Biological and Therapeutic Insights from the Cancer Genome

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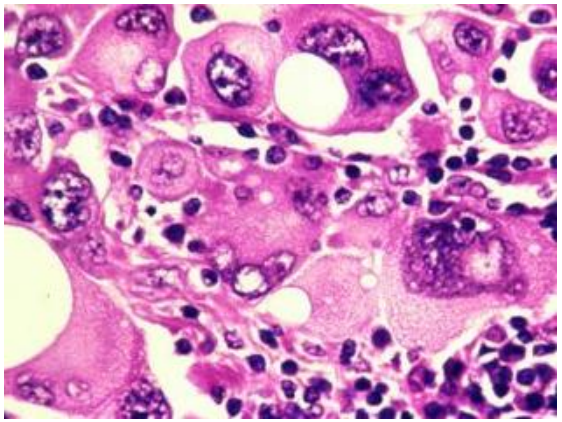
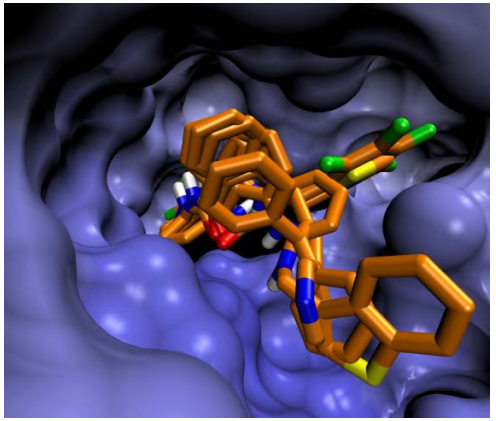




Cancer is a disease of the genome

- Theodor Boveri (1914)
- Chromosomal defects lead to abnormal cell proliferation

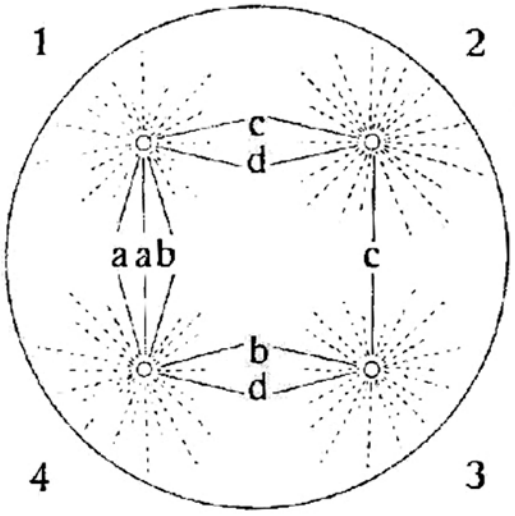
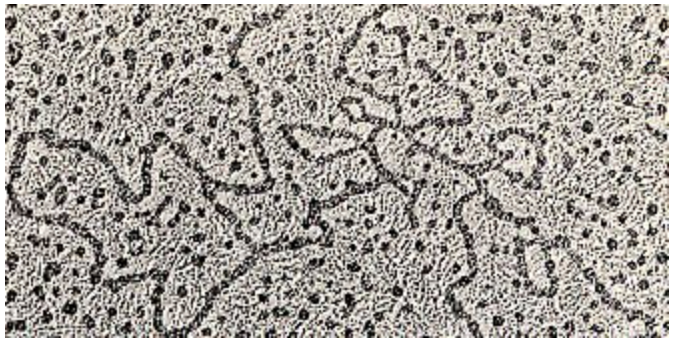
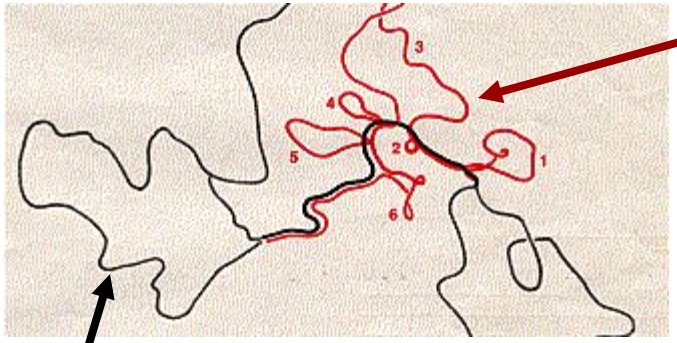


Fig. A.

Concerning the origin of malignant tumors. T. Boveri
J. Cell Sci. doi:10.1242/jcs.025742
(translated 2008)

Cancer arises from alterations in normal cellular genes





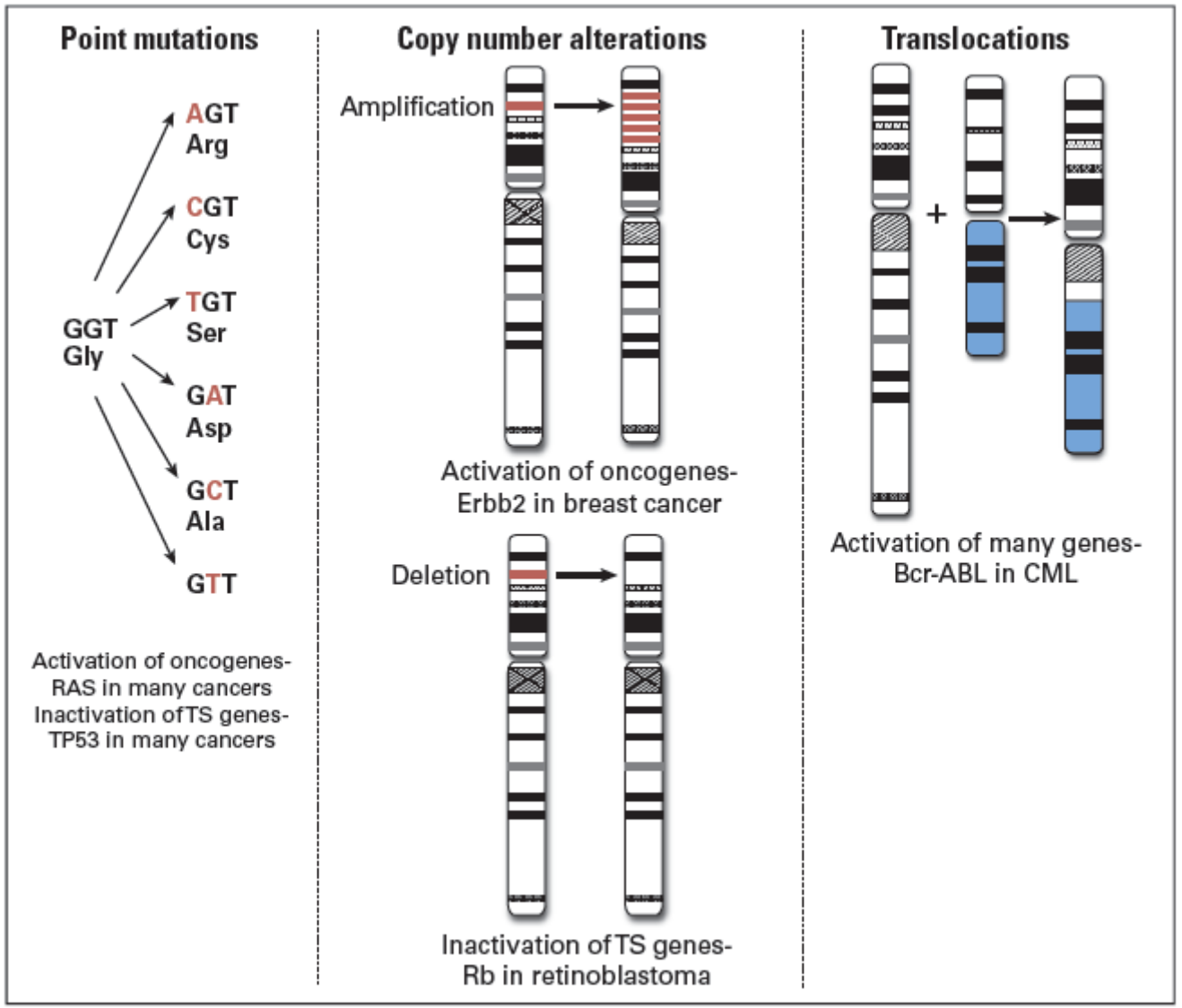
src probe

Normal avian genomic DNA

- Transforming src sequences from the Rous Sarcoma Virus are present in the DNA from normal cells.

Stehelin, Dominique, Varmus, Bishop, & Vogt, *Nature* 260, no. 5547 (1976): 170-173.

Major Categories of Tumor Genomic Alterations



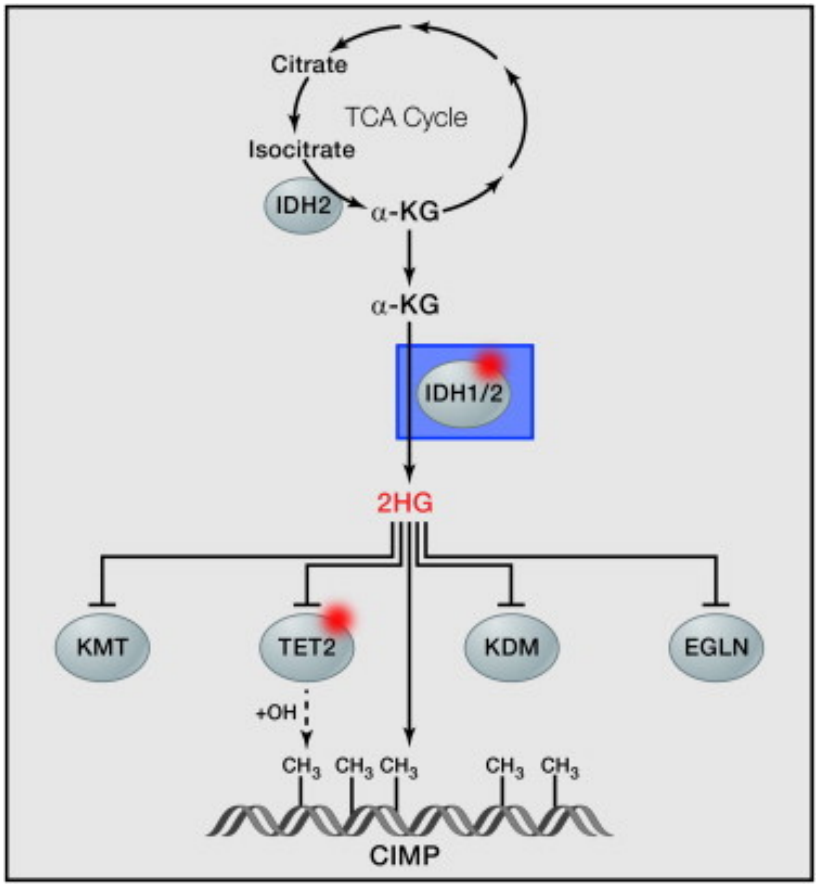
MacConaill & Garraway, *J. Clin. Oncol.* (2010)

Cancer Genome Insights

Insights into biology

Insights into precision medicine

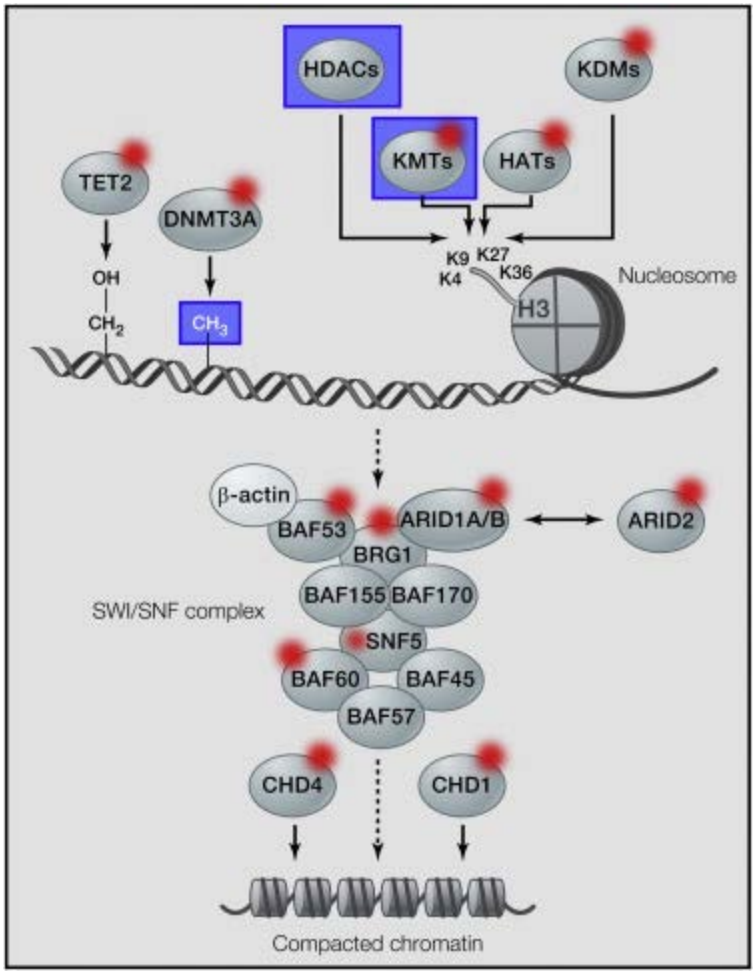
Fundamental insights from cancer genome sequencing - 1



Garraway and Lander, *Cell* (2013)

- **Recurrent *IDH1/2* mutations in GBM and AML link genetics to cancer metabolism**

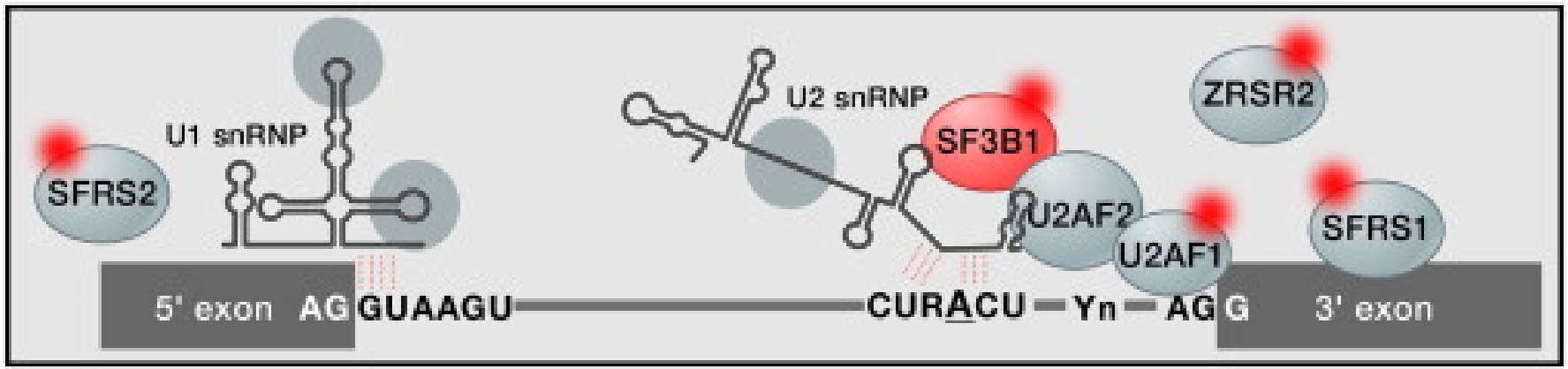
Fundamental insights from cancer genome sequencing - 2



