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Pioneering Personalized Diagnostics

Clinical Next-Gen Sequencing for Solid Tumors: What, How, Why and When?

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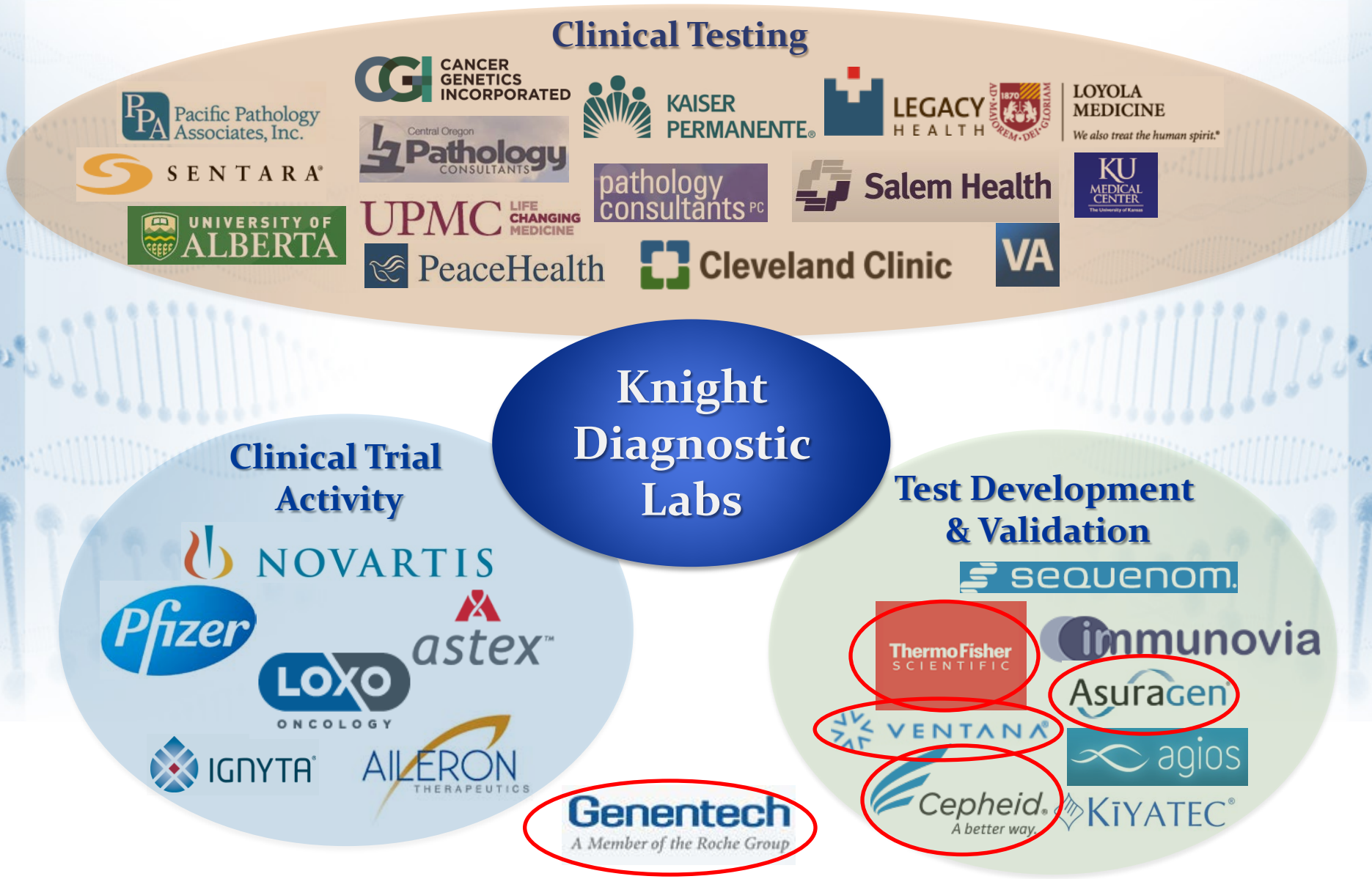
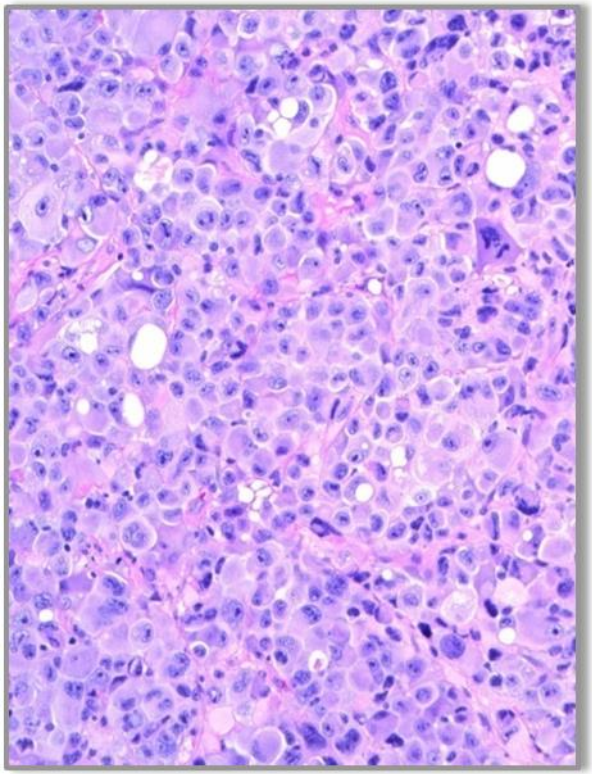


Individualized Cancer Medicine

- 42 year old male with ‘glioblastoma’ treated with surgery, temozolomide and radiation
- Bone and lymph node mets appeared at 20 months (what is this tumor?)
- Admitted to OHSU to manage pain, monitor pending cord compression
- Another round of chemo failed
- *BRAF* V600E mutation identified
- Patient started on dabrafenib
- Excellent clinical response

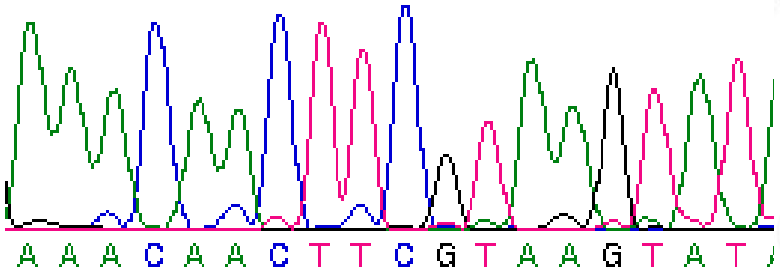


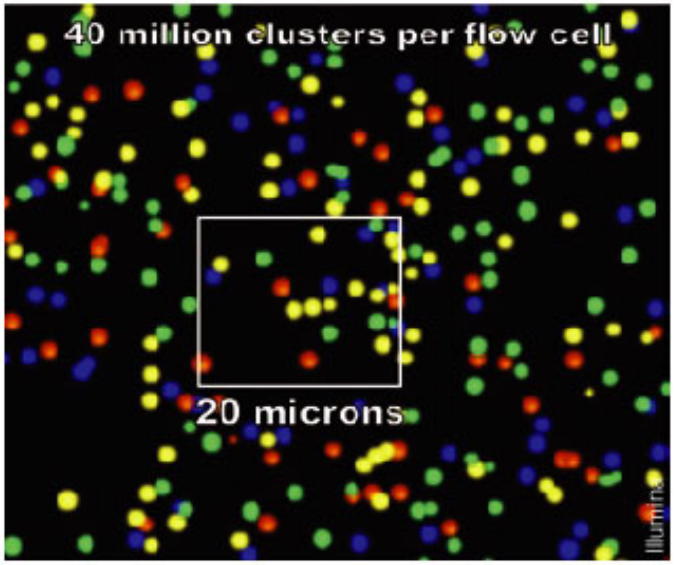
Topics

- Brief intro to next-gen sequencing
- Squeezing NGS data from tiny samples
- NSCLC as a model for routine molecular subtyping
  - Mutations
  - Copy number changes
  - Fusion gene detection
- Interpretation of sequence alterations

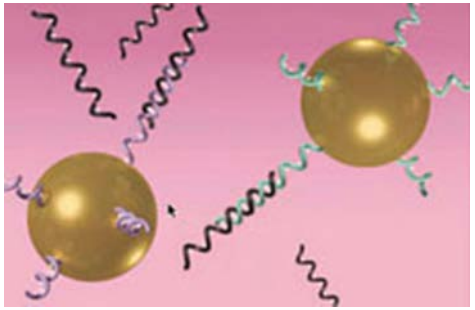
Next-Generation DNA Sequencing

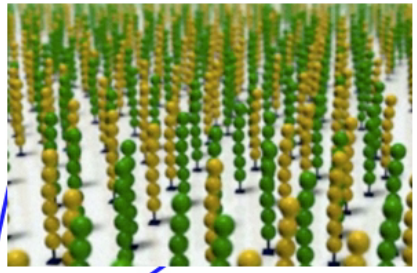
- Massively parallel sequencing (many sequencing reactions performed simultaneously)

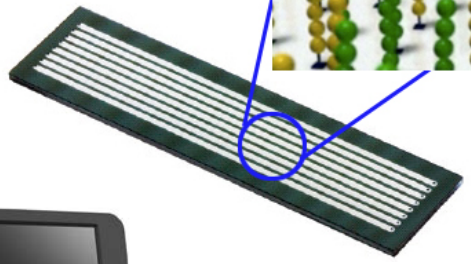


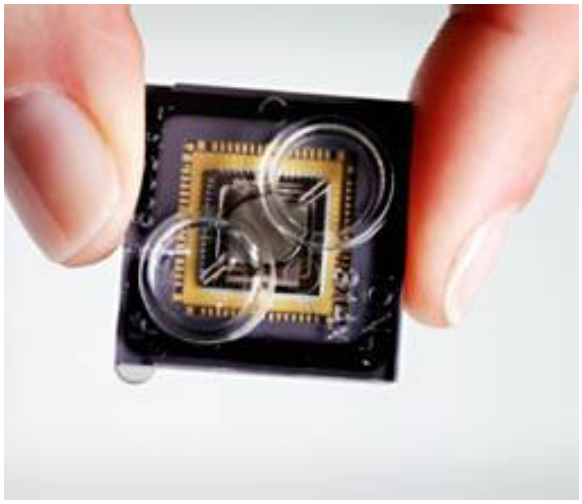


- ATP
- CTP
- GTP
- TTP

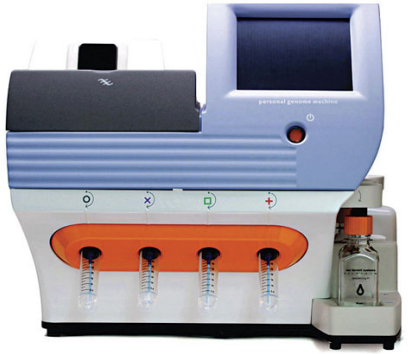






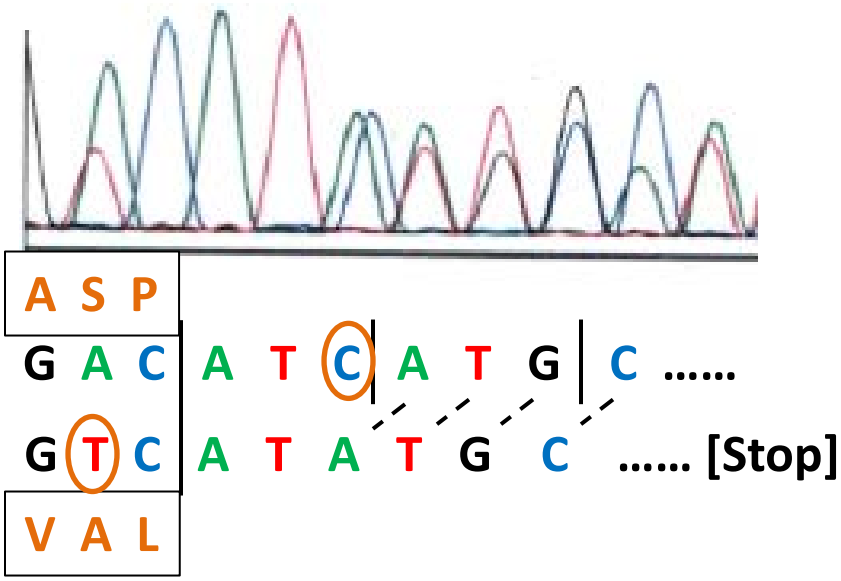


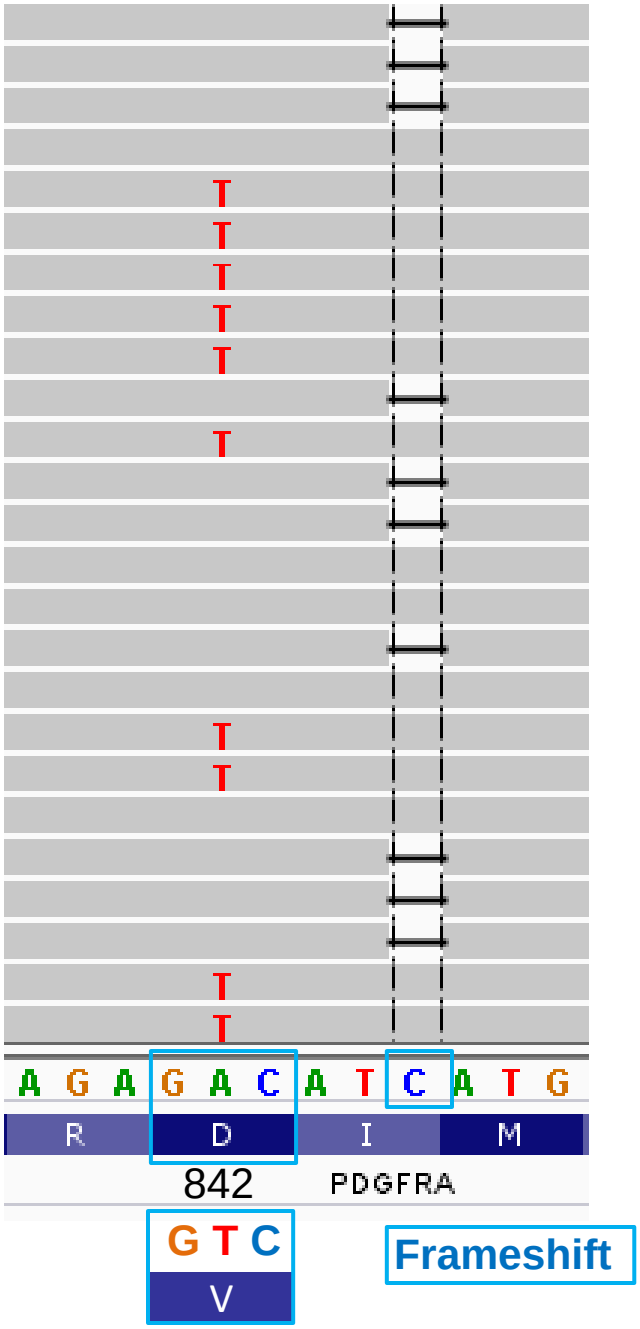
Illumina NextSeq 500



Ion Torrent PGM

Gastrointestinal Stromal Tumor (GIST)

