Defining the Genomic Landscape of Canine Cancers

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Conflict of Interest Disclosure

I have financial interest, arrangement or affiliation with:

Name of Organization	**Relationship**
FidoCure	Scientific Advisory Board, consultant, grant funding
Merck Animal Health	Consultant

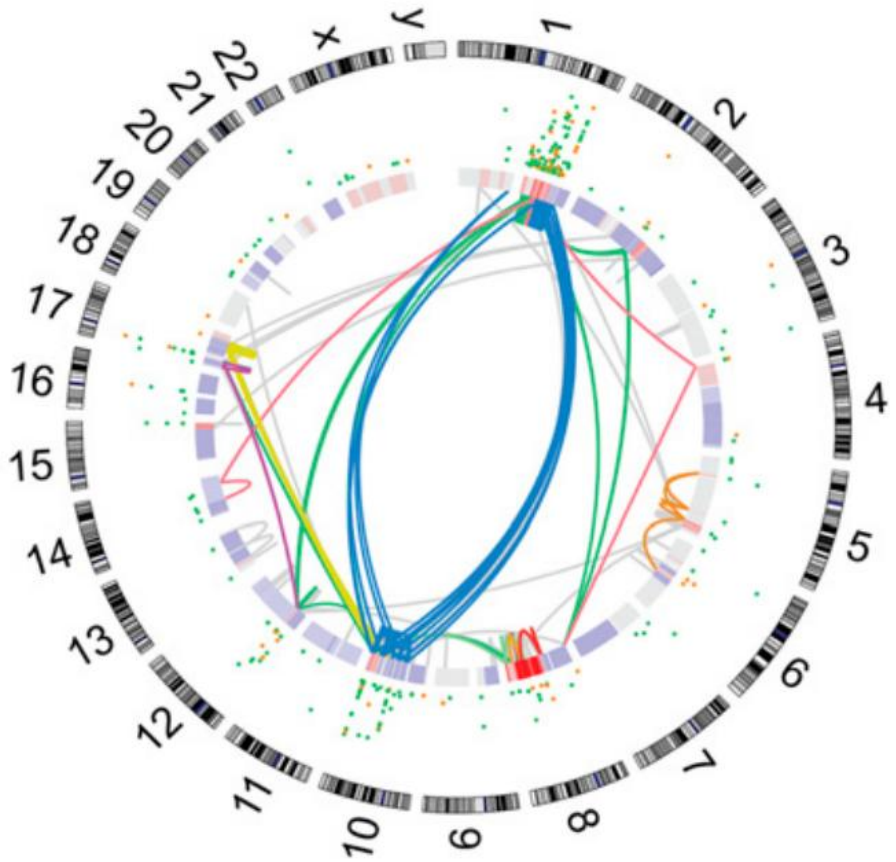
Learning Objectives

In this session, new methodologies being used to characterize canine cancers at the genomic level will be reviewed including various sequencing techniques, the role of liquid biopsy as a non-invasive diagnostic and monitoring tool, and currently available diagnostic platforms. The application of this knowledge to more effectively treat canine cancers with targeted therapies will be discussed.

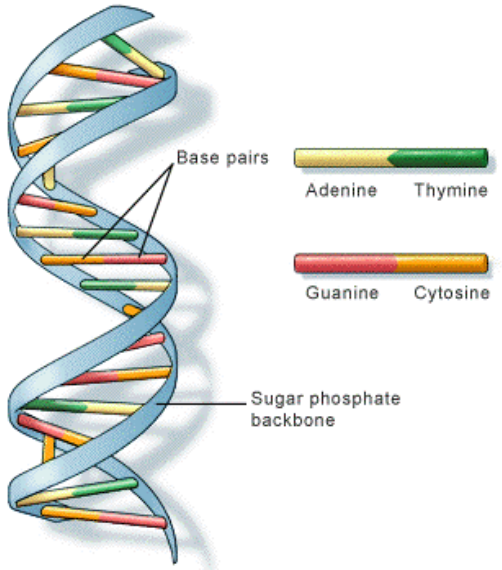
Learning Objectives:

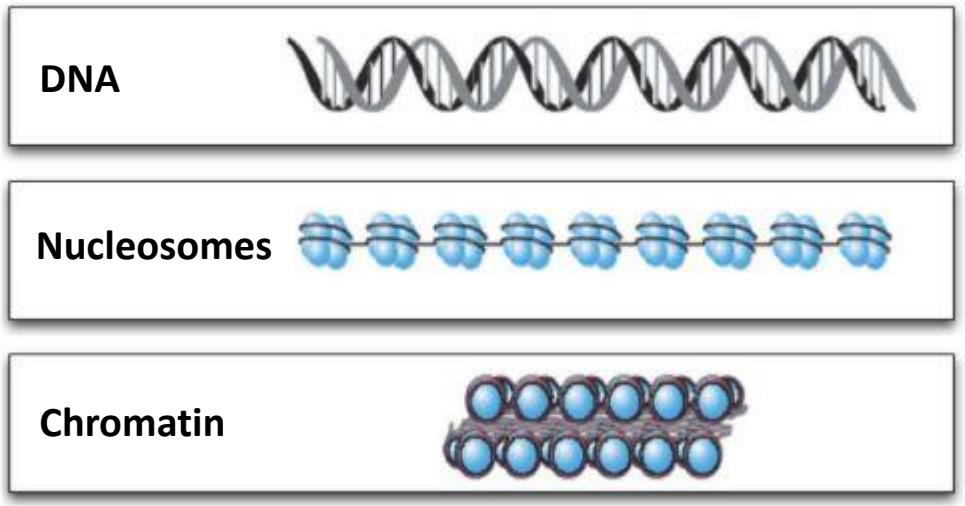
1. Understand the advancements in methods and tools being used to define the genetic drivers of canine cancer.
2. Understand the principles of liquid biopsy and its utility for cancer screening and monitoring.
3. Understand how information derived from genomic analysis of canine cancers can be used to facilitate application of targeted therapies.

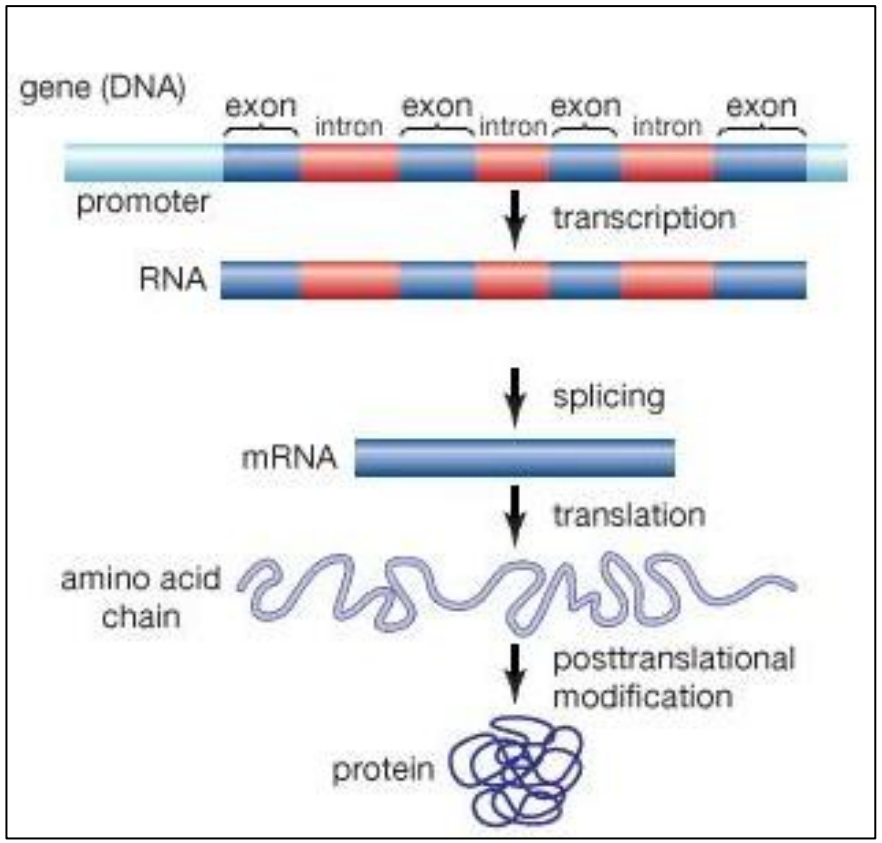
Dissecting the Genomic Landscape of Cancer



Normal DNA structure and gene expression



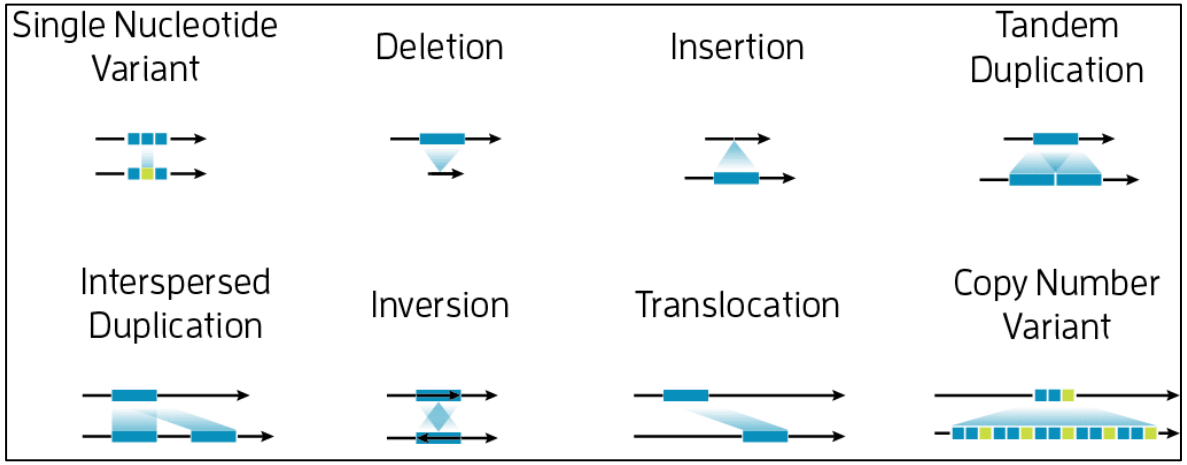




DNA Mutations and Cancer

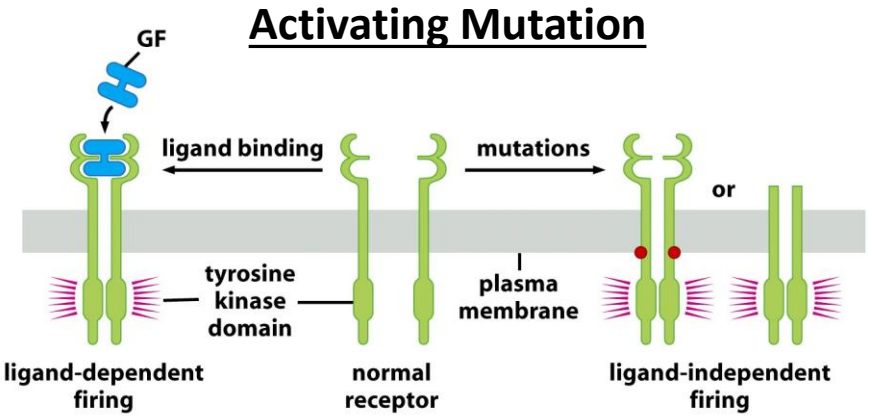
DNA can be disrupted in many ways in tumor cells.

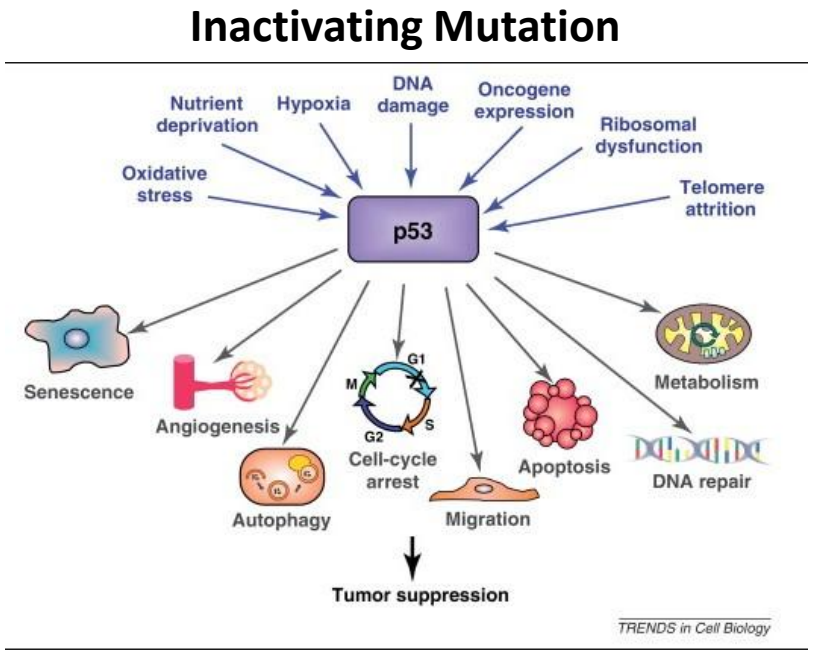
- **Single Nucleotide Variants or Polymorphisms (SNVs/SNPs):** these are a single base pair substitution that do not change the *number* of nucleotides in the DNA sequence
- **Insertions and Deletions (Indels):** these involve fewer than 50 base pairs and may substantially alter the subsequent protein structure by creating stop codons, etc.
- **Structural Variants (SVs):** these consist of changes in the DNA greater than 50 base pairs in size (deletions, insertions, translocations, etc.).



Effects of Genomic Changes on Cell Function

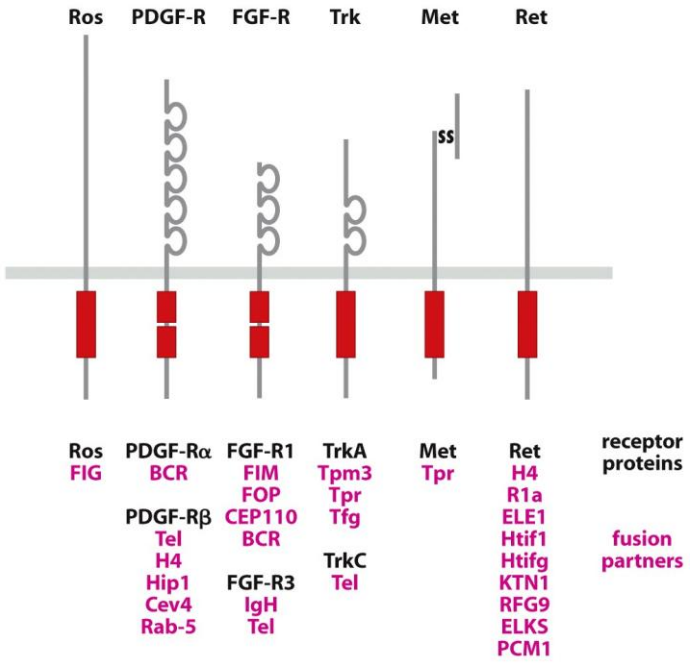
- Mutations may activate the protein resulting in unregulated cell signaling and proliferation.
- Mutations may inactivate a protein resulting in the loss of capacity for cells to die.
- Changes in gene copy number can lead to massive overexpression of a protein and activation of a signaling pathway.



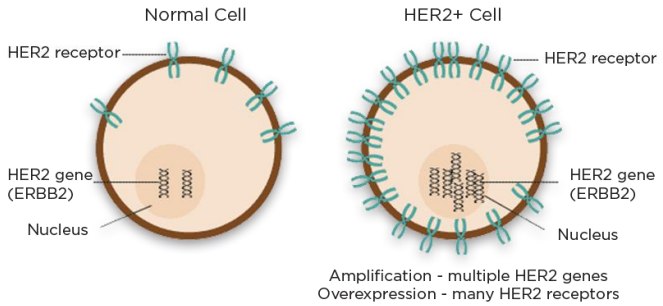


Examples of Genomic Disruption in Cancer

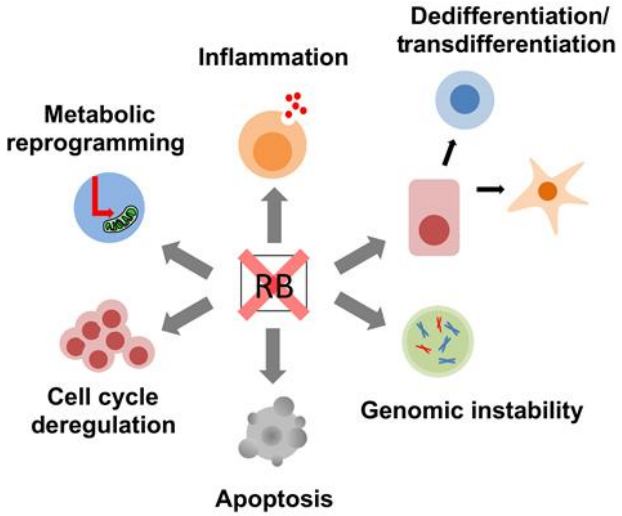
Translocations/Fusion Proteins



Overexpression

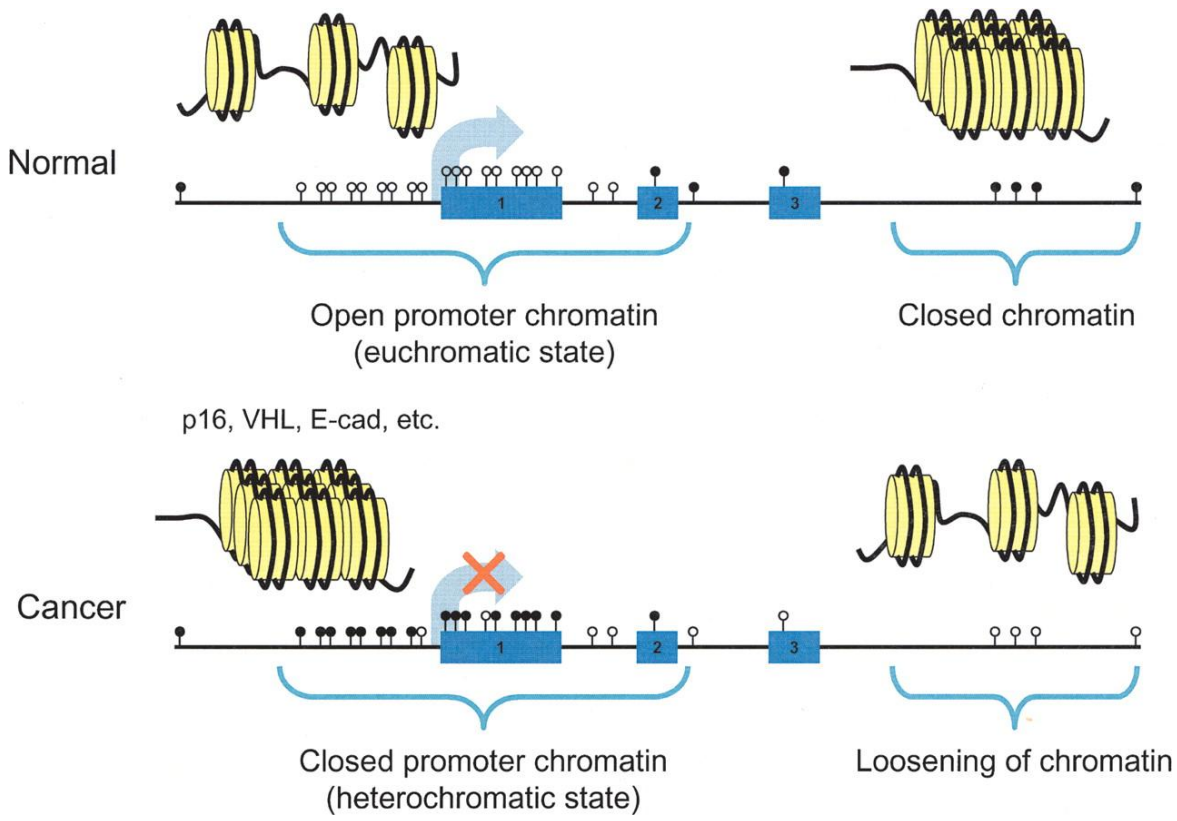


Inactivation



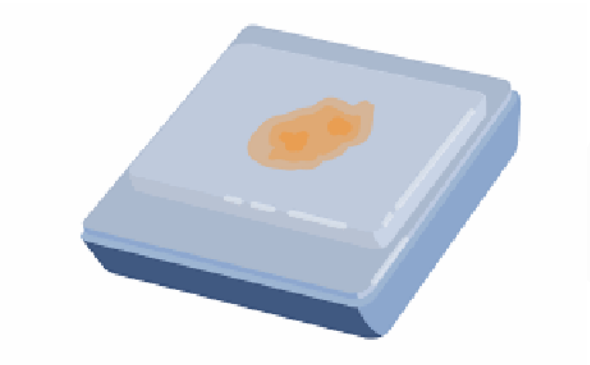
Epigenetic Alterations of DNA and Cancer

- Epigenetic changes occur when parts of the DNA or the support structure for DNA (histones) are altered.
- These alterations interfere with the winding of DNA on histones or blocks binding of enzymes/factors necessary for gene expression.
- Epigenetic changes affect a variety of cell signaling pathways and influence the ability of tumor cells to evade the immune system, along with disrupting several other key biologic processes that ultimately promote tumor growth.