Garraway and Lander, *Cell* (2013)

- **Mutations that disrupt chromatin remodeling and DNA methylation occur in many cancers**

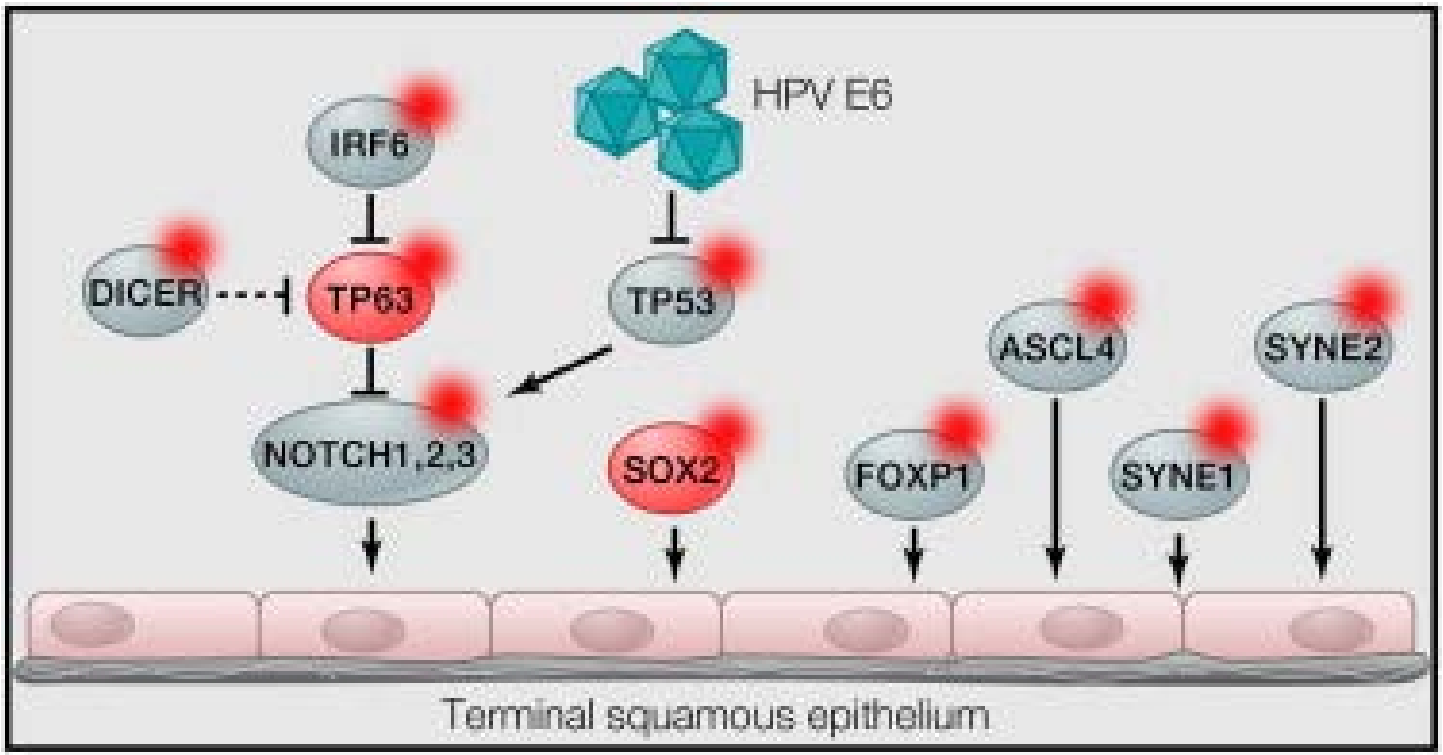
Fundamental insights from cancer genome sequencing - 3



- **Mutations that disrupt mRNA splicing occur in multiple cancer types**

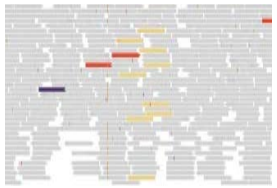
Garraway and Lander, *Cell* (2013)

Fundamental insights from cancer genome sequencing - 4



- **Many mutations (~30%) disrupt Notch signaling and squamous differentiation in head/neck cancer** (Stransky et al., *Science* 2011)
- **Mutations that may dysregulate squamous differentiation occur in 44% of lung squamous cancer** (TCGA, *Nature* 2012)

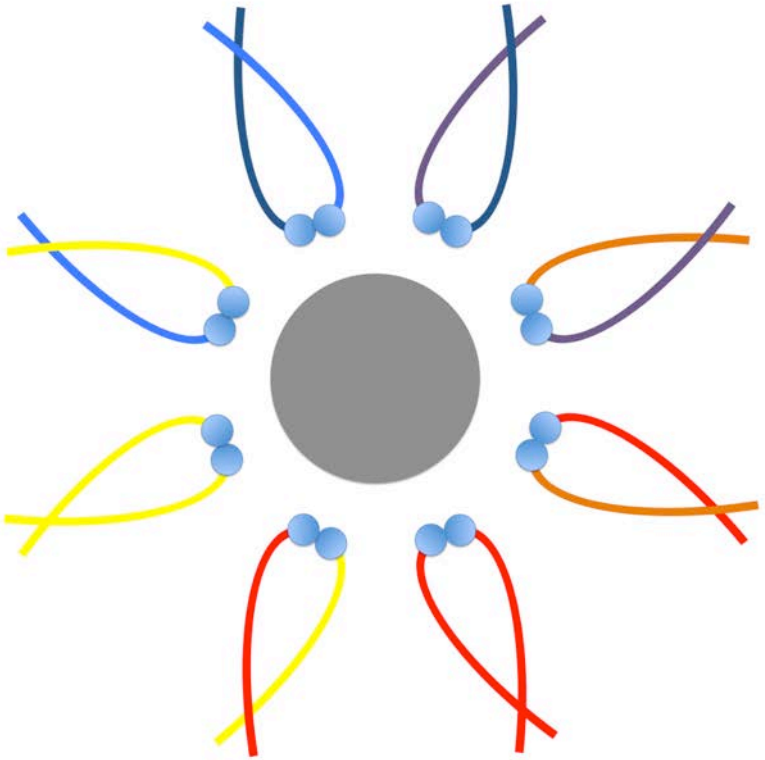
“Chains” of rearrangements in prostate cancer genomes



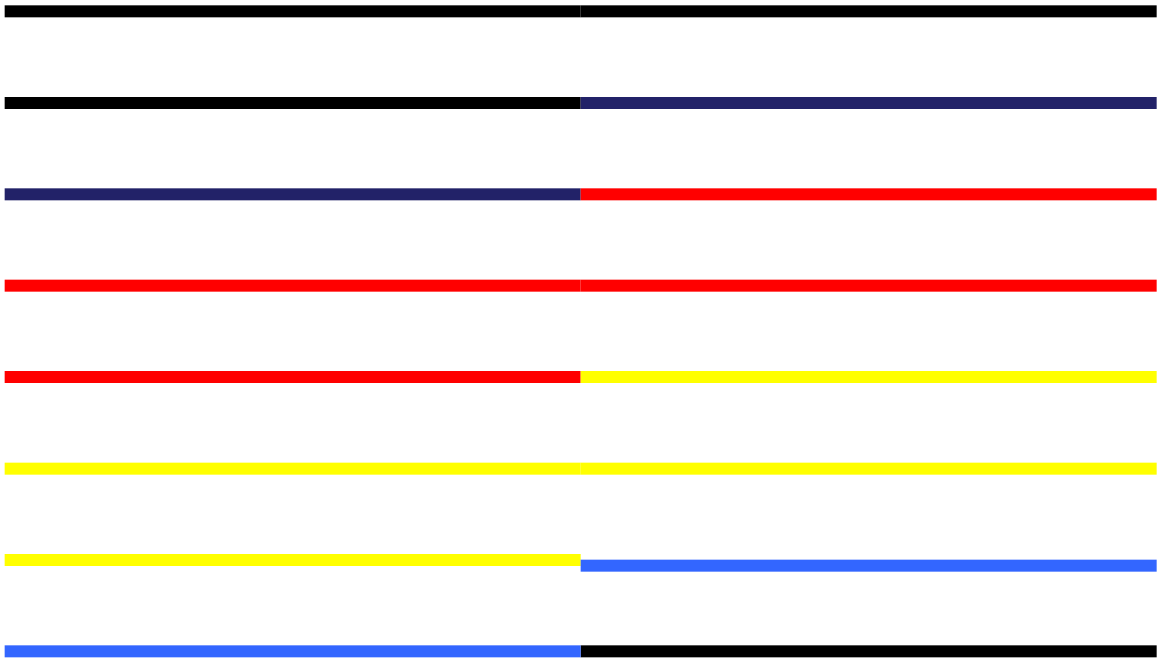
Berger *et al.*, *Nature,* 2011

Generation of “closed chains” in ETS-positive prostate cancers

Generation of “closed chains” in ETS-positive prostate cancers



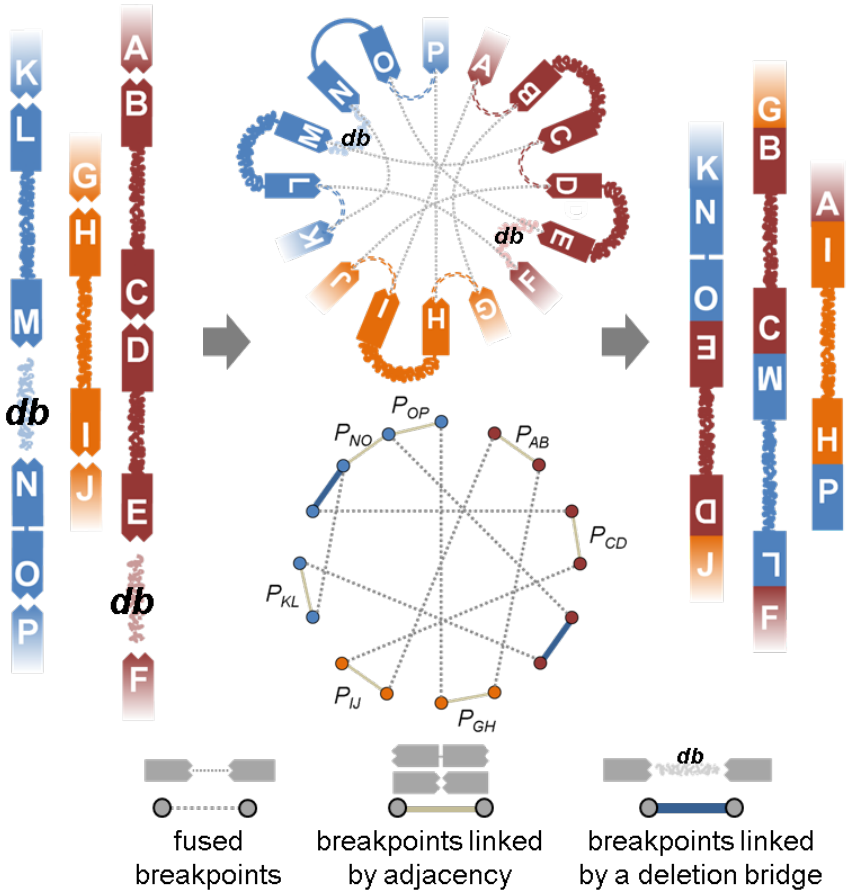
Generation of “closed chains” in ETS-positive prostate cancers