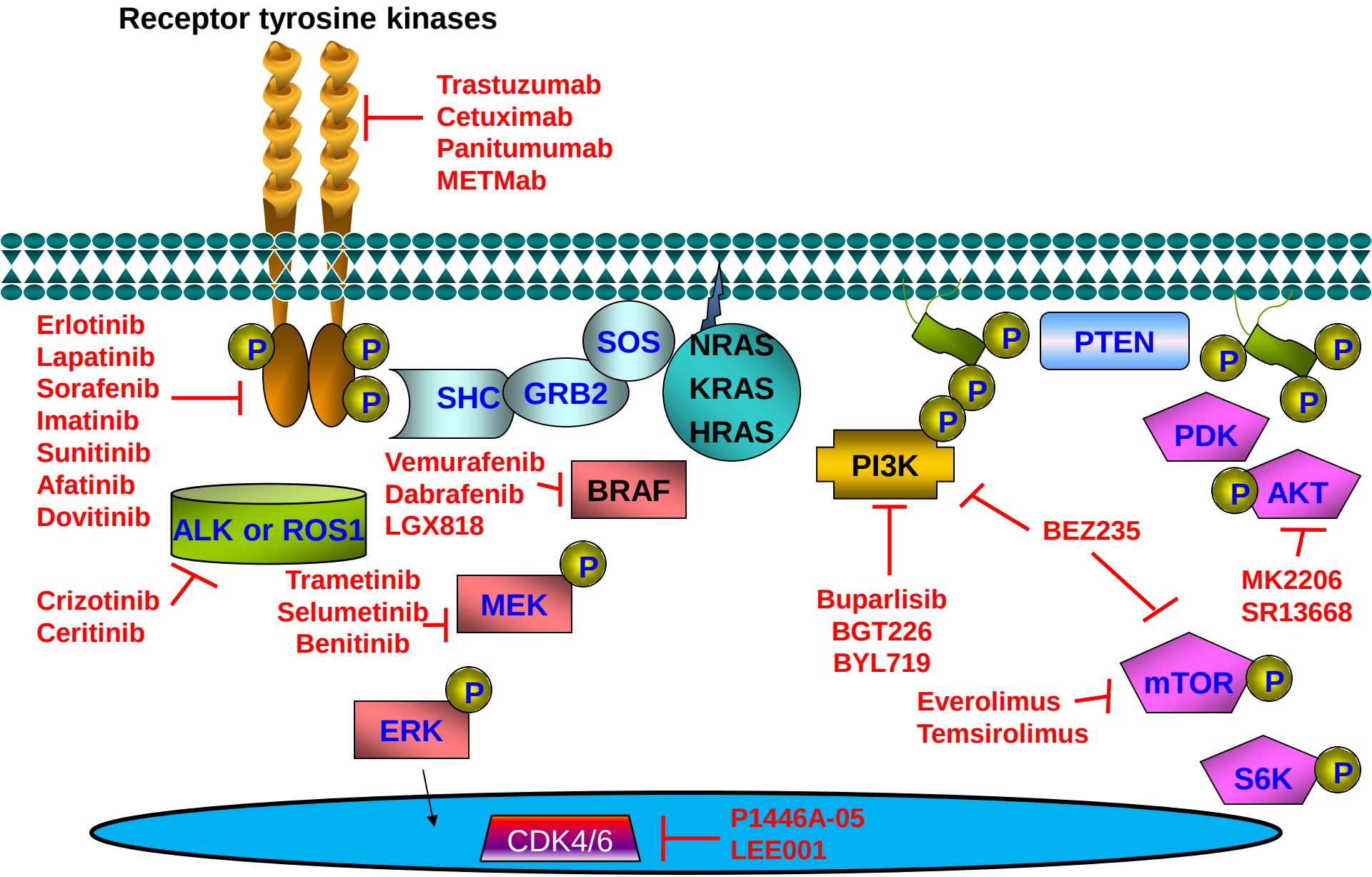




PDGFRA D842V *and* M844fs*16

Known GIST driver mutation Truncates kinase domain

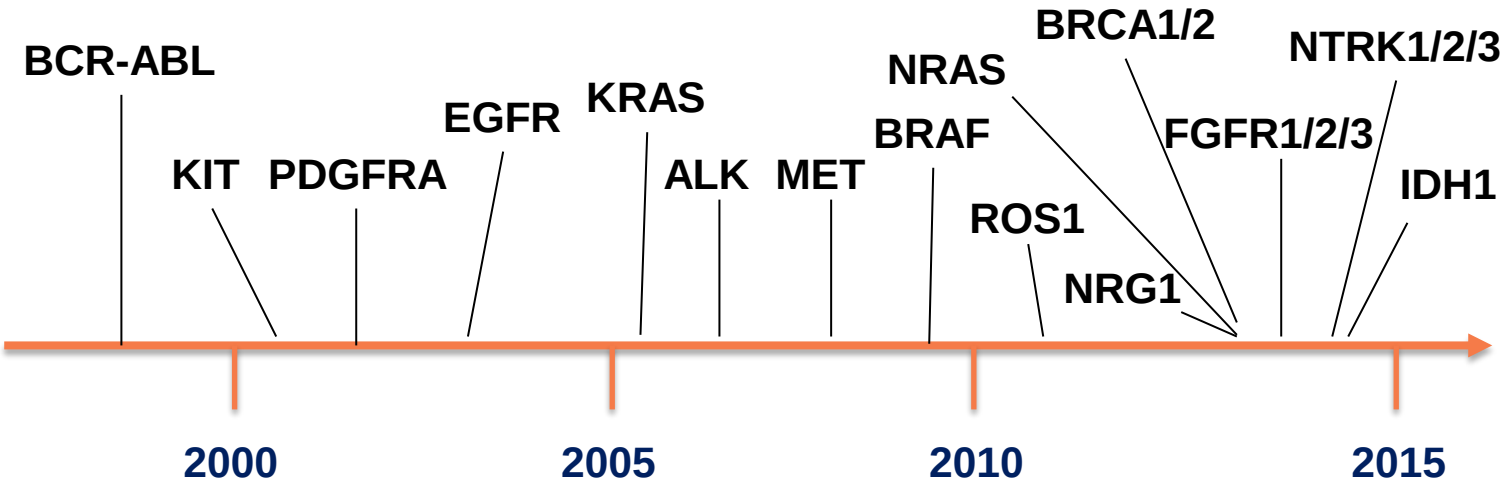
>500 Targeted Therapeutics in Development



Clinically Informative Genes For Solid Tumors

Molecular targets for which:

- FDA-approved therapies are (or likely will be) available
- Molecular testing is required for treatment



Other genes of interest:

- ERBB2, MAP2K1, PIK3CA, AKT1, mTOR, Rictor, TSC1/2

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GeneTrails™ Next-Gen Tests for Cancer

Panel	# Genes	Availability
General solid tumor panel	37	Available
Gene fusion panel for solid tumors	20	Available
Colorectal cancer panel	3*	Available
GI stromal tumor panel	23	Available
AML / MDS panel	42	Available
AML / Lymphoma panel	76	Available
New solid tumor panel	130	April, 2016
Leukemia fusion gene panel	??	Q2 2016

Preparing a Sequencing Library

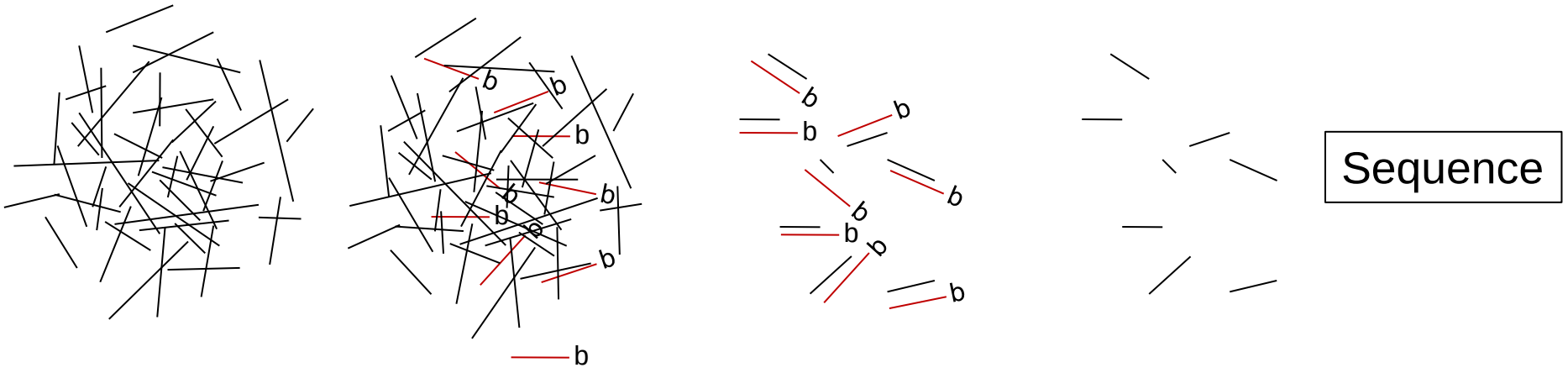
- Hybridization-Capture Approach -

~50-500 ng Genomic DNA

Hybridize to biotinylated probes for desired genes/exons

Purify hybridized RNA probes with magnetic beads

Treat with Rnase and add adapters



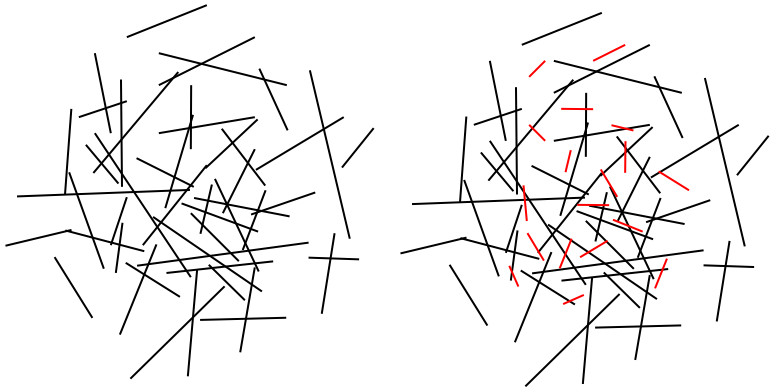
Preparing a Sequencing Library

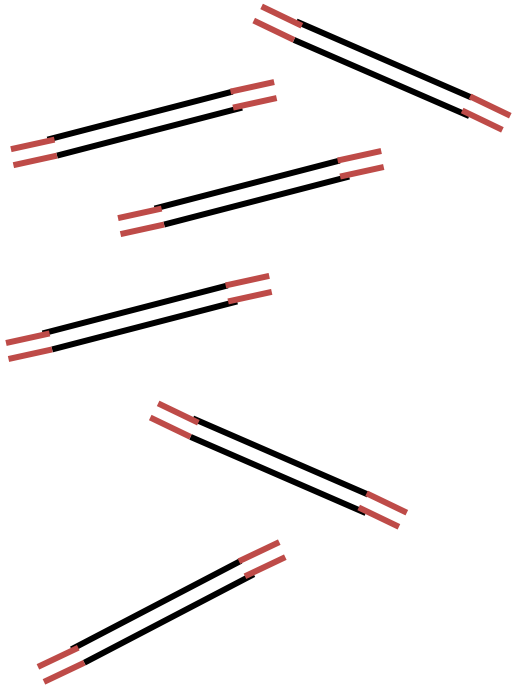
- Amplicon-Based Approach -

10-20 ng
Genomic DNA

Add PCR primers
to genes/exons of interest

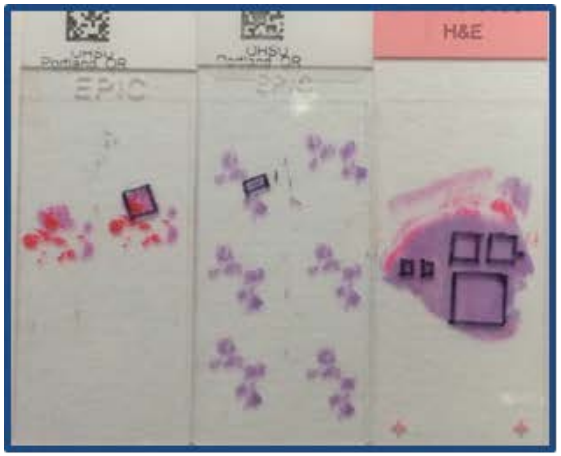
Amplify by PCR
Add adapters and barcodes





Sequence

NGS Workflow



Dissection

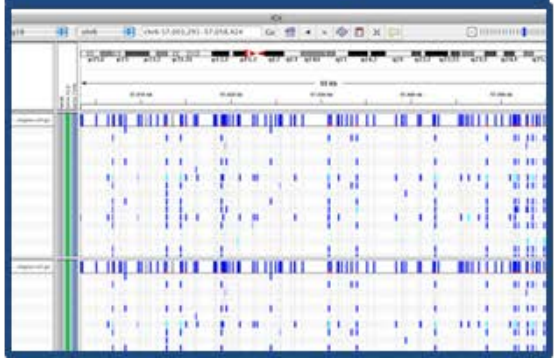
Standard Extraction

Direct Extraction

Library construction

Sequencing

Data analysis

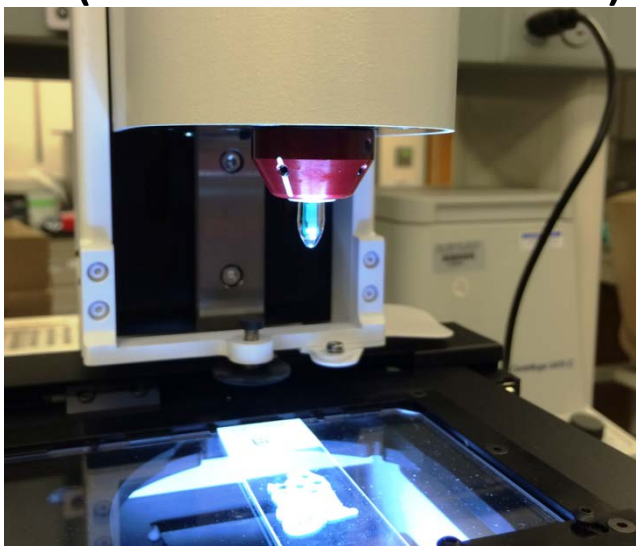


Standard Extraction

- Deparaffinize in mineral oil
- Remove H20 phase
- Add proteinase K
- Heat to 56^{o}C overnight
- Centrifuge briefly
- Purify nucleic acid using mini-column (requires washing and elution steps)
- Measure concentration of purified material

Validating Direct Extraction Method

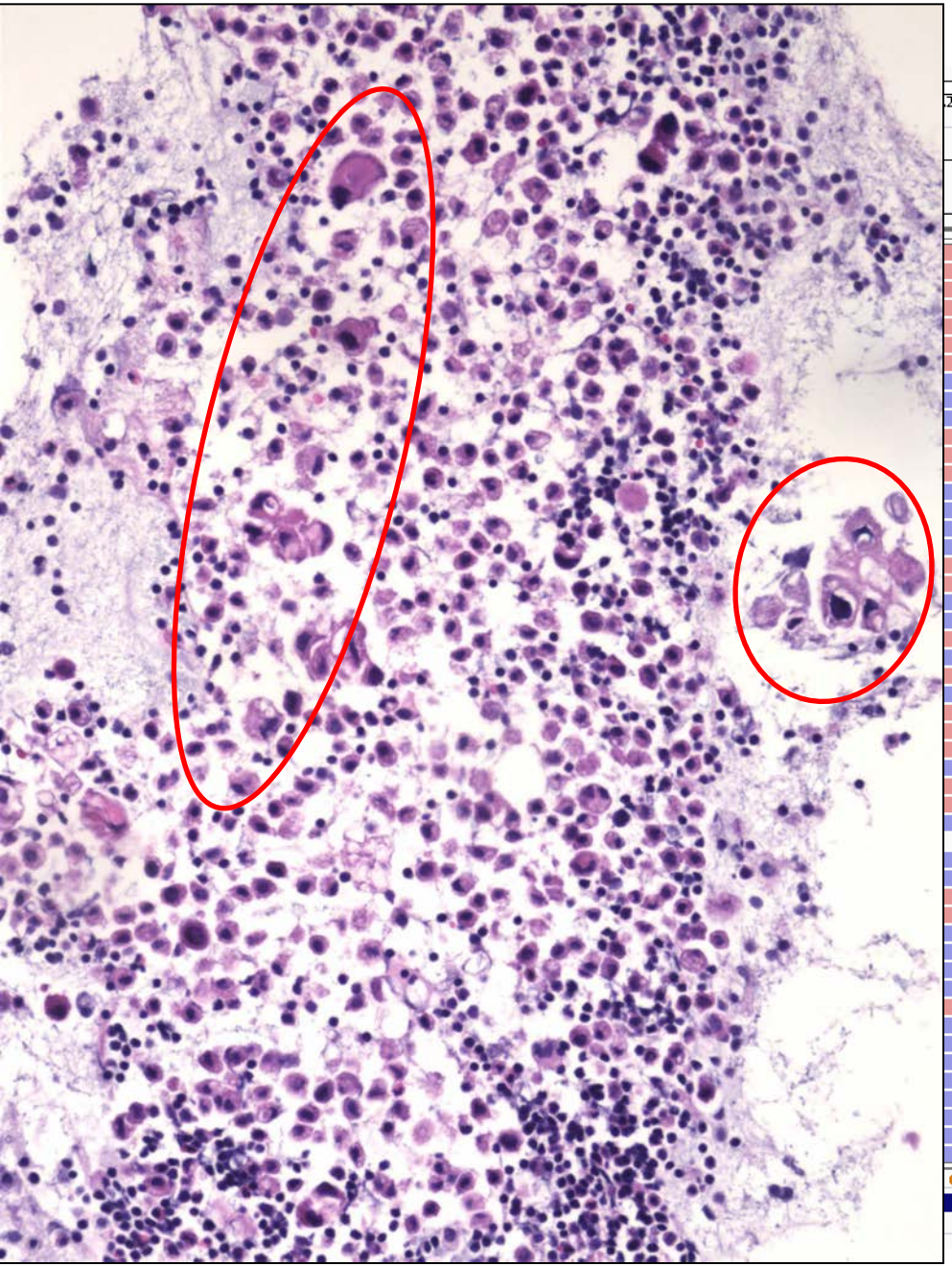
- Multiple tumor types:
  - SQCC, lung adca, colon adca, melanoma, GIST, astrocytoma, low grade B-cell lymphoma
- Varying areas of dissection (5 micron sections):
  - 64 mm^2
  - 32 mm^2
  - 25 mm^2
  - 16 mm^2
  - 4 mm^2
  - 2 mm^2