Ting et al. Genes Dev. 2006

Methods to Define Tumor Genome

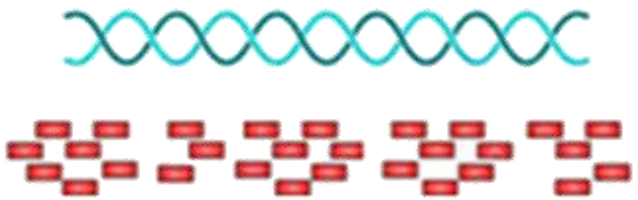


Methods to Evaluate DNA from Tissue Biopsy

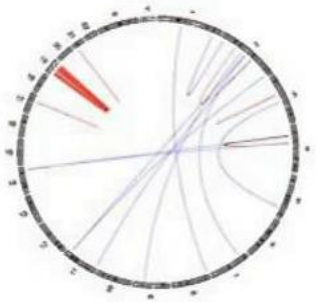
FROZEN

FFPE

Whole genome sequencing

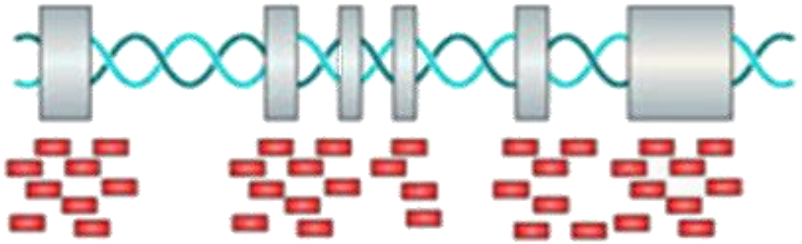


All genes, translocations and non-coding DNA

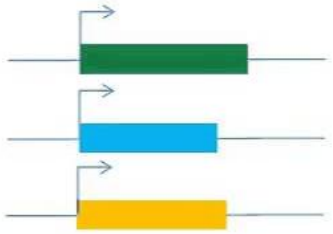


Lower coverage

Whole exome sequencing

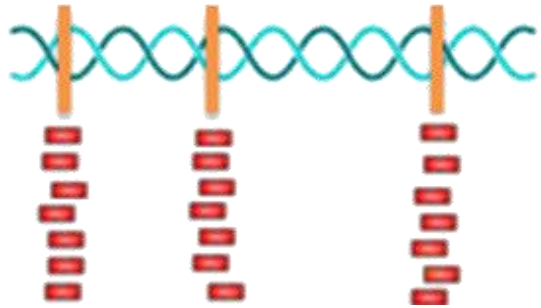


22,000 genes



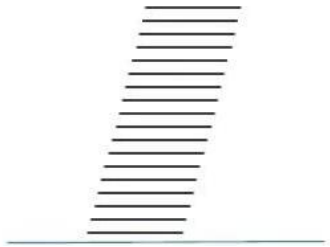
Intermediate coverage

Targeted sequencing



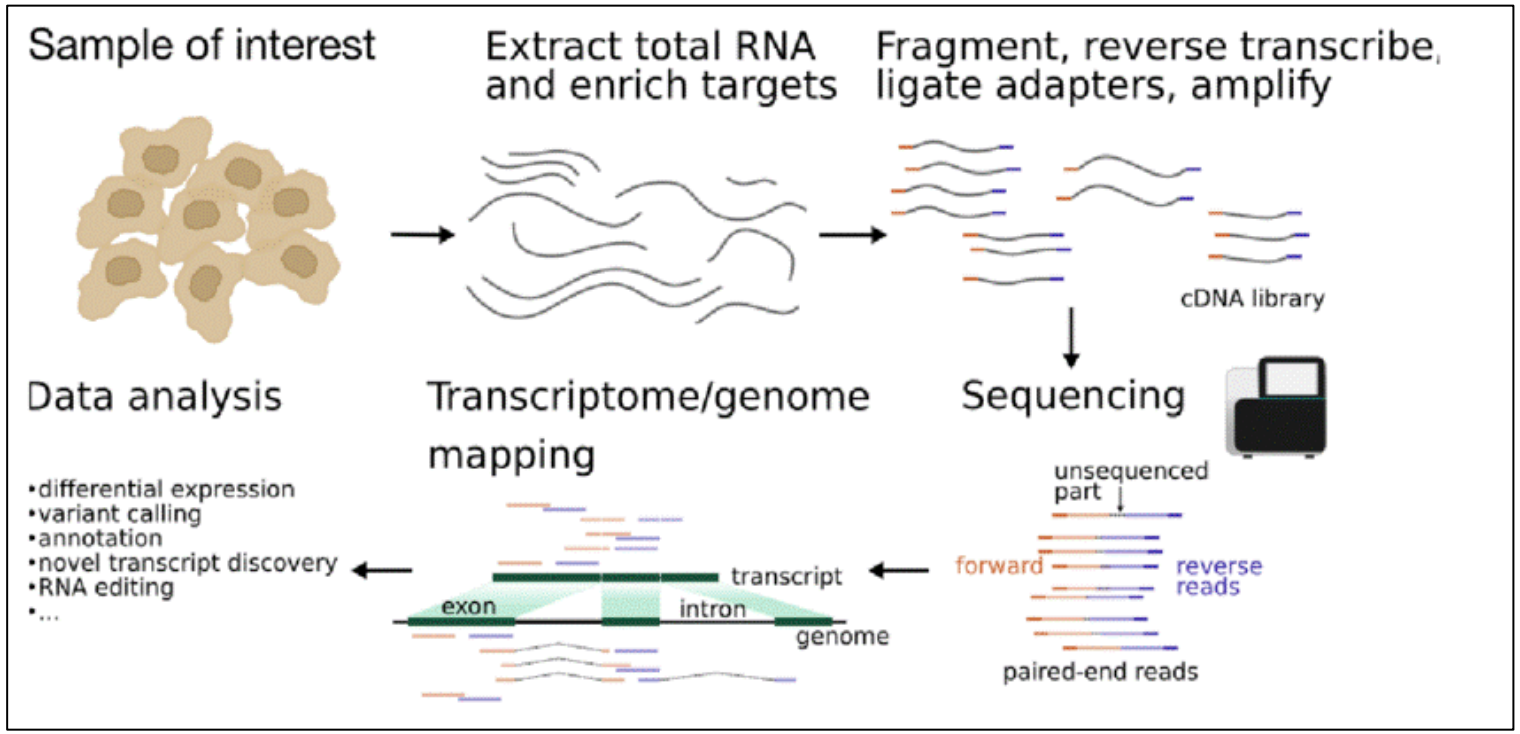
40-400 genes

High coverage

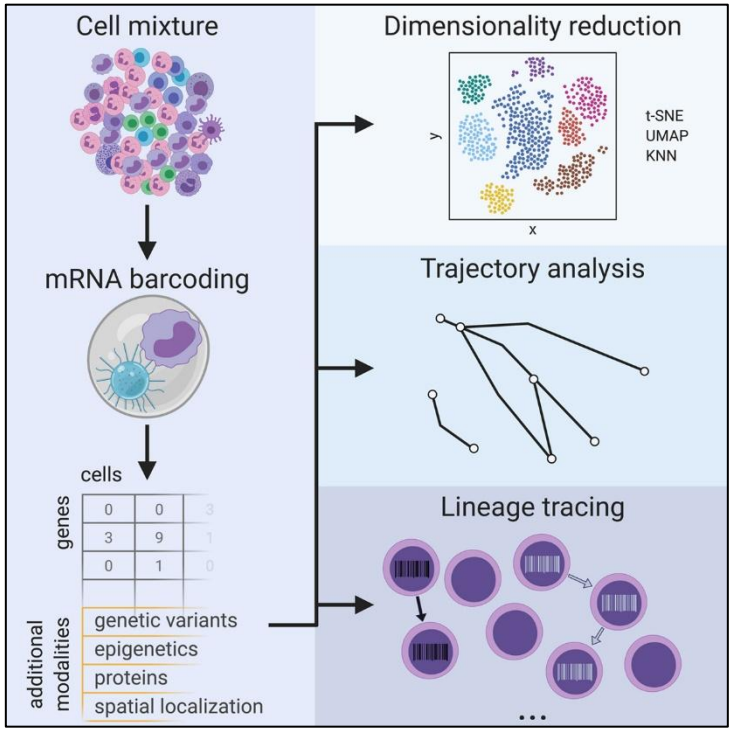


Methods to Evaluate RNA from Tissue

RNA Seq (frozen)



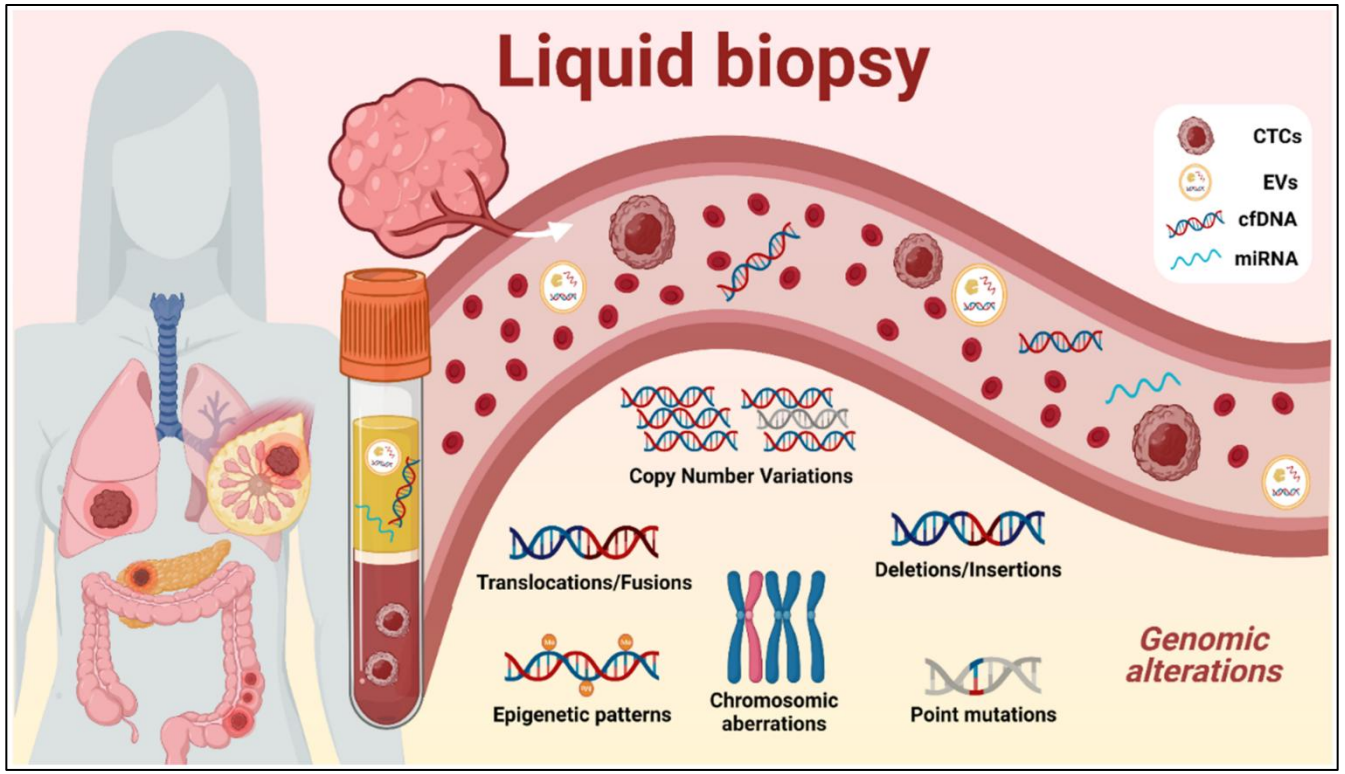
scRNA Seq (fresh/FFPE)



Acota et al, 2021; Van de Berge et al, 2018

Methods to Evaluate Tumor Genomics: Liquid Biopsy

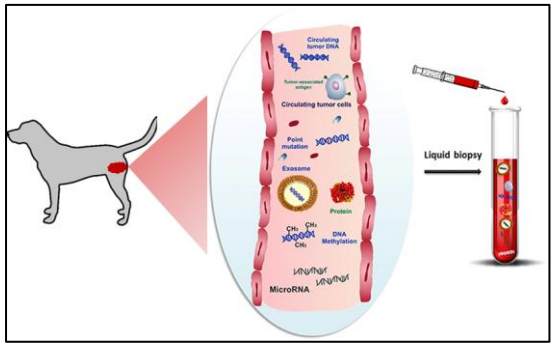
- **Tumors shed several key elements into the bloodstream including RNA, DNA, exosomes and other components.**
- **Methods have been developed to capture and analyze these various components, creating a mechanism for *liquid biopsy (blood biopsy).***



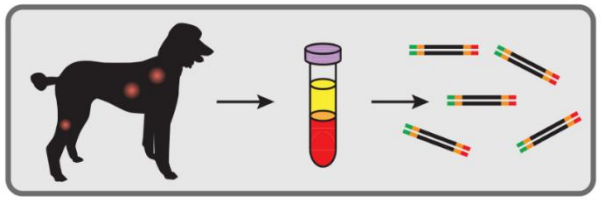
Palacin-Aliana, Biomedicines, 2021

Methods to Evaluate Tumor Genomics: Liquid Biopsy

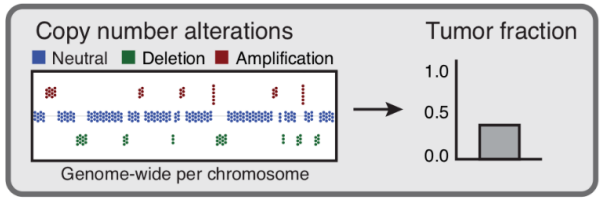
- **Blood is collected into special tubes that stabilizes cells and plasma.**
- **The cell-free DNA (cfDNA) in the plasma is a mix of normal DNA and DNA derived from the tumor (ctDNA); ctDNA can be quantified and analyzed.**

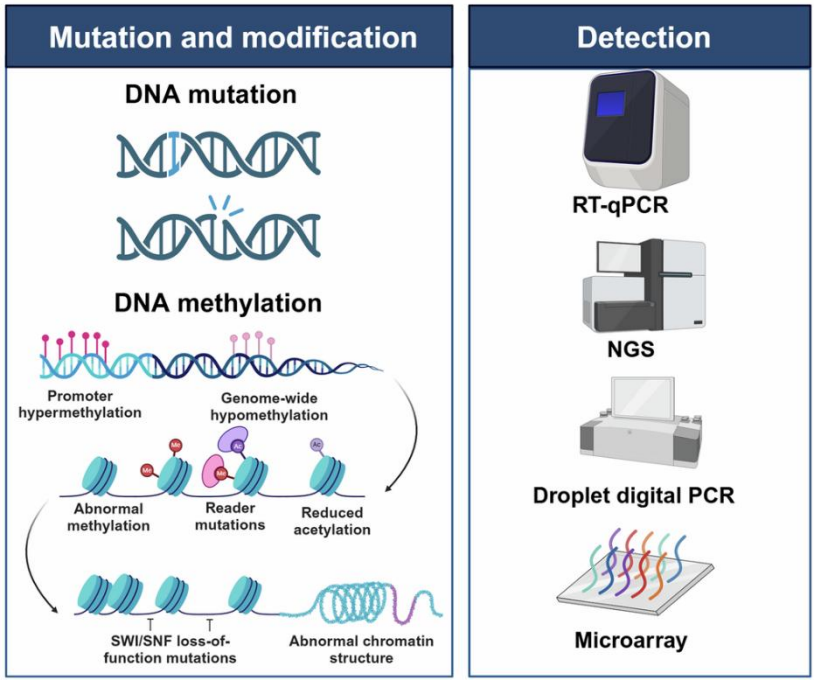


1) Cell-free DNA library construction



2) Ultra low-pass whole-genome sequencing (0.1x)

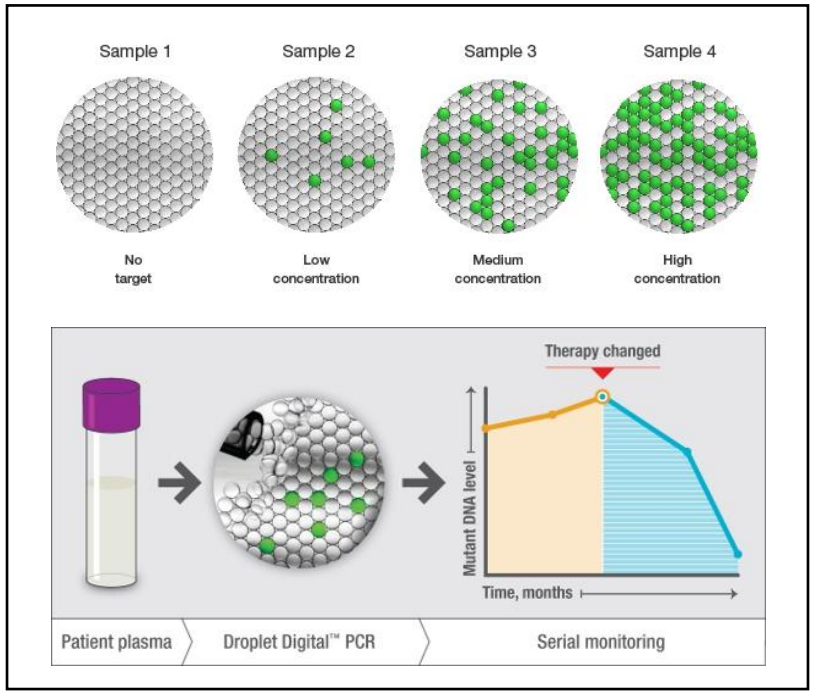


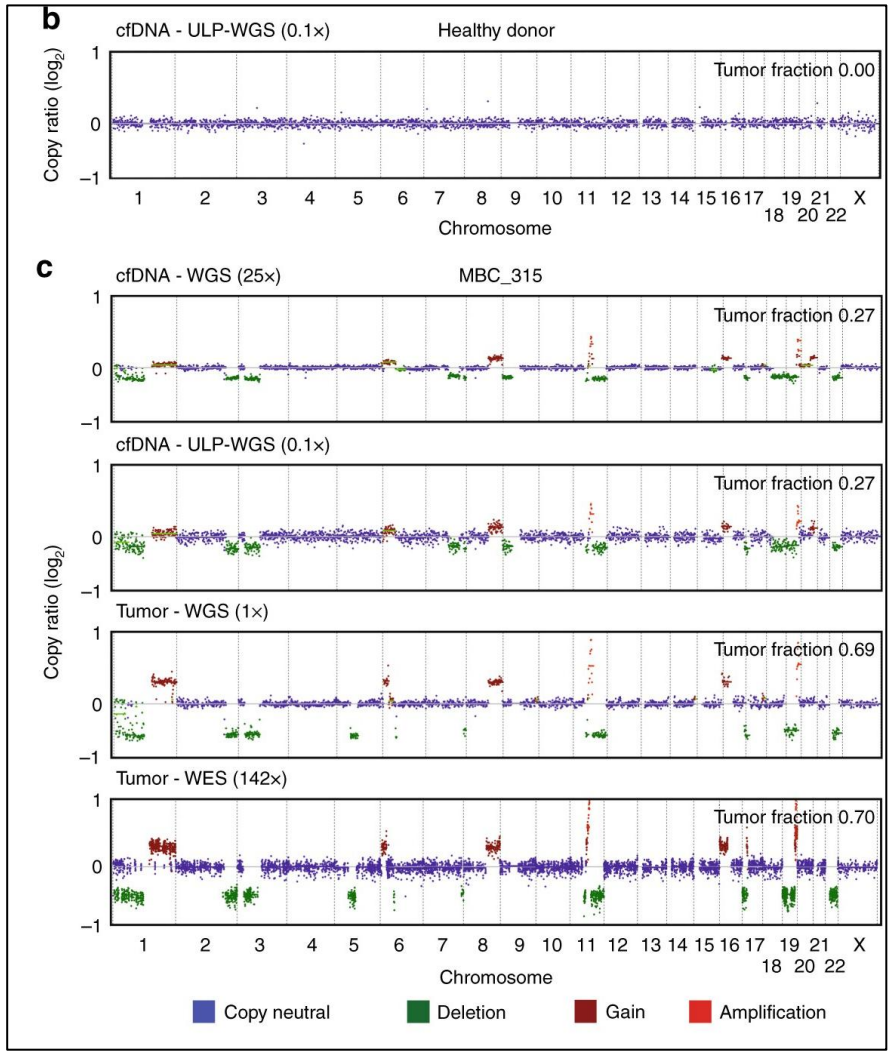


Adapted from Adalsteinsson, et al., Nat Comm, 2017; Ma et al, Signal Transduct Target Ther, 2024

Liquid Biopsy Application: ctDNA

- **Typical ctDNA analysis gives an estimate of copy number variation in the sample; there should be no extra copies in the absence of cancer.**
- **If the fraction of ctDNA is large enough then WES and/or WGS can be performed which often gives more information.**
- **ctDNA can also be used to quantify a specific target using ddPCR.**





Adalsteinsson, et al., Nat Comm, 2017

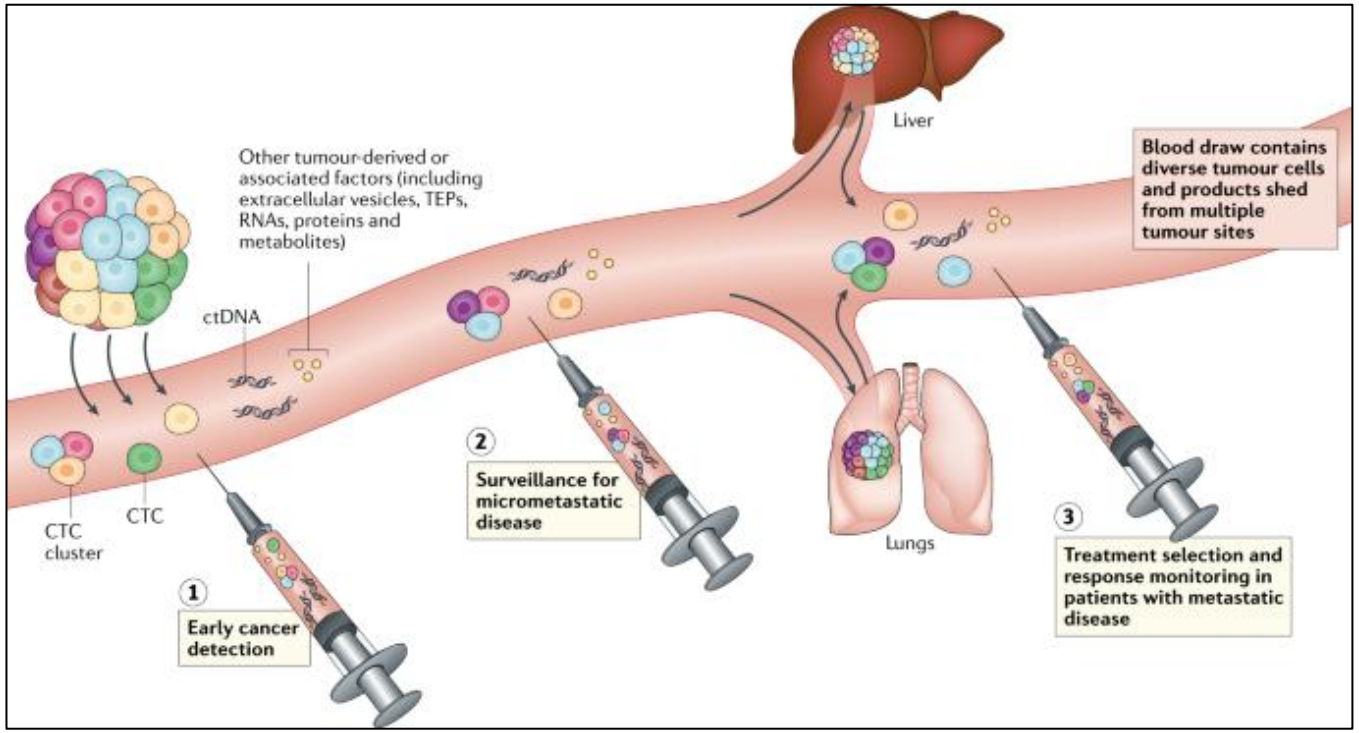
Tissue Biopsy vs Liquid Biopsy to Define Cancer Genome

Standard Tissue Biopsy

- Invasive
- Longer time for procedure
- Higher cost
- Constrained by sample quality
- Not compatible with ease of monitoring
- Can readily analyze whole genome and RNA

Liquid Biopsy

- Minimally invasive
- Shorter time for procedure
- Lower cost
- More comprehensive information about tumor in all locations
- Ease of repeated sampling
- Doesn't detect large structural changes
- Quantity and quality of ctDNA or other tumor related components may be limiting



Ignatiadis et al. Nature Reviews Clinical Oncology 2021

Liquid Biopsy: FDA Approved

GUARDANT360

73-Gene Panel

Complete Sequencing of Covered Exons*

Point Mutations (SNVs) (73 Genes)							Indels (23 Genes)		Amplifications (CNVs) (18 Genes)		Fusions (6 Genes)
AKT1	ALK	APC	AR	ARAF	ARID1A	ATM	ATM	APC	AR	BRAF	ALK
BRAF	BRCA1	BRCA2	CCND1	CCND2	CCNE1	CDH1	ARID1A	BRCA1	CCND1	CCND2	FGFR2
CDK4	CDK6	CDKN2A	CTNNB1	DDR2	EGFR	ERBB2	BRCA2	CDH1	CCNE1	CDK4	FGFR3
ESR1	EZH2	FBXW7	FGFR1	FGFR2	FGFR3	GATA3	CDKN2A	EGFR	CDK6	EGFR	NTRK1
GNA11	GNAQ	GNAS	HNF1A	HRAS	IDH1	IDH2	ERBB2	GATA3	ERBB2	FGFR1	RET
JAK2	JAK3	KIT	KRAS	MAP2K1	MAP2K2	MAPK1	KIT	MET	FGFR2	KIT	ROS1
MAPK3	MET	MLH1	MPL	MTOR	MYC	NF1	MLH1	MTOR	KRAS	MET	
NFE2L2	NOTCH1	NPM1	NRAS	NTRK1	NTRK3	PDGFRA	NF1	PDGFRA	MYC	PDGFRA	
PIK3CA	PTEN	PTPN11	RAF1	RB1	RET	RHEB	PTEN	RB1	PIK3CA	RAF1	
RHOA	RIT1	ROS1	SMAD4	SMO	STK11	TERT**	SMAD4	STK11			
TP53	TSC1	VHL					TP53	TSC1			
						** includes TERT promoter region	VHL				

*Exons selected to maximize detection of known somatic mutations. List available upon request

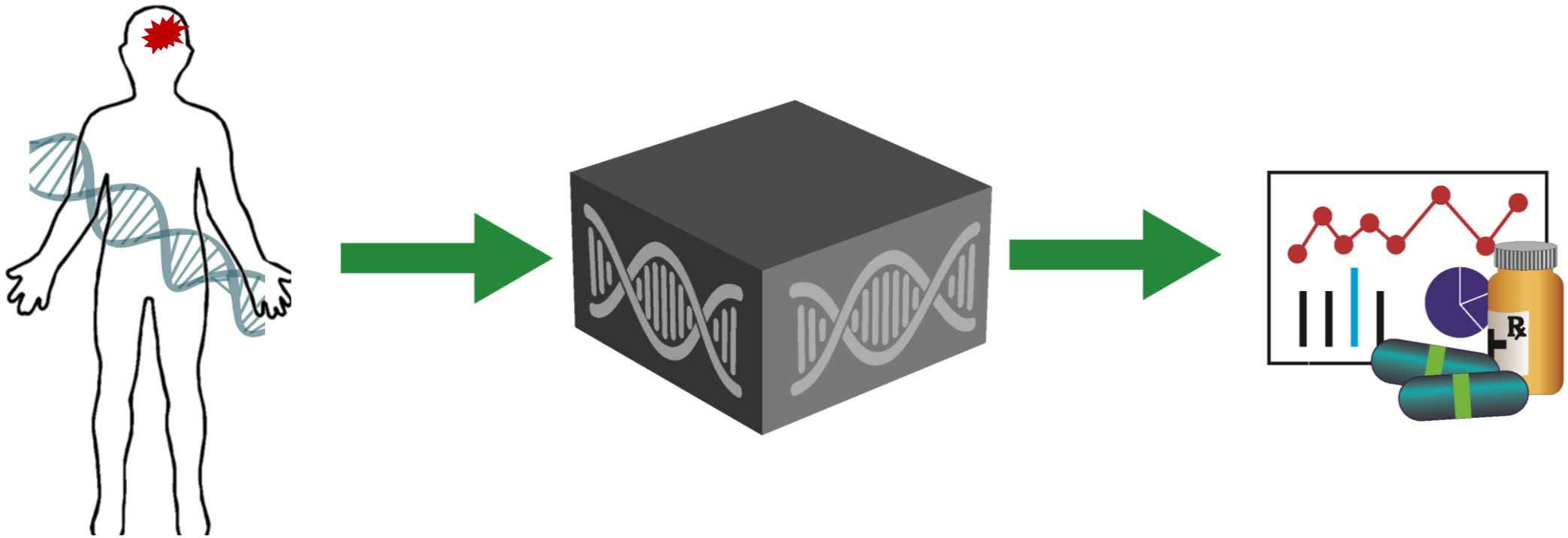
- **Both Guardant and FoundationOne are examples of liquid biopsy platforms approved by the FDA**
- **These use a targeted exome sequencing panel to identify mutations across genes commonly known to be mutated in a variety of human solid cancers**

FoundationOne Liquid CDx FDA-Approved Gene List[1]

As part of its FDA-approved intended use, FoundationOne Liquid CDx interrogates 311 genes, including 309 genes with complete exonic (coding) coverage and 2 genes with only select non-coding coverage (indicated with *). **Select genes and select exons (indicated in bold) are captured with increased sensitivity.**

