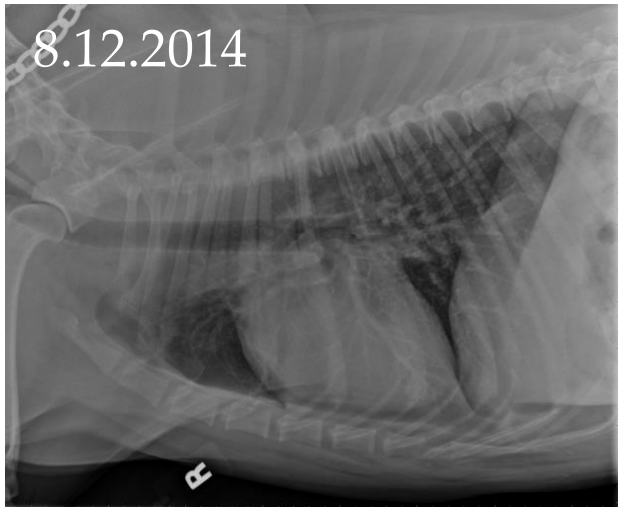
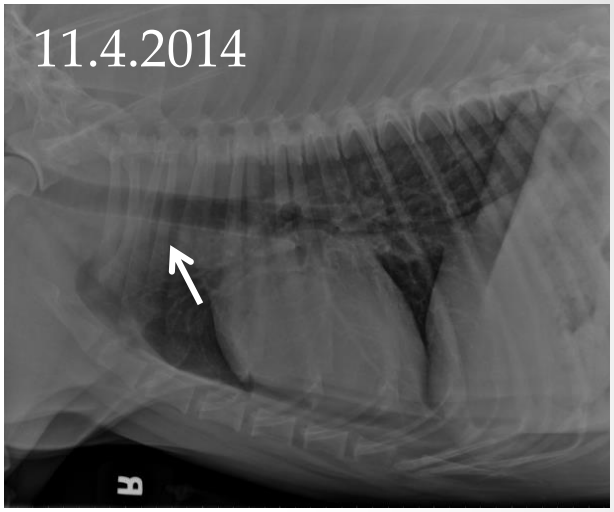
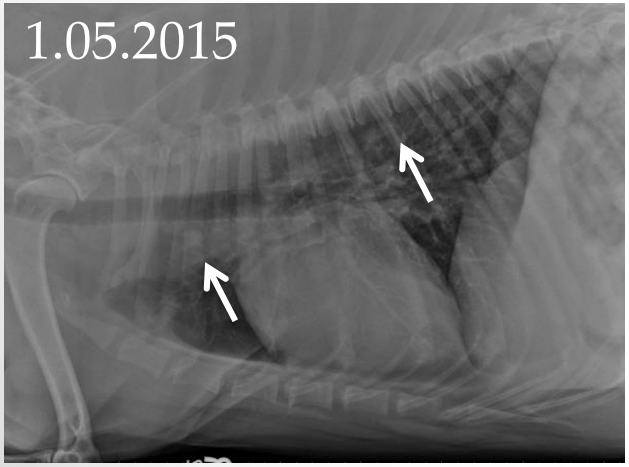
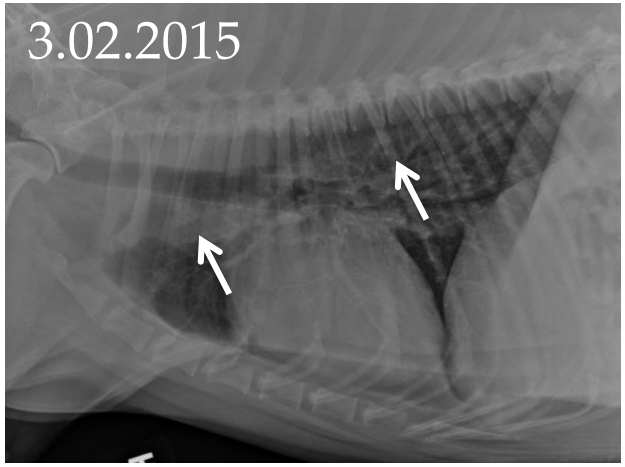
Inflammatory infiltrates versus progressive pulmonary metastatic disease following ADXS31-164 immune therapy?

