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

Nicola Mason, B.Vet.Med, PhD (Immunology), DACVIM (Internal Medicine)  
Associate Professor, Departments of Pathobiology and Clinical Studies,  
School of Veterinary Medicine,  
University of Pennsylvania

pmason@vet.upenn.edu  
215.898.3996
I have the following disclosures* related to my presentation:

Employee: University of Pennsylvania

Grants/Research contracts: Advaxis Inc., Aratana Therapeutics, Abramson Cancer Foundation, Morris Animal Foundation, Canine Health Foundation

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
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

- 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

Sequence identity with canine HER2
- EC1 89%
- EC2 93%
- IC1 98%
ADXS31-164 administered in the setting of minimal residual disease prevents metastatic disease and prolongs overall survival.

**Overall survival**
- MST: 956 days
- n=18

**Disease Free Interval**
- DFI: 615 days
- n=18

**MOA to prevent metastatic disease**
- HER2-specific CD4+ T cells
- HER2-specific CD8+ T cells
- Constitutive expression of FasL
- IL-12
- Listeria
- INNATE
- ADAPTIVE
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

2 x 8 Gy

Percent survival

Time (days)

n=57

Overview of Pilot Study Timeline

Median Duration of Pain Relief with RT alone = 70 days
Median Survival Time with RT alone = 136 days

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

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

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

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

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 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
Stable clinical disease following RT+ADXS31-164

386-007  7.11.2014
386-011  05.19.2015

6.02.2015
9.03.2014
Lameness scores

0=clinically sound, 1=barely detectable lameness, 2=mild lameness, 3=moderate lameness, 4=severe lameness, 5=non weight bearing
Inflammatory infiltrates versus progressive pulmonary metastatic disease following ADXS31-164 immune therapy?
K-M curves and autopsy findings of euthanized dogs

<table>
<thead>
<tr>
<th>Dog</th>
<th>Metastatic lesion location</th>
<th>HER2 status of metastatic lesions</th>
<th>Time to Progression (days)</th>
<th>Overall Survival (days)</th>
<th>Reason for euthanasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>002</td>
<td>Primary: Right Proximal Humerus Metastatic sites: Lungs, Liver, Spleen, Omentum, Right Kidney, Right 5th rib, Vertebrae (T5, L1, and L5) and Subcutis</td>
<td>HER2+ Scores pending</td>
<td>238</td>
<td>285</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>004</td>
<td>Primary: Right Distal Radius Metastatic sites: Right pre-scapular LN, Right Axillary L.N, Lungs, Heart, Spleen</td>
<td>HER2+ Scores pending</td>
<td>62</td>
<td>62</td>
<td>Metastatic disease</td>
</tr>
<tr>
<td>008</td>
<td>Primary: Left Distal Radius Metastatic sites: Lungs</td>
<td>HER2+ Scores pending</td>
<td>113</td>
<td>187</td>
<td>Pathologic fracture</td>
</tr>
<tr>
<td>009</td>
<td>Primary: Right Proximal Humerus Metastatic sites: None</td>
<td>None</td>
<td>57</td>
<td>57</td>
<td>Pathologic fracture</td>
</tr>
<tr>
<td>014</td>
<td>Unknown – no autopsy</td>
<td>Unknown</td>
<td>90</td>
<td>115</td>
<td>Pathologic fracture</td>
</tr>
</tbody>
</table>
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 milieu
    - Tregs & MDSC
    - Cytokines
    - IDO, Arginase I
Summary, Conclusions and Future Directions

• ADXS31-164 administration in dogs
  • Repeat administrations of up to $3.3 \times 10^9$ 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
    o 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
  o Understanding the mechanism of action of ADXS31-164 – is HER2 a relevant target?
    • Evaluation of CTCs for HER2 expression
  o Understanding differences between primary and metastatic tumor microenvironments
  o Evaluation of ADXS31-164 as neo-adjuvant therapy
Acknowledgements

Mason Lab
Josephine Gnanandarajah
Kazim Panjwani
Georges Habineza-Ndikuyeze
Anita Gaurnier-Hausser
Aliza Schmidt

Radiology/Radiation Oncology
Jenn Reetz, Ana Caceres, Wil Mai, Lili Duda, Steph Corsi, Susan Mendez

Cardiology
Maggie Machen, Dani Laughlin, Mel Hezzell,
Chloe Thorn

Surgical team
Cara Blake, Kim Agnello, Jeff Runge, Christa Cioffi,
Jacob Rubin

Microbiology
Shelley Rankin, Donna Maloney

Pathology
Julie Engiles, Falon Gray, Amy Durham
Juli Burns & Jackie Ferracone

Nurses and Staff
Sam Kean, Victoria Enders, Jen Prendergast, Ali McKenna, Chantal Reme, Ashley Deese, Amanda Ashley, Lila Sierra

Yvonne Paterson
Matt Seavey
Reshma Singh
George Gunn

Anu Wallecha
Robert Petit
Chris French

Funding sources
Advaxis Inc
Abramson Cancer Center
Aratana Therapeutics
Morris Animal Foundation
Skippy Frank Translational Medicine Fund
Richard Lichter Canine Foundation

Referring Oncologists
Kate Vickery, MJ Hamilton, Kevin Choy,
McFadden, Conor McNeil, Carrie Hume, Marlene Hauck, Emma Warry, Bridget Urie, Jennifer Locke,
Kathy Kazmierski, Pascale Salah, Paula Levine, Lisa Van der Gagg, Christine Mullen, and many more…