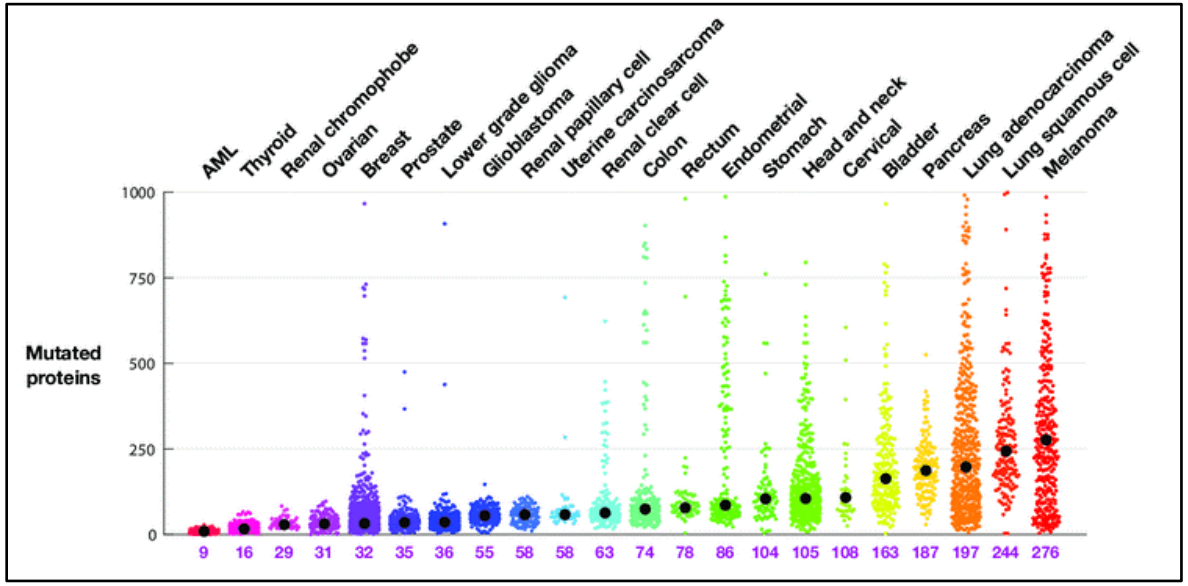
ABL1 [Exons 4-9]
ACVR1B
AKT1 [Exon 3]
AKT2
AKT3
ALK [Exons 20-29]
ALOX12B
AMER1 (FAM123B)
APC
AR
ARAF [Exons 4,5,7, 11,13,15,16]
ARFRP1
ARID1A
ASXL1
ATM
ATR
ATRX
AURKA
AURKB
AXIN1
AXL
BAP1
BARD1
BCL2
BCL2L1
BCL2L2
BCL6
BCOR
BCORL1
BRAF [Exons 11-18]
BRCA1 (introns 2, 7, 8, 12, 16, 19, 20)
BRCA2 (intron 2)
BRD4
BRIP1
BTG1
BTG2
BTK [Exons 2,15]
C11orf30 (EMSY)
C17orf39 (GID4)
CALR
CARD11
CASP8
CBFB
CBL
CCND1
CCND2
CCND3
CCNE1
CD22
CD274 (PD-L1)
CD70
CD79A
CD79B
CDC73
CDH1
CDK12
CDK4
CDK6
CDK8
CDKN1A
CDKN1B
CDKN2A
CDKN2B
CDKN2C
CEBPA
CHEK1
CHEK2
CIC
CREBBP
CRKL
CSF1R
CSF3R
CTCF
CTNNA1
CTNNB1 [Exon 3]
CUL3
CUL4A
CXCR4
CYP17A1
DAXX
DDR1
DDR2 [Exons 5,17,18]
DIS3
DNMT3A
DOT1L
EED
EGFR
EP300
EPHA3
EPHB1
EPHB4
ERBB2
ERBB3 [Exons 3,6,7,8,10,12,20, 21,23,24,25]
ERBB4
ERCC4
ERG
ERRFI1
ESR1 [Exons 4-8]
EZH2 [Exons 4,10,17,18]
FAM46C
FANCA
FANCC
FANCG
FANCL
FAS
FBXW7
FGF10
FGF12
FGF14
FGF19
FGF23
FGF3
FGF4
FGF6
FGFR1
FGFR2
FGFR3 [Exons 7, 9 (alternative designation exon 10), 14, 18]
FGFR4
FH
FLCN
FLT1
FLT3 [Exons 14,15,20]
FOXL2
FUBP1
GABRA6
GATA3
GATA4
GATA6
GNA11 [Exons 4,5]
GNA13
GNAQ [Exons 4,5]
GNAS [Exons 1,8]
GRM3
GSK3B
H3F3A
HDAC1
HGF
HNF1A
HRAS [Exons 2,3]
HSD3B1
ID3
IDH1 [Exon 4]
IDH2 [Exon 4]
IGF1R
IKBKE
IKZF1
INPP4B
IRF2
IRF4
IRS2
JAK1
JAK2 [Exons 14]
JAK3 [Exons 5,11,12,13,15,16]
JUN
KDM5A
KDM5C
KDM6A
KDR
KEAP1
KEL
KIT [Exons 8,9,11,12,13,17]
KLHL6
KMT2A (MLL)
KMT2D (MLL2)
KRAS
LTK
LYN
MAF
MAP2K1 (MEK1) [Exons 2,3]
MAP2K2 (MEK2) [Exons 2-4,6,7]
MAP2K4
MAP3K1
MAP3K13
MAPK1
MCL1
MDM2
MDM4
MED12
MEF2B
MEN1
MERTK
MET
MITF
MKNK1
MLH1
MPL [Exon 10]
MRE11A
MSH2
MSH3
MSH6
MSTIR
MTAP
MTOR [Exons 19,30,39,40, 43-45,47,48,53,56]
MUTYH
MYC
MYCL (MYCL1)
MYCN
MYD88 [Exon 4]
NBN
NF1
NF2
NFE2L2
NFKBIA
NKX2-1
NOTCH1
NOTCH2
NOTCH3
NPM1 [Exons 4-6,8,10]
NRAS [Exons 2,3]
NSD3 (WHSC1L1)
NTSC2
NTRK1 [Exons 14,15]
NTRK2
NTRK3 [Exons 16,17]
PALB2
PARK2
PARP1
PARP2
PARP3
PAX5
PBRM1
PDCD1 (PD-1)
PDCD1LG2 (PD-L2)
PDGFRA [Exons 12,18]
PDGFRB [Exons 12-21,23]
PDK1
PIK3C2B
PIK3C2G
PIK3CA [Exons 2,3,5-8,10,14,19,21] (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)
PIK3CB
PIK3R1
PIM1
PMS2
POLD1
POLE
PPARG
PPP2R1A
PPP2R2A
PRDM1
PRKAR1A
PRKCI
PTCH1
PTEN
PTPN11
PTPRO
QKI
RAC1
RAD21
RAD51
RAD51B
RAD51C
RAD51D
RAD52
RAD54L
RAF1 [Exons 3,4,6,7,10,14,15,17]
RARA
RB1
RBM10
REL
RET [Exons 11,13-16]
RICTOR
RNF43
ROS1 [Exons 31,36-38,40]
RPTOR
SDHA
SDHB
SDHC
SDHD
SETD2
SF3B1
SGK1
SMAD2
SMAD4
SMARCA4
SMARCB1
SMO
SNCAIP
SOCS1
SOX2
SOX9
SPEN
SPOP
SRC
STAG2
STAT3
STK11
SUFU
SYK
TBX3
TEK
TERC* (ncRNA)
TERT* (Promoter)
TET2
TGFBR2
TIPARP
TNFAIP3
TNFRSF14
TP53
TSC1
TSC2
TYRO3
U2AF1
VEGFA
VHL
WHSC1
WT1
XPO1
XRCC2
ZNF217
ZNF703

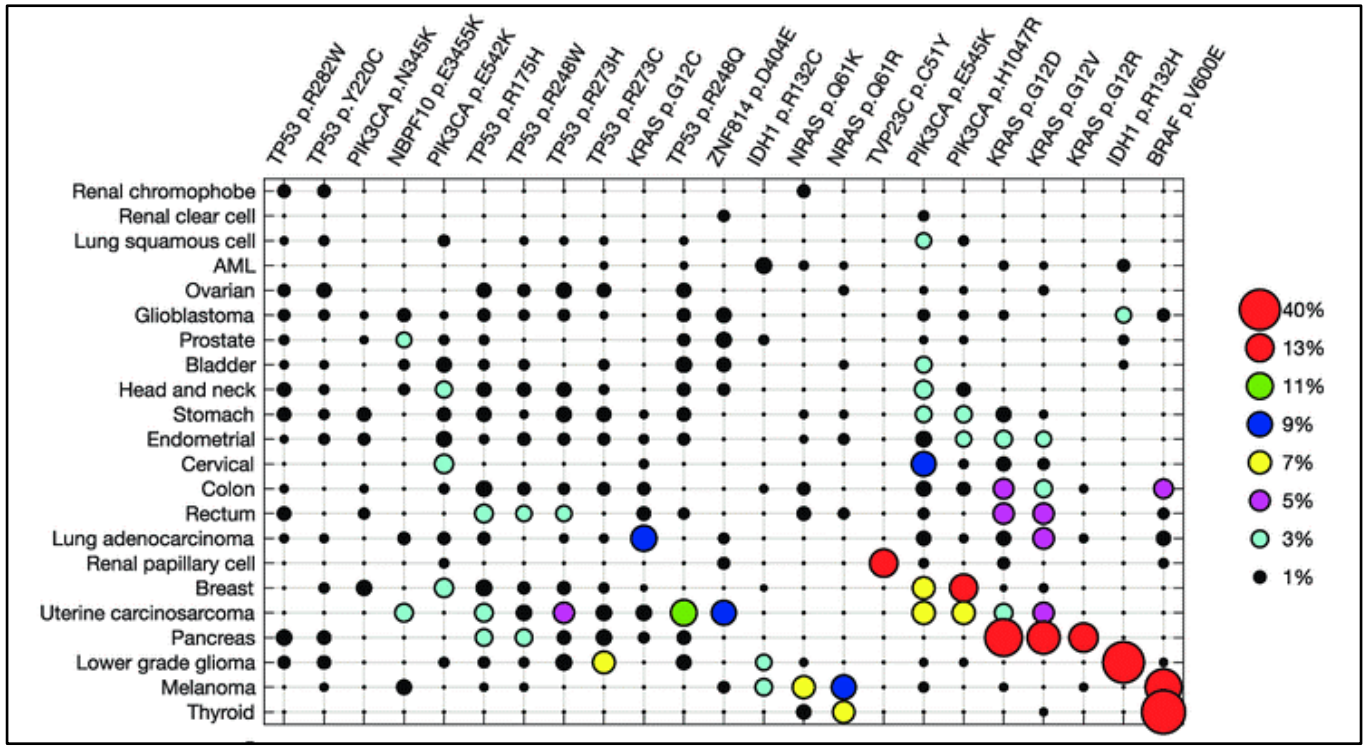
Leveraging the Human Cancer Genome to Improve Treatment



Genomic Landscape of Human Cancers

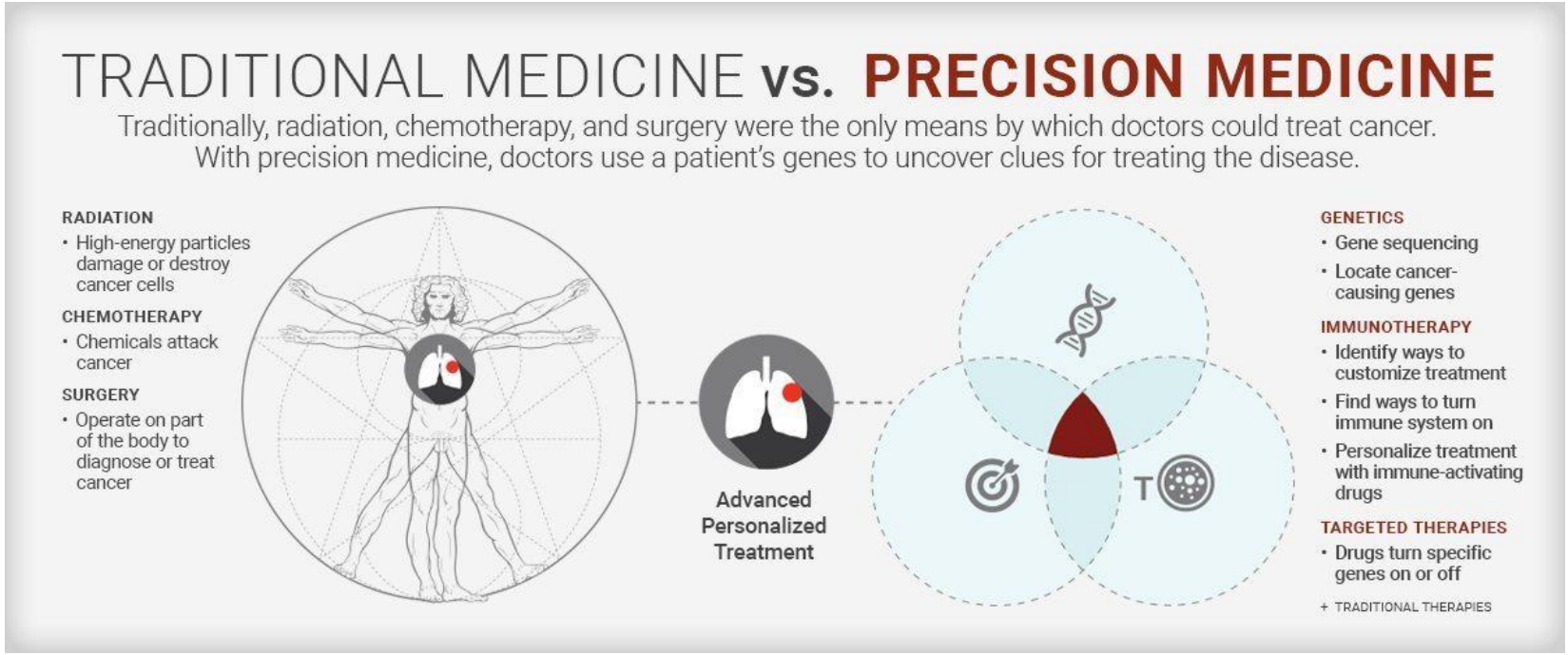
- Analysis of human cancer genomes has provided key insights into drivers of tumor development and metastasis.
- Human cancers are also now routinely characterized at the genomic level to find their therapeutic vulnerabilities.
- These are then used to help choose which chemotherapy agents, small molecule inhibitors and immunotherapies may be most likely to work.
- The genomic analysis is also used to help understand the mechanisms that lead to therapeutic resistance.

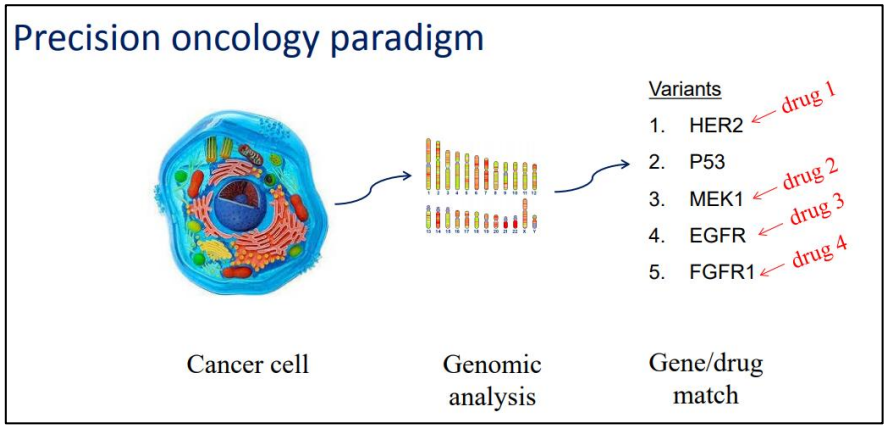


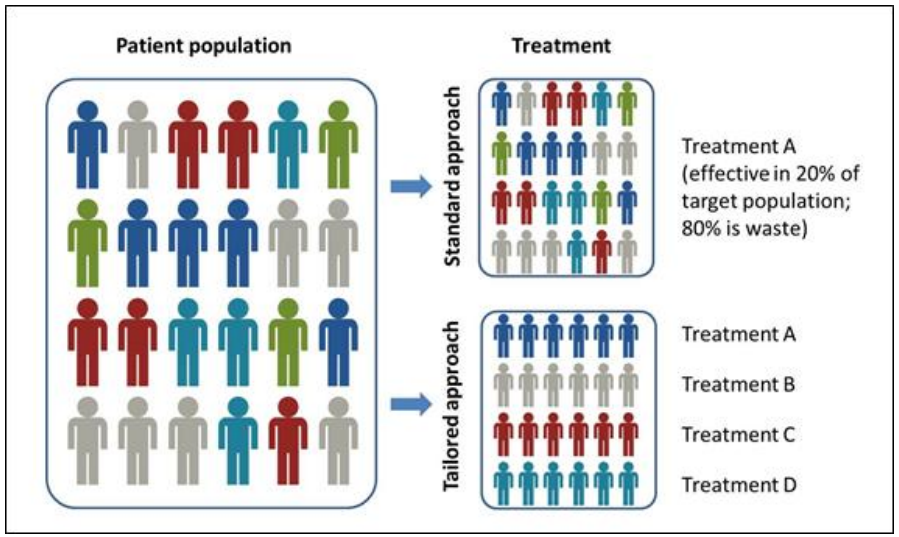


Castle et al, Frontiers in Immunology, 2019

Using Genomic Information to Drive Treatment







https://healthmatters.nyp.org/precision-medicine/; Intermountain Healthcare
https://www.cpgr.org.za/precision-medicine-in-south-africa-a-cost-benefit-analysis

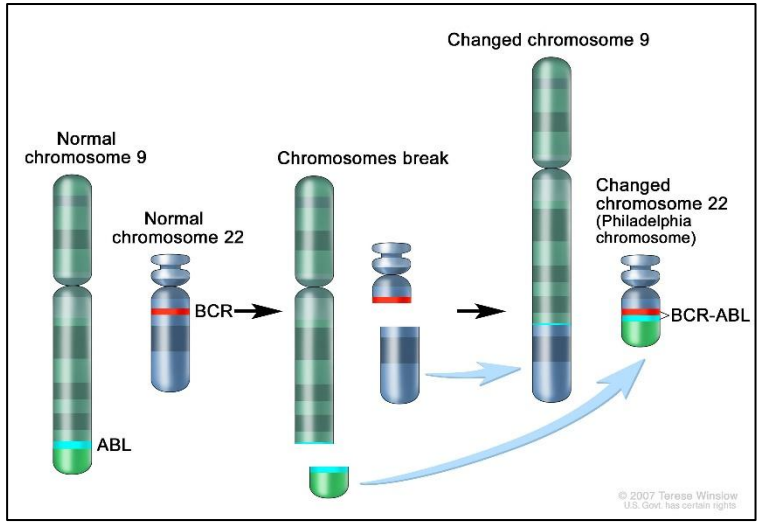
Drugs for Precision Medicine: Small Molecule Inhibitors

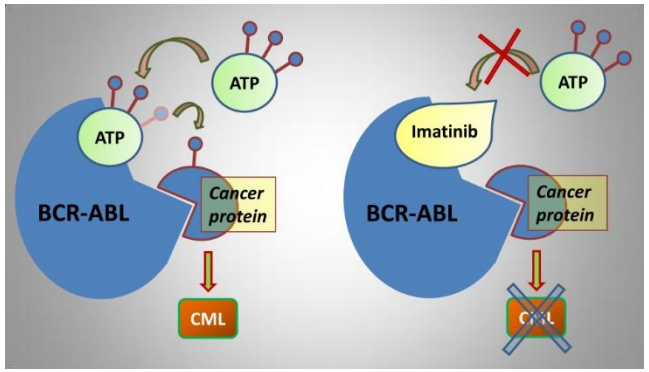
- Small molecules typically designed to target a specific protein and block its function.
- Usually given orally and usually work at low micromolar or nanomolar concentrations.
- Toxicities result from blocking pathways in normal cells and are typically readily reversible.
- Inhibitors can block a single pathway/protein or sometimes multiple pathways/proteins.
- Well over 100+ small molecules have been approved for human use by the FDA.

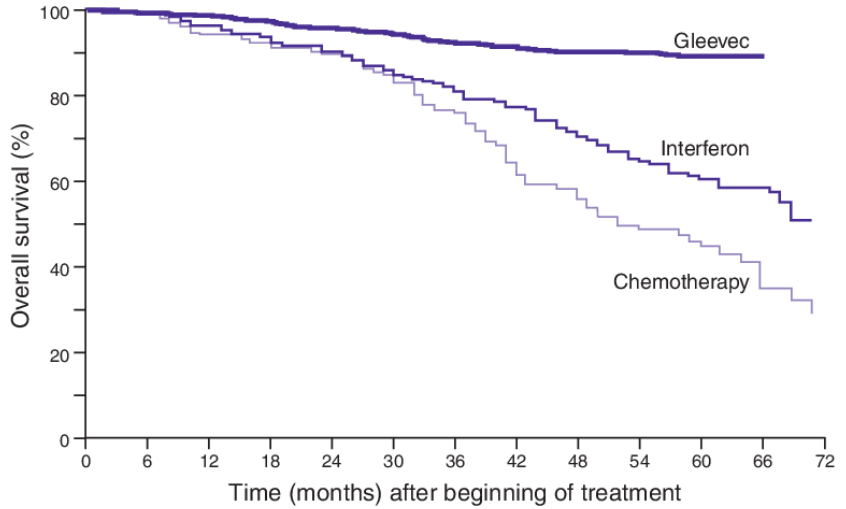
Approved Inhibitors	Approved Date	Primary Targets	Inhibitor of Type	PDB Entry	Reversible/ Irreversible
Imatinib	2001/05	ABL/PDGFR/c-KIT	II	1OPJ	Reversible
Gefitinib	2003/05	EGFR	I	4I22	Reversible
Erlotinib	2004/11	EGFR	I	4HJO	Reversible
Sorafenib	2005/12	VEGFR/PDGFR etc	II	4ASD	Reversible
Sunitinib	2006/01	KIT/PDGFR etc	I	2Y7J	Reversible
Dasatinib	2006/06	ABL/SRC etc	I	3QLG	Reversible
Lapatinib	2007/03	EGFR/Her2	I	1XKK	Reversible
Nilotinib	2007/10	ABL/KIT etc	II	3GP0	Reversible
Pazopanib	2009/10	c-KIT/FGFR etc	I	-	Reversible
Vandetanib	2011/04	VEGFR/EGFR etc	I	2IVU	Reversible
Crizotinib	2011/08	ALK/ROS1	I	3ZBF	Reversible
Vemurafenib	2011/08	BRAF	I	3OG7	Reversible
Ruxolitinib	2011/11	JAK1/JAK2	I	4U5J	Reversible
Axitinib	2012/01	VEGFR etc	I	4AGC	Reversible
Bosutinib	2012/09	ABL/SRC	I	4OTW	Reversible
Regorafenib	2012/09	VEGFR etc	II	-	Reversible
Tofacitinib	2012/11	JAK1/JAK3	I	3LXN	Reversible
Cabozantinib	2012/11	c-MET/VEGFR2 etc	II	-	Reversible
Ponatinib	2012/12	ABL	II	4C8B	Reversible
Trametinib	2013/05	MEK1	III	-	Reversible
Dabrafenib	2013/05	BRAF	I	4XV2	Reversible
Afatinib	2013/07	EGFR	I	4G5J	Irreversible
Ibrutinib	2013/11	BTK	I	4IFG	Irreversible
Ceritinib	2014/04	ALK	I	4MKC	Reversible
Idelalisib	2014/07	PI3Kd	I	4XE0	Reversible
Nintedanib	2014/10	VEGFR etc	I	3C7Q	Reversible
Palbociclib	2015/02	CDK4/CDK6	I	2EUF	Reversible
Lenvatinib	2015/02	VEGFR1/2/3	I	3WZD	Reversible
Cobimetinib	2015/11	MEK	III	4AN2	Reversible
Osimertinib	2015/11	EGFR	I	4ZAU	Irreversible
Alectinib	2015/12	ALK	I	5XV7	Reversible
Ribociclib	2017/03	CDK4/CDK6	I	5L2T	Reversible
Brigatinib	2017/04	ALK/EGFR	I	5J7H	Reversible
Midostaurin	2017/04	FLT3 etc	I	4NCT	Reversible
Neratinib	2017/06	EGFR/HER2	I	2JIV	Irreversible
Abemaciclib	2017/09	CDK4/CDK6	I	5L2S	Reversible
Copanlisib	2017/09	PI3Ka/PI3Kd	I	5G2N	Reversible
Acalabrutinib	2017/10	BTK	I	-	Irreversible
Fostamatinib	2018/04	SYK	I	3FQS	Reversible
Baricitinib	2018/05	JAK1/2	I	4W9X	Reversible
Binimetinib	2018/06	MEK	III	4U7Z	Reversible
Encorafenib	2018/06	BRAF	I	-	Reversible

Small Molecule Inhibitors in CML: Imatinib

- Over 90% of patients with Chronic Myelogenous Leukemia (CML) have a genomic translocation causing a fusion of two genes BCR and ABL.
- This fusion creates constitutive signaling in the cell and drives the development CML.
- Imatinib (Gleevec) was developed to bind to BCR-ABL and block ATP binding, thereby preventing phosphorylation and signaling through this fusion protein.
- The use of imatinib has markedly improved outcomes for patients with CML.