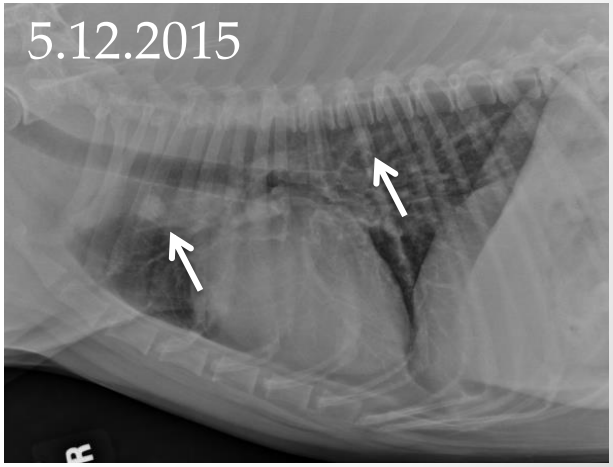




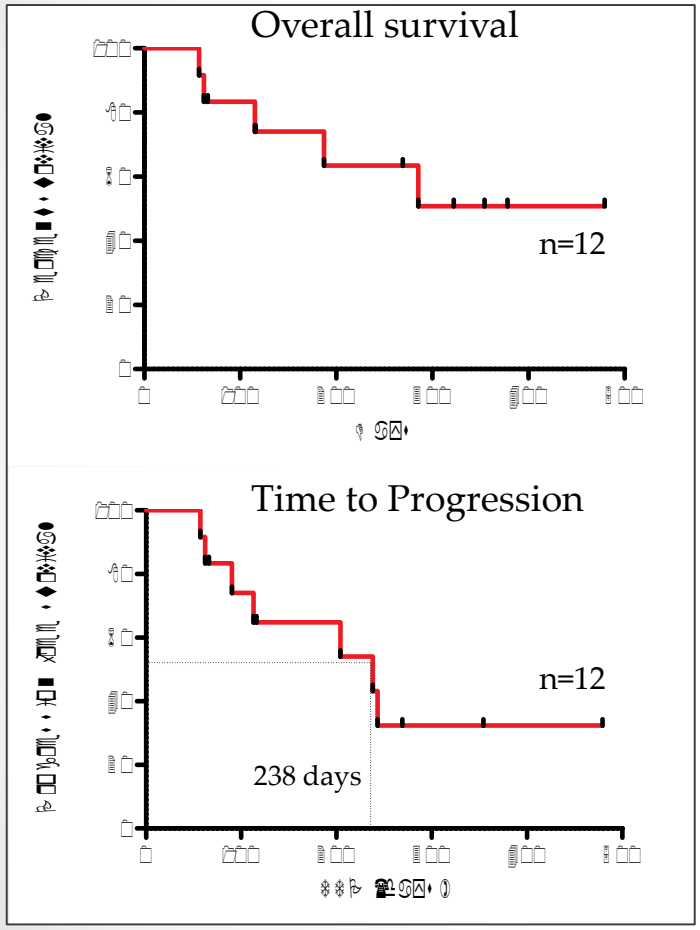








K-M curves and autopsy findings of euthanized dogs



Dog	Metastatic lesion location	HER2 status of metastatic lesions	Time to Progression (days)	Overall Survival (days)	Reason for euthanasia
002	Primary: Right Proximal Humerus Metastatic sites: Lungs, Liver, Speen, Omentum, Right Kidney, Right 5th rib, Vertebrae (T5, L1, and L5) and Subcutis	HER2+ Scores pending	238	285	Metastatic disease
004	Primary: Right Distal Radius Metastatic sites: Right pre-scapular LN, Right Axillary LN, Lungs, Heart, Spleen	HER2+ Scores pending	62	62	Metastatic disease
008	Primary: Left Distal Radius Metastatic sites: Lungs	HER2+ Scores pending	113	187	Pathologic fracture
009	Primary: Right Proximal Humerus Metastatic sites: None	None	57	57	Pathologic fracture
014	Unknown – no autopsy	Unknown	90	115	Pathologic fracture

