Development of a Personalized Medicine Pipeline for Treatment of Sarcomas

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David Hsu, Jason Somarelli – Duke
Rachael Thomas, Hiro Mochizuki, Betsy Scholl – NCSU
A Cross Species Genomic Analysis of Osteosarcoma: Implications for Novel Therapeutic Approaches

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**CHALLENGE**

- Few treatment advances have been made for sarcoma patients in recent decades.
- Lack of advance is primarily due to paucity of access to patient samples, which impedes research progress, combined with very limited studies of such cases.

**OPPORTUNITY**

- While uncommon in humans, sarcomas represent >15% of all canine malignancies.

**FOCUS**

- Osteosarcoma
APPROACH

Dogs with naturally occurring sarcomas represent a unique comparative “model” that has several advantages over studying human sarcoma patients:

1. The complete oncologic progression occurs within one to two years.
2. Dogs are usually treated without adjuvant therapies, thereby limiting the number of confounding variables.
3. Pet dogs have an intact immune system and share the same environment as humans.

These advantages make dogs an attractive species in which to develop a personalized medicine pipeline to match tumor mutations to drug efficacy.
# How do canine and human osteosarcoma compare?

<table>
<thead>
<tr>
<th>Variable</th>
<th>Canine</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence in U.S.</td>
<td>&gt; 10,000 diagnosed cases/year</td>
<td>~ 800 diagnosed cases/year</td>
</tr>
<tr>
<td>Mean age of onset</td>
<td>Geriatric (7 year)</td>
<td>Adolescent (14 year)</td>
</tr>
<tr>
<td>Gender</td>
<td>1.5 female to 1 male</td>
<td>1.5 female to 1 male</td>
</tr>
<tr>
<td>Primary tumor site</td>
<td>77% long bones Metaphyseal Distal radius &gt; proximal humerus Distal femur &gt; tibia</td>
<td>90% long bones Metaphyseal Distal femur &gt; proximal tibia Proximal humerus</td>
</tr>
<tr>
<td>Histologic high grade</td>
<td>95%</td>
<td>85-90%</td>
</tr>
<tr>
<td>Chemotherapy naïve metastatic rate</td>
<td>90% before 12 months</td>
<td>80% before 24 months</td>
</tr>
<tr>
<td>Metastatic sites</td>
<td>Lung &gt; bone &gt; soft tissue</td>
<td>Lung &gt; bone &gt; soft tissue</td>
</tr>
<tr>
<td>Chemotherapy impact</td>
<td>Significant</td>
<td>Significant</td>
</tr>
</tbody>
</table>

SURVIVAL OF PATIENTS WITH LOCALIZED OSTEOSARCOMA

- 1960s – single agent chemo
- 1980s - combination chemo
- 2004 - multiple intensified chemo regimens
- Pre-chemo
Surveyed over 10,000 cases of canine osteosarcoma

- 45% Purebred
- 55% Mix

- 51% Female
- 49% Male
Surveyed over 10,000 cases of canine osteosarcoma

3.6% (1 in 28)
Our earlier studies have demonstrated similarities in genomic changes detected by whole genome profiling of canine and human osteosarcoma


Our earlier studies have demonstrated similarities in genomic changes detected by whole genome profiling of canine and human osteosarcoma.
Human osteosarcoma
Canine osteosarcoma
How do canine and human osteosarcoma compare?
A Cross Species Genomic Analysis of Osteosarcoma: Implications for Novel Therapeutic Approaches

Development of a Personalized Medicine Pipeline for Treatment of Sarcomas

Rare disease, Genetically complex, Stalled progress...

How do we identify new targets?

9/10 new cancer drugs stall, Current models aren’t predictive, Treatment not precise...

How do we screen new candidate drugs more effectively?
Treatment of Cancer

Patient Care

Clinical Trial

Preclinical Work

Humans

Cells

Mice
These two projects together work cooperatively to create a new paradigm:

The “MDH” (Mouse to Dog to Human) Pipeline

A Cross Species Genomic Analysis of Osteosarcoma: Implications for Novel Therapeutic Approaches

Development of a Personalized Medicine Pipeline for Treatment of Sarcomas
Generation of Sarcoma Xenografts

Establish cell lines
Exome sequencing
Drug screen

Identify candidate drugs
Mouse Clinical Trial
Dog Clinical Trial

The Mouse to Dog to Human (MDH) Pipeline
A Cross Species Genomic Analysis of Osteosarcoma

[Identifying a Target]
Hypothesis: The identification of conserved genetic variation across species would provide a unique perspective to view the determinants of osteosarcoma biology.

CNV variations in 2 dogs and 4 mice with OS compared to published human sequence data (Perry et al., PNAS 2014)

Suggested that **ATRX** variations are conserved across species.
What is ATRX?
A Cross Species Genomic Analysis of Osteosarcoma

ATRX = Alpha Thalassaemia Retardation Syndrome, X-linked

Gibbons, Orphanet Journal of Rare Diseases, 2006
ATRX has two highly conserved domains:

- SWI/SNF helicase domain
  - Chromatin remodeling
- ADD domain
  - DNA methylation patterns
  - Tethering proteins to chromatin

Argentaro et al., *PNAS* 2007
ATRX forms dimers with DAXX...

...Deposits histone variant H3.3...

...This happens in GC-rich regions of the genome, including pericentric, ribosomal, and telomeric repeat sequences.

Voon et al., *Nucleic Acids Research*, 2016
ATRX- Is it Important in Malignancy

- ATRX required for genome stability
  - Suppressor of ALT pathway
  - Regulator of gene expression and function
  - Important tumor suppressor
- Mutations in ATRX identified in over 15 types of cancers, including neuroblastomas, pancreatic neuroendocrine tumors, and a variety of sarcomas.