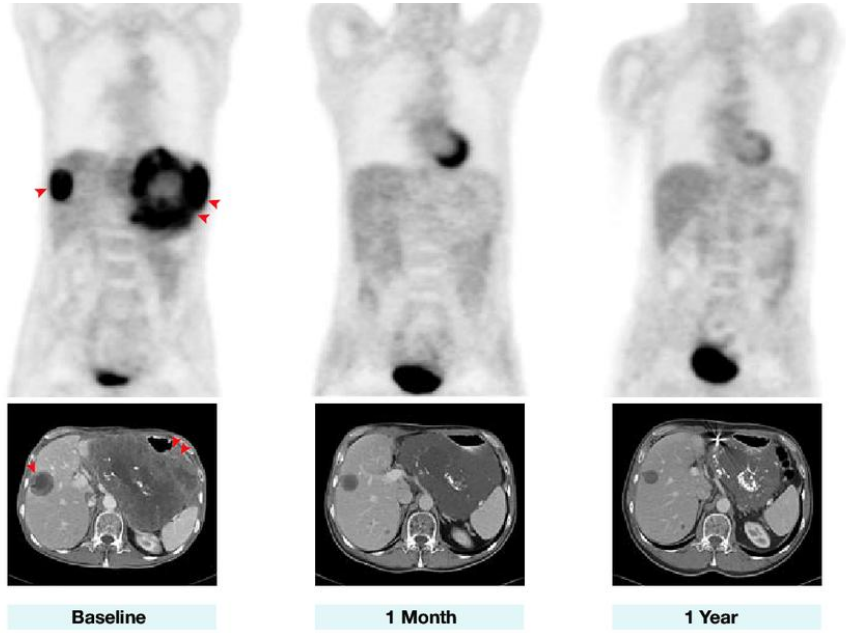


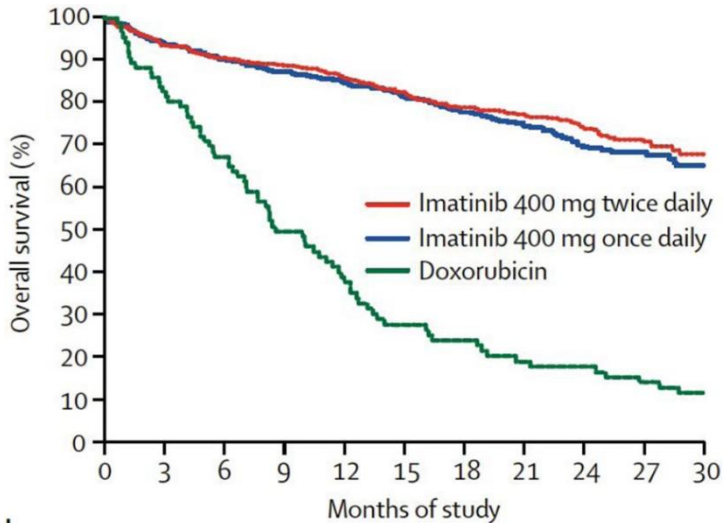


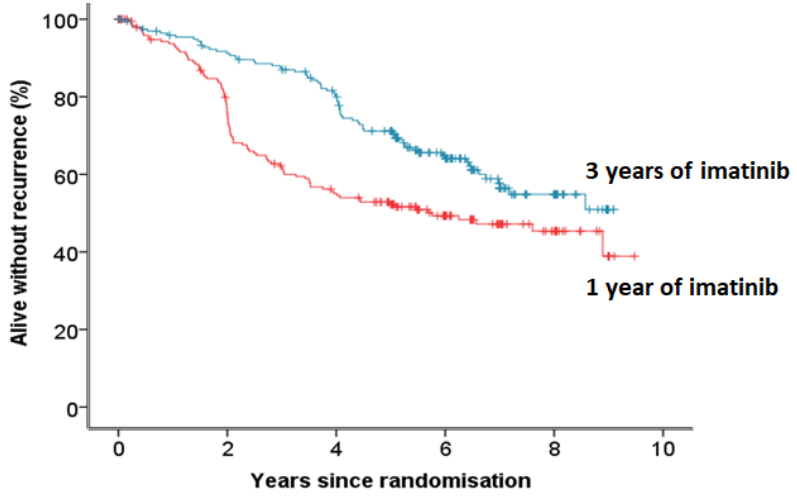
Guenova, Modern Therapy of CML; 2013Druker, Nature Medicine, 2009

Small Molecule Inhibitors in GIST: Imatinib

- Imatinib also blocks signaling of the receptor kinases KIT and PDGFR.
- Human patients with gastrointestinal stromal tumor (GIST) often have mutations in either KIT or PDGFR that cause constitutive receptor activation and drive tumor growth.
- As with CML, imatinib has markedly improved outcomes for patients with GIST.



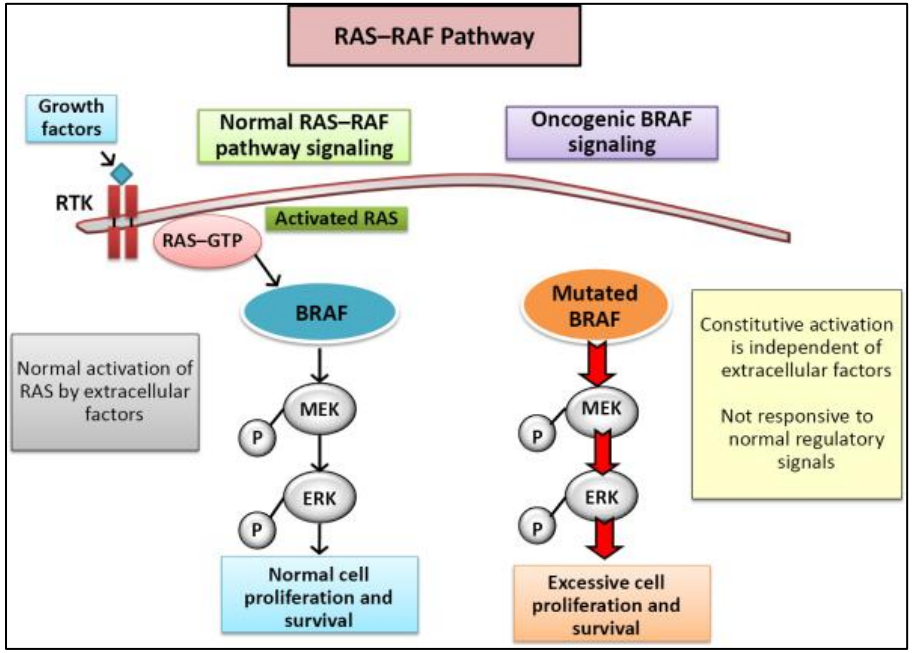


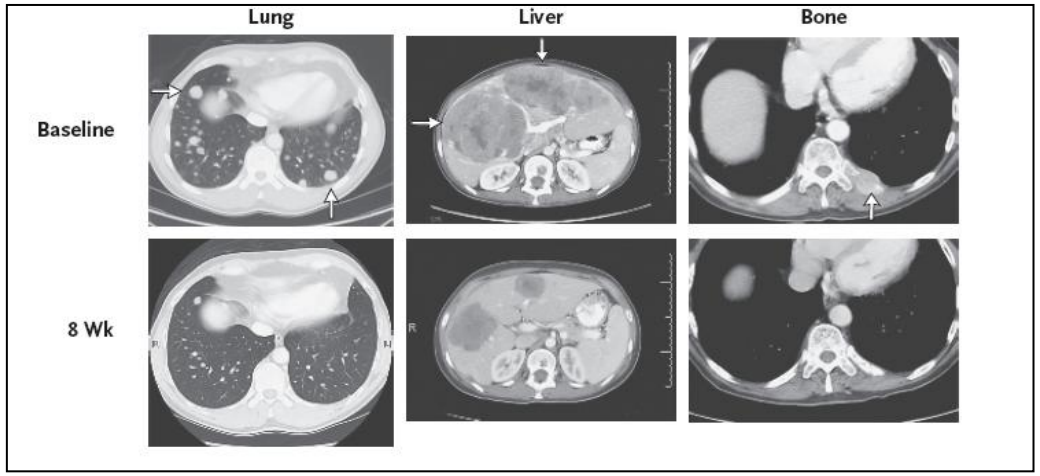


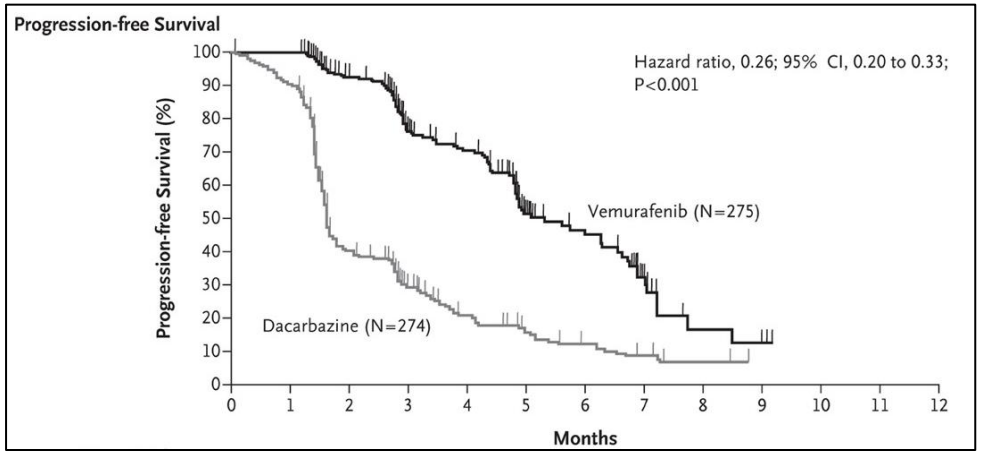
Balachandran, Adv in Surgery 2014; Van Den Abbeele, The Oncologist 2008

Small Molecule Inhibitors in Melanoma: Vemurafenib

- Approximately 60% of patients with cutaneous melanoma possess a specific mutation in the cytoplasmic kinase BRAF that causes persistent cell signaling.
- Vemurafenib was developed to specifically target mutant BRAF in melanoma.
- Response to treatment is rapid, although resistance tends to development rapidly through a variety of mechanisms including upregulation of signaling pathways upstream and downstream of BRAF.



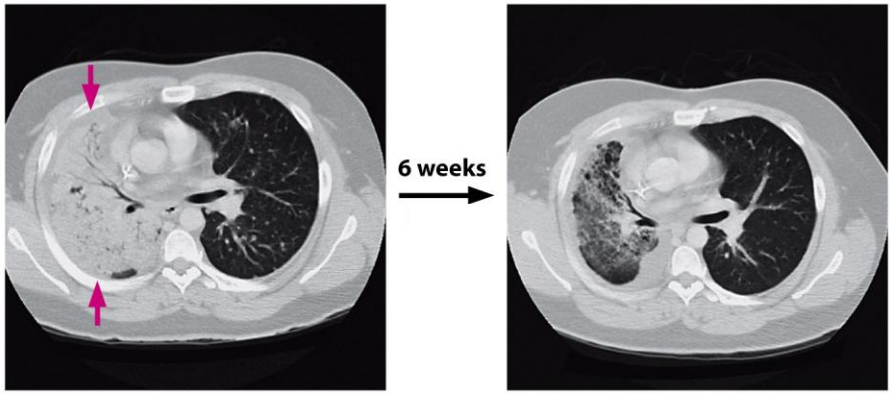


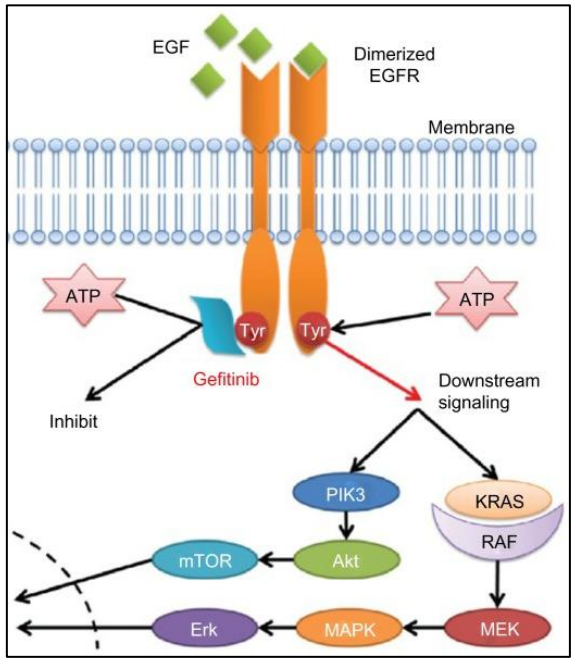


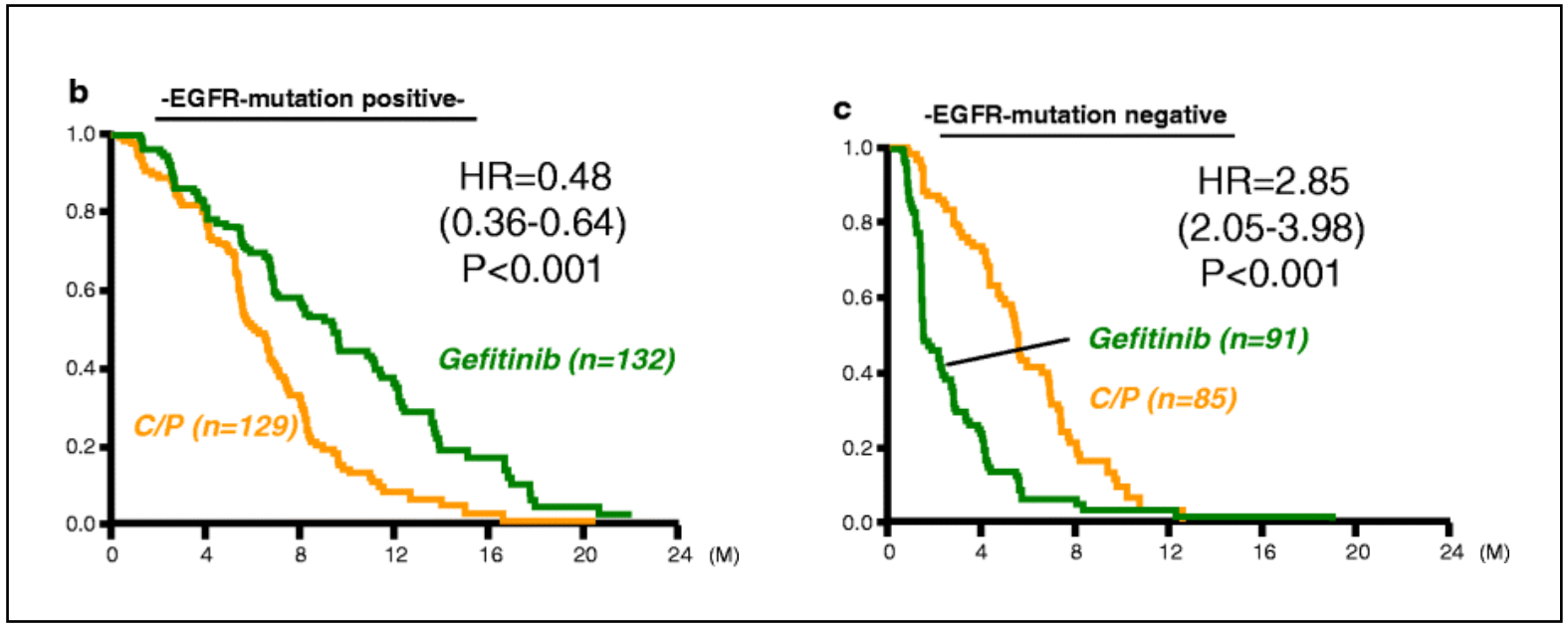
Sosman et al, NEJM, 2012; Muñoz-Couselo, ATM, 2015; Chapman NEJM, 2011

Small Molecule Inhibitors in Lung Cancer: Gefitinib

- A portion of patients with non-small cell lung cancer have a mutation in the EGFR that causes activation of the receptor.
- Gefitinib (Iressa) blocks mutant EGFR, interrupting signaling and causing tumor cell death.
- Patients with EGFR mutation had better outcomes with gefitinib versus chemotherapy.



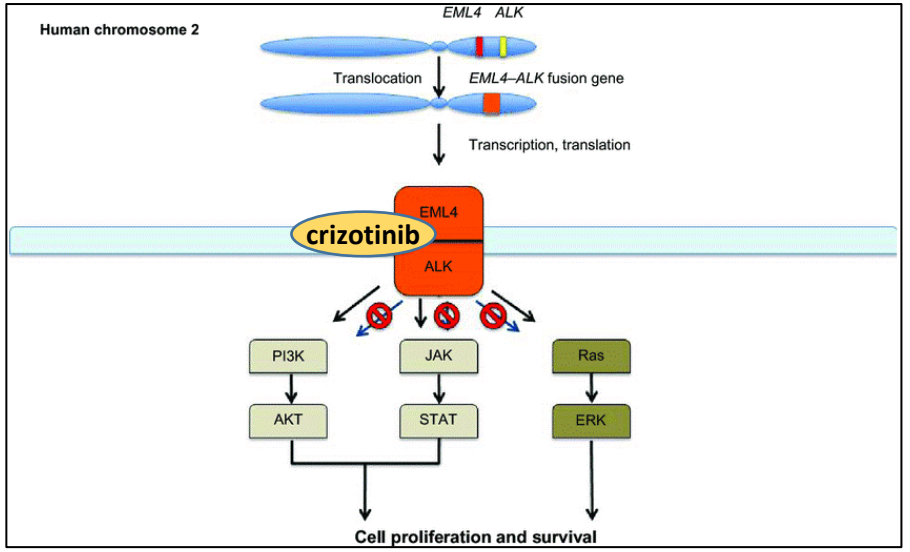


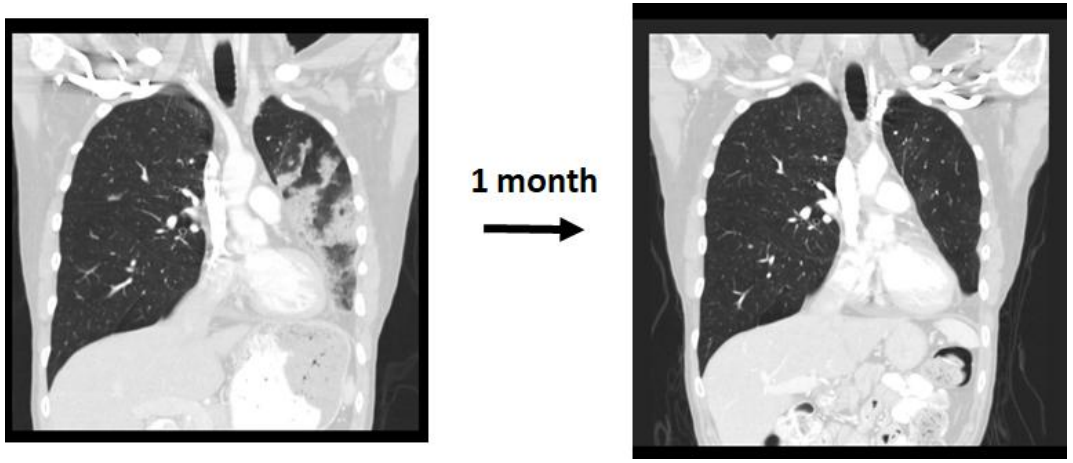


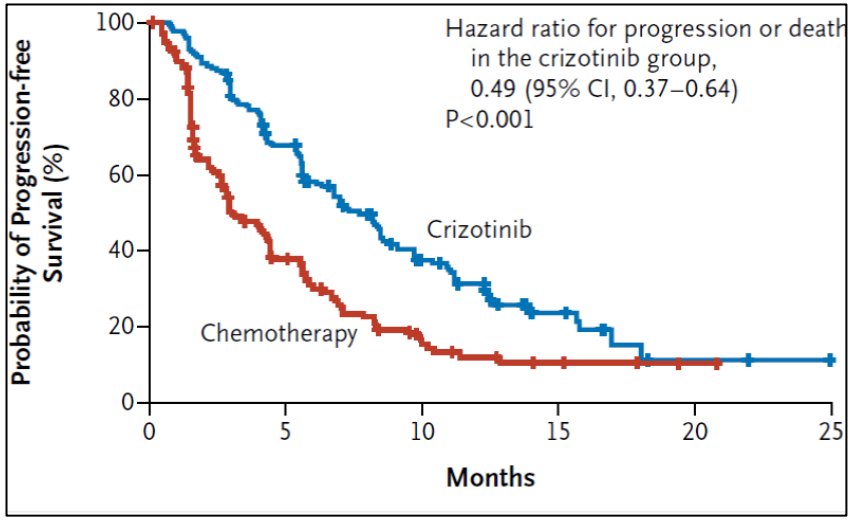
Kobayashi, Targeted Oncology, 2013; Kuribayashi Journal of Cancer Biology and Research 2013

Small Molecule Inhibitors in Lung Cancer: Crizotinib

- A small percentage of patients with non-small cell lung cancer have a fusion in EML4-ALK, causing constitutive ALK signaling.
- Crizotinib is a small molecule inhibitor that was developed to block the fusion protein.
- Patients with this translocation had better outcomes when treated with crizotinib compared to chemotherapy.

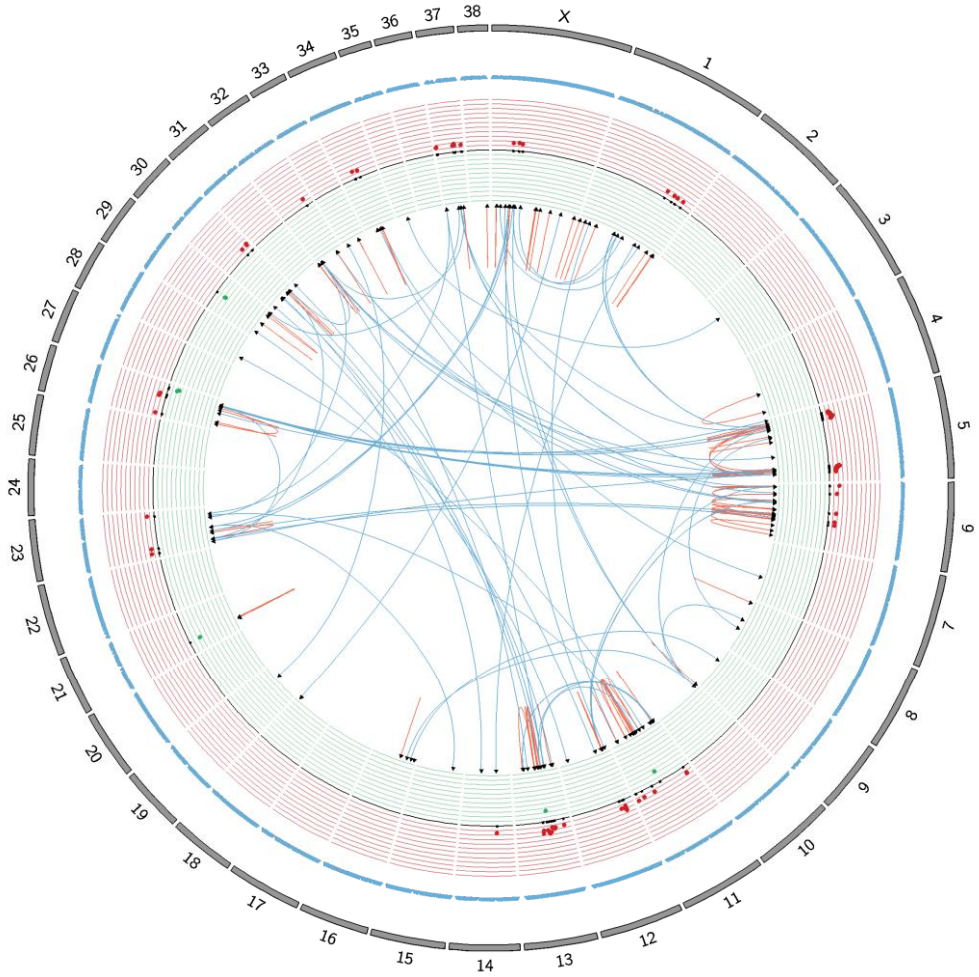






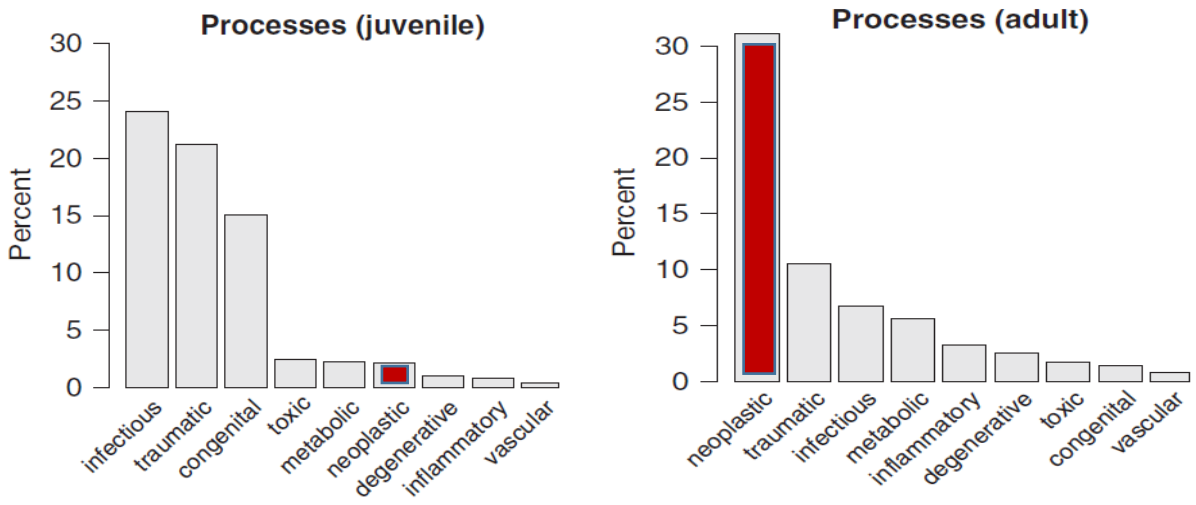
Kobayashi, Targeted Oncology, 2013; Kuribayashi Journal of Cancer Biology and Research 2013

