NC STATE UNIVERSITY The Center for Comparative Medicine and Translational Research

One Medicine, full circle

CCMTR

NC STATE UNIVERSITY

INSTITUTE OF MEDICINE

OF THE NATIONAL ACADEMIES

Advising the nation • Improving health

The Role of Clinical Studies for Pets with Naturally Occurring Tumors in Translational Cancer Research

June 8-9, 2015

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

Matthew Breen PhD C.Biol. FSB

Oscar J. Fletcher Distinguished Professor of Comparative Oncology Genetics

Dept. of Molecular Biomedical Sciences, College of Vet. Medicine, NC State University

Center for Comparative Medicine and Translational Research, NCSU

Center for Human Health and the Environment, NCSU

Lineberger Comprehensive Cancer Center, UNC-Chapel Hill

BREEN LAB @ NCSU

One Medicine

"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


SPONTANEOUS
CANCERS
COLLEGE OF VETERINARY MEDICINE NORTH CAROLINA STATE UNIVERSITY
BREEN LAB @ NCSU

COLLEGE OF VETERINARY MEDICINE NORTH CAROLINA STATE UNIVERSITY

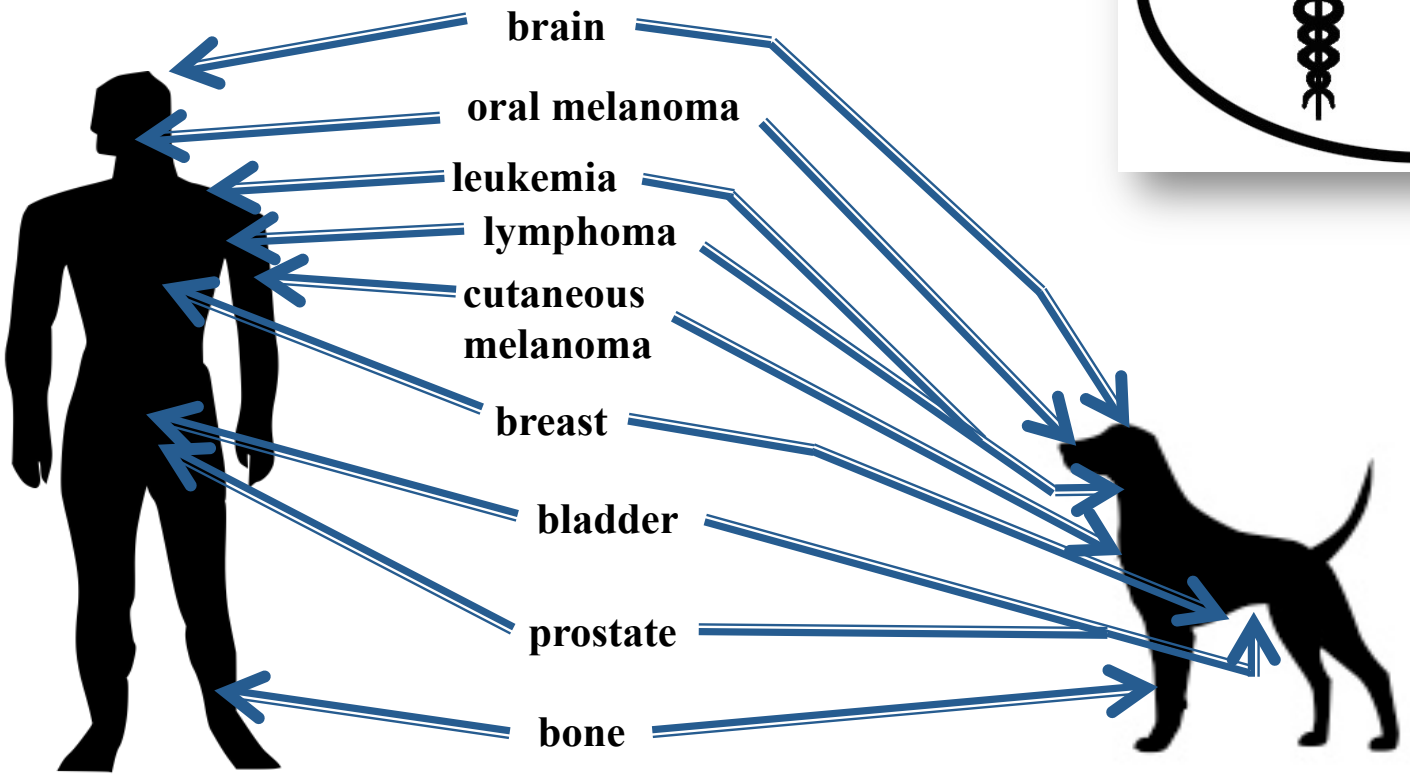
BREEN LAB @ NCSU

Canine cancer – breed predisposition

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



~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

CANINE COMPARATIVE ONCOLOGY & GENOMICS CONSORTIUM

CCOGC

ANY QUESTIONS?

HOME | ABOUT | SERVICES | PUBLICATIONS | CONTACT

PURCHASING TISSUES FROM OUR BANK

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.

FIND OUT MORE

TISSUES WE CAN COLLECT FOR YOU

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.

FIND OUT MORE

ANNOUNCEMENTS

- Canine Comparative Oncology and Genomics Consortium and the Pfizer-CCOGC Biospecimen Repository Announces the Sale of 1000 Biospecimens in First Year and Completion of Quality Control and Assessment of Repository

MAST CELL TUMORS

Mast cell tumors are the most common skin tumor in dogs. They can occur at any location and affect any age or breed of dog. However breeds such as the Boxer,...

More Info...

Learn about another cancer

SEARCH

GO

USEFUL LINKS

- Morris Animal Foundation
- AKC Canine Health Foundation
- Veterinary Cancer Society
- ACVIM