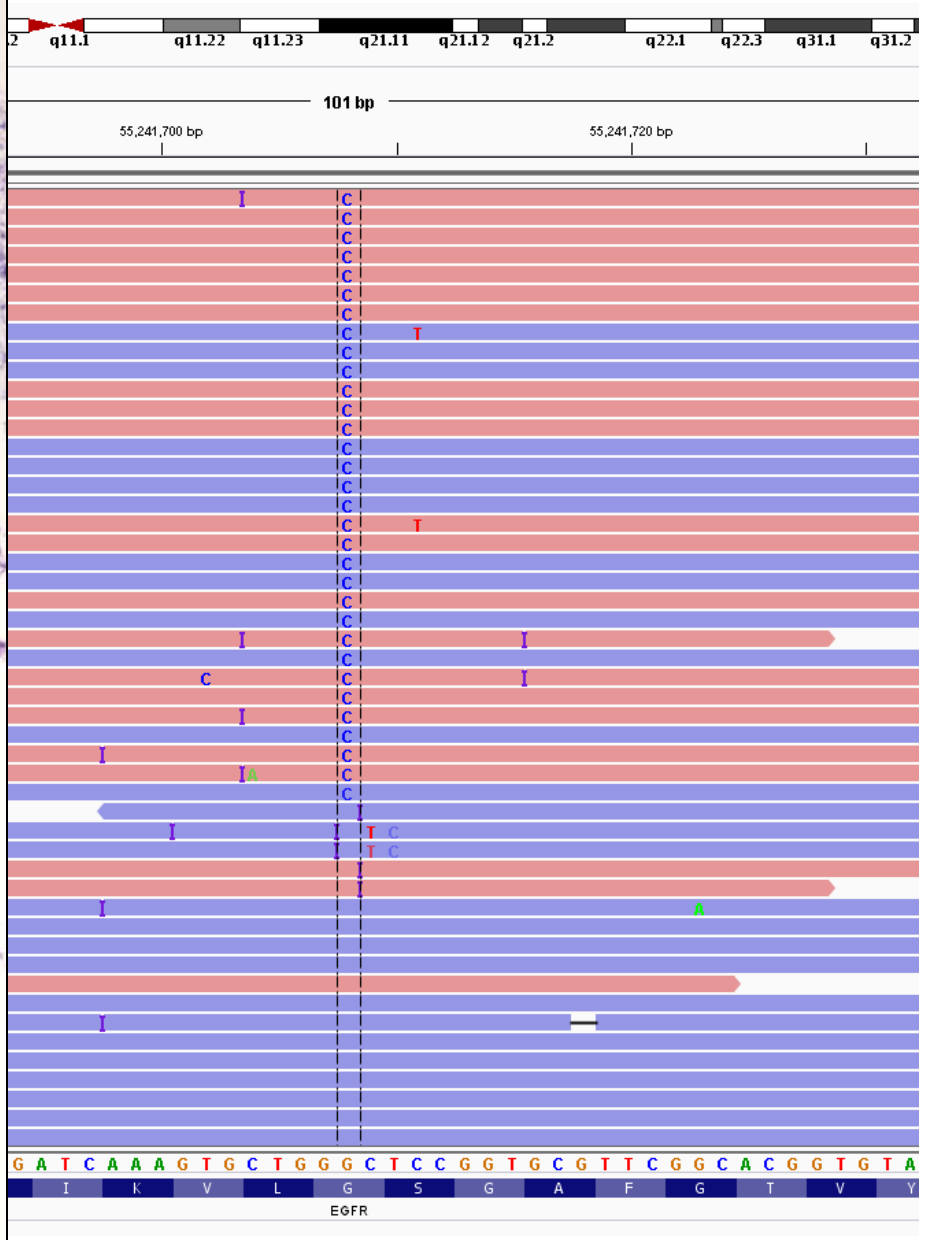
- Samples sequenced by NGS on Ion Torrent system
- Results 100% concordant with original sequence data

Kang et al. AMP Abstract 2015

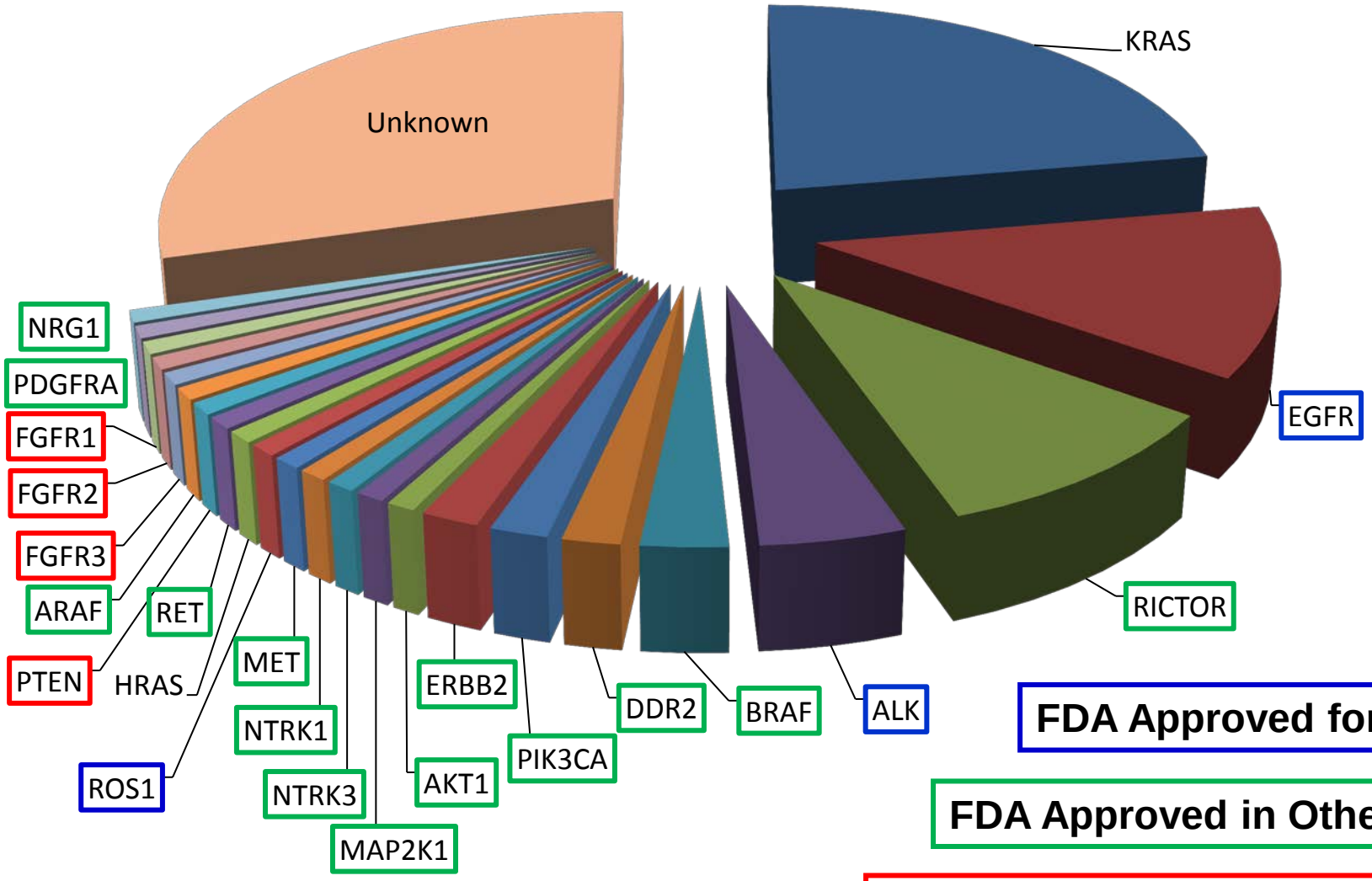
Pleural Fluid – 58 year old F



EGFR p.G719A



Molecular Subtypes of Lung Cancer 2016



FDA Approved for Lung Ca

FDA Approved in Other Tumors

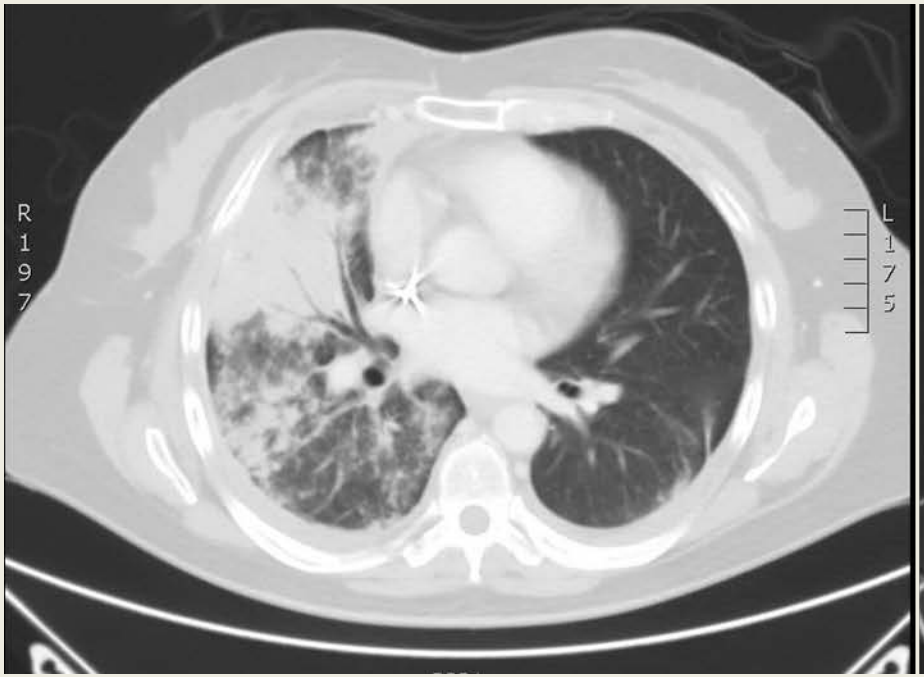
Drugs are in Clinical Trials

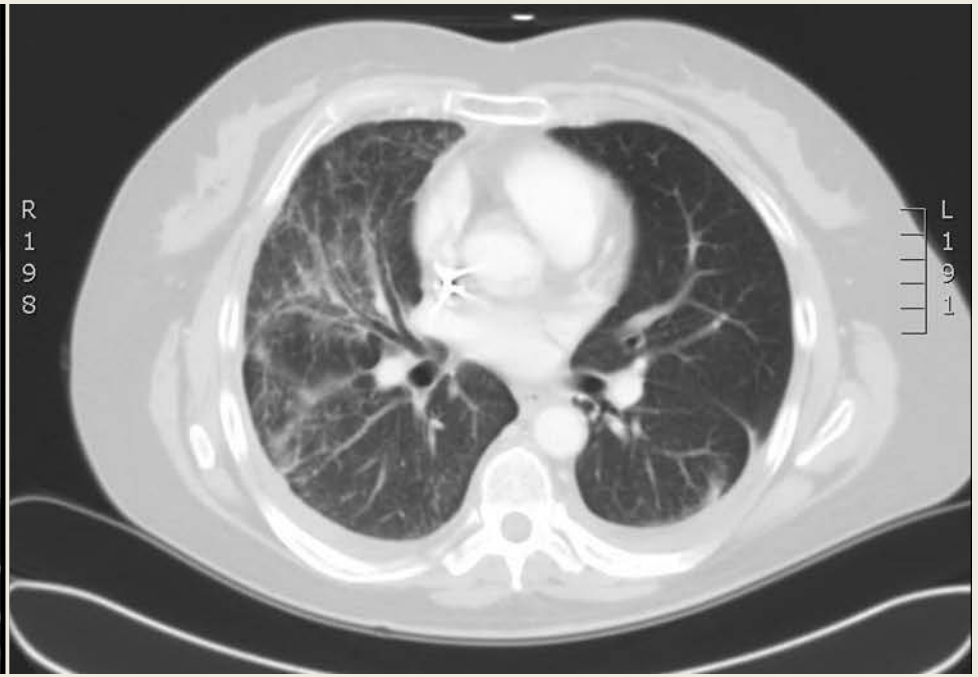
NSCLC Case Example


http://www.oregonlive.com/health/index.ssf/2012/12/new_oregon_health_science_univ_1.html

- 47 y/o woman with bronchioalveolar carcinoma dx'd in Aug 2010
- Genotyping in July 2012:
  - **$BRAF^{V600E}$ mutation + MET amplif**
- Phase I study combining BRAF + MEK inhibitors: 6 month response

Baseline **2 months**





BAC With $BRAF^{V600E}$ + MET amplification

2010 • EGFR inhibitor (erlotinib) failed

2011 • Chemotherapy (pemetrexed) worked for >1 year

2012 (OHSU) • Targeting $BRAF^{V600E}$ worked (really well!), but for only 6 months

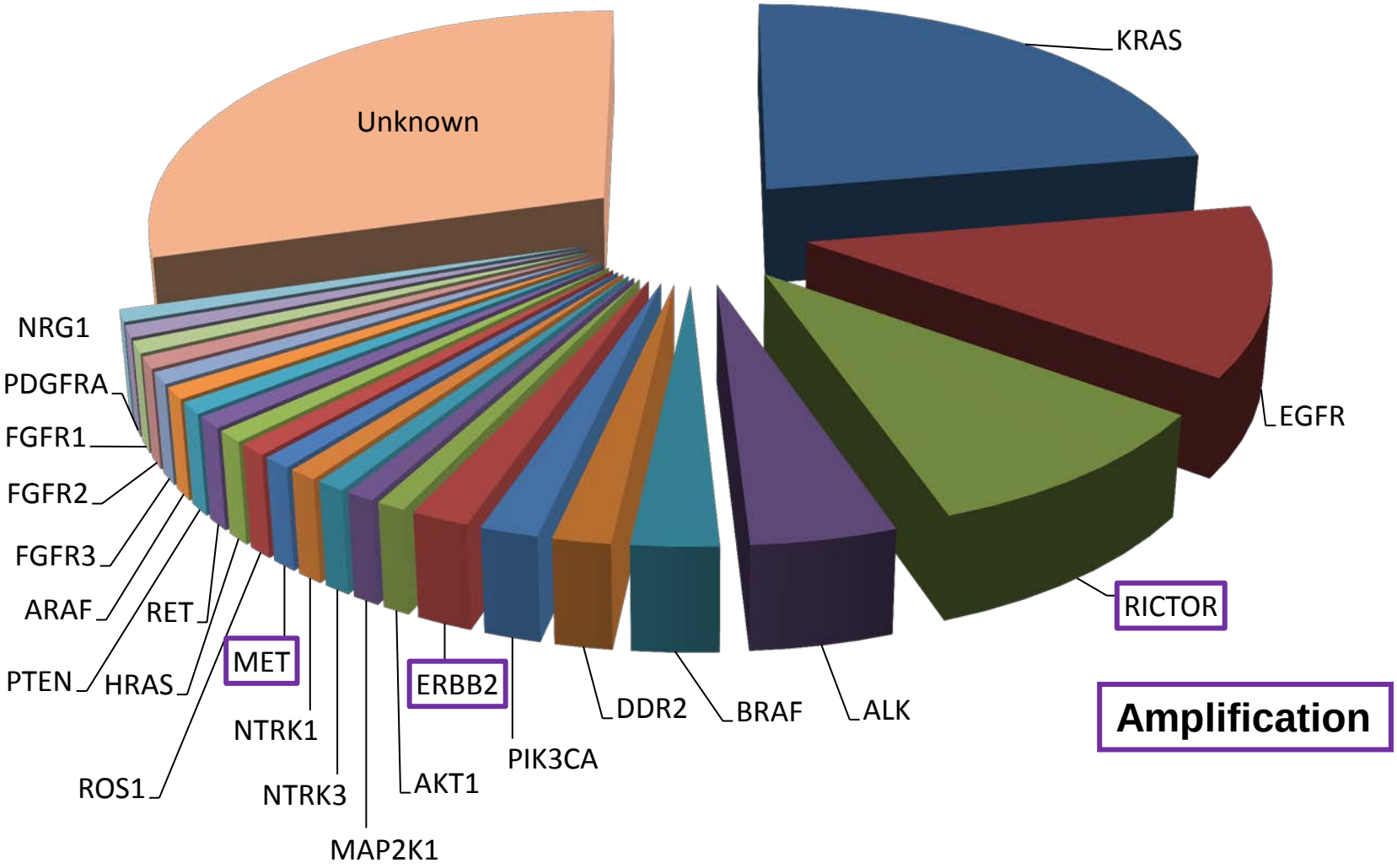
2013 • Phase 1 CTO trial didn't work (not truly targeted)

