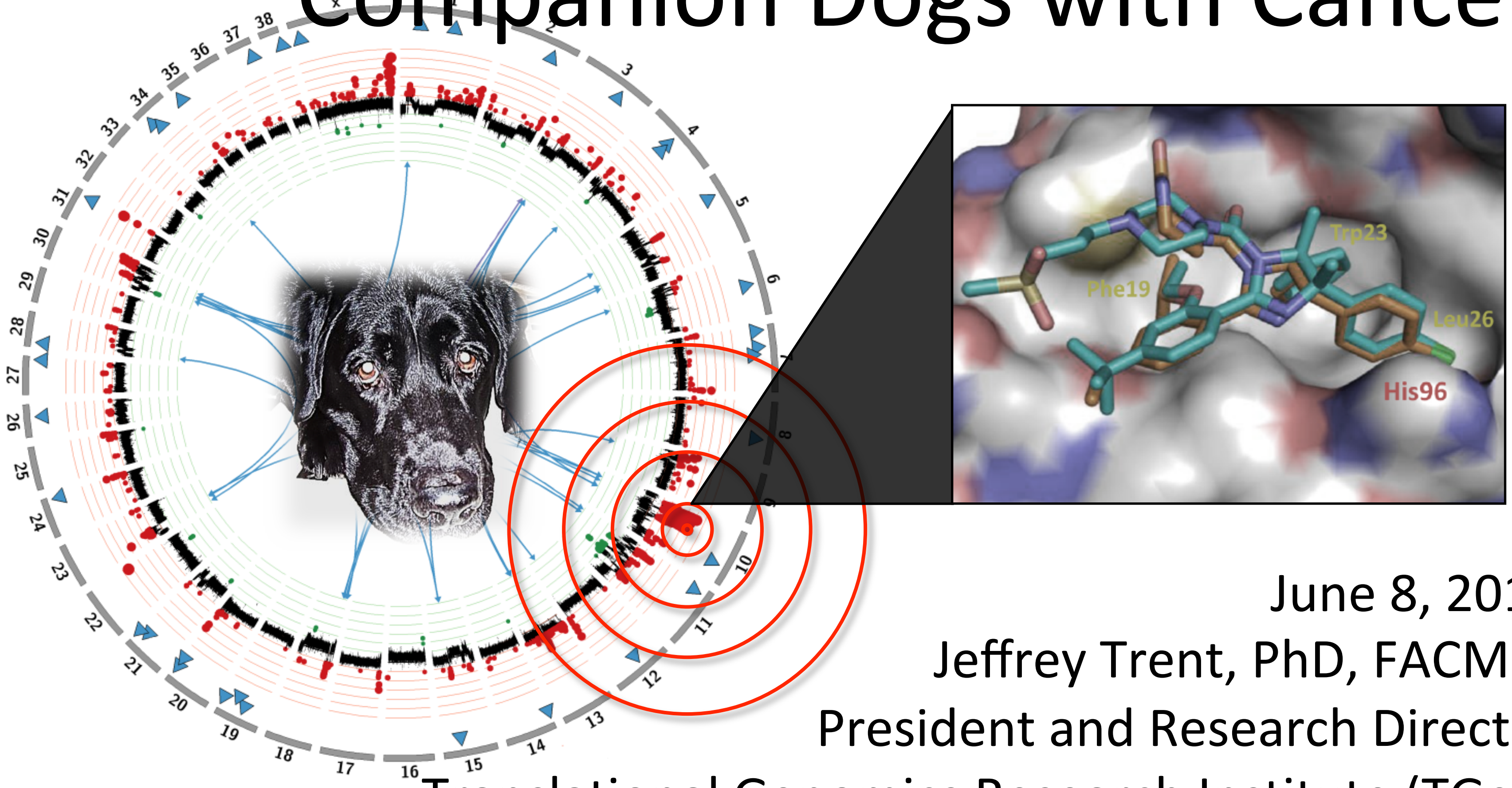
Enabling Precision Medicine for Companion Dogs with Cancer



June 8, 2015

Jeffrey Trent, PhD, FACMG.

President and Research Director

Translational Genomics Research Institute (TGen)

a non-profit biomedical research Institute

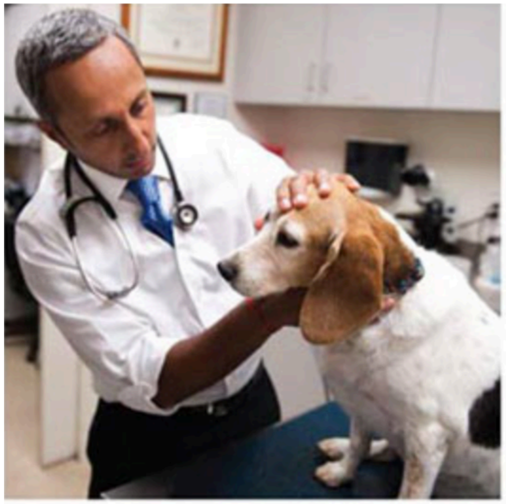
Deploying Investigational Agents, Biomarkers and Genomics within Canine Clinical Trials: From IND to Biology

Pat LoRusso,
D.O.

Yale

Will Hendricks,
Ph.D.

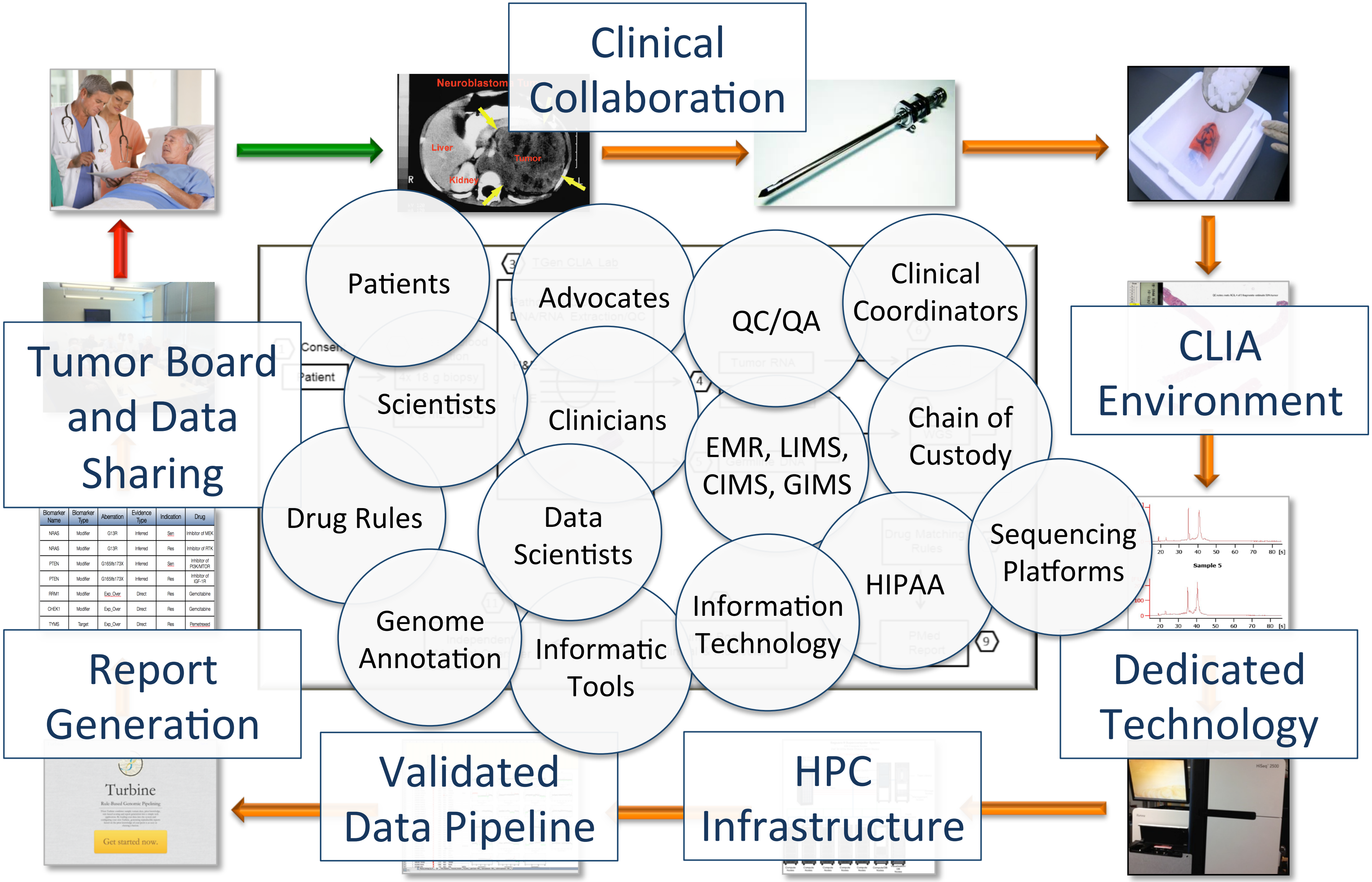
tgen



Chand Khanna
D.V.M.Ph.D

NATIONAL CANCER INSTITUTE

- Precision Medicine in Human Cancer
- Canine Cancer Genomic Landscapes
- Emerging Canine PMed Tools
- Opportunities in Comparative Oncology



Clinical Collaboration

CLIA Environment

Dedicated Technology

HPC Infrastructure

Validated Data Pipeline

Report Generation

Tumor Board and Data Sharing

Biomarker Name	Biomarker Type	Aberration	Evidence Type	Indication	Drug
NRAS	Modifier	G13R	Inferred	Sen	Inhibitor of MEK
NRAS	Modifier	G13R	Inferred	Res	Inhibitor of RTK
PTEN	Modifier	G165fs173X	Inferred	Sen	Inhibitor of PI3K/MTOR
PTEN	Modifier	G165fs173X	Inferred	Res	Inhibitor of IGF-1R
RRM1	Modifier	Exp_Over	Direct	Res	Gemcitabine
CHEK1	Modifier	Exp_Over	Direct	Res	Gemcitabine
TYMS	Target	Exp_Over	Direct	Res	Pemetrexed

Patients

Advocates

QC/QA

Clinical Coordinators

Scientists

Clinicians

EMR, LIMS, CIMS, GIMS

Chain of Custody

Drug Rules

Data Scientists

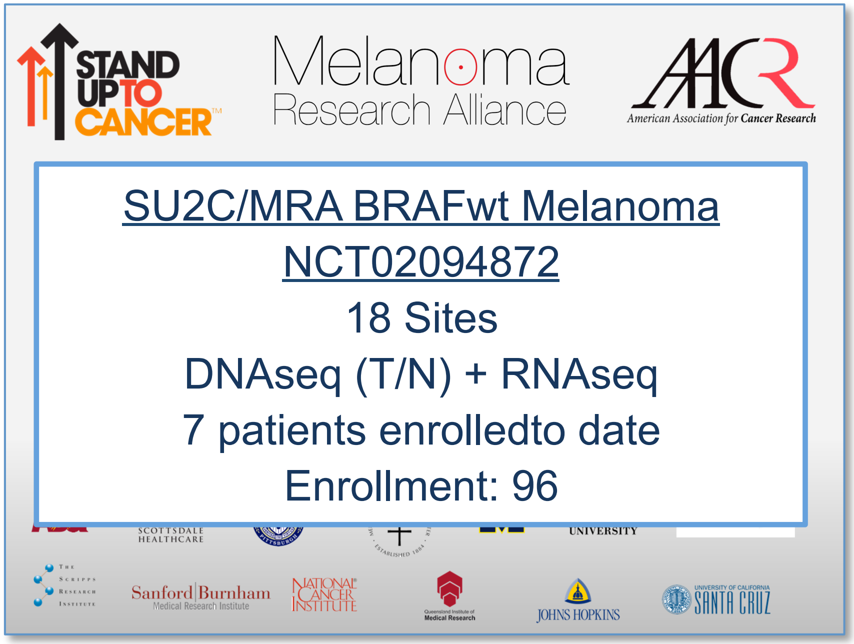
HIPAA

Sequencing Platforms

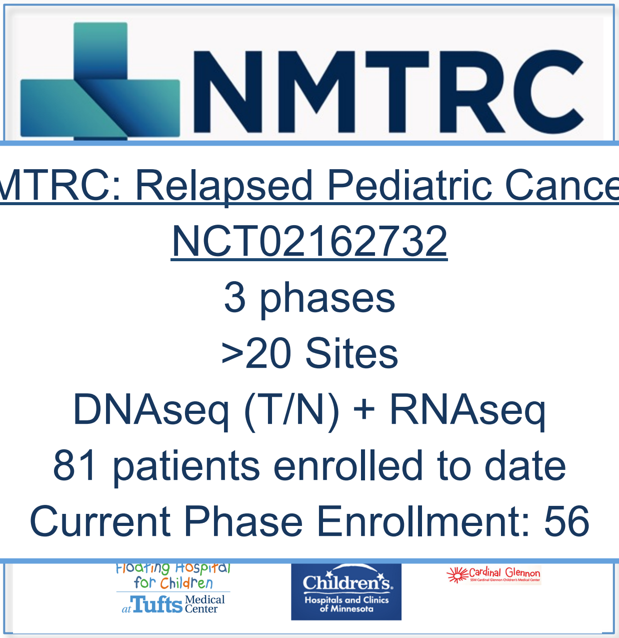
Genome Annotation

Informatic Tools

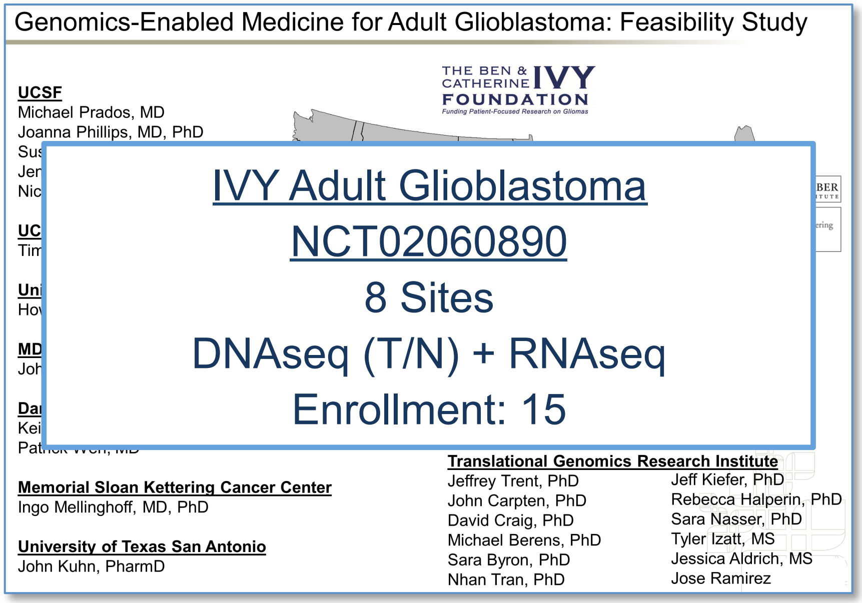
Information Technology



SU2C/MRA BRAFwt Melanoma
NCT02094872
18 Sites
DNAseq (T/N) + RNAseq
7 patients enrolledto date
Enrollment: 96



NMTRC: Relapsed Pediatric Cancers
NCT02162732
3 phases
>20 Sites
DNAseq (T/N) + RNAseq
81 patients enrolled to date
Current Phase Enrollment: 56



Genomics-Enabled Medicine for Adult Glioblastoma: Feasibility Study

UCSF
Michael Prados, MD
Joanna Phillips, MD, PhD
Sus
Jen
Nic

UC
Tim

Uni
Hov

MD
Joh

Da
Kei
Pat

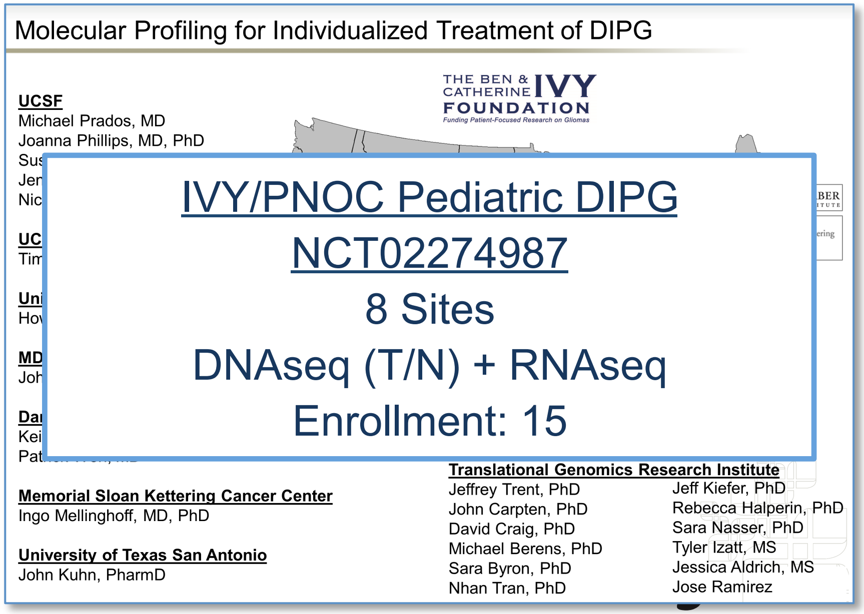
Memorial Sloan Kettering Cancer Center
Ingo Mellinghoff, MD, PhD

University of Texas San Antonio
John Kuhn, PharmD

Translational Genomics Research Institute

Jeffrey Trent, PhD	Jeff Kiefer, PhD
John Carpten, PhD	Rebecca Halperin, PhD
David Craig, PhD	Sara Nasser, PhD
Michael Berens, PhD	Tyler Izatt, MS
Sara Byron, PhD	Jessica Aldrich, MS
Nhan Tran, PhD	Jose Ramirez

IVY Adult Glioblastoma
NCT02060890
8 Sites
DNAseq (T/N) + RNAseq
Enrollment: 15



Molecular Profiling for Individualized Treatment of DIPG

UCSF
Michael Prados, MD
Joanna Phillips, MD, PhD
Sus
Jen
Nic

UC
Tim

Uni
Hov

MD
Joh

Da
Kei
Pat

Memorial Sloan Kettering Cancer Center
Ingo Mellinghoff, MD, PhD

University of Texas San Antonio
John Kuhn, PharmD

Translational Genomics Research Institute

Jeffrey Trent, PhD	Jeff Kiefer, PhD
John Carpten, PhD	Rebecca Halperin, PhD
David Craig, PhD	Sara Nasser, PhD
Michael Berens, PhD	Tyler Izatt, MS
Sara Byron, PhD	Jessica Aldrich, MS
Nhan Tran, PhD	Jose Ramirez