www.CCOGC.net

CCOGC

COLLEGE OF VETERINARY MEDICINE NORTH CAROLINA STATE UNIVERSITY

BREEN LAB @ NCSU

CANINE COMPARATIVE ONCOLOGY & GENOMICS CONSORTIUM

CCOGC

- 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

Colorado State University

University of Wisconsin

Michigan State University

Tufts University

UC-Davis

Ohio State University

University of Tennessee

University of Missouri

Active CCOGC collection sites

Inactive collection site

CANINE COMPARATIVE ONCOLOGY & GENOMICS CONSORTIUM

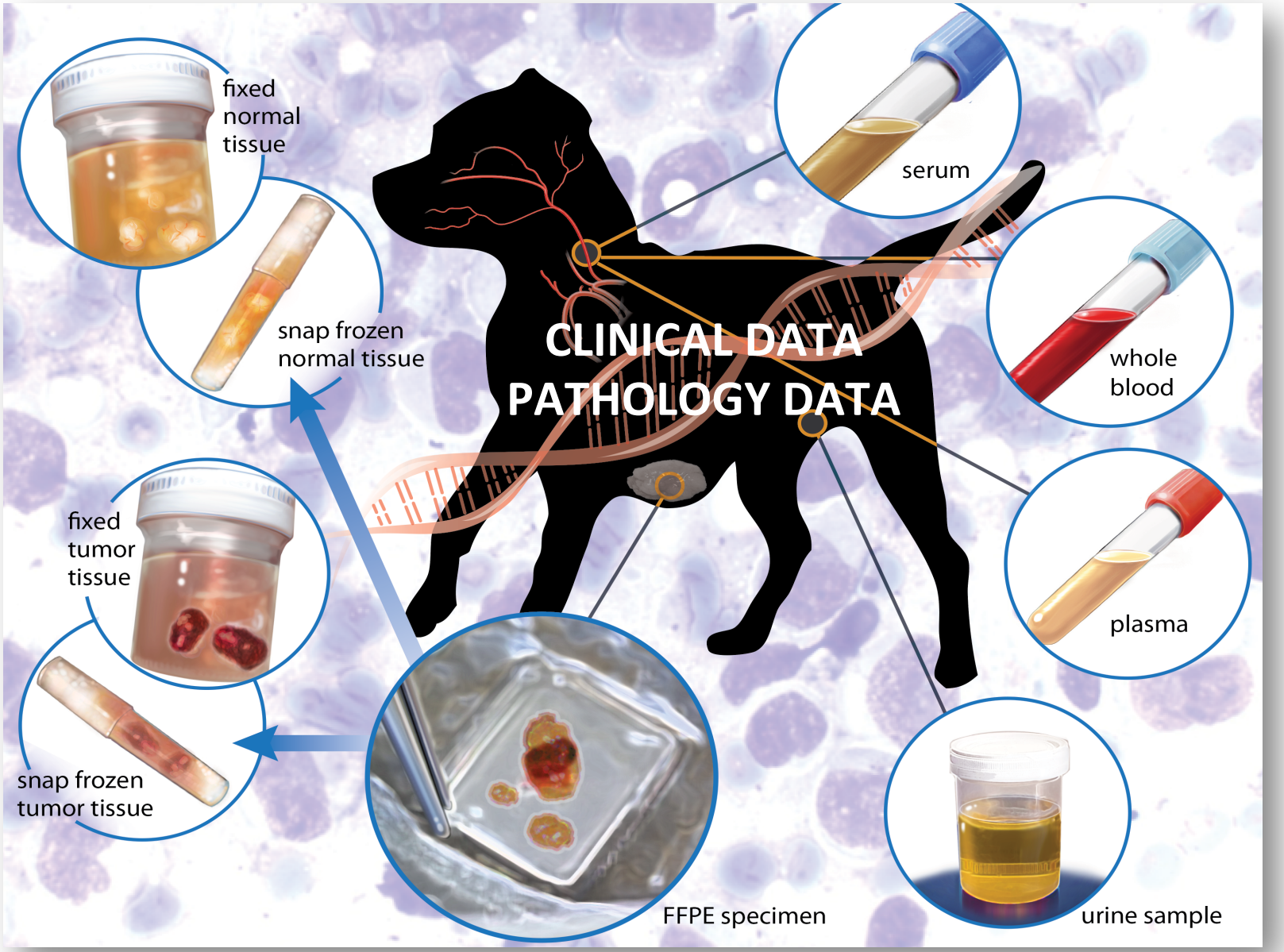
CCOGC

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



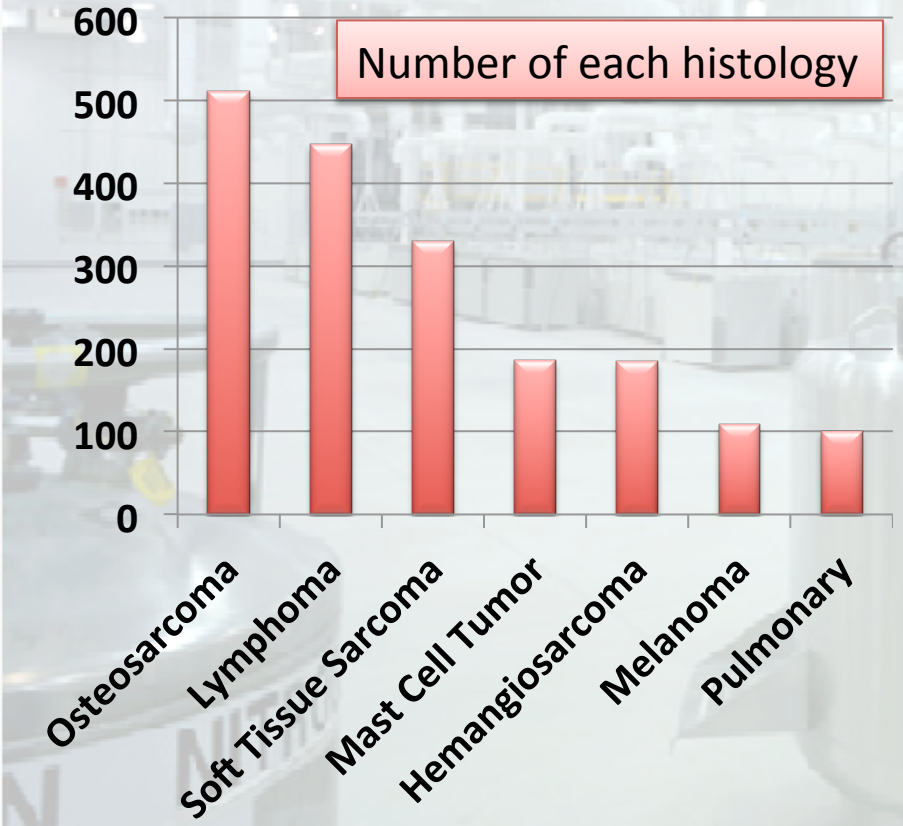
Collaboration with pet owners/parents

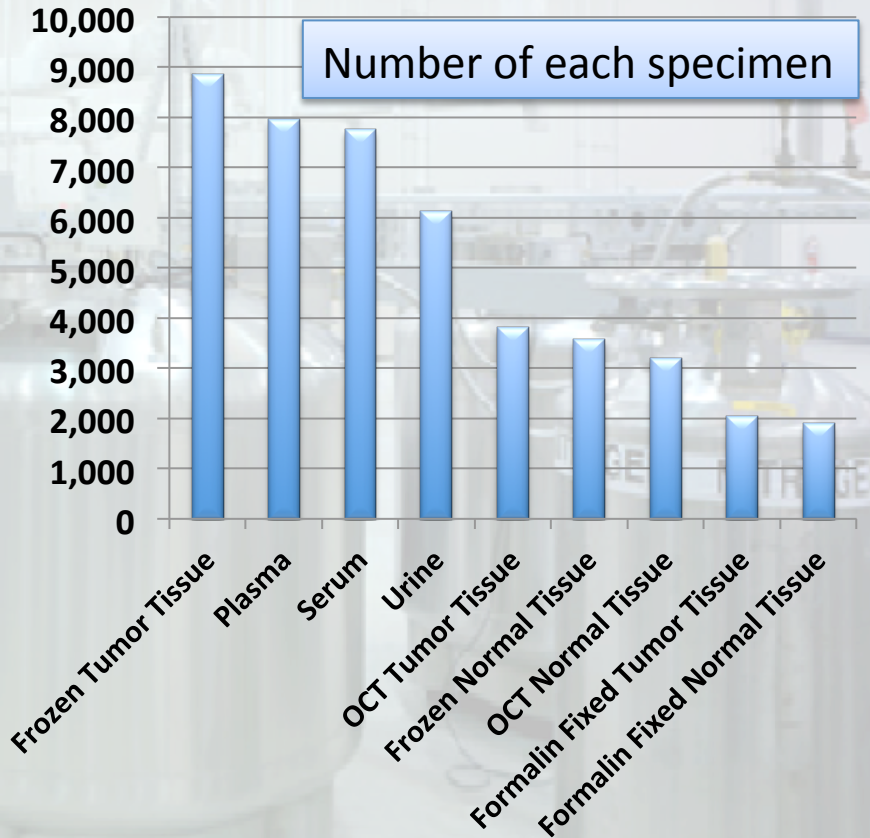
Academic-citizen based collaboration

CANINE COMPARATIVE ONCOLOGY & GENOMICS CONSORTIUM

CCOGC

- ~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

Rachael Thomas [1,2], Mark Simpson [3], Hiroyuki Mochizuki [1], Christina Williams [1], Kelsey Poorman [1], Katie Kennedy [1], Christina Mazcko [4], Jaime F. Modiano [5,6] and Matthew Breen [1,2,7]

[1] Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC; [2] Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC; [3] Laboratory of Cancer Biology and Genetics & [4] Comparative Oncology Program, Center for Cancer Research, National Cancer Institute, Bethesda, MA; [5] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, 5; [6] Masonic Cancer Center, University of Minnesota, Minneapolis, MN; [7] Cancer Genetics, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill

Presented at AACR, The Translational Impact of Model Organisms on Cancer, Nov 2013

CANINE COMPARATIVE ONCOLOGY & GENOMICS CONSORTIUM

CCOGC

Pathology review – diagnosis verification

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

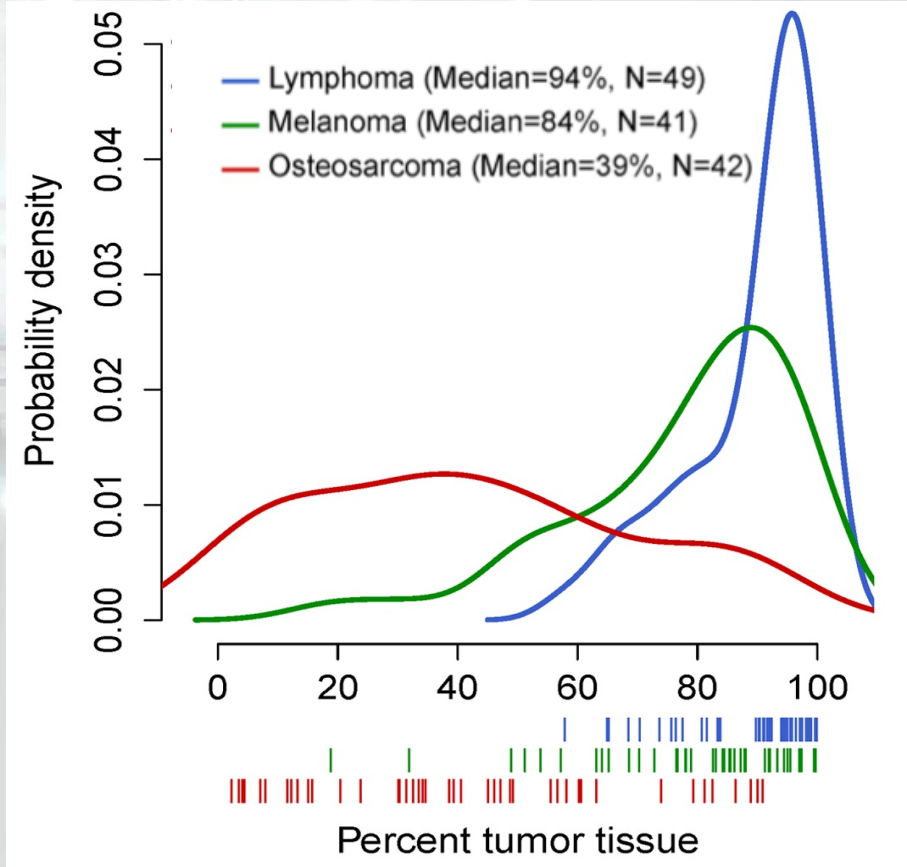
Outcome of pathology re-review of 331 cases distributed across each of the seven tumor histologies.

CANINE COMPARATIVE ONCOLOGY & GENOMICS CONSORTIUM

Pathology review –
tumor purity

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

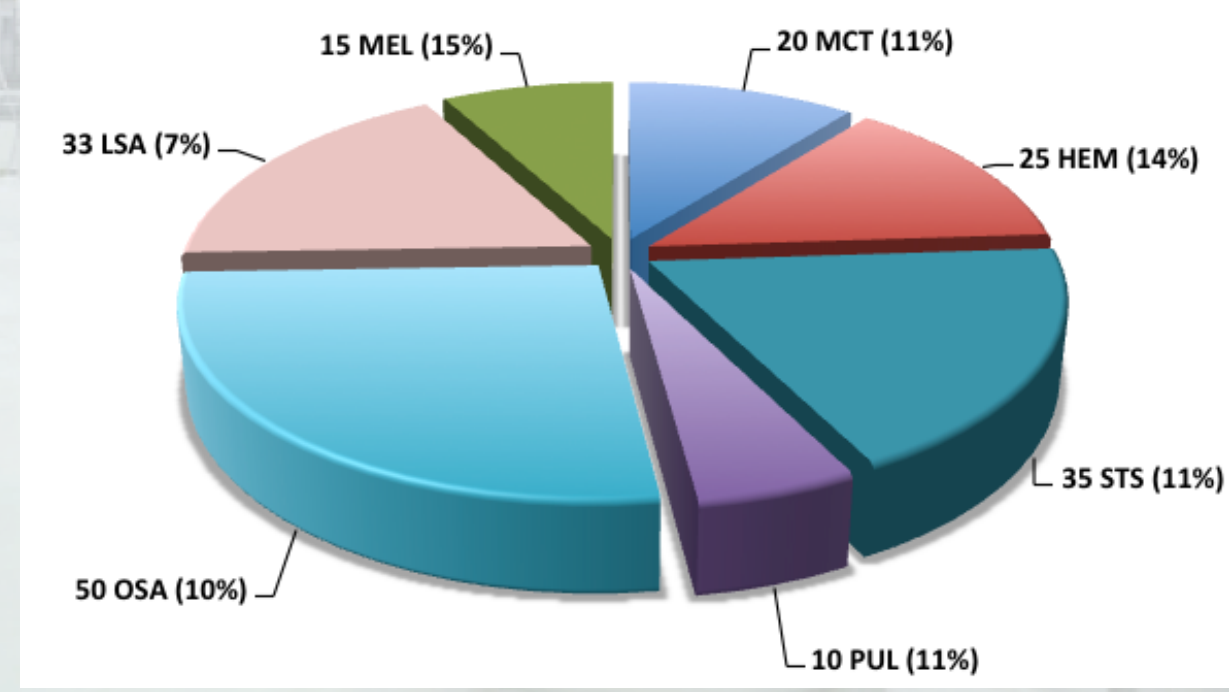
- **Lymphomas consistently exhibited a high proportion of tumor**
- **Melanomas were more variable**
- **Osteosarcoma cases showed extensive variation.**



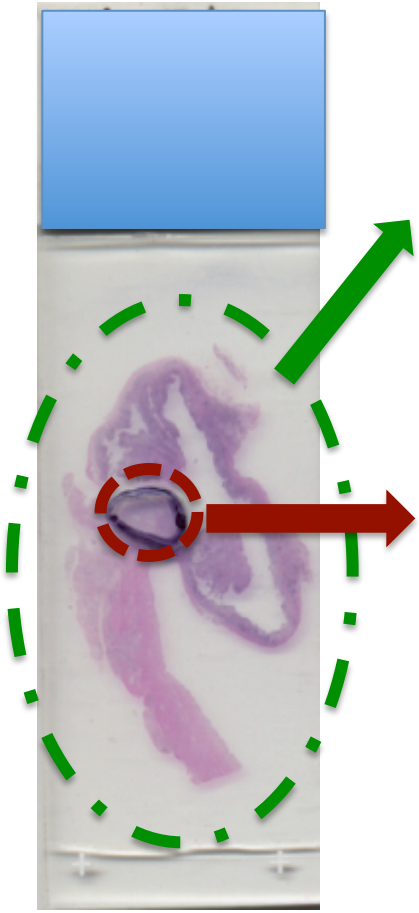
CANINE COMPARATIVE ONCOLOGY & GENOMICS CONSORTIUM