Canine Cancer Genomics

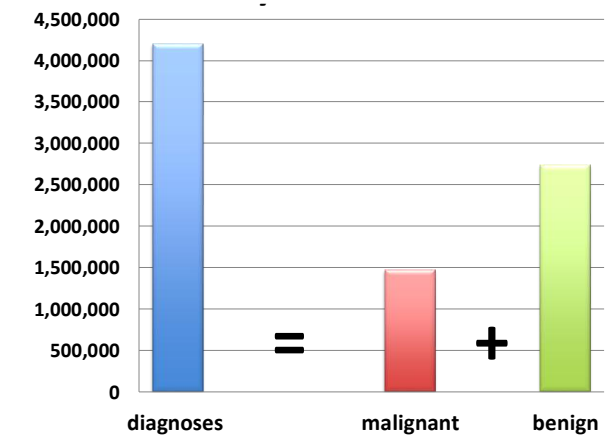


Canine Cancer Genomics

Causes of Death



Yearly Cancer Incidence



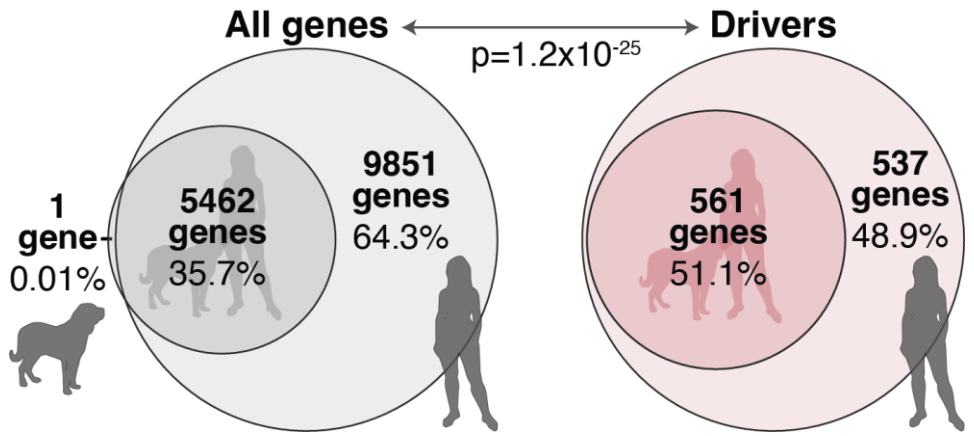
2,500 cases/100,000 dogs
500 cases/100,000 people

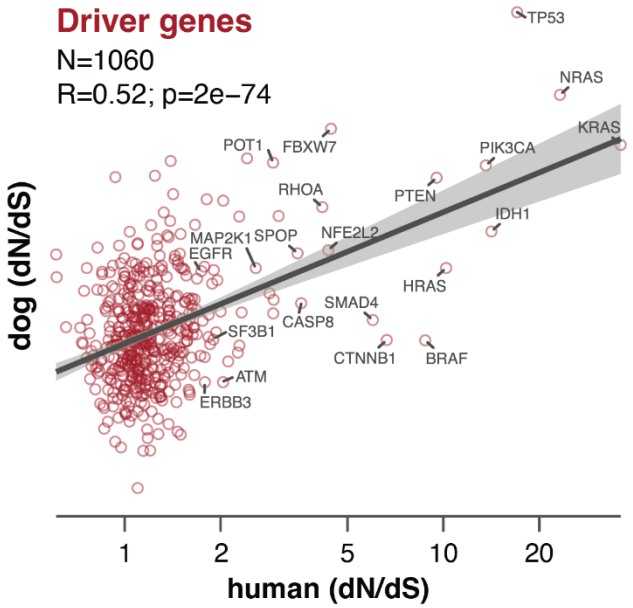
Historical barriers

- **Lack of knowledge regarding canine tumor genomics**
- **Incomplete canine genome builds with inadequate annotation**
- **Few tools to interrogate and analyze genomics data**
- **Poor connectivity with other genomics resources (humans, murine, etc) to facilitate comparative studies**

Spontaneous Canine Cancer as a Translational Model

GENE	CANINE	HUMAN
	OSTEOSARCOMA	
TP53	24-83% somatic mutations; 18% LOH[23-29]	**OS:** 20%-90% mutations; 24-42% LOH[30-33] Many other cancers
PTEN	30% CN loss[34,35]	**OS:** CN loss[36]; many other cancers
DLG2	56% CN Loss[37]	**OS:** 42-52% CN Loss[37]
DMD	50% intragenic deletions and CN loss[29]	**Olfactory neuroblastoma:** 86% deletions[38] **GIST:** 31% deletions[39,40] **Melanoma:** Homozygous deletions[41]
SETD2	21-42% SNV and CN Loss[28,29]	**OS:** Single CN Loss and SNVs[42] **Renal Cell CA:** 15.6% SNVs[43] **ALL:** 12% SNVs[44] **Glioma:** 15% SNVs[45]
MYC	38-40% CN gain[29,34]	**OS:** 39% CN Gain[34,46]; many other cancers
	B CELL LYMPHOMA	
TRAF3	44% somatic mutations[47]	**DLBCL:** 9% somatic mutations[47]
MYC-IgH Translocation	B-cell LSA[48]	Burkitt lymphoma[48]
	HEMANGIOSARCOMA	
TP53	59.6% SNV[49,50]	**AS:** 20 – 35% SNV[51-53], many other cancers
PIK3CA	29.8% SNV[49,50]	**AS:** 21%, SNV, primarily breast AS[52] **Breast Cancers:** 8-40% SNV[54] **Colonic CA:** 32% SNV[54] **Glioblastoma:** 27% SNV[54]
PTEN	50% deletions[55]	**AS:** Mutation in hepatic AS[56]; many others
PLCG1	1/20 (0.05%) SNV[50]	**AS:** 9 – 17% SNV[52,53]
	UROTHELIAL CARCINOMA	
BRAF	67-87% activating V595E mutation[57,58]	**Melanoma:** 66% V600E mutation[59] **Thyroid CA:** 25-82.3% V600E mutations[60] **Colonic CA:** 10-15% V600E mutations[61]
	MAST CELL TUMOR	
KIT	30% ITDs and point mutations (exons 8, 9, 11 and 12)[62,63]	**GIST:** > 65% activating mutations (exons 9 & 11)[64,65]



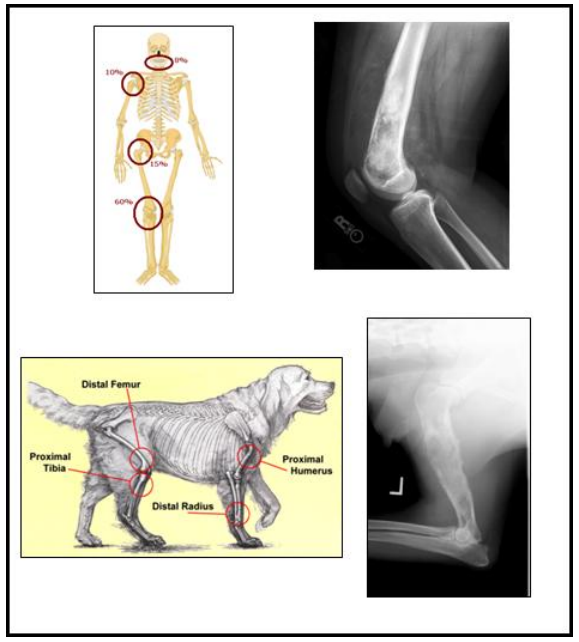


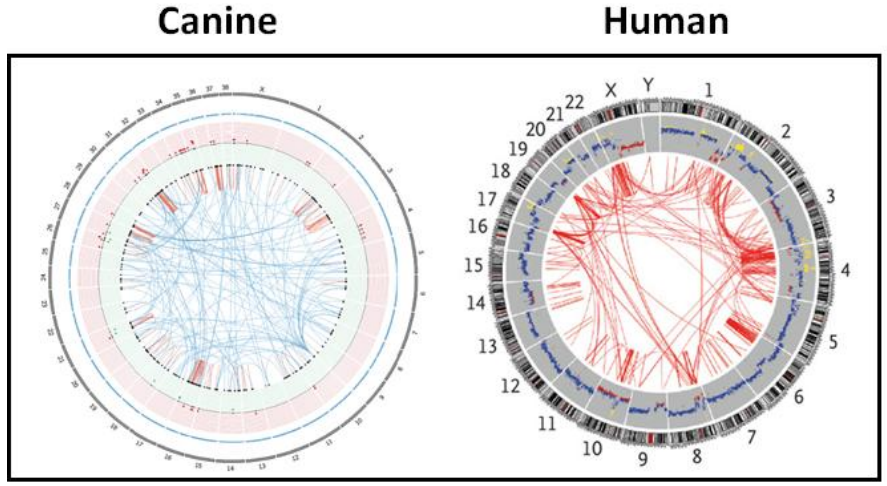
Karlsson et al, unpublished

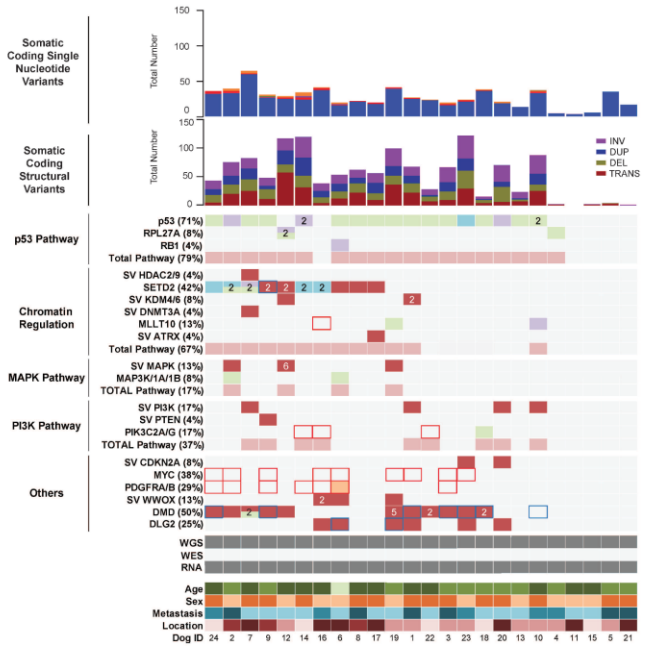
Genomic Landscapes of Human and Canine OS are similar

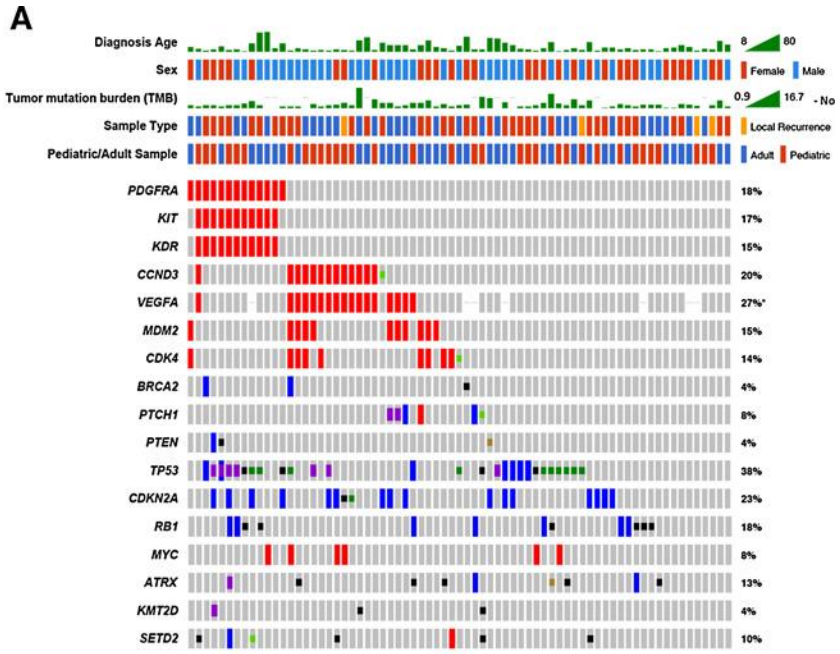
OS Genome is structurally complex

- Complex somatic rearrangements
- Localized hypermutation
- Frequent copy number aberrations
- Relatively few point mutations



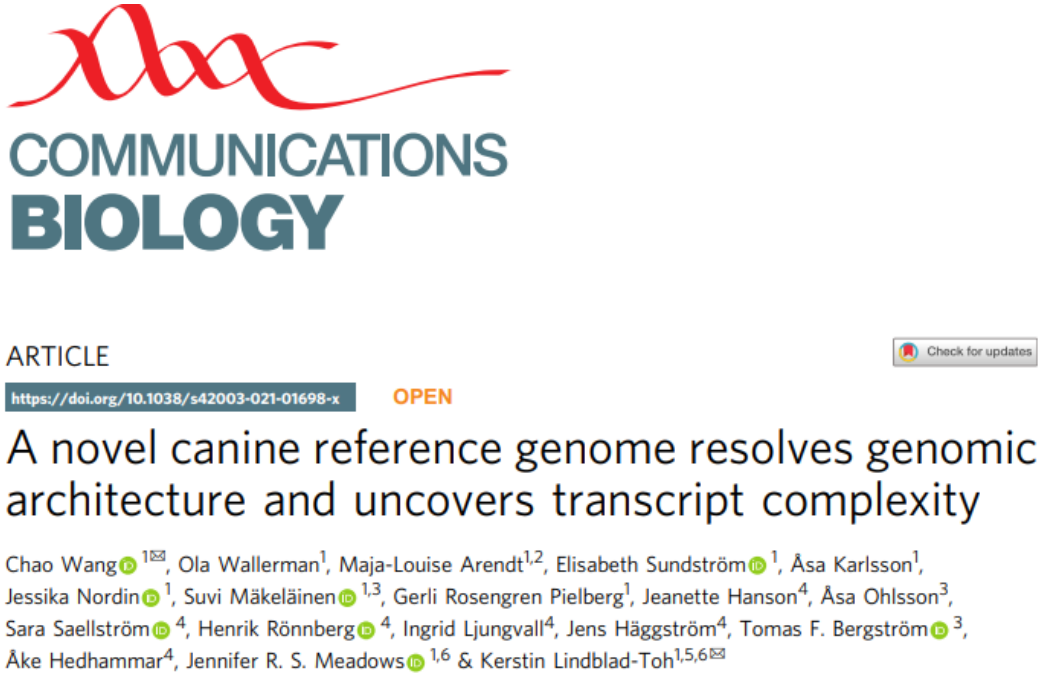






Suehara et al, 2019; Sayles et al. 2018; Perry et al. 2014; Chen et al. 2014; Gardner et al, 2020

Better Canine Genome Build: canFam4


COMMUNICATIONS BIOLOGY

ARTICLE

https://doi.org/10.1038/s42003-021-01698-x OPEN

Check for updates

A novel canine reference genome resolves genomic architecture and uncovers transcript complexity

Chao Wang[1✉], Ola Wallerman[1], Maja-Louise Arendt[1,2], Elisabeth Sundström[1], Åsa Karlsson[1], Jessika Nordin[1], Suvi Mäkeläinen[1,3], Gerli Rosengren Pielberg[1], Jeanette Hanson[4], Åsa Ohlsson[3], Sara Saellström[4], Henrik Rönnberg[4], Ingrid Ljungvall[4], Jens Häggström[4], Tomas F. Bergström[3], Åke Hedhammar[4], Jennifer R. S. Meadows[1,6] & Kerstin Lindblad-Toh[1,5,6✉]

	canFam4	canFam3
Sum_length	2.48 Gb	2.41 Gb
n_seq.	2198	3268
N50	64.30 Mb	63.24 Mb
n_N50	15	15
N90	32.14 Mb	38.81 Mb
n_N90	35	34
n_Gap	585	23,876
N_Gap_per_1Mb	0.24	9.91

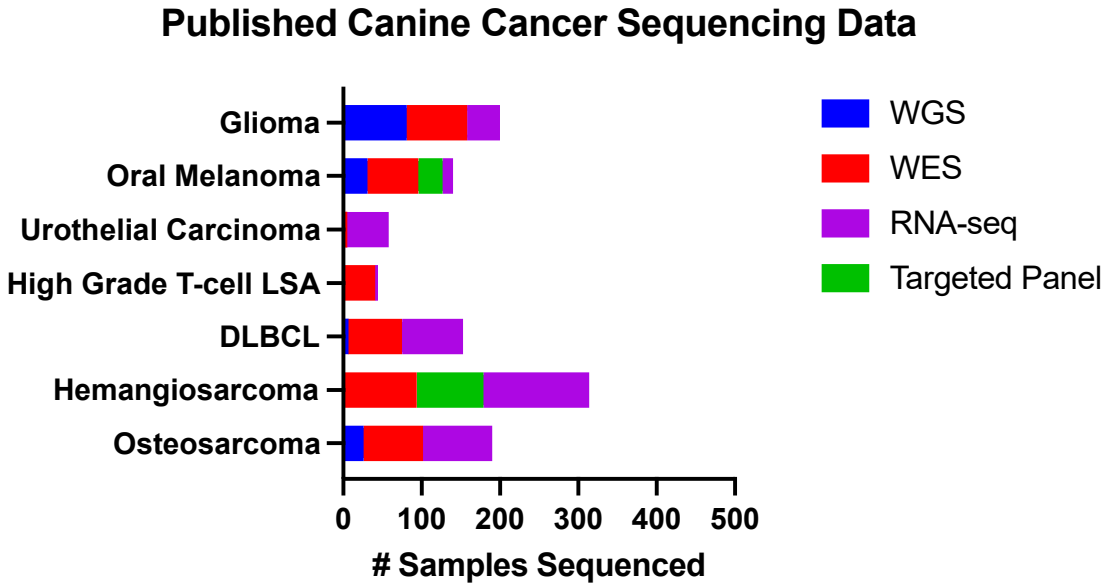
CanFam4 has chromosome length scaffolds and contiguity increased 55-fold over canFam3.1.

Annotation with generated and existing long and short read RNA-seq, miRNA-seq and ATAC-seq revealed that 32.1% of lifted over canFam3.1 gaps harbored previously hidden functional elements, including promoters, genes and miRs.

Key genomic regions were completed, including the Dog Leucocyte Antigen (DLA), T Cell Receptor (TCR) and 366 COSMIC cancer genes.

Advances in Canine Cancer Genomics

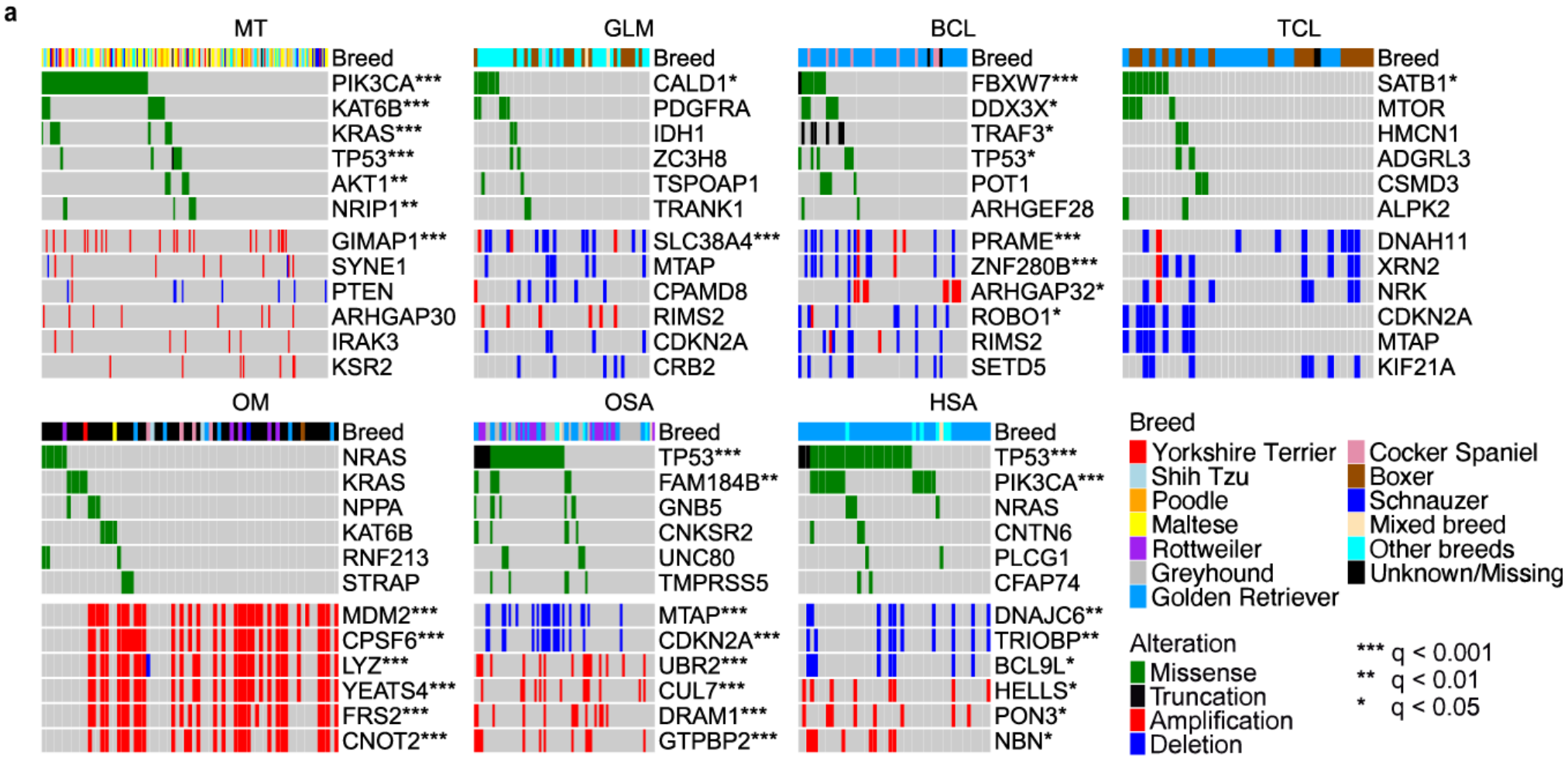
- >600 NGS/genetic studies have been undertaken in dogs over past 10 years.
- Several of these have been completed with the purpose of beginning to identify opportunities for precision medicine in canine cancer.
- While the landscape of canine tumor genome profiles still being charted, clear opportunities to begin integrating targeted therapy have been identified.



Alsaihati et al. Nat Comm. 2021

Re-Analysis of Published Canine Cancer Genomics Data

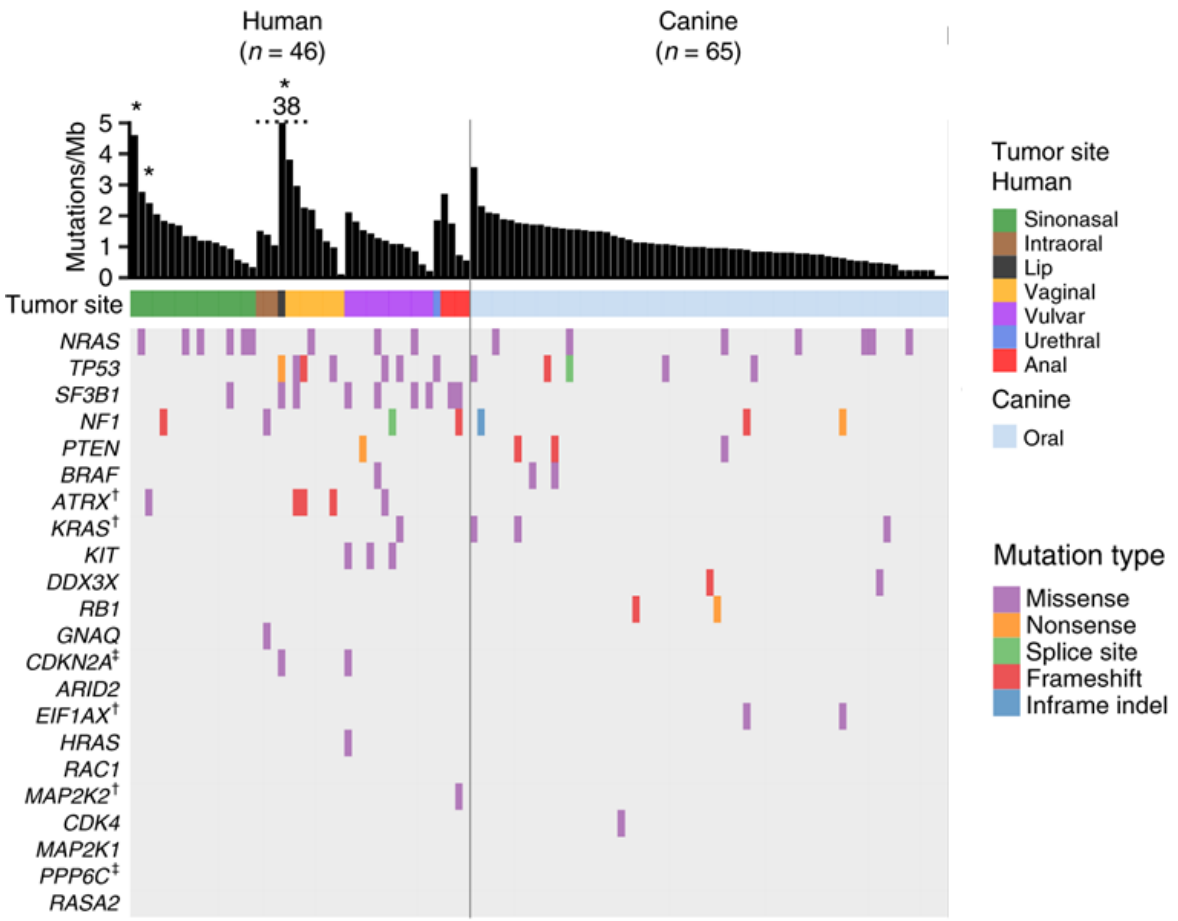
WES (1316 paired T/N) and WGS (172 paired T/N) from > 7 tumor types and > 35 breeds



Alsaihati et al. Nat Comm. 2021

Leveraging Human Precision Oncology

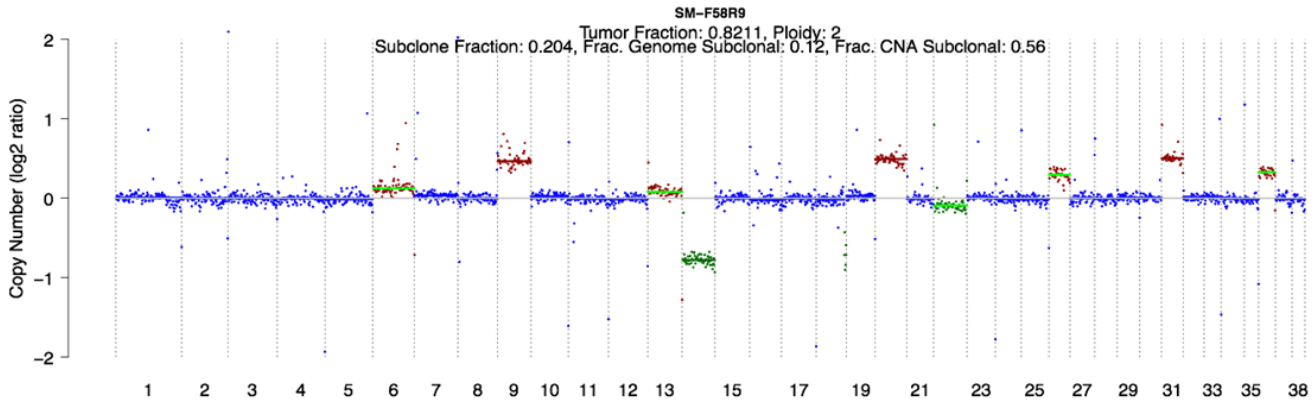
- Understanding the canine tumor genome provides a gateway for identifying new targets for therapy.
- Comparing the genomic landscapes of canine and human cancers permits us to leverage what is known about the human counterpart.
- For example, human and canine mucosal melanomas demonstrated some key similarities, including the presence of NRAS mutations (oncogene, activating) and PTEN mutations (tumor suppressor, inactivating).