IVY/PNOC Pediatric DIPG
NCT02274987
8 Sites
DNAseq (T/N) + RNAseq
Enrollment: 15

Integrating Genomic Profiling and Therapeutic Recommendation

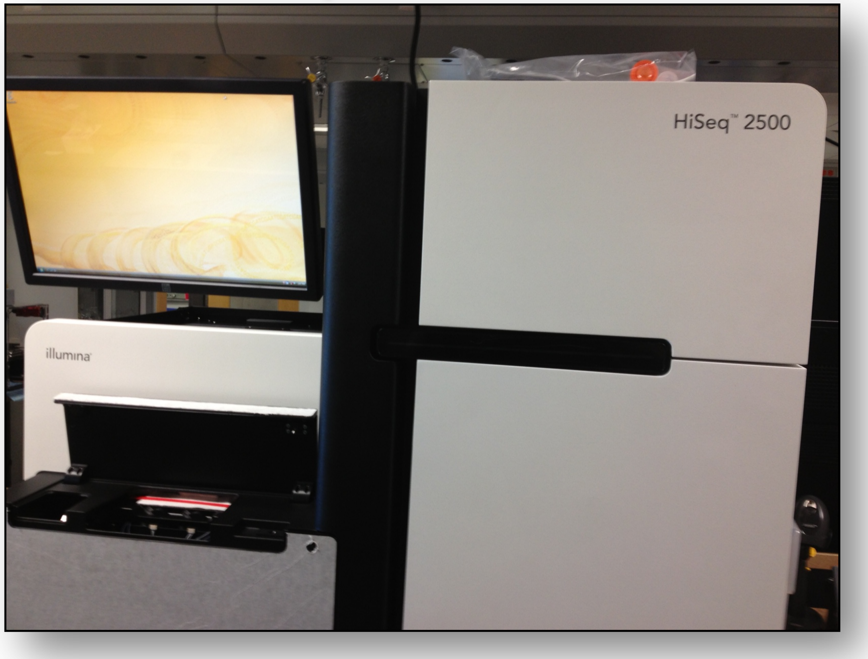
John Carpten

David Craig

- **Deep exome sequencing** to identify low level mutations in heterogeneous samples
- **Long insert whole genome sequencing** to identify structural events and to accurately measure copy number changes
- **Sequencing matched normal DNA** from patient can identify important cancer predisposition genes
- **RNA-sequencing**
  - Confirm mutations
  - Can identify oncogenic fusions
  - Differential expression; isoforms, neoantigens

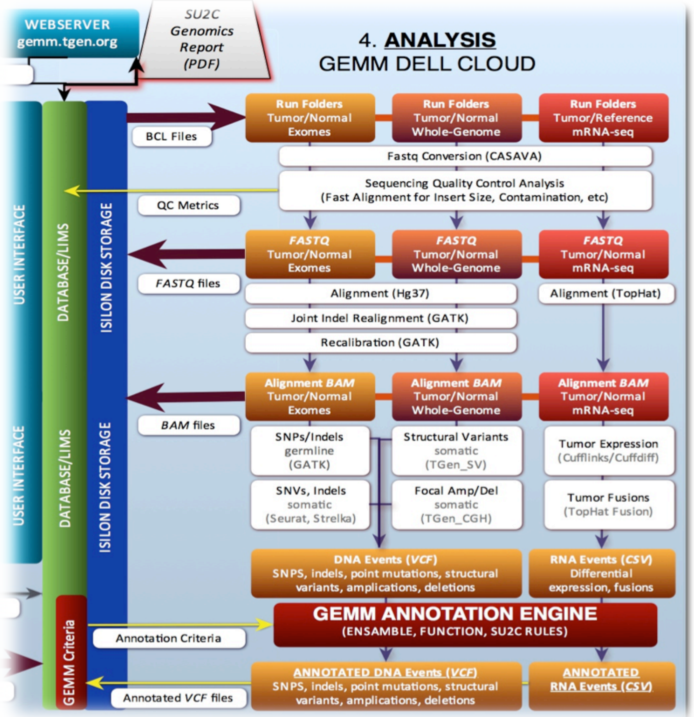
Dell-enabled HPC Infrastructure @ TGen

Illumina HiSeq 2500 – 27 hour run time

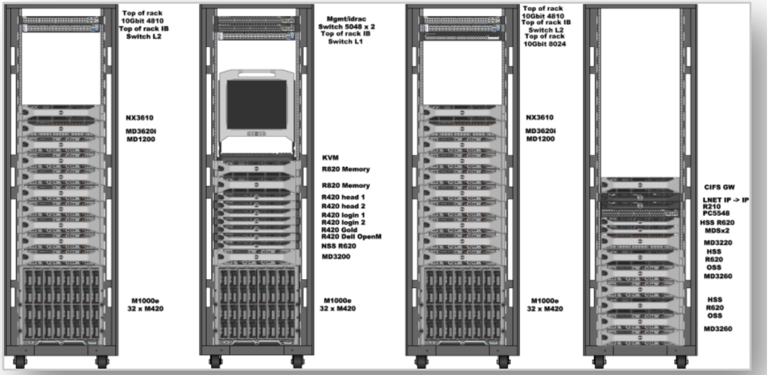


2011- 1M CPUhrs/yr
7.2014 1M CPUhrs/mo

Production pipeline: 2.5 days
Research pipeline: 1 day



Dell Clinical Cluster
2000 Cores
300 TB Lustre File system
54 Gbps Infiniband
10 Gbps Ethernet

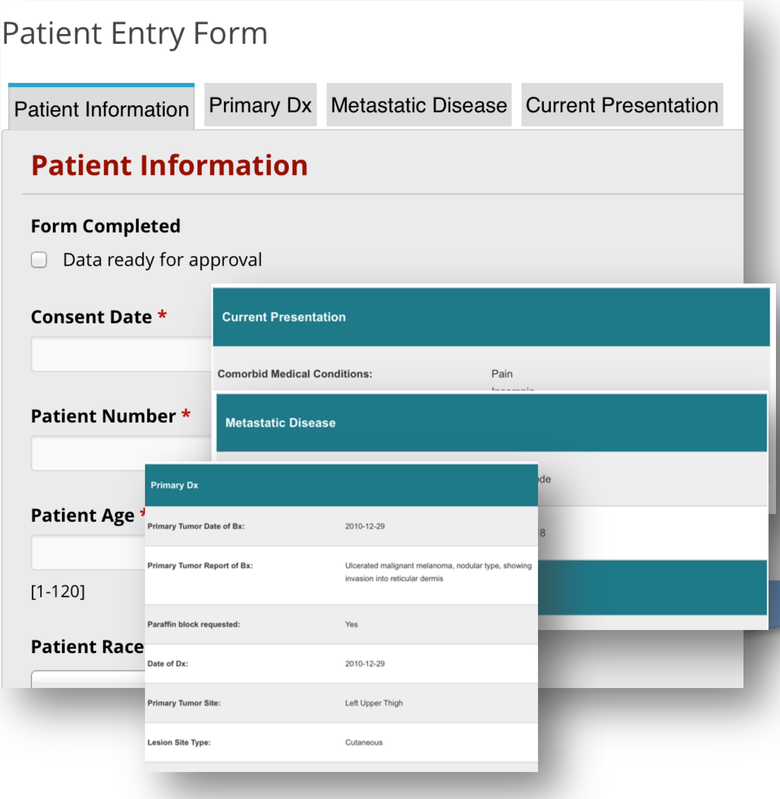


CLINICAL DATA INTEGRATION

In Depth Clinical Fields JSON or XML ←→ RNA/DNA Genome-wide VCFs/BAMs

Clinical: XML/JSON

Genomic: VCF/BAM



Patient Entry Form

Patient Information

Form Completed

☐ Data ready for approval

Consent Date *

Patient Number *

Patient Age *

[1-120]

Patient Race

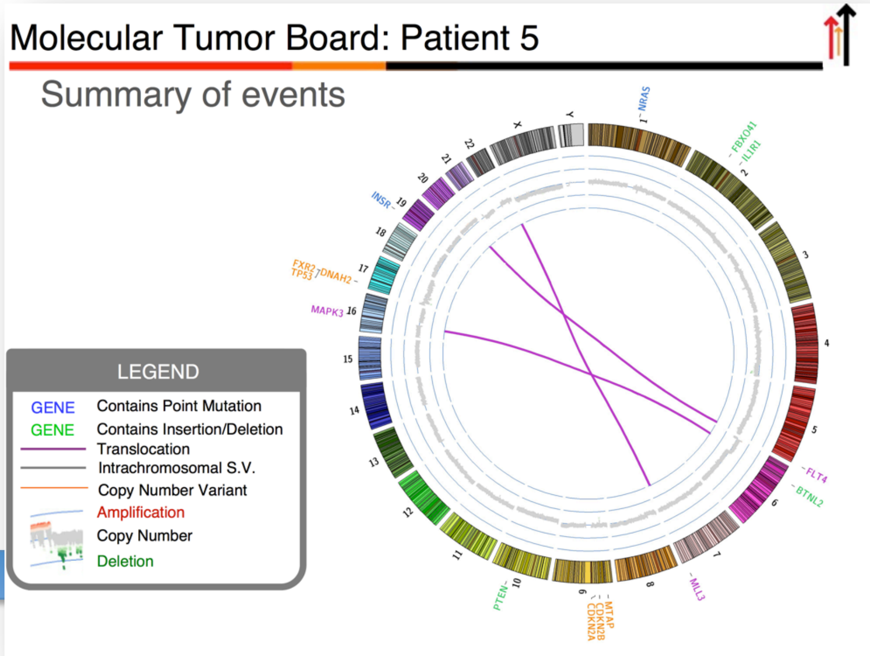
Current Presentation

Comorbid Medical Conditions:	Pain

Metastatic Disease

Primary Dx

Primary Tumor Date of Bx:	2010-12-29
Primary Tumor Report of Bx:	Ulcerated malignant melanoma, nodular type, showing invasion into reticular dermis
Paraffin block requested:	Yes
Date of Dx:	2010-12-29
Primary Tumor Site:	Left Upper Thigh
Lesion Site Type:	Cutaneous



Molecular Tumor Board: Patient 5

Summary of events

LEGEND

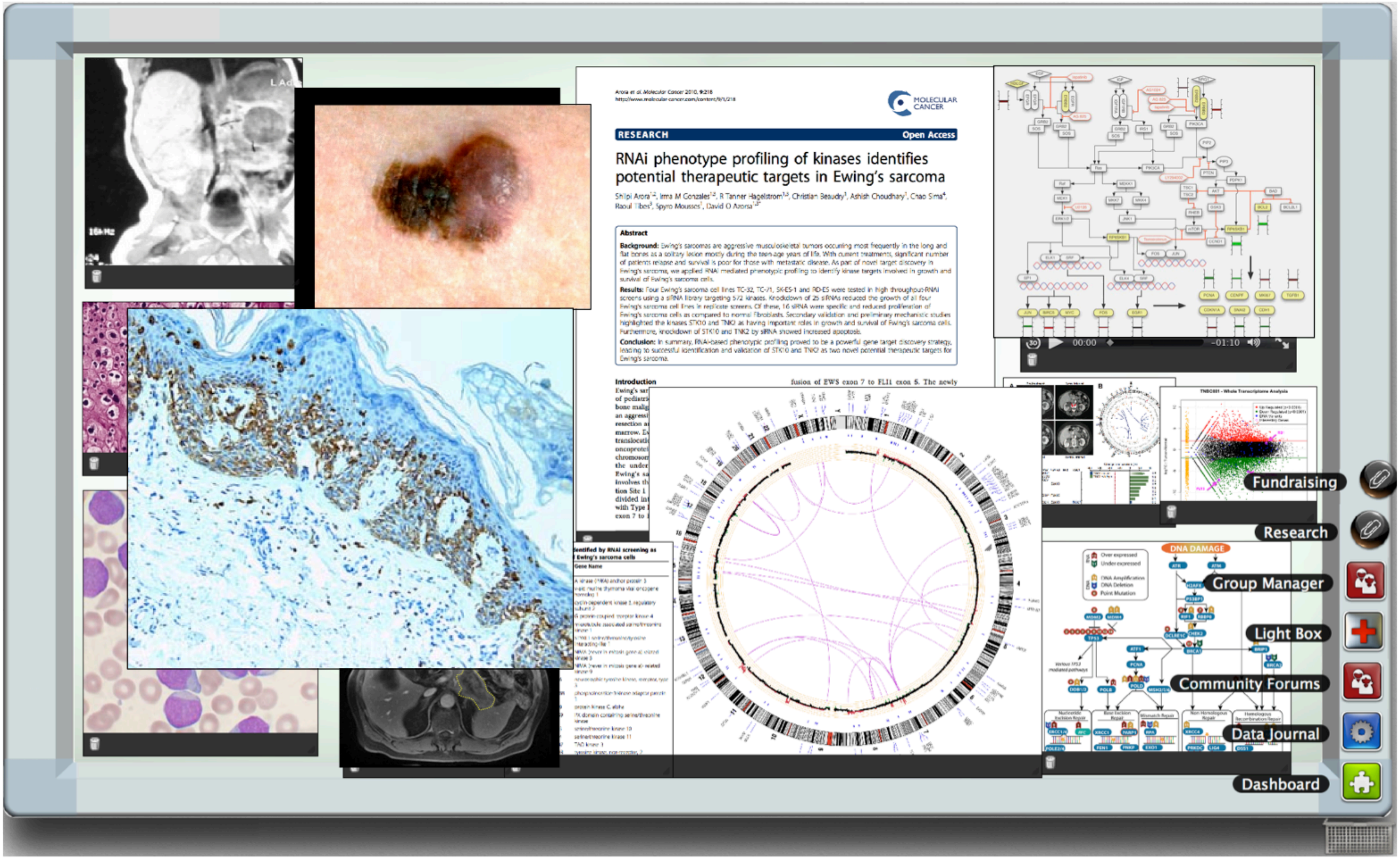
GENE	Contains Point Mutation
GENE	Contains Insertion/Deletion
—	Translocation
—	Intrachromosomal S.V.
—	Copy Number Variant
	Amplification Copy Number Deletion

Somatic: cBioPorta/ICGC, **Germline:** dbGap

Clinical Collaboration Applications for Accessing Distributed Data Across Federated Network

All sites use a secure HIPPA compliant, cloud enabled, web accessible dashboard

real-time display of multi-media files (PDF, pictures, video, audio). Users experience the same configuration of information being presented (before, during, and after the meeting)



Arora et al. Molecular Cancer 2010, 9:218
http://www.molecular-cancer.com/content/9/1/218

MOLECULAR CANCER

RESEARCH Open Access

RNAi phenotype profiling of kinases identifies potential therapeutic targets in Ewing's sarcoma

Shilpi Arora[1,2], Irma M Gonzales[1,2], R Tanner Hagelstrom[1,2], Christian Beaudry[1], Ashish Choudhary[1], Chao Sima[4], Raoul Tibes[3], Spyro Mousses[1], David O Azorsa[1,2*]

Abstract

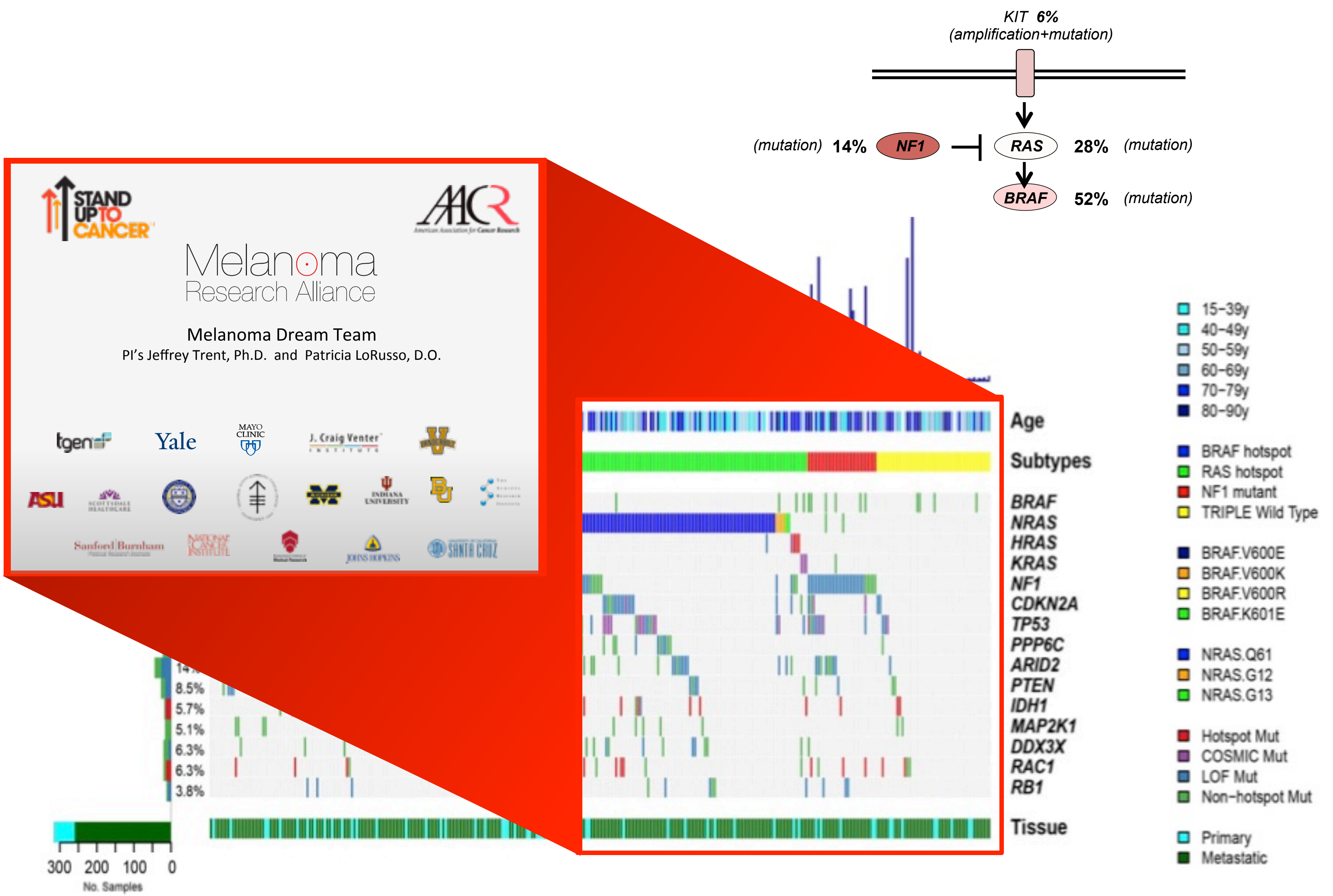
Background: Ewing's sarcomas are aggressive musculoskeletal tumors occurring most frequently in the long and flat bones as a solitary lesion mostly during the teen-age years of life. With current treatments, significant number of patients relapse and survival is poor for those with metastatic disease. As part of novel target discovery in Ewing's sarcoma, we applied RNAi mediated phenotypic profiling to identify kinase targets involved in growth and survival of Ewing's sarcoma cells.

