CENTER FOR CANCER RESEARCH

Connecting the Cancer Community

Cancer Genomics & Precision Medicine in the 21st Century

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NATIONAL CANCER INSTITUTE

Outline

- **Define terms**
- **Describe vision for how genetic characterization of tumors will change treatment paradigms for cancer in the future**
- **Describe ongoing clinical trials currently using this approach**
- **Describe potential difficulties/pitfalls**

Terms/Vocabulary

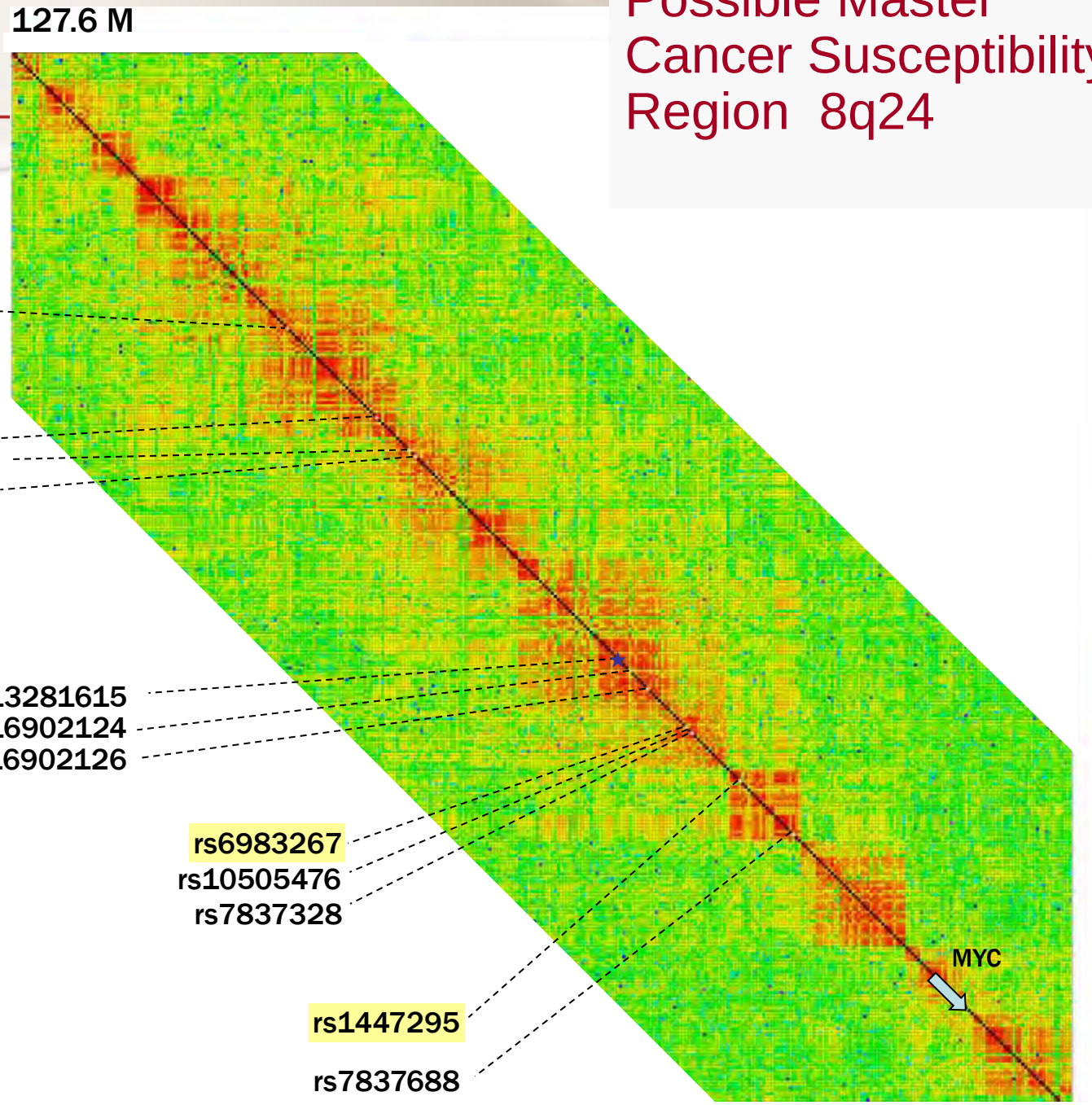
- **Single Nucleotide Polymorphisms (SNP)**
  - Variation in single base in DNA in germline, most common variants in genome (over 50 million identified)
  - SNP arrays interrogate the entire genome-uses DNA from germ-line (blood)
- **Used in Genome Wide Association Studies (GWAS)**
  - Typically uses SNP arrays to compare populations (with disease or not)
  - Determines risk or susceptibility to some state

Terms (con't)

- **RNA expression profiles-determines global messenger RNA expression in a sample-using hybridization of mRNA to a Chip**
- **Methylation arrarys-determines global methylation of the genome-an epigenetic change typically inserts a methyl group at CpG islands in DNA and alters transcription-using hybridization of DNA to a Chip**
- **Massively parallel sequencing-allows for rapid sequencing of entire exome (WES) on whole genome (WGS) or cDNA (RNA-seq)**