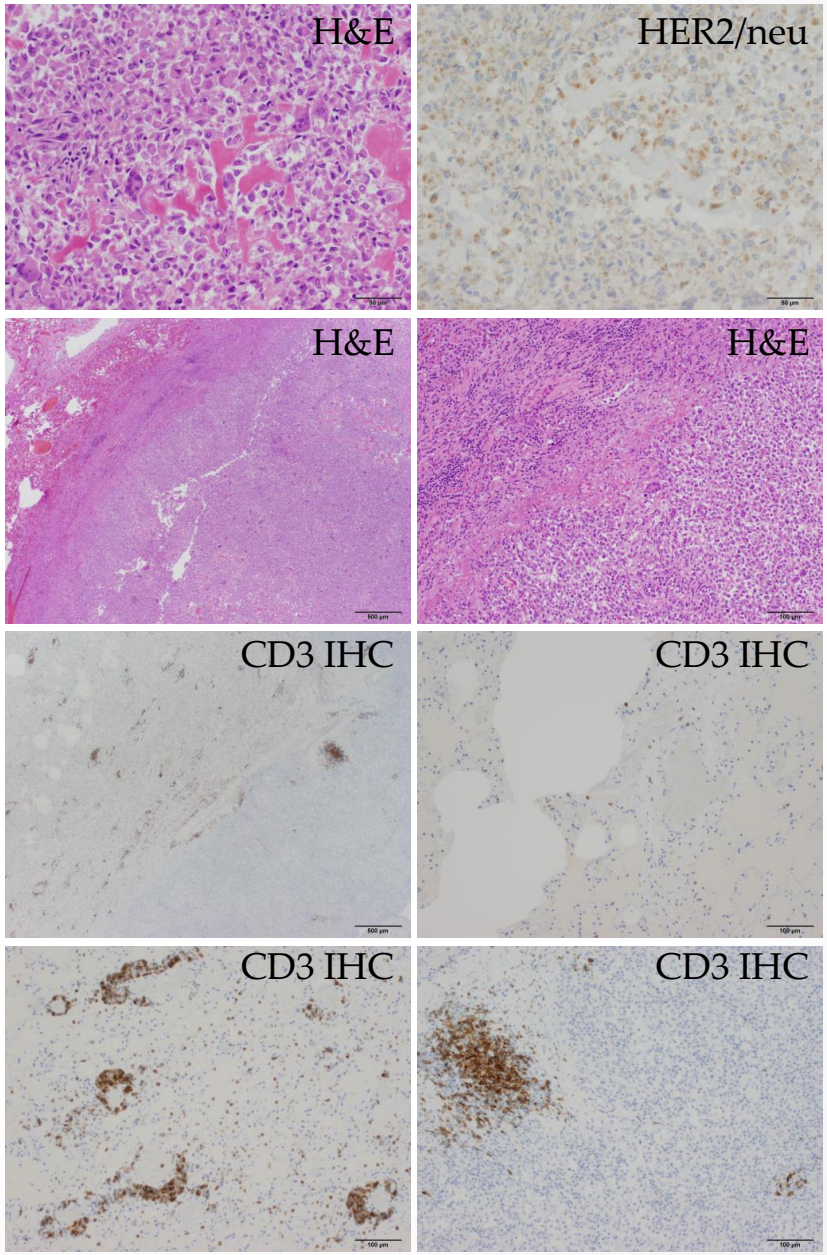
Lymphocytic recruitment to metastatic lung lesions