Chromosomal deletions reveal additional chains

Sylvan Baca

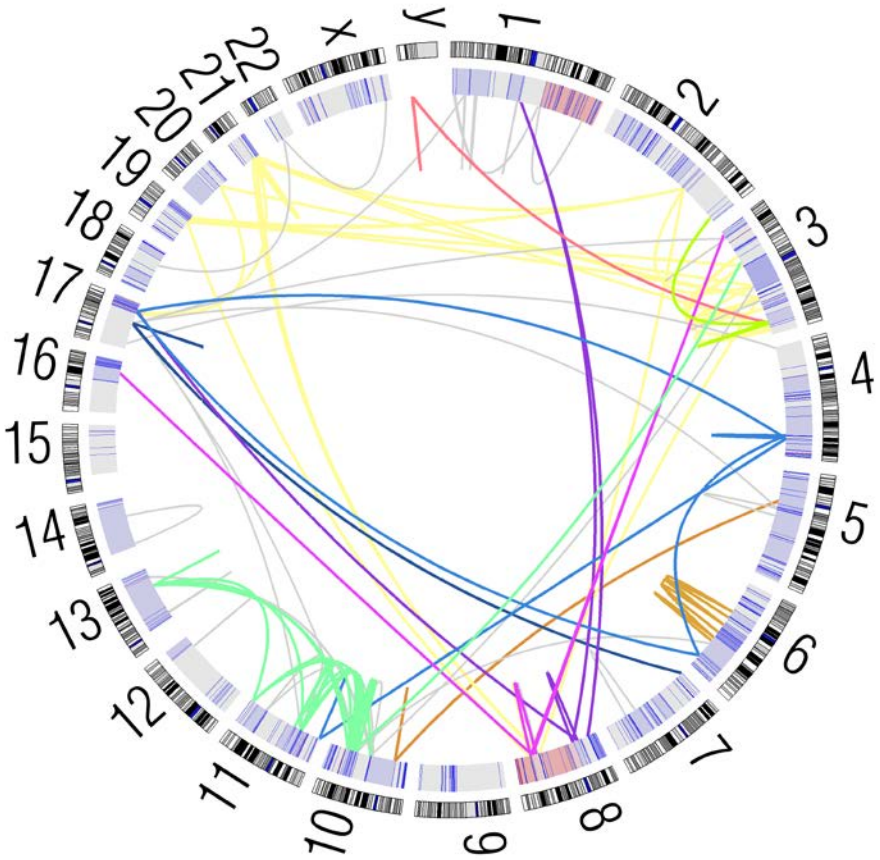
ChainFinder Algorithm



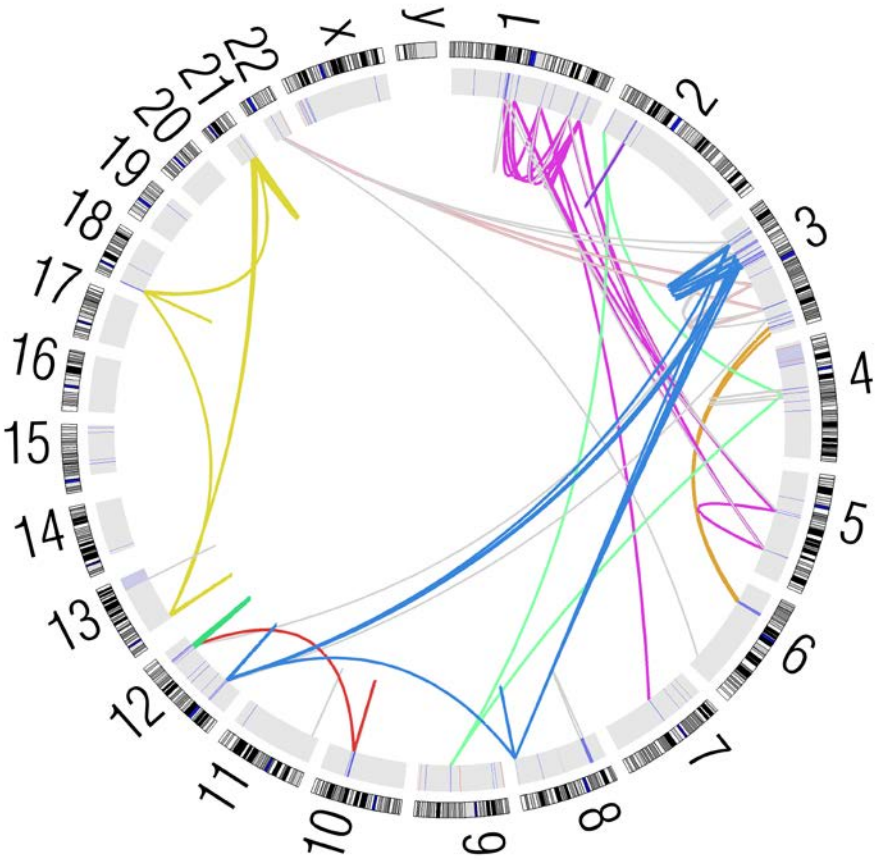
- Create a graph representation of rearrangement breakpoints (nodes) and chromosomal deletion segments (edges)
- Search the graph for sets of connected breakpoint nodes that are statistically unlikely to have arisen independently

Baca at al., *Cell* (2013)

Chained rearrangements are common in prostate cancer ("**chromoplexy**")



P07-4941

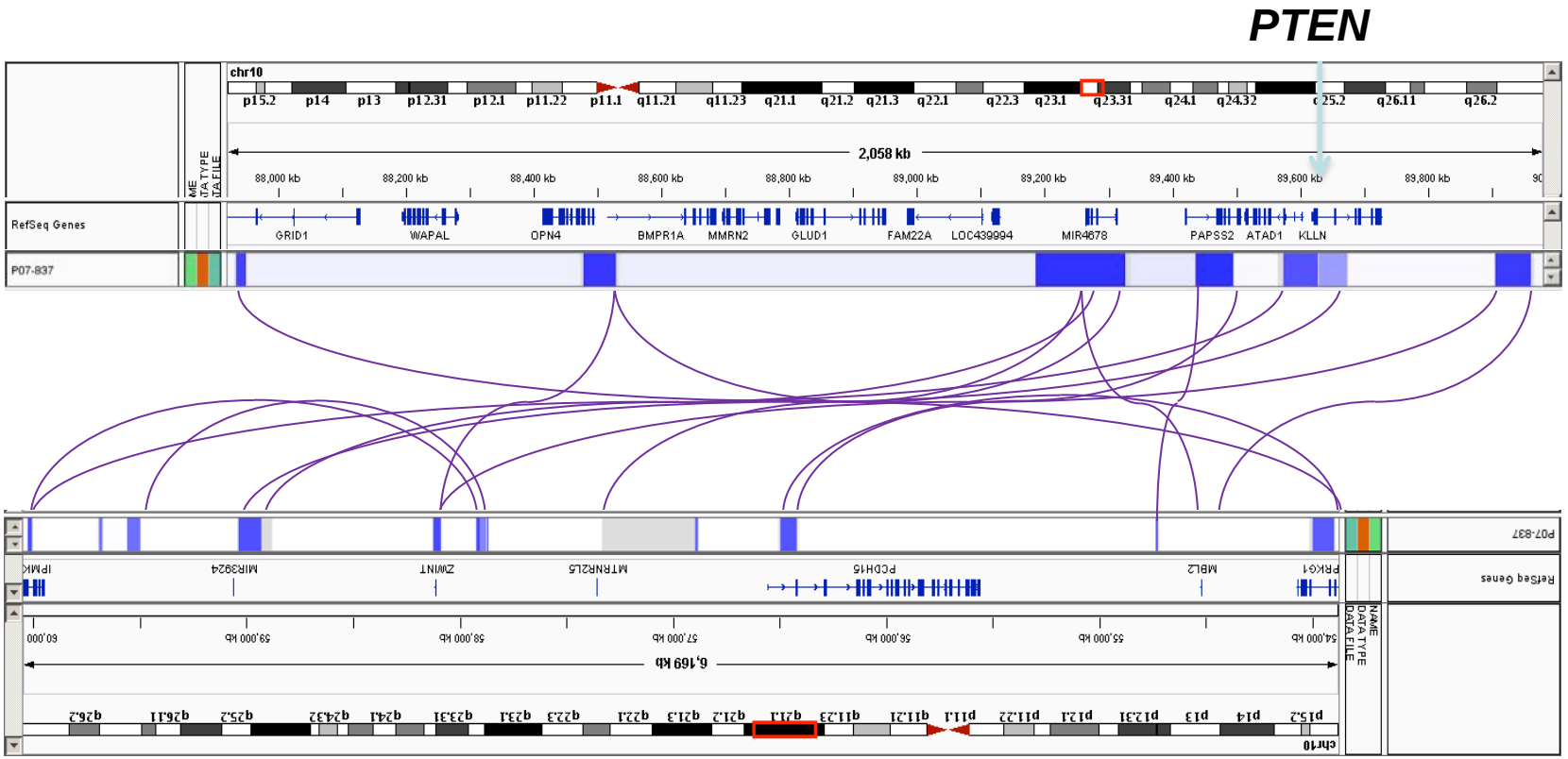


P09-1042

84% of tumors have ≥ 1 chain
65% of tumors have ≥ 2 chains
Some chains exhibit subclonality

Baca at al., *Cell* (2013)

Cancer genes are often disrupted by chromoplexy



Two sections of chromosome 10q

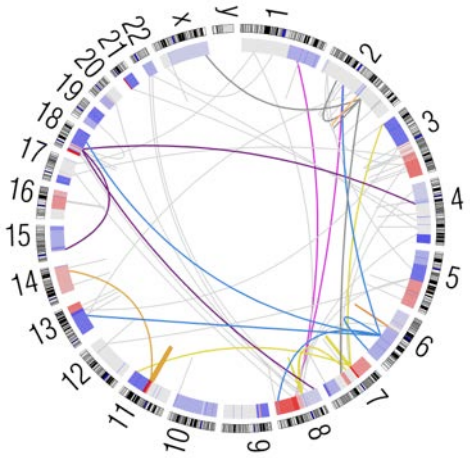
Baca at al.,
Cell (2013)

Cancer genes are often disrupted by chromoplexy

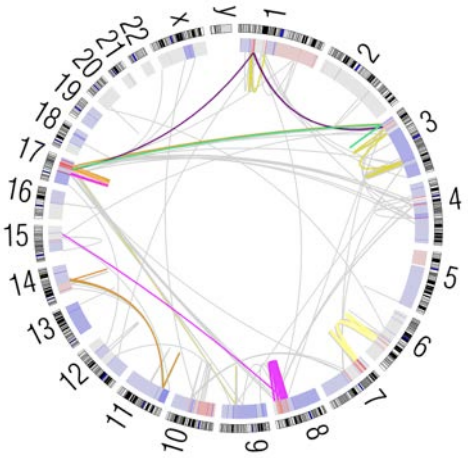
Gene	# tumors with disruption by chromoplexy
ERG (fusion with *TMPRSS2*)	15 (of 26 fusion-positive cases)
PTEN	10
NKX3-1	4
TP53	3
CDKN1B	2
RB1	2

Baca at al., *Cell* (2013)