• Phase 1 AURKA + docetaxol had modest effect (not truly targeted)

• MET inhibitor (crizotinib) didn't work

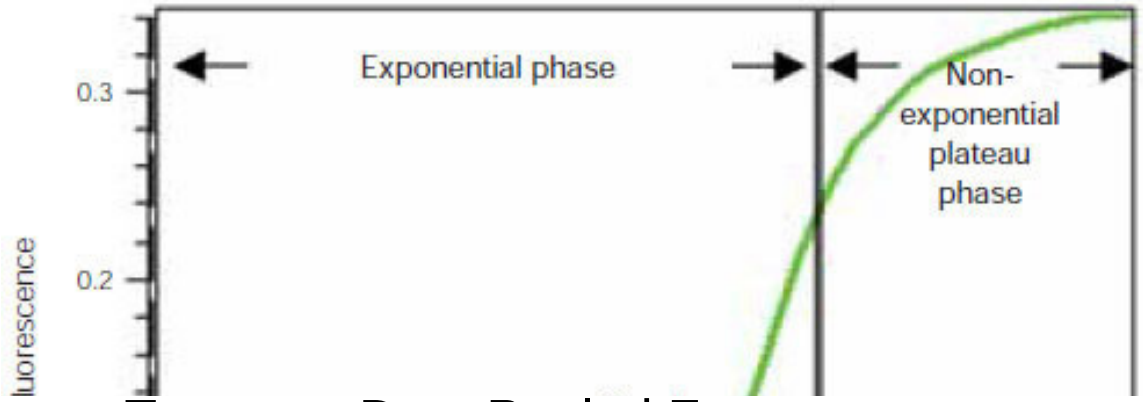
2014 • Re-targeting BRAF worked for ~3 months

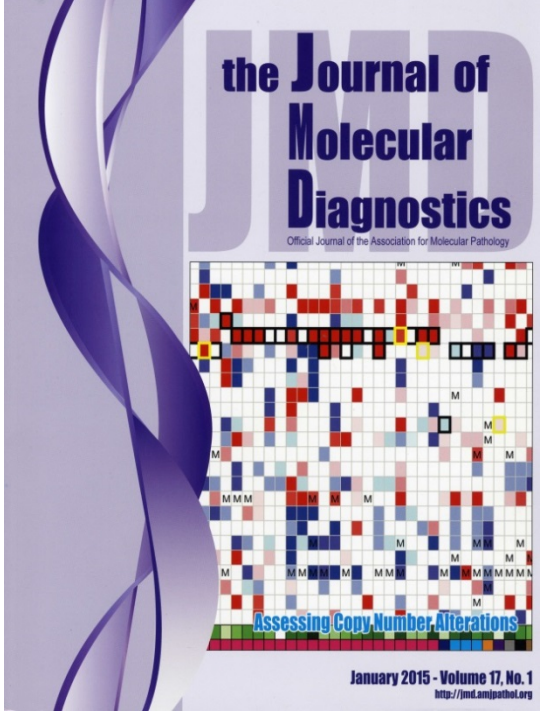
Molecular Subtypes of Lung Cancer 2016



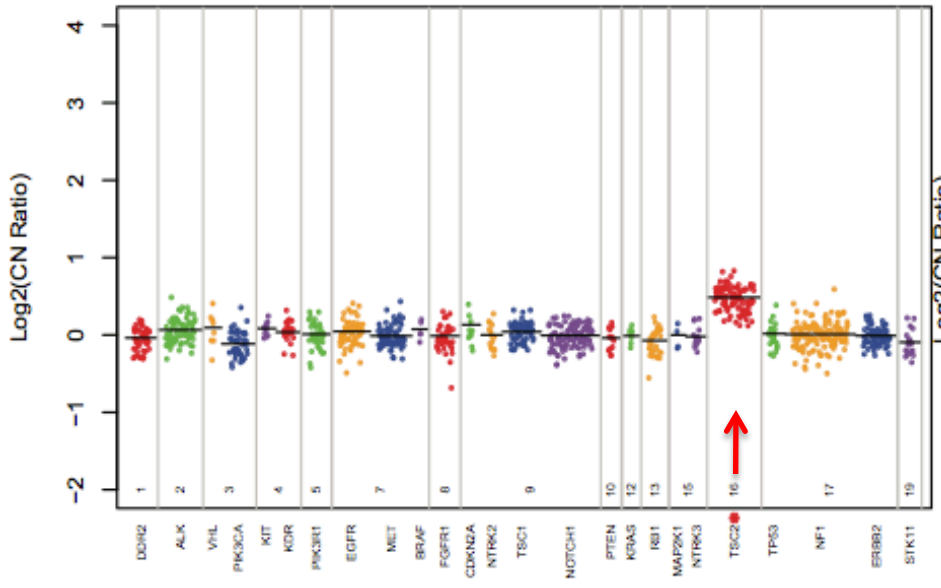
Assessing Copy Number Alterations in Targeted, Amplicon-Based Next-Generation Sequencing Data

Catherine Grasso,* Timothy Butler,* Katherine Rhodes,† Michael Quist,* Tanaya L. Neff,*‡ Stephen Moore,‡§ Scott A. Tomlins,¶ Erica Reinig,‖ Carol Beadling,*‡ Mark Andersen,† and Christopher L. Corless*‡‖

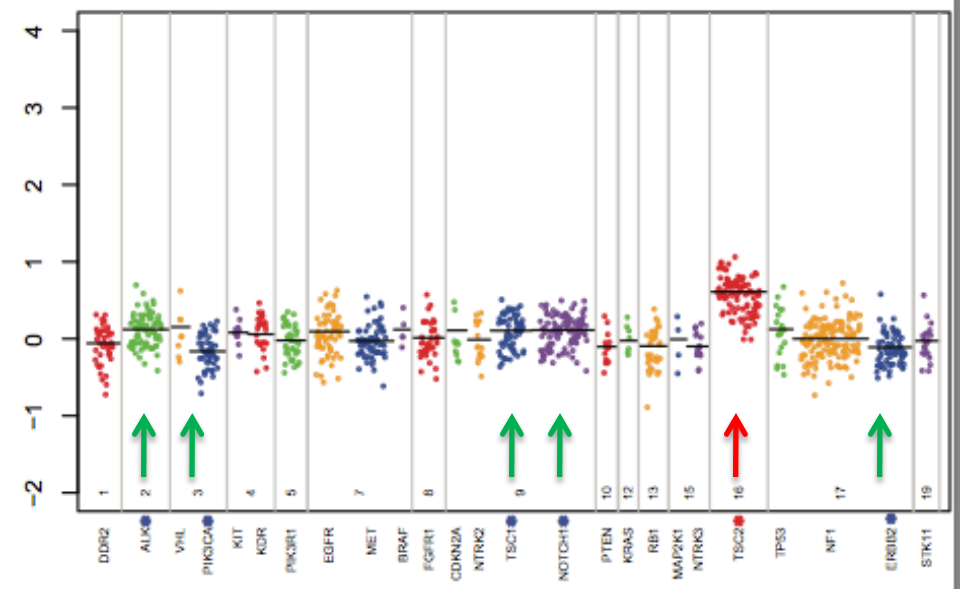




Tumor *vs* Data Pooled From 13 Non-matched Normals

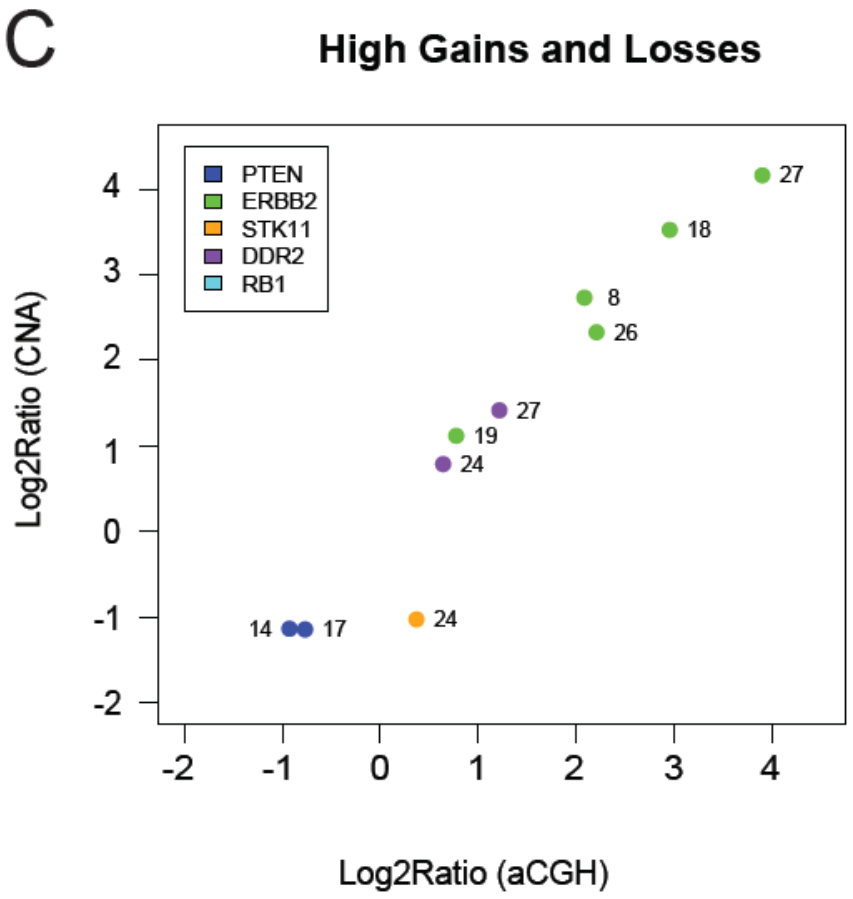


Tumor *vs* Matched Normal

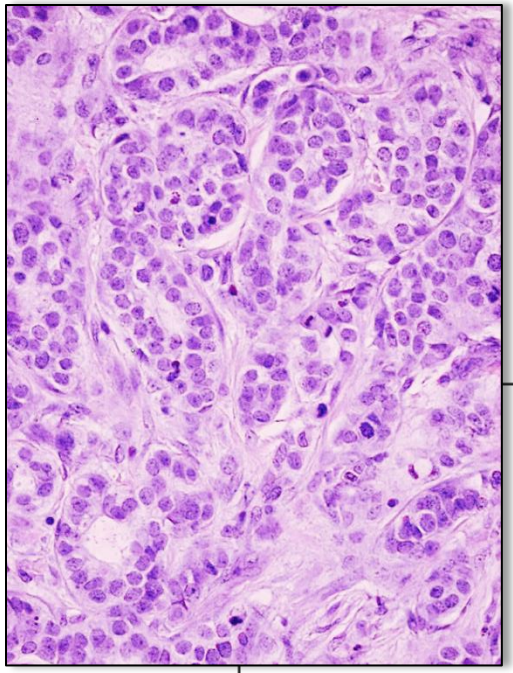


Validating a Copy Number Algorithm

- Correlation with whole exome
- Correlation with FISH (87% agreement)
- Correlation with array CGH

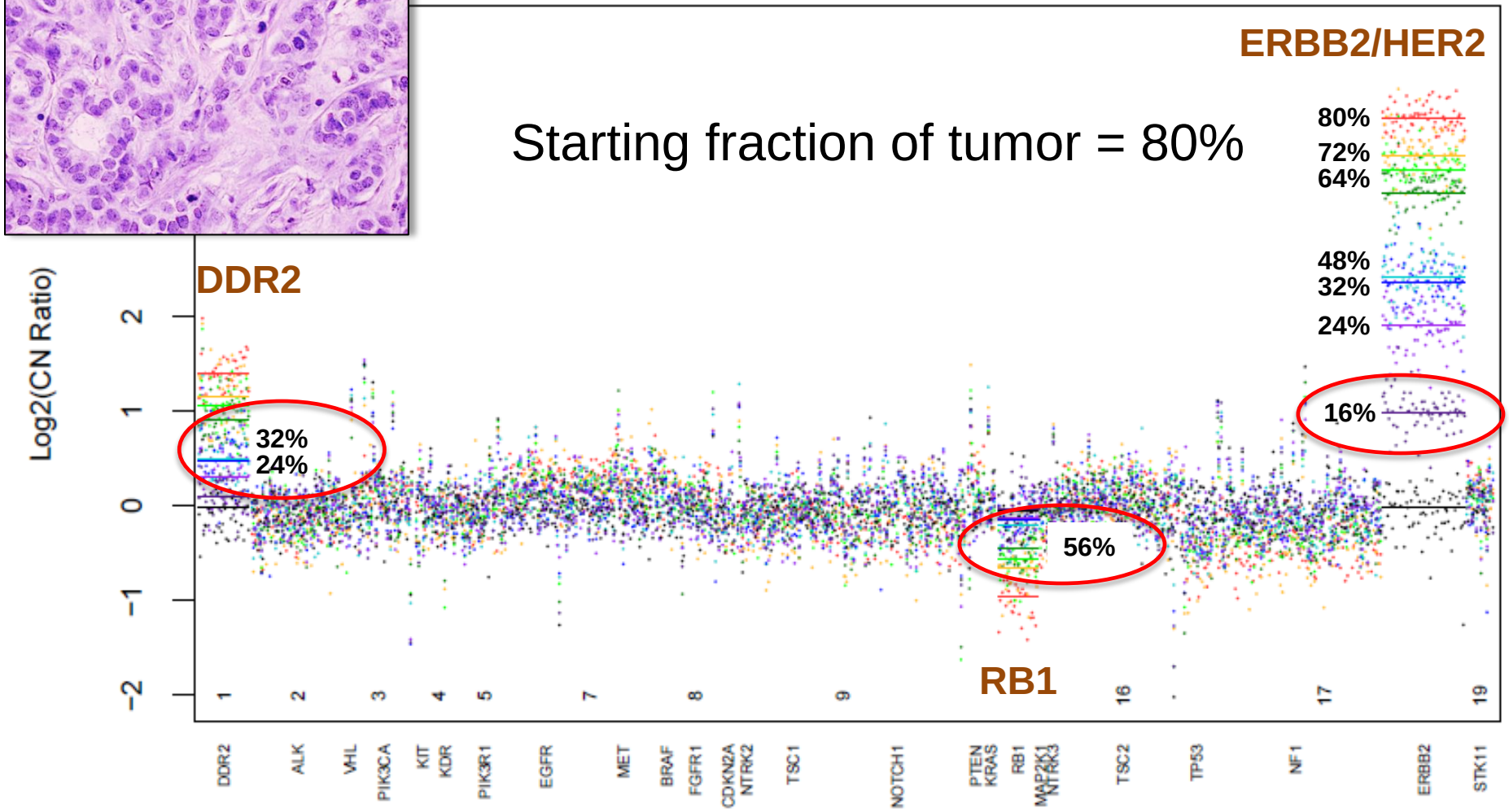


Grasso et al. J Mol Diagn. 2015 Jan;17(1):53-63.