GWAS

Possible Master Cancer Susceptibility Region 8q24



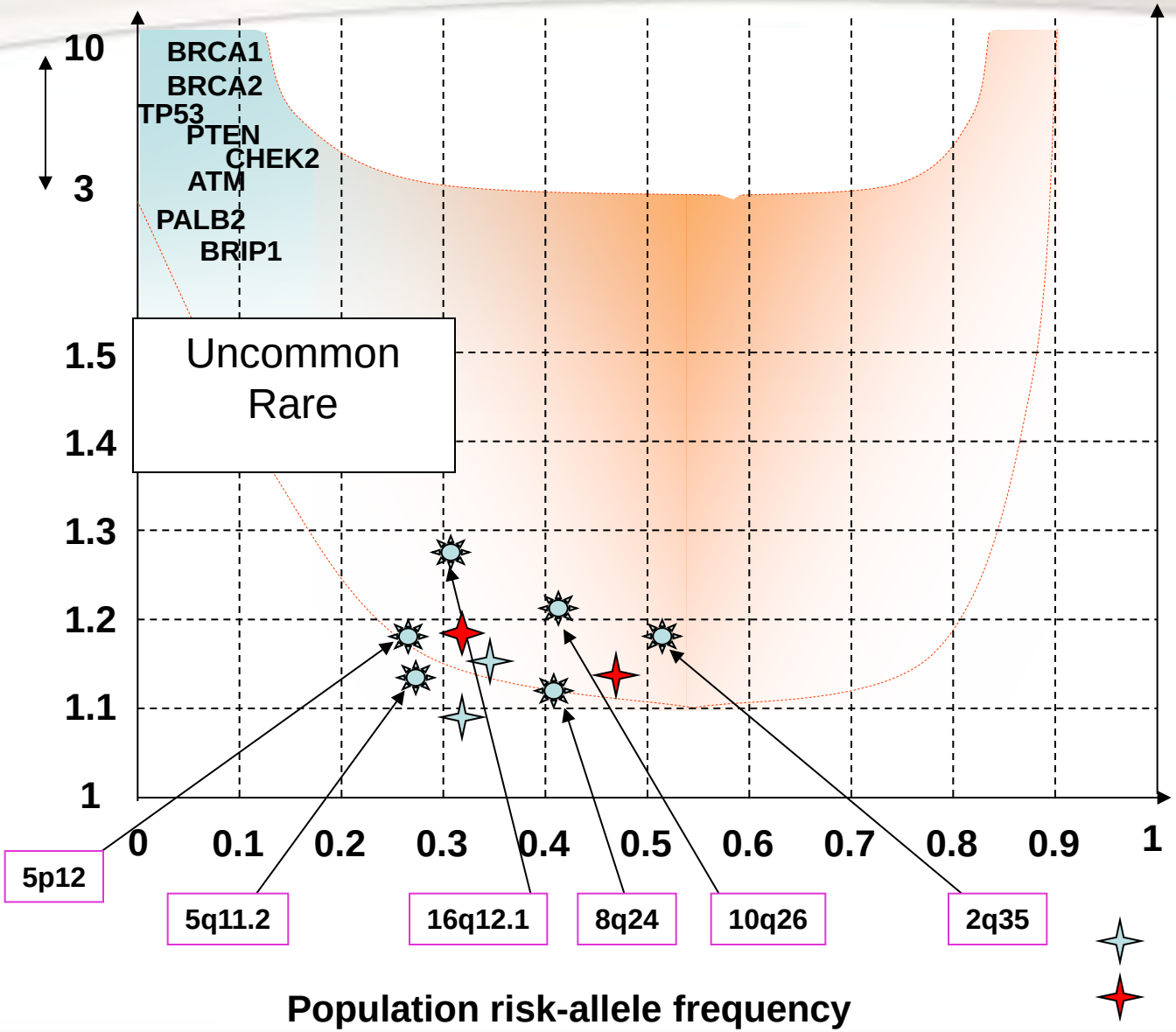
Region 2
Prostate only

Breast only

Region 3
Prostate & Colon

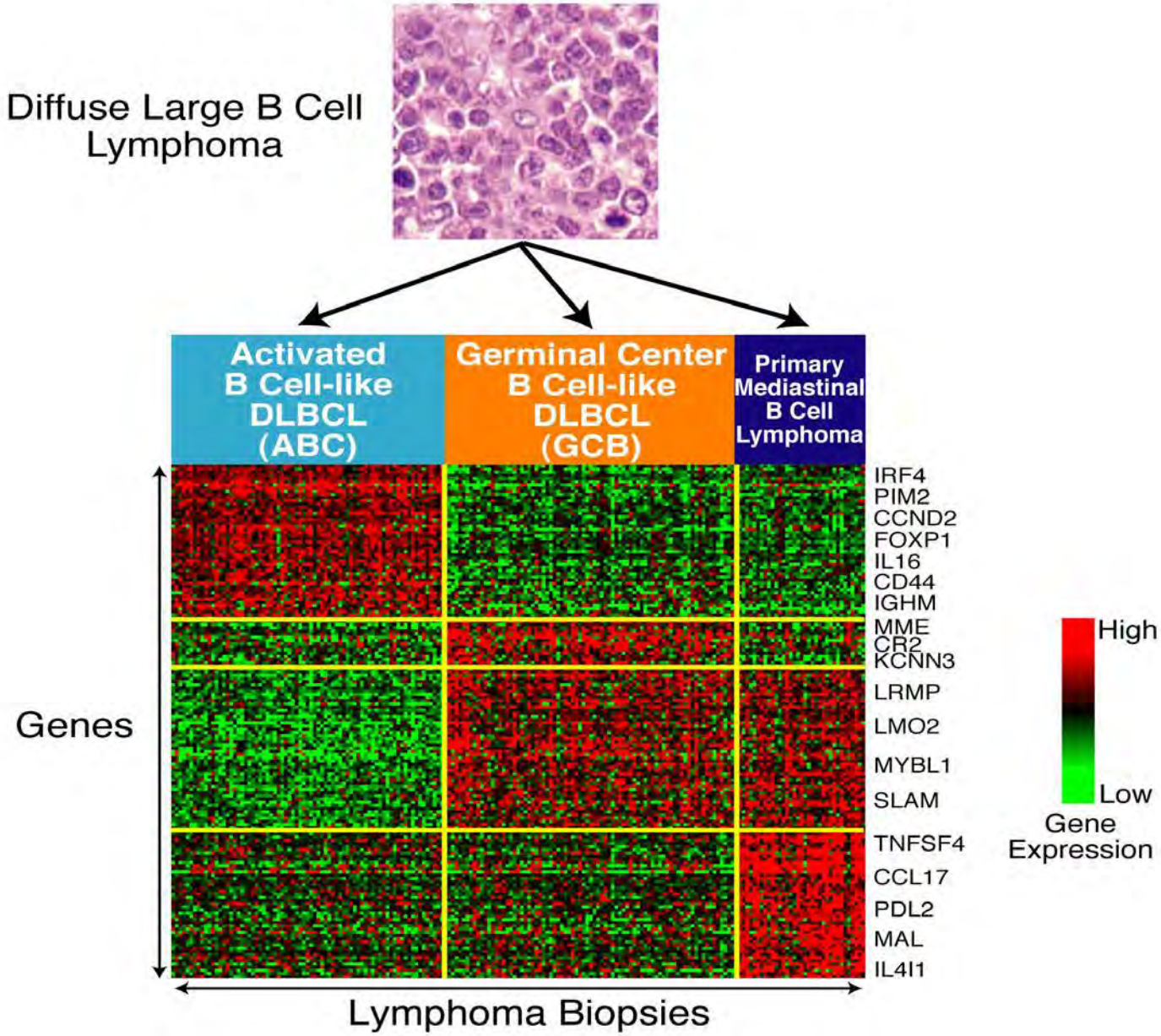
Region 1
Prostate only

Genetic Predisposition to Breast Cancer European Population



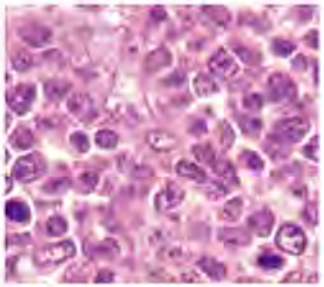
New Loci
CGEMS
CGEMS/BCAC

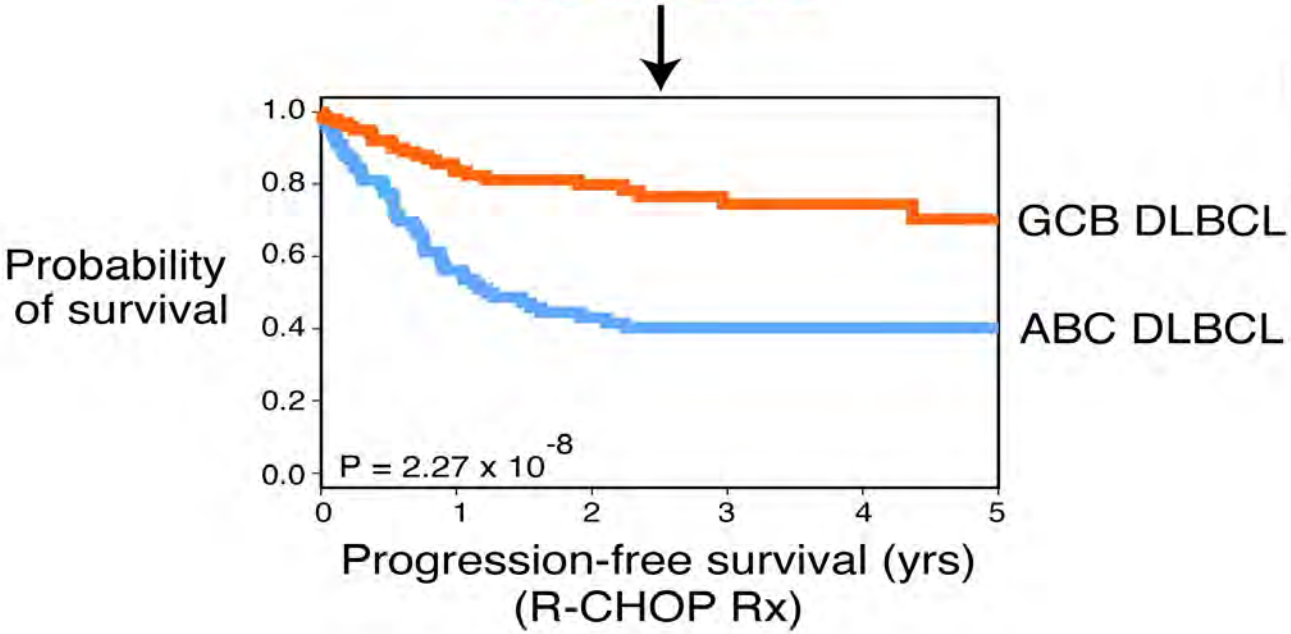
Dissecting Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling



Dissecting Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

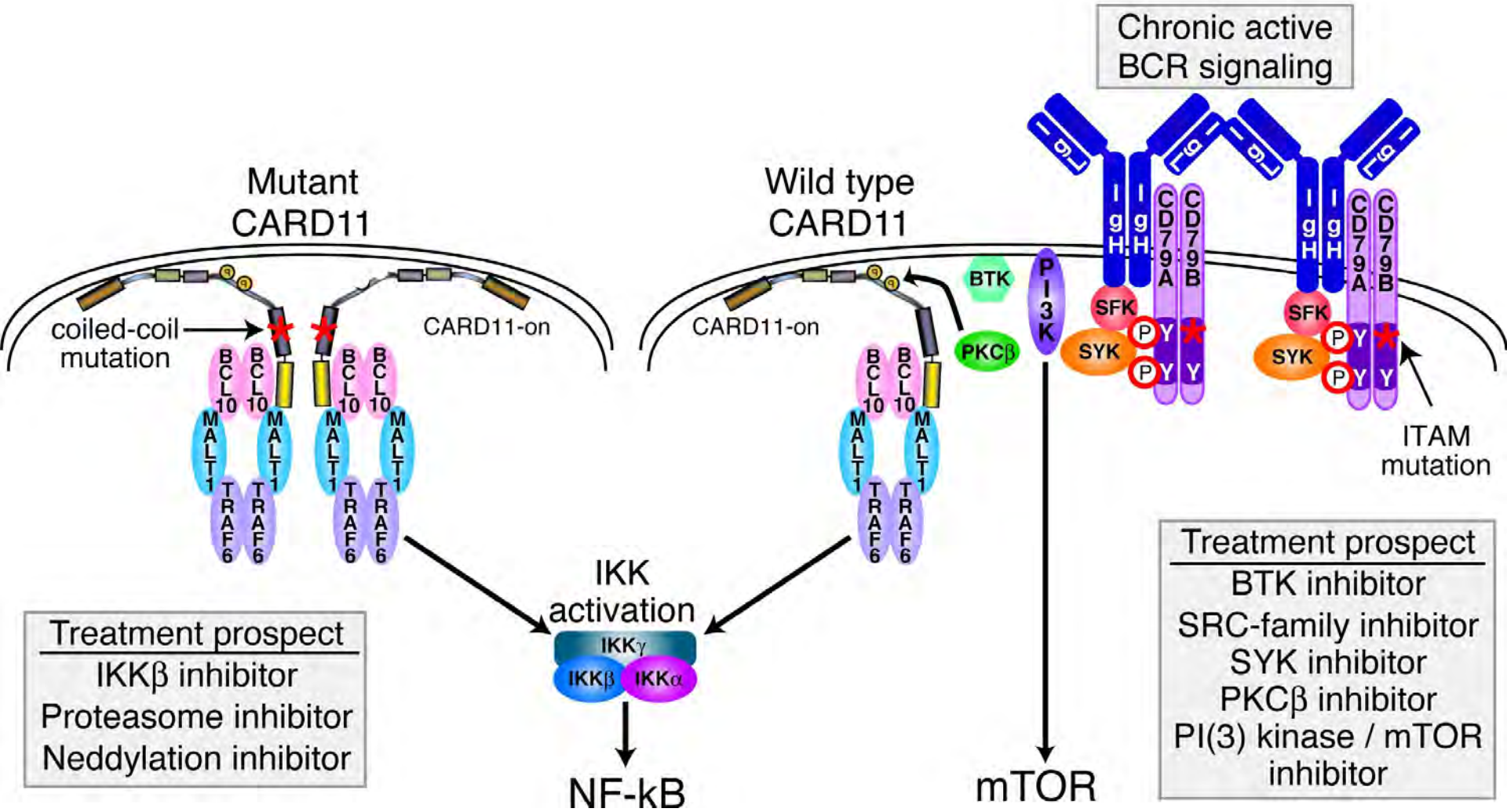
Diffuse Large B Cell Lymphoma



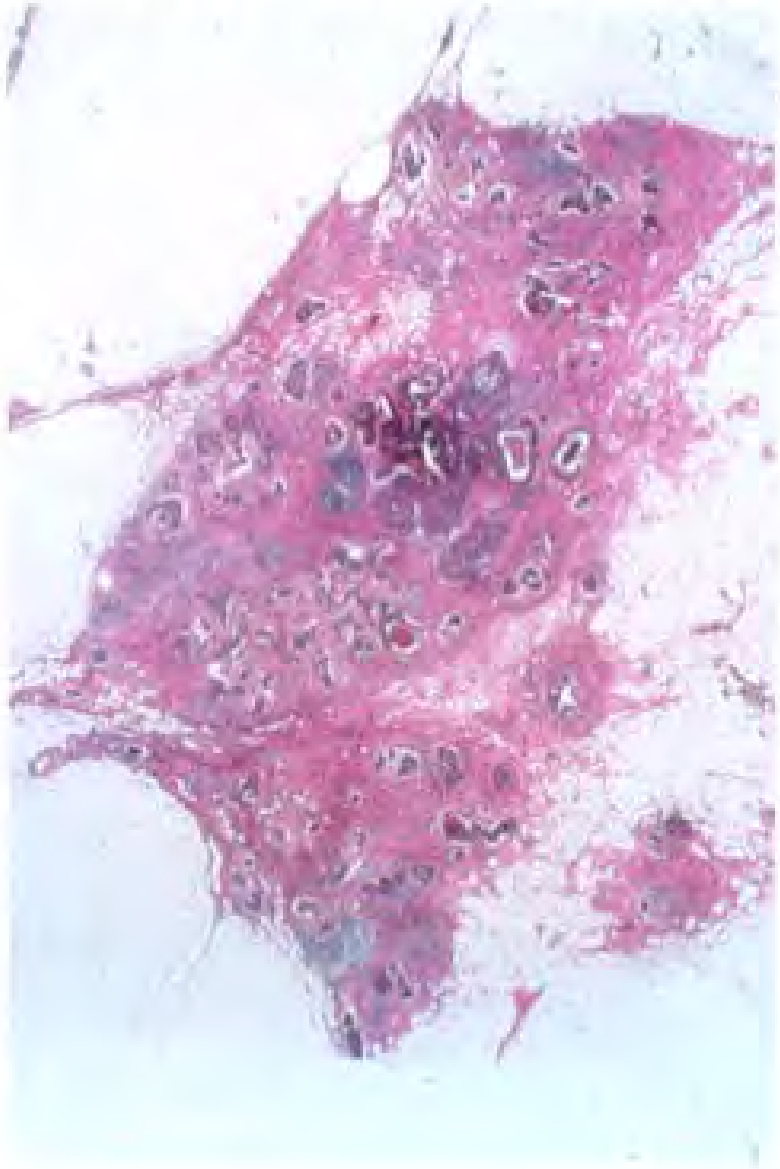


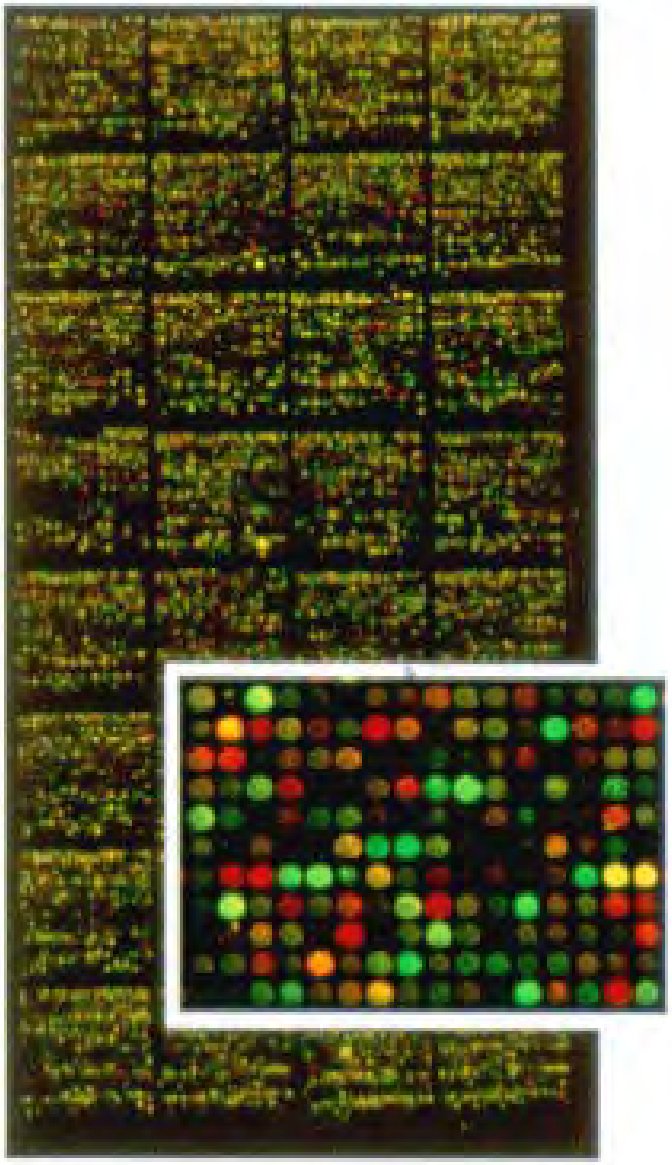
	3-year progression-free survival
GCB DLBCL	75%
ABC DLBCL	40%

Different Therapeutic Strategies for Subsets of ABC DLBCL Based on Characteristics of Mutations Activating NF-kB

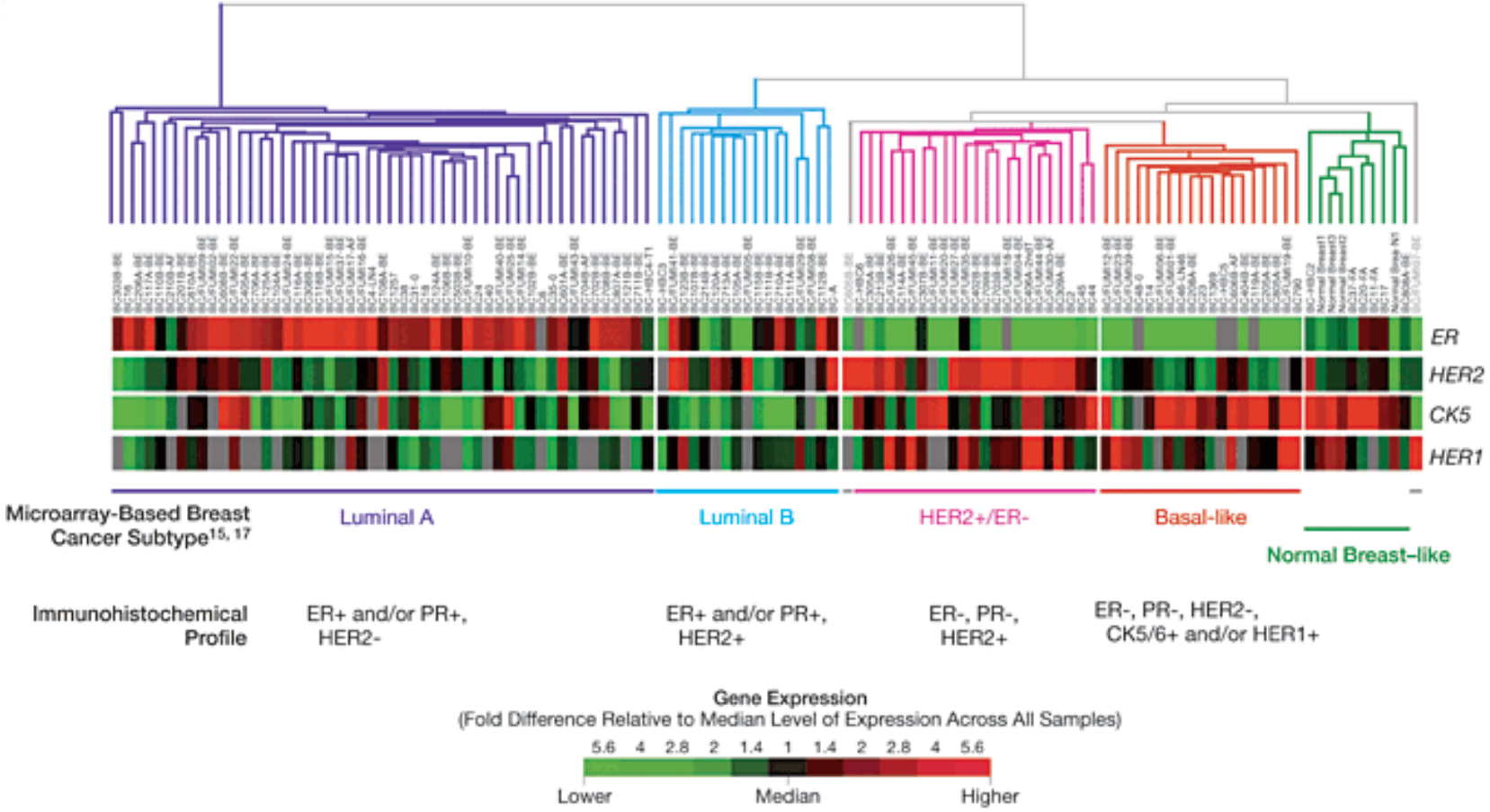


Two images of a breast cancer





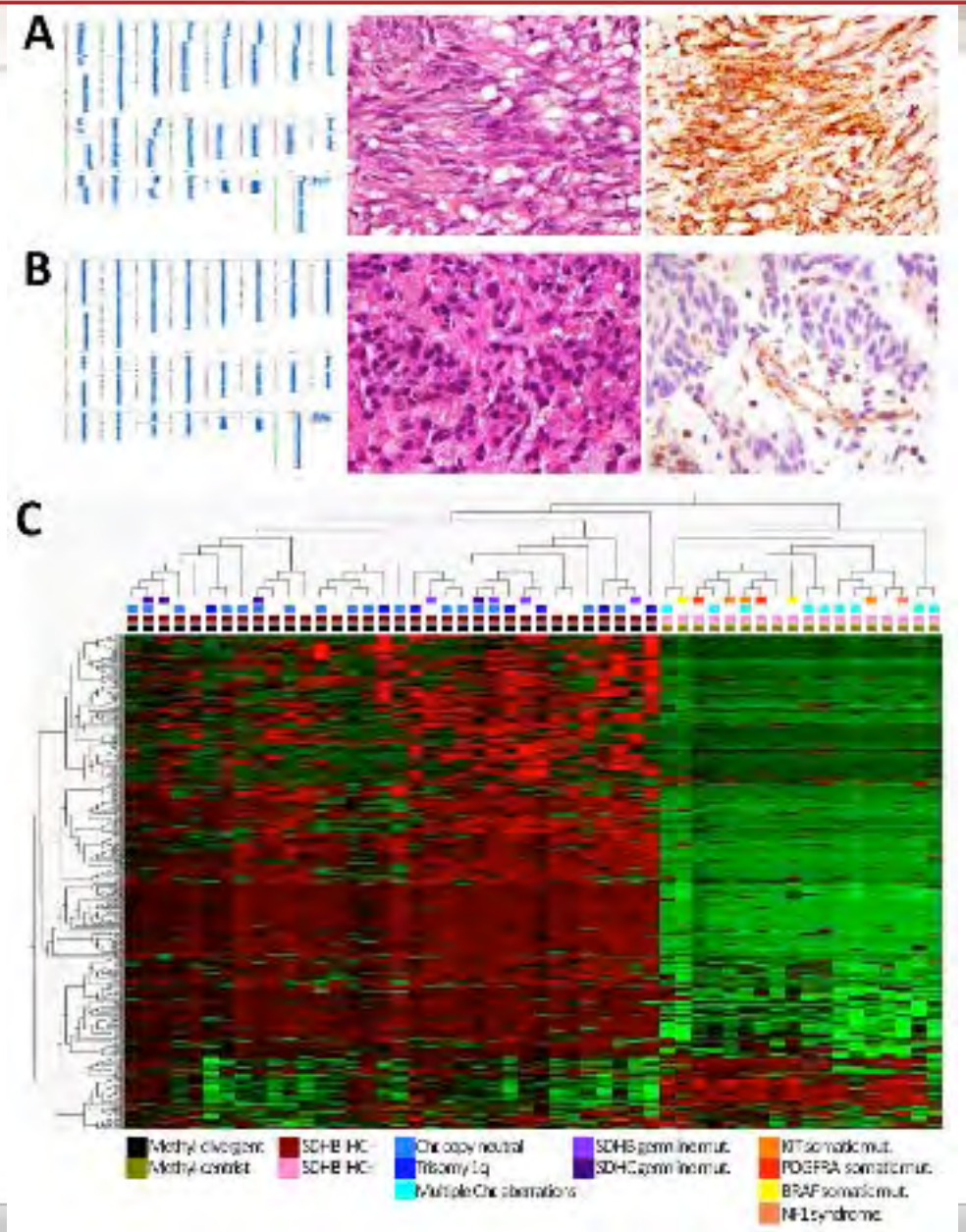
Expression profile Identification of Breast Tumor Intrinsic Subtypes