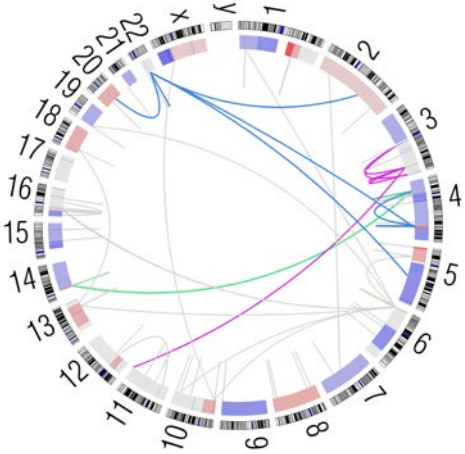
Chromoplexy in other tumor lineages



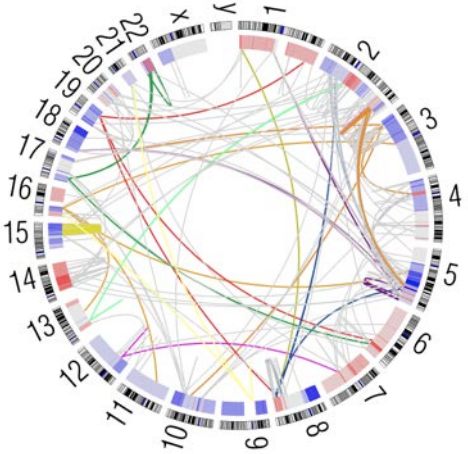
Head and neck squamous



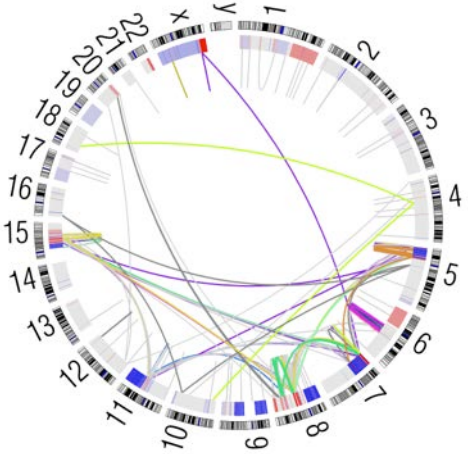
Breast



Lung adenocarcinoma



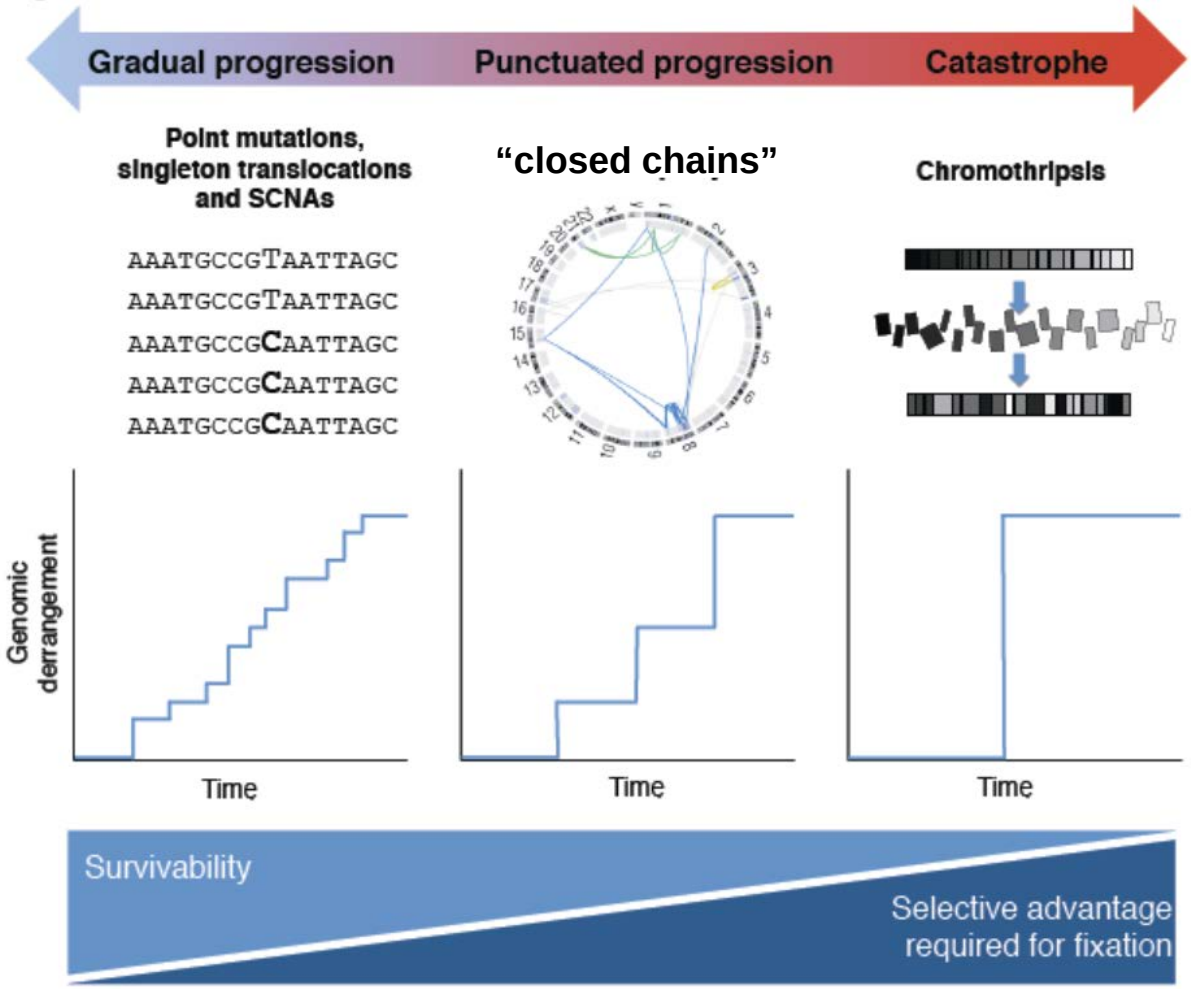
Lung adenocarcinoma



Melanoma

Fundamental insights from cancer genome sequencing - 5

- **A continuum model for tumor evolution**

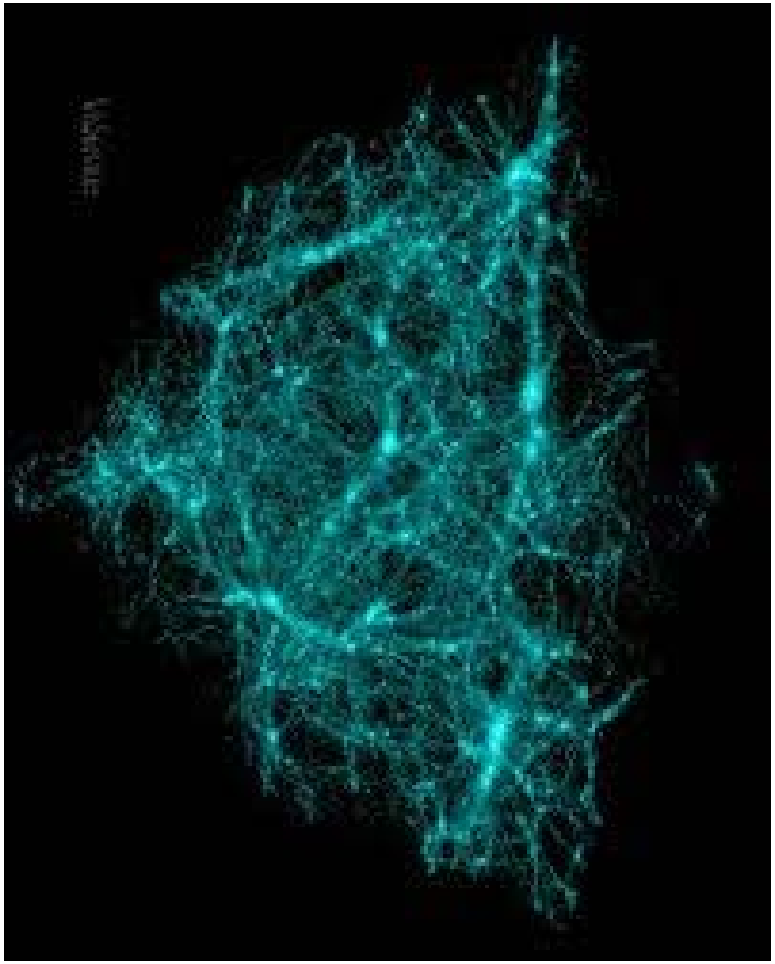


Berger et al., *Nature* (2011)

Baca at al., *Cell* (2013)

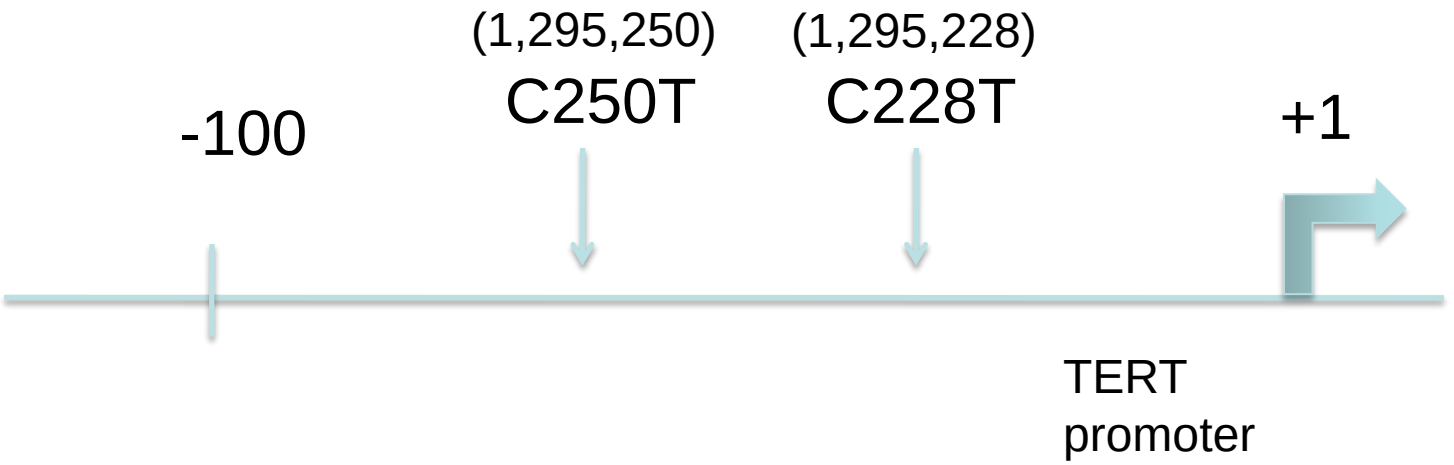
The “dark matter” of the cancer genome

- Regions of the genome that we cannot easily interpret
- Examples:
  - regulatory regions
  - intergenic regions
  - repeat-rich DNA
  - “non-focal” copy number alterations

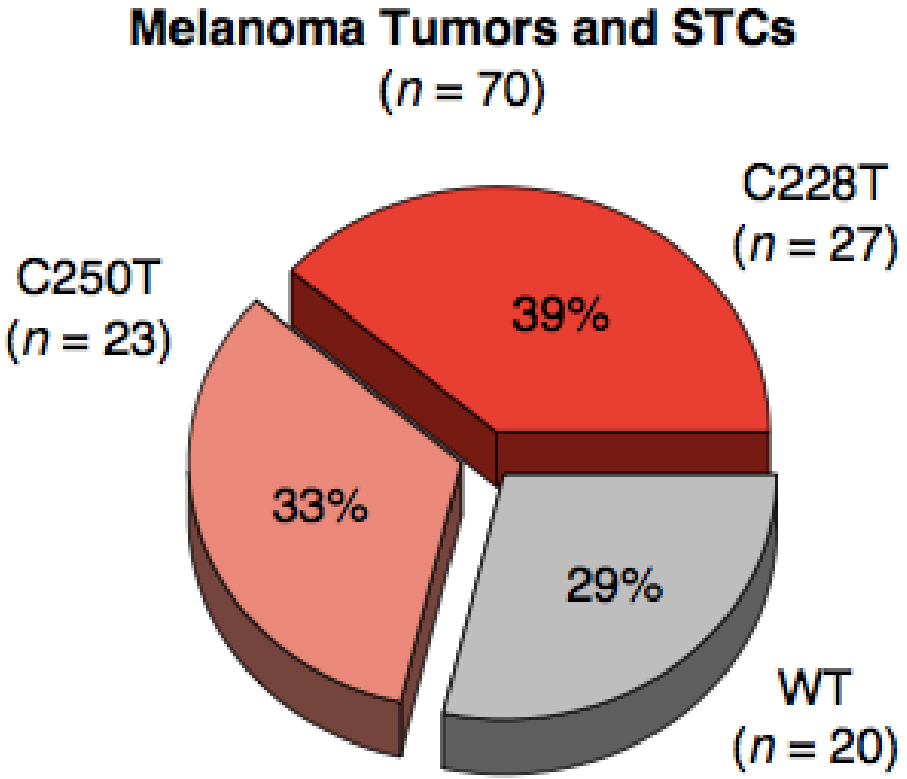


Identification of two recurrent mutations in the *TERT* promoter

- 17 of 19 (89%) melanomas had one of two mutations within 100bp of the transcription start site of the *TERT* promoter
- Both are C to T transitions (indicative of UV damage)
- Mutations were mutually exclusive

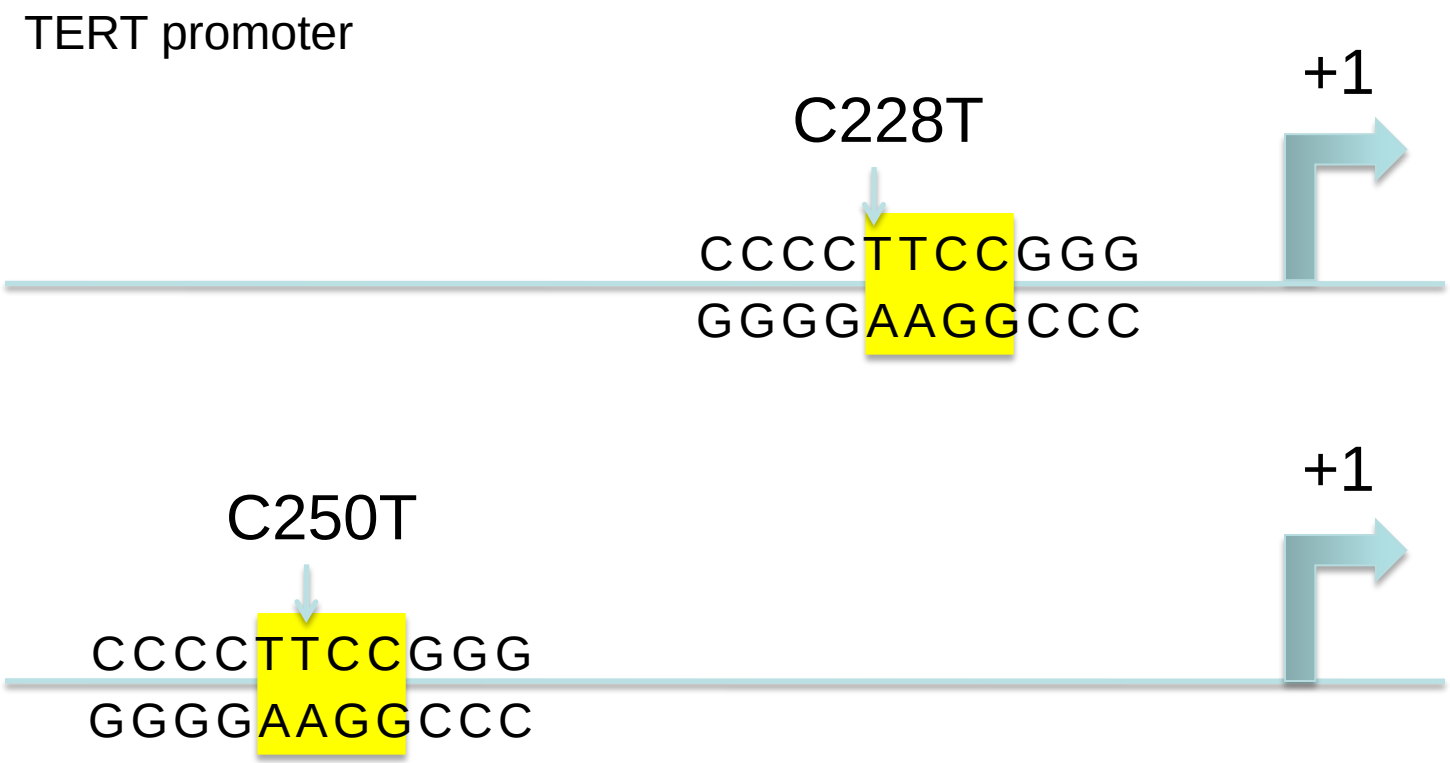


50 of 70 (71%) harbor TERT promoter mutations



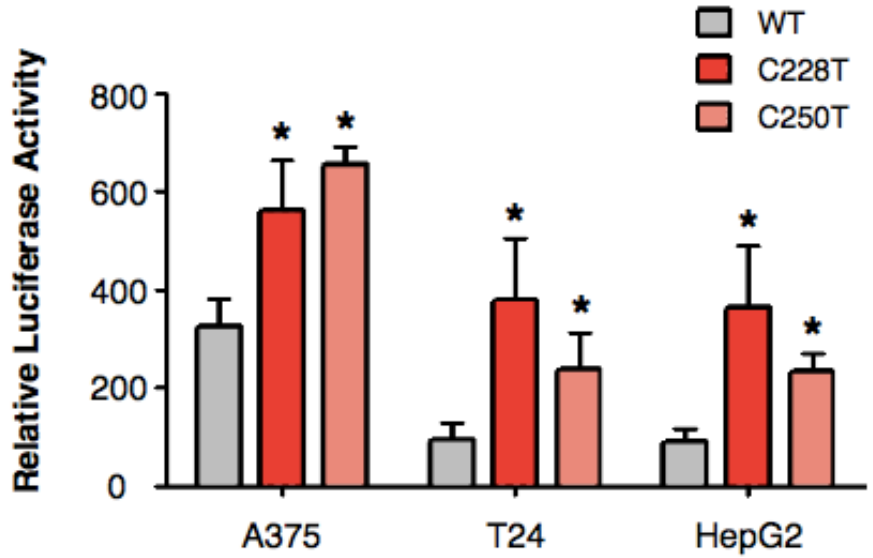
Huang, Hodis et al., *Science* (2013)

C228T and C250T create consensus ETS sites (GGAA/T)

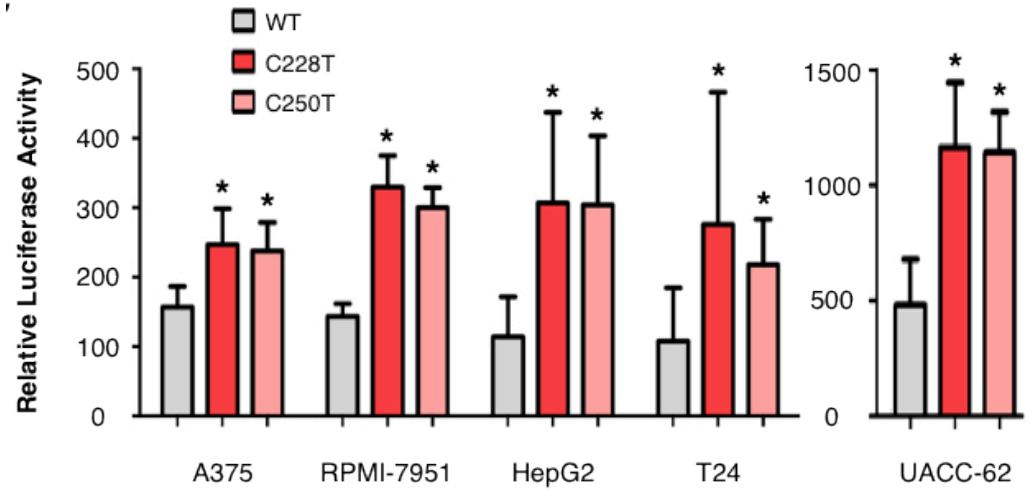


Huang, Hodis et al., *Science* (2013)

C228T and C250T mutations augment transcriptional activity from the TERT promoter