Results: Four Ewing's sarcoma cell lines TC-32, TC-71, SK-ES-1 and RD-ES were tested in high throughput-RNAi screens using a siRNA library targeting 572 kinases. Knockdown of 25 siRNAs reduced the growth of all four Ewing's sarcoma cell lines in replicate screens. Of these, 16 siRNA were specific and reduced proliferation of Ewing's sarcoma cells as compared to normal Fibroblasts. Secondary validation and preliminary mechanistic studies highlighted the kinases STK10 and TNK2 as having important roles in growth and survival of Ewing's sarcoma cells. Furthermore, knockdown of STK10 and TNK2 by siRNA showed increased apoptosis.

Conclusion: In summary, RNAi-based phenotypic profiling proved to be a powerful gene target discovery strategy, leading to successful identification and validation of STK10 and TNK2 as two novel potential therapeutic targets for Ewing's sarcoma.

Introduction

fusion of EWS exon 7 to FLI1 exon 5. The newly



STAND UP TO CANCER

AACR American Association for Cancer Research

Melanoma
Research Alliance

Melanoma Dream Team
PI's Jeffrey Trent, Ph.D. and Patricia LoRusso, D.O.

Treatment of non-BRAF^{V600E} Metastatic Melanoma

Cancer Genome Atlas Research Network. Watson IR, Chin L, Gershenwald, J et al. (In Press Cell)

Project Phases for SU2C Genomics Medicine in Melanoma (G.E.M.M) Trial

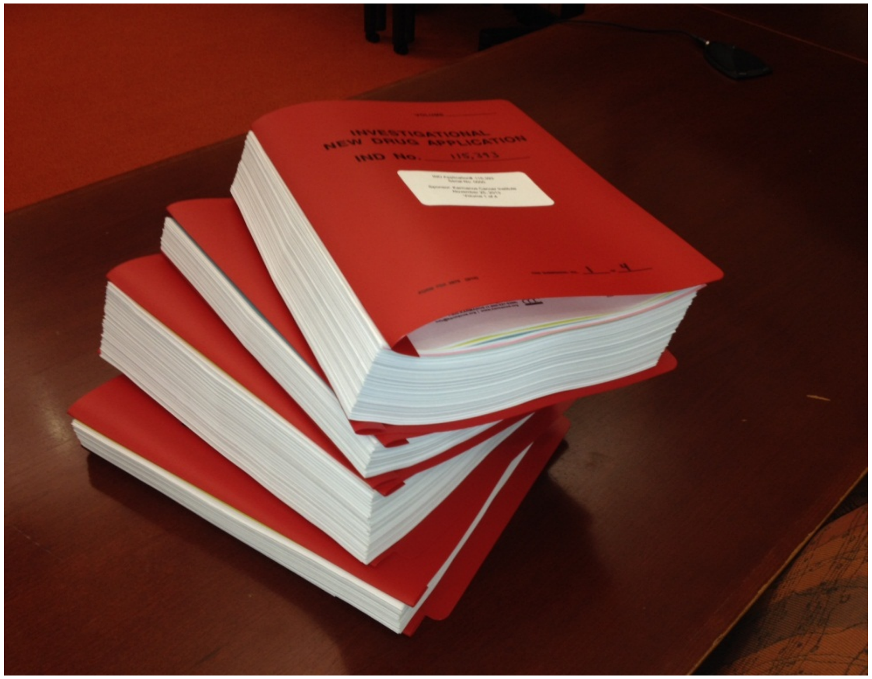
- Clinical Trial Development (with FDA)
- Molecular Platform optimization/validation
- FDA mandated Pilot Clinical Trial (n=5)
- Formal FDA Review
- Approved IND as a Randomized Clinical Trial

Primary Objective:

- Best Overall Response Rate (BORR): targeted treatment vs. physician's choice of standard therapy

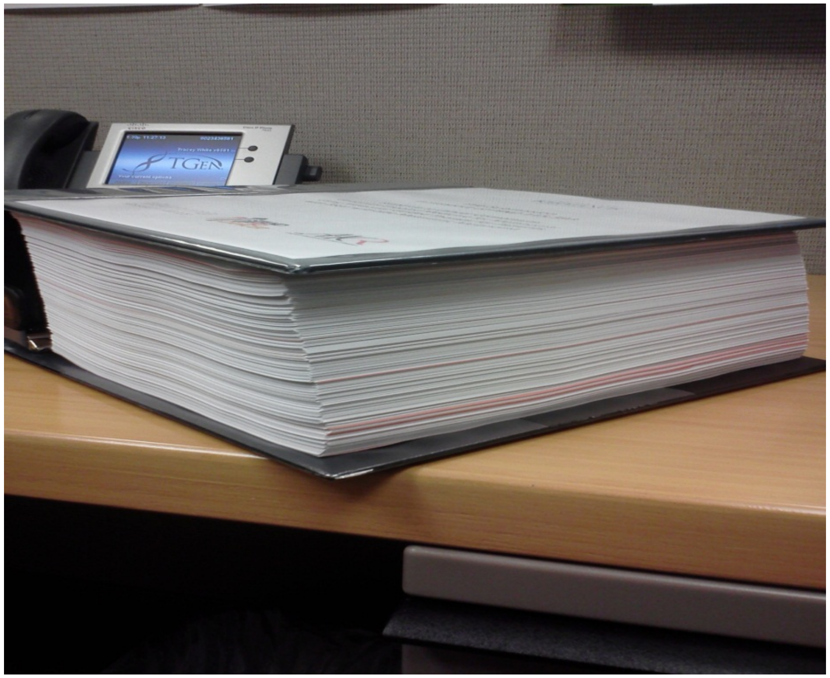
FDA IND and Pre-Submissions – 2,443 Pages of Documentation

Investigational New Drug Application



Number of pages = 1,517

"Pre-Submission" - alternative to the
Investigational New Device Exemption



Number of pages = 926

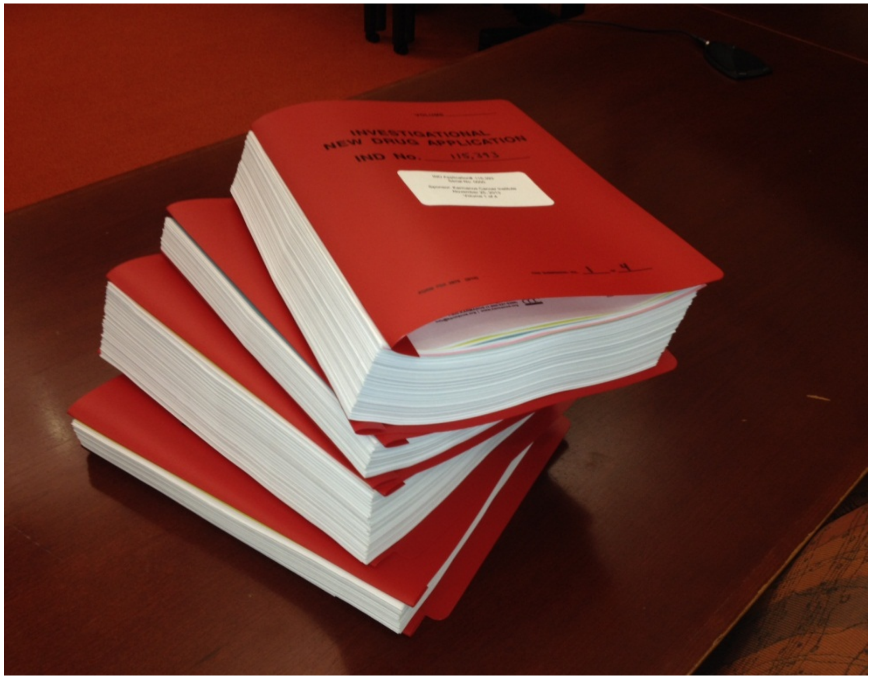
Project Phases Developed over 3 YEARS!!

Melanoma Research Alliance

SU2C

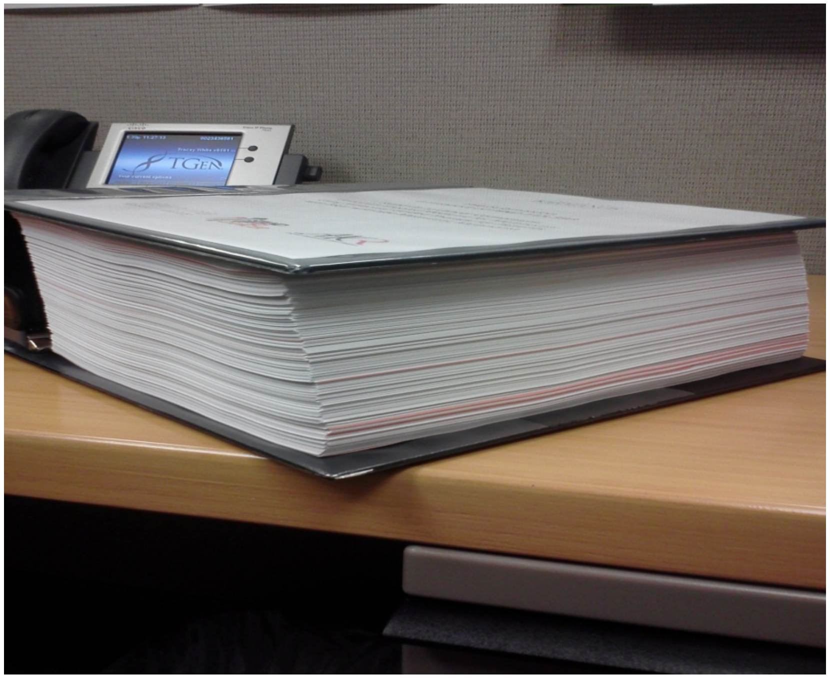
FDA IND and Pre-Submissions – 2,443 Pages of Documentation

Investigational New Drug Application



Number of pages = 1,517

"Pre-Submission" - alternative to the Investigational New Device Exemption



Number of pages = 926

TRIAL IS CURRENTLY OPEN AND ENROLLING!

Yale, Mayo (3), UMI, IU

Melanoma Research Alliance

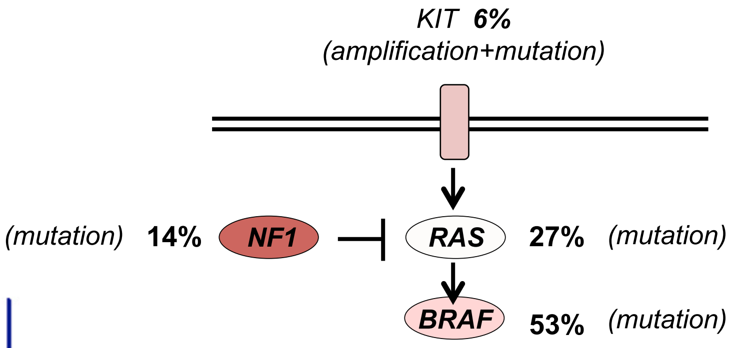
Available Investigational Agents with Phase II dosing:

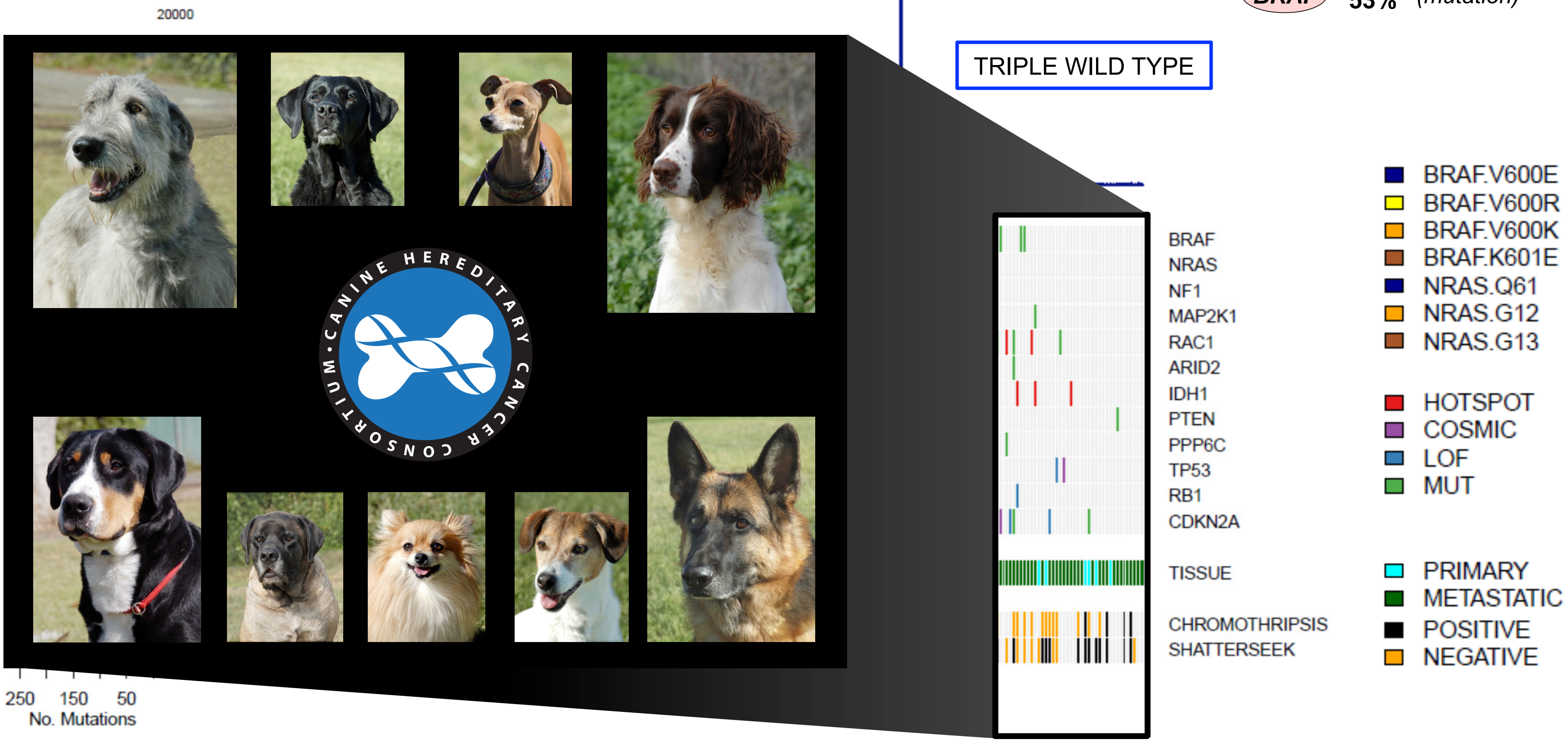
Company Name:	**Drug Name:**	**Target:**	**Route**
Millennium	MLN8237	Aurora A kinase	Oral
	MLN9708	proteasome protease inhibitor	Oral
Pfizer	PF-00299804	pan-erbB	Oral
	PD-0332991	CDK 4/6 inhibitor	Oral
Plexxikon	PLX3397	FMS, Kit and Fit3-ITD	Oral
Exelixis	XL184	Mulit-kinase (VEGFR2, Met, FLT3, Tie2, Kit and Ret)	Oral
Novartis/Array	MEK162	MEK 1/2	Oral
Novartis	BGJ398		Oral
Other	Ongoing efforts at securing additional agents	Single and combination	N/A

Melanoma TCGA

Cancer Genome Atlas Research Network.
Watson IR, Chin L, Gershenwald, J et al. (2014)

- Cutaneous melanomas
- Non-glabrous skin
- Mainly regional metastases
- Total accrual ~300



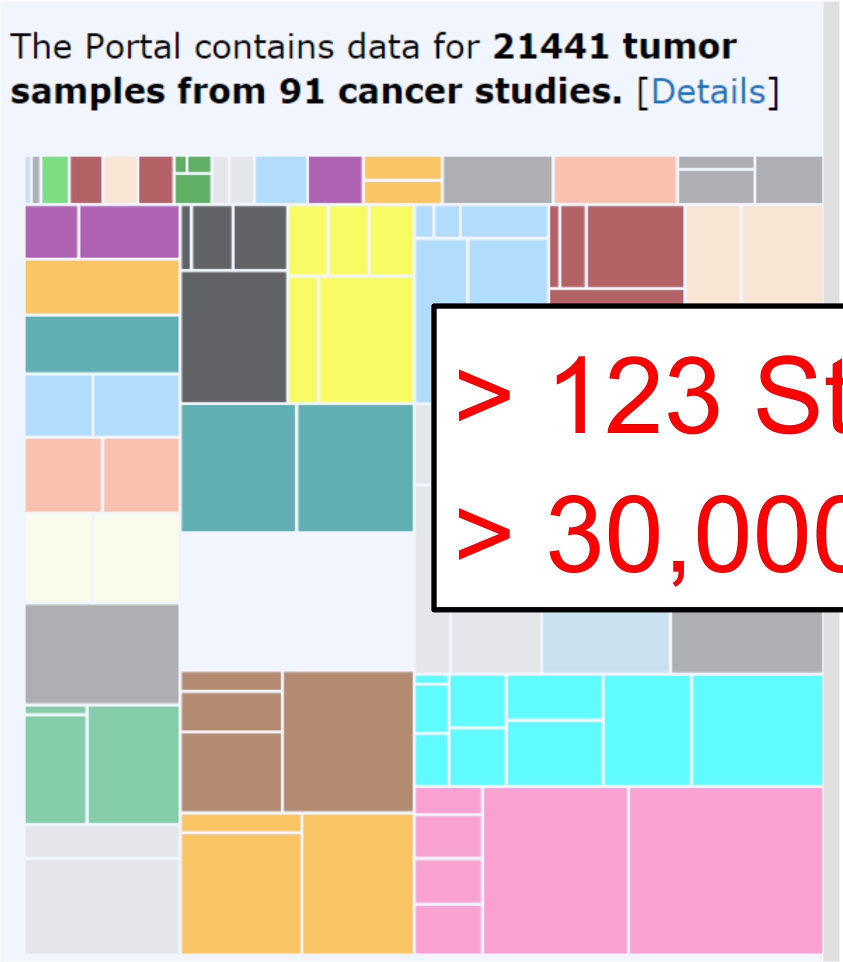


Deep Interrogation of Canine Melanoma as a Comparative Model to Improve Cancer Therapeutics for Triple Wild Type Human Melanoma