Pulmonary nodule
Metastatic HER2^{+} OSA

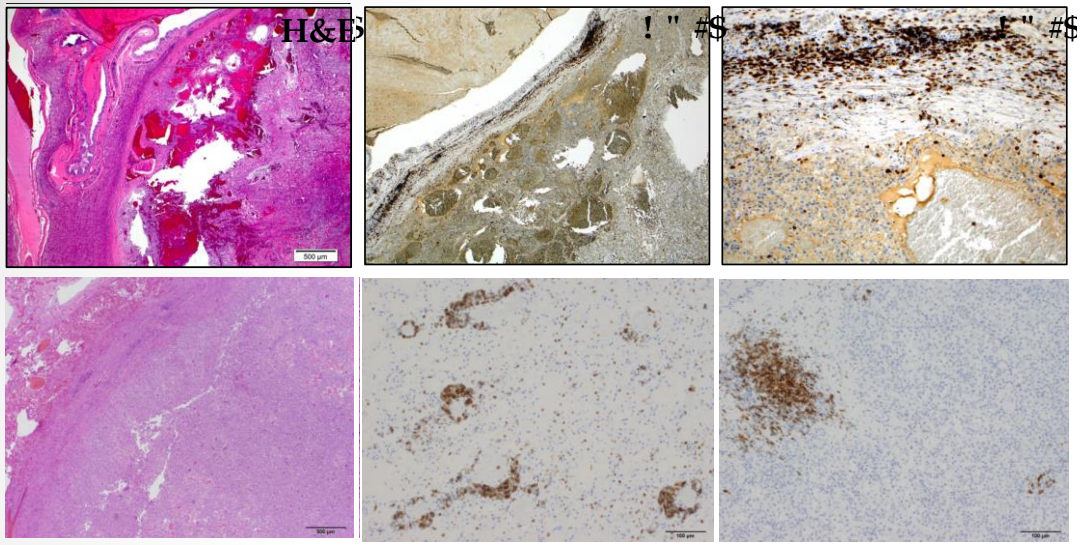
Lymphocytic recruitment

CD3^{+} lymphocytes specifically associated with nodule

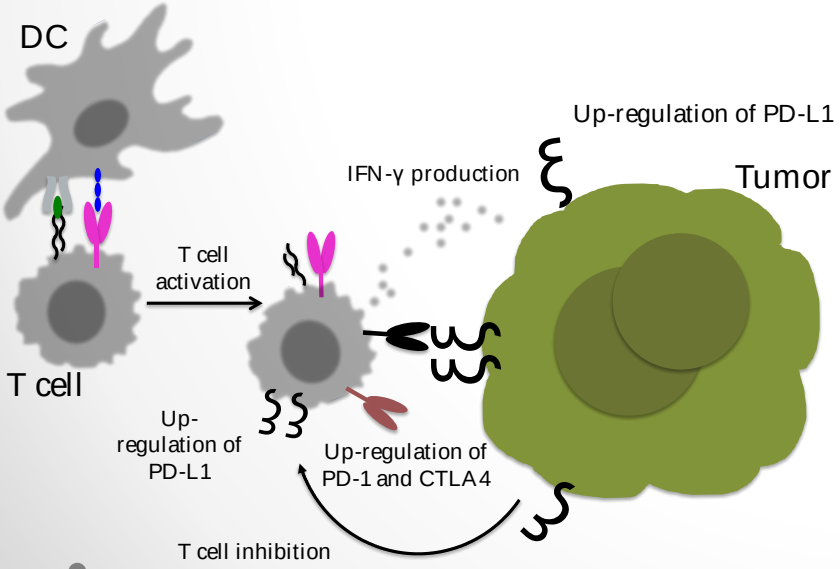
Perivascular cuffing CD3^{+} lymphocytes adjacent to nodule

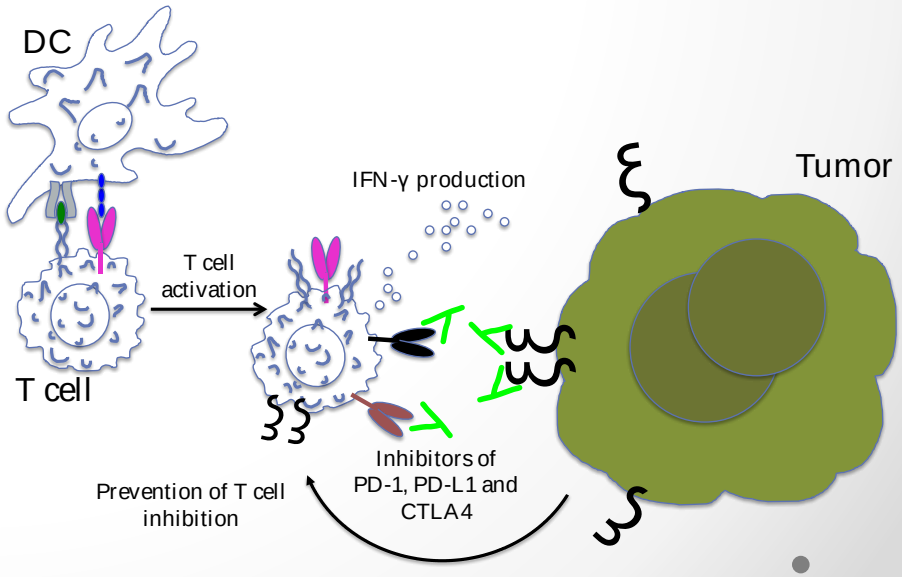


Physical and Functional T cell Inhibition Reduces Efficacy in Metastatic Disease



- Physical barriers
  - Fibrous capsule
  - High intra-tumoral pressure
- Functional barriers
  - Immune Checkpoints
  - Immune suppressive mileu
    - Tregs &MDSC
    - Cytokines
    - IDO, Arginase I





Summary, Conclusions and Future Directions

- ADXS31-164 administration in dogs
  - Repeat administrations of up to 3.3 x 10^9 CFU are well tolerated
  - Breaks peripheral tolerance to HER2/neu and perhaps primes an effective memory response
  - Delay or prevents metastatic disease when administered in MRD
    - Innate versus Adaptive Immune response
  - Delays clinical and radiographic progression of OSA when used following palliative RT
- Disease Progression is not associated with loss of HER2/neu
  - Sequencing required to determine presence of HER2 mutations
- Effective control or elimination of pre-existing metastatic disease will likely require combination therapies that address the tumor microenvironment
  - Combination therapy with checkpoint inhibitors
  - Combination therapy with FAP targeting CAR T cells
- Future Directions
  - Understanding the mechanism of action of ADXS31-164 – is HER2 a relevant target?
    - Evaluation of CTCs for HER2 expression
  - Understanding differences between primary and metastatic tumor microenvironments
  - Evaluation of ADXS31-164 as neo-adjuvant therapy

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Pathology
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Aratana Therapeutics
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Richard Lichter Canine Foundation

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