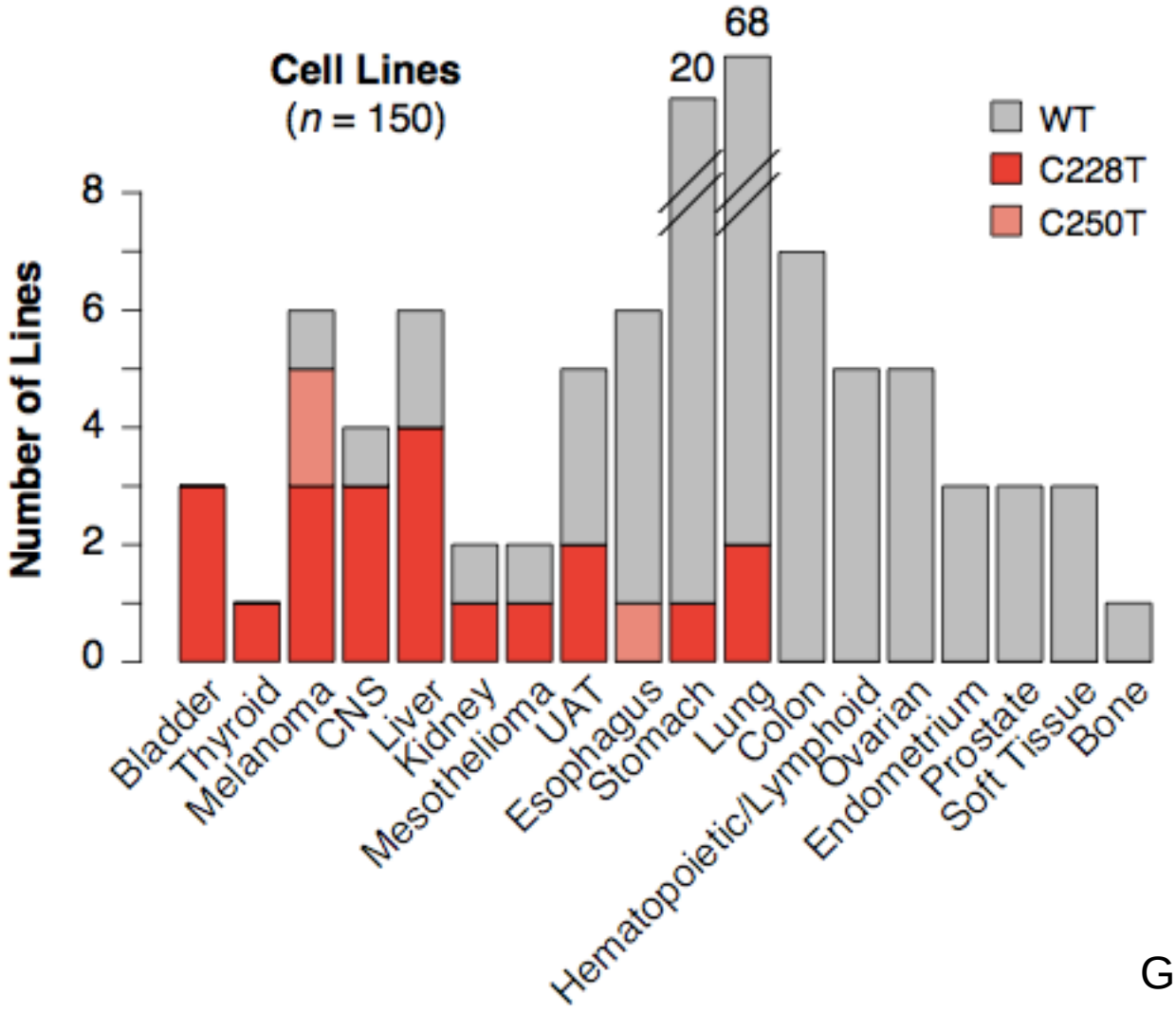
* p< 0.01



* p< 0.05

Mary Jue Xu

Recurrent TERT promoter mutations in cancer cell lines



Gregory Kryukov

Cancer Genome Insights

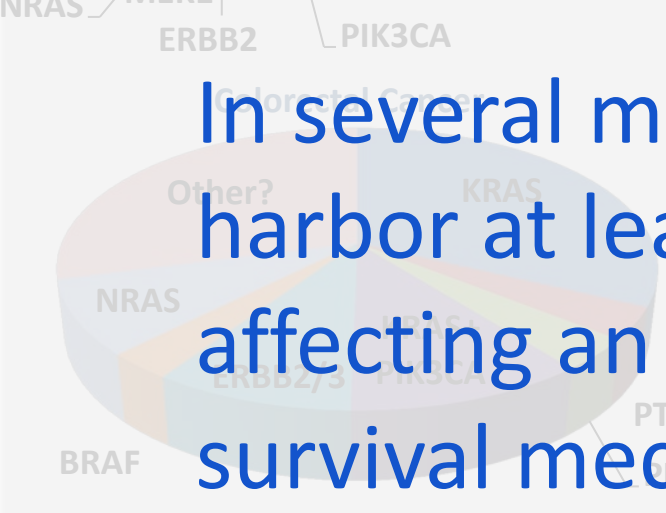
Insights into biology

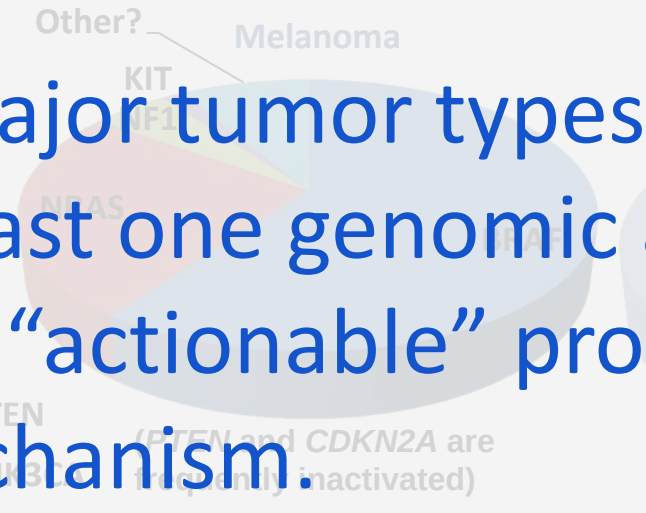
Insights into precision medicine

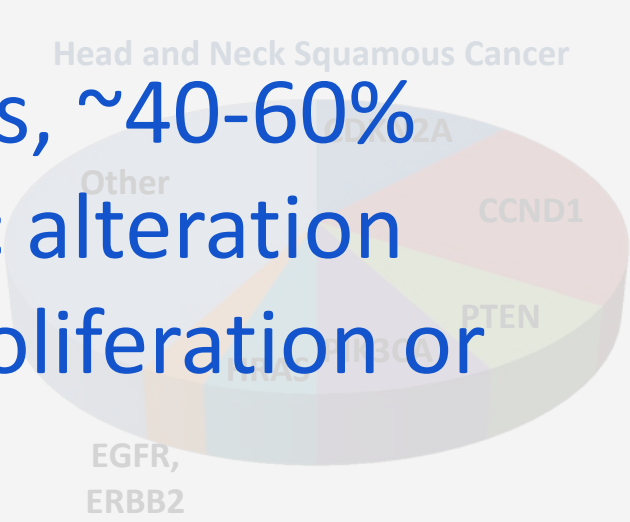
Genomics-Driven Cancer Medicine: Guiding Principles

<u>Principle #1</u>: Molecular pathways involved in tumor survival and progression are often activated by genetic alterations.

Garraway, *J. Clin. Oncol.*, 2013







In several major tumor types, ~40-60% harbor at least one genomic alteration affecting an “actionable” proliferation or survival mechanism.

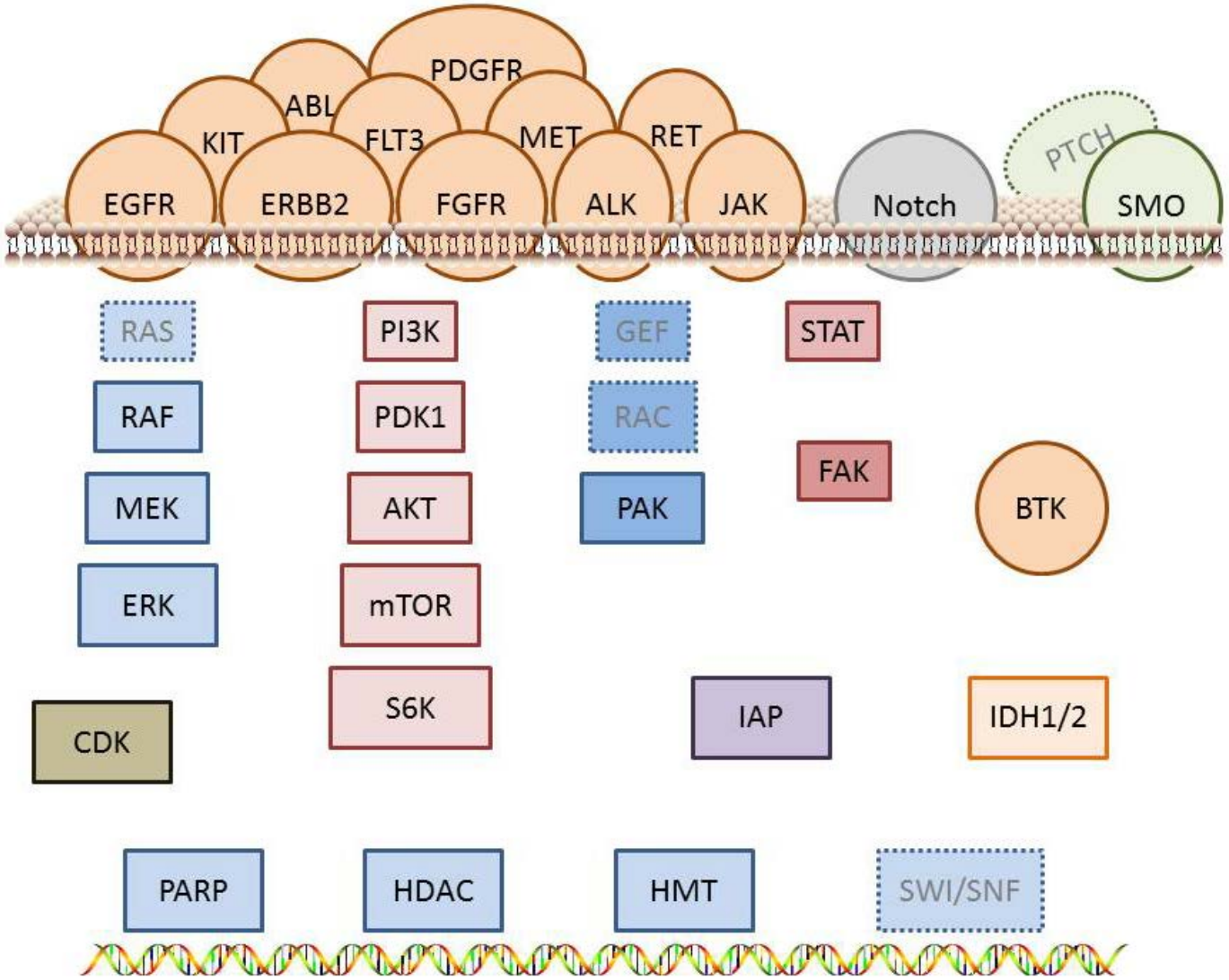
Genomics-Driven Cancer Medicine: Guiding Principles

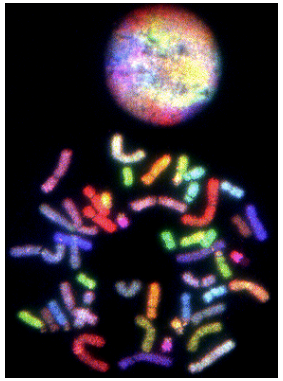
Principle #1: Molecular pathways involved in tumor survival and progression are often activated by genetic alterations.

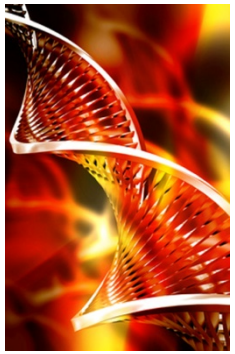
Principle #2: Anticancer agents targeting many oncogenic pathways have entered clinical trials.

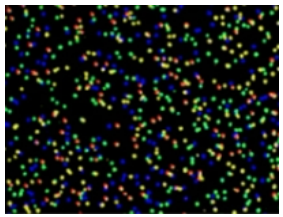
Garraway, *J. Clin. Oncol.*, 2013

Spectrum of Targeted Anticancer Agents in Clinical Development

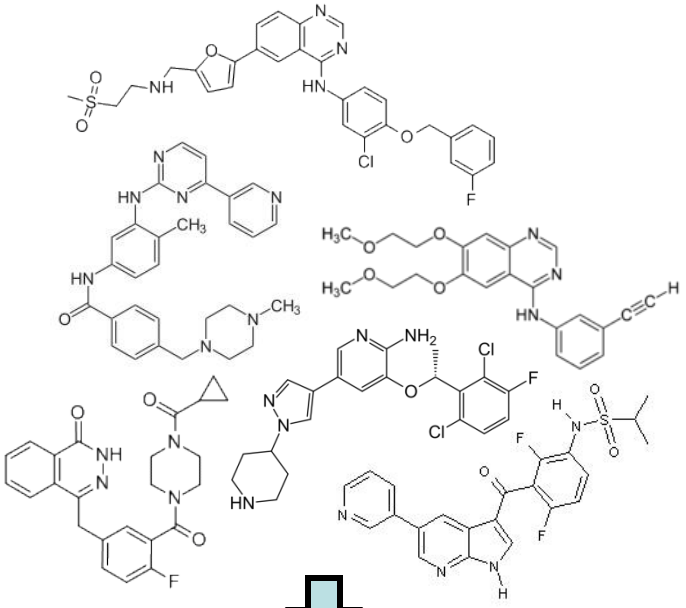


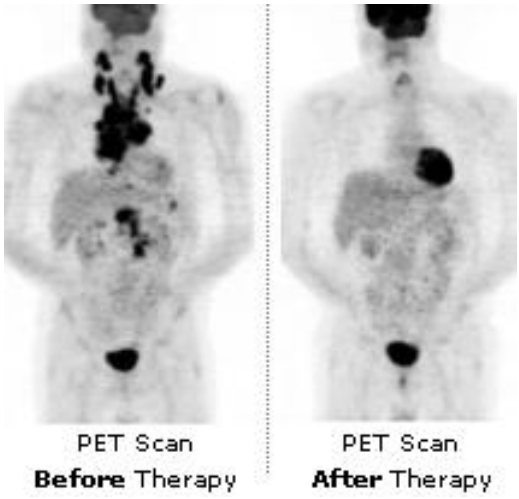






GENOMIC STUDIES





TUMOR DEPENDENCY → **RATIONAL THERAPEUTICS** → **TUMOR RESPONSE (advanced disease)**