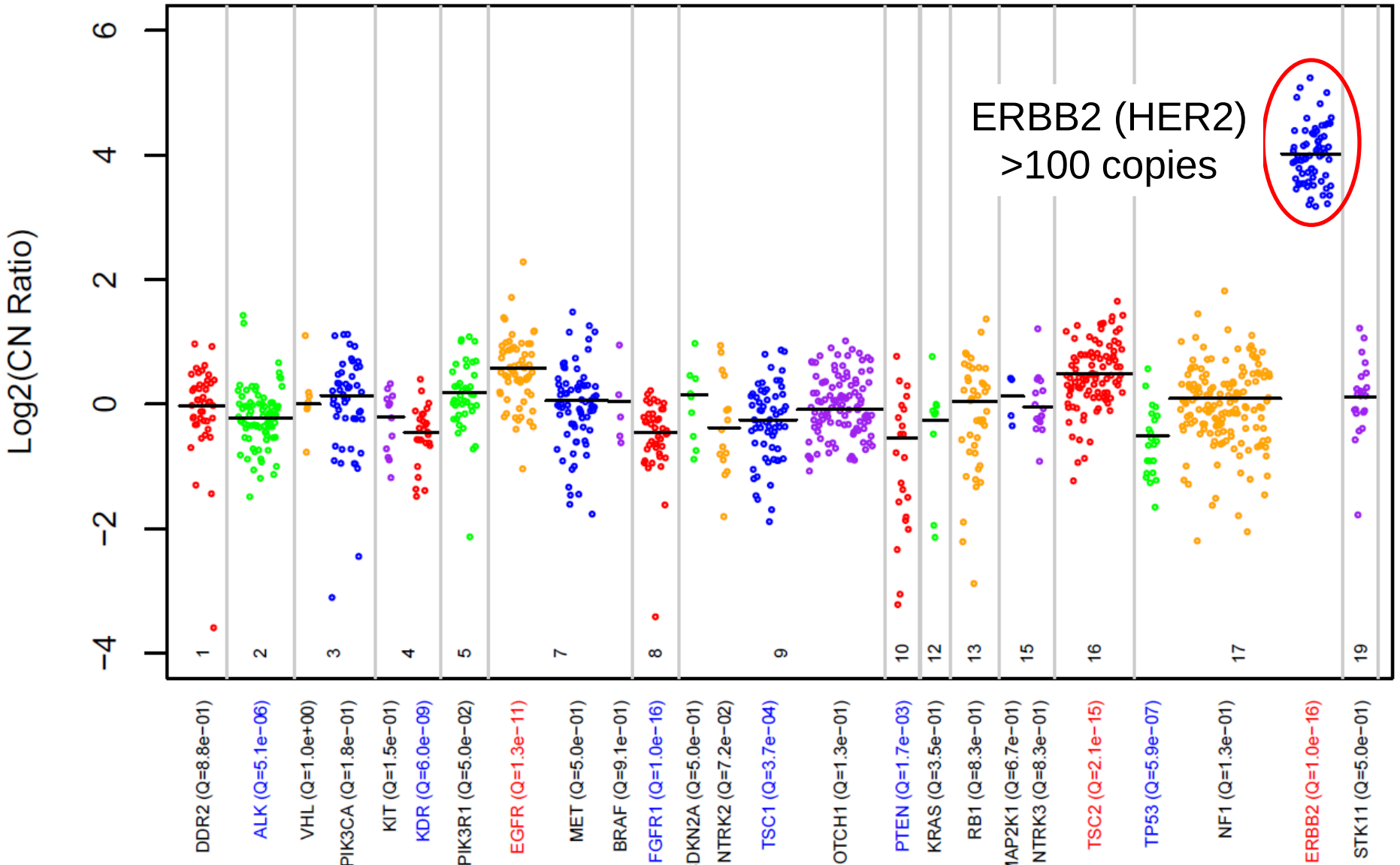
Sensitivity for Copy Number Alterations

Dilution with Matched Normal DNA

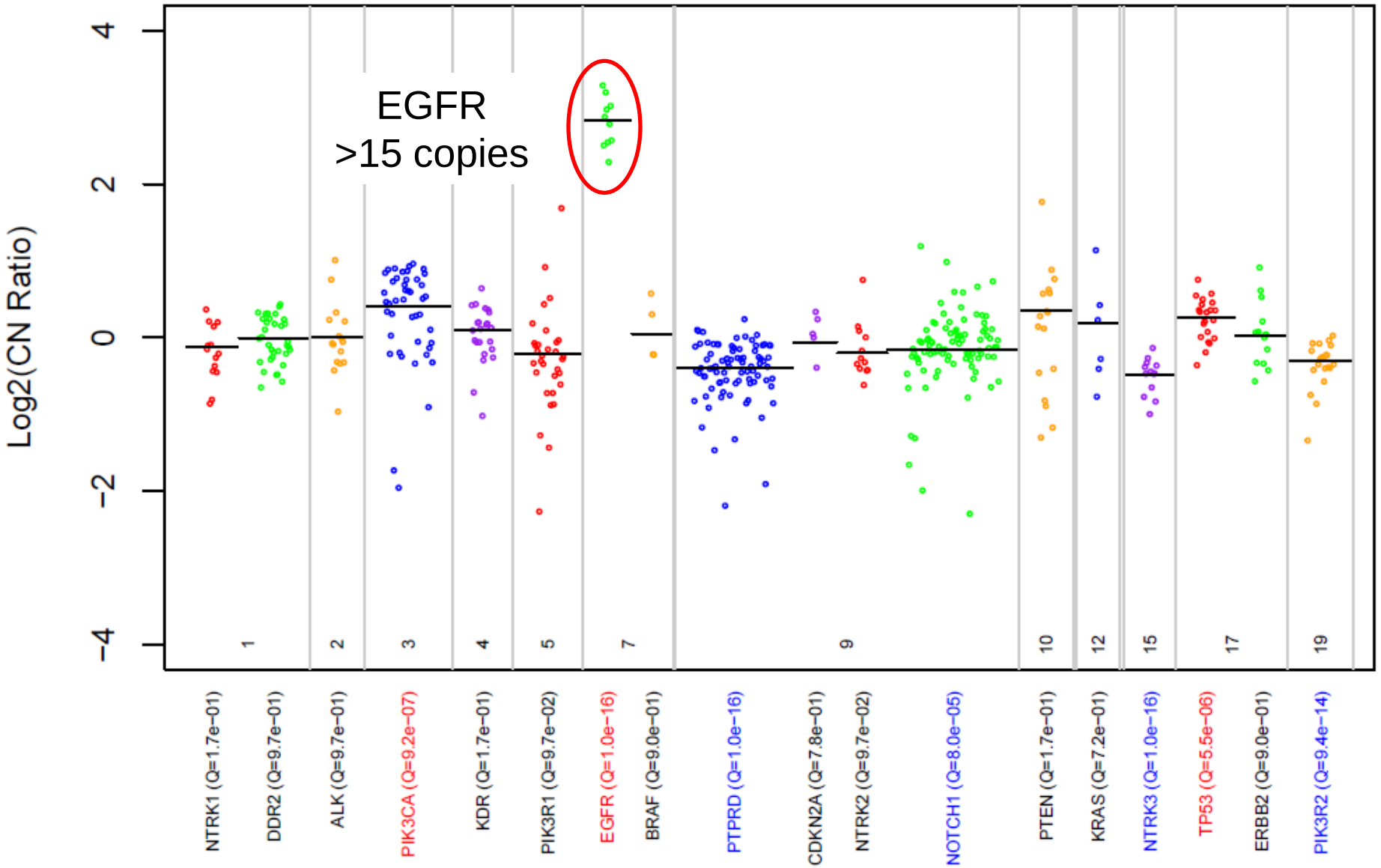


Grasso et al. J Mol Diagn. 2015 Jan;17(1):53-63.

