Use and availability of canine cancer tissue banks in translational research – the dog as a model for accelerating gene discovery.

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"Between animal and human medicine there is no dividing line – nor should there be. The object is different but the experience obtained constitutes the basis of all medicine."

Rudolf Virchow (1821-1902)

How do we apply this to cancer research?
The traditional cancer model
1. Cancer is a genetic disease

2. Certain breeds of dog are highly affected by certain cancers (naturally occurring)

3. Indicates that breeds of dog have an inherited predisposition to cancers

4. Same environment as human

POWERFUL OPPORTUNITY = identification of genetic factors in the dog will simultaneously offer major factors contributing to advancing cancer research in humans
One Medicine

oral melanoma
leukemia
lymphoma
cutaneous melanoma
breast
bladder
prostate
bone

~1.66 million diagnoses each year
(~500 cases/100,000 population)

~4.2 million diagnoses each year
(~5,300 dogs/100,000 population)

One Pathogenesis
Why do we need biobanks?

- Research involving canine genetic or genomic information analyzed using biological specimens from well annotated patients is key to understanding complex diseases including cancer.

- These data are key to advancing cancer detection, diagnosis, prognosis, intervention, treatment, and prevention.

- For maximum efficiency, establishment and sharing of resources is needed, comprising canine biological samples and information derived from their analysis.
Requirements of effective biobanks

Key elements (SOPs)

SPECIMEN SELECTION AND STORAGE
Fit for purpose
High quality preparation, storage and retrieval

SPECIMEN ACCESS
Specimens and patient data accessible for research over time

SPECIMEN USE
Data sharing plan
Comparative approach is highly dependent on access to high quality cancer specimens.

Canine cancer patient biospecimens are available for scientific use through the Canine Comparative Oncology and Genomics Consortium (CCOGC) and the Pfizer-CCOGC Biospecimen Repository.

The Pfizer-CCOGC Biospecimen Repository currently houses over 2,000 patient samples across seven spontaneously arising cancer histologies.

By utilizing our current tissue collection structure for our bank, we can tailor collections for the specific needs of your unique research. After submission of a brief on-line application, there will be follow up contact by the CCOGC to refine your request. We will assess the availability within our network, prepare Standard Operating Procedures and a budget. After your approval and a contract process, tissue collection will begin specifically designed for your research.

Canine Biobank
All samples were stored in the canine biobank. The Canine Biobank is a number of tissues that are kept at -80°C in liquid nitrogen. These samples are curated in a searchable database.

At Year 2012-2013 robust had samples from about 50 different breeds. Since each sample is split up into different parts, we now have 30,000 samples.

Since we store data and samples in this way, we can use the samples for multiple projects. If we already have samples for your project, we have no need to search for new dogs. In this way, we minimize the amount of new samples need to be taken.

The Canine Biobank is a huge thank you to Traveller Club and The Insurance company Agio who have helped this project since 2008.

More Info...

Learn about another cancer
One time start-up costs $1.7 million
- $1.1M Pfizer, $0.6M AKC-CHF, $0.5M MAF

Physical infrastructure

Database development and management

Sample collection (>2,000 patients)

Quality and assurance
Compliance is essential for consistency and quality

**Pfizer-CCOGC Biospecimen Repository Standard Operating Procedures**

**Purpose**

1. **Background**
2. **Criteria for specimen banking**
   - 2.1 Protocol Definitions
   - 2.2 Clinical Assessment
3. **Tissue Collections and Processing**
   - 3.1 Selections and Submission of Tissue Embedded in Paraffin Blocks
   - 3.2 Selections and Submission of Frozen Tissues
   - 3.3 Collection of Whole Blood for Nucleic Acid Extraction
   - 3.4 Collections and Submission of Whole Blood for Genomic DNA
   - 3.5 Collections and Submission of Serum
   - 3.6 Collections and Submission of Plasma
   - 3.7 Collections and Submission of Urine
4. **Patients and Specimen Labeling**
5. **Shipping**
6. **Sample Collection Supplies**
7. **Database Entry**
8. **Sample Processing Flow Chart**
One patient, many opportunities

CLINICAL DATA

PATHOLOGY DATA

fixed normal tissue

serum

whole blood

plasma

urine sample

fixed tumor tissue

snap frozen normal tissue

snap frozen tumor tissue

FFPE specimen
Collaboration with pet owners/parents

Academic-citizen based collaboration
~60,000 specimens from ~1,900 cancer bearing patients averaging 30 vials/patient