Cancer Genome Atlas Research Network. Watson IR, Chin L, Gershenwald, J et al. TCGA Workshop, 2012

- Precision Medicine in Human Cancer
- **Canine Cancer Genomic Landscapes**
- Emerging Canine PMed Tools
- Opportunities in Comparative Oncology

cBioPortal
for Cancer Genomics



The Portal contains data for **21441 tumor samples from 91 cancer studies.** [Details]

ICGC Data Portal

Donor Distribution by Primary Site

> 123 Studies

> 30,000 Tumors

Cancer projects	55
Cancer primary sites	18
Donors	12,807
Simple somatic mutations	12,942,642
Mutated genes	57,517

Type	Genetic basis - prior studies	# NGS Studies	Platform and Samples	Notable Findings	Reference
Mast cell tumor	KIT	0	n/a	n/a	n/a
Soft tissue sarcoma	Various	1	7 Exomes	- 16 mutations/tumor - Few CNVs - NF1, MLL3, PTCH1, MDM4, ATP7B, AIG1	*Roberts et al, 2014*
Lymphoma	TP53, MYC, KIT, RB1, PTEN, CDKN2A, NRAS	1	84 NHL - 5 exome	44% TRAF3	*Bushell et al, 2015*
Osteosarcoma	CDKN2A, TP53, RB, PTEN				n/a
Mammary carcinoma	BRCA1/BRCA2		- 2 ctDNA - 20 ddPCR	5	*Beck et al, 2013*
			12 cases - 3 WGS - 10 RNAseq - 4 exome	- 0-907 SNVs/tumor - Large CNVs - PTEN, BRAF, PIM1, CCND3, ZFAND3-MGAM, POLD1, BRCA1, IGFR2, FOXC2, DLG2, USH2A	*Liu et al , 2014*
TCC	Unknown	1	4 RNAseq 62 Sanger	80% BRAF V595E	*Decker et al, 2015*
CTVT	LINE1 insertion upstream of , MYC, TP53	1	2 WGS, FISH	- Clonal, Aneuploid/Diploid - 2118 SVs, 1.9M SNVs - MYC, CDKN2A, SETD2, ERG	*Murchison et al, 2014*

< 10 Studies

< 50 Tumors

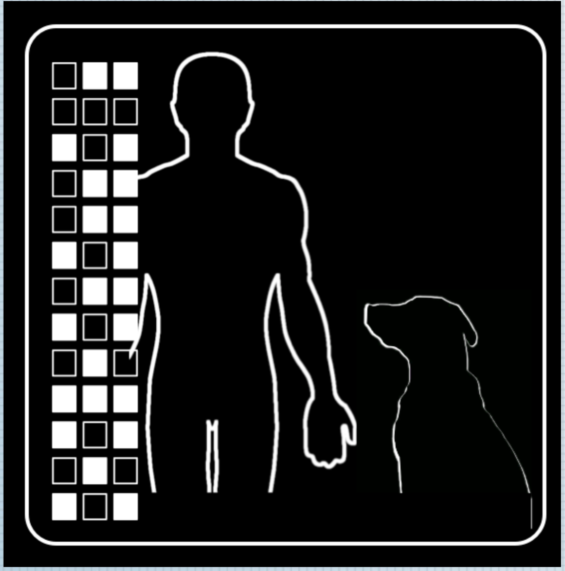
The Canine Hereditary Cancer Consortium (CHCC)

An unprecedented alliance of researchers, veterinarians, physicians, and owners

"From Bark to Bedside"

CANIS LUPIS FAMILIARIS

Establishing a new paradigm for tackling complex canine disease

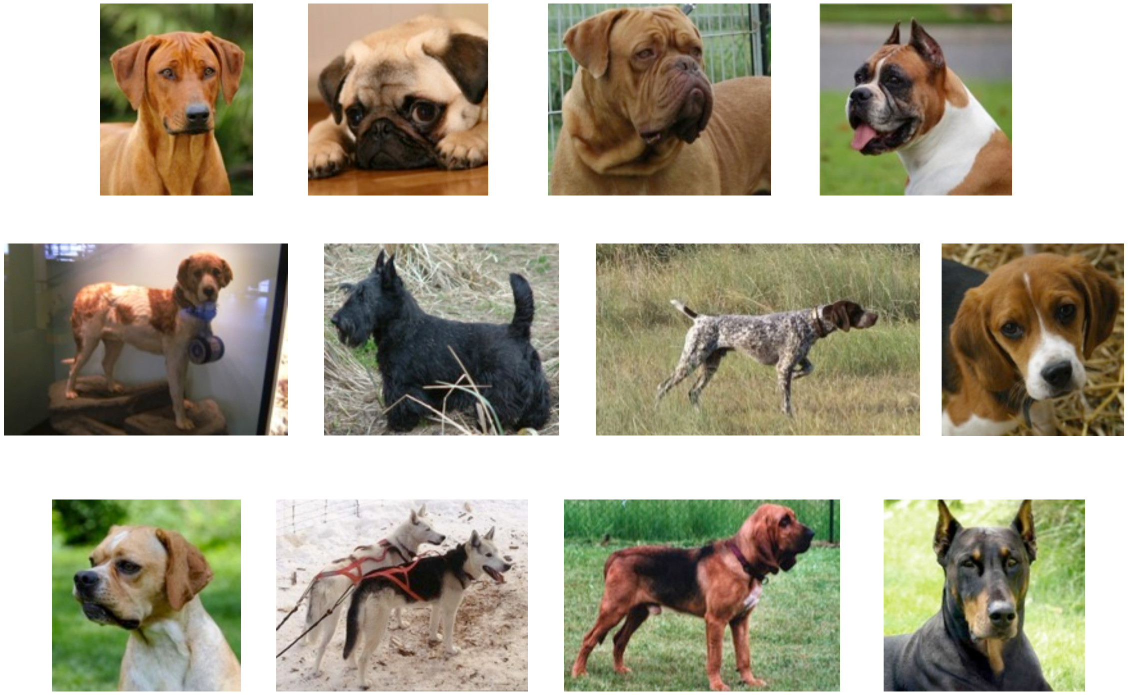


UC2 CA148149 NCI/NIH

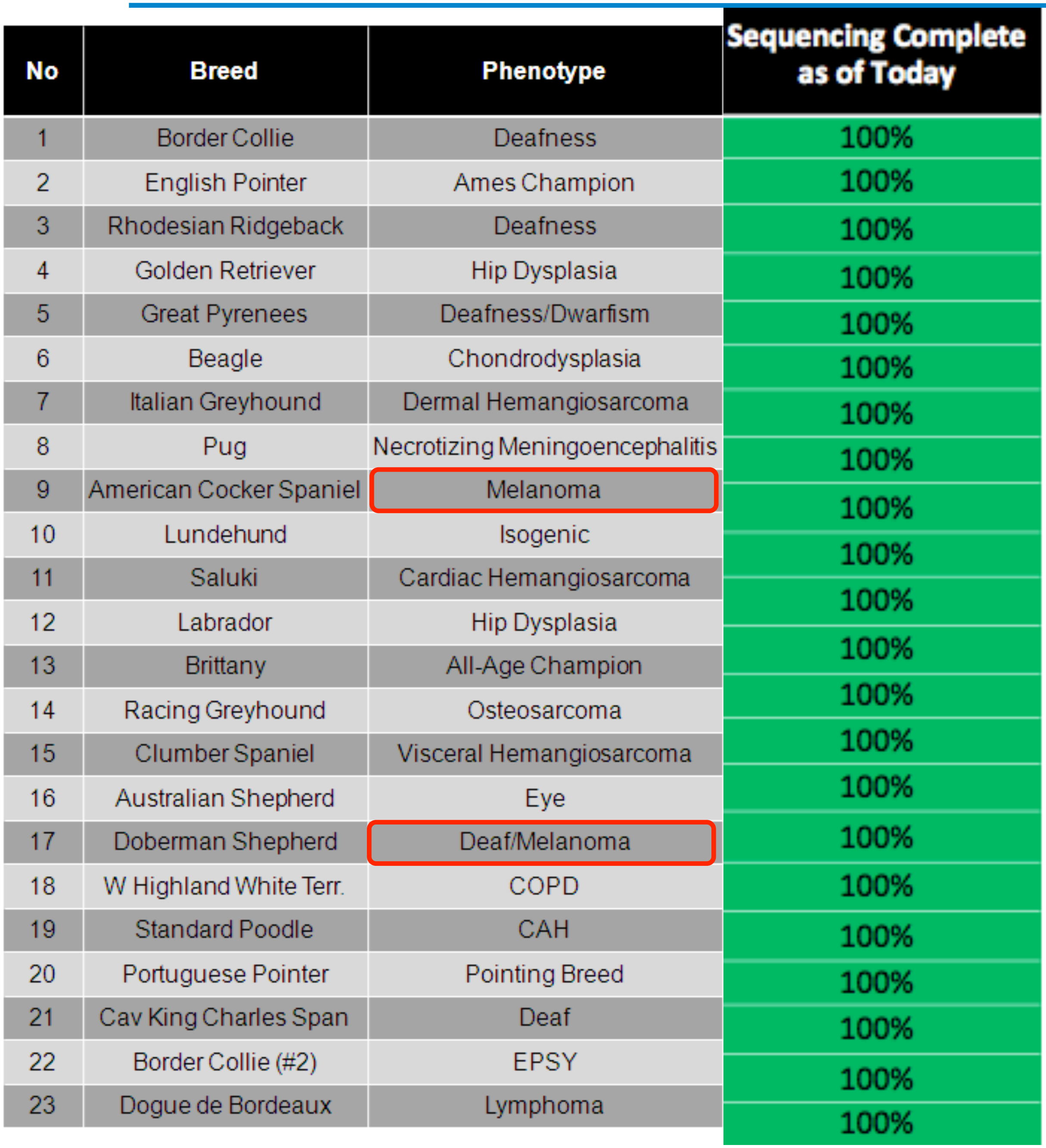
Beginning with Canine Genome Sequencing
WGS (Illumina)

- ~25 dogs sequenced at TGen (Matt Huentleman)
  - The database of variants from unaffected dogs can be subtracted in silico from the variants of the affected dog to identify putative causal variants under the association peak or potentially a somatic mutation

Cocker Spaniel with Melanoma



Canine Genome Sequencing Project - Phase I



No	Breed	Phenotype	Sequencing Complete as of Today
1	Border Collie	Deafness	100%
2	English Pointer	Ames Champion	100%
3	Rhodesian Ridgeback	Deafness	100%
4	Golden Retriever	Hip Dysplasia	100%
5	Great Pyrenees	Deafness/Dwarfism	100%
6	Beagle	Chondrodysplasia	100%
7	Italian Greyhound	Dermal Hemangiosarcoma	100%
8	Pug	Necrotizing Meningoencephalitis	100%
9	American Cocker Spaniel	Melanoma	100%
10	Lundehund	Isogenic	100%
11	Saluki	Cardiac Hemangiosarcoma	100%
12	Labrador	Hip Dysplasia	100%
13	Brittany	All-Age Champion	100%
14	Racing Greyhound	Osteosarcoma	100%
15	Clumber Spaniel	Visceral Hemangiosarcoma	100%
16	Australian Shepherd	Eye	100%
17	Doberman Shepherd	Deaf/Melanoma	100%
18	W Highland White Terr.	COPD	100%
19	Standard Poodle	CAH	100%
20	Portuguese Pointer	Pointing Breed	100%
21	Cav King Charles Span	Deaf	100%
22	Border Collie (#2)	EPSY	100%
23	Dogue de Bordeaux	Lymphoma	100%

MISSOURI
Robert Schnabel
Gary Johnson
Dennis O'Brien

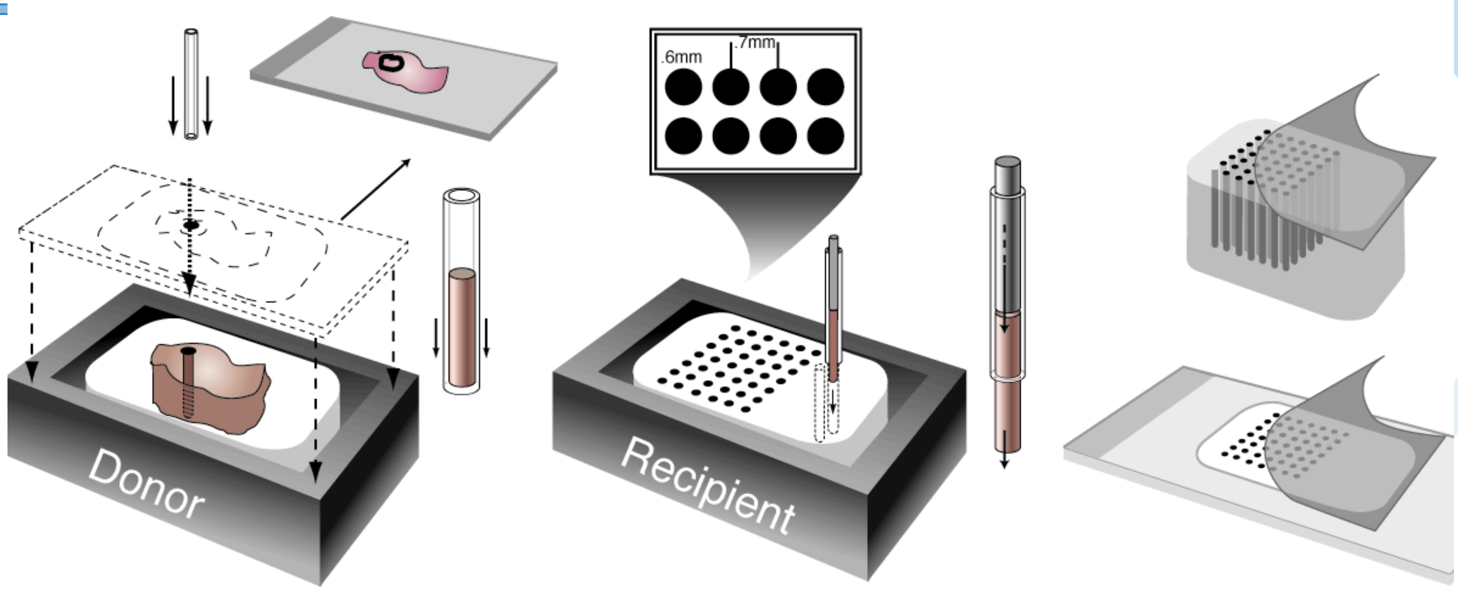
UCLA
Robert Wayne
John Novembre

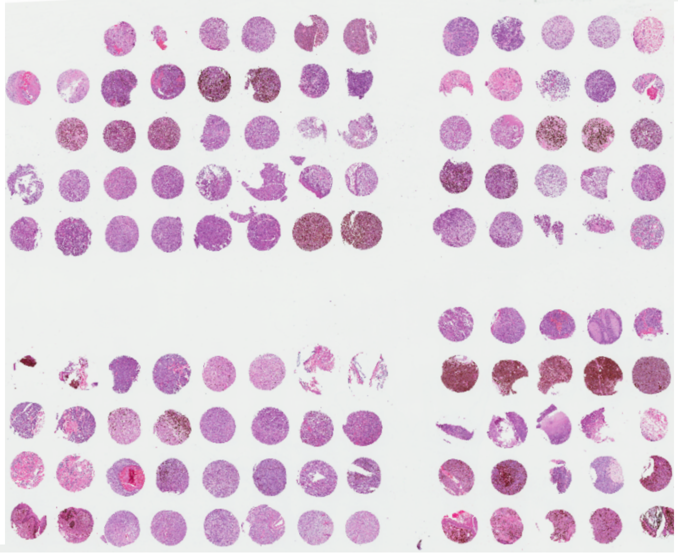
TEXAS A&M
William Murphy

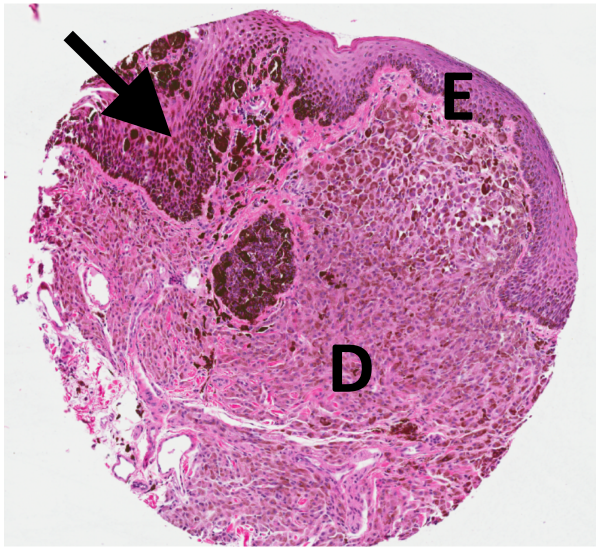
NHGRI
Elaine Ostrander
Brian David, Post-Doc

These groups these groups + ours have been sharing whole genome data to create a detailed database of breed specific variants.