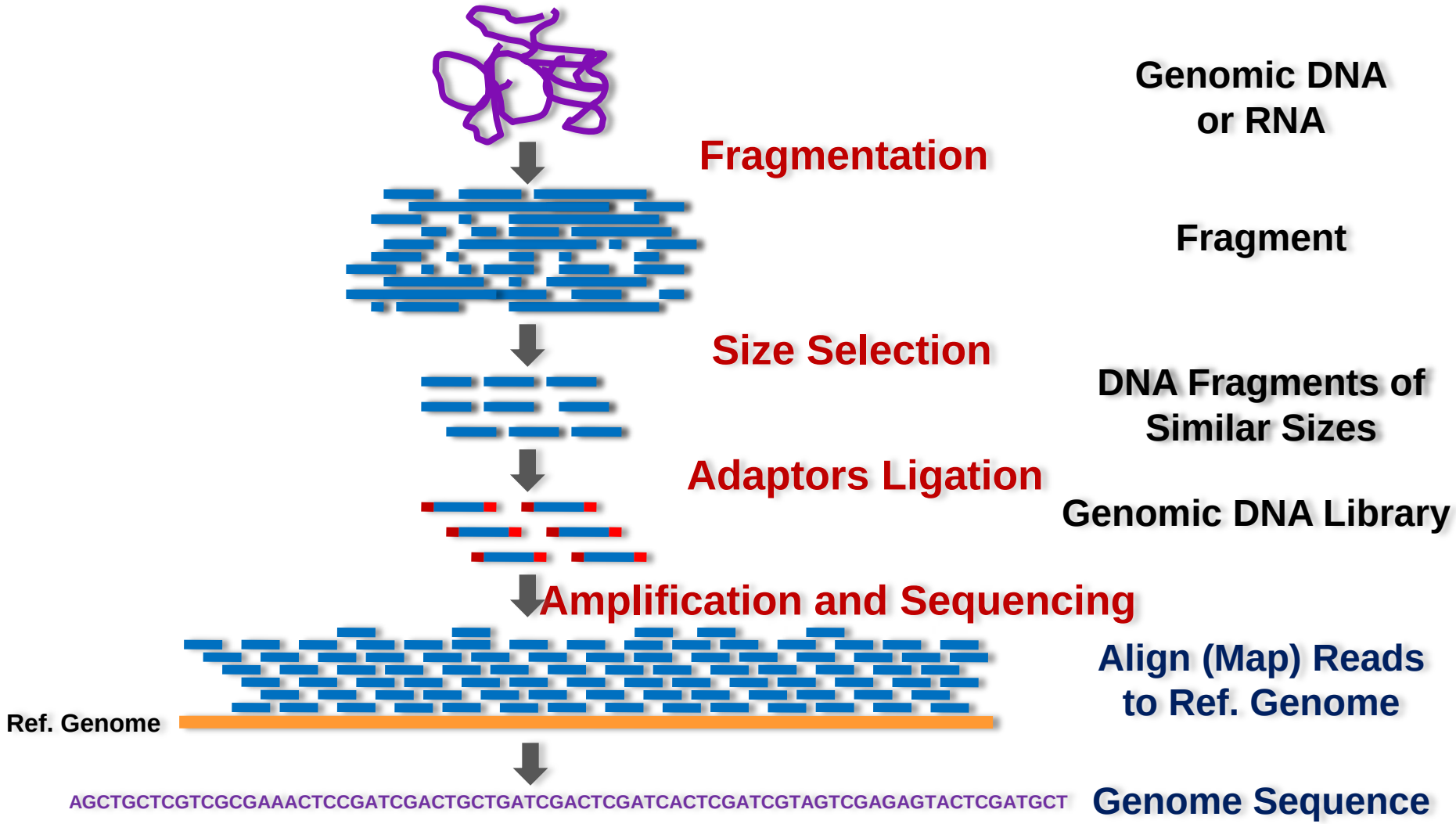
Carey, L. A. et al. JAMA 2006;295:2492-2502.

Copyright restrictions may apply.

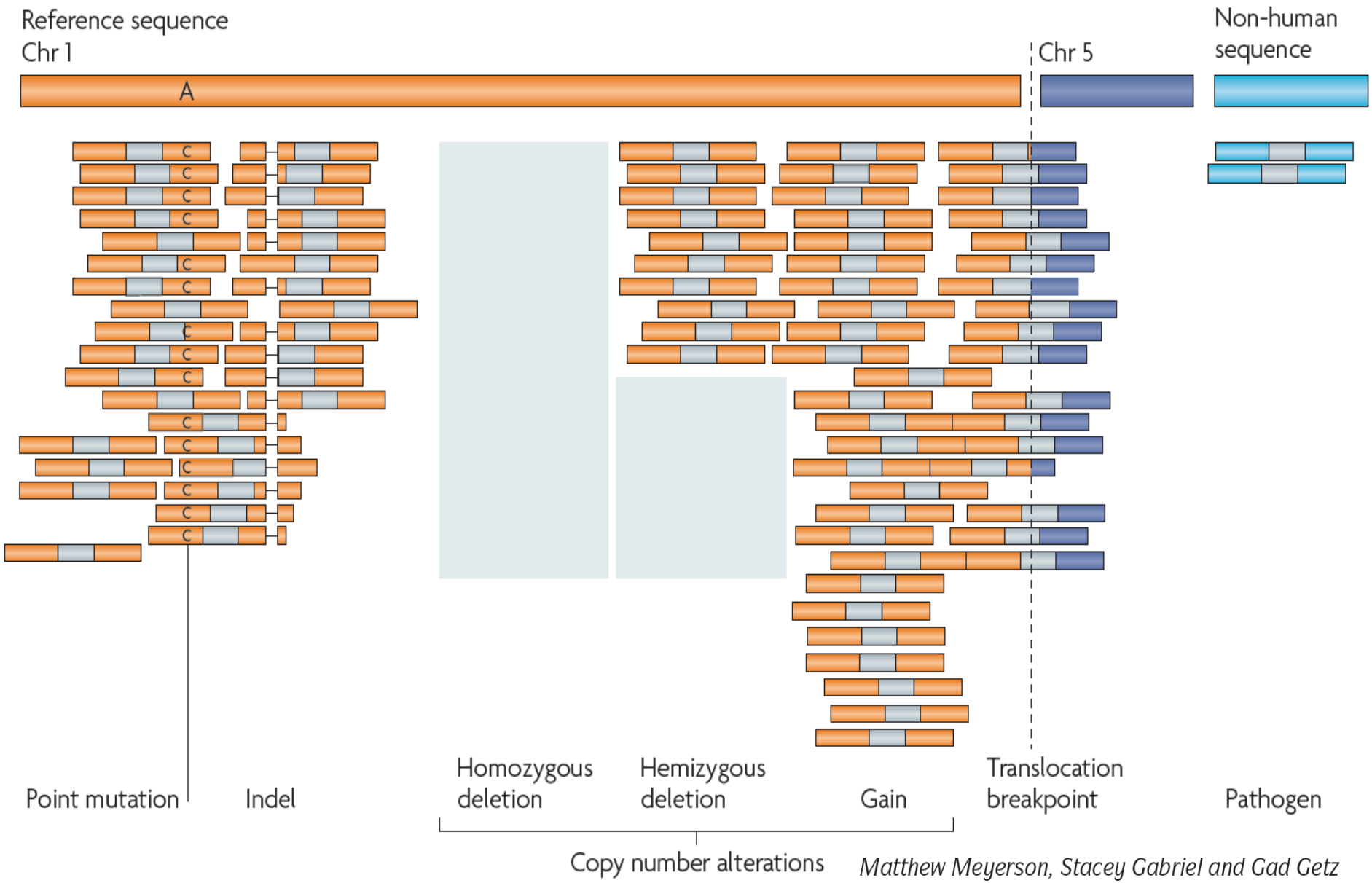
SDH Deficient GIST Have Global Hypermethalyation



Massively Parallel Sequencing
(Next-Generation Sequencing)



Types of Alterations that can be Detected using

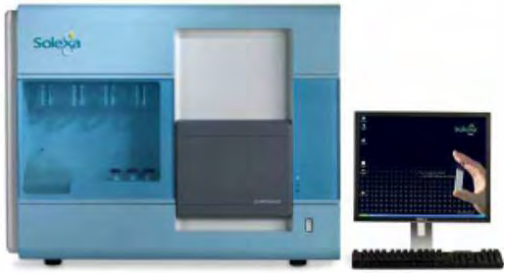


Matthew Meyerson, Stacey Gabriel and Gad Getz

NATURE REVIEWS | GENETICS VOLUME 11 | OCTOBER 2010

Next Generation Sequencing will Identify Other Driver Mutations and Enable Individualized Therapy for Cancer Therapy