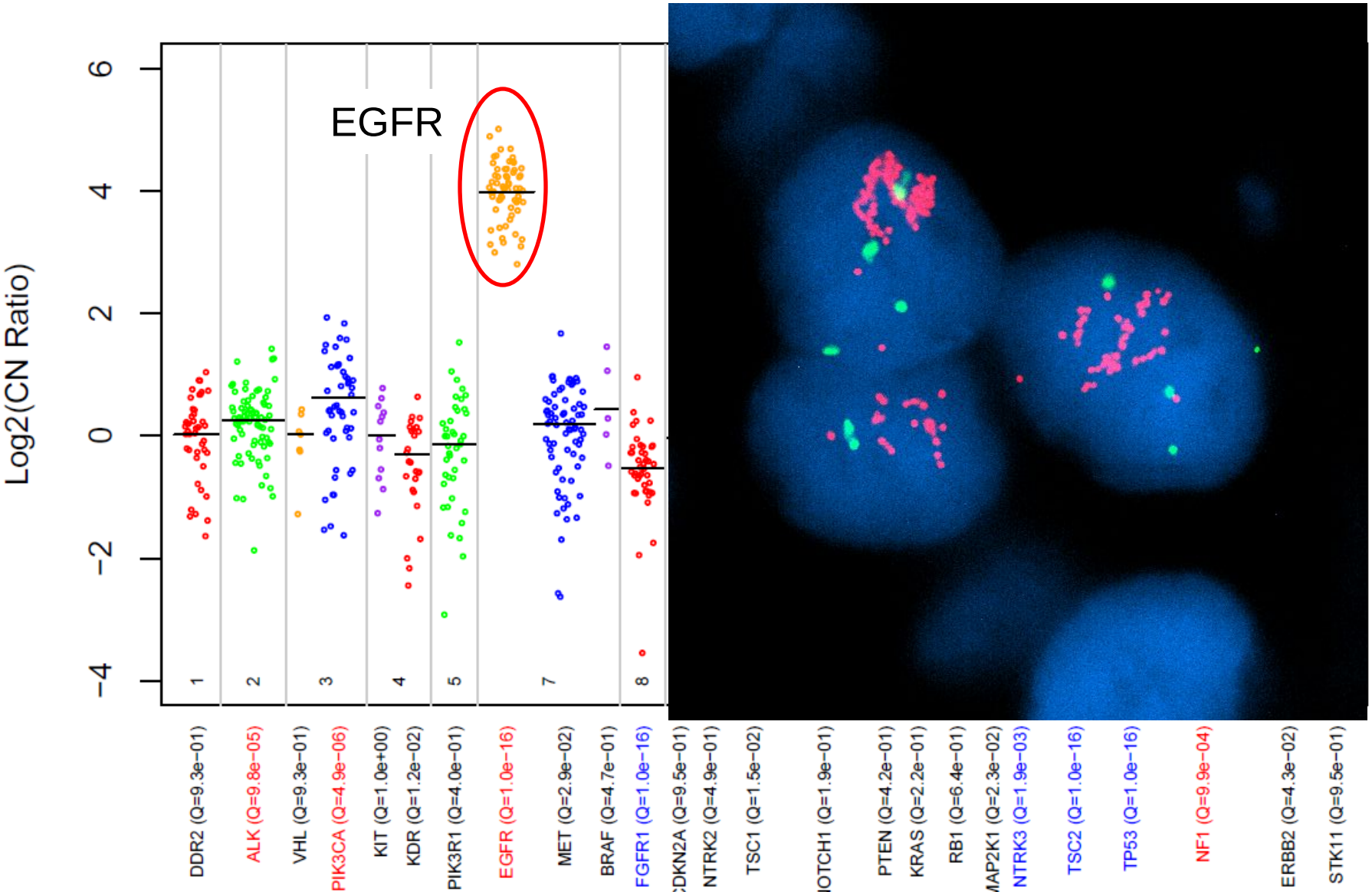
Lung Adenocarcinoma



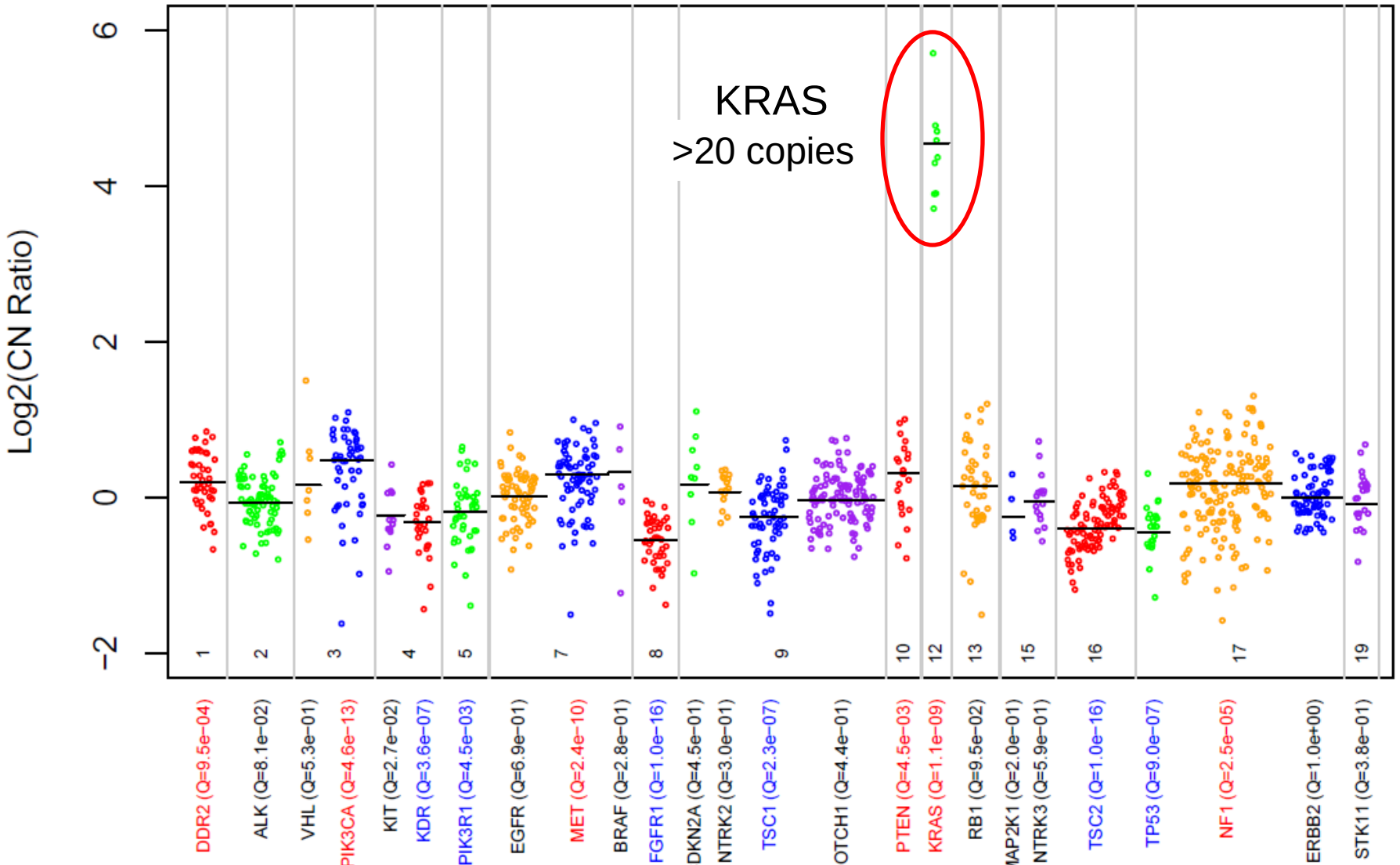
Lung Squamous Carcinoma



EGFR Amplification in Metastatic Breast Carcinoma



KRAS Amplification in Ampullary Adenocarcinoma

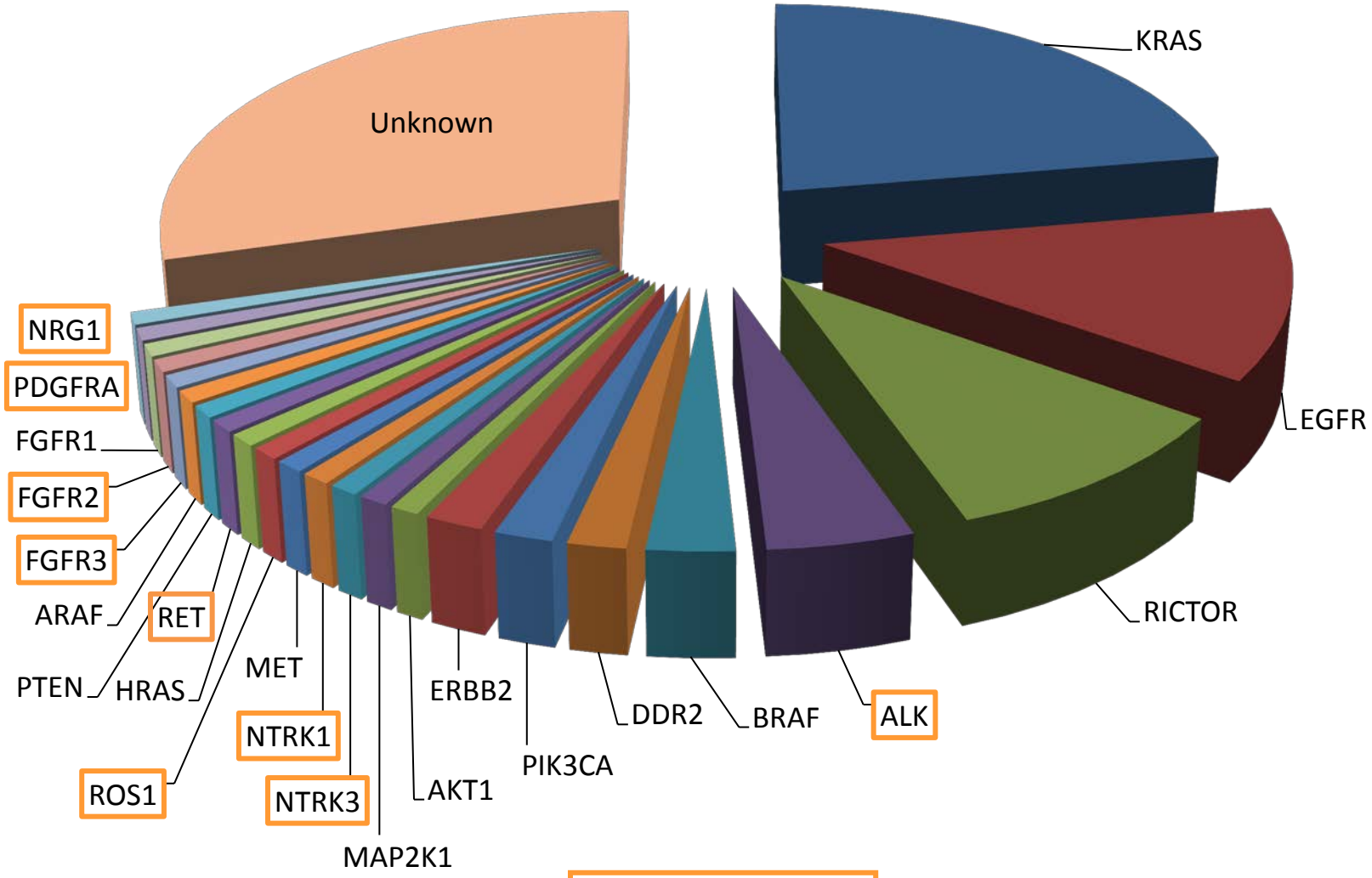


Common Gene Amplifications in GI Malignancies

Site	Gene	Cases	Copy Number
Esophagus	KRAS	21% (6/29)	9 – 100
	HER2 (ERBB2)	17% (5/29)	6 - 48
Gallbladder	KRAS	11% (1/9)	20
Bile duct	HER2 (ERBB2)	11% (1/9)	17
Colon	KRAS	2% (2/105)	6 - 10
	HER2 (ERBB2)	1% (1/105)	17

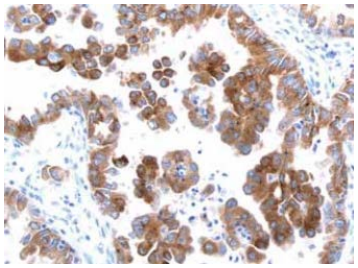
Brinkerhoff et al. J Mol Diag 2014, Vol.16(6), p.747

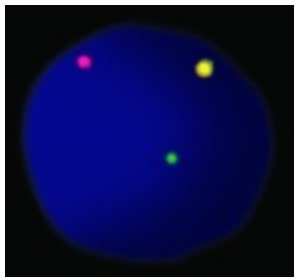
Molecular Subtypes of Lung Cancer 2016

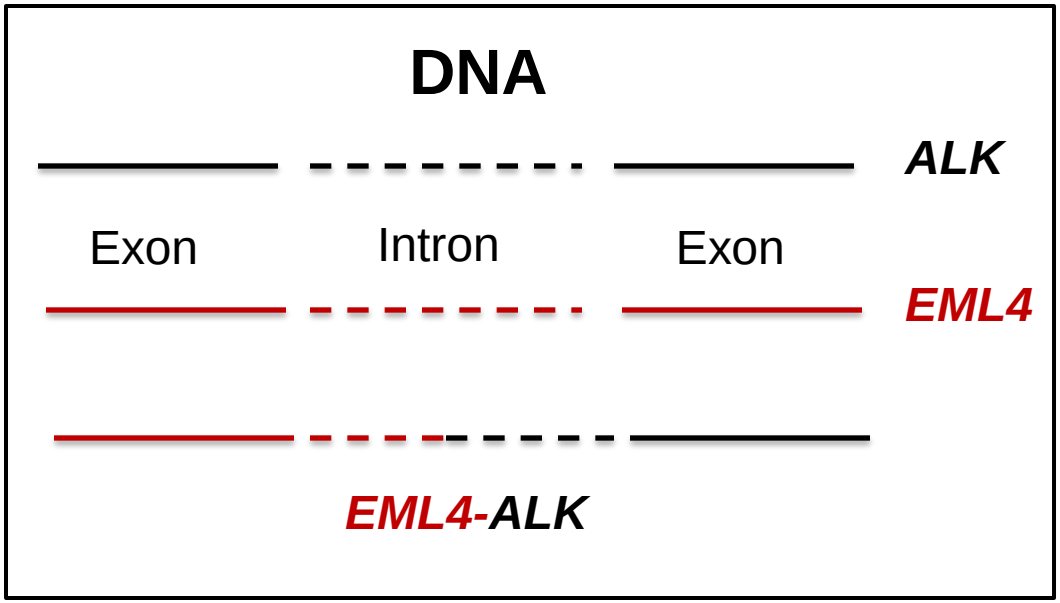


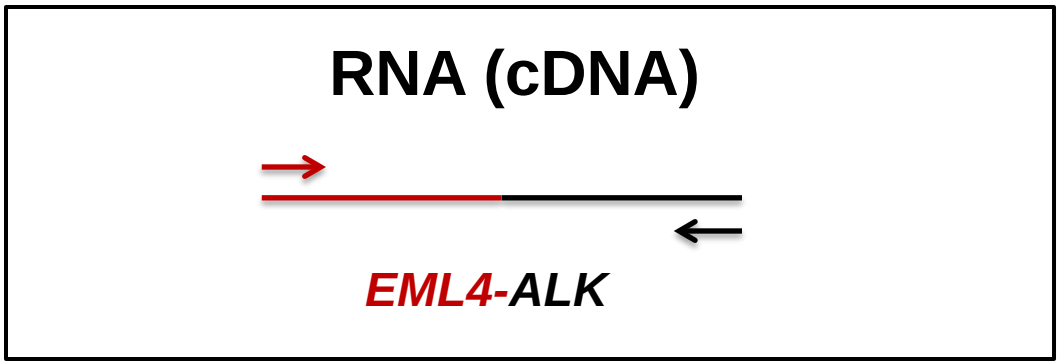
Detecting Actionable Gene Fusions

Kinase Fusion	IHC	FISH	NGS
ALK	Yes	Yes	Yes
BRAF	No	Yes	Yes
FGFR1	No	Yes	Yes
FGFR2	No	Yes	Yes
FGFR3	No	Yes	Yes
MET	No	Yes	Yes
NTRK1	Yes	Yes	Yes
NTRK3	Yes	Yes	Yes
PGDFRA	No	Yes	Yes
RET	No?	Yes	Yes
ROS1	Yes	Yes	Yes









TECHNICAL ADVANCE

A Multiplexed Amplicon Approach for Detecting Gene Fusions by Next-Generation Sequencing

ATIC/ALK	AGTRAP/BRAF	FGFR2/BICC1	NIN/PDGFRB	CD74/ROS1
C2orf44/ALK	AKAP9/BRAF	FGFR2/CASP7	ESRP1/RAF1	CCDC6/ROS1
CARS/ALK	FCHSD1/BRAF	FGFR2/CCDC6	RAF1/MSS51	CEP85L/ROS1
CLTC/ALK	FAM131B/BRAF	FGFR2/CIT	SRGAP3/RAF1	EZR/ROS1
EML4/ALK	KIAA1549/BRAF	FGFR2/KIAA1967	AFAP1/RET	GOPC/ROS1
FN1/ALK	SLC45A3/BRAF	FGFR2/OFD1	CCDC6/RET	LRIG3/ROS1
FN1/ALK	EGFR variant III	SLC45A3/FGFR2	ELKS/RET	KDELR2/ROS1
KIF5B/ALK	EGFR/PSPH	FGFR3/BAIAP2L1	ERC1/RET	SDC4/ROS1
KLC1/ALK	CAND1/EGFR	FGFR3/TACC3	GOLGA5/RET	SLC34A2/ROS1
MSN/ALK	EGFR/SEPT14	TPR/MET	HOOK3/RET	TFG/ROS1
NPM1/ALK	EGFR/SLC12A9	MIR548F1/MET	HTIF/RET	TPM3/ROS1
PPFIBP1/ALK	BAG4/FGFR1	BCAN/NTRK1	KIF5B/RET	
SEC31A/ALK	CPSF6/FGFR1	CD74/NTRK1	PARG/RET	
SQSTM1/ALK	ERLIN2/FGFR1	MPRIP/NTRK1	PCM1/RET	
STRN/ALK	FGFR1/ZNF703	MIR548F1/NTRK1	PRKAR1A/RET	
TFG/ALK	FGFR1/PLAG1	NFASC/NTRK1	NCOA4/RET	
TPM3/ALK	FGFR1/TACC1	TFG/NTRK1	RET/RFG9	
TPM4/ALK	FGFR1/ZNF703	TPM3/NTRK1	TRIM24/RET	
TRAF1/ALK	FGFR2/AHCYL1	TPR/NTRK1	TRIM27/RET	
VCL/ALK	FGFR2/AFF3	SCAF11/PDGFRA	TRIM33/RET	

Highly targetable fusions:

- ALK
- ROS1
- RET
- EGFR
- BRAF
- FGFR1/2/3
- MET
- NTRK1
- NRG1
- PDGFRA
- PDGFRB

Next-gen sequencing:

- Identify the fusion partners
- Sensitivity down to 1%

Advantages: small footprint; easy to interpret the data

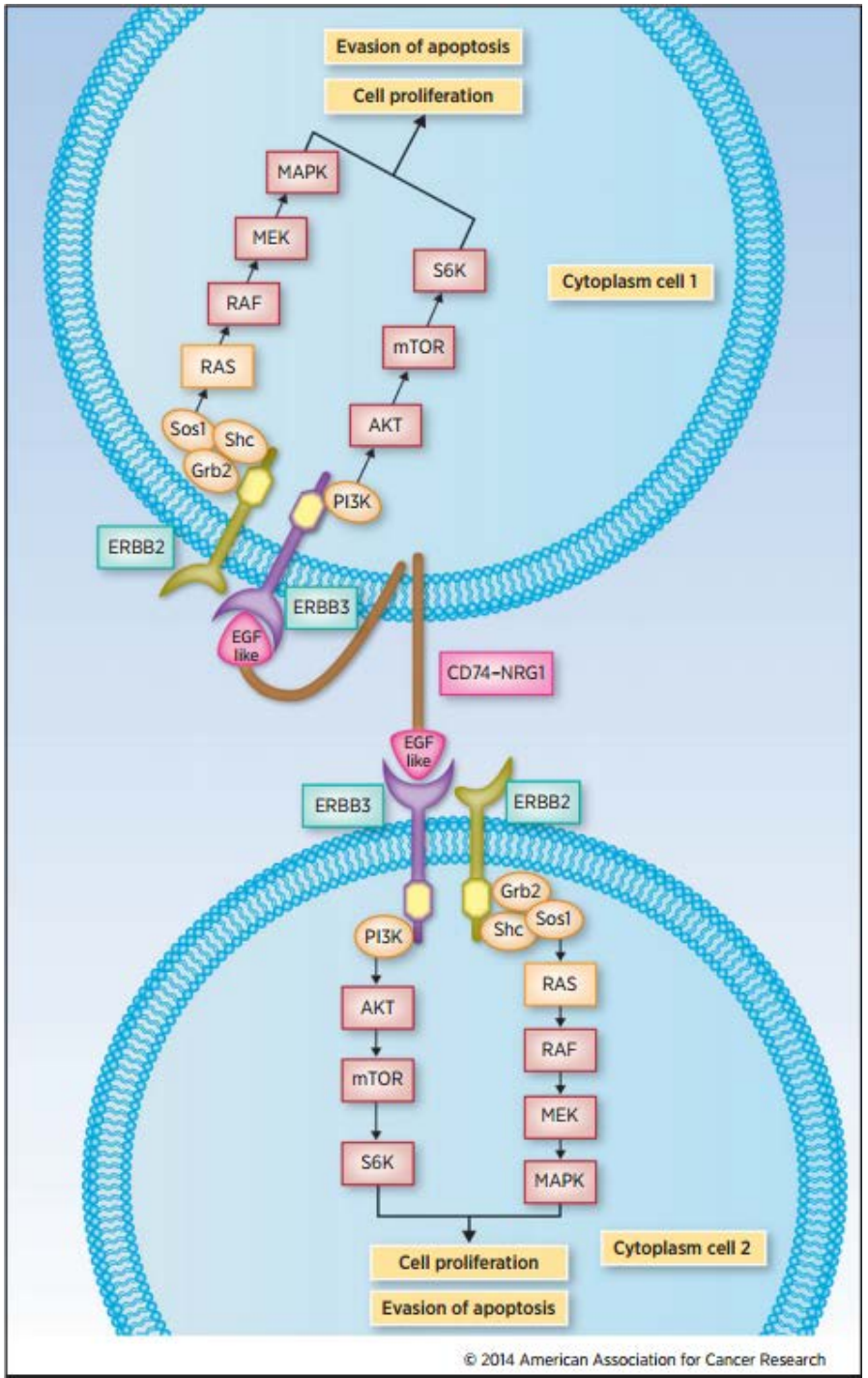
Disadvantage: need to know the partner gene

Beadling et al. J Mol Diagn. 2016 Mar;18(2):165-175.