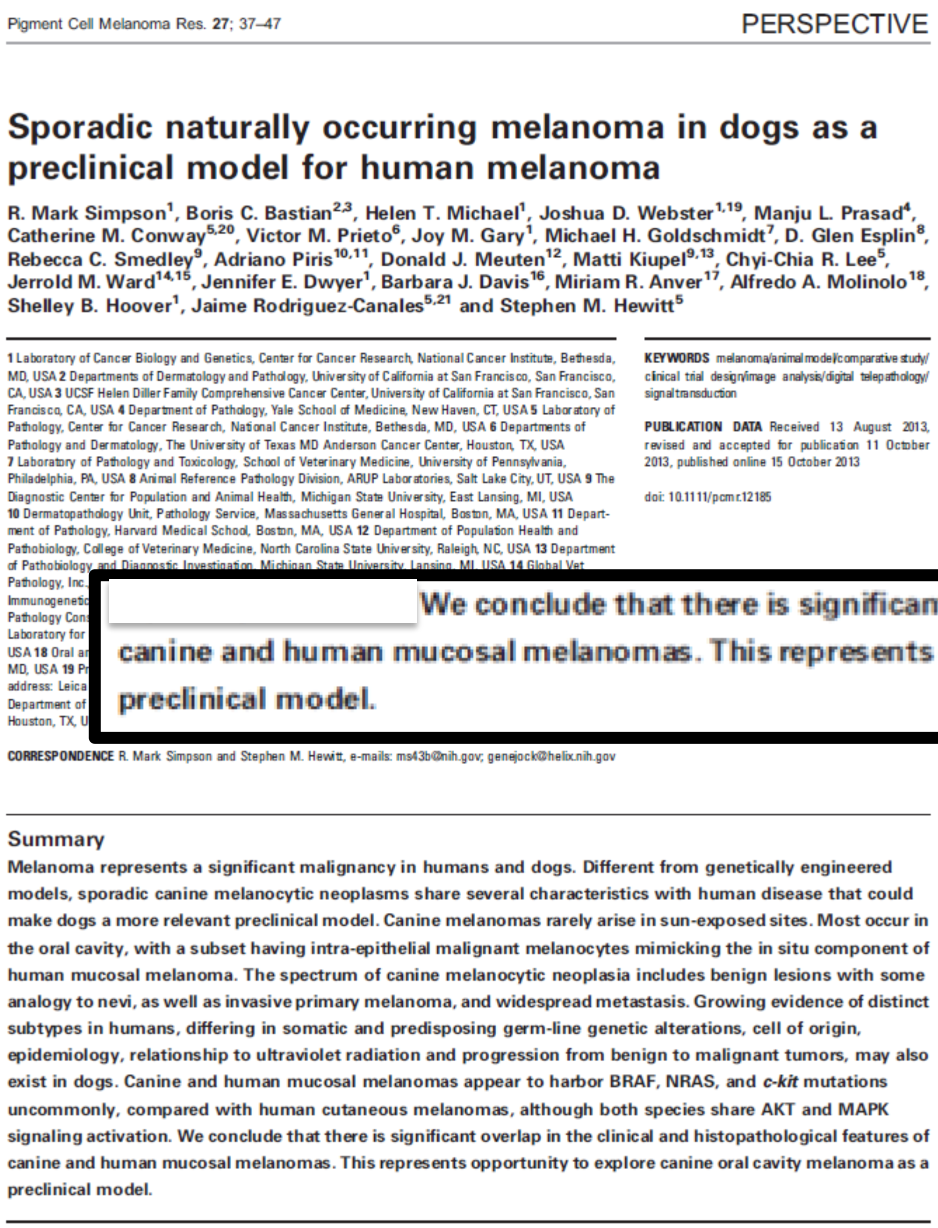
Canine Tissue Microarrays (Dr. Barb Davis)







- Primary, invasive mucosal melanoma is seen arising from the epidermis (E) on the upper left (arrow) and filling the dermis (D) with large epithelioid melanoma cells



Pigment Cell Melanoma Res. **27**; 37–47

PERSPECTIVE

Sporadic naturally occurring melanoma in dogs as a preclinical model for human melanoma

R. Mark Simpson[1], Boris C. Bastian[2,3], Helen T. Michael[1], Joshua D. Webster[1,19], Manju L. Prasad[4], Catherine M. Conway[5,20], Victor M. Prieto[6], Joy M. Gary[1], Michael H. Goldschmidt[7], D. Glen Esplin[8], Rebecca C. Smedley[9], Adriano Piris[10,11], Donald J. Meuten[12], Matti Kiupel[9,13], Chyi-Chia R. Lee[5], Jerrold M. Ward[14,15], Jennifer E. Dwyer[1], Barbara J. Davis[16], Miriam R. Anver[17], Alfredo A. Molinolo[18], Shelley B. Hoover[1], Jaime Rodriguez-Canales[5,21] and Stephen M. Hewitt[5]

1 Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA **2** Departments of Dermatology and Pathology, University of California at San Francisco, San Francisco, CA, USA **3** UCSF Helen Diller Family Comprehensive Cancer Center, University of California at San Francisco, San Francisco, CA, USA **4** Department of Pathology, Yale School of Medicine, New Haven, CT, USA **5** Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA **6** Departments of Pathology and Dermatology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA
7 Laboratory of Pathology and Toxicology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA **8** Animal Reference Pathology Division, ARUP Laboratories, Salt Lake City, UT, USA **9** The Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI, USA
10 Dermatopathology Unit, Pathology Service, Massachusetts General Hospital, Boston, MA, USA **11** Department of Pathology, Harvard Medical School, Boston, MA, USA **12** Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA **13** Department of Pathobiology and Diagnostic Investigation, Michigan State University, Lansing, MI, USA **14** Global Vet
Pathology, Inc.
Immunogenetic
Pathology Cons
Laboratory for
USA **18** Oral ar
MD, USA **19** Pr
address: Leica
Department of
Houston, TX, U

CORRESPONDENCE R. Mark Simpson and Stephen M. Hewitt, e-mails: ms43b@nih.gov; genejock@helix.nih.gov

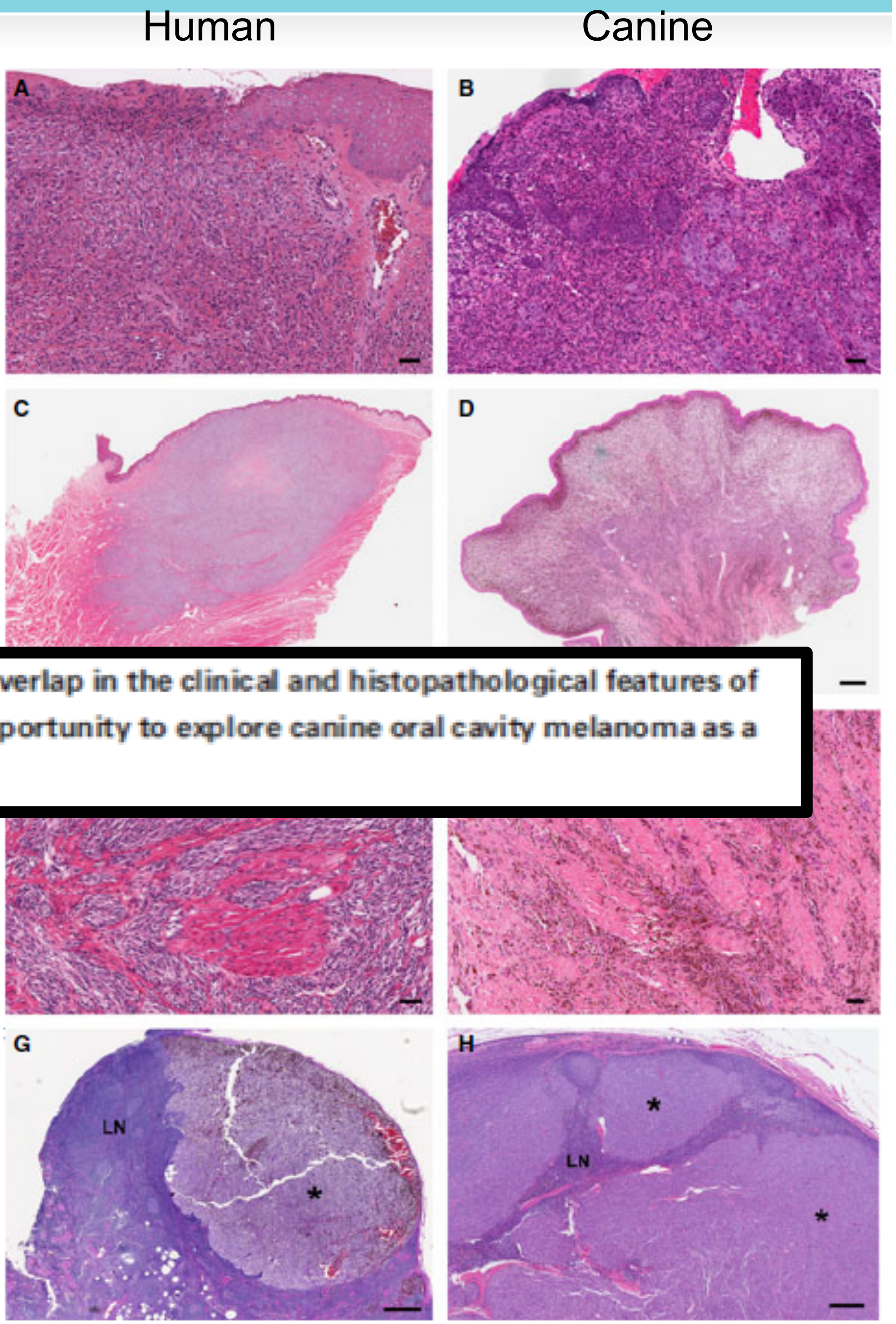
KEYWORDS melanoma/animal model/comparative study/clinical trial design/image analysis/digital telepathology/signal transduction

PUBLICATION DATA Received 13 August 2013, revised and accepted for publication 11 October 2013, published online 15 October 2013

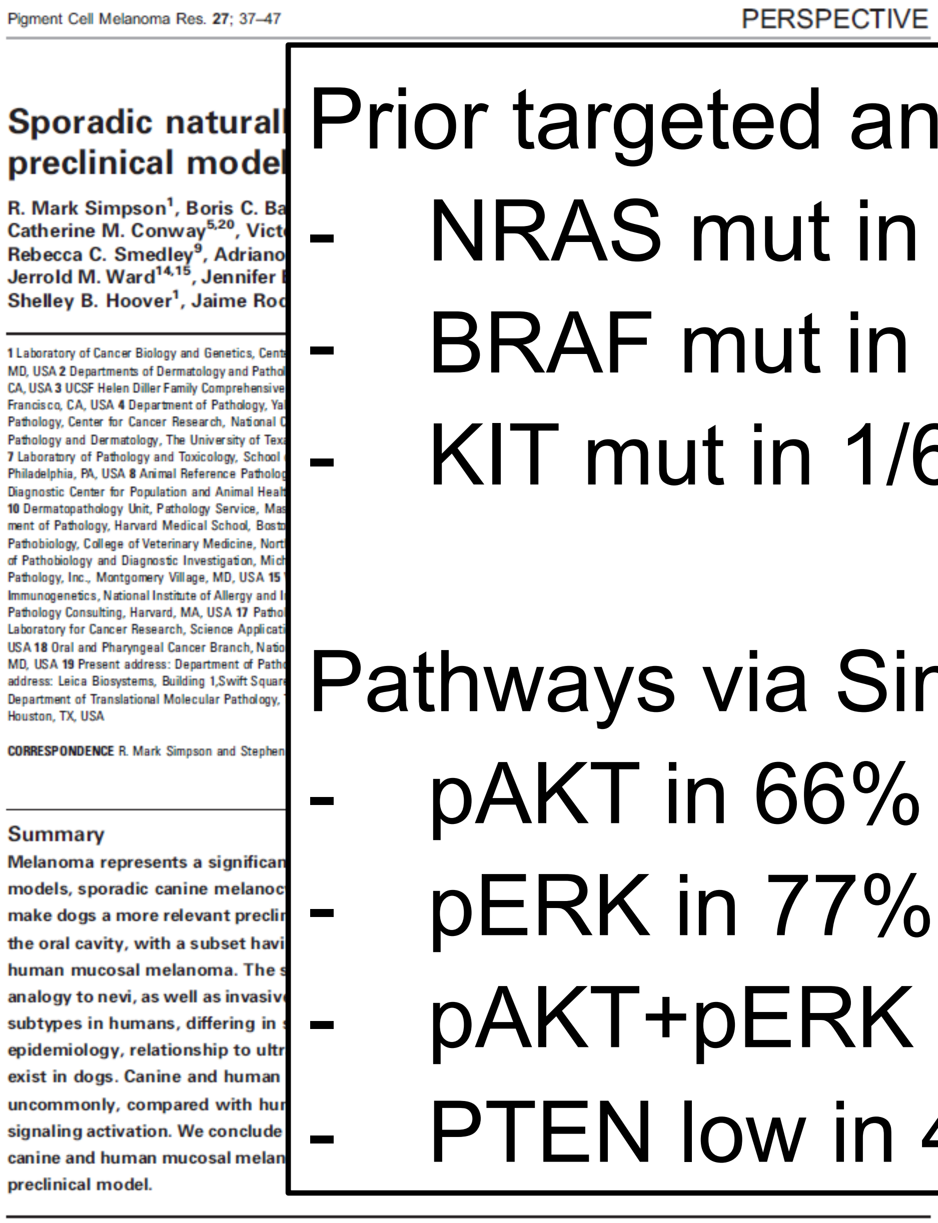
doi: 10.1111/pcmr.12185

Summary

Melanoma represents a significant malignancy in humans and dogs. Different from genetically engineered models, sporadic canine melanocytic neoplasms share several characteristics with human disease that could make dogs a more relevant preclinical model. Canine melanomas rarely arise in sun-exposed sites. Most occur in the oral cavity, with a subset having intra-epithelial malignant melanocytes mimicking the in situ component of human mucosal melanoma. The spectrum of canine melanocytic neoplasia includes benign lesions with some analogy to nevi, as well as invasive primary melanoma, and widespread metastasis. Growing evidence of distinct subtypes in humans, differing in somatic and predisposing germ-line genetic alterations, cell of origin, epidemiology, relationship to ultraviolet radiation and progression from benign to malignant tumors, may also exist in dogs. Canine and human mucosal melanomas appear to harbor BRAF, NRAS, and *c-kit* mutations uncommonly, compared with human cutaneous melanomas, although both species share AKT and MAPK signaling activation. We conclude that there is significant overlap in the clinical and histopathological features of canine and human mucosal melanomas. This represents opportunity to explore canine oral cavity melanoma as a preclinical model.



We conclude that there is significant overlap in the clinical and histopathological features of canine and human mucosal melanomas. This represents opportunity to explore canine oral cavity melanoma as a preclinical model.



Pigment Cell Melanoma Res. **27**; 37–47

PERSPECTIVE

Sporadic naturall
preclinical model

R. Mark Simpson[1], Boris C. Ba
Catherine M. Conway[5,20], Victo
Rebecca C. Smedley[9], Adriano
Jerrold M. Ward[14,15], Jennifer
Shelley B. Hoover[1], Jaime Roo

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MD, USA **2** Departments of Dermatology and Pathol
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Francisco, CA, USA **4** Department of Pathology, Yal
Pathology, Center for Cancer Research, National C
Pathology and Dermatology, The University of Texa
7 Laboratory of Pathology and Toxicology, School
Philadelphia, PA, USA **8** Animal Reference Patholog
Diagnostic Center for Population and Animal Healt
10 Dermatopathology Unit, Pathology Service, Mas
ment of Pathology, Harvard Medical School, Bosto
Pathobiology, College of Veterinary Medicine, Nort
of Pathobiology and Diagnostic Investigation, Mich
Pathology, Inc., Montgomery Village, MD, USA **15**
Immunogenetics, National Institute of Allergy and I
Pathology Consulting, Harvard, MA, USA **17** Patho
Laboratory for Cancer Research, Science Applicati
USA **18** Oral and Pharyngeal Cancer Branch, Natio
MD, USA **19** Present address: Department of Patho
address: Leica Biosystems, Building 1,Swift Square
Department of Translational Molecular Pathology,
Houston, TX, USA

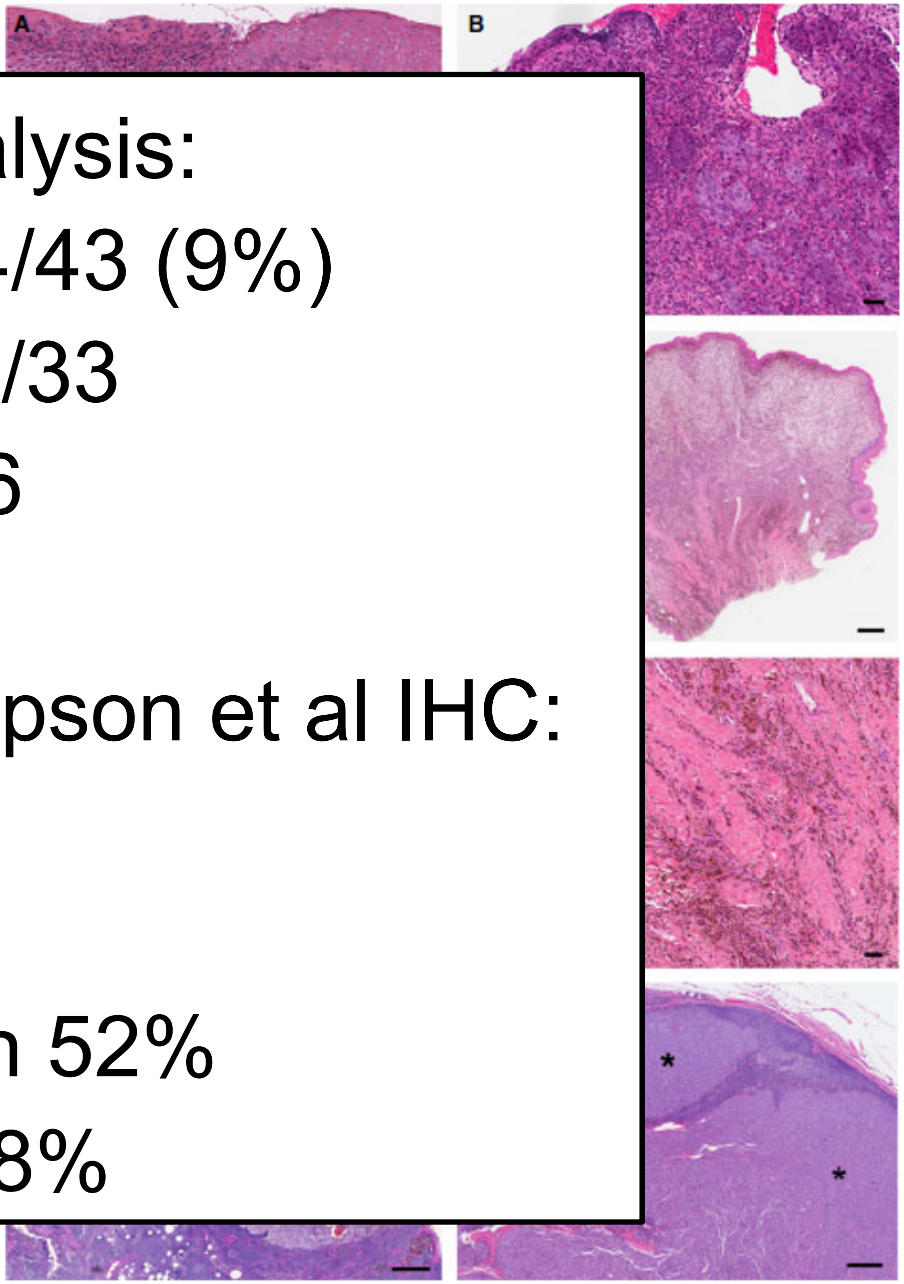
CORRESPONDENCE R. Mark Simpson and Stephen

Summary

Melanoma represents a significan
models, sporadic canine melanoc
make dogs a more relevant preclin
the oral cavity, with a subset havi
human mucosal melanoma. The s
analogy to nevi, as well as invasiv
subtypes in humans, differing in
epidemiology, relationship to ultr
exist in dogs. Canine and human
uncommonly, compared with hur
signaling activation. We conclude
canine and human mucosal melan
preclinical model.