Nucleic acid QC

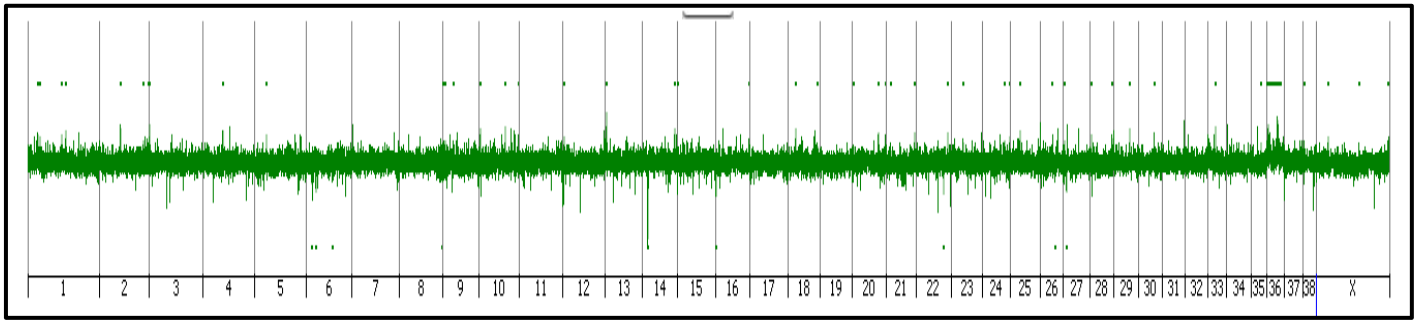
188 cases, selected at random, from the biorepository to provide proportional representation of each tumor histology and submitting institution

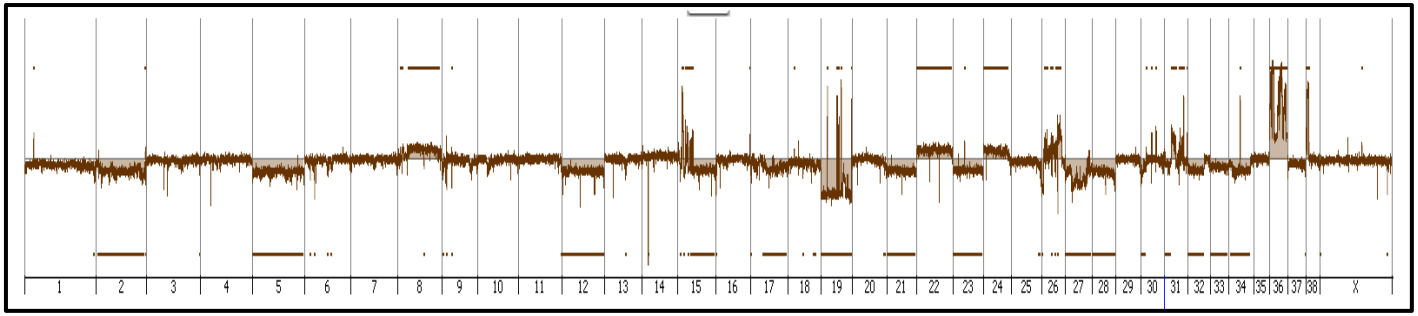


The importance of pathologic review



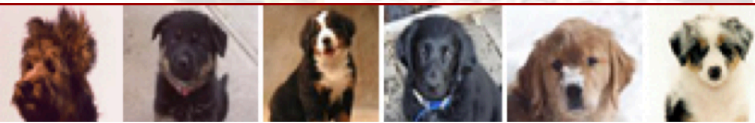
Canine bladder mass with Inflammation





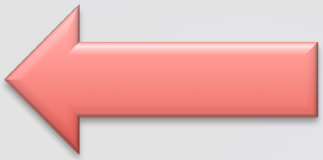
DNA source	BRAF mutation analysis	
	Mutation fraction by ddPCR	Sanger sequencing
Full size section	1.2%	negative
Macrodissected section	12.2%	negative

How are we using canine well defined cancer patient specimens to accelerate cancer research for the benefit of dogs and people?

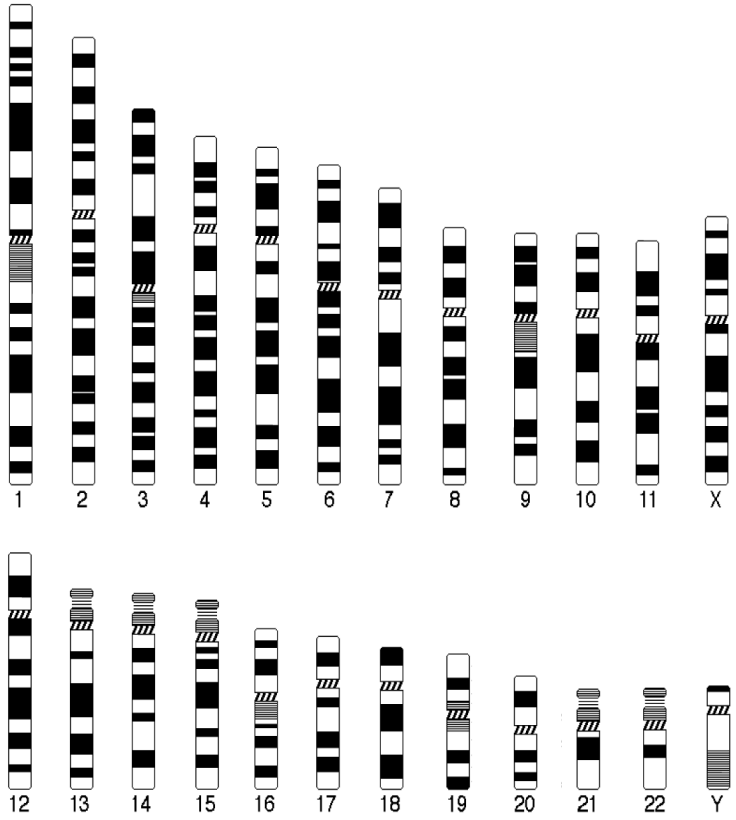


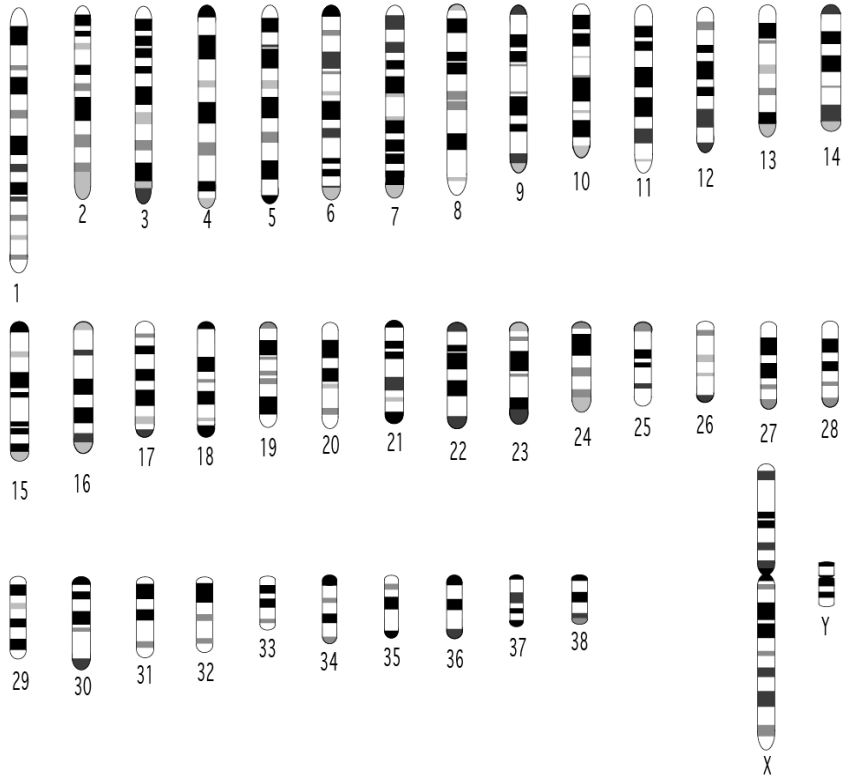
How are we approaching comparative oncology using the dog as a model?

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



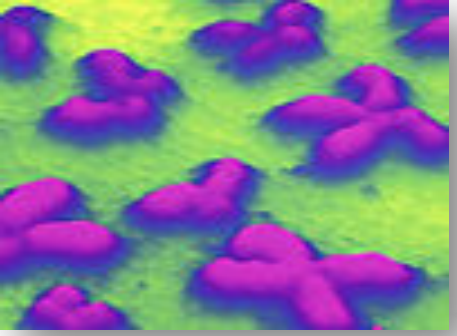


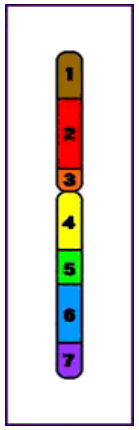
46 chromosomes

22 autosomal pairs + sex chromosomes

78 chromosomes

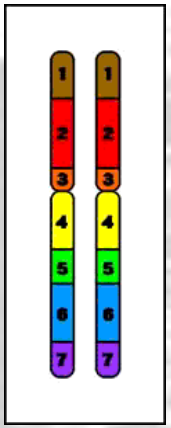
38 autosomal pairs + sex chromosomes

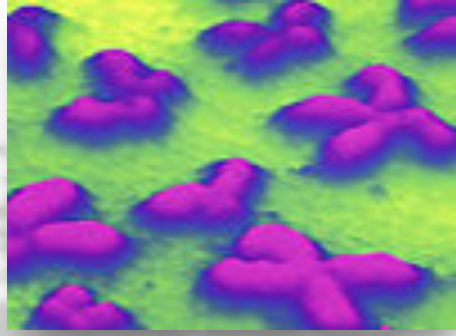


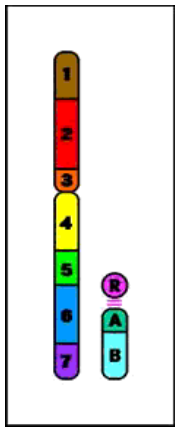


pericentric inversion

insertion/ duplication



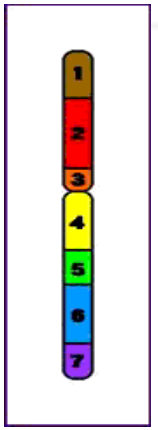


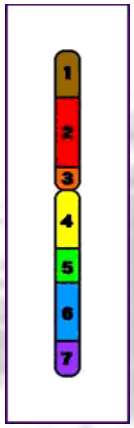


reciprocal translocation

Nature's biological filing cabinets become rearranged during cancers

interstitial deletion

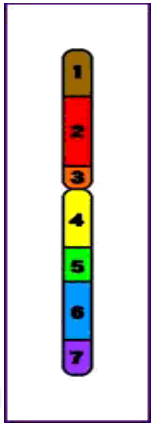




paracentric inversion

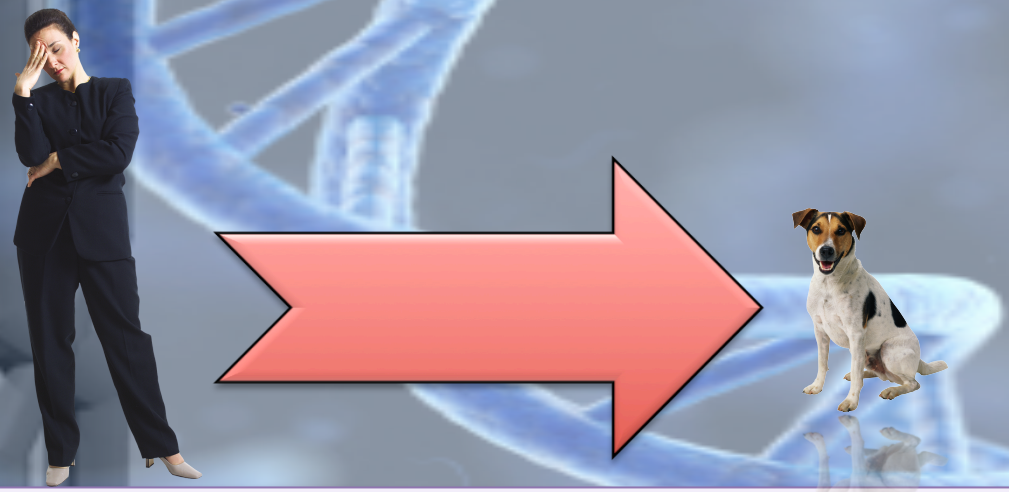
….. some genes are missing, some genes are duplicated, and in certain cases some genes move to new locations where they do not get along well with their new neighbors