Roche / 454
Genome Sequencer FLX
Titanium



Illumina / GAII/HQ
2500
Whole Genome 48 hrs

PacBio RS
Ion Torrent

Life Technologies
SOLiD v4

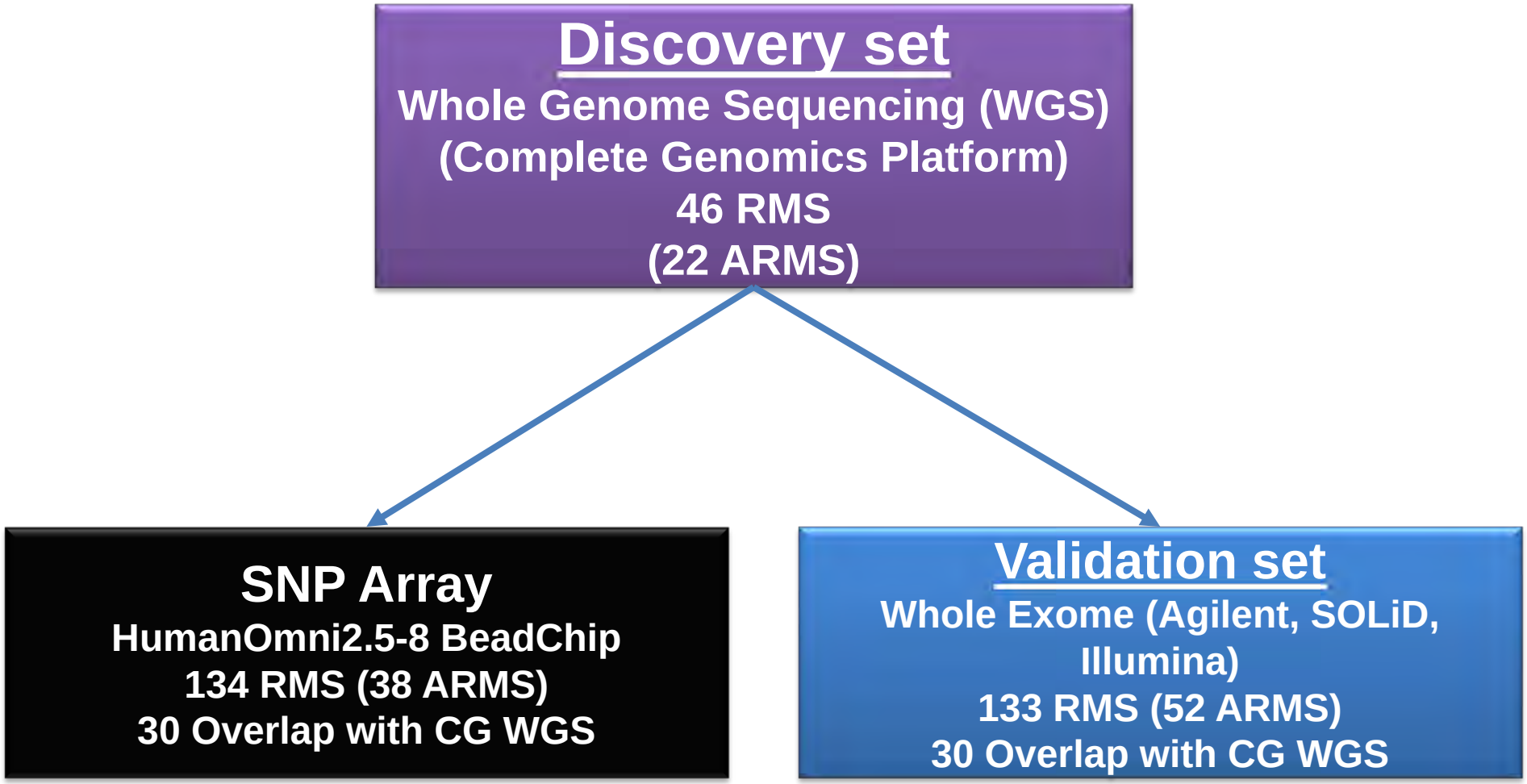
Life
Technologies
5500 XL

Life
Technologies
Ion Torrent

Life
Technologies
Proton
1 Genome 2 hrs

Helicos
HeliScope

Comprehensive Analysis of the Rhabdomyosarcoma genome: Study Design

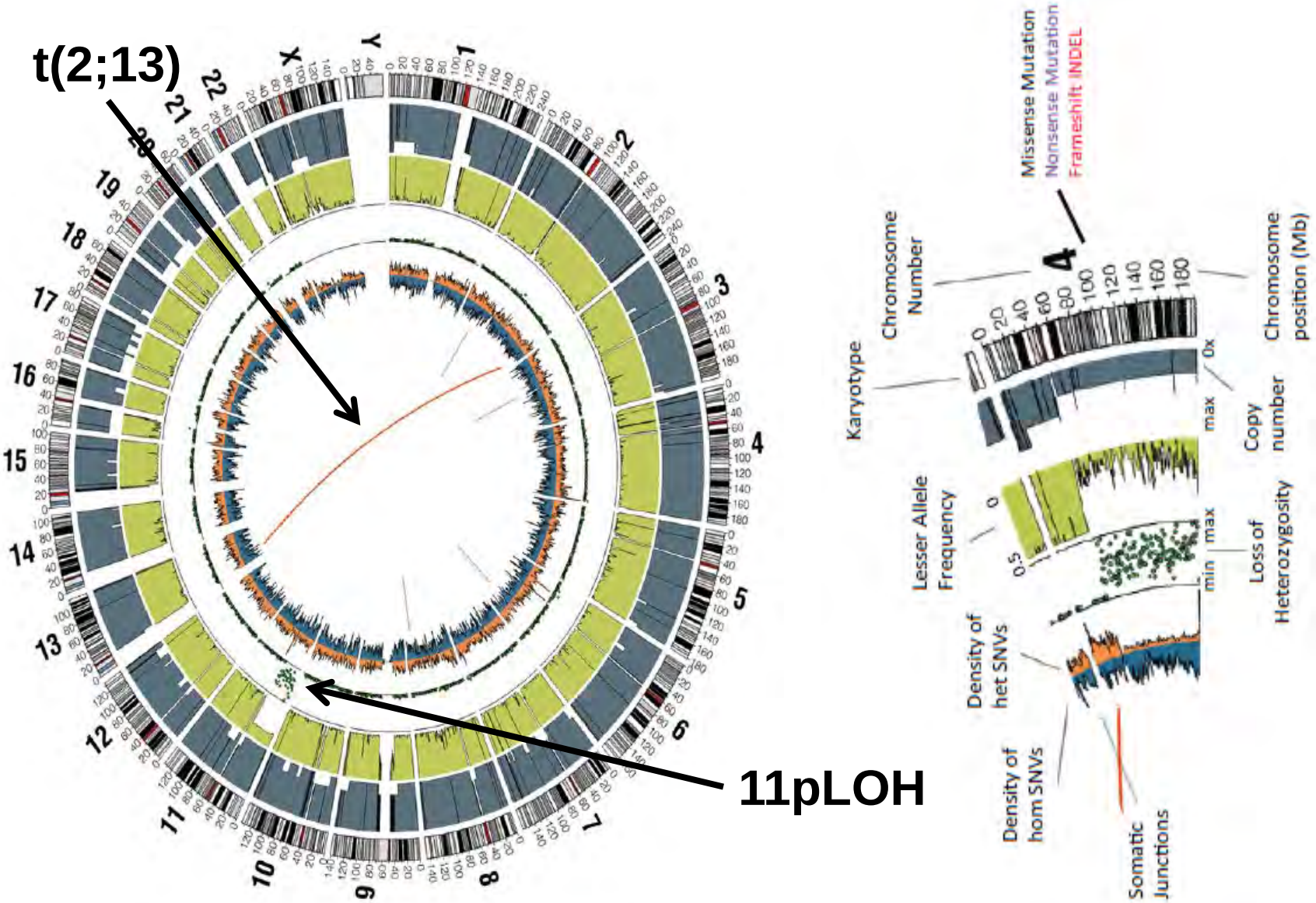


2/46 Samples had Aberrant Fusions
Incorrect Diagnosis

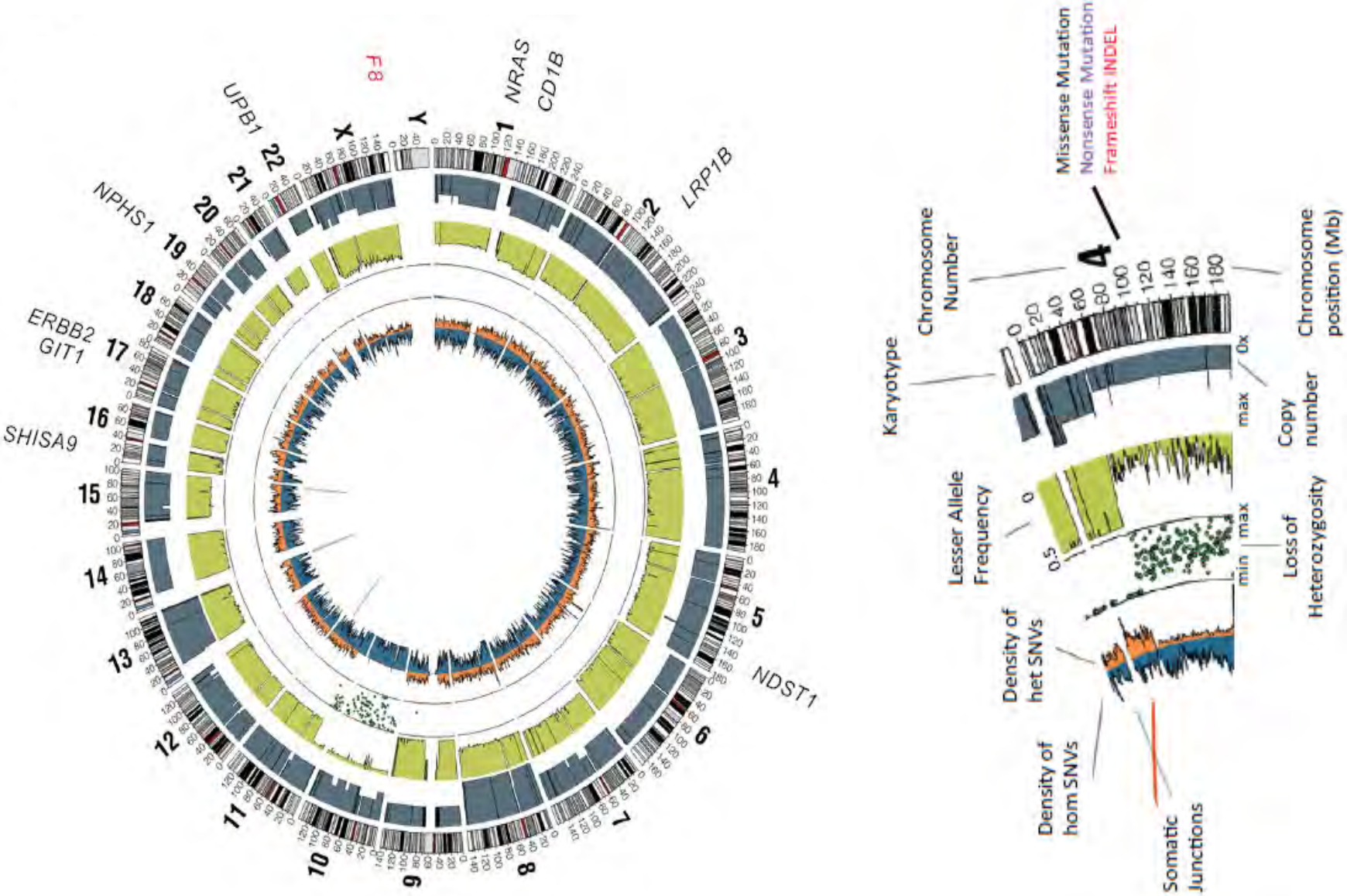
1. **Sample 1** –Original Histology: Sarcoma, Un differentiated RMS, Not Otherwise Specified. Had ALK-NPM1 fusion by whole genome sequencing; Review of Histology = Hematological malignancy- most likely misdiagnosed ALCL

2. **Sample 2** - Original Histology: Consistent with RMS Presence of RET-NCO4 fusion by whole genome sequencing which has been reported in papillary thyroid carcinoma. Likely misdiagnosed or sample label error from source

Typical Fusion Positive ARMS; Fusion Gene, Low Aneuploidy, Low Somatic Mutation rate