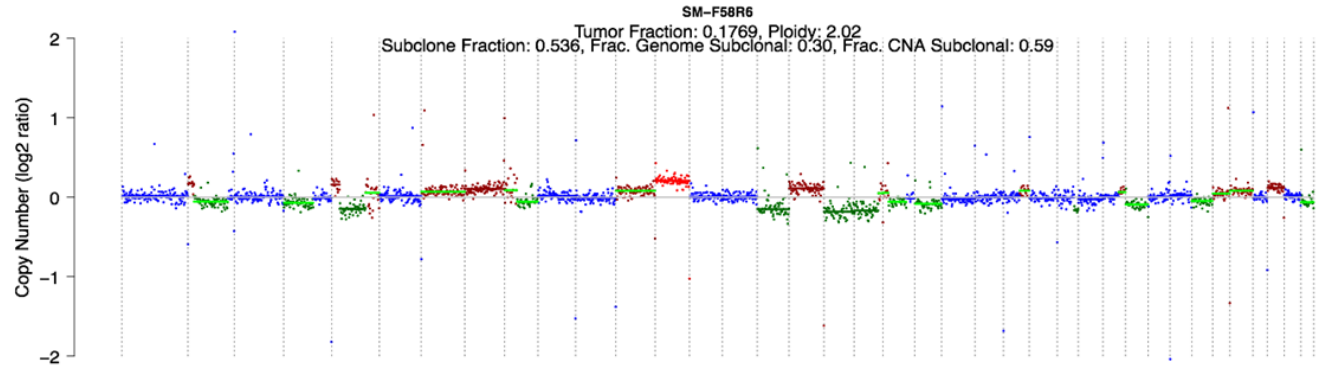
Wong et al, Nature Communications, 2019

Liquid Biopsy and Canine Cancers

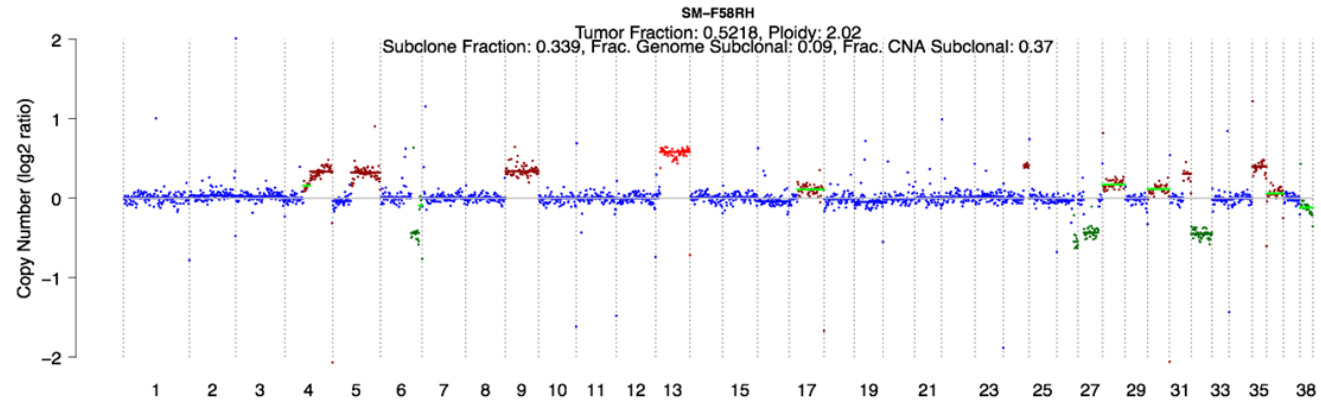
Lymphoma



Osteosarcoma

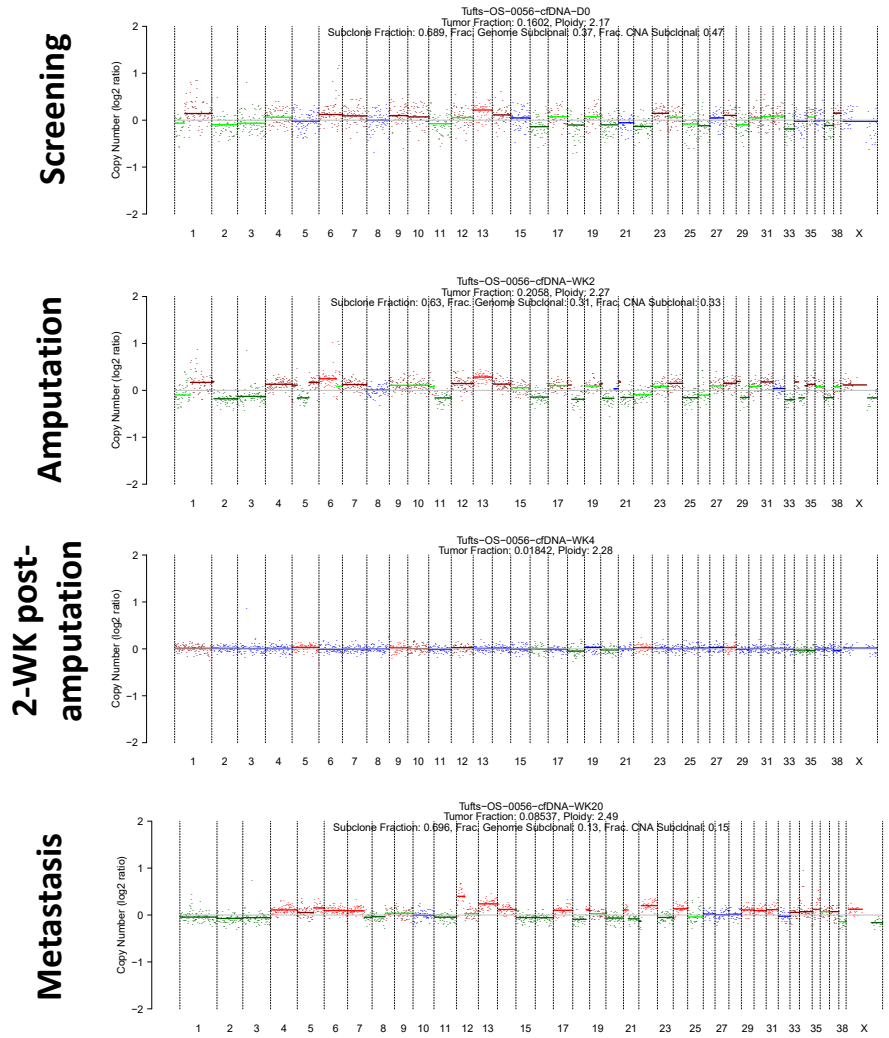


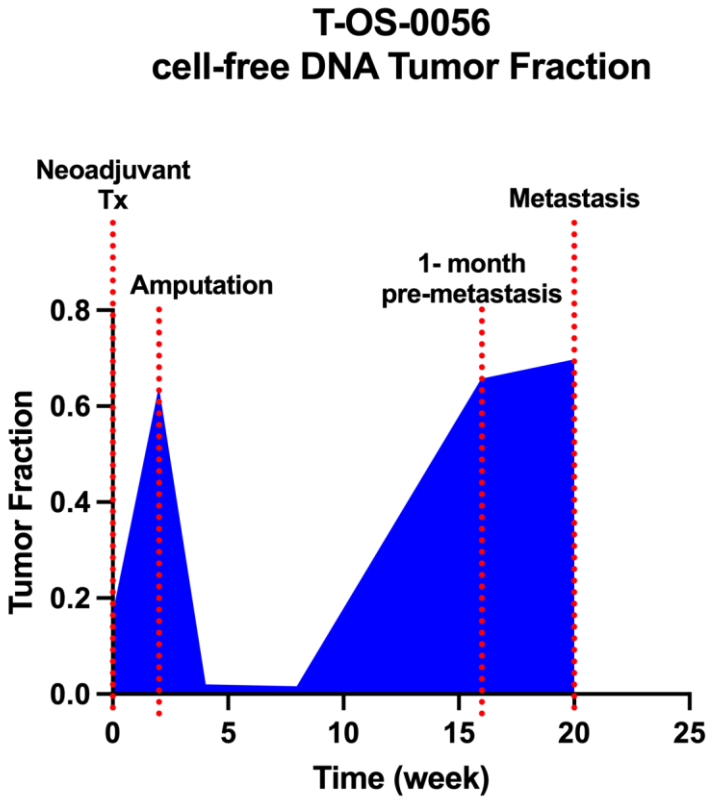
Hemangiosarcoma



Courtesy of Kate Megquier DVM, PhD

Liquid biopsy studies for disease monitoring



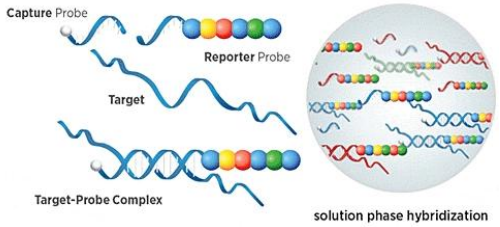


Courtesy of Heather Gardner

Partnerships to enhance genomics

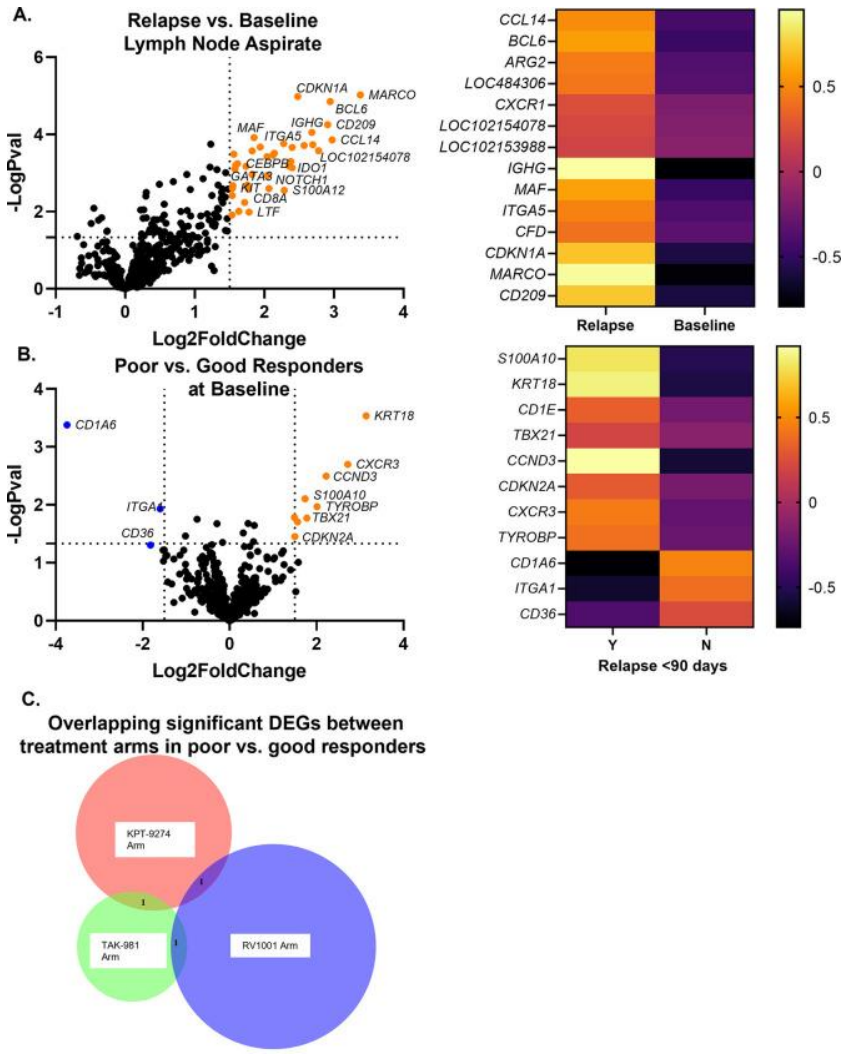
nanoString®

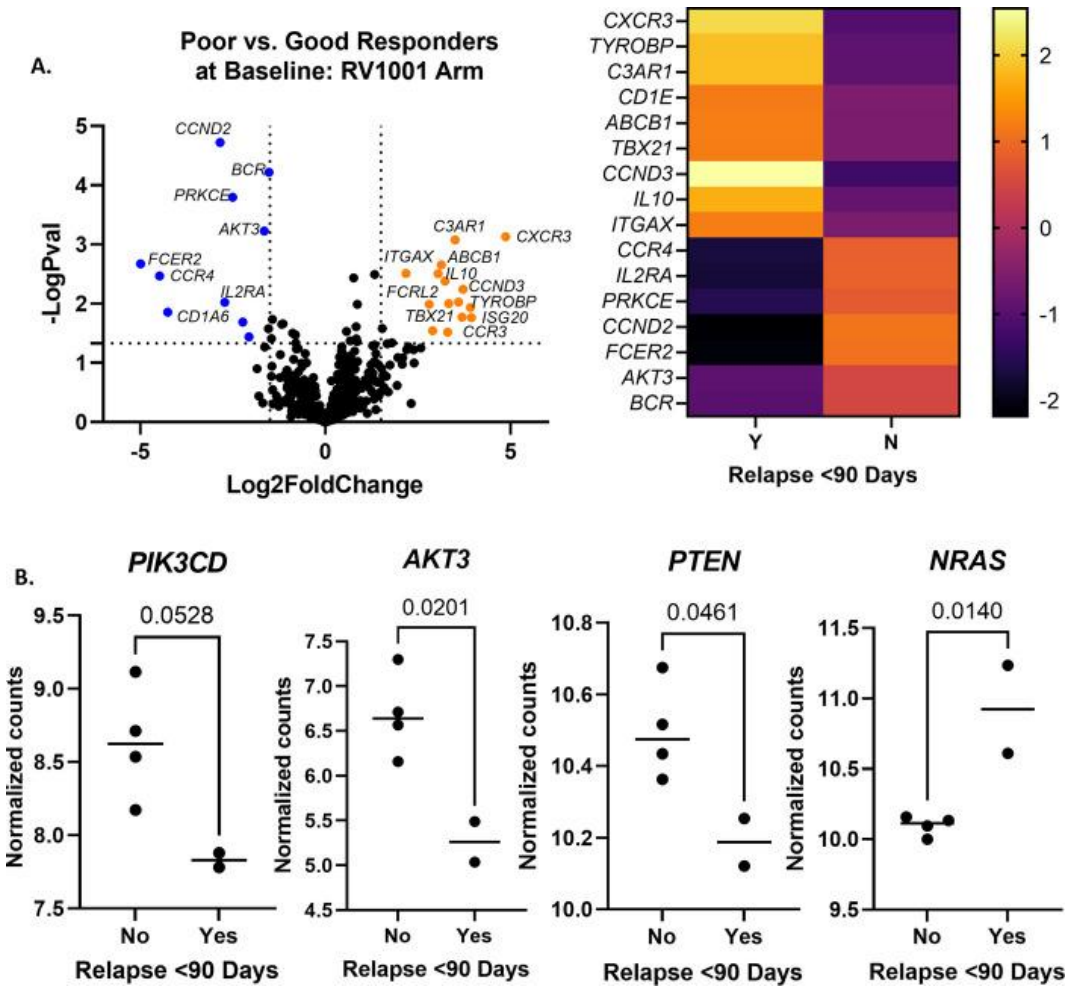
nCounter



- 800 canine genes for pan-cancer immune response studies.
- Companion to the nCounter Human IO 360™ and Human PanCancer Immune Profiling panel currently in use with human clinical trials
- Significant overlapping content designed for directly comparing human and canine immune response.

nCounter IO Panel: Genes associated with outcome in dogs with lymphoma

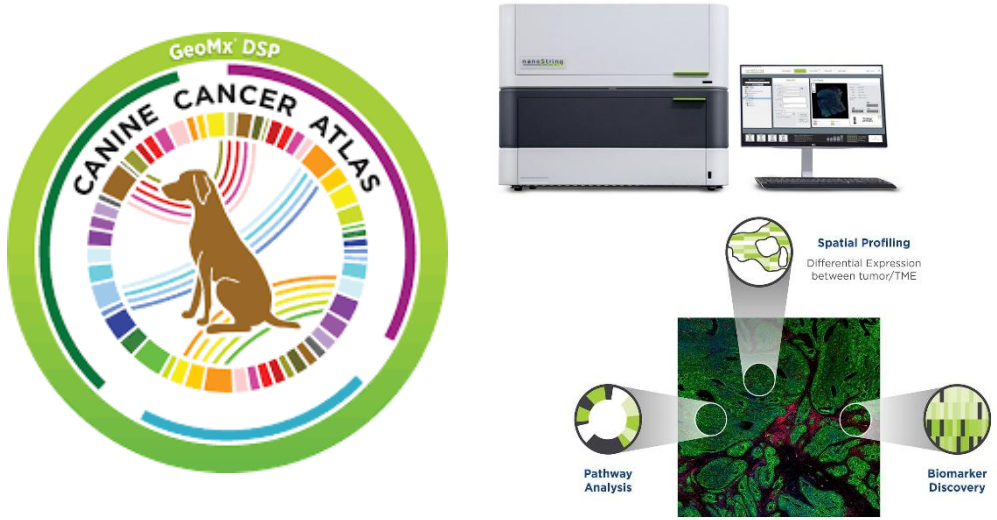




Dittrich et al, 2023

Partnerships to enhance genomics

Spatial Transcriptomics (GeoMx)

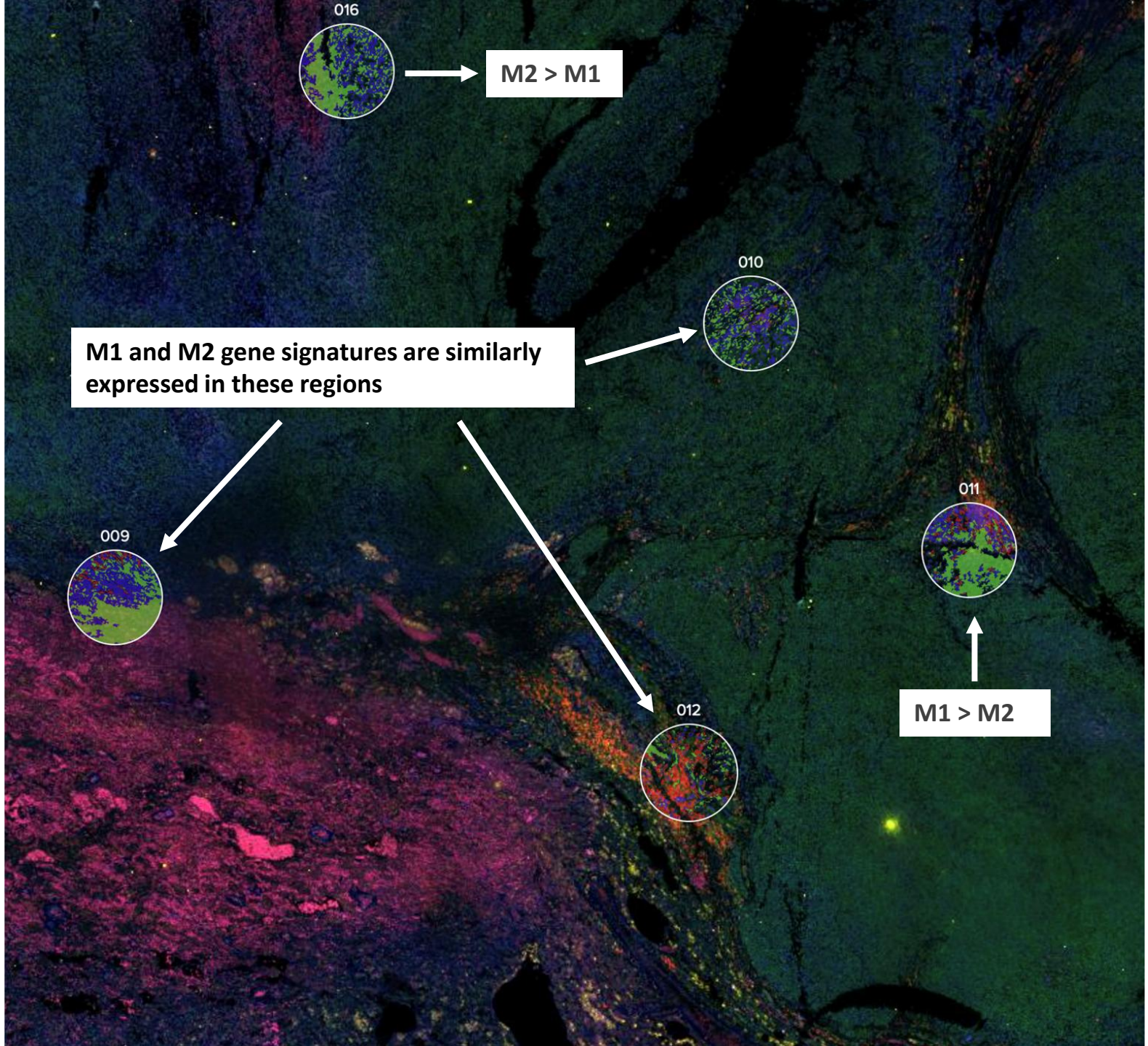


- Spatially profile 1,962 genes and 48 control targets across 110 annotated pathways involved in the canine immune response to IO treatments
- Tumor specific content for sevearl canine cancers including melanoma, osteosarcoma, lymphoma, urothelial carcinoma, and glioblastoma
- Overlapping content with Canine IO Panel

GeoMx Canine Cancer Atlas

Canine Osteosarcoma

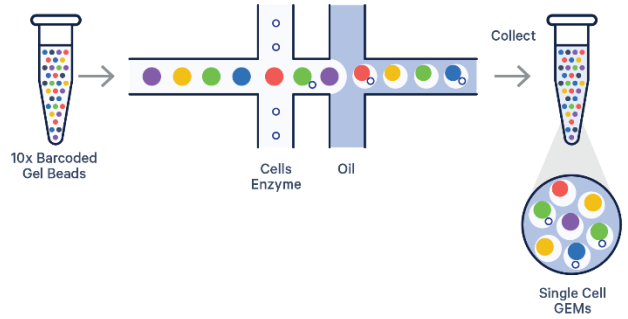
- M1/M2 gene signatures were equally expressed in most ROIs
- M1 or M2 gene signatures predominated in only select ROIs
- These data are concordant with similar data from humans demonstrating regional differences in macrophage phenotypes across the tumor microenvironment.