deletion



Now we have the reagents to ask some key questions

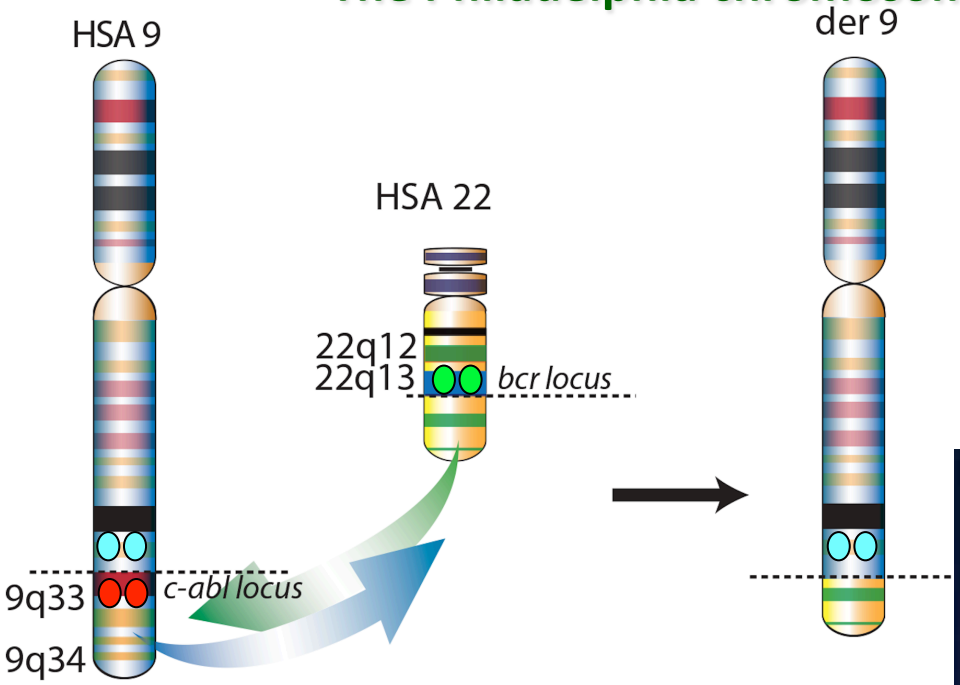
Q. Are there evolutionarily related chromosome aberrations that suggest an ancestral mechanism of pathogenetically significant events?



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)



- ABL
- BCR
- HSA 9q32 SLP

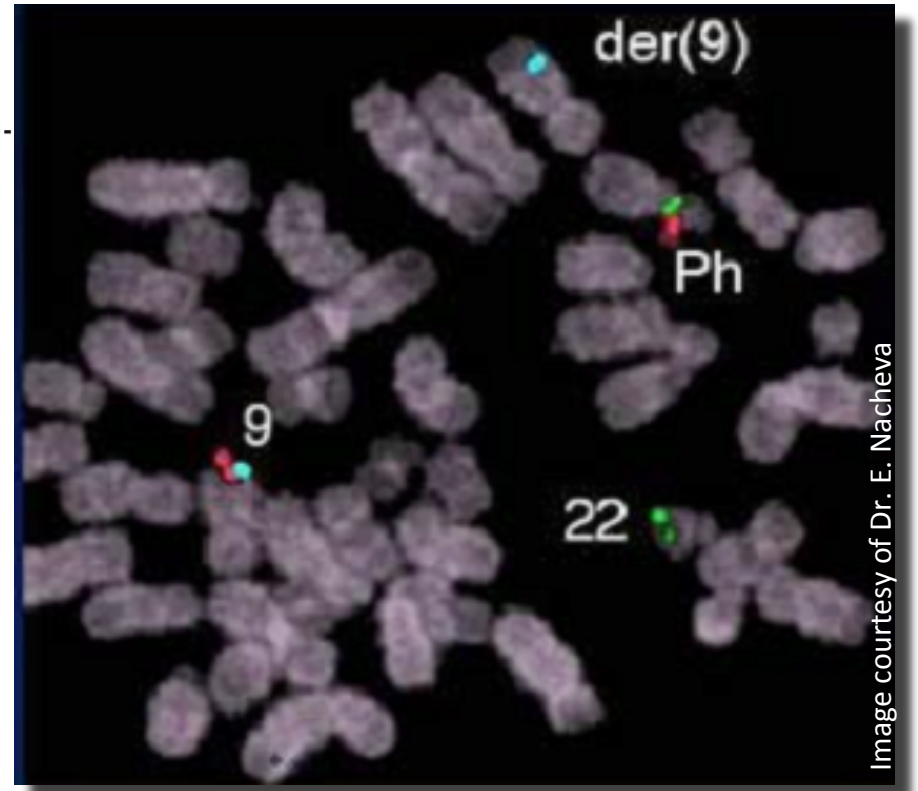
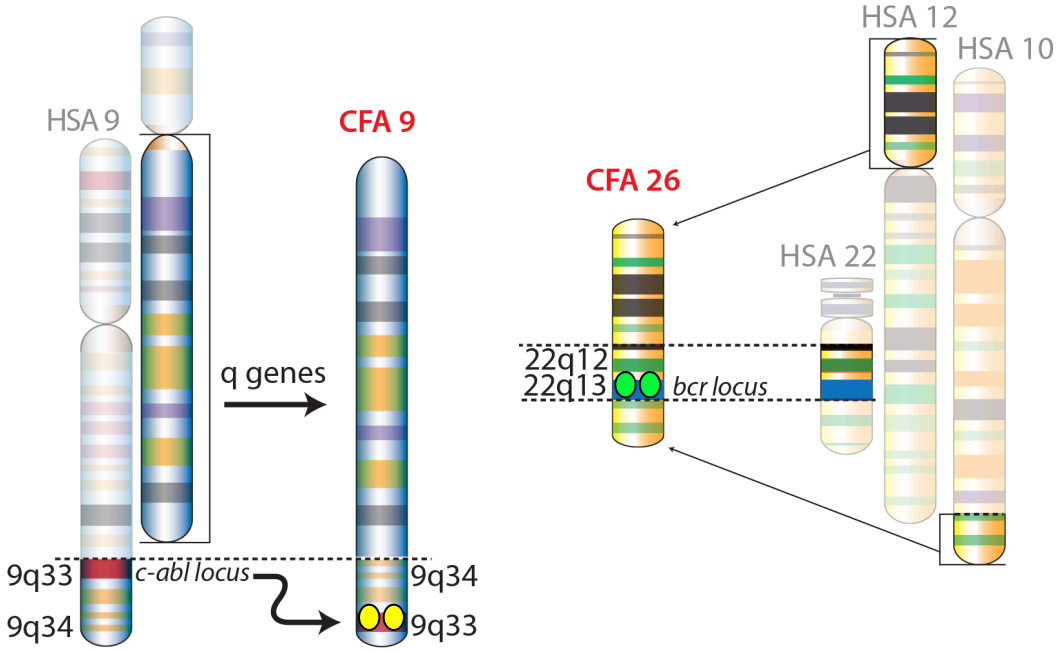
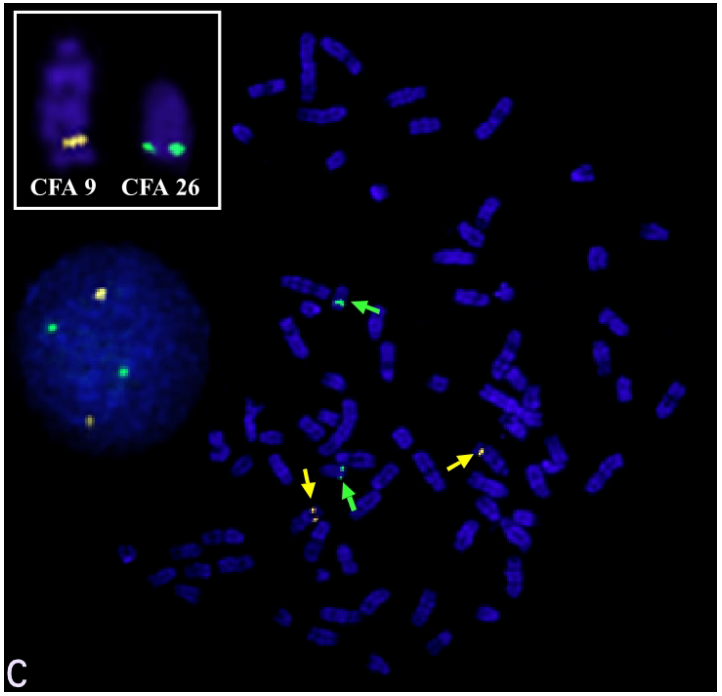