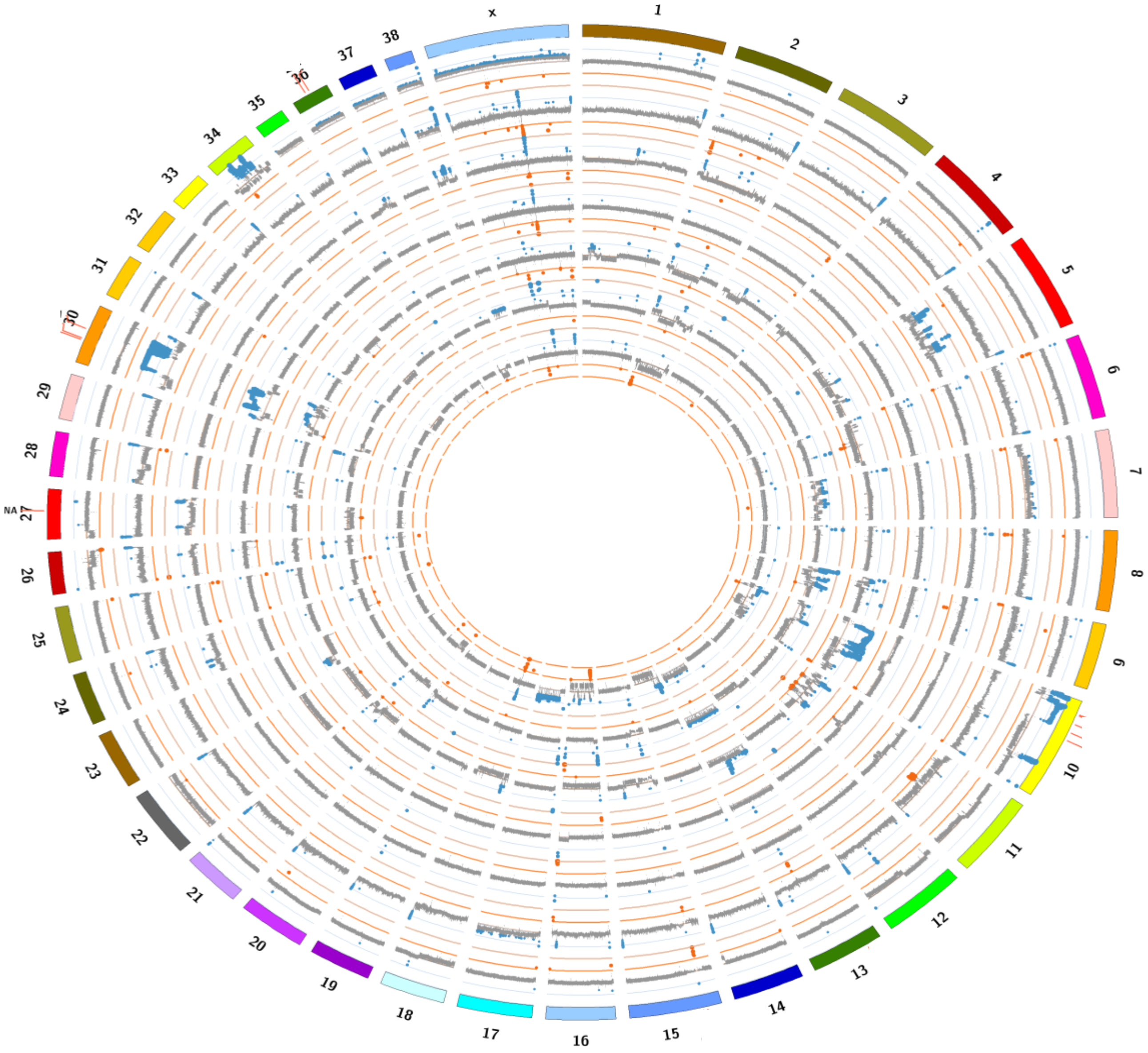
Prior targeted analysis:
- NRAS mut in 4/43 (9%)
- BRAF mut in 0/33
- KIT mut in 1/66

Pathways via Simpson et al IHC:
- pAKT in 66%
- pERK in 77%
- pAKT+pERK in 52%
- PTEN low in 48%



Simpson et al, PCMR, 2014

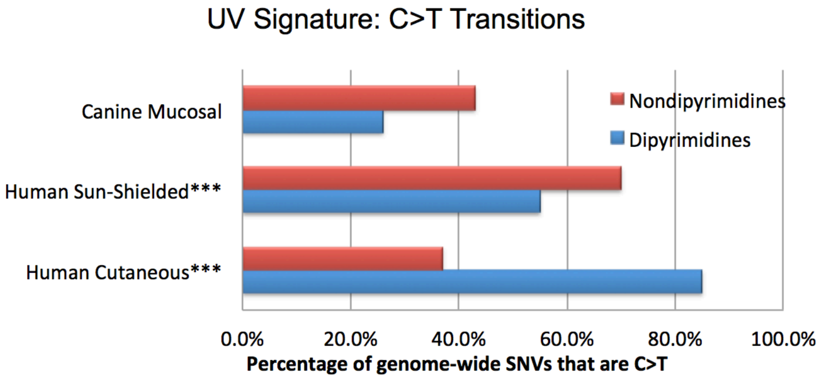
2/3 of canine mucosal tumors are "quadruple wild-type" (BRAFwt, RASwt, KITwt, NF1wt)

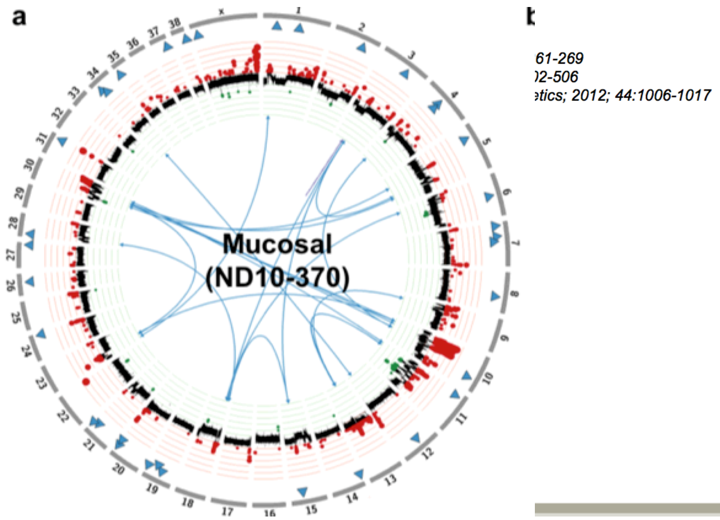


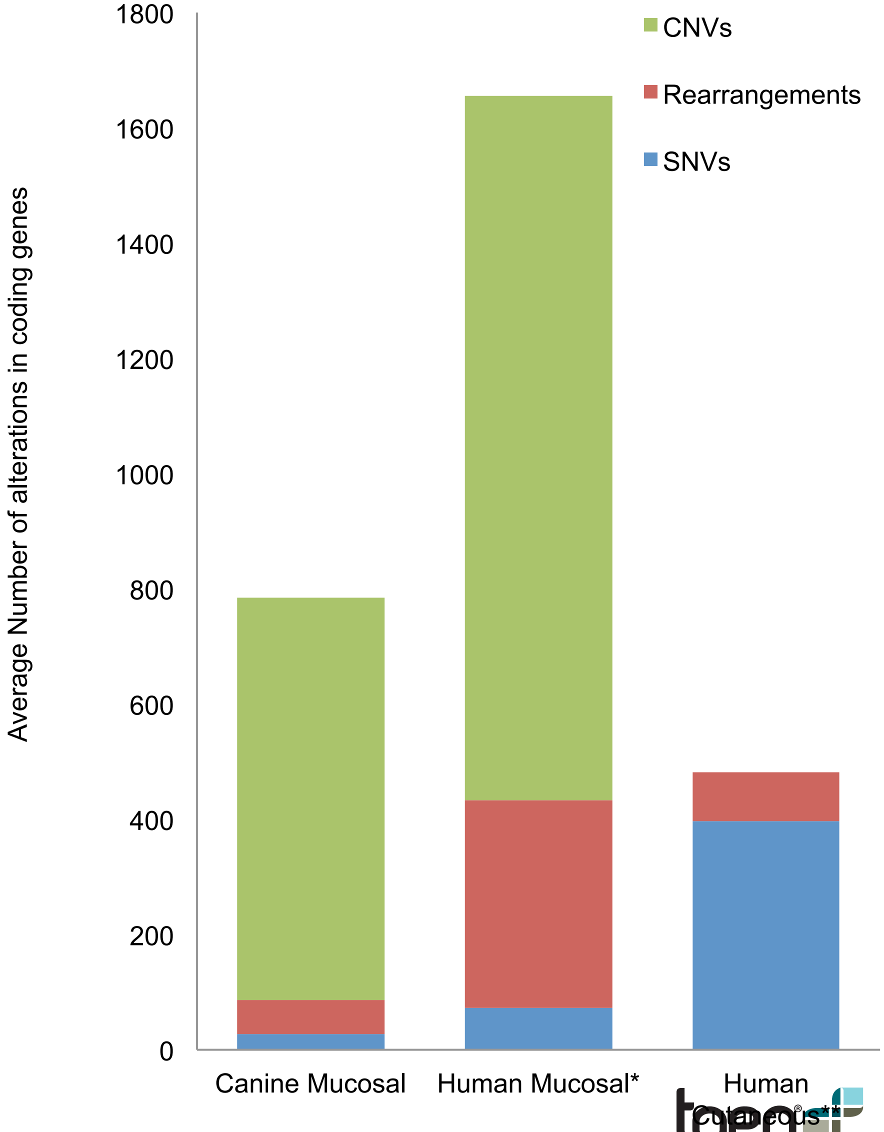
Canine Mucosal Melanoma Mirrors Human Mucosal Melanoma with CNV's >>SNV's and no UV-Signature

Mutation Rate

	Average SNVs per Mb
Canine Mucosal	1.1
Human Mucosal*	2.6
Human Cutaneous**	25.7







*adapted from Furney et al. J Pathol 2013; 230:261-269

**adapted from Berger et al. Nature 2012; 485:502-506

Therapeutic Implications of Genomic Landscapes

MEK and mTOR Inhibition

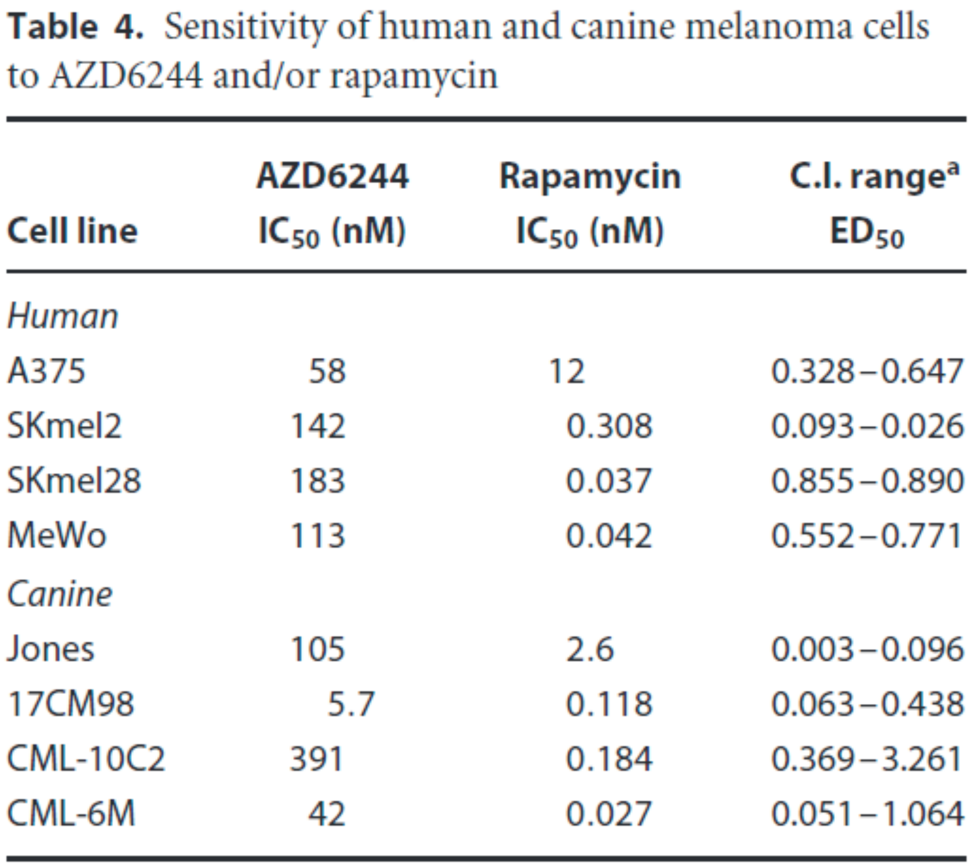
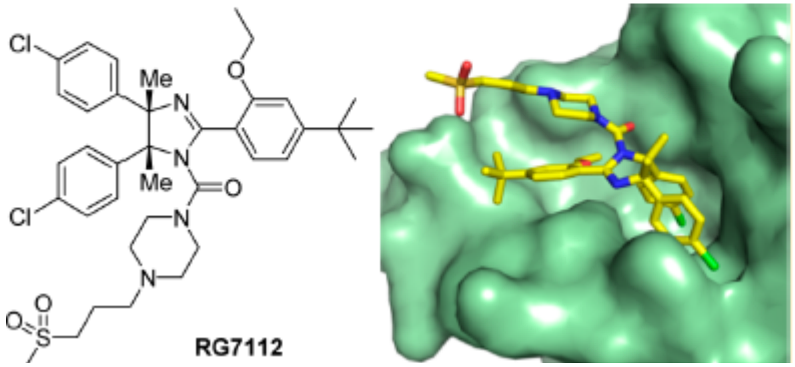


Table 4. Sensitivity of human and canine melanoma cells to AZD6244 and/or rapamycin

Cell line	AZD6244 IC_{50} (nM)	Rapamycin IC_{50} (nM)	C.I. range[a] ED_{50}
Human			
A375	58	12	0.328–0.647
SKmel2	142	0.308	0.093–0.026
SKmel28	183	0.037	0.855–0.890
MeWo	113	0.042	0.552–0.771
Canine			
Jones	105	2.6	0.003–0.096
17CM98	5.7	0.118	0.063–0.438
CML-10C2	391	0.184	0.369–3.261
CML-6M	42	0.027	0.051–1.064

MDM2 Inhibition



RG7112

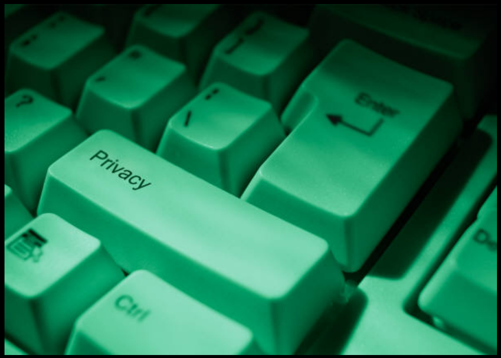
24% of cases with amplification, exclusive with TP53 mutation

17% of cases positive for MDM2 by IHC (Barb Davis)

Fowles et al, Vet Comp Onc, 2014; Vu et al, ACS Med Chem, 2013

- Precision Medicine in Human Cancer
- Canine Cancer Genomic Landscapes
- **Emerging Canine PMed Tools**
- Opportunities in Comparative Oncology

Continuous Reach-Through to Canine Patients



A life comprehensively recorded (and somewhat less comprehensively regulated) should more rapidly allow integration of genomic and clinical data