ERBB2 (breast cancer)	Trastuzumab, Lapatinib	weeks-months
KIT (GIST)	Imatinib	months-years
EGFR (lung cancer)	Erlotinib	months
SMO (BCC)	GDC0449, LDE225	months
ALK (lung cancer)	Crixotinib	months
BRAF (melanoma)	Vemurafenib, dabrafenib, trametinib	Months-1 yr
…	…	…

Genomics-Driven Cancer Medicine: Guiding Principles

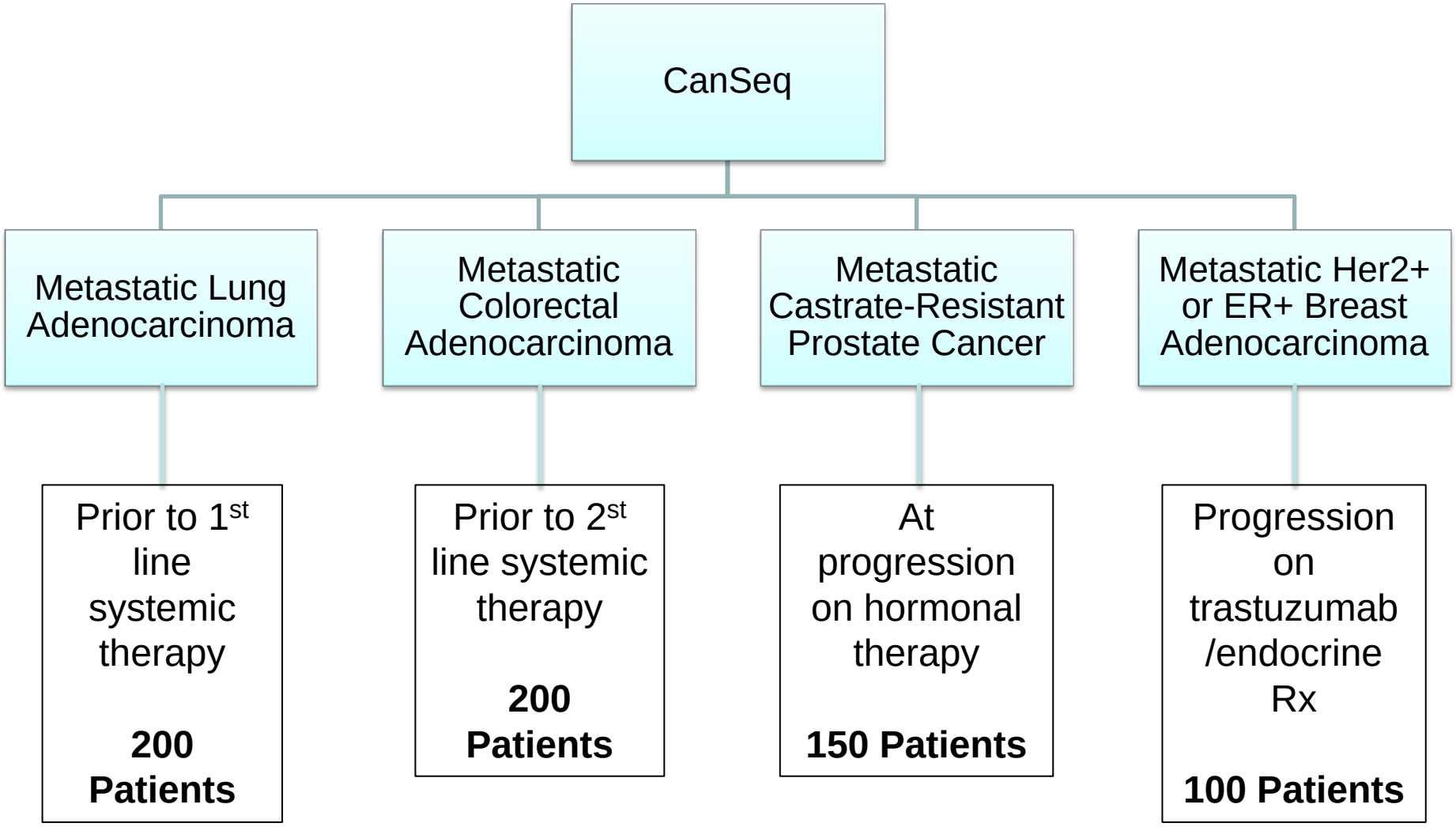
Principle #1: Molecular pathways involved in tumor survival and progression are often activated by genetic alterations.

Principle #2: Anticancer agents targeting many oncogenic pathways have entered clinical trials.

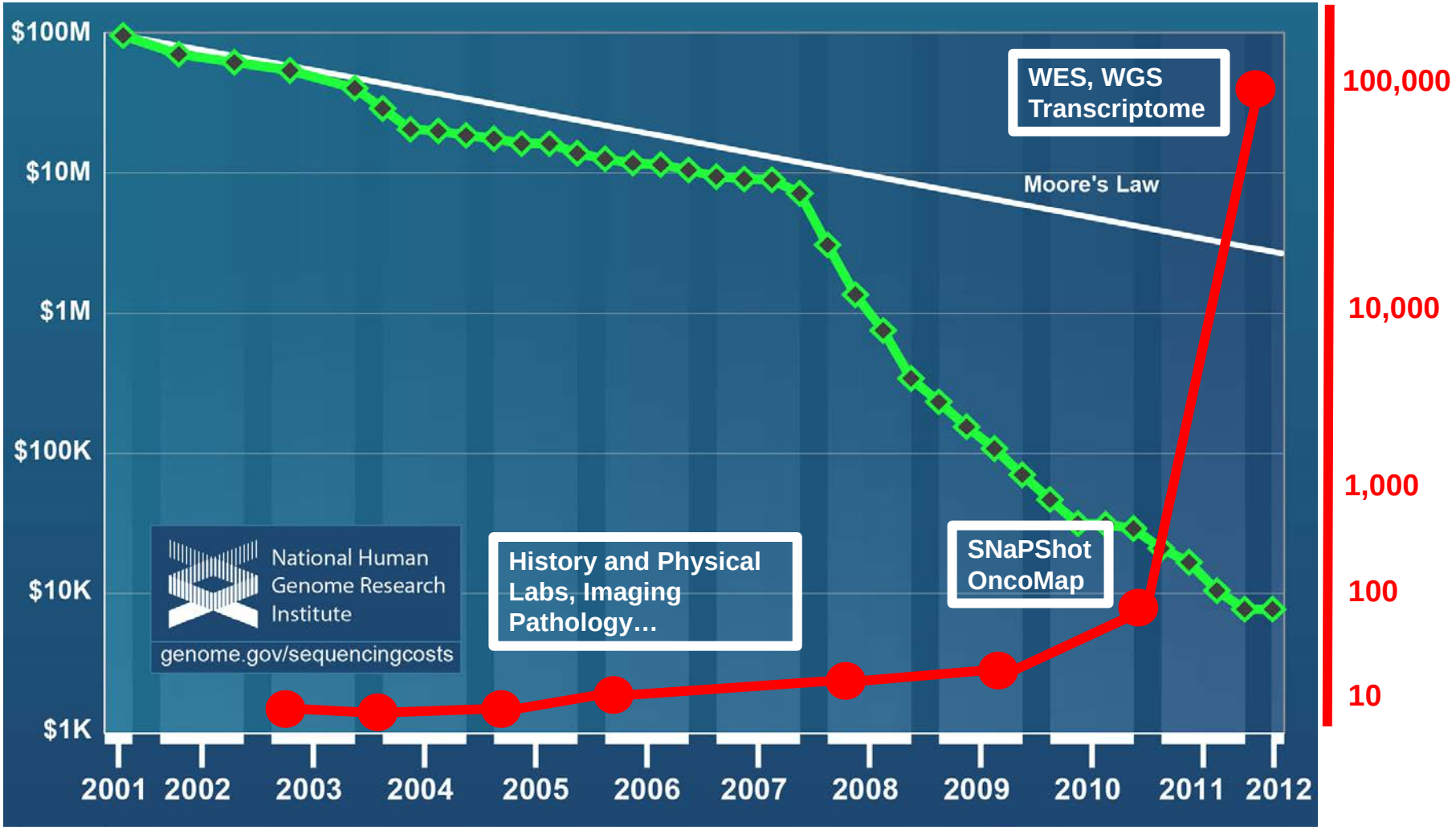
Principle #3: Genomics technologies enable robust tumor genomic profiling in the clinical arena.

CanSeq: Prospective Whole Exome Sequencing

Prospective whole-exome sequencing on patients at DFCI/BWH with return of clinically actionable results to clinical care team



Big Data in Oncology



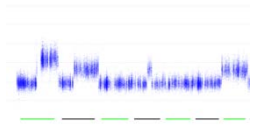
Source: NHGRI

Precision Heuristics for Interpreting the Alteration Landscape (PHIAL)

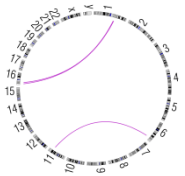
Mutations …ACC… …TAG…

Insertion/ Deletions …TCG… …AACCC…

Copy Number Alterations



Rearrangements



PHIAL

Investigate Clinical Actionability

Investigate Biological Relevance

Cancer Genes

Cancer Pathways

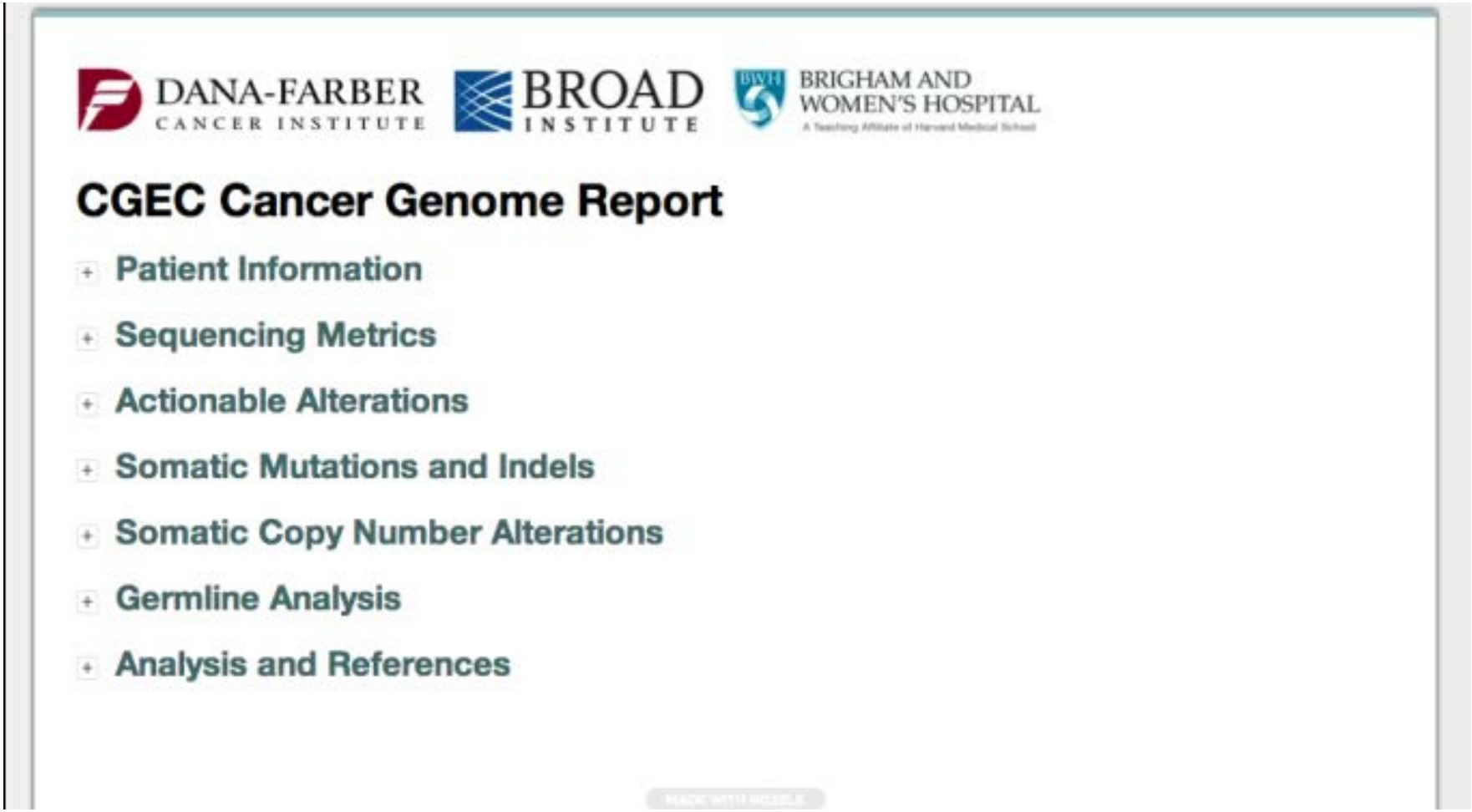
COSMIC

Synonymous Variants

"May it be a light to you in dark places, when all other lights go out."
Galadriel, *The Fellowship of the Ring* (Tolkein)

Eli Van Allen

Evaluating Actionable Alterations



Eli Van Aller

Evaluating Actionable Alterations

Actionable Table and Details

Table 4. Actionable findings with details, sorted by actionability score

Gene	Alteration	Variant	Coverage	Allelic_fraction	Tier	Trials
KRAS	p.A146V	Missense_Mutation	248	0.61	Actionable: Tier 2-A, Plausibly Actionable, Tier 1-B(R), Prognostic/Diagnostic-B	Click here
STK11	p.G279fs	Frame_Shift_Del	23	0.48	Plausibly Actionable: Tier 1-C, 1-D, and 2-B	Click here
ATM	p.K208fs	Frame_Shift_Ins	39	0.36	Plausibly Actionable: Tier 2-B	Click here
BCL6	p.E419V	Missense_Mutation	112	0.53	Theoretically Actionable: Tier 2-E	Click here

KRAS p.A146V: Activating mutations in KRAS are among the most common genetic alterations in human tumors. KRAS mutations play a central role in tumor progression in multiple cancer types, and have been implicated in poor prognosis and resistance to therapy.

KRAS alterations are common across numerous malignancies. Activating KRAS mutations are found in 15 to 30% of all patients with non-small cell lung cancer (NSCLC).