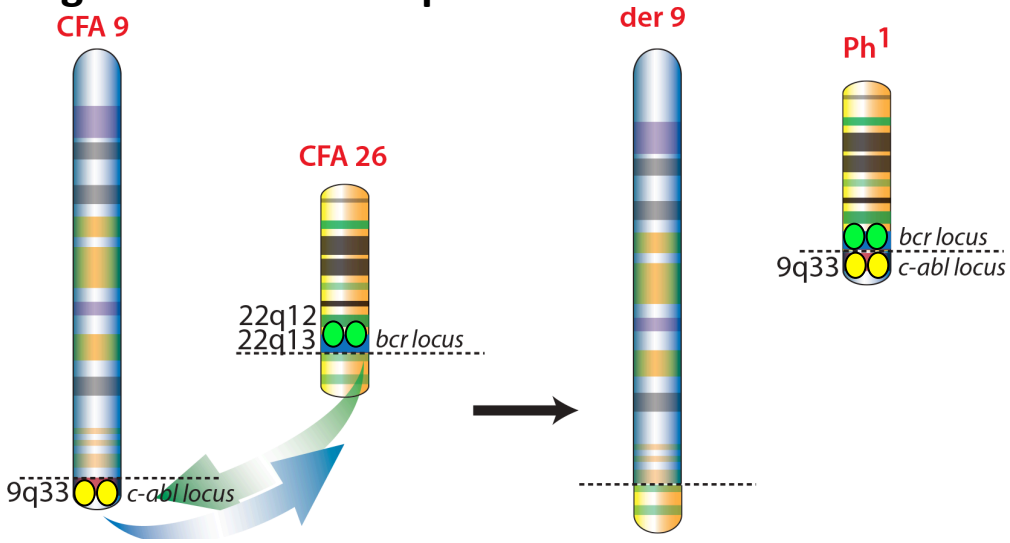
Image courtesy of Dr. E. Nacheva

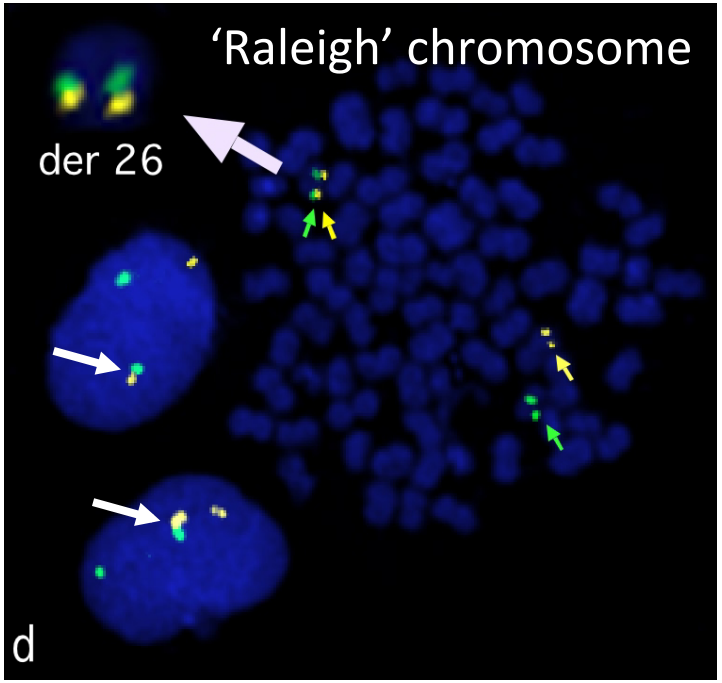
Comparative chromosome alignment





Dog chromosomes – predicted translocation





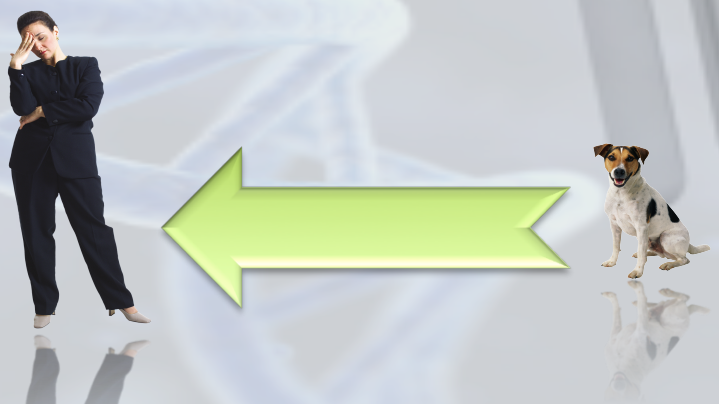
Q. Are there evolutionarily related chromosome aberrations which suggest an ancestral mechanism of pathogenetically significant events ?

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

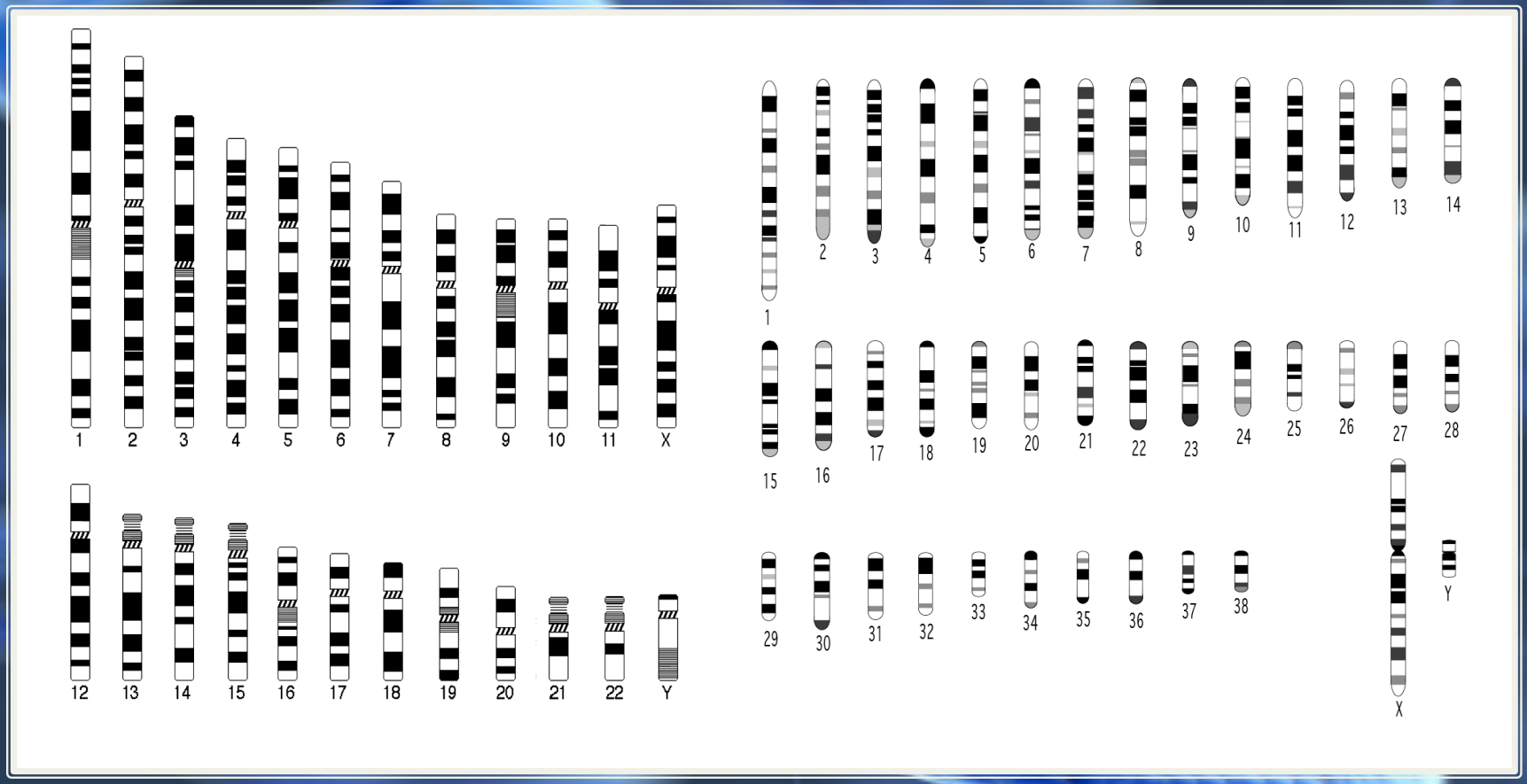
VETERINARY PERSPECTIVE = same pathogenetic mechanism = same treatments ?

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



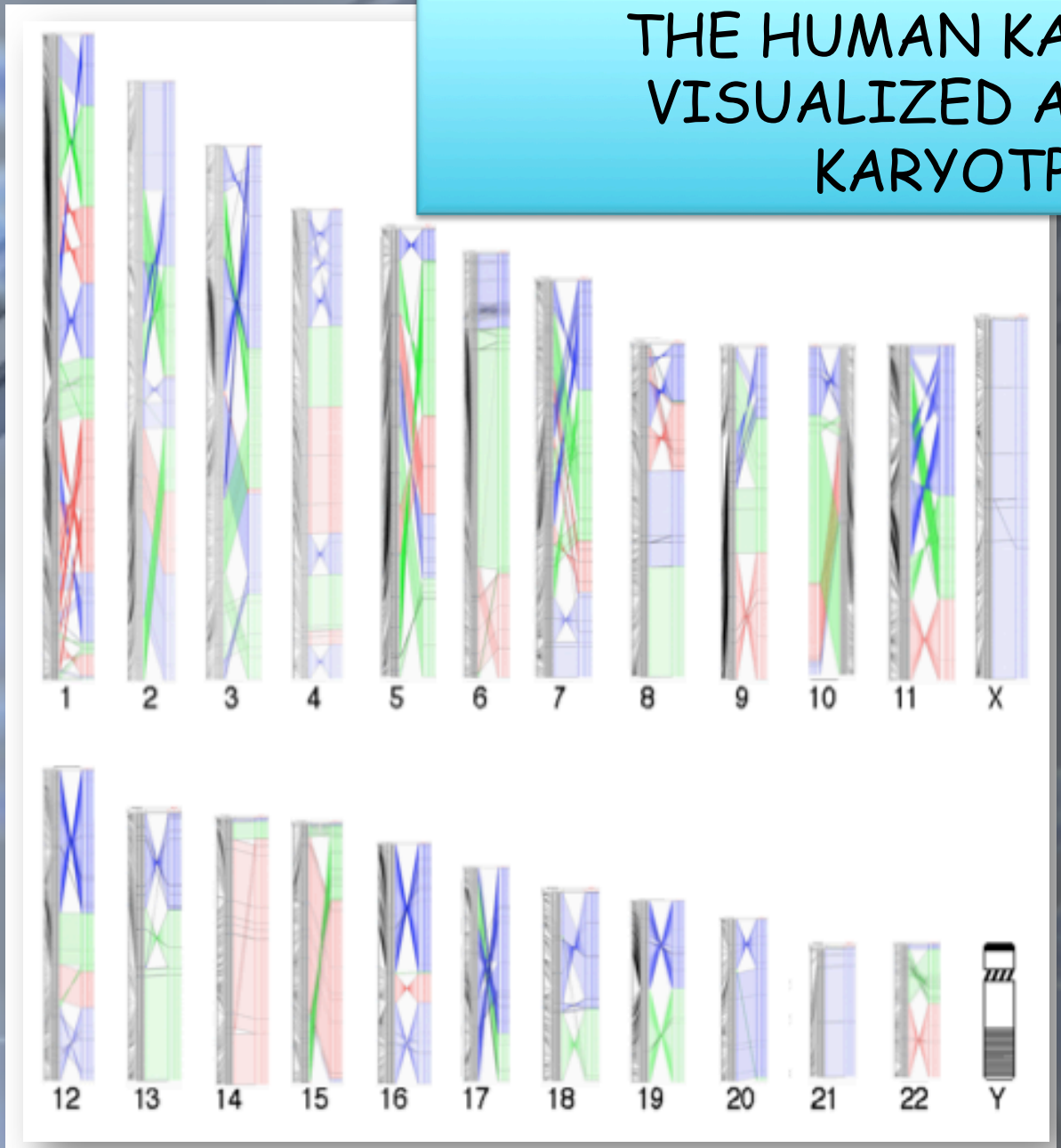
Contrasting architecture of the dog and human genomes offers tremendous potential to refine regions of significance