Members of the Comparative Oncology Trials Consortium

Auburn University
Auburn, AL

Colorado State University
Ft. Collins, CO

Kansas State University
Manhatten, KS

Michigan State University
East Lansing, MI

North Carolina State University
Raleigh, NC

Purdue University
West Lafayette, IN

Texas A&M University
College Station, TX

The Ohio State University
Columbus, OH

Tufts University
North Grafton, MA

University of California
Davis, CA

University of Florida
Gainesville, FL

University of Georgia
Athens, GA

University of Guelph
Guelph, ON Canada

University of Illinois
Urbana, IL

University of Minnesota
St. Paul, MN

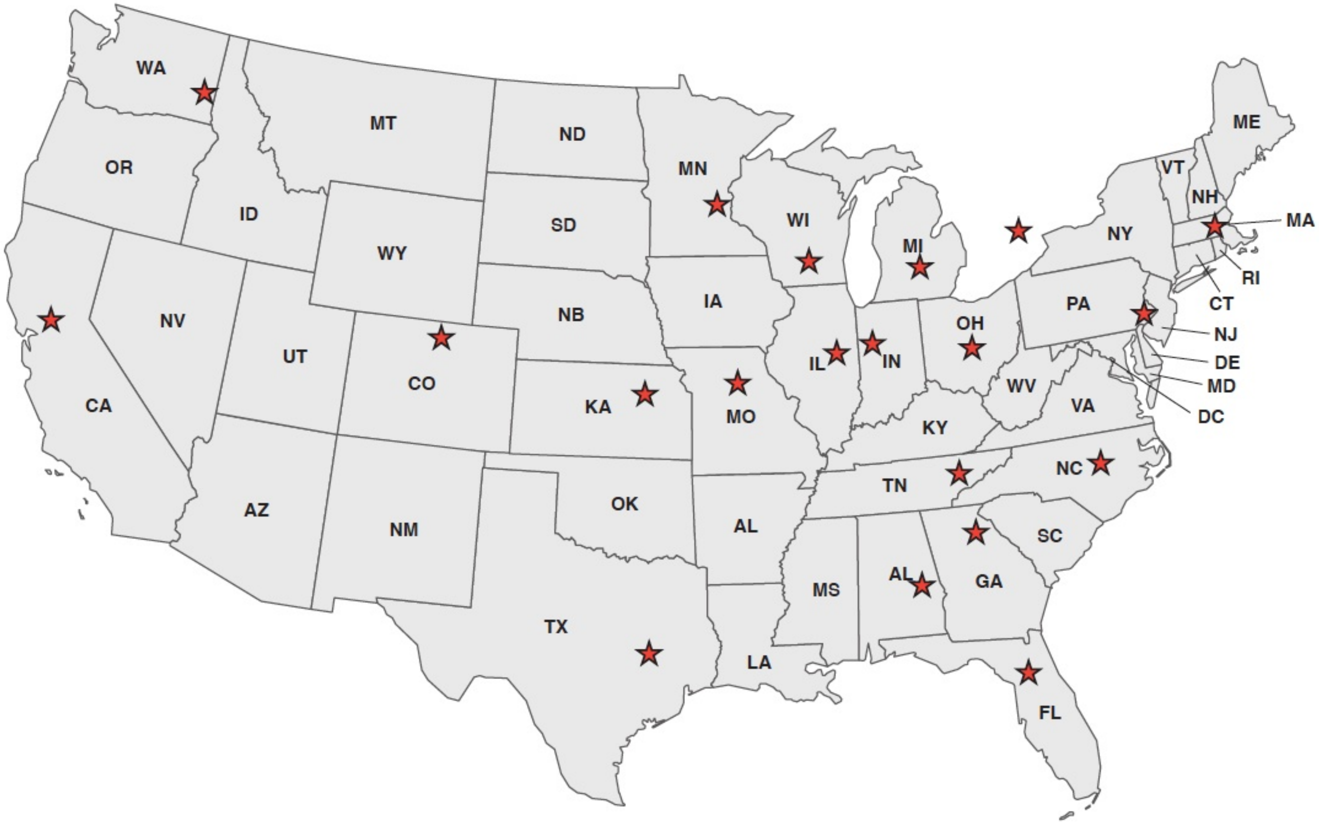
University of Missouri
Columbia, MO

University of Pennsylvania
Philadelphia, PA

University of Tennessee
Knoxville, TN

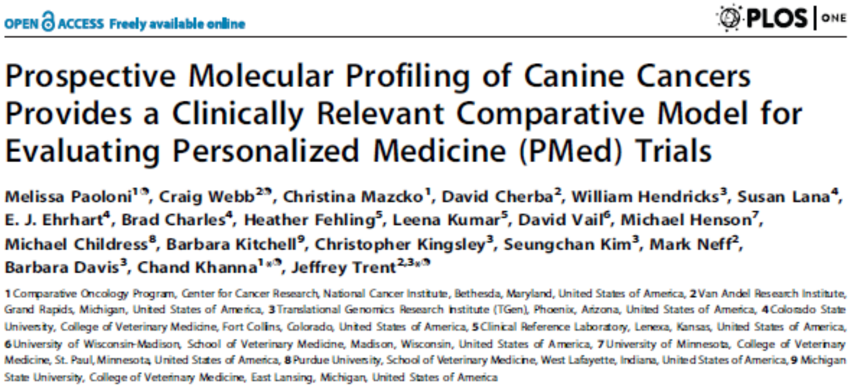
University of Wisconsin
Madison, WI

Washington State University
Pullman, WA



Dr. Amy LeBlanc

COTC016

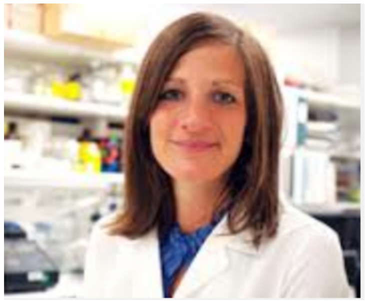

OPEN ACCESS Freely available online

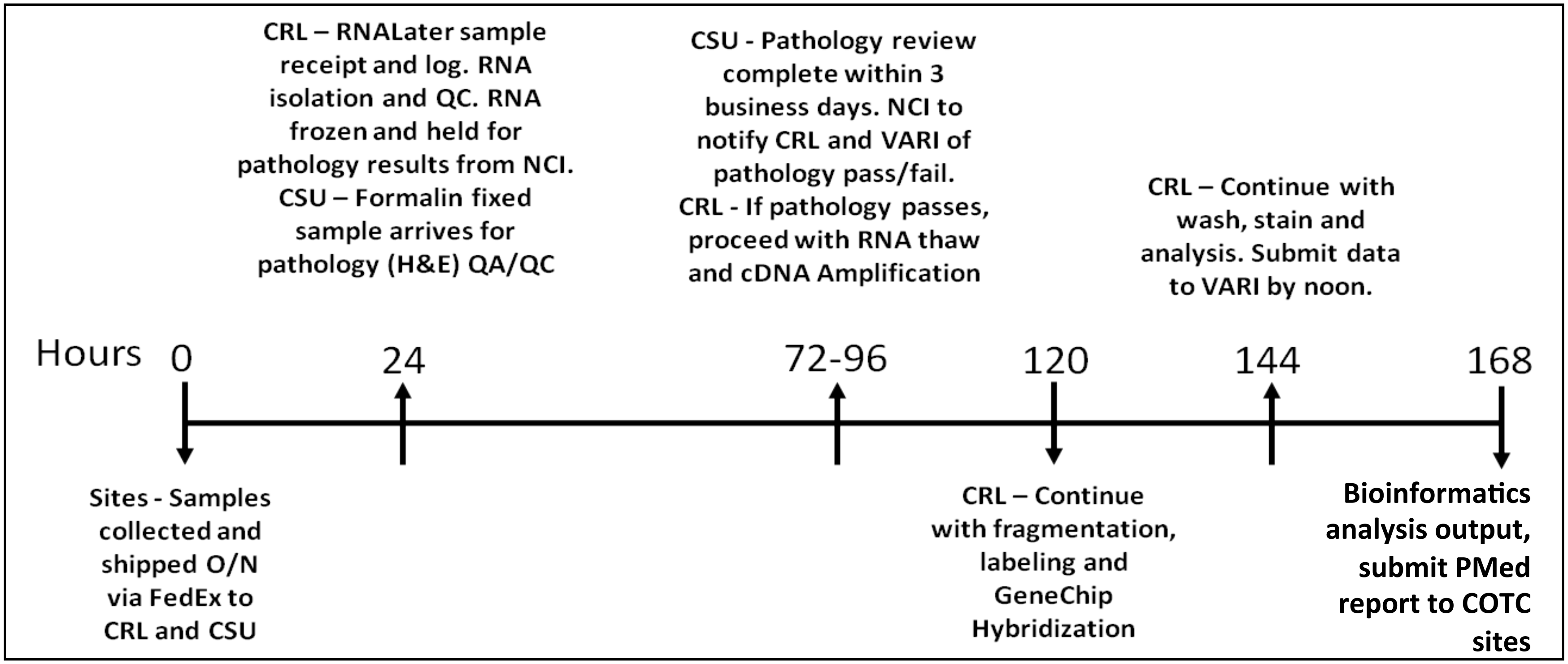
PLOS | ONE

Prospective Molecular Profiling of Canine Cancers Provides a Clinically Relevant Comparative Model for Evaluating Personalized Medicine (PMed) Trials

Melissa Paoloni[1¶], Craig Webb[2¶], Christina Mazcko[1], David Cherba[2], William Hendricks[3], Susan Lana[4], E. J. Ehrhart[4], Brad Charles[4], Heather Fehling[5], Leena Kumar[5], David Vail[6], Michael Henson[7], Michael Childress[8], Barbara Kitchell[9], Christopher Kingsley[3], Seungchan Kim[3], Mark Neff[2], Barbara Davis[3], Chand Khanna[1*¶], Jeffrey Trent[2,3*¶]

1 Comparative Oncology Program, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, United States of America, 2 Van Andel Research Institute, Grand Rapids, Michigan, United States of America, 3 Translational Genomics Research Institute (TGen), Phoenix, Arizona, United States of America, 4 Colorado State University, College of Veterinary Medicine, Fort Collins, Colorado, United States of America, 5 Clinical Reference Laboratory, Lenexa, Kansas, United States of America, 6 University of Wisconsin-Madison, School of Veterinary Medicine, Madison, Wisconsin, United States of America, 7 University of Minnesota, College of Veterinary Medicine, St. Paul, Minnesota, United States of America, 8 Purdue University, School of Veterinary Medicine, West Lafayette, Indiana, United States of America, 9 Michigan State University, College of Veterinary Medicine, East Lansing, Michigan, United States of America




Hours

0

Sites - Samples collected and shipped O/N via FedEx to CRL and CSU

24

CRL – RNALater sample receipt and log. RNA isolation and QC. RNA frozen and held for pathology results from NCI. CSU – Formalin fixed sample arrives for pathology (H&E) QA/QC

72-96

CSU - Pathology review complete within 3 business days. NCI to notify CRL and VARI of pathology pass/fail. CRL - If pathology passes, proceed with RNA thaw and cDNA Amplification

120

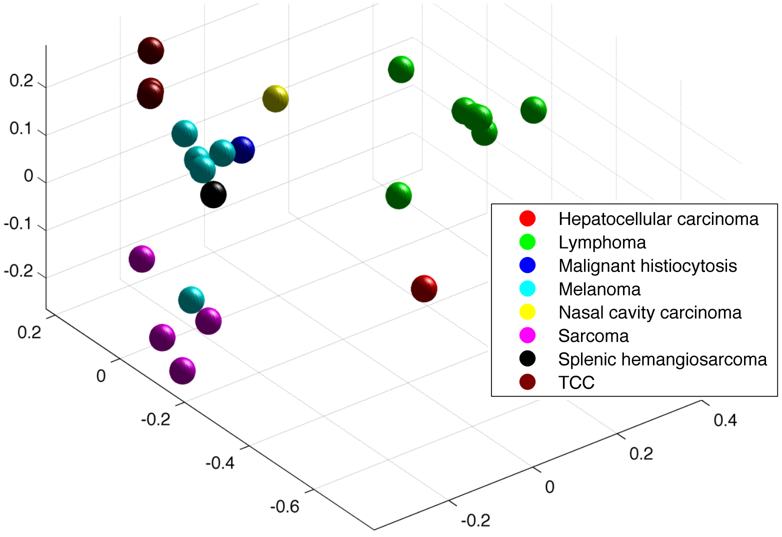
CRL – Continue with fragmentation, labeling and GeneChip Hybridization

144

CRL – Continue with wash, stain and analysis. Submit data to VARI by noon.

168

Bioinformatics analysis output, submit PMed report to COTC sites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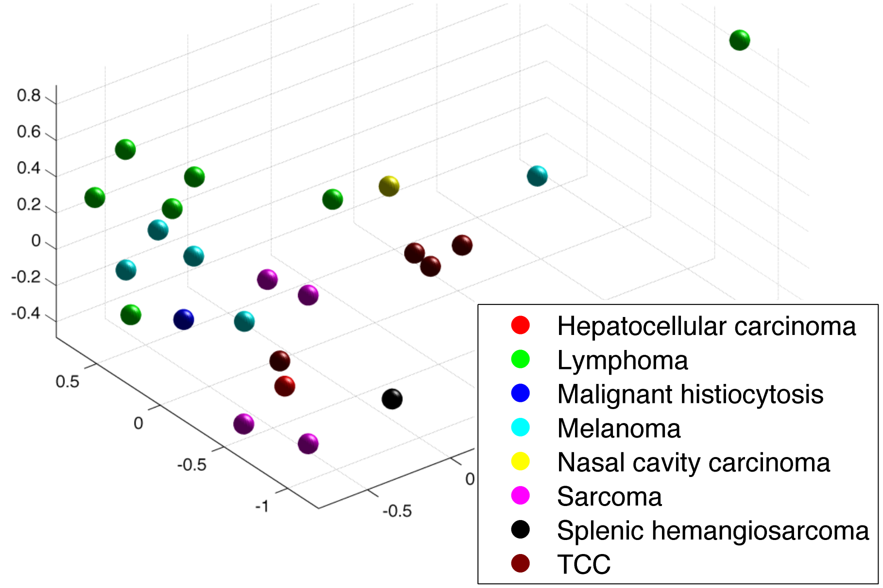
● Hepatocellular carcinoma
● Lymphoma
● Malignant histiocytosis
● Melanoma
● Nasal cavity carcinoma
● Sarcoma
● Splenic hemangiosarcoma
● TCC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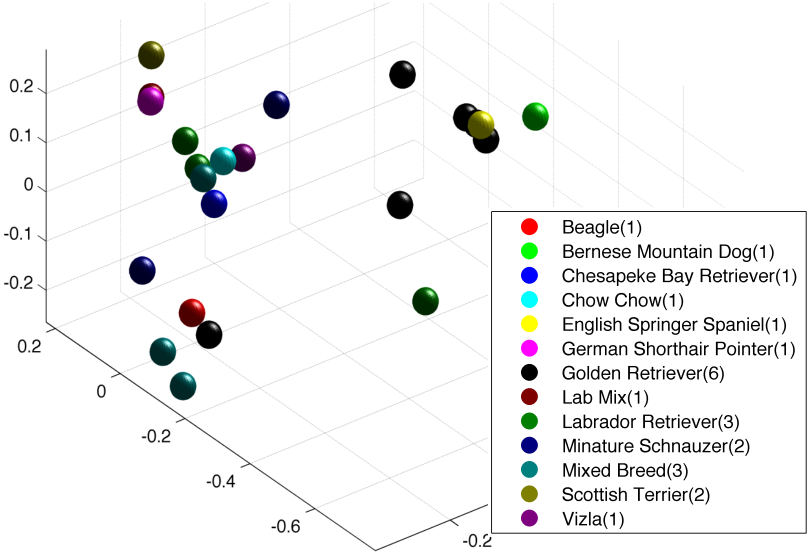
● Hepatocellular carcinoma
● Lymphoma
● Malignant histiocytosis
● Melanoma
● Nasal cavity carcinoma
● Sarcoma
● Splenic hemangiosarcoma
● TCC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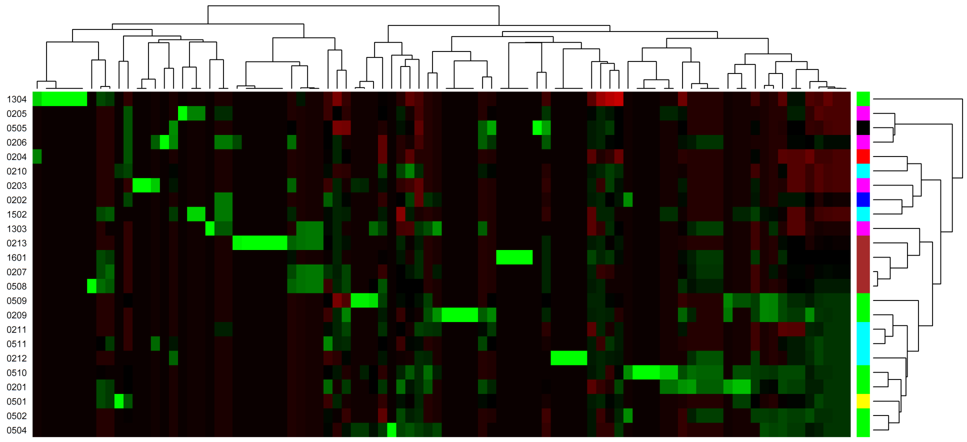
● Beagle(1)
● Bernese Mountain Dog(1)
● Chesapeke Bay Retriever(1)
● Chow Chow(1)
● English Springer Spaniel(1)
● German Shorthair Pointer(1)
● Golden Retriever(6)
● Lab Mix(1)
● Labrador Retriever(3)
● Minature Schnauzer(2)
● Mixed Breed(3)
● Scottish Terrier(2)
● Vizla(1)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0508
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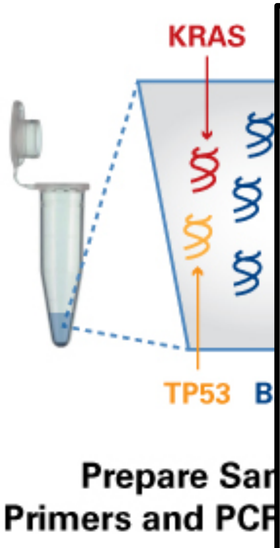
0511
0212
0510
0201
0501
0502
0504

Canine-Specific Resources

» CanFam 3 exomes in development (Roche, Agilent others)

» Targeted amplicon sequencing developed at TGen via Raindance/Illumina



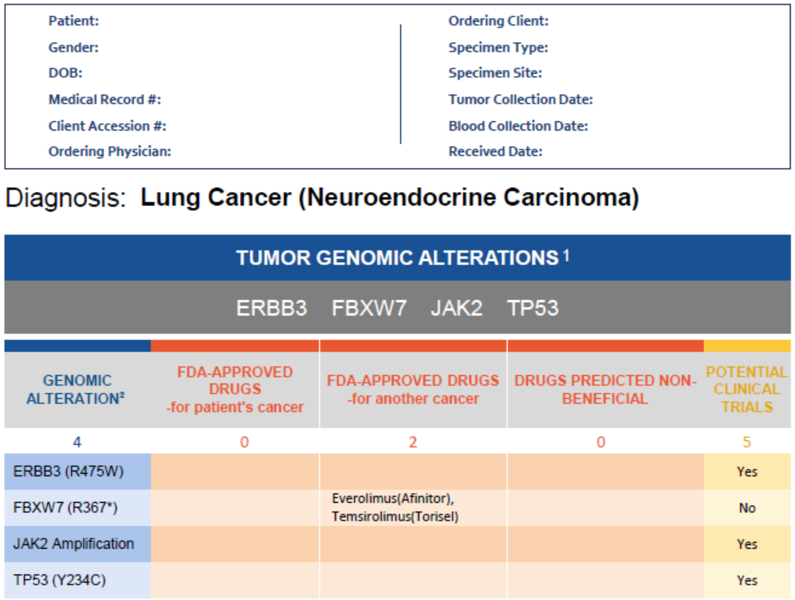
TGen developed drug rule database

- Literature-derived
- Actively curated
- Lifted to canine, supplemented with canine-specific rules

Canine s

ABL1	BCL6	EGFR	FGFR2	HRAS	KIT	NRAS	RB1	STK11
AKT1	BRAF	ERBB2	FGFR3	IDH1	KRAS	PDGFRA	RET	TP53
ALK	CDH1	ERBB4	FLT3	IDH2	MET	PIK3CA	SMAD4	VHL
APC	CDKN2A	EZH2	GNAQ	JAK2	MLH1	PTEN	SMARCB1	
ATM	CSF1R	FBXW7	GNAS	JAK3	NOTCH1	PTPN11	SMO	
BCL2	CTNNB1	FGFR1	HNF1A	KDR	NPM1	PTPRJ	SRC	