This alteration has rarely been found in other cancer types. This alteration has only been reported in 15 colorectal cancer cases in the COSMIC database. An additional 68 cases of A146T have been reported in colorectal cancer in the COSMIC database. However, one systematic study of exon 4 mutations in conorectal cancer demonstrated the presence of A146 mutations in 5% of colon cancers.

This alteration is a known activating mutation, though may be less potent than the more common codon 12 and 13 mutations.

Activating mutations in KRAS predict poor survival in patients with NSCLC, though these studies have generally only included codon 12 and 13 mutations. Activating mutations in KRAS may predict sensitivity to inhibitors of the RAS/RAF/MEK/ERK pathway. Preclinical studies have shown that MEK inhibitors, in particular, may be effective for KRAS mutant tumors, and these agents are in clinical trials for patients with KRAS mutant cancers. Activating KRAS mutations may also predict resistance to anti-EGFR therapies.

STK11 p.G279fs: STK11 is a well-known tumor suppressor (also known as LKB1) that is commonly inactivated in several cancers. Germline mutations in STK11 cause Puetz-Jeghers Syndrome (PJS).

This gene has been implicated in NSCLC. In addition, it is commonly seen in conjunction with KRAS mutations

This gene has been implicated in NSCLC. This specific alteration has not been reported in the COSMIC database for NSCLC, though inactivating mutations in STK11 are common in this tumor type, ocurring at a rate of 5-15% of NSCLC. They commonly co-occur with KRAS mutations.

This alteration is likely inactivating, since it is a frameshift mutation that occurs at codon 279 out of 434.

Loss of STK11 activates the MTOR pathway and therefore may predict sensitivity to inhibitors of this pathway. Preclinical evidence suggests that MTOR

Eli Van Aller

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Reporting Results to Clinicians

DANA-FARBER CANCER INSTITUTE | BROAD INSTITUTE | BRIGHAM AND WOMEN'S HOSPITAL

CanSeq Cancer Genome Report

Patient ID: xxxxxxxx
DOB: xxxxx
Diagnosis: Lung Adenocarcinoma

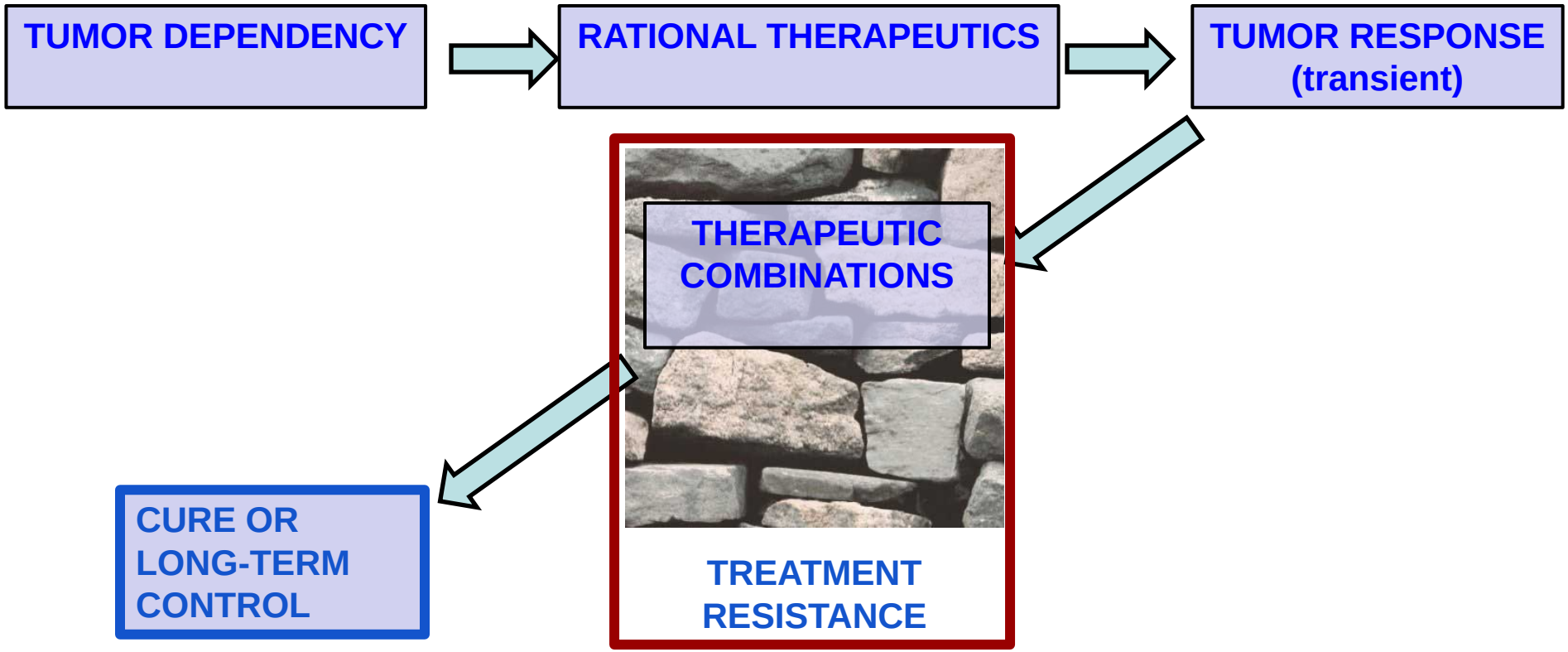
ACTIONABLE SOMATIC ALTERATIONS

Alteration	Action / Agent	FDA Approved?	Level of Evidence	Validated by:
KRAS A146V	MEK Inhibitors Resistance to EGFR inhibitors Poor prognosis		Eligibility Criteria Limited Clinical Theoretical	IonTorrent Seq
STK11 G279fs	Everolimus Temsirolimus mTOR Inhibitors Dasatinib FAK inhibitors	Yes Yes Yes	Other tumor type Other tumor type Pre-clinical Pre-clinical Pre-clinical	IonTorrent Seq
ATM K208fs	PARP inhibitors		Pre-clinical	IonTorrent Seq

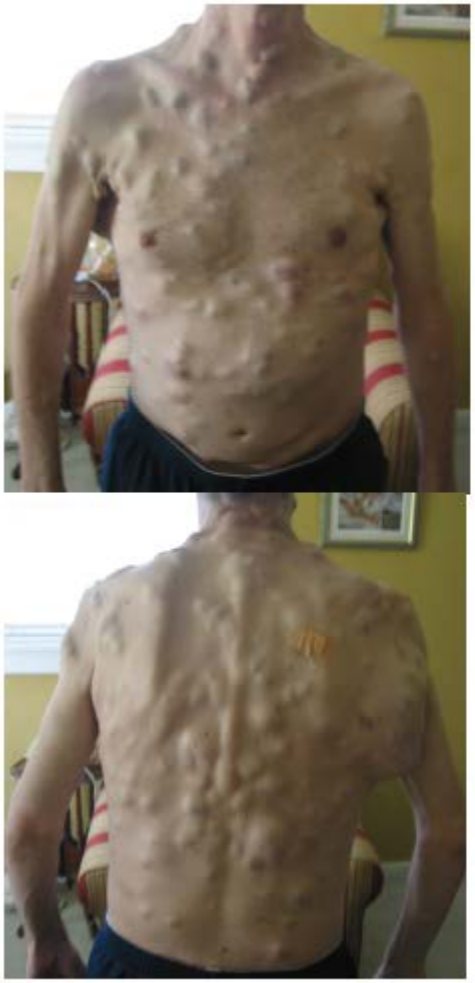
***KRAS* A146V**

- Activating mutations in KRAS are among the most common genetic alterations in human tumors. KRAS mutations play a central role in tumor progression in multiple cancer types, and have been implicated in poor prognosis and resistance to therapy.
- KRAS alterations are common across numerous malignancies. Activating KRAS mutations are found in 15–30% of all patients with non-small cell lung cancer (NSCLC).
- **This alteration is a known activating mutation**, though may be less potent than the more common codon 12 and 13 mutations (PMID: 20570890).
- **This alteration has not been reported in the COSMIC database for NSCLC.** Furthermore, A146 mutations in KRAS were not found in 2 studies comprised 449 cases of NSCLC in which KRAS was sequenced in its entirety (PMID: 18948947, 18632602).
- **This alteration has rarely been found in other cancer types**. This alteration has only been reported in 15 colorectal cancer cases in the COSMIC database. An additional 68 cases of A146T have been reported in colorectal cancer in the COSMIC database. However, one systematic study of exon 4 mutations in colorectal cancer demonstrated the presence of A146 mutations in 5% of colon cancers (PMID: 20570890).
- **Activating mutations in KRAS predict poor survival in patients with NSCLC**, though these studies have generally only included codon 12 and 13 mutations.
- **Activating mutations in KRAS may predict sensitivity to inhibitors of the RAS/RAF/MEK/ERK pathway.** Preclinical studies have shown that MEK inhibitors, in particular, may be effective for KRAS mutant tumors, and these agents are in clinical trials for patients with KRAS mutant cancers.
- **Activating KRAS mutations may also predict resistance to anti-EGFR therapies.**

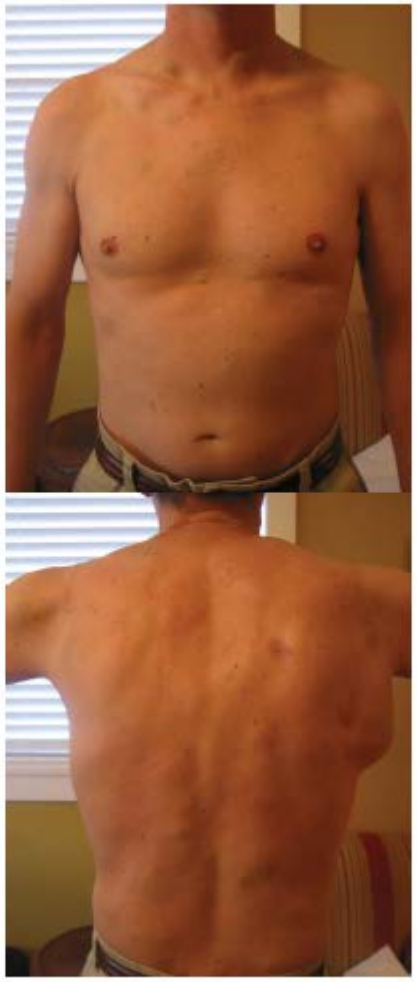
A “Critical Path” to Effective Cancer Treatment



Clinical Response and Resistance to RAF or MEK Inhibition in Melanoma



October, 2009



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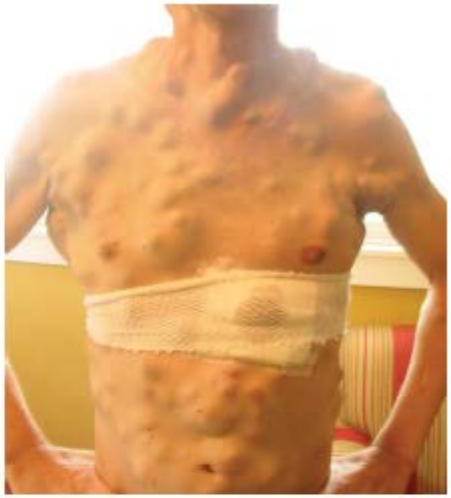
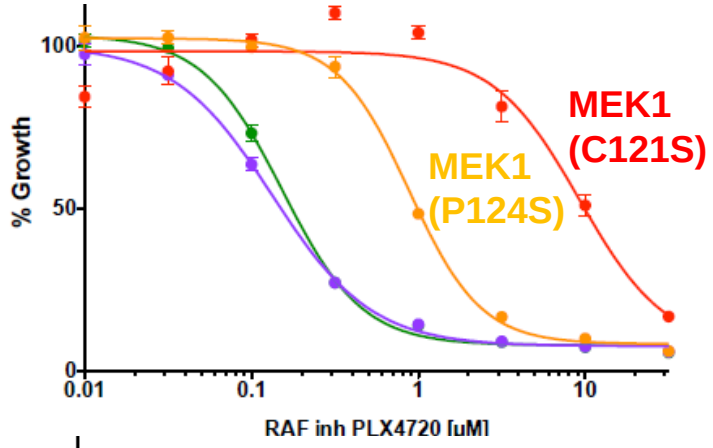
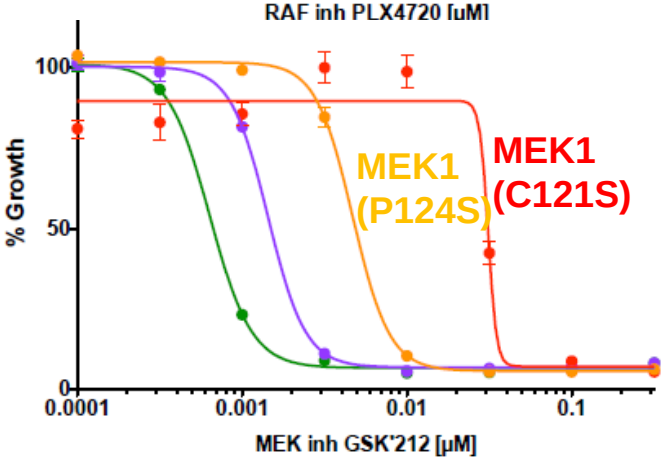
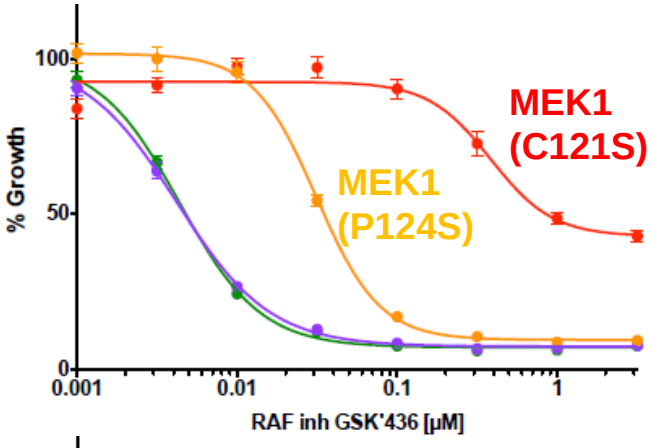