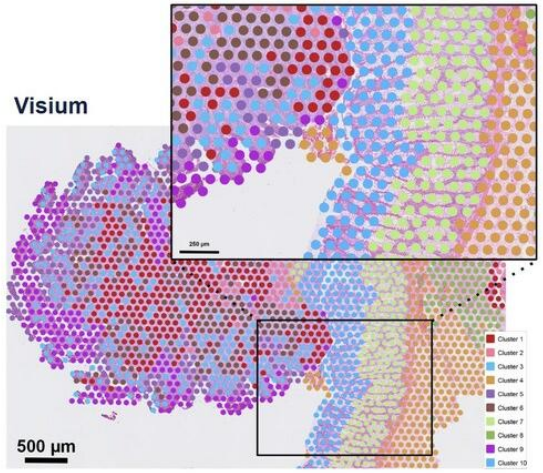
Gardner/London, unpublished

Partnerships to enhance genomics

Single Cell RNA Sequencing

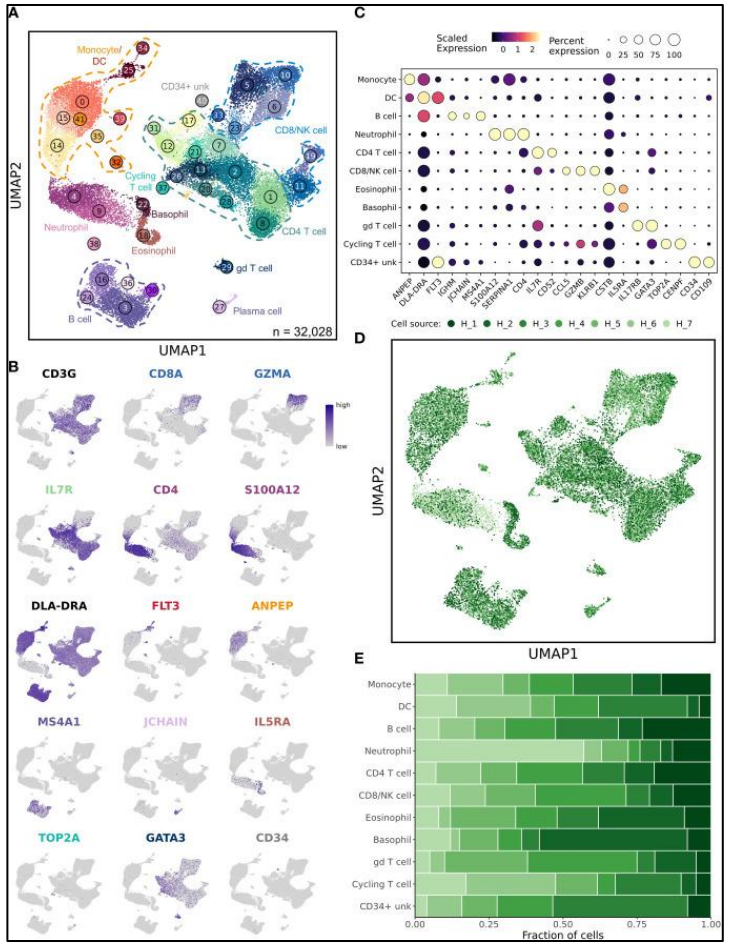


Spatial Transcriptomics (Vizium)

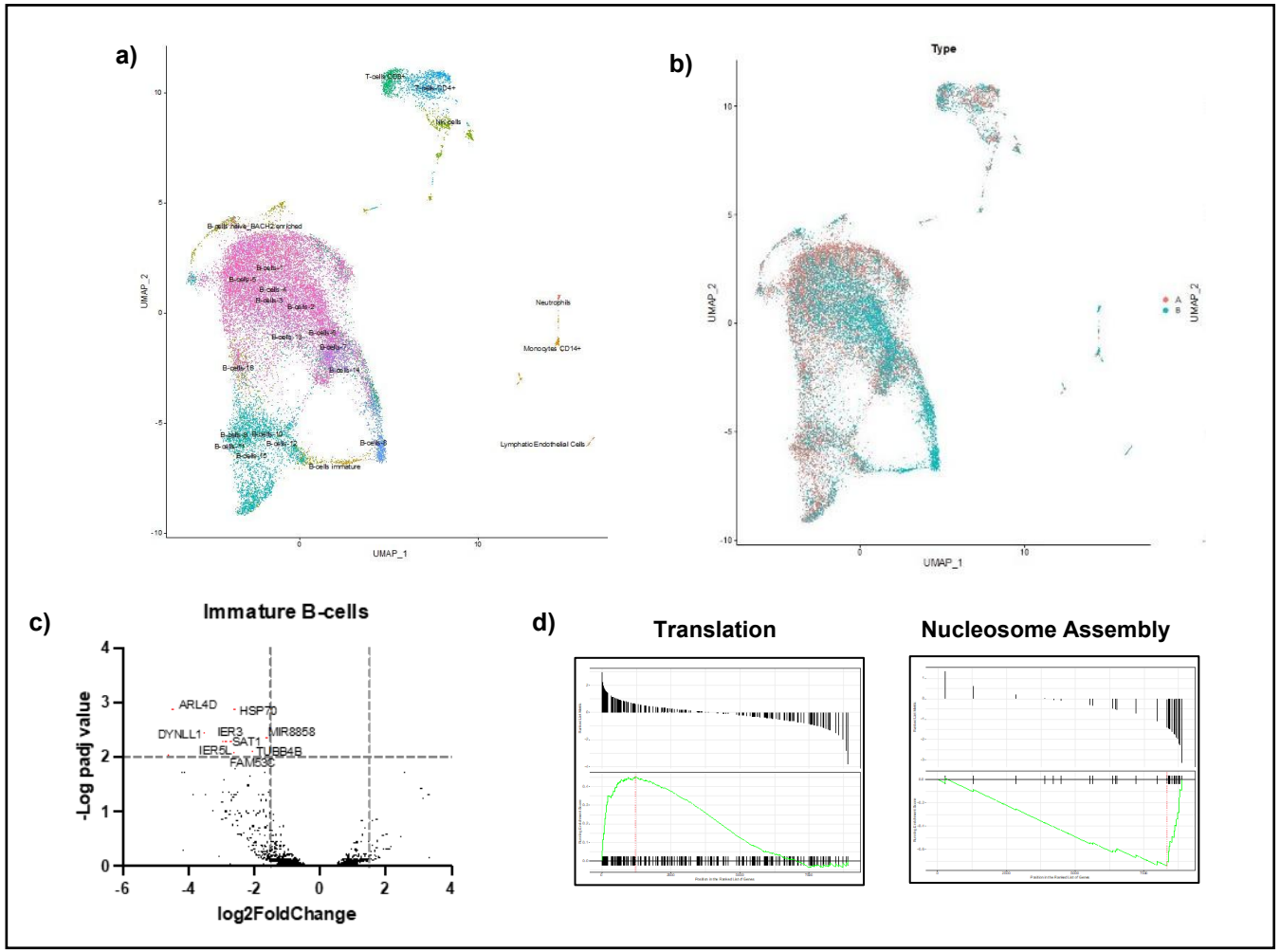


- **Historically, these platforms have been limited to using live single cells and frozen tissue due to the lack of canine specific reagents**
- **10X has partnered to develop canine specific panel that enables use of formalin fixed specimens for both platforms to interrogate gene expression profiles**

Single cell RNAseq of canine normal and neoplastic cells



Unsupervised clustering reveals 42 unique cell populations in healthy dog leukocytes.

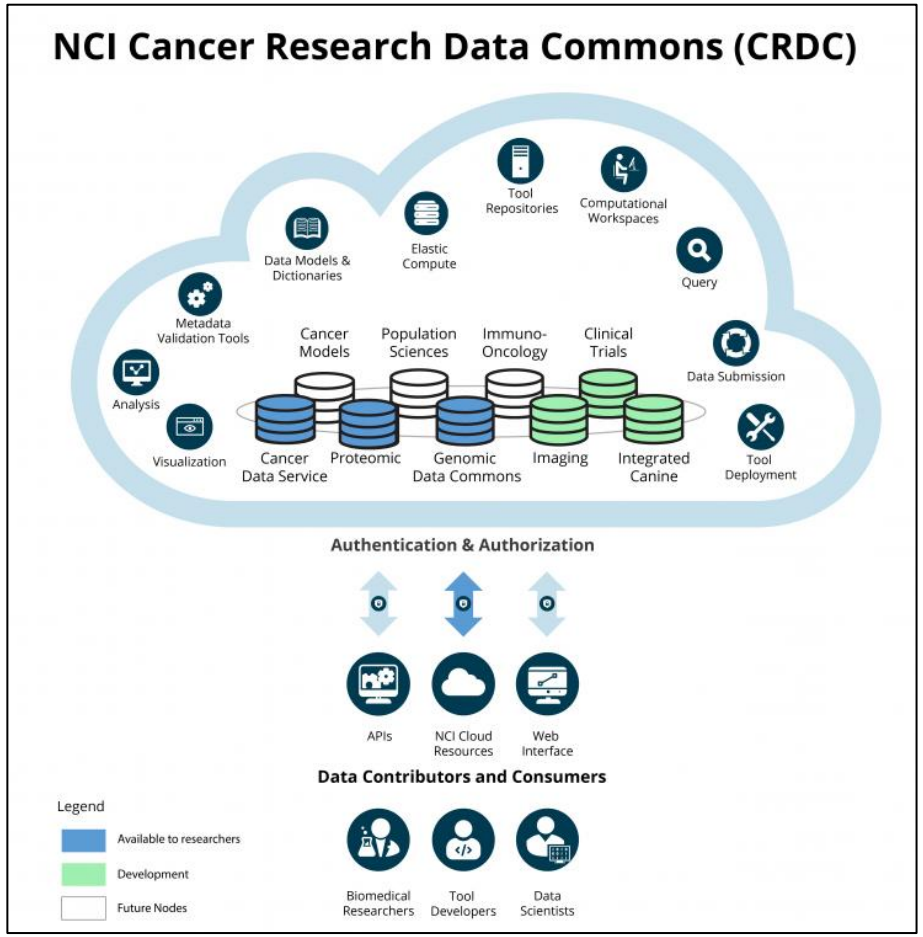


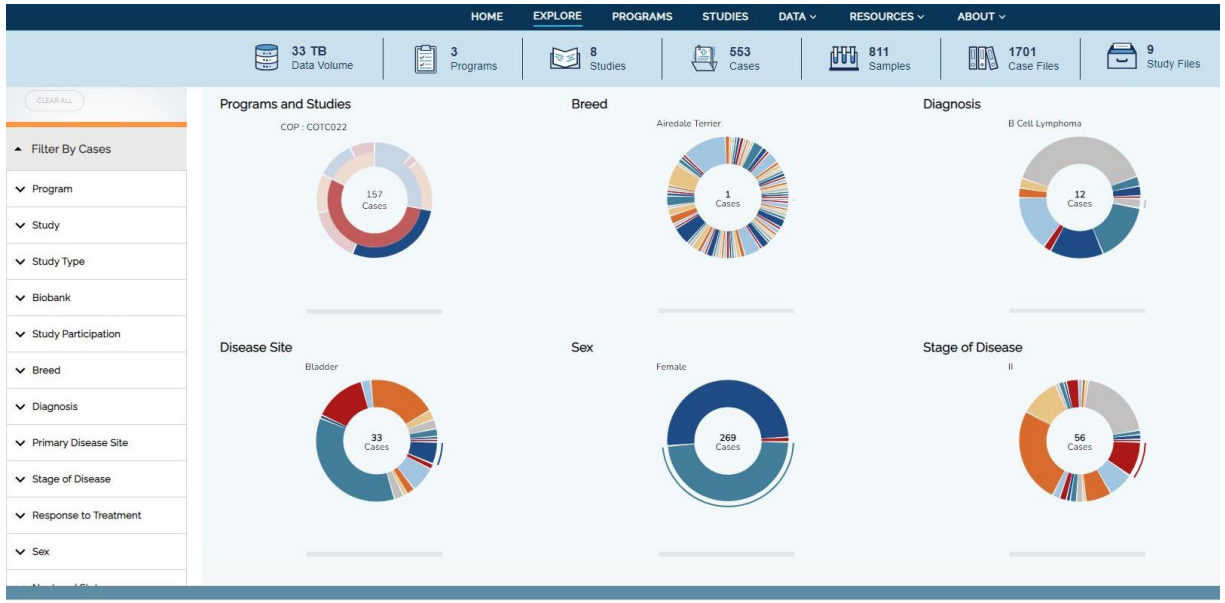
Rapid shift in gene expression in malignant B cells within 2 hours following treatment (*pre*: orange; *post*: blue)**.**

Ammons et al, 2023

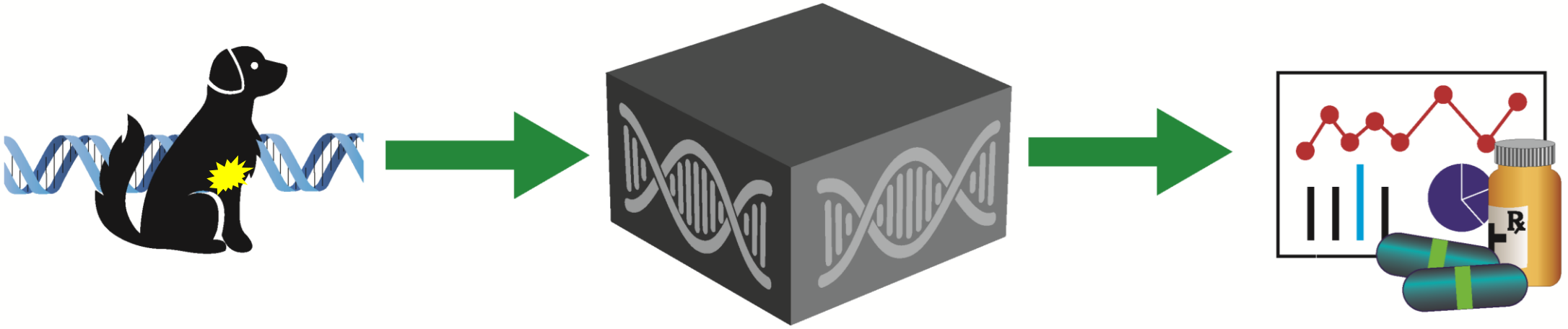
Gardner, unpublished

Integrated Canine Data Commons





Leveraging the Canine Cancer Genome to Improve Treatment



Small Molecule Inhibitors in Veterinary Medicine

Small molecule inhibitors have been developed for use in veterinary medicine

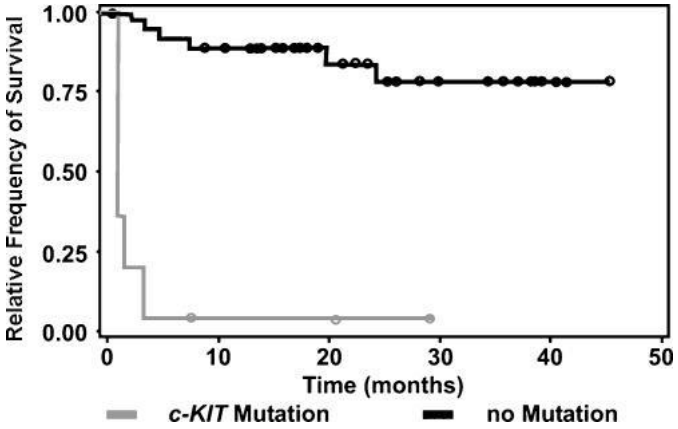
- **Toceranib (Palladia): multi-targeted to KIT, VEGR, PDGFR:**
- **Verdinexor (Laverdia): XPO1 inhibitor**

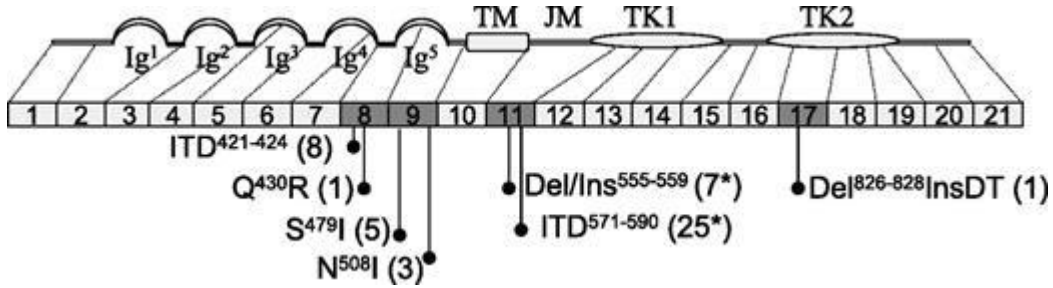
Several approved human inhibitors are now being used to treat canine cancers based genomic data

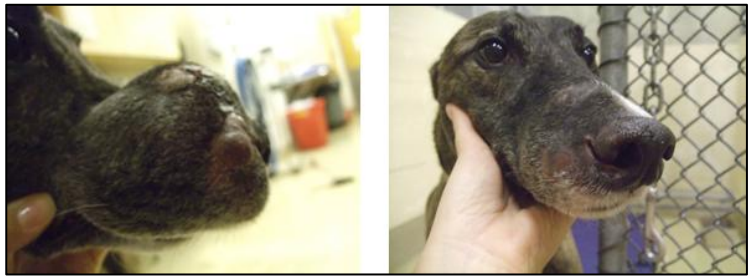
- **Trametinib: MEK inhibitor: histiocytic sarcoma (PTPN11), bladder cancer BRAF**
- **Rapamycin: mTOR inhibitor: hemangiosarcoma, mammary tumors (PI3Kα mutation)**
- **Dasatinib: SRC inhibitor: osteosarcoma (DMD mutation)**
- **Vemurafenib: BRAF inhibitor: bladder cancer (BRAF)**
- **Imatinib: KIT/PDGFR inhibitor: mast cell tumor, GIST (KIT)**
- **Sorafenib: multitargeted: hepatocellular carcinoma**
- **Lapatinib: EGFR inhibitor: bronchoalveolar carcinoma**
- **Acalabrutinib: BTK inhibitor: B cell lymphoma**
- **Vorinostat: HDAC inhibitor: hemangiosarcoma, other cancers**

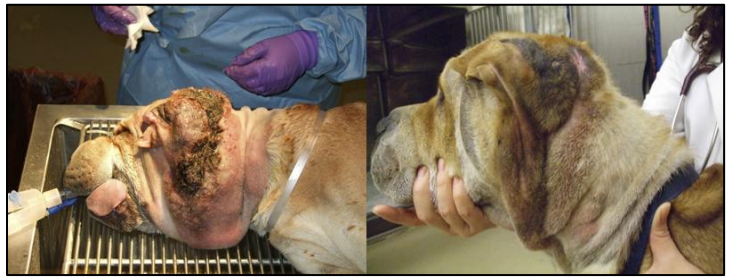
KIT mutations in Canine Mast Cell Tumors

- Mutations in KIT occur in canine mast cell tumors consisting of short insertions (short internal tandem duplications).
- These cause uncontrolled KIT signaling and contribute to aggressive tumor behavior; are associated with a worse prognosis.
- The single agent response rate of MCT with KIT exon 11 mutation to toceranib is over 70%.
- Imatinib exhibits similar activity in this setting.



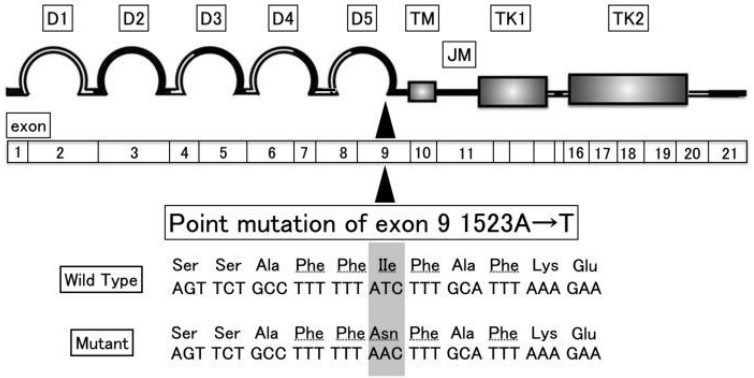


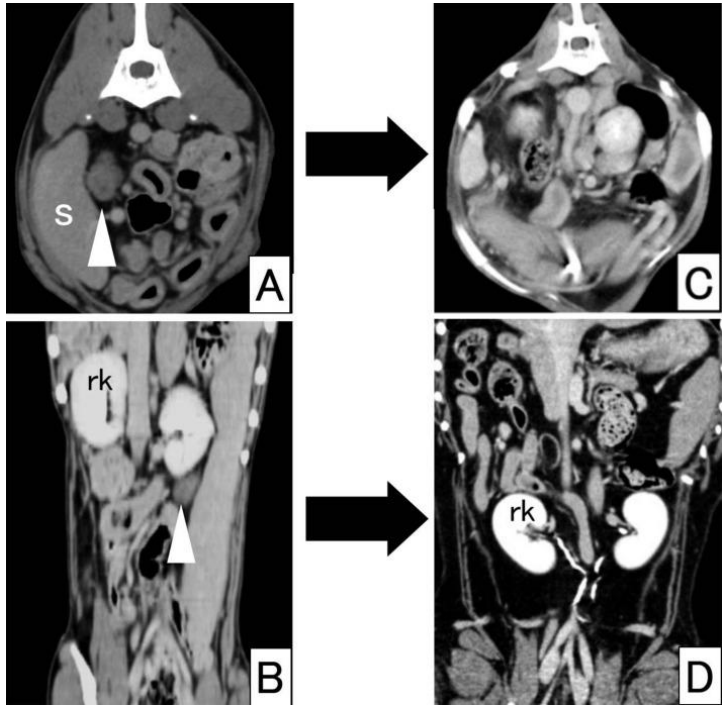




KIT mutations in Canine GIST

- **Mutations in KIT have been identified in canine GIST.**
- **Imatinib has been used both in the setting of macroscopic and microscopic disease in a limited number of cases with promising results.**

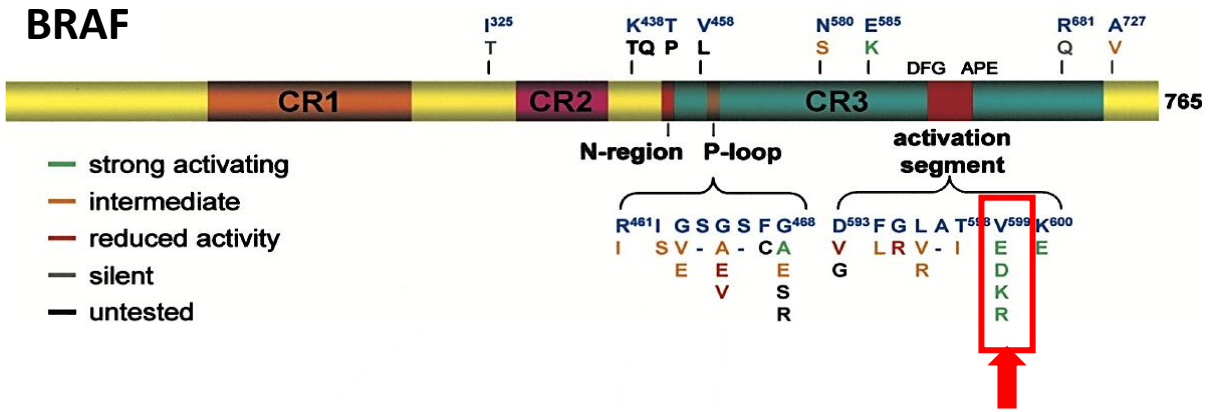


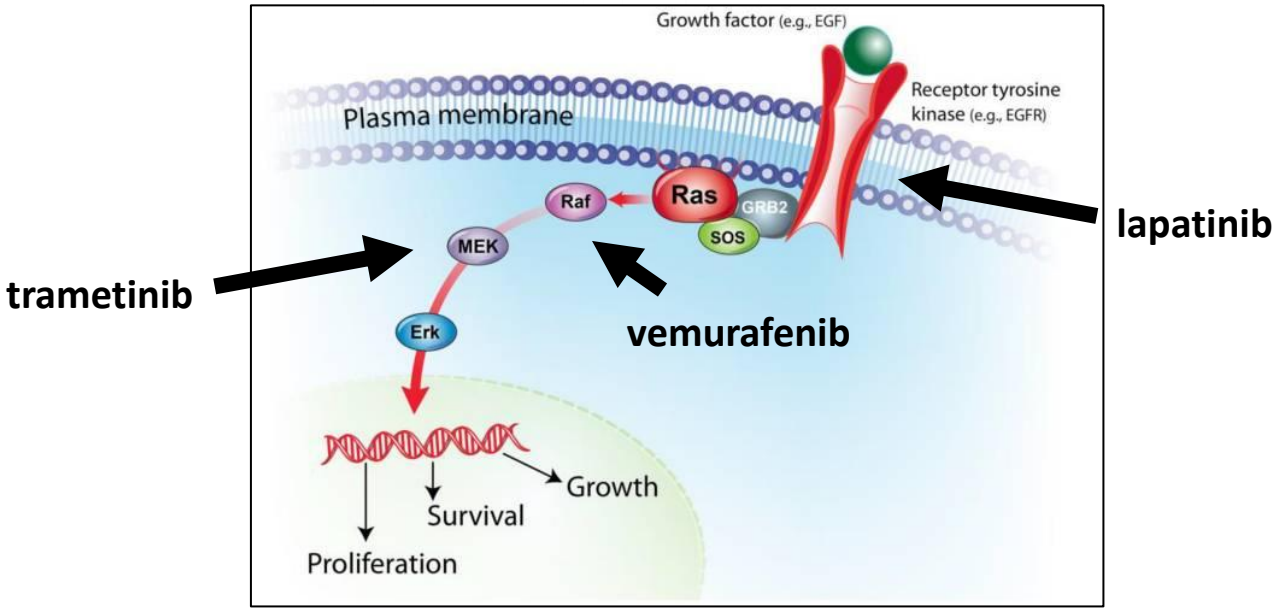


Takeuchi et al, 2015

Point Mutation in BRAF in Canine TCC

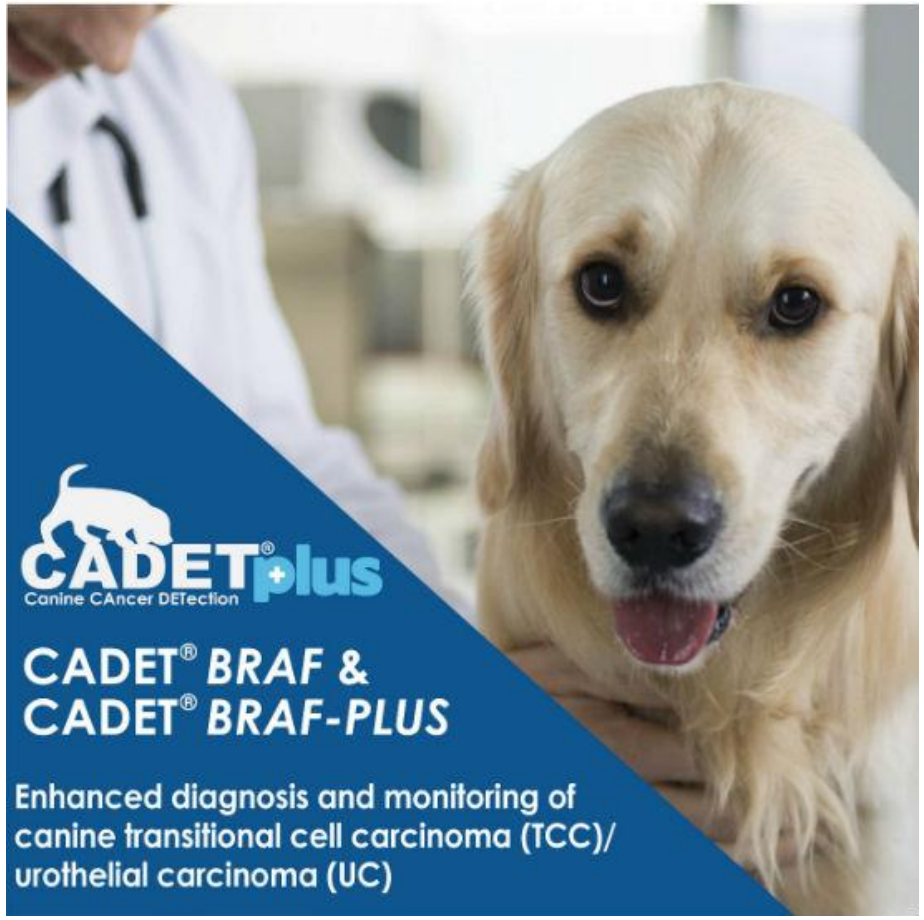
- Over 80% of canine transitional cell carcinomas of the bladder possess activating mutations in the oncogene BRAF.
- The mutation in BRAF is analogous to the BRAF mutation found commonly in human cutaneous malignant melanoma and some other human carcinomas (thyroid, colon).
- A recent trial of vemurafenib in dogs demonstrated some clinical efficacy (38% partial response rate).
- Data generated with TCC cell lines in dogs suggests that inhibition of upstream (EGFR, lapatinib) and downstream (MEK, trametinib) elements of the signaling pathway may be more effective in controlling disease.