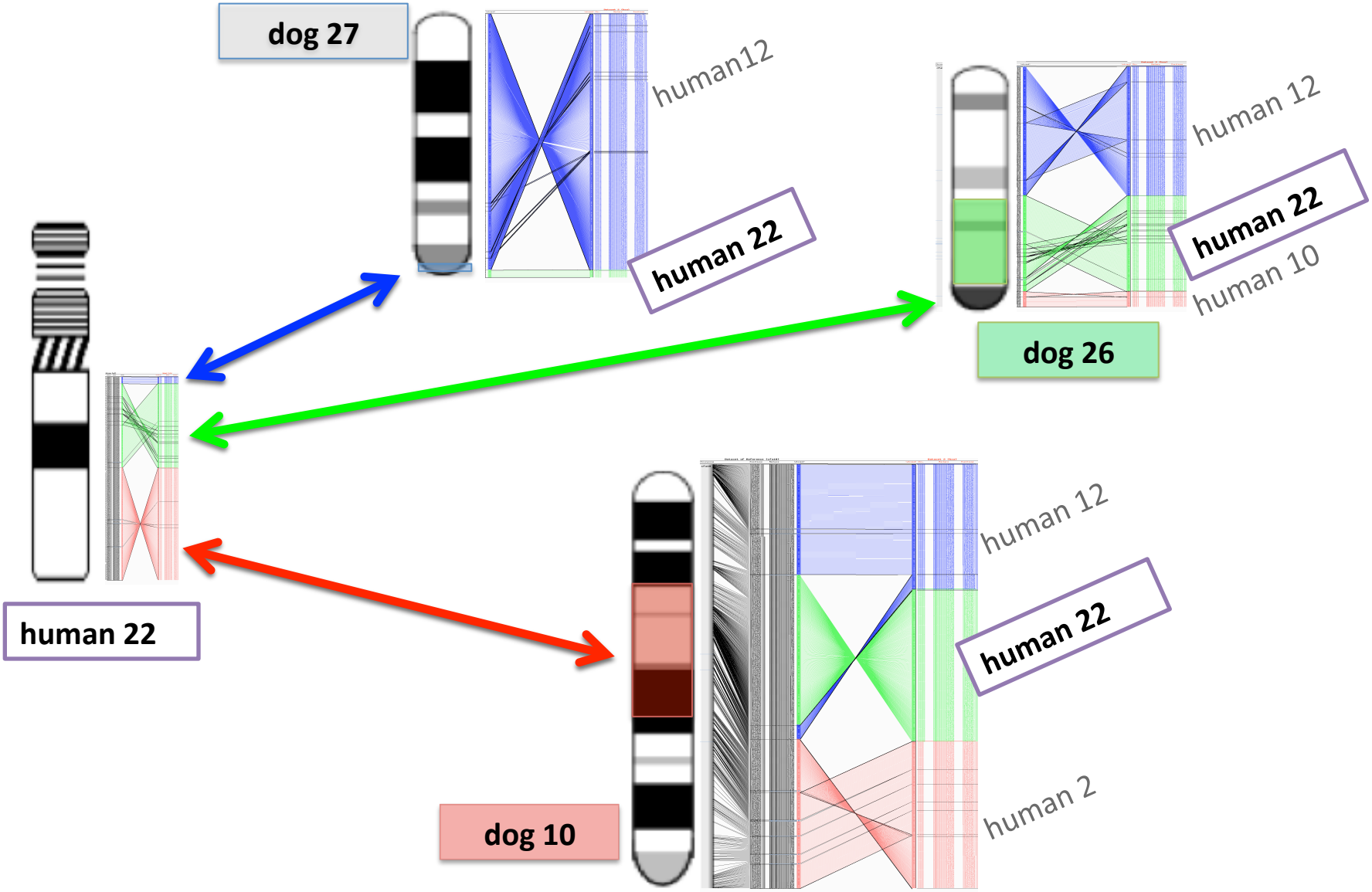
HUMAN 2n = 46
bi-armed autosomes
range from 247Mb - 47Mb

DOMESTIC DOG 2n = 78
single-armed autosomes
range from 125Mb - 26Mb

THE HUMAN KARYOTYPE VISUALIZED AS A DOG KARYOTPYE



Comparative genomic map of human 22 and the domestic dog



***'Putting our heads together'*: insights into genomic conservation between human and canine intracranial tumors**

Rachael Thomas · Shannon E. Duke · Huixia J. Wang · Tessa E. Breen · Robert J. Higgins · Keith E. Linder · Peter Ellis · Cordelia F. Langford · Peter J. Dickinson · Natasha J. Olby · Matthew Breen

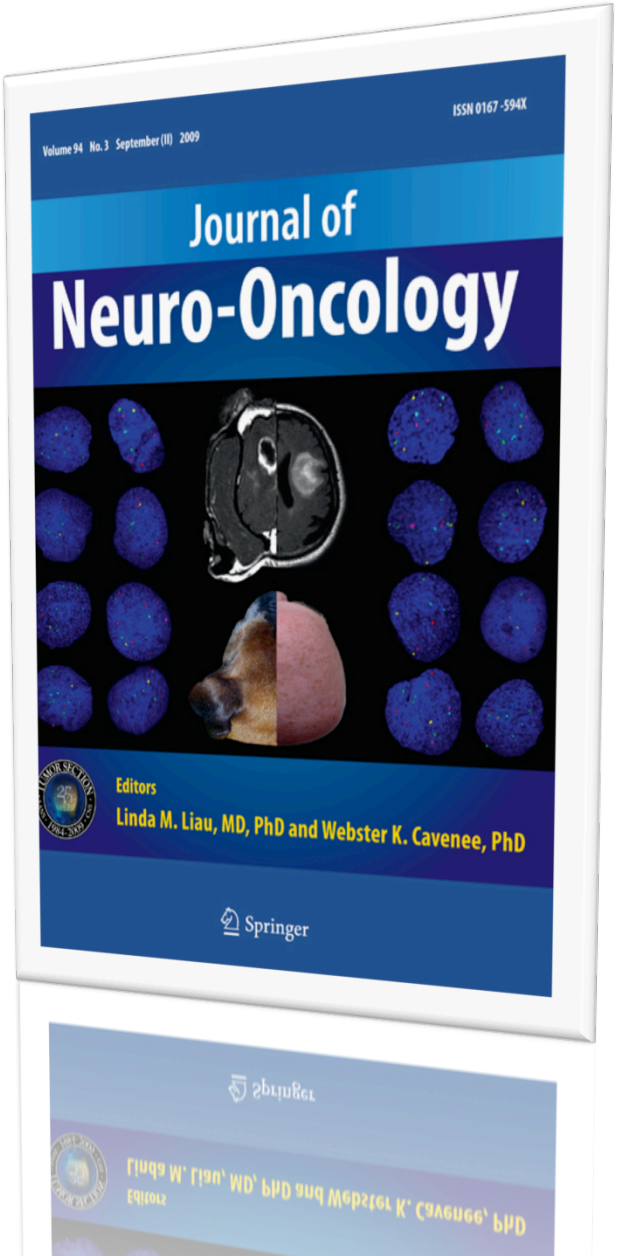
Cytogenetics of meningiomas

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

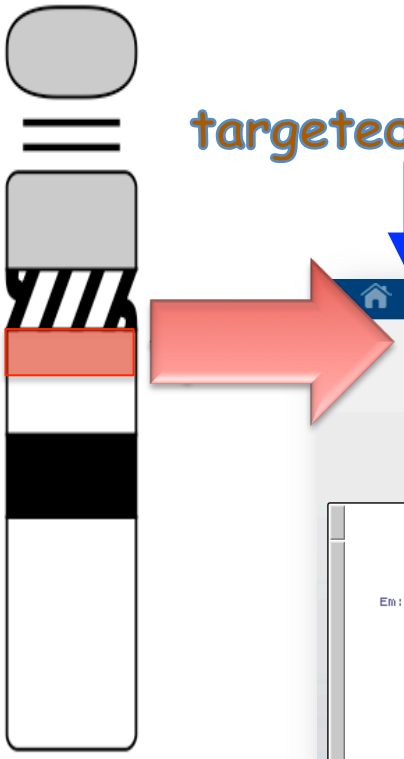
- A hallmark genomic imbalance of meningiomas is either the partial (del(22)(q12)) or **total deletion of chromosome 22**

  = 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)



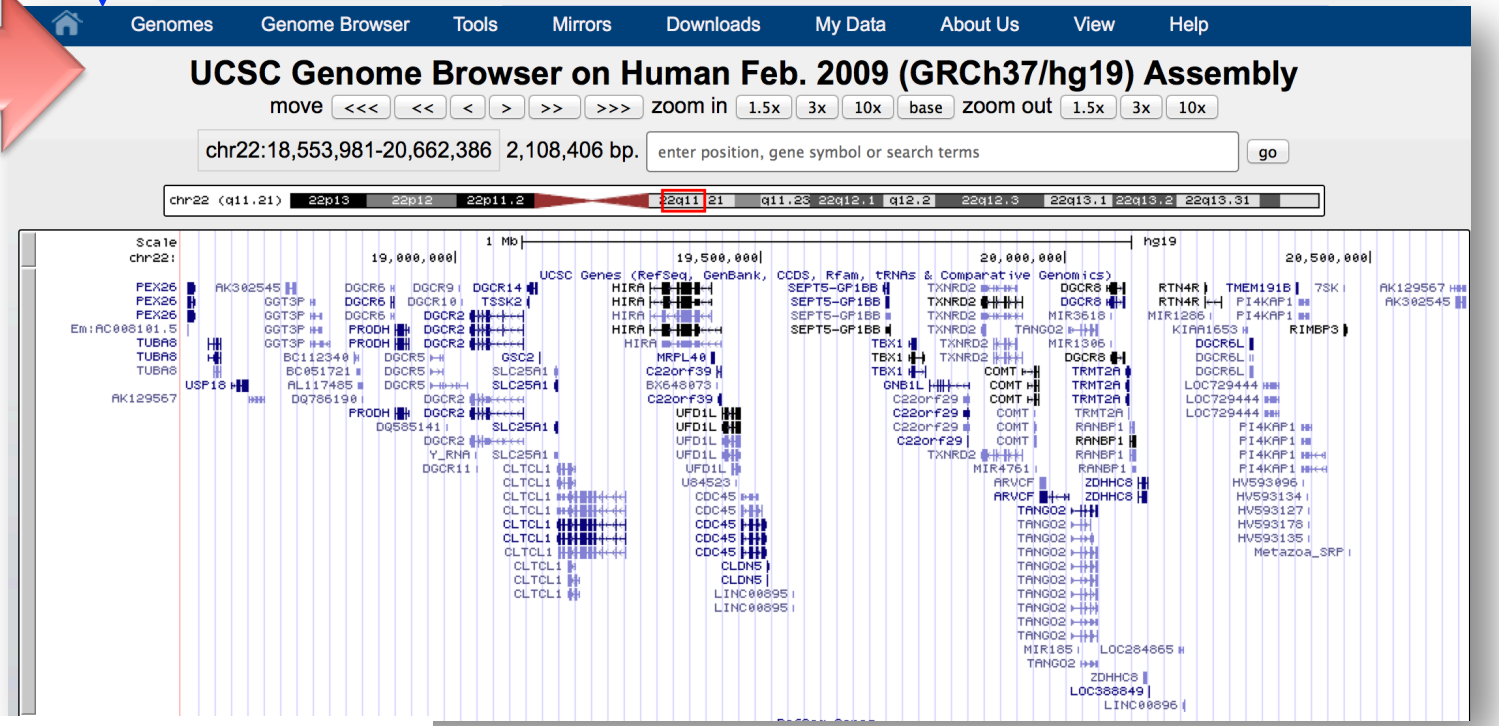
Evaluation of canine meningioma data reduces candidate region of interest ~25 fold



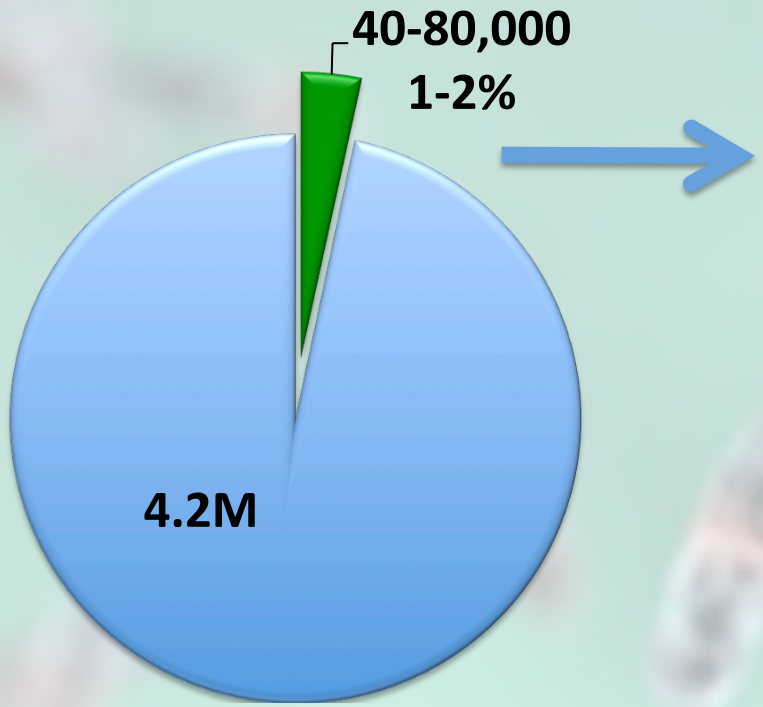
human 22
50 Mb

targeted analysis

50Mb to 2Mb - 550 genes to <10



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 ?