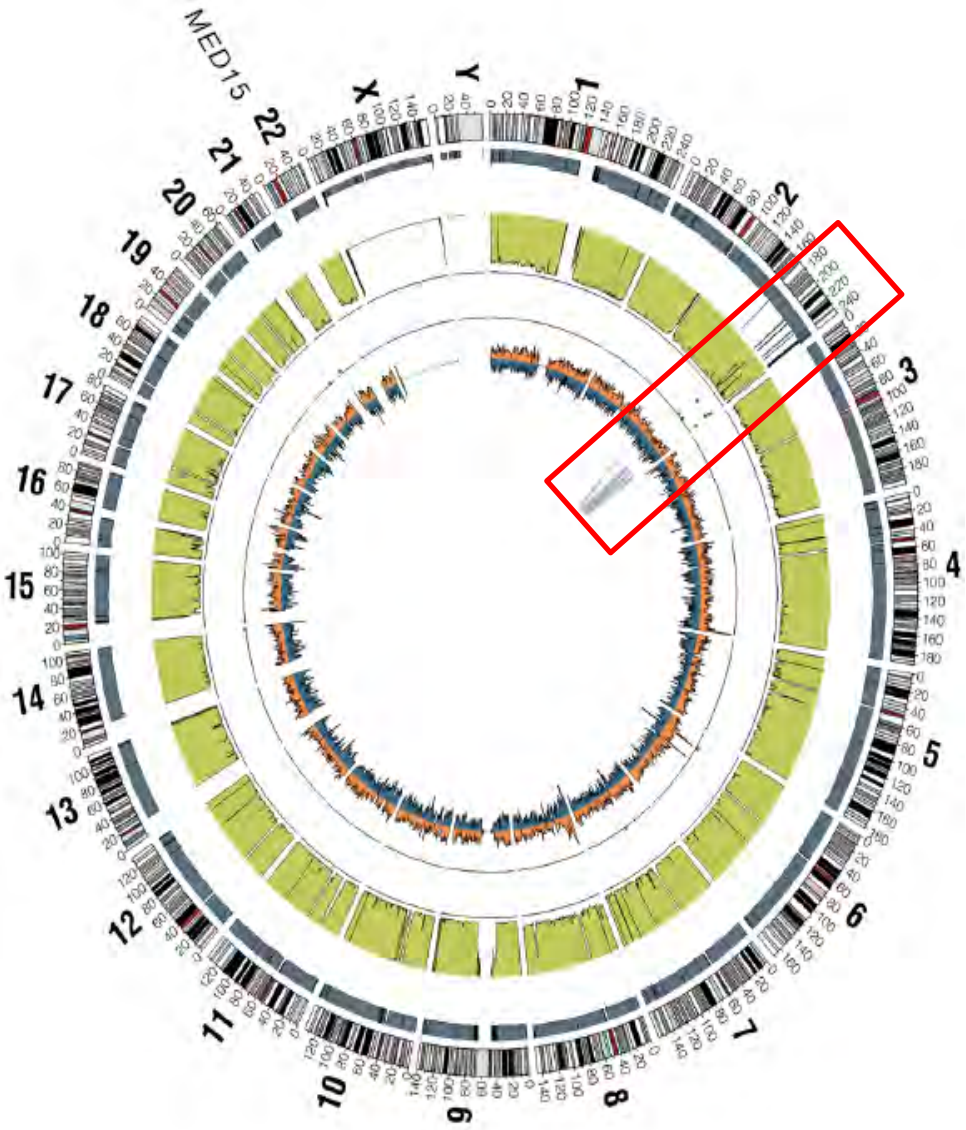


Typical ERMS; Higher Aneuploidy, Higher Somatic Mutation rate



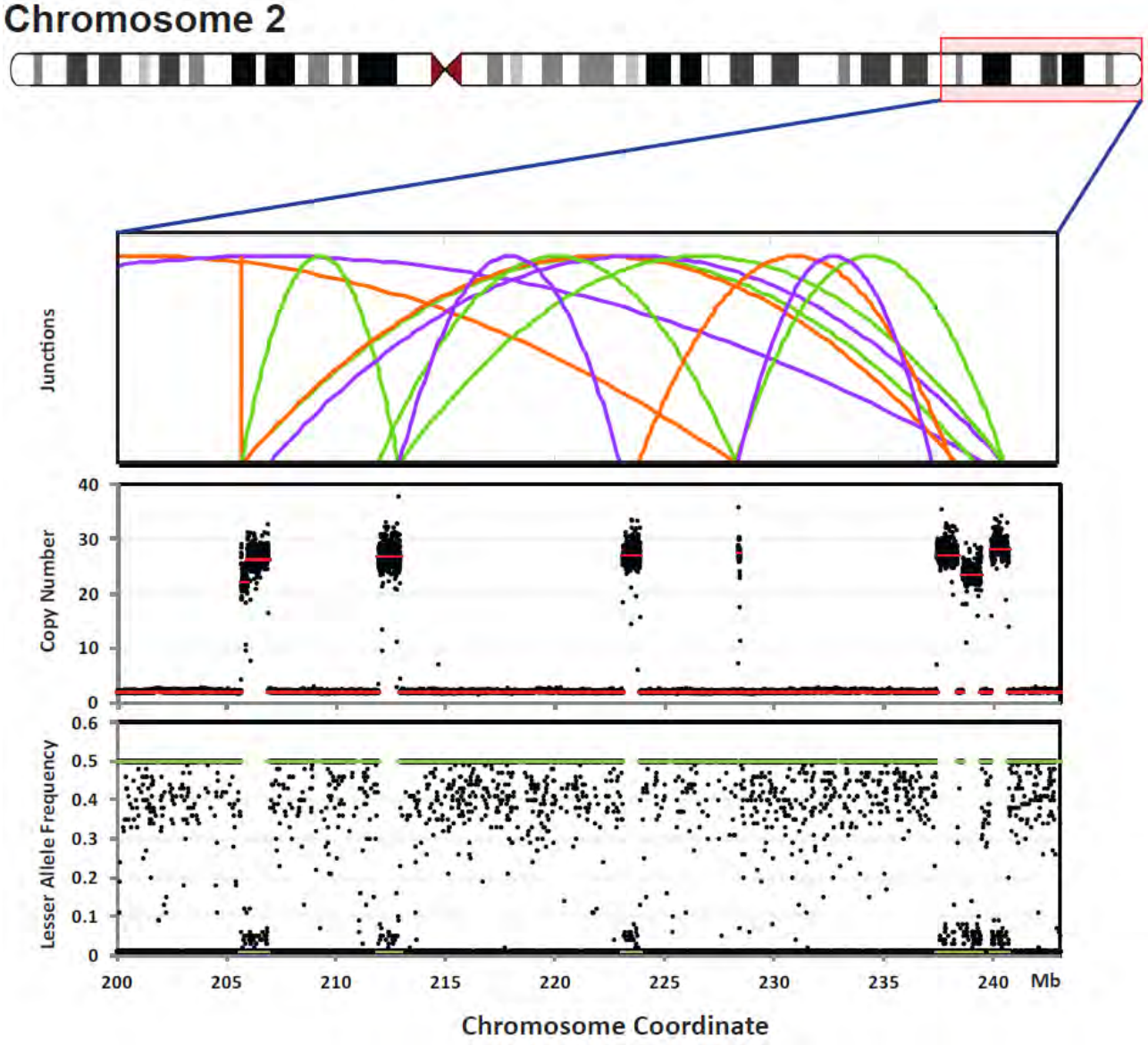
ARMS-Fusion Gene Detection:

- 22-ARMS by Histology
- 14-PAX3-FOXO1
- 3-PAX7-FOXO1
- 1-PAX3-NCOA1
- 1-Novel
- 3-Fusion Negative

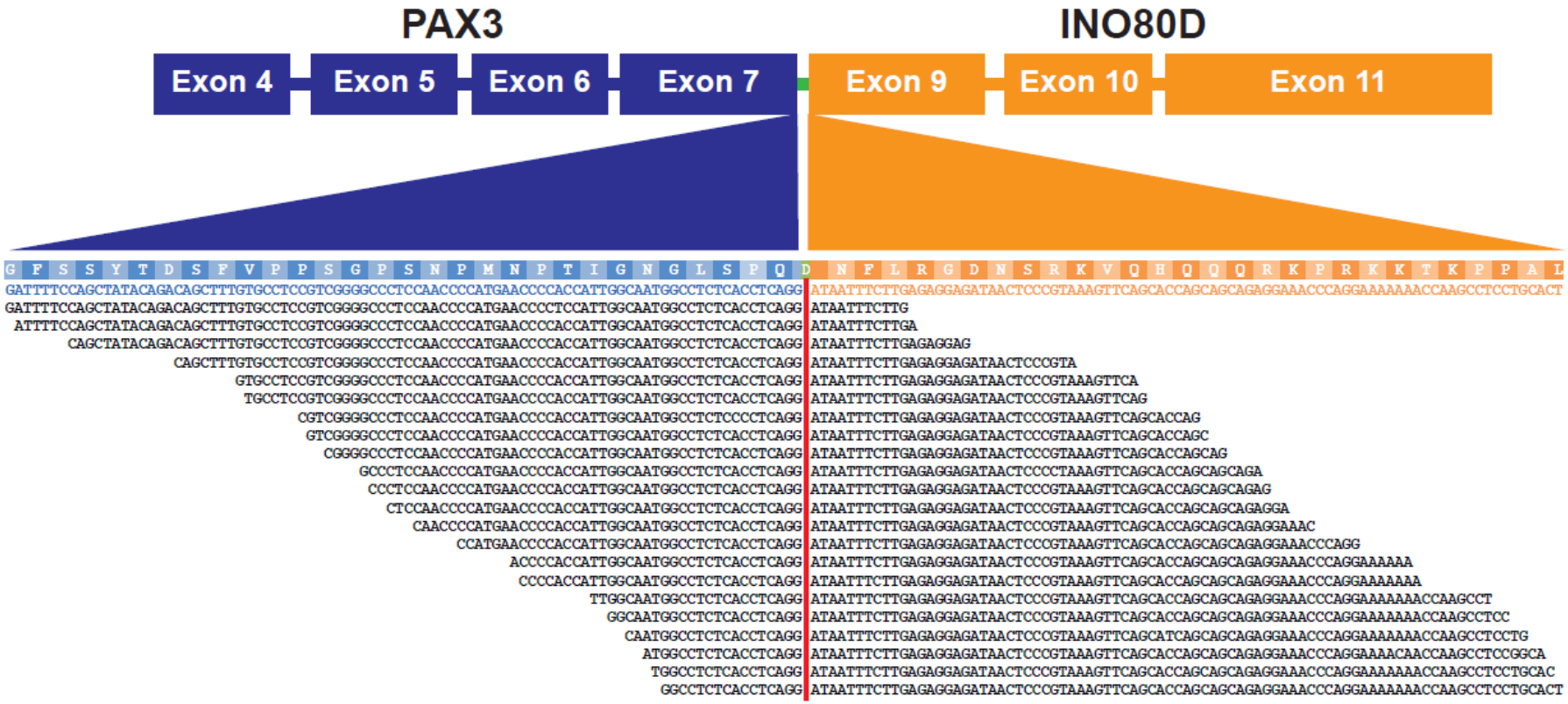


Fusion Negative ARMS Shows massive 2q Rearrangement with in-Frame *PAX3-INO80D*

Purple:tail-to-head Green: head-to-tail junction (possibly tandem duplication) Orange: tail-to-tail junction or head-to-head junction (inversion)