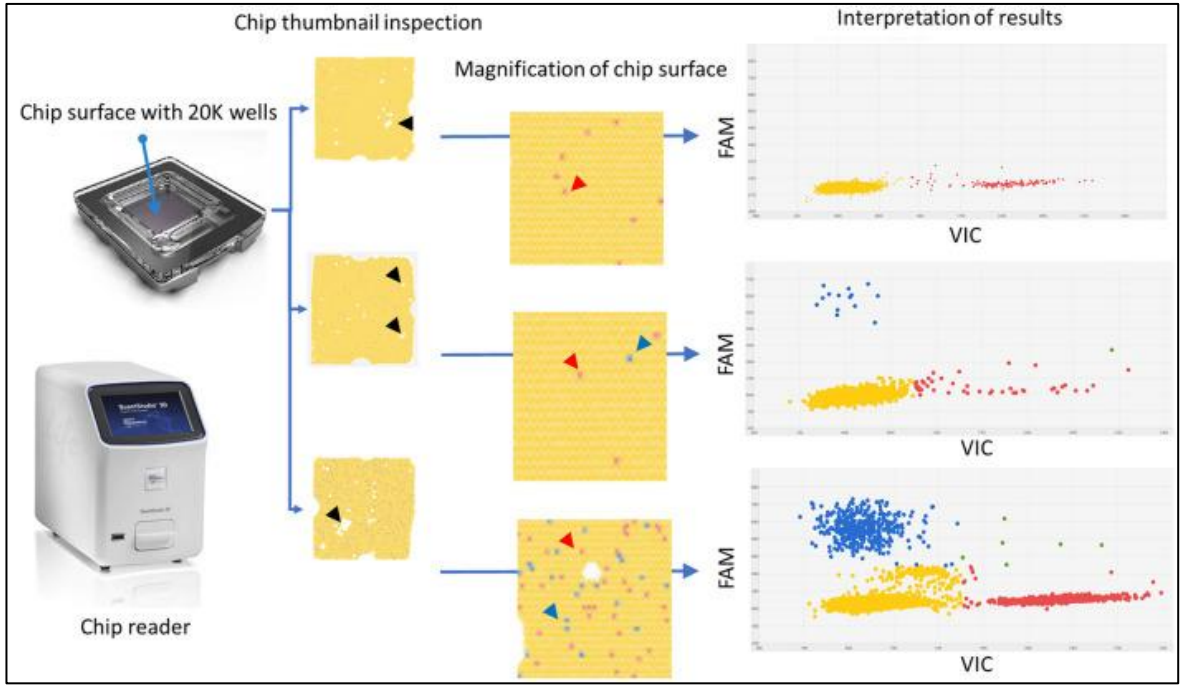




Decker et al. Mol Cancer Res 2015

Liquid Biopsy Bladder Cancer Test





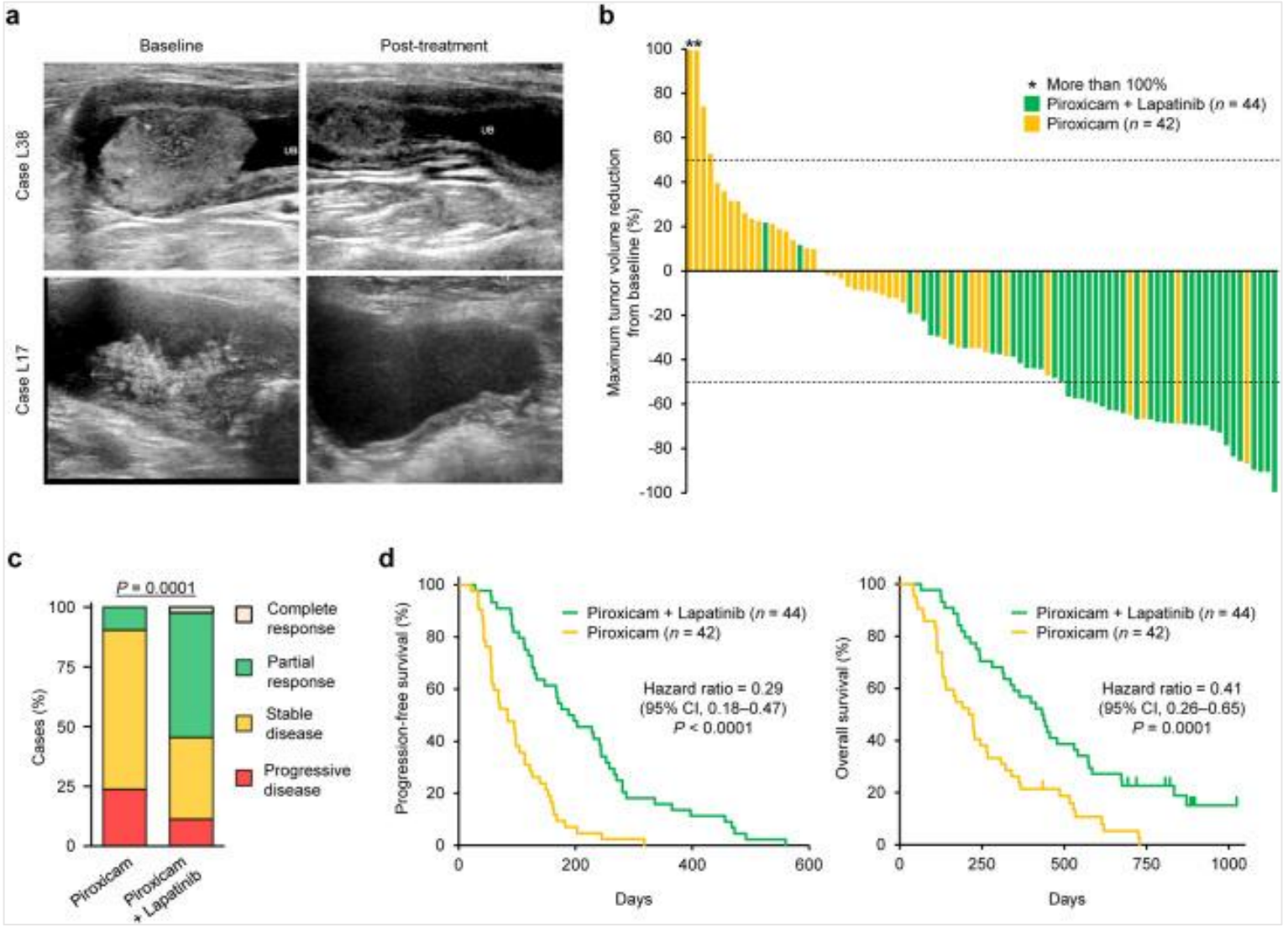
Lapatinib in Canine TCC

(a) Representative ultrasonographic images of bladder masses in dogs treated with lapatinib and piroxicam.; mass was completely absent 12 weeks after treatment.

(b) Waterfall plot showing the maximum percentage of tumor burden reduction from baseline in dogs treated with lapatinib and piroxicam or piroxicam alone.

(c) Clinical responses in dogs treated with lapatinib and piroxicam or piroxicam alone.

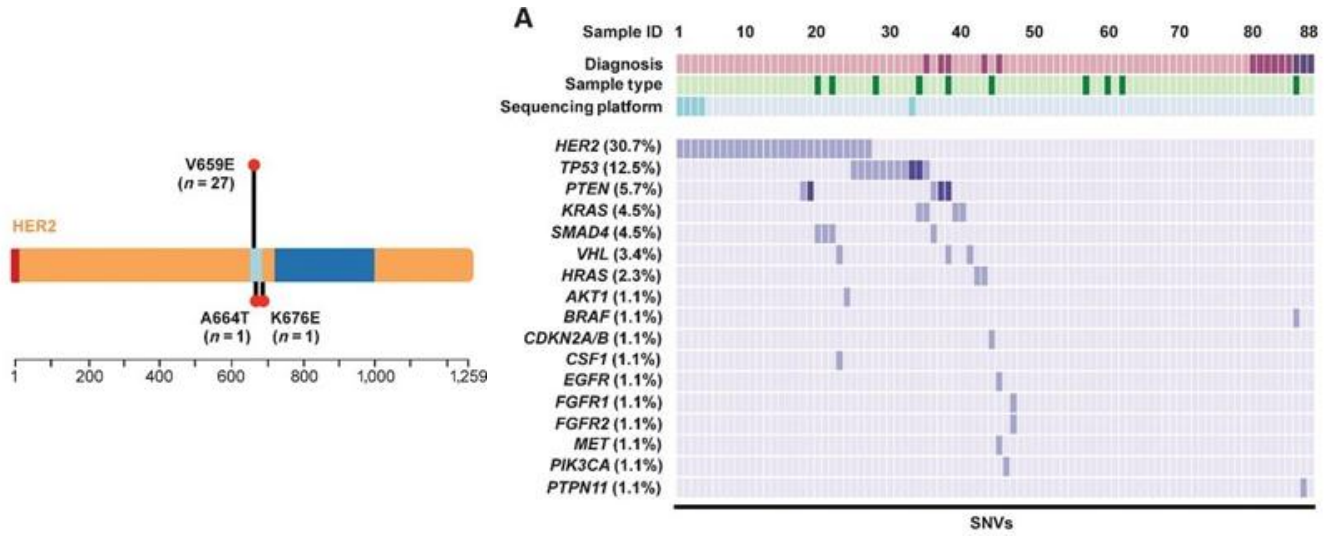
(d) (d) Progression-free survival (left) and overall survival (right) in dogs treated with lapatinib and piroxicam or piroxicam alone

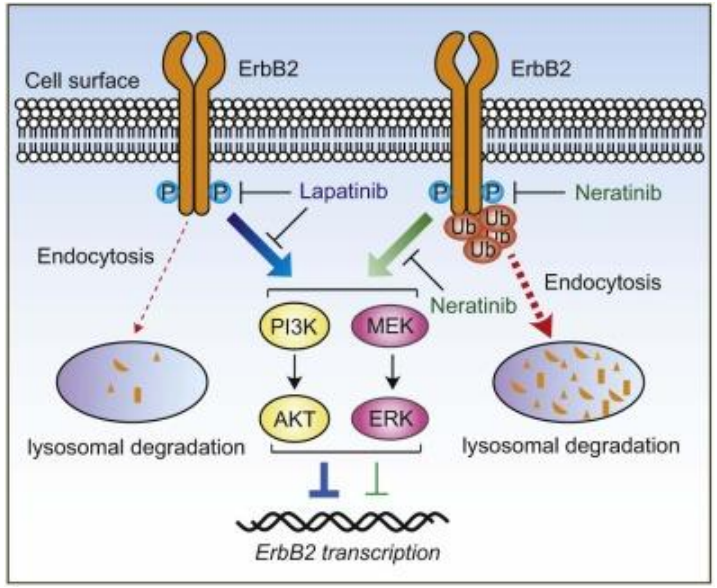


Maeda et al, 2022

Point Mutation in EGFR in Canine Lung Cancer

- Point mutations in the HER2/neu were detected in 38% of canine pulmonary adenocarcinomas (28/74), but none in adenosquamous (0/11) or squamous cell (0/3) carcinomas.
- The majority (93%) of HER2 mutations were hotspot V659E transmembrane domain (TMD) mutations comparable to mutations at this same site in human cancers (lung, gastric).
- The mutations in canine HER2 are activating and represent a target for lapatinib and neratinib, both EGFR inhibitors.



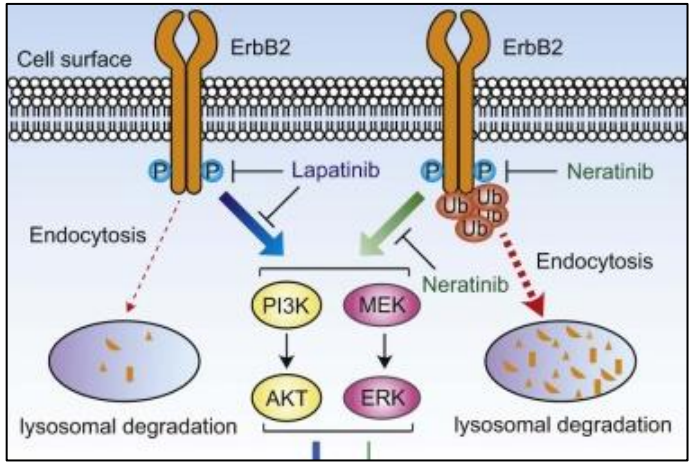


Lorch et al. Clin Cancer Res 2019; Zhang Cancer Letters, 2016

Nouveau
11 yr old Golden Retriever

Gene	Mutations
TSC1	Pro588Ala
SETD2	Thr301fs
PIK3CA	Glu418Lys
ERBB2	Val659Glu
BRCA2	Asn2929Asp (SNP)

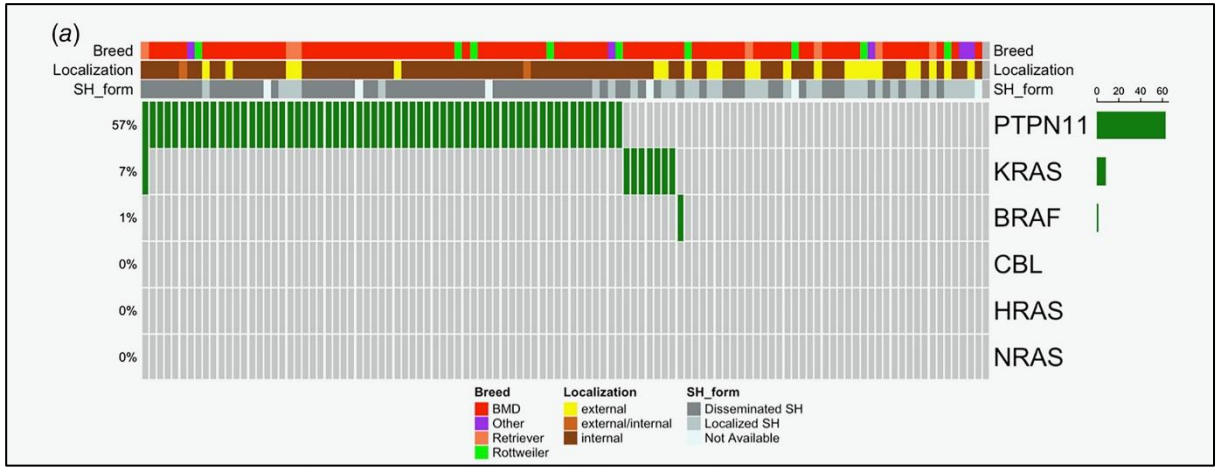
- Diagnosed with pulmonary carcinoma.
- Analysis revealed an activating mutation in ERBB2.
- Started on lapatinib.
- Partial response (over 50% reduction) in tumor size.
- Lived 14 months post diagnosis.

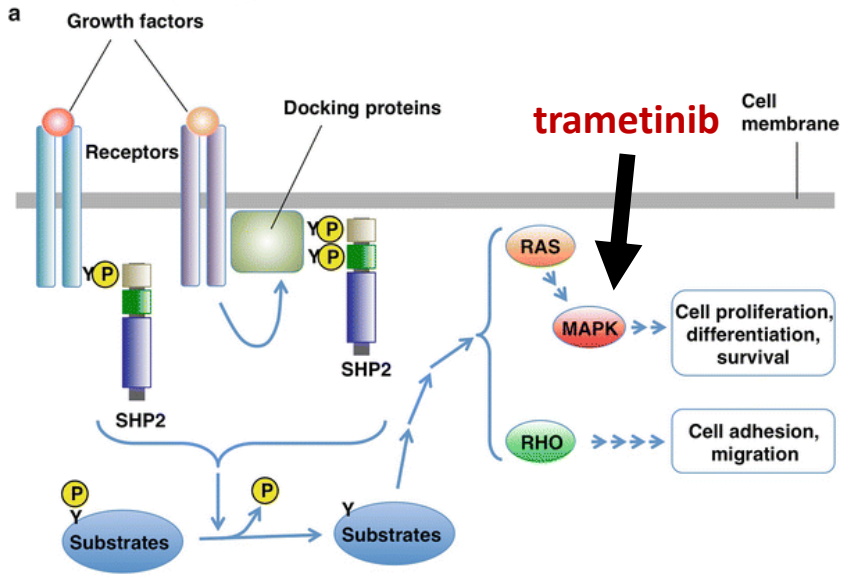


FidoCure®

PTPN11 (SHP2) Mutation in Canine Histiocytic Sarcoma

- PTPN11 codes for a tyrosine phosphatase important for regulating cell signaling.
- Mutations in PTPN11 concentrated at hotspots common to human cancers were identified in dogs with systemic and/or aggressive histiocytosis (56.75%; 63/111) .
- Drugs targeting the MAPK pathway were effective in blocking proliferation of canine histiocytic sarcoma cell lines.
- Trametinib likely has utility in treating a substantial subset of histiocytic sarcoma patients.





Decker et al. Mol Cancer Res 2015

Gracie
6 yr old American Pitbull

August 2018:

- PE: firm mass at the dorsomedial aspect of the left quadriceps muscle (~7.5 cm in diameter).
- Underwent surgical debulking; mass was very invasive and complete resection not possible.

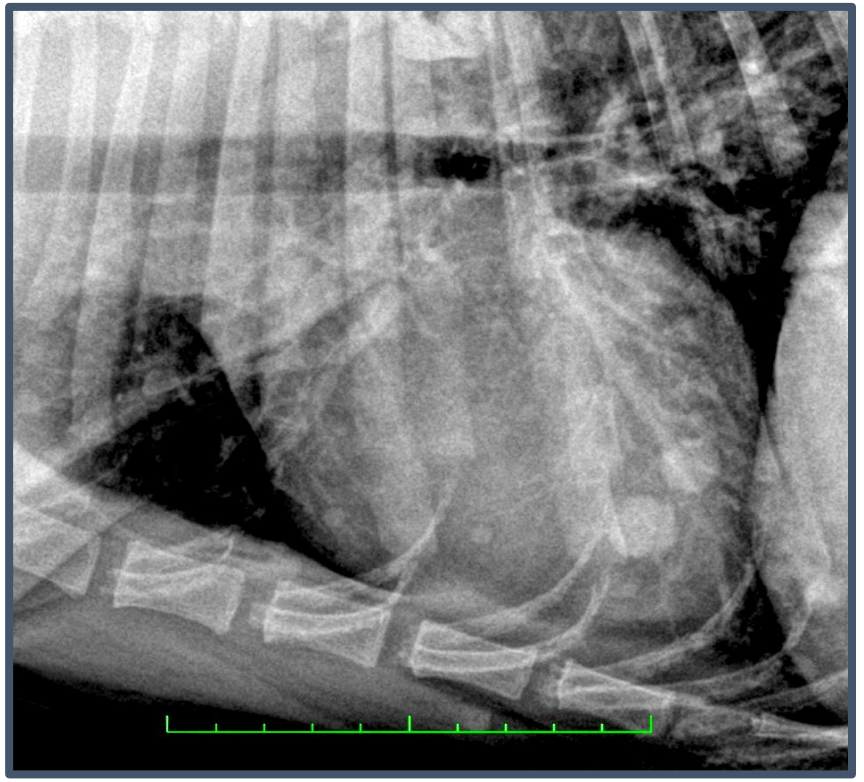
Histopathology: Poorly differentiated incompletely excised sarcoma

January 2019:

- Tumor recurred locally; owners elected referral to oncologist

February 2019

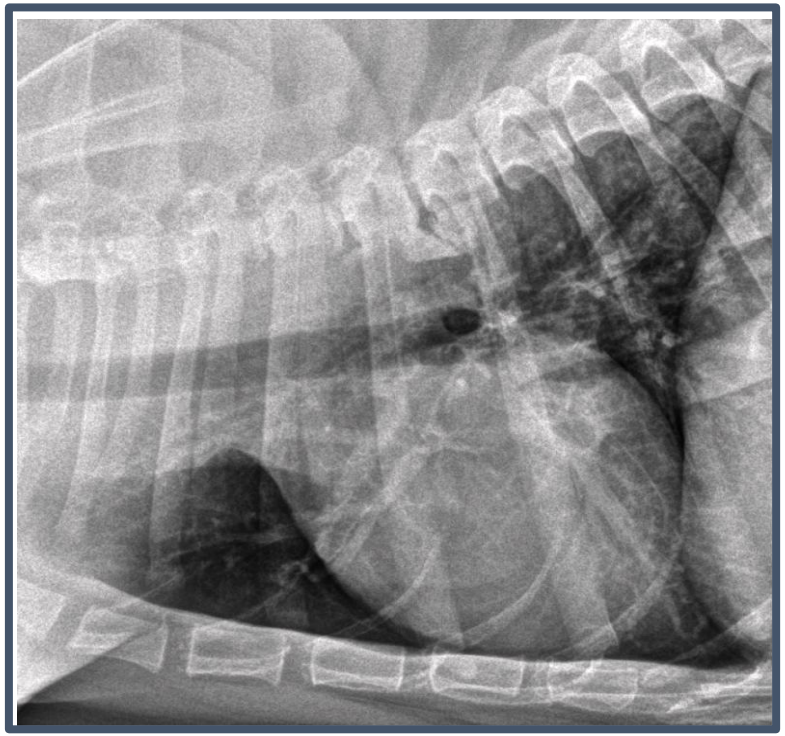
- Non-resectable mass and multifocal metastatic disease to the lungs.
- Tissue samples sent to FidoCure® for analysis.



FidoCure®

Gene	Mutations
PDGFRB	Ala1064_Asp1065insAlaAsp
CREBBP	Pro2112_Gln2113del
BRCA2	Met3332_Lys3333insIleLys
RTP2	His97Tyr
NOTCH1	Ser1163Asn - SNP
KMT2C	Arg1623Cys
VEGF, MEK1, MEK2 and MYC overexpressed	

Date	Primary Tumor	Thoracic Rads
Feb 2019	7.5 cm x 7.5 cm	multiple mets
March 2019	7 cm x 5.5 cm	NED
May 2019	2.5 cm x 2.5 cm	NED
July 2019	2.0 cm x 2.3 cm	NED



- Began trametinib (MEK inhibitor), imatinib (PDGFR inhibitor) and rapamycin (mTOR inhibitor) through FidoCure.
- Had complete resolution of pulmonary mets, and partial response of primary tumor
- Disease recurred in October 2019; euthanized November 2019.

FidoCure®

Conclusions

- A detailed understanding the human tumor genomic landscape has dramatically altered treatment strategies.
- Over 100 small molecule inhibitors are now approved to treat human cancers, and their use is typically guided by patient specific tumor mutations/genetic alterations.
- Veterinary oncology has begun leveraging this information to treat affected patients using both FDA approved veterinary drugs as well as available human small molecule inhibitors.
- Companies are now offering tests that can be used to guide patient specific treatments. These include screening for known mutations, broader exome sequencing of tumor biopsies to capture additional mutations and liquid biopsy analysis for cancer detection.
- Effective application of both small molecule inhibitor and immunotherapy approaches to canine cancer will continue to improve as larger sets of canine cancer genomic data are curated.