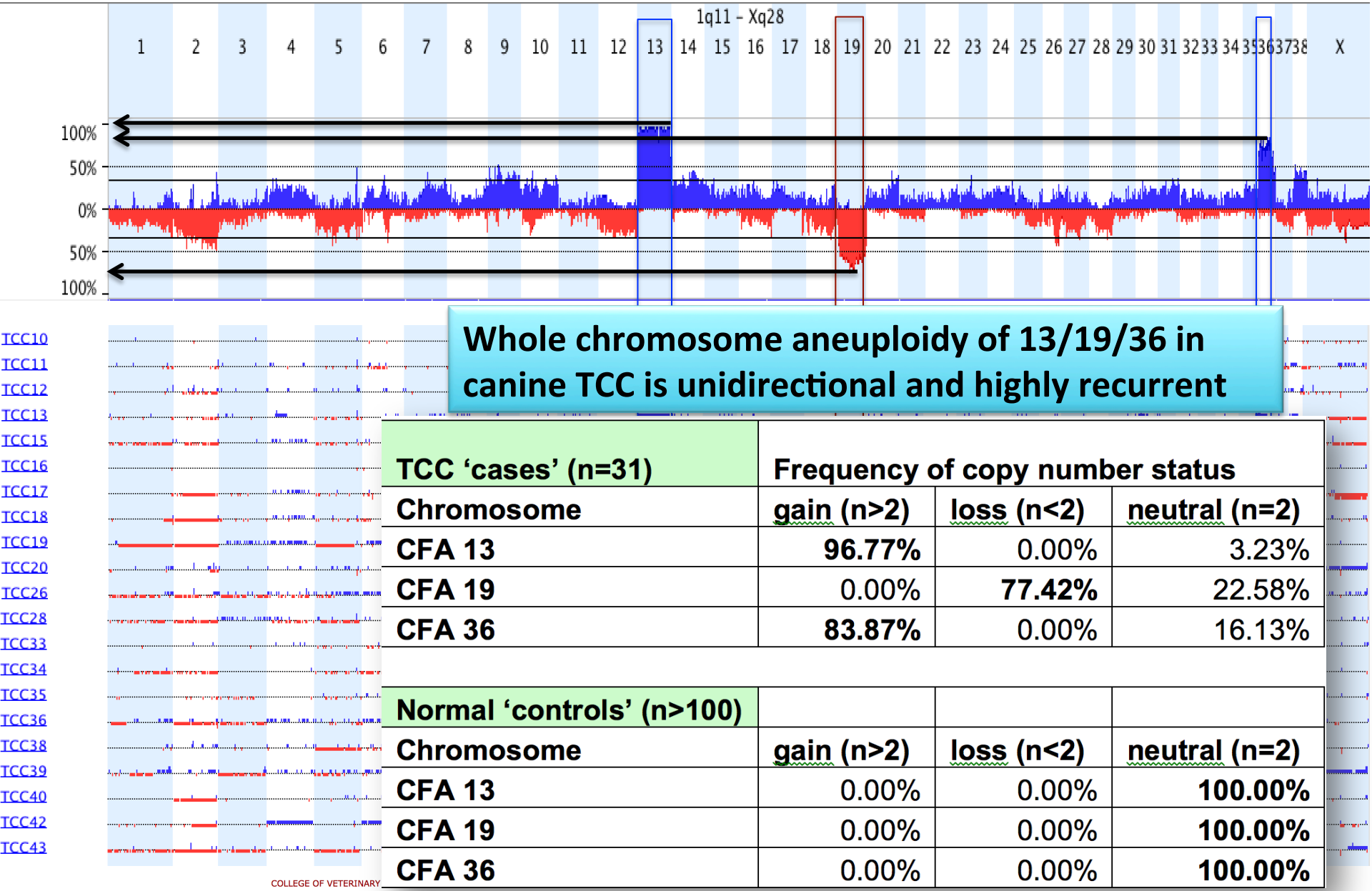
biopsy- only way to conclusively diagnose and obtained by traumatic catheterization or cystoscopy, both of which carry risk of seeding = ***reluctance to biopsy***

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

TCC 'cases' (n=31)	Frequency of copy number status		
Chromosome	**gain (n>2)**	**loss (n<2)**	**neutral (n=2)**
CFA 13	**96.77%**	0.00%	3.23%
CFA 19	0.00%	**77.42%**	22.58%
CFA 36	**83.87%**	0.00%	16.13%

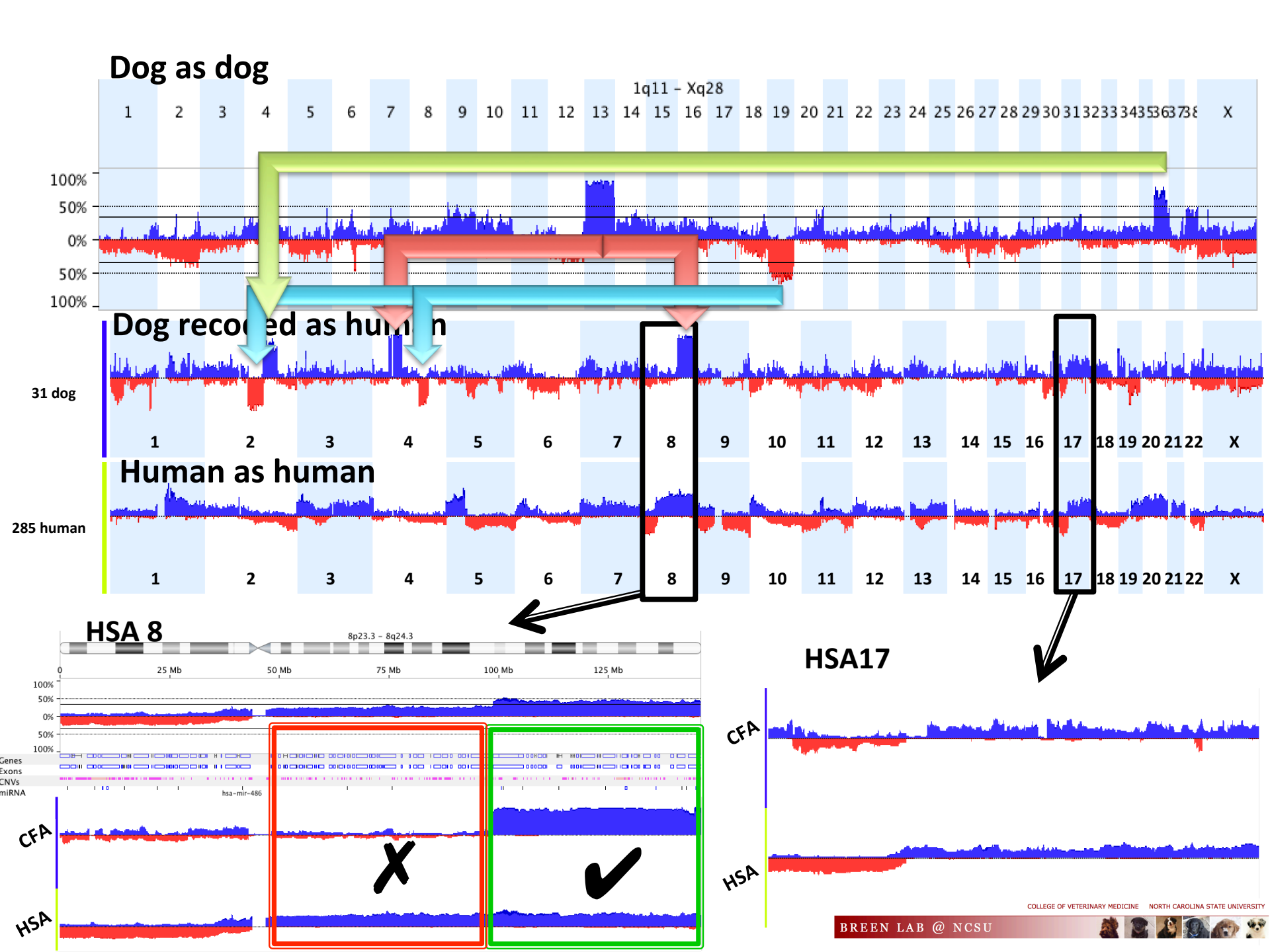
Normal 'controls' (n>100)			
Chromosome	**gain (n>2)**	**loss (n<2)**	**neutral (n=2)**
CFA 13	0.00%	0.00%	**100.00%**
CFA 19	0.00%	0.00%	**100.00%**
CFA 36	0.00%	0.00%	**100.00%**

“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

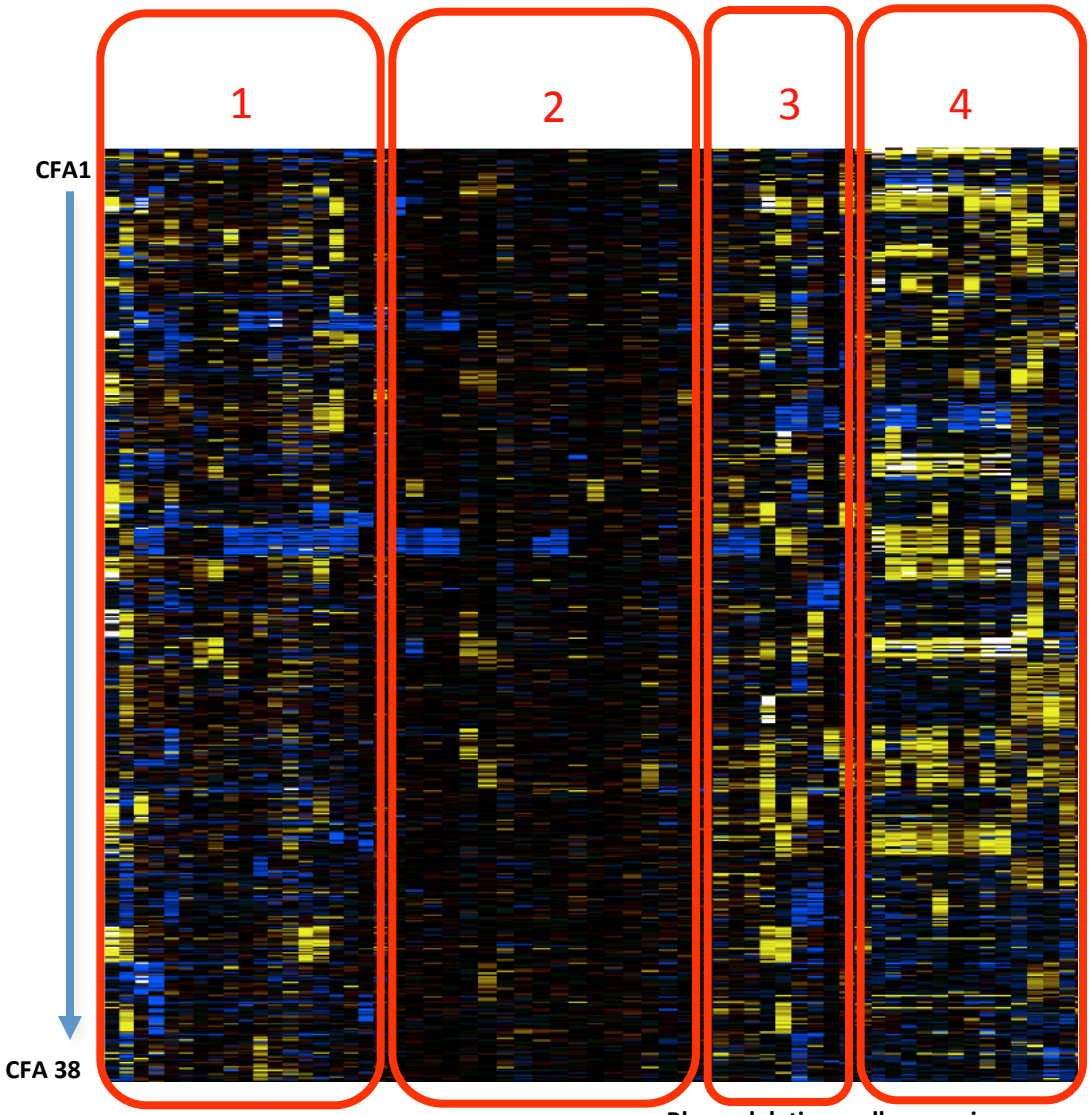
Received: 10 January 2015 / Revised: 7 February 2015 / Accepted: 10 February 2015