>100 breeds  
52% male:48% female
Quality control & quality assurance of canine biological specimens available through the Pfizer-CCOGC biospecimen repository for comparative oncology studies

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Presented at AACR, The Translational Impact of Model Organisms on Cancer, Nov 2013
### Pathology review – diagnosis verification

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Number of cases assessed</th>
<th>Number (%) of diagnoses corroborated</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT</td>
<td>50</td>
<td>47 (94%)</td>
</tr>
<tr>
<td>HEM</td>
<td>50</td>
<td>32 (64%)</td>
</tr>
<tr>
<td>STS</td>
<td>49</td>
<td>44 (90%)</td>
</tr>
<tr>
<td>PUL</td>
<td>50</td>
<td>46 (92%)</td>
</tr>
<tr>
<td>OSA</td>
<td>42</td>
<td>42 (100%)</td>
</tr>
<tr>
<td>LSA</td>
<td>49</td>
<td>49 (100%)</td>
</tr>
<tr>
<td>MEL</td>
<td>41</td>
<td>35 (85%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>331</td>
<td>295 (89%)</td>
</tr>
</tbody>
</table>

Outcome of pathology review of 331 cases distributed across each of the seven tumor histologies.
Pathology review –
tumor purity

Assessment of 132 cases according to the percentage of tumor versus stromal contamination contained within each specimen.

• Lymphomas consistently exhibited a high proportion of tumor
• Melanomas were more variable
• Osteosarcoma cases showed extensive variation.
Nucleic acid QC

188 cases, selected at random, from the biorepository to provide proportional representation of each tumor histology and submitting institution
The importance of pathologic review

<table>
<thead>
<tr>
<th>DNA source</th>
<th>BRAF mutation analysis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutation fraction by ddPCR</td>
<td>Sanger sequencing</td>
</tr>
<tr>
<td>Full size section</td>
<td>1.2%</td>
<td>negative</td>
</tr>
<tr>
<td>Macrodissected section</td>
<td>12.2%</td>
<td>negative</td>
</tr>
</tbody>
</table>
How are we using canine well defined cancer patient specimens to accelerate cancer research for the benefit of dogs and people?
How are we approaching comparative oncology using the dog as a model?

Identification of inherited and somatic changes

molecular cytogenetics
gene expression
case-control GWAS
exome sequencing of tumor-normal pairs
WGS of tumor-normal pairs
Human and dog genome organization

46 chromosomes
22 autosomal pairs + sex chromosomes

78 chromosomes
38 autosomal pairs + sex chromosomes
Nature’s biological filing cabinets become rearranged during cancers

..... some genes are missing, some genes are duplicated, and in certain cases some genes move to new locations where they do not get along well with their new neighbors.
Now we have the reagents to ask some key questions

Q. Are there evolutionarily related chromosome aberrations that suggest an ancestral mechanism of pathogenetically significant events?

Rationale: look for well described human cytogenetic changes in canine cancers
One example of many

The Philadelphia chromosome - der(22)t(9;22)(q34;q11)

Tyrosine kinase

BCR-ABL FUSION

Image courtesy of Dr. E. Nacheva
Comparative chromosome alignment

Dog chromosomes – predicted translocation

‘Raleigh’ chromosome
Q. Are there evolutionarily related chromosome aberrations which suggest an ancestral mechanism of pathogenetically significant events?

A. YES .... but what could this mean for human and dog patients?

**VETERINARY PERSPECTIVE** = same pathogenetic mechanism = same treatments?

**MEDICAL PERSPECTIVE** = differential organization of the dog genome may narrow key regions of the genome associated with cancers

Q. CAN WE USE THE GENOME OF THE DOG TO IDENTIFY CANCER GENES THUS FAR ‘HIDDEN’ IN THE HUMAN GENOME
Contrasting architecture of the dog and human genomes offers tremendous potential to refine regions of significance.
THE HUMAN KARYOTYPE VISUALIZED AS A DOG KARYOTYPE
Comparative genomic map of human 22 and the domestic dog
Cytogenetics of meningiomas

Among the first solid tumors recognized as having cytogenetic alterations (Mark et al. 1972)

• A hallmark genomic imbalance of meningiomas is either the partial (del(22)(q12)) or total deletion of chromosome 22
  = up to a 50Mb deletion with over 500 genes