Canine Cancer Panel



Patient:		**Ordering Client:**	
Gender:		**Specimen Type:**	
DOB:		**Specimen Site:**	
Medical Record #:		**Tumor Collection Date:**	
Client Accession #:		**Blood Collection Date:**	
Ordering Physician:		**Received Date:**	

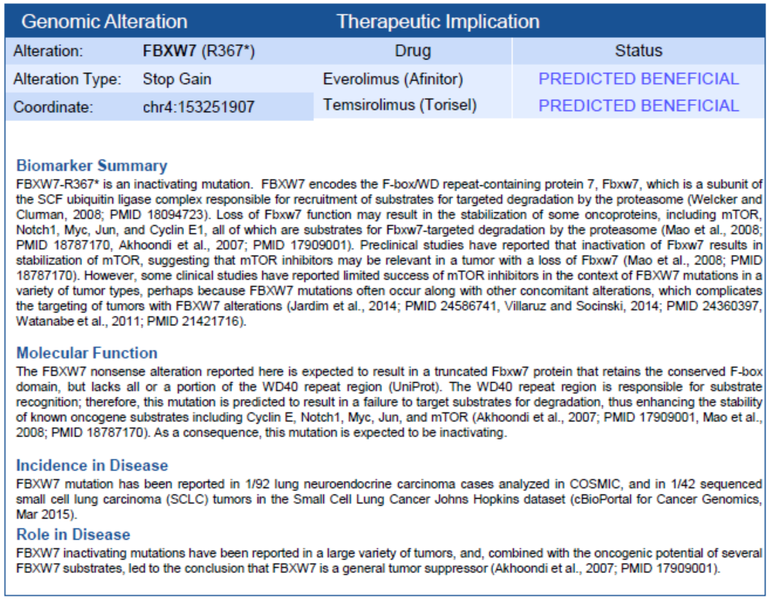
Diagnosis: **Lung Cancer (Neuroendocrine Carcinoma)**

TUMOR GENOMIC ALTERATIONS [1]

ERBB3 FBXW7 JAK2 TP53

GENOMIC ALTERATION[2]	FDA-APPROVED DRUGS -for patient's cancer	FDA-APPROVED DRUGS -for another cancer	DRUGS PREDICTED NON-BENEFICIAL	POTENTIAL CLINICAL TRIALS
4	0	2	0	5
ERBB3 (R475W)				Yes
FBXW7 (R367*)		Everolimus(Afinitor), Temsirolimus(Torisel)		No
JAK2 Amplification				Yes
TP53 (Y234C)				Yes

Canine Cancer Panel – Genomic Alterations Detail



Genomic Alteration		Therapeutic Implication	
Alteration:	**FBXW7** (R367*)	Drug	Status
Alteration Type:	Stop Gain	Everolimus (Afinitor)	PREDICTED BENEFICIAL
Coordinate:	chr4:153251907	Temsirolimus (Torisel)	PREDICTED BENEFICIAL

Biomarker Summary

FBXW7-R367* is an inactivating mutation. FBXW7 encodes the F-box/WD repeat-containing protein 7, Fbxw7, which is a subunit of the SCF ubiquitin ligase complex responsible for recruitment of substrates for targeted degradation by the proteasome (Welcker and Clurman, 2008; PMID 18094723). Loss of Fbxw7 function may result in the stabilization of some oncoproteins, including mTOR, Notch1, Myc, Jun, and Cyclin E1, all of which are substrates for Fbxw7-targeted degradation by the proteasome (Mao et al., 2008; PMID 18787170, Akhoondi et al., 2007; PMID 17909001). Preclinical studies have reported that inactivation of Fbxw7 results in stabilization of mTOR, suggesting that mTOR inhibitors may be relevant in a tumor with a loss of Fbxw7 (Mao et al., 2008; PMID 18787170). However, some clinical studies have reported limited success of mTOR inhibitors in the context of FBXW7 mutations in a variety of tumor types, perhaps because FBXW7 mutations often occur along with other concomitant alterations, which complicates the targeting of tumors with FBXW7 alterations (Jardim et al., 2014; PMID 24586741, Villaruz and Socinski, 2014; PMID 24360397, Watanabe et al., 2011; PMID 21421716).

Molecular Function

The FBXW7 nonsense alteration reported here is expected to result in a truncated Fbxw7 protein that retains the conserved F-box domain, but lacks all or a portion of the WD40 repeat region (UniProt). The WD40 repeat region is responsible for substrate recognition; therefore, this mutation is predicted to result in a failure to target substrates for degradation, thus enhancing the stability of known oncogene substrates including Cyclin E, Notch1, Myc, Jun, and mTOR (Akhoondi et al., 2007; PMID 17909001, Mao et al., 2008; PMID 18787170). As a consequence, this mutation is expected to be inactivating.

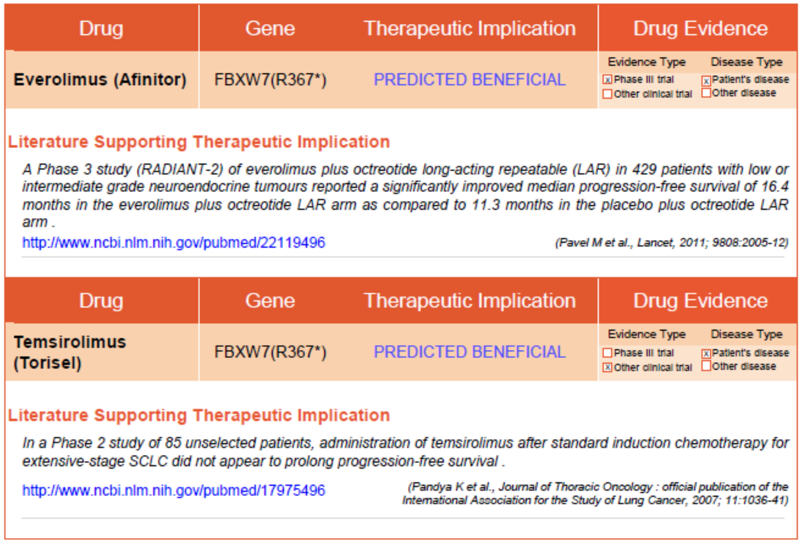
Incidence in Disease

FBXW7 mutation has been reported in 1/92 lung neuroendocrine carcinoma cases analyzed in COSMIC, and in 1/42 sequenced small cell lung carcinoma (SCLC) tumors in the Small Cell Lung Cancer Johns Hopkins dataset (cBioPortal for Cancer Genomics, Mar 2015).

Role in Disease

FBXW7 inactivating mutations have been reported in a large variety of tumors, and, combined with the oncogenic potential of several FBXW7 substrates, led to the conclusion that FBXW7 is a general tumor suppressor (Akhoondi et al., 2007; PMID 17909001).

Canine Cancer Panel – Drug Evidence Detail



Drug	Gene	Therapeutic Implication	Drug Evidence	
			Evidence Type	Disease Type
Everolimus (Afinitor)	FBXW7(R367*)	PREDICTED BENEFICIAL	☒ Phase III trial ☐ Other clinical trial	☒ Patient's disease ☐ Other disease

Literature Supporting Therapeutic Implication

A Phase 3 study (RADIANT-2) of everolimus plus octreotide long-acting repeatable (LAR) in 429 patients with low or intermediate grade neuroendocrine tumours reported a significantly improved median progression-free survival of 16.4 months in the everolimus plus octreotide LAR arm as compared to 11.3 months in the placebo plus octreotide LAR arm .

http://www.ncbi.nlm.nih.gov/pubmed/22119496 *(Pavel M et al., Lancet, 2011; 9808:2005-12)*

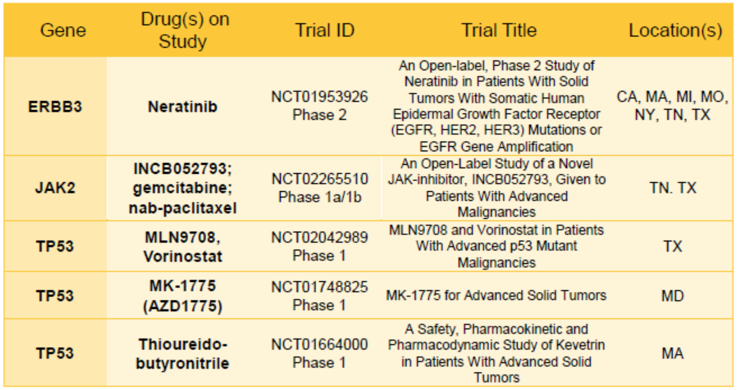
Drug	Gene	Therapeutic Implication	Drug Evidence	
			Evidence Type	Disease Type
Temsirolimus (Torisel)	FBXW7(R367*)	PREDICTED BENEFICIAL	☐ Phase III trial ☒ Other clinical trial	☒ Patient's disease ☐ Other disease

Literature Supporting Therapeutic Implication

In a Phase 2 study of 85 unselected patients, administration of temsirolimus after standard induction chemotherapy for extensive-stage SCLC did not appear to prolong progression-free survival .

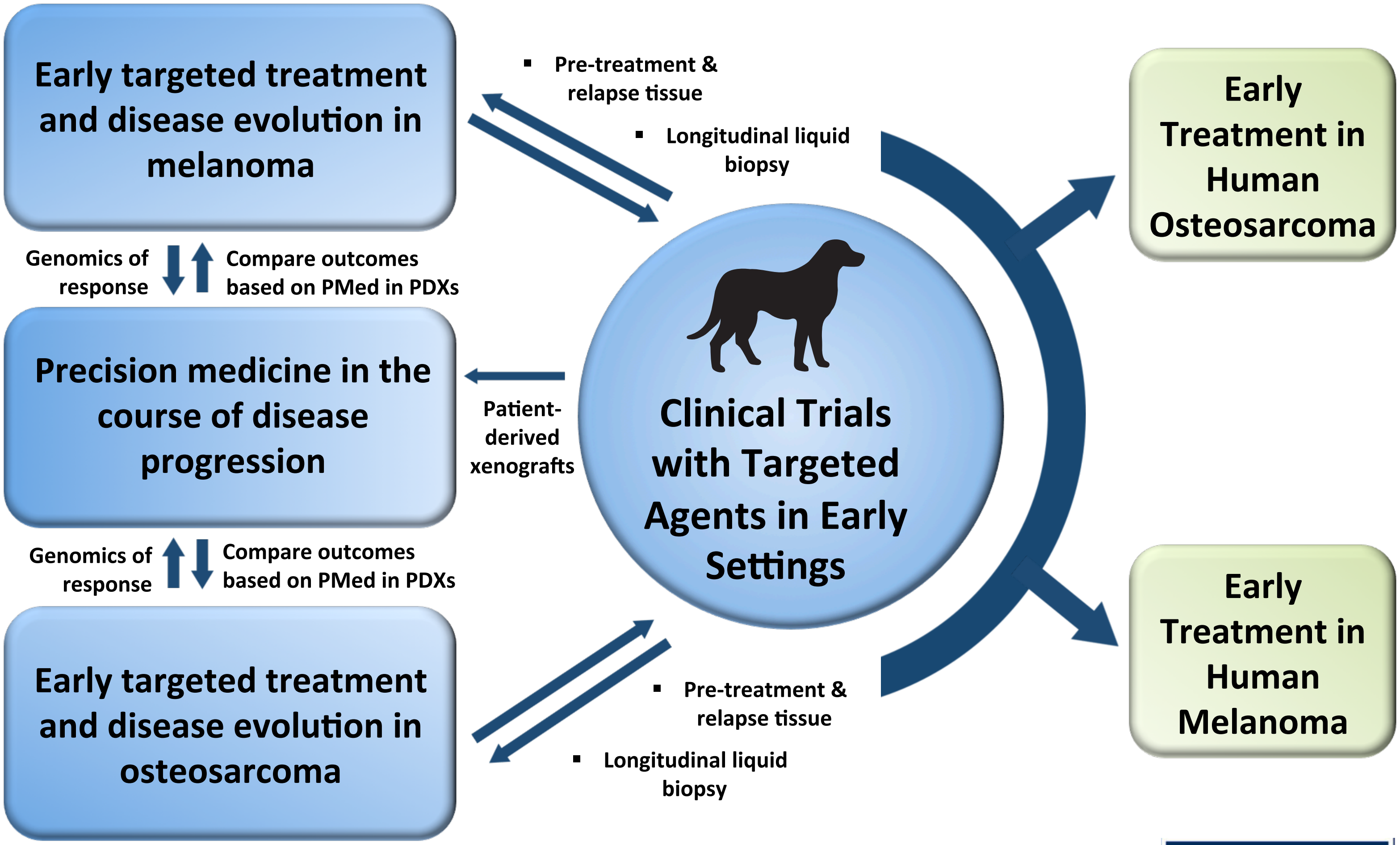
http://www.ncbi.nlm.nih.gov/pubmed/17975496 *(Pandya K et al., Journal of Thoracic Oncology : official publication of the International Association for the Study of Lung Cancer, 2007; 11:1036-41)*

Canine Cancer Panel – Clinical Trials Detail



Gene	Drug(s) on Study	Trial ID	Trial Title	Location(s)
ERBB3	**Neratinib**	NCT01953926 Phase 2	An Open-label, Phase 2 Study of Neratinib in Patients With Solid Tumors With Somatic Human Epidermal Growth Factor Receptor (EGFR, HER2, HER3) Mutations or EGFR Gene Amplification	CA, MA, MI, MO, NY, TN, TX
JAK2	**INCB052793; gemcitabine; nab-paclitaxel**	NCT02265510 Phase 1a/1b	An Open-Label Study of a Novel JAK-inhibitor, INCB052793, Given to Patients With Advanced Malignancies	TN. TX
TP53	**MLN9708, Vorinostat**	NCT02042989 Phase 1	MLN9708 and Vorinostat in Patients With Advanced p53 Mutant Malignancies	TX
TP53	**MK-1775 (AZD1775)**	NCT01748825 Phase 1	MK-1775 for Advanced Solid Tumors	MD
TP53	**Thioureido-butyronitrile**	NCT01664000 Phase 1	A Safety, Pharmacokinetic and Pharmacodynamic Study of Kevetrin in Patients With Advanced Solid Tumors	MA

- Precision Medicine in Human Cancer
- Canine Cancer Genomic Landscapes
- Emerging Canine PMed Tools
- Opportunities in Comparative Oncology



Early targeted treatment and disease evolution in melanoma

- Pre-treatment & relapse tissue
- Longitudinal liquid biopsy

Genomics of response

Compare outcomes based on PMed in PDXs

Precision medicine in the course of disease progression

Patient-derived xenografts

Genomics of response

Compare outcomes based on PMed in PDXs

Early targeted treatment and disease evolution in osteosarcoma

- Pre-treatment & relapse tissue
- Longitudinal liquid biopsy

Clinical Trials with Targeted Agents in Early Settings

Early Treatment in Human Osteosarcoma

Early Treatment in Human Melanoma

OHIO STATE

THE UNIVERSITY OF TEXAS
MD Anderson ~~Cancer~~ Center
Making Cancer History®

NATIONAL CANCER INSTITUTE®

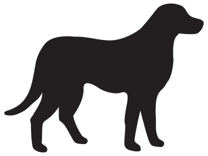
Yale
UNIVERSITY

tgen®

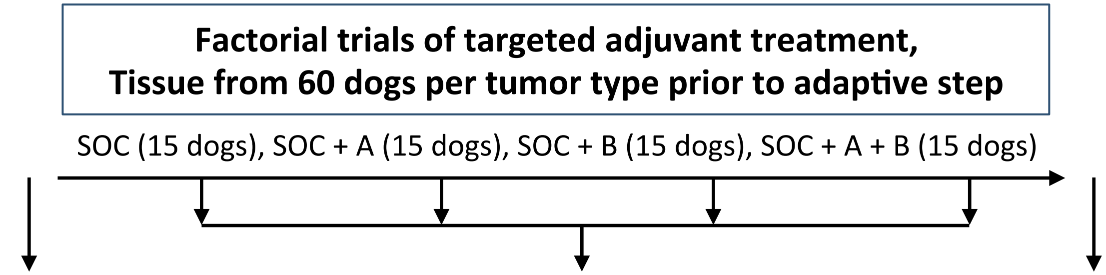
Colorado State University
College of Veterinary Medicine and Biomedical Sciences

J. Craig Venter™
INSTITUTE

Clinical Trial Sample Flowchart for Correlative Biology



Canine patient with primary melanoma or osteosarcoma. Surgical resection to no evidence of disease at COTC site



Factorial trials of targeted adjuvant treatment,
Tissue from 60 dogs per tumor type prior to adaptive step

SOC (15 dogs), SOC + A (15 dogs), SOC + B (15 dogs), SOC + A + B (15 dogs)

Pre-treatment surgical specimen and blood

Core B COTC

- Pathology
- PK/PD

Core C Genomics

- LI-WGS
- Exome-seq
- mRNA-seq

Projects 1 and 2 SA3

- Longitudinal ctDNA analysis

Project 3

- PDXs

Longitudinal blood

Core B COTC

- PK/PD

Projects 1 and 2 SA3

- Longitudinal ctDNA analysis

Relapse tissue

Core B COTC

- PK/PD

Core C Genomics

- LI-WGS
- Exome-seq
- mRNA-seq

Projects 1 and 2 SA3

- Longitudinal ctDNA analysis

Project 3

- PDXs

Precision Medicine Studies

Rare childhood disorders

DORRANCE CENTER FOR RARE CHILDHOOD DISORDERS

Multiple Myeloma

MMRF MULTIPLE MYELOMA Research Foundation

Adult Glioblastoma

THE BEN & CATHERINE IVY FOUNDATION
Funding Patient-Focused Research on Gliomas

Breast Cancer

SIDEOUT FOUNDATION

Pediatric brain tumor

Pacific Pediatric Neuro-Oncology Consortium

UCSF

Melanoma

STAND UP TO CANCER

AACR American Association for Cancer Research

Melanoma Research Alliance

Neuroblastoma

Michael & Susan Dell FOUNDATION

Lung cancer

STAND UP TO CANCER

Childhood Cancer

McDonald Hyundai Hope Foundation

Rare Cancers

NATIONAL FOUNDATION FOR CANCER RESEARCH
Research for a Cure

Partners & Collaborators

SCOTTSDALE HEALTHCARE

MAYO CLINIC

CHOC Children's
Children's Hospital of Orange County

Children's National

Memorial Sloan Kettering Cancer Center

USC

OHIO STATE

DANA-FARBER CANCER INSTITUTE

The Children's Hospital of Philadelphia

Queensland Institute of Medical Research

THE UNIVERSITY OF TEXAS MD Anderson ~~Cancer~~ Center

THE UNIVERSITY OF UTAH

Van Andel Research Institute

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