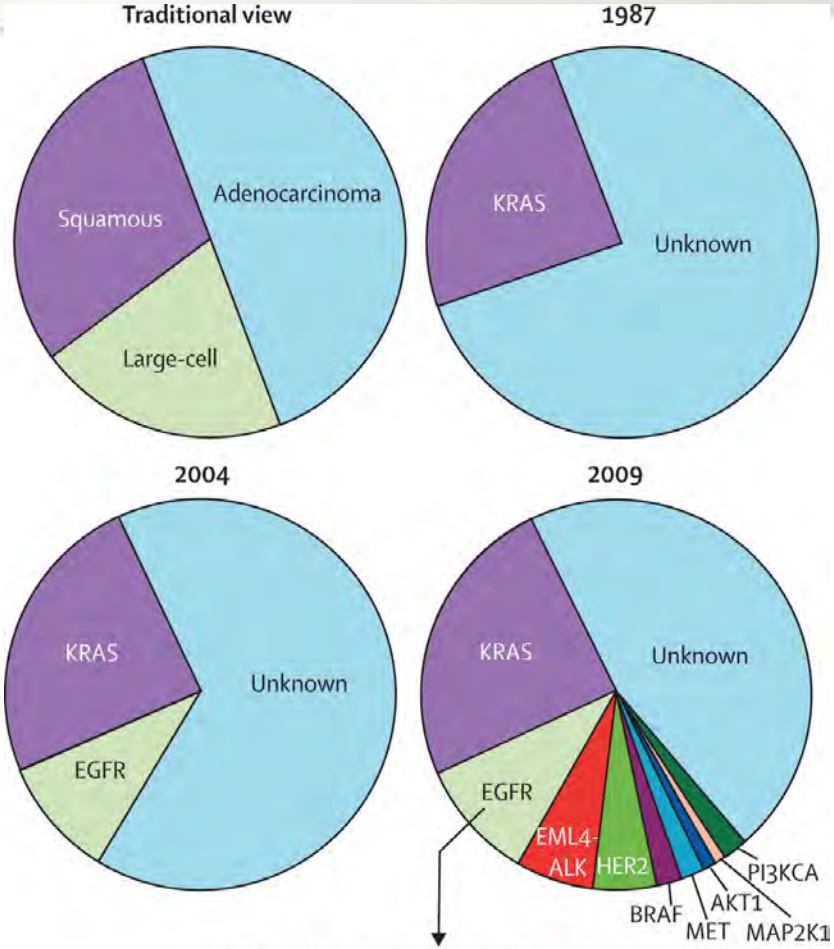


RNAseq Confirms Expression of *PAX3-INO80D* Novel Fusion Transcript



HOW HAS THIS CHANGED CANCER TREATMENT?

Change in View of Lung Cancer



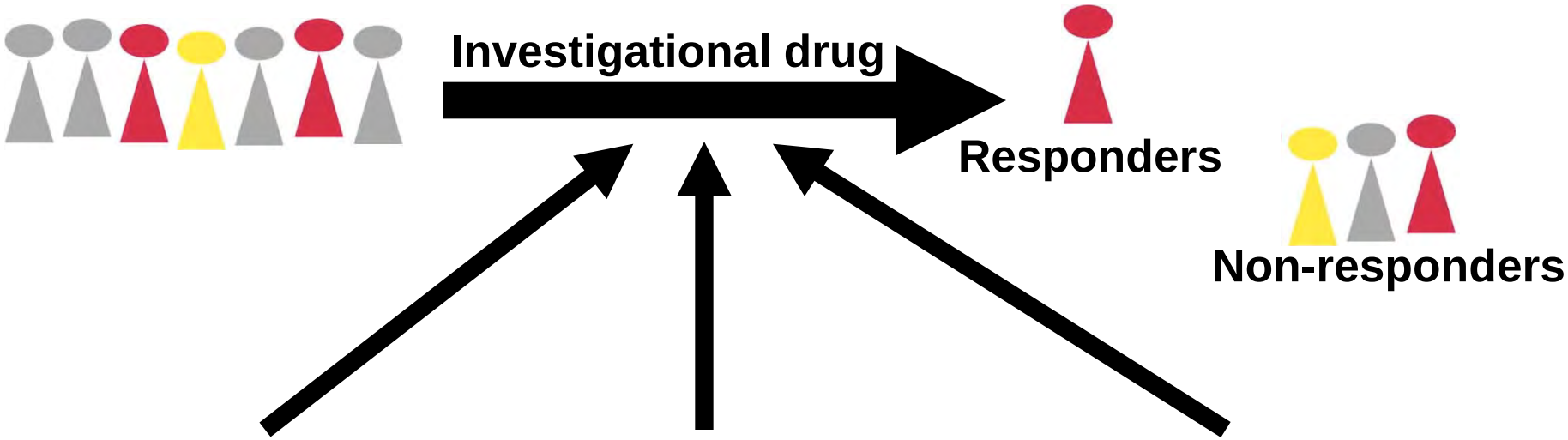
- **Mutations associated with drug sensitivity**
  EGFR Gly719X, exon 19 deletion, Leu858Arg, Leu861Gln
- **Mutations associated with primary drug resistance**
  EGFR exon 20 insertions
- **Mutations associated with acquired drug resistance**
  EGFR Thr790Met, Asp761Tyr, Leu747Ser, Thr854Ala

Shifting the Paradigm

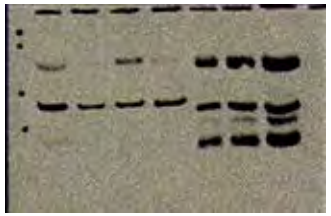
Previous Approach	New Practices
Descriptive medicine	Understanding of disease mechanisms
Empirical diagnosis	Mechanism-based diagnosis/treatment
Grouped by Organ Site	Sub-grouped by molecular/biological classification
Uniform treatment	Individualized treatment
Retrospectively diagnose disease	Prospectively evaluate relative disease risk
Acute care	Early detection and intervention

Toward Precision Medicine

Put more science into clinical trials

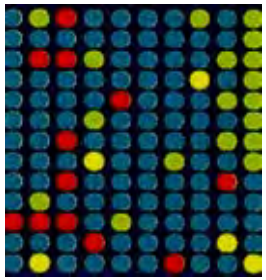


Pharmaco dynamic measurements



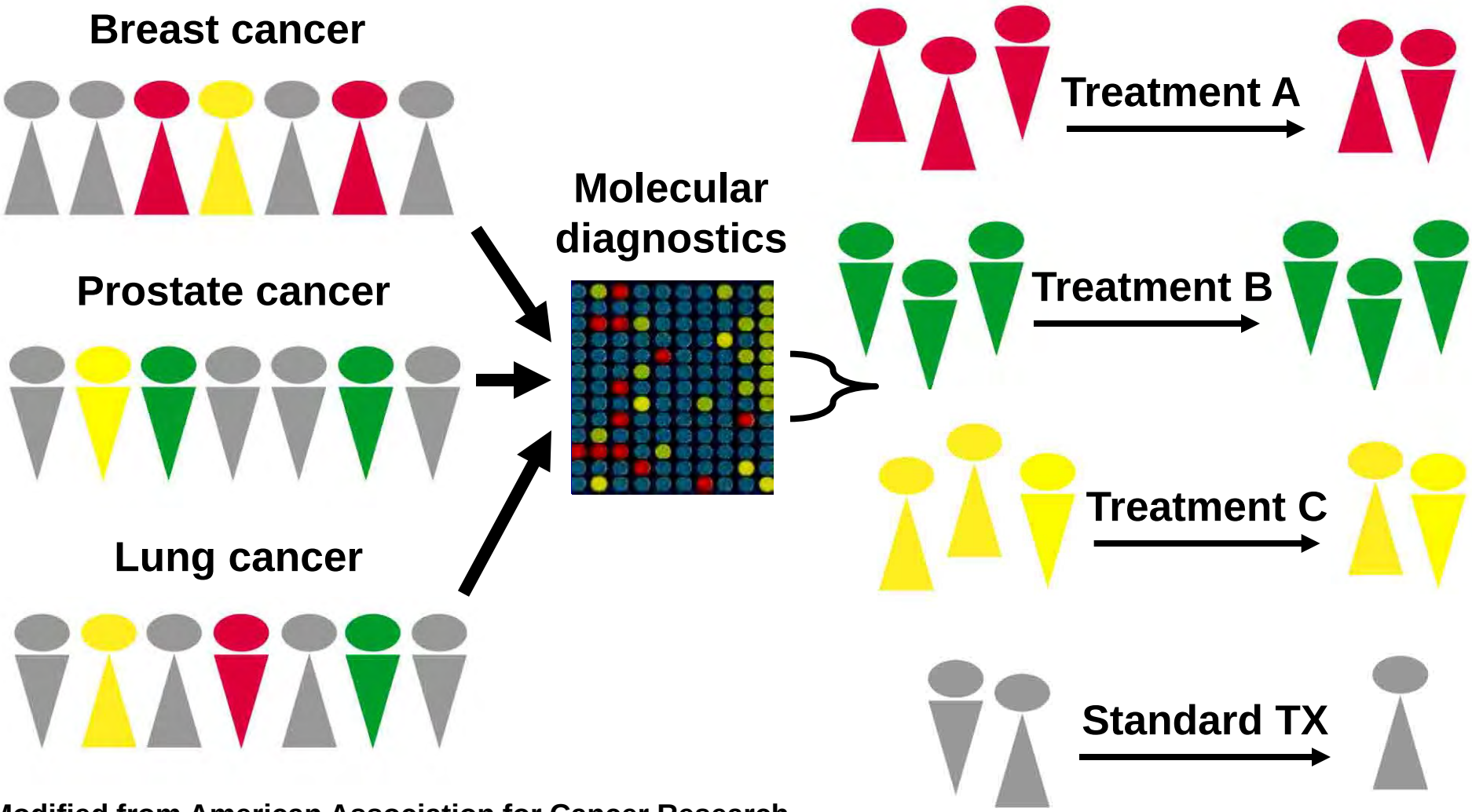
Molecular diagnostics: candidate approach