• Loss of chromosome 22 more often occurs in low grade meningiomas (implies key early event?)
• Deletions frequently involve the region HSA 22q12 (NF2)
Evaluation of canine meningioma data reduces candidate region of interest ~25 fold

50Mb to 2Mb - 550 genes to <10

targeted analysis

human 22
50 Mb
Canine TCC/UC (bladder cancer)

SYMPTOMS of urothelial cancer in the dog are shared with variety of other urinary tract conditions e.g. bladder infection/inflammation, bladder stones

APPROACHES to diagnosis currently:
- cytology - can be misleading, relies on abnormal cells which may be shed due to other conditions
- imaging - tumor, inflammation?
- biopsy - only way to conclusively diagnose and obtained by traumatic catheterization or cystoscopy, both of which carry risk of seeding = reluctance to biopsy

Diagnostic challenge
( >2.5M cases of UTI p.a)

Demand for a FC-urine diagnostic for TCC
oaCGH profiling of canine TCC – cohort analysis

Whole chromosome aneuploidy of 13/19/36 in canine TCC is unidirectional and highly recurrent

<table>
<thead>
<tr>
<th>TCC ‘cases’ (n=31)</th>
<th>Frequency of copy number status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome</td>
<td>gain (n&gt;2)</td>
</tr>
<tr>
<td>CFA 13</td>
<td>96.77%</td>
</tr>
<tr>
<td>CFA 19</td>
<td>0.00%</td>
</tr>
<tr>
<td>CFA 36</td>
<td>83.87%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Normal ‘controls’ (n&gt;100)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome</td>
<td>gain (n&gt;2)</td>
</tr>
<tr>
<td>CFA 13</td>
<td>0.00%</td>
</tr>
<tr>
<td>CFA 19</td>
<td>0.00%</td>
</tr>
<tr>
<td>CFA 36</td>
<td>0.00%</td>
</tr>
</tbody>
</table>
“Genomic Recoding”

are people really like their dogs?

Canine urothelial carcinoma: genomically aberrant and comparatively relevant

S. G. Shapiro · S. Raghunath · C. Williams · A. A. Motsinger-Reif · J. M. Cullen · T. Liu · D. Albertson · M. Ruvolo · A. Bergstrom Lucas · J. Jin · D. W. Knapp · J. D. Schiffman · M. Breen

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### Compile the data

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>n=</th>
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<tbody>
<tr>
<td>Lymphoma</td>
<td>350</td>
</tr>
<tr>
<td>Leukemia</td>
<td>175</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>125</td>
</tr>
<tr>
<td>Intracranial</td>
<td>100</td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>110</td>
</tr>
<tr>
<td>Histiocytic malignancies</td>
<td>130</td>
</tr>
<tr>
<td>Melanoma</td>
<td>100</td>
</tr>
<tr>
<td>Mast cell</td>
<td>200</td>
</tr>
<tr>
<td>Urogenital carcinoma</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>1,280</td>
</tr>
</tbody>
</table>
Cluster analysis of genome-wide DNA copy number changes in a series of canine cancer patients.

Do any of these recurrent aberrations correlate with subtype and/or prognosis in dogs?

YES

Are there corresponding cytogenetic lesions in human patients?

YES
Genomics toolbox

DNA copy number
Cytogenetic analysis
gene expression

SNP genotyping
Exome sequencing
Whole genome sequencing

Animal cancers

lymphoma
osteosarcoma
intracranial tumors
soft tissue sarcoma
hemangiosarcoma
mast cell tumors
melanoma
leukemia
UG carcinoma

Human cancers

Detailed pathology and clinical outcome associated cases = opportunities for diagnostic and prognostic signatures
On the path to effective cancer therapies, the keys to unlocking some of these puzzles may be walking right beside us.
Cancer across life: Peto’s paradox and the promise of comparative oncology

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Natasha Olby - Veterinary Neurologist
Chris Mariani - Veterinary Neurologist
Steven Suter - Clinical Veterinary Oncologist
Dahlia Nielsen - Statistical Geneticist
Alison Motsinger-Reif - Biostatistician

Key collaborators (Dog, human and marine mammal)

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Bill Kisseberth, Guillermo Couto - Ohio State U, USA
Pete Dickensen, UC Davis, USA
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Elaine Ostrander et al - NIH
Frances Gulland, Marine Mammal Center, CA
Sea Lion Cancer Consortium
Mona Rosenberg - Veterinary Cancer Group, CA
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... and the thousands of dog parents and their veterinarians who have submitted specimens to help this research.