Lovejoy et al., *PLoS Genet*, 2012
Heaphy et al., *Science*, 2011
Bower et al., *PLoS ONE*, 2012
Heat map showing variable patterns of ATRX expression in human osteosarcoma samples in the SARC database
A Cross Species Genomic Analysis of Osteosarcoma

[Loading the Toolbox]
• Established 8 different OS cell lines

<table>
<thead>
<tr>
<th>4 Human</th>
<th>4 Canine</th>
</tr>
</thead>
<tbody>
<tr>
<td>143b</td>
<td>Abrams</td>
</tr>
<tr>
<td>MG63</td>
<td>Moresco</td>
</tr>
<tr>
<td>SAOS2</td>
<td>D17</td>
</tr>
<tr>
<td>U2OS</td>
<td>D418</td>
</tr>
</tbody>
</table>
A Cross Species Genomic Analysis of Osteosarcoma

- Established baseline ATRX expression

<table>
<thead>
<tr>
<th>Ladder</th>
<th>hFOB</th>
<th>143B</th>
<th>MG63</th>
<th>SAOS2</th>
<th>U-2 OS</th>
<th>Abrams</th>
<th>Moresco</th>
<th>D17</th>
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ATRX = 280 kDa

α-tubulin = 50 kDa
• Developed siRNAs for knockdown
IF: ATRX siRNA Knockdown- 143B

<table>
<thead>
<tr>
<th>NS</th>
<th>DAPI</th>
<th>ATRX</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
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<table>
<thead>
<tr>
<th>siRNA 5</th>
<th>DAPI</th>
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IF: ATRX siRNA Knockdown - Abrams

NS

siRNA 2

siRNA 4
qPCR Results for siRNA Knockdown of ATRX

### 143B ATRX siRNA Knockdown

<table>
<thead>
<tr>
<th></th>
<th>24 hour</th>
<th>48 hour</th>
<th>72 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>s5</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>s6</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>s11</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

### Abrams ATRX siRNA Knockdown

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>s2</td>
<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>s4</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>s2</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
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A Cross Species Genomic Analysis of Osteosarcoma
Recall that ATRX is a suppressor of the Alternative Lengthening of Telomeres (ALT) pathway ...

Could ATR inhibitors be considered as a therapy for ATRX-mutated osteosarcoma?
AZD6738 (AstraZeneca) +/- ATRX knockdown 143B human osteosarcoma
AZD6738 (AstraZeneca) +/- ATRX knockdown 143B human osteosarcoma

[Diagram showing percent cell inhibition with AZD6738 and ATRX knockdown conditions.]
AZD6738 (AstraZeneca) +/- ATRX knockdown 143B human osteosarcoma

A Cross Species Genomic Analysis of Osteosarcoma
Development of a Personalized Medicine Pipeline

[Loading the Toolbox]
• Established pathway for engrafting canine sarcomas as PDX
Development of a Personalized Medicine Pipeline

Operating Room

Surgical Pathology

Formalin Fixed Paraffin Embedded (FFPE)

Fresh Frozen Block

Blood

Viable Cells

[Loading the Toolbox]
Development of a Personalized Medicine Pipeline

[Loading the Toolbox]

Viable Cells

Cell Lines

Patient Derived Xenografts
• We adapted that pathway to canine sarcomas
Canine PDX

- 26 canine sarcomas have been implanted into NSG mice.
- 20 of them successfully engrafted (77%)
- Latency 49-279 days
Canine PDX

D418 - Osteosarcoma
Is the pathology similar in xenografts to primary tumors?

D401 primary
PCR with dog or mouse primaries confirms canine specificity

Mouse primers  Dog primers

gDNA  -  -  -
Mouse fibroblasts
Dog D17 cells
Dog D418 cells
Development of a Personalized Medicine Pipeline

[Initiating a Drug Screen]
Development of a Personalized Medicine Pipeline

- Exome sequencing
- Validate in PDXs
- Dog clinical trials

Human/dog cell line drug screen

Common drugs
Human

Dog

Human

Dog
Proportion of live cells

Cell line
- 143B
- Abrams
- D17
- D418
- MG-63
- MG-60
- Moresco
- SAOS2
- U2OS

Chemotherapeutics:
- Daunorubicin hydrochloride
- Idarubicin hydrochloride
- Doxorubicin hydrochloride
- Daunomycin
- Methotrexate
- Plicamycin
- Paclitaxel
- Ixabepilone
- Mitoxantrone
- Docetaxel
- Cabazitaxel
- Valrubicin
- Vinblastine sulfate
- Vinorelbine tartrate
- Vincristine sulfate
- DMSO
- Bortezomib
- Carfilzomib
- Pralatrexate
- Omacetaxine mepesuccinate
- Epirubicin hydrochloride
- Cisplatin
Histogram of average % Killing when binned by 312 drug target classes in the drug library.

This is as we would expect: the mean hovers around 0-10% killing – most drugs do nothing – and a relatively rare few are extremely effective.
This shows average and standard deviation (error bars) for % killing across the top 20 drug targets.

This only includes targets that have multiple drugs as confirmation of the importance of the overall target class.

Note proteasome inhibitors as #2!!!
Summary

• This is strong collaboration getting stronger, thanks to C3O funding.

• Our MDH (Mouse to Dog to Human) Platform is the only one of its kind.

• The combination of cross-species genomic analysis and cross-species drug screening may be a path forward to finding new treatments.