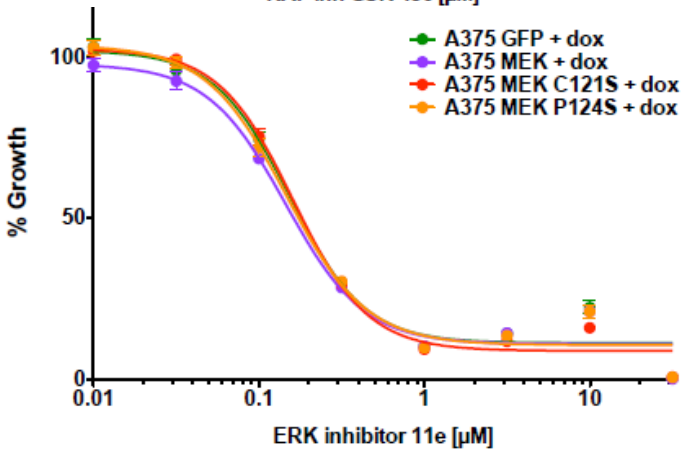
March, 2010

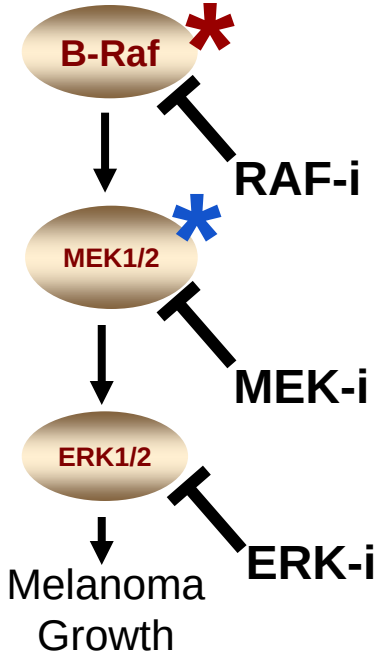
MEK1 mutations and resistance to RAF/MEK inhibition







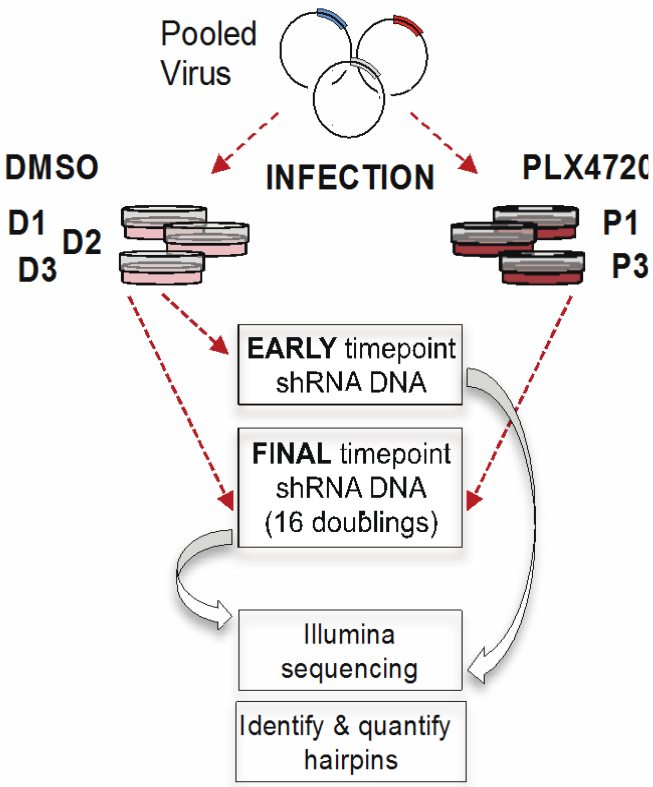




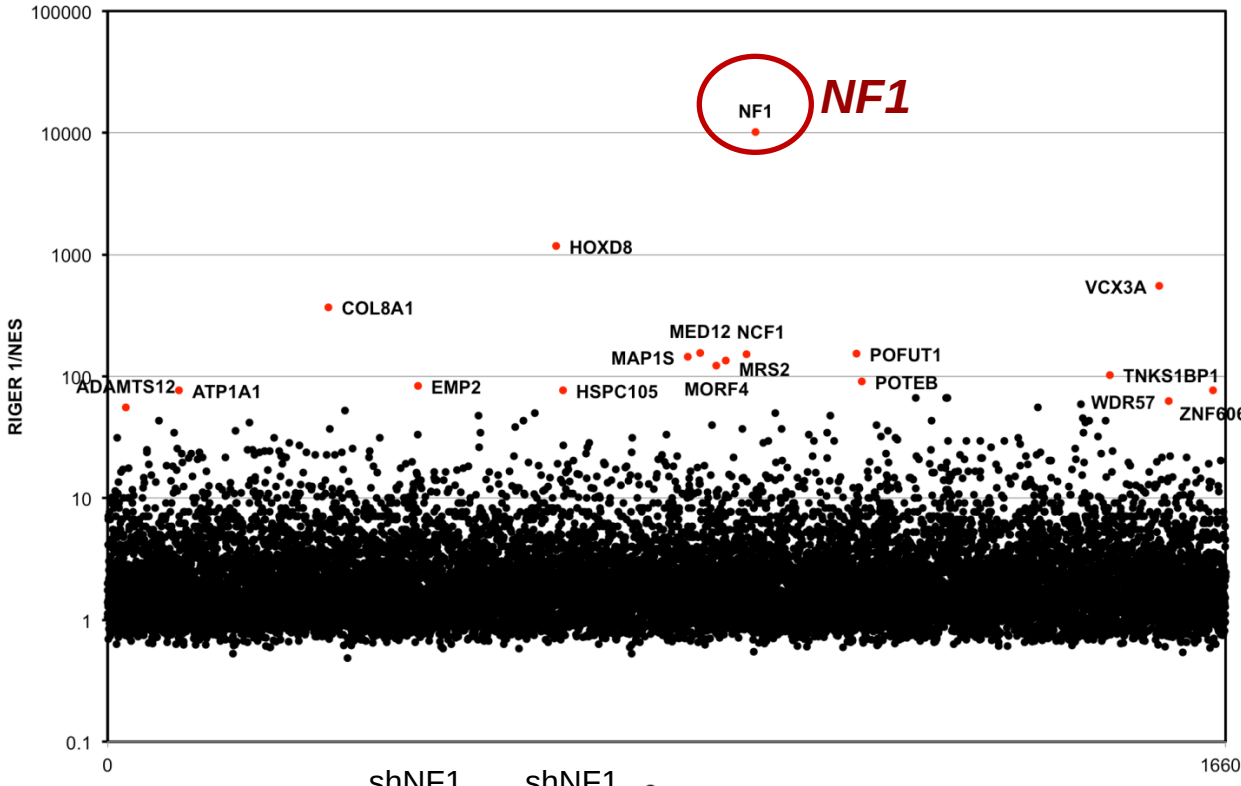
Eva Goetz, unpublished

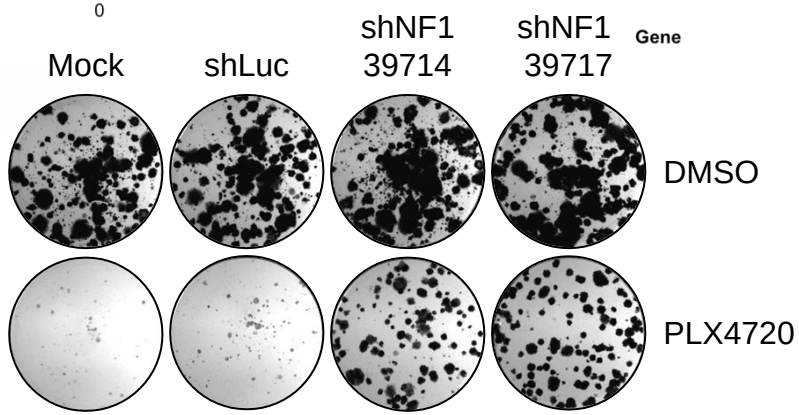
Emery et al., *PNAS* (2009), Wagle et al., *JCO* (2011)

Genome-scale loss-of-function screens for resistance to RAF inhibition



- 90,000 shRNAs
- Targeting 16,600 genes





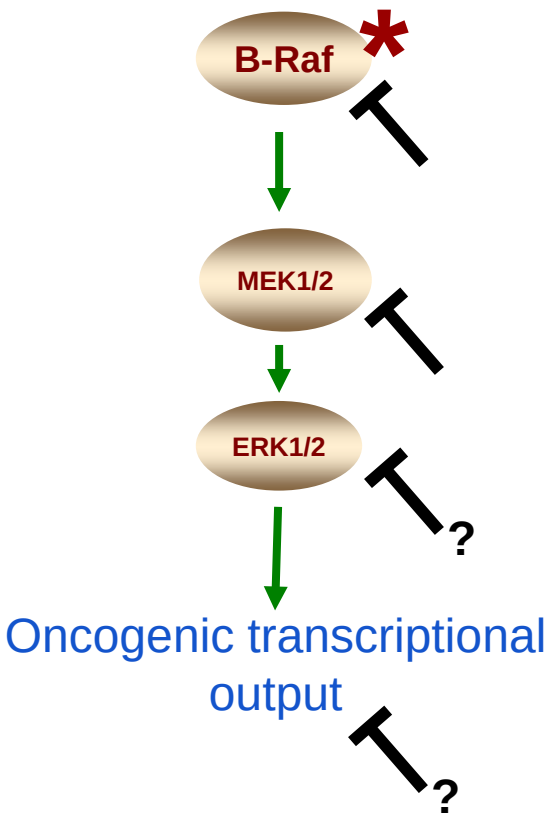
Steven Whittaker

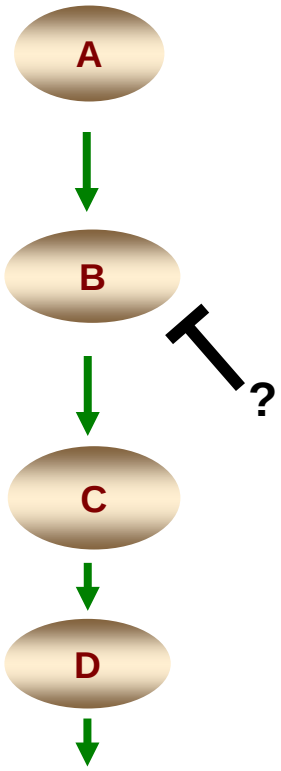
NF1 mutations in patients with intrinsic and acquired resistance to vemurafenib

Patient	PFS (months)	Resistance	cDNA	Protein	Candidate splice motif	Splice motif sequence	Site broken?
15	1.5	De novo	c.135C>T	p.N45N	Enhancer	ATCAAT	Yes
45	5	Acquired	c.4023G>A	p.Q1341Q	Splice site	AACCTCCTTCAGAT	Yes
46	2.5	De novo	c.7248C>T	p.R2450*	N/A	N/A	N/A
50	2	De novo	c.3018C>T	p.V1006V	Enhancer	ATGGTC	Yes

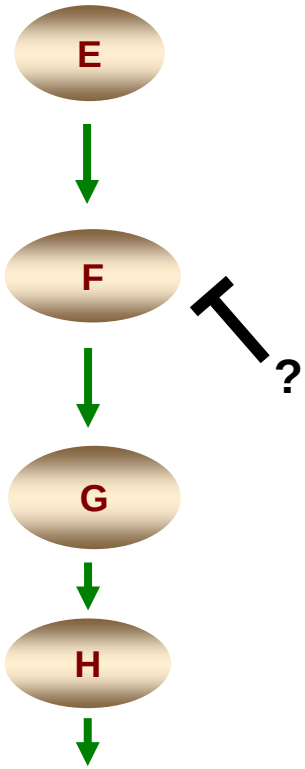
Steven Whittaker
Eli Van Allen
Nikhil Wagle

Approaches to Therapeutic Combinations in Melanoma



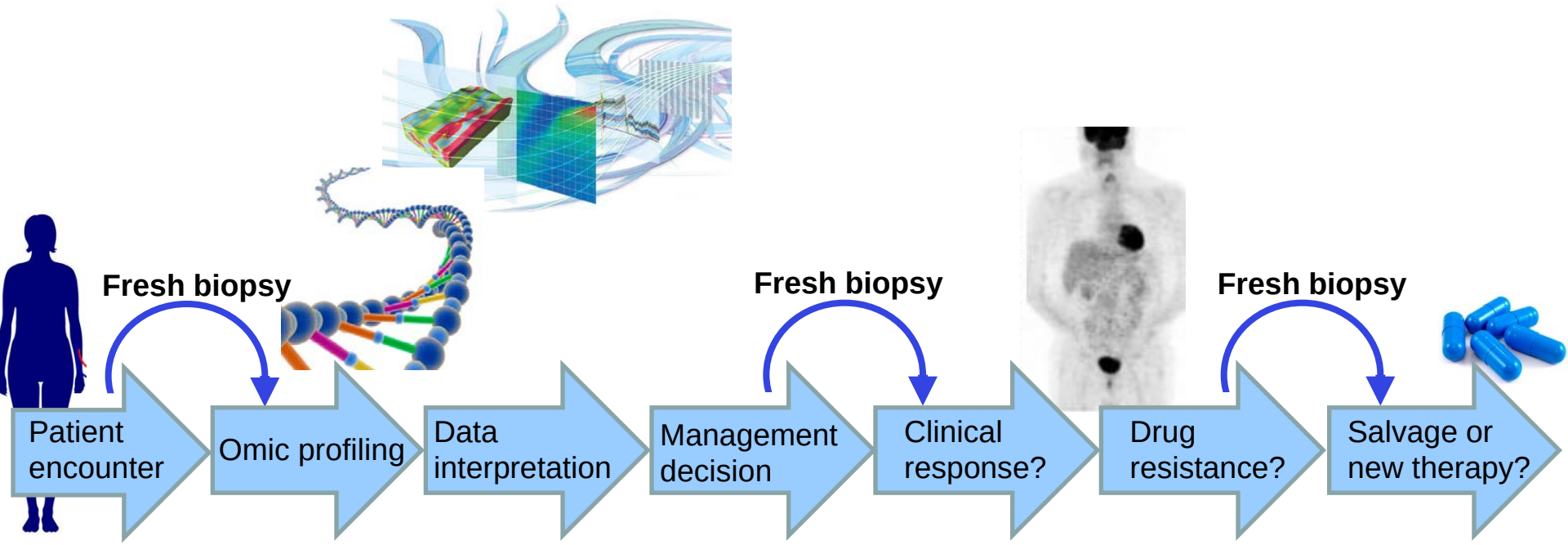


PI3 kinase pathway?
GPCR pathway?



GEF/GTPase/PAK signaling?
Immune checkpoint mechanisms

The Engine of Precision Cancer Medicine



The Cancer Genomics Vision: Looking Forward

- Completing the mutational atlas for primary tumors
- Expanding the atlas beyond primary tumors
  - Metastases
  - Following relapse to therapy
- Systematic functional annotation
- Systematic clinical implementation
- Worldwide data sharing

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Kohlberg Foundation
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Novartis, Inc.
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