Dog as dog
1q11 – Xq28
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 X
100%
50%
0%
50%
100%
Dog recoded as human
31 dog
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
Human as human
285 human
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X
HSA 8
8p23.3 – 8q24.3
0 25 Mb 50 Mb 75 Mb 100 Mb 125 Mb
100%
50%
0%
50%
100%
Genes
Exons
CNVs
miRNA
hsa-mir-486
CFA
HSA
✗
✔
HSA17
CFA
HSA
COLLEGE OF VETERINARY MEDICINE NORTH CAROLINA STATE UNIVERSITY
BREEN LAB @ NCSU

Compile the data

Lymphoma	n= 350
Leukemia	n= 175
Osteosarcoma	n= 125
Intracranial	n= 100
Hemangiosarcoma	n= 110
Histiocytic malignancies	n= 130
Melanoma	n= 100
Mast cell	n= 200
Urogenital carcinoma	n= 100
	n=1,280

Cluster analysis of genome-wide DNA copy number changes in a series of canine cancer patients.



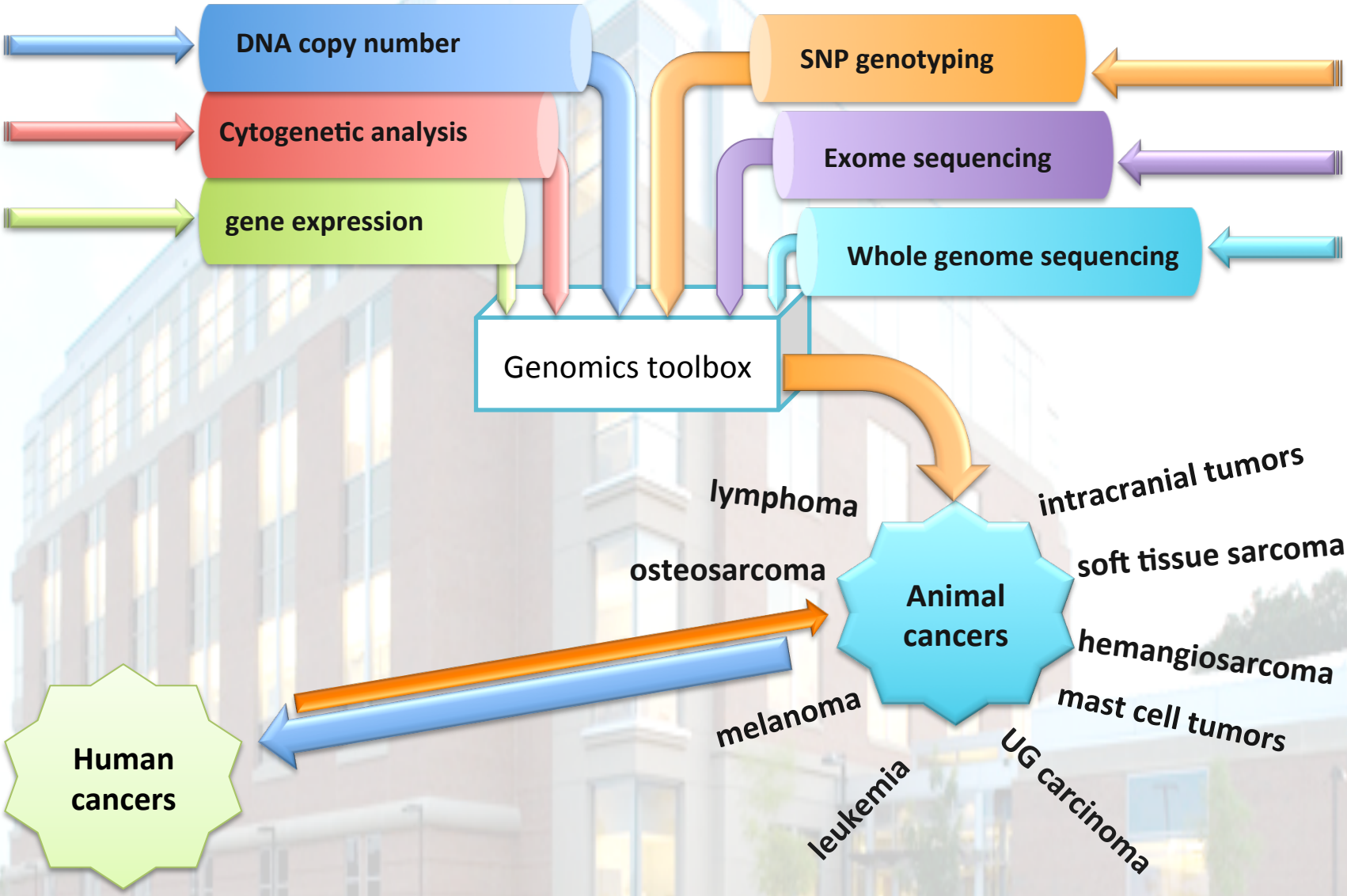
Blue = deletion, yellow = gain

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

YES

Are there corresponding cytogenetic lesions in human patients ?

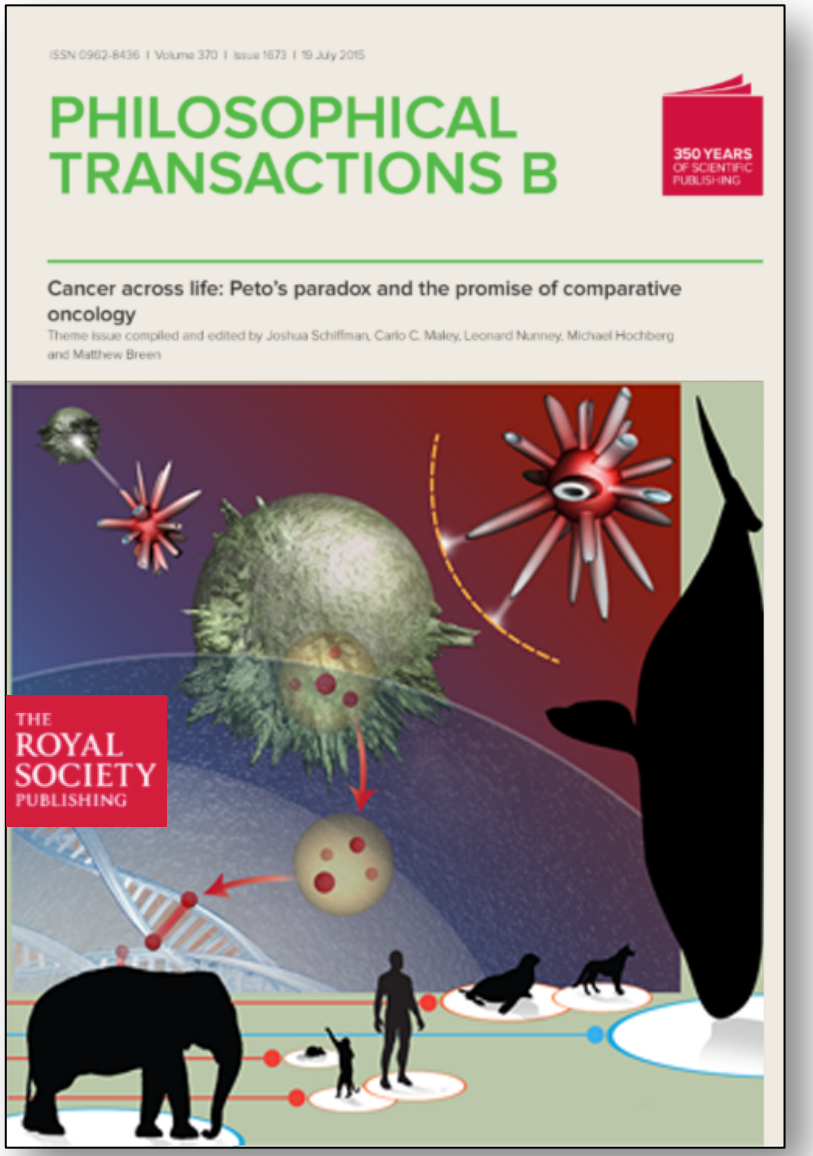
YES



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

Compiled and edited by Joshua D. Schiffman, Carlo C. Maley, Leonard Nunney, Michael Hochberg and Matthew Breen

Vol 370: 19th July 2015

On-line June 8th 2015

Acknowledgements

NCSU

Shannon Becker- Graduate student
Katie Kennedy– Graduate student
Hiro Mochizuki – Post Doc
Lauren Poe – Research Assistant
Sharanya Raghunath – Post Doc
Sarah Roode – DVM/PhD student
Daniel Rotroff – Post Doc
Susan Shapiro – DVM/PhD student
Rachael Thomas- Research Assistant Professor
Christina Williams - Research Associate

Luke Borst – Veterinary Pathologist
John Cullen – Veterinary Pathologist
Keith Linder – Veterinary Pathologist
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)

Kerstin Lindblad-Toh et al – Broad Institute
Jaime Modiano et al - U. Minnesota, USA
Josh Schiffman et al Huntsman CI, USA
Anne Avery, Sue Lana et al – ACC, CSU, USA
Bill Kisseberth, Guillermo Couto– Ohio State U, USA
Pete Dickensen, UC Davis, USA
Ted Valli - VDx Veterinary Diagnostics CA, USA
Jim Cerhan, Susan Slager et al - Mayo Clinic, USA
Debbie Knapp, Purdue University, USA
Chand Khanna, Stephen Hewitt – NCI
Elaine Ostrander et al - NIH
Frances Gulland, Marine Mammal Center, CA
Sea Lion Cancer Consortium
Mona Rosenberg – Veterinary Cancer Group, CA
Scott Moroff/Phil Bergman– VCA/Antech Diagnostics
Jimmy Jin, Michael Ruvolo, Anne Lucas, Agilent Technologies

Acknowledgements

NCSU

Shannon Becker- Graduate student
Katie Kennedy- Graduate student
Hiro Mochizuki – Post Doc
Lauren Poe – Research Assist
Sharanya D
S
D
Su
Ro
Ch

Luk
Joh
Keit
Nat 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)

Kerstin Lindblad-Toh et al
Jaime
USA
Stephen Hewitt – NCI
Elaine Ostrander et al - NIH
Frances Gulland, Marine Mammal Center, CA
Sea Lion Cancer Consortium
Mona Rosenberg – Veterinary Cancer Group, CA
Scott Moroff/Phil Bergman– VCA/Antech Diagnostics
Jimmy Jin, Michael Ruvolo, Anne Lucas, Agilent Technologies

…. and the thousands of dog parents and their veterinarians who have submitted specimens to help this research.

Thank you

Matthew_Breen@ncsu.edu