NRG1 Fusions in NSCLC

- In 2014, two groups identified NRG1 gene fusions in cases of invasive mucinous adenocarcinoma of the lung
- Both groups showed that the fusions lead to activation of HER3:HER2 signaling

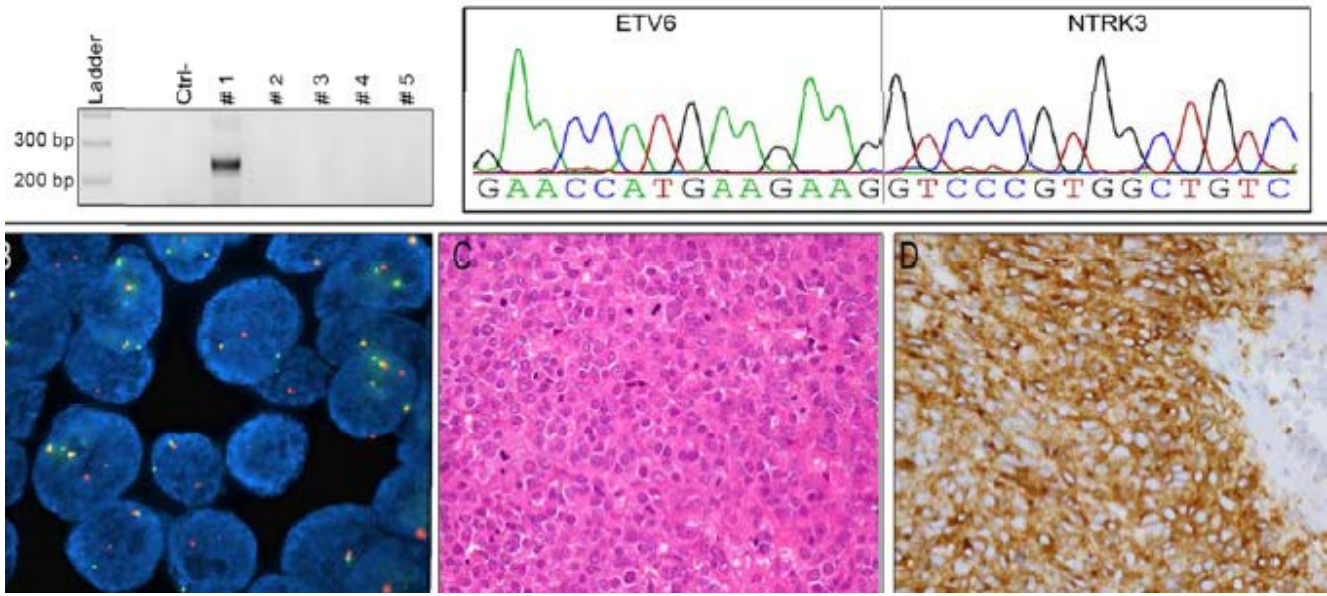
- We recently identified NRG1 fusions in two cases of lung adenoca
- Both patients are being treated with afatinib off-label
- First follow-up CT has shown a marked response

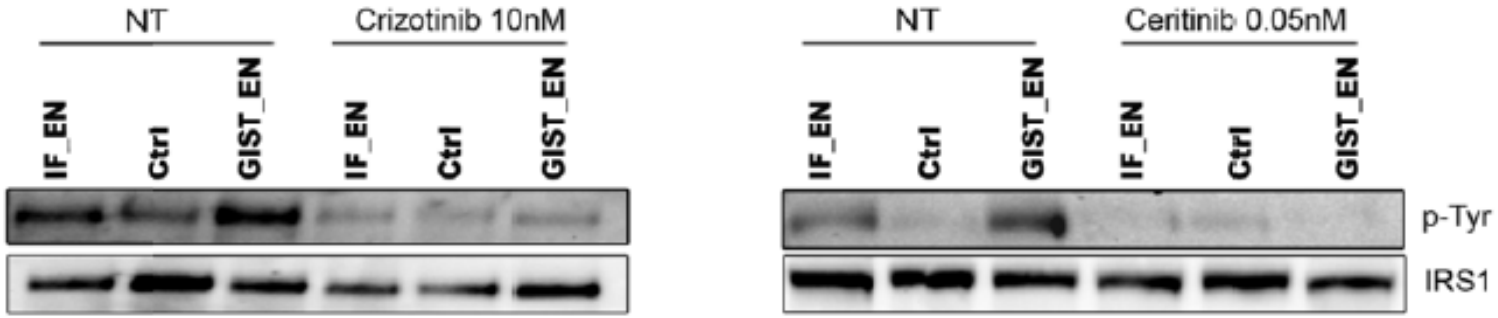


Clin Cancer Res; 21(9) May 1, 2015
Cancer Discov*; 4(4); 1–8. ©2014 AACR*.
Clin Cancer Res. 2014 Jun 15;20(12):3087-93.

Kinase Fusions in GISTs

- 44 yr male
- 5 cm rectal tumor
- CD117 positive
- Wild-type for:
  - KIT, PDGFRA
  - BRAF, SDH
- 34 mitoses / 50 mm^2





Brenca et al. J Pathol. 2015 Nov 26.

Variant List From a 37-Gene Panel

68 Yr Old Male With Head & Neck SQCC

Chrom	Position_Start	Position_End	Ref	Variant	Type	Consequence	Zygosity	Var_Freq	Gene	p_AA_change
chr4	55968089	55968089	T	G	SNP	nonsynonymous	Het	28	KDR	p.K747N
chr9	21971096	21971096	C	A	SNP	stop gain	Het	48	CDKN2A	p.E88*
chr17	7577548*	7577548	G	C	SNP	stop gain	Het	23	TP53	p.M234*

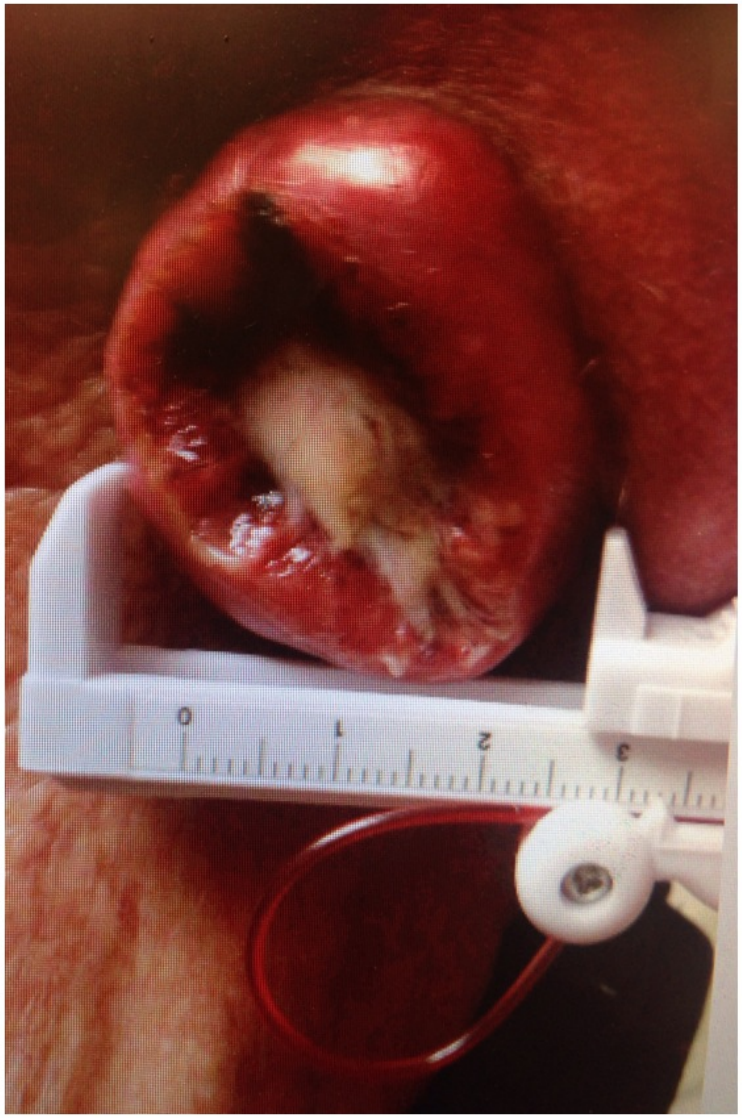
Gene	Amino acid Change	% Allele	Interp.
KDR	K747N	28%	Het
CDKN2A	E88*(stop)	48%	Hom
TP53	M234*(stop)	23%	Het

Tumor in Sample = 50% of cells

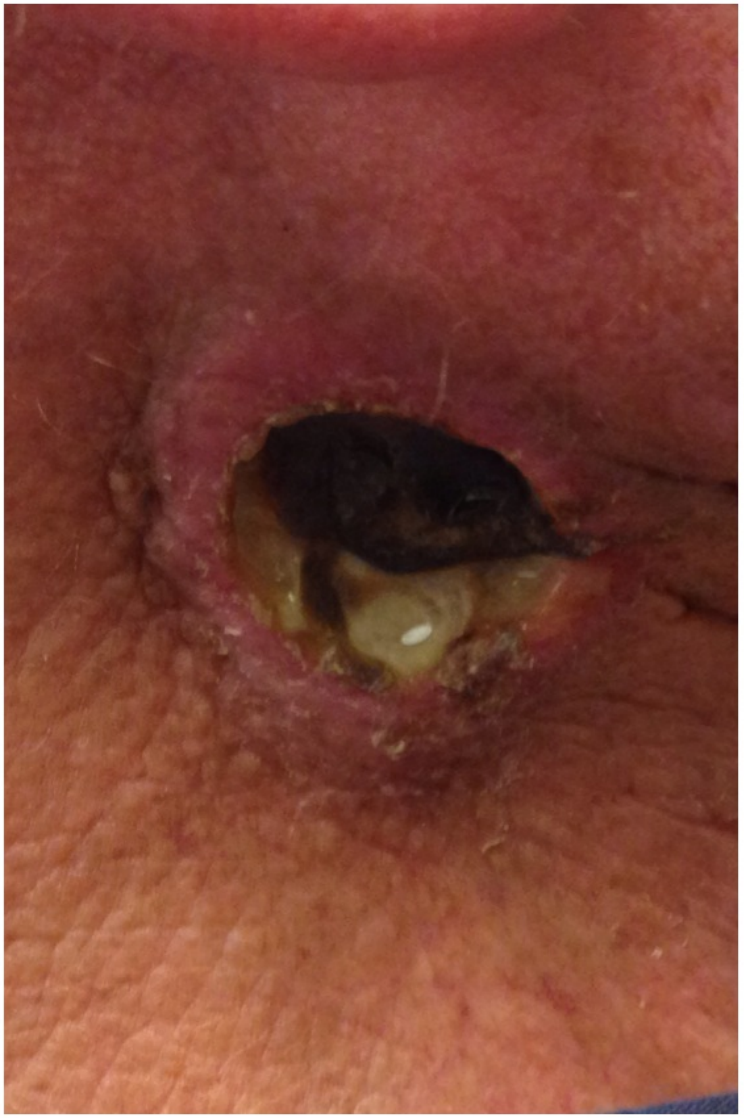
KDR encodes VEGFR2

68 yr Old Male with Head & Neck SQCC

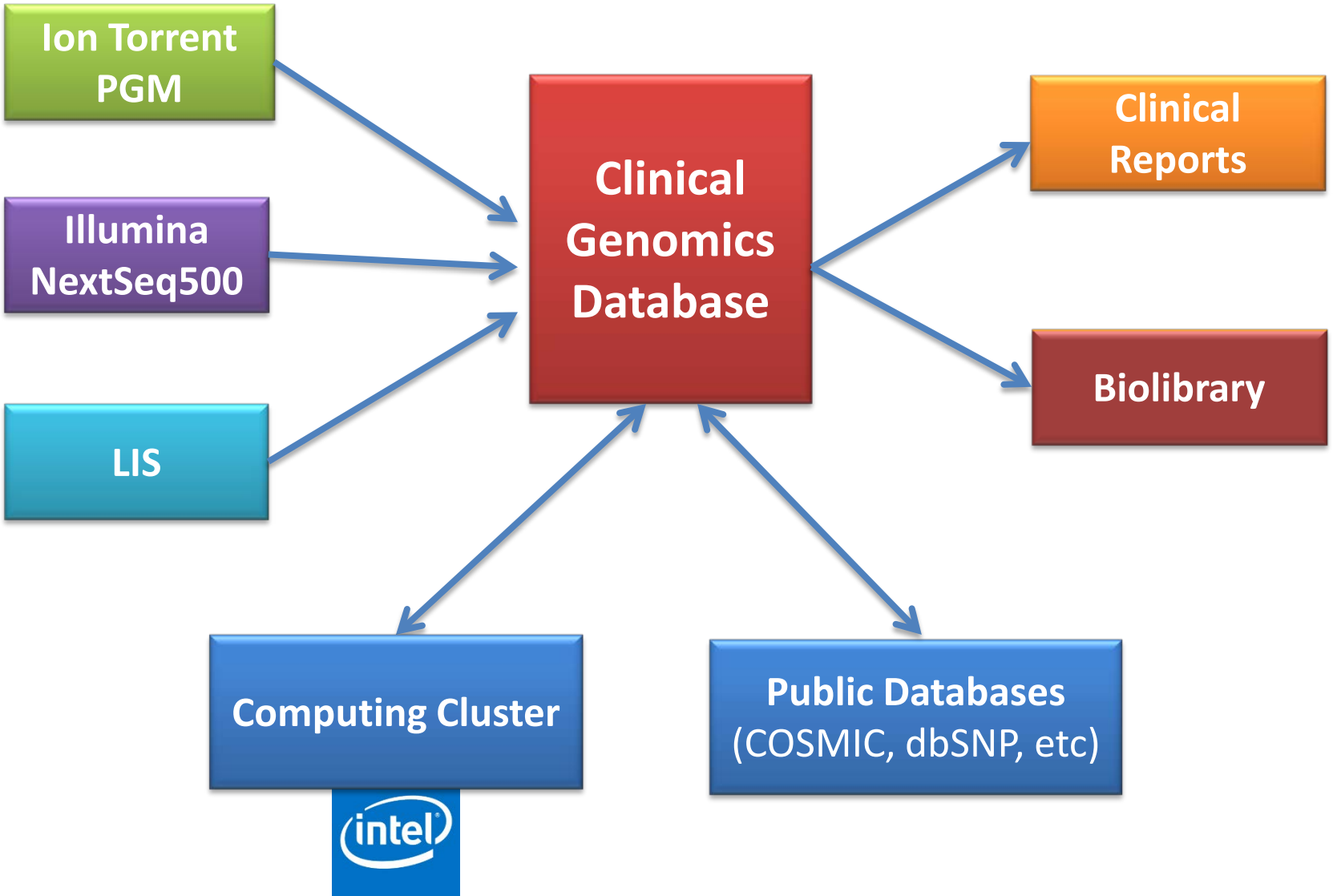
Before Treatment



Treatment Day 15
TKI With VEGFR2 Activity



Data Workflow



Specimen tested: DNA-15-01334
Reported diagnosis: Ductal carcinoma

GeneTrails® Solid Tumor Panel

Positive for PIK3CA p.R88Q and p.H1047R. Both of these mutations cause activation of PI3 kinase signaling. Consideration might be given to a trial of a PI3 kinase inhibitor.

Positive for CDKN2A copy number loss. Genomic alterations affecting the CDKN2A gene, which encodes the p16 tumor suppressor protein, are common in carcinomas and contribute to dysregulation of the cell cycle. Pre-clinical studies suggest that tumors with loss of CDKN2A may be sensitive to CDK4/6 inhibitors.

Positive for TP53 copy number loss. The TP53 gene encodes a tumor suppressor protein that responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. TP53 gene alterations, including mutations and loss of heterozygosity, are associated with a variety of human cancers.

Positive for RET p.S649L. This alteration has not been reported and its clinical significance is unknown.

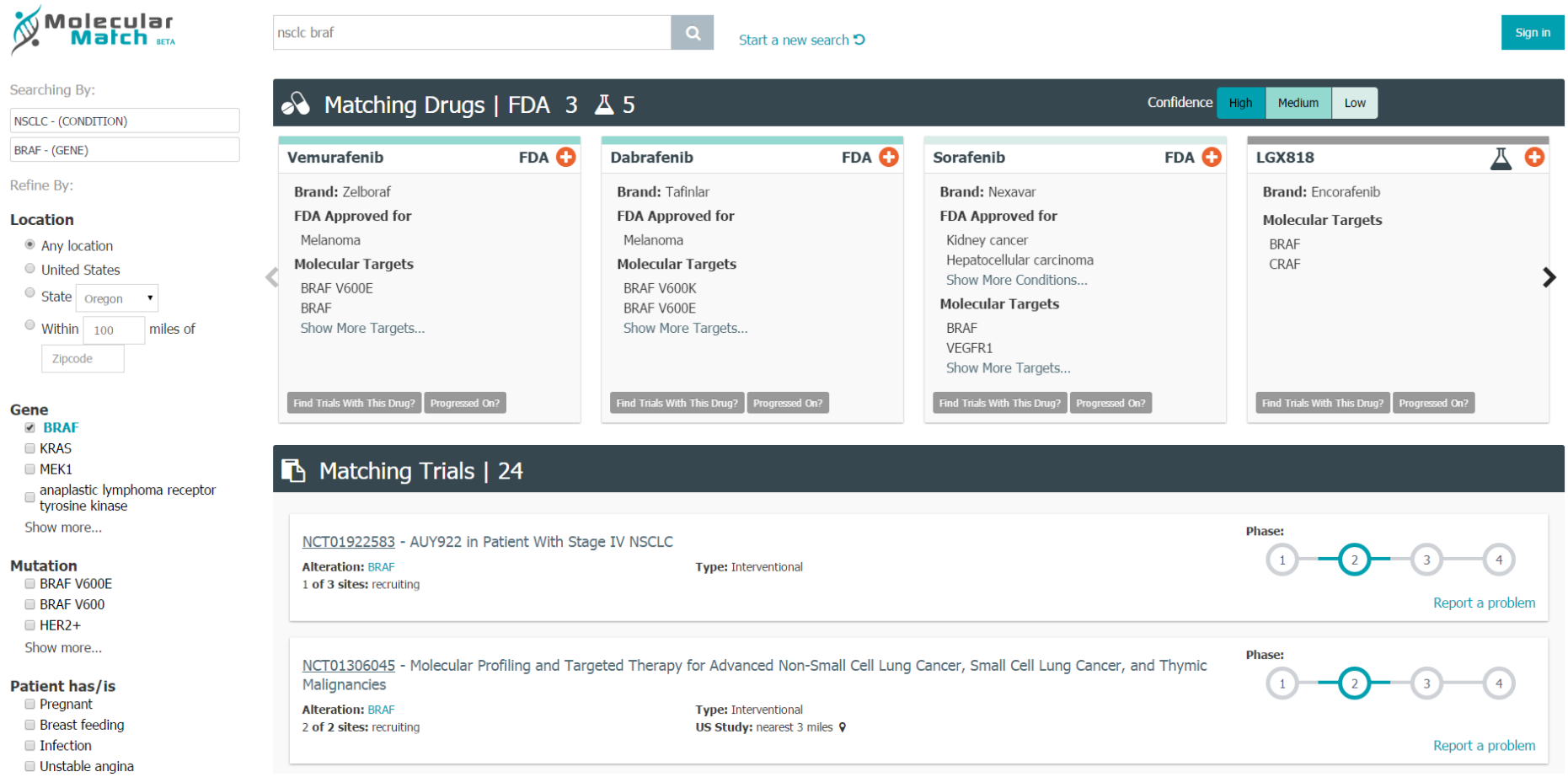
Possible Clinical Trials

Additional details for this case available at https://www.MolecularMatch.com

NCT02187783 - Modular Phase II Study to Link Targeted Therapy to Patients With Pathway Activated Tumors: Module 8 - LEE011 for Patients With CDK4/6 Pathway Activated Tumors
Phase: Phase 2 **Location(s):** AL, AK, AZ, AR, CA, CO, CT, FL, GA, HI, IL, IN, IA, LA, MD, MI, MN, MO, NV, NJ, NM, NY, NC, OH, OR, PA, RI, SC, SD, TN, TX, UT, VA, WA, WI
Gene(s): CCND1, CCND3, CDK4, CDK6, CDKN2A
Genotype(s): CDK4 Amplification , CCND3 Amplification, CCND1 Amplification
Drug(s): Lee-011

NCT01449058 - A Phase Ib Open-label, Multi-center, Dose Escalation and Expansion Study of Orally Administered MEK162 Plus BYL719 in Adult Patients With Selected Advanced Solid Tumors
Phase: Phase 1/2 **Location(s):** CA, FL, IL, MA, NY, TX, UT
Gene(s): PTEN, PIK3R1, KRAS, BRAF, MAP2K1, PI3K, NRAS, PIK3CA
Genotype(s): NF1 Loss, PTEN Loss
Drug(s): Byl719, Mek-162

Finding Trials: MolecularMatch.com



KRAS p.A146T

Var Freq:	9.8	Tech Variant Comment:	Low Frequency
dbSNP:	rs121913527	COSMIC:	COSM1165198 COSM19404

Gene Significance:	This mutation causes activation of KRAS signaling.

Significance Rule	Variant: Clinical Significance	Last Reviewed On
KRAS: p.A146T for All Diagnoses	Suspected pathogenic	2014-10-08 12:05:40.0

Therapy Summary:	No approved therapies found.

Related Therapies (3)

Adenocarcinoma: Colon		resistance	cetuximab		late trials
Any nonsynonymous - missense on CCDS8702.1 between(inclusive) 146-146	Adenocarcinoma: Colon	sensitivity	MEK inhibitors + PI3K pathway inhibitors	preclinical	22392911
Any nonsynonymous -					23325582
Adenocarcinoma: Colon		resistance	panitumumab		late trials

Results:

Tumor Content	Diagnosis	Specimen Description	Specimen Source
80%	Malignant Meningioma	Left Skull Base Tumor	S13-102966 A1

Actionable	Applicable	Unknown Significance
		KDR_Q472H, PAX5_A322T

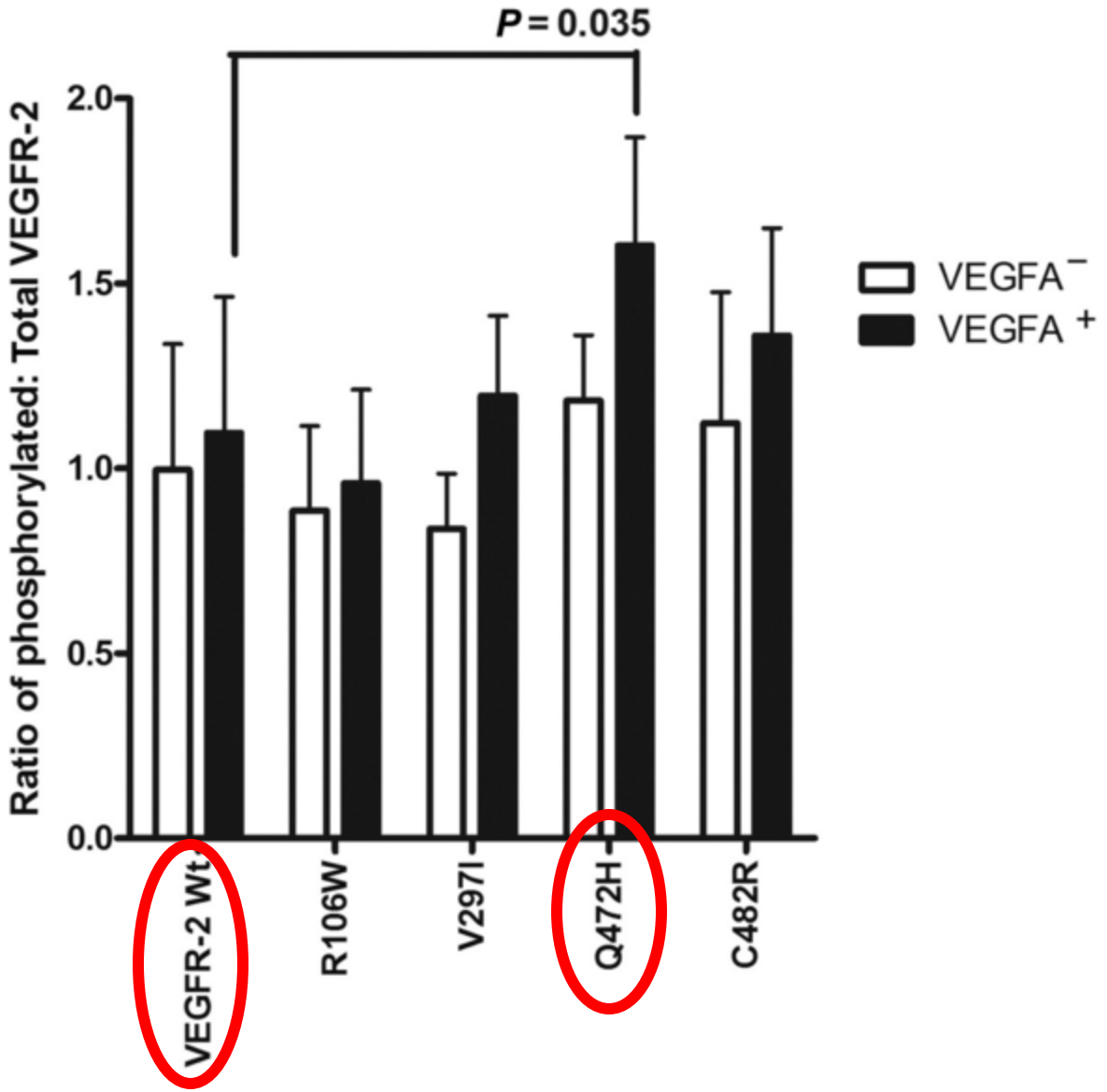
Biomarkers Sequenced (targeted regions)

ABL1 (8)	AKT1 (3)	ALK (10)	APC (1))	ASXL1 (1)	ATM (15)	BRAF (2)
CBL (2)	CDH1 (3)	CDKN2A1 (1)	CSF1R (2)	CSF3R (4)	CTNNB1 (1)	DNMT3A (22)
DPYD (2)	EGFR (9)	ERBB2 (3)	ERBB4 (8)	EZH2 (19)	FBXW7 (5)	FGFR1 (9)
FGFR2 (9)	FGFR3 (8)	FGFR4 (8)	FLT3 (5)	GNA11 (4)	GNAQ (4)	GNAS (2)
HNF1A (2)	HRAS (2)	IDH1 (1)	IDH2 (1)	IGF1R (6)	JAK2 (5)	JAK3 (2)
KDR (12)	KIT (10)	KMT2A (5)	KRAS (3)	MAP3K9 (4)	MLH1 (1)	MPL (1)
MYD88 (1)	NOTCH1 (2)	NPM1 (1)	NRAS (2)	PAX5 (9)	PDGFRA (5)	PHF6 (8)
PIK3CA (13)	PTEN (6)	PTPN11 (8)	RB1 (8)	RET (9)	RUNX1 (8)	SF3B1 (12)
SMAD4 (8)	SMARCB1 (3)	SMO (5)	SRC (1)	STK11 (5)	TET2 (9)	TP53 (4)
TPMT (3)	TYMS (1)	UGT1A1 (1)	VHL (2)	WT1 (3)		

KDR_Q472H (NM_002253.2:c.1416A>T) ← 20% of the general population!

This is a common polymorphism that is predicted to result in a missense protein variant. This mutation is considered to be activating because it results in increased phospho-activation in response to VEGF-A stimulation and greater microvessel density in cell-based studies (PMID:21712447), however the clinical implication of this finding is unclear. There are currently no drugs approved for the treatment of this tumor type with KDR (VEGFR-2) mutations. There are no clinical data to indicate that KDR mutations in this tumor type are predictive of therapeutic response. However, several approved and investigational drugs target VEGFR-2. One potential approach to consider includes use of VEGFR-2 inhibitors. This treatment approach is based on the availability of investigational and approved drugs that inhibit VEGFR-2 and data in cell-based studies.

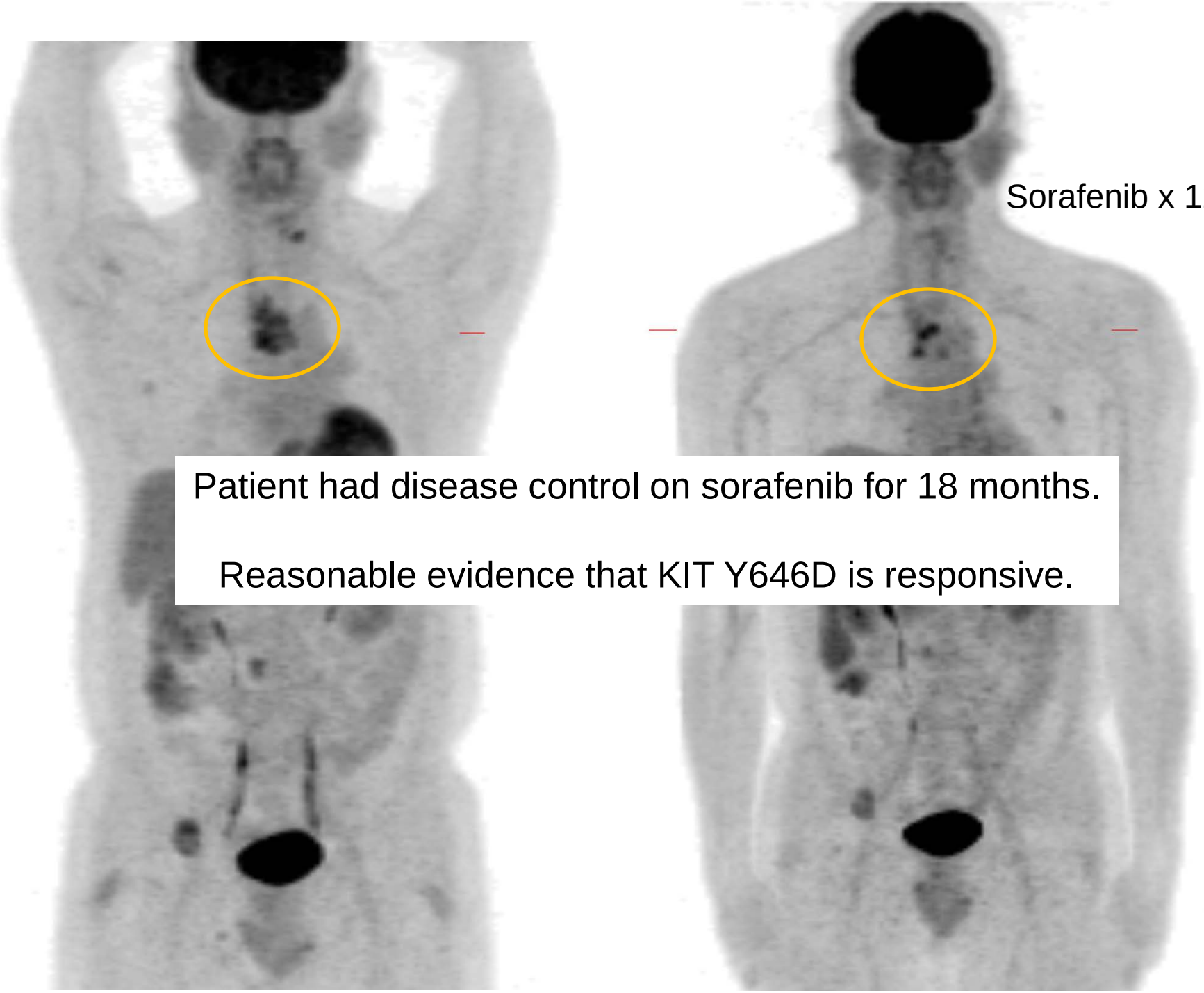
VEGFR2 Phosphorylation in Transfected HEK293 Cells



"We need to provide knowledge, not just data"

Dr. Kojo Elenitoba-Johnson, Nov. 2013

- 40 year old male with thymic carcinoma and no good treatment options
- Tumor sequenced by a commercial laboratory (200+ gene panel)
  - No actionable mutations reported
  - Variants of unknown significance at very bottom of report included "*KIT* Y646D" (kinase domain)
  - Close to K642E (known hotspot)
- Literature review: 6 case reports of KIT-mutant thymic carcinomas responding to KIT inhibitors
- Recommendation: try a KIT inhibitor



Sorafenib x 12 days

Patient had disease control on sorafenib for 18 months.

Reasonable evidence that KIT Y646D is responsive.

Summary

- NGS provides a convenient approach to assessing important targets in solid tumors
  - Panels need not be enormous
  - CNV can be assessed, but tumor content is critical
  - RNAseq is useful for detecting gene fusions
- Sequence variants must be interpreted with caution, and be built on a solid understanding of cancer biology and current treatment options

Acknowledgements

Members of the Knight Diagnostic Laboratories

Support From

- GIST Cancer Research Fund
- The LifeRaft Group
- BP Lester Foundation
- Knight Cancer Institute
- Novartis Pharma

Whole Exome Sequencing

- Benefit offered to Intel patients/spouses with advanced cancer
  - Tumor and germline DNA sequenced
  - Analysis performed using GATK-based pipeline licensed from the Broad Institute
  - Current contract is for 100 patients (2015-2016)