Molecular diagnostics: unbiased approach



Modified from American Association for Cancer Research

Precision Medicine



Modified from American Association for Cancer Research

Cancer is a disease of the genome

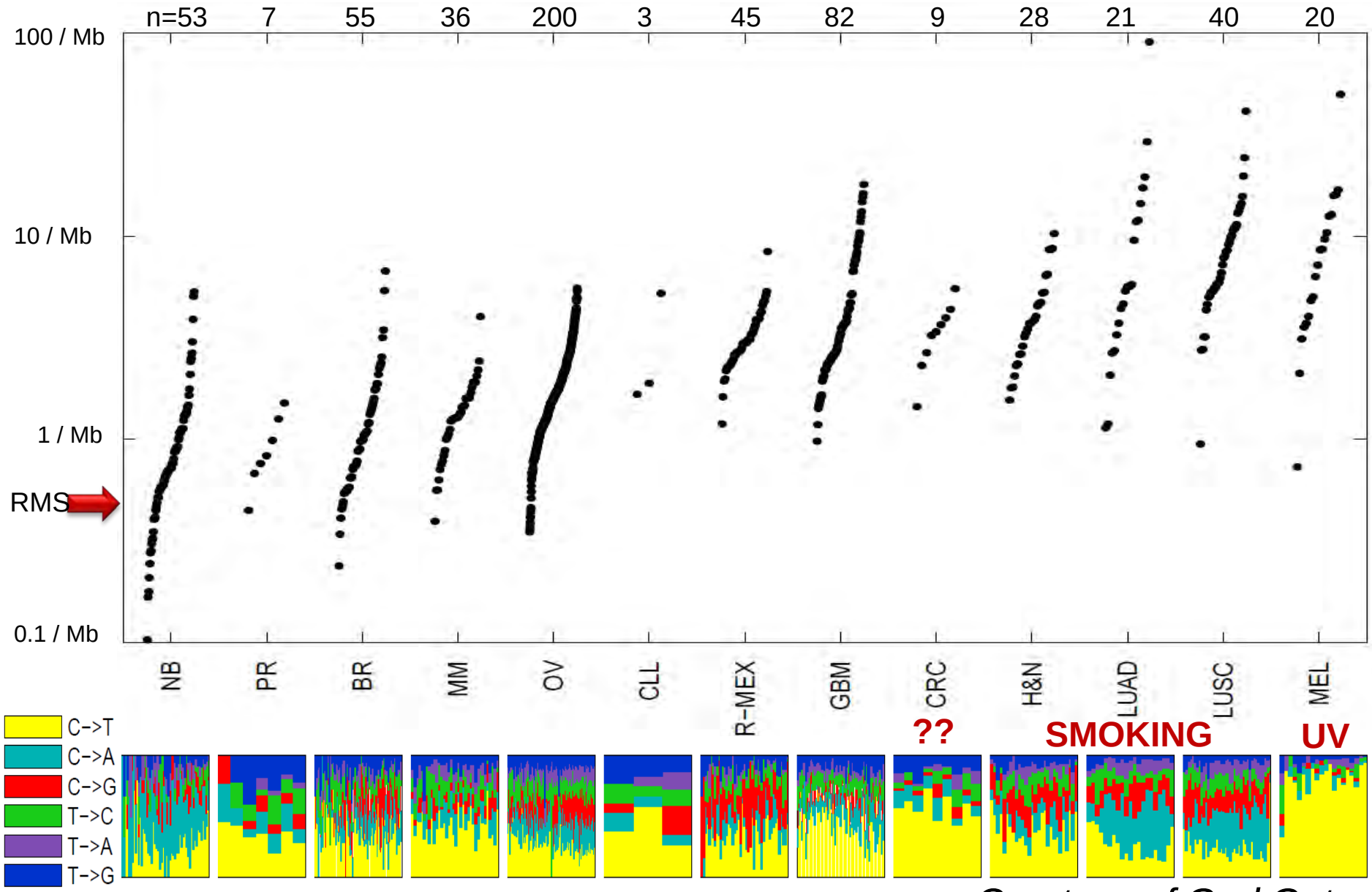
- **Therefore, if we precisely define the cancer genome, we will understand and cure cancer**
  - Why we must be cautious about such statements
- **Definitions**
- **Founder mutations-first genomic mutation**
  - These are often lesions that lead to genomic/chromosomal instability (p53, RB, etc.) and are often not fully transforming
- **Driver mutations-these are mutations that are required for expression of fully transformed phenotype**

Cancer is a disease of the genome (cont)

- Driver mutations are the mutations we would like to target and inhibit their function

- **Passenger mutations-these mutations are "collateral damage" resulting from genomic instability and are not required for maintaining the transformed phenotype, therefore are "noise" in the system**
- **Since most cancers are rapidly evolving biologic entities, it is a major task to sort out "drivers" from "passengers", and these may change over time**

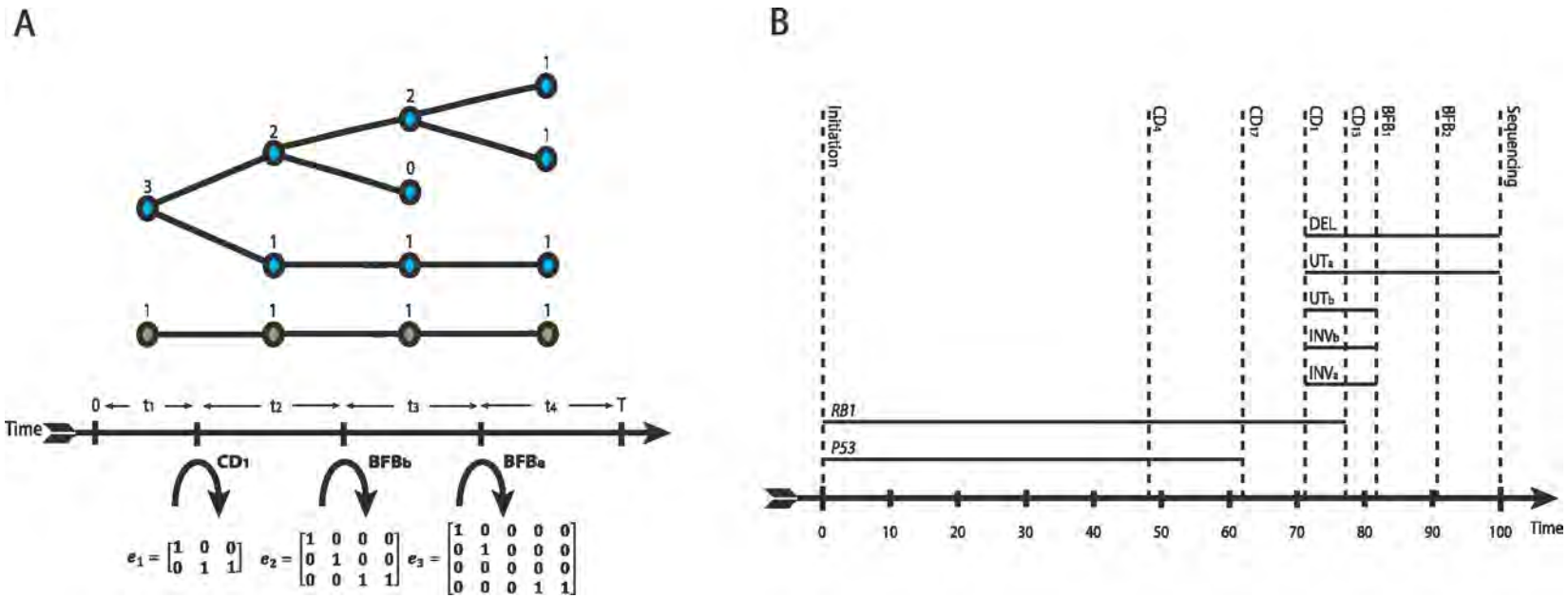
Mutation Rates across Cancers are not Uniform



Courtesy of Gad Getz

Clonal Evolution

Founder RB1 and p53 mutations followed by additional mutations



Greenman C D et al. Genome Res. 2012;22:346-361

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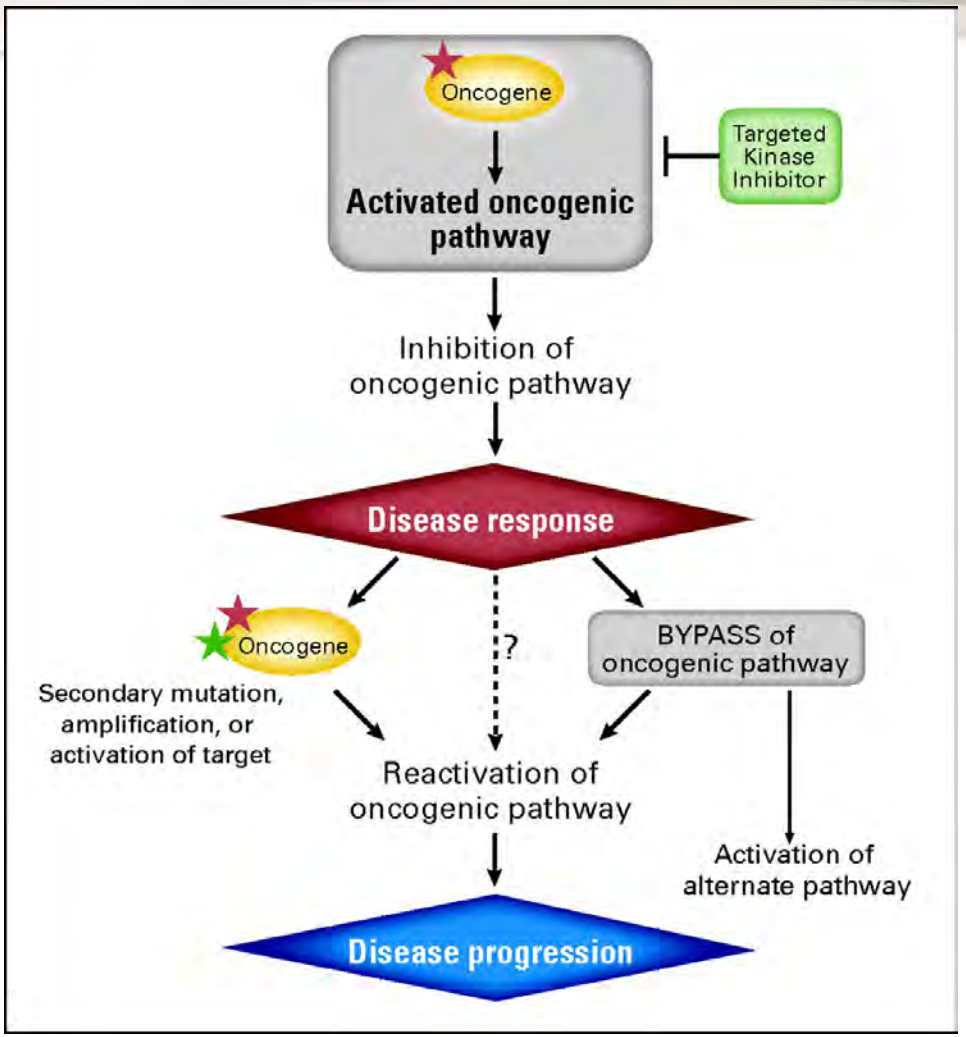
Signaling pathways are not 1-way

- **Driver mutations in signaling pathways (kinases) are components of highly integrated "wiring" that is not a one way flow of information**
  - Because these are critically important for normal cell functions, these are **highly regulated** pathways
- **Perturbation of a single component of will lead to activation of other components due to feedback activation or loss of feedback repression**

Kinase oncogene dependence and principles of drug resistance.



Wagle N et al. JCO 2011;29:3085-3096

©2011 by American Society of Clinical Oncology

Example of Vemurafenib

- **50-60% of melanoma patients have driver mutations in BRAF (V600E)**
- **At doses of vemurafenib that inhibit 90% of B-RAF activity, most patients respond rapidly with tumor shrinkage**
- **Median duration of response is less than 12 months due to resistance**
- **What are the mechanisms of resistance?**

Example-BRAF (V600E) mutations in colon cancer

- ***Unresponsiveness of colon cancer to BRAF (V600E) inhibtion through feedback activation of EGFR*** ***Prahallad A, et al. Nature Jan 26 2012***
- **Mechanism-appears to be inhibition of BRAF leads to inhibition of MEK and ERK, leading to reduced phosphatase activity of CDC25C, leading to reduced dephosphorylation of EGFR, leading to increased activation and EGFR signaling**

Neuroblastoma

- One of the SRBCT
- Derived from primordial neural crest cells destined to become sympathetic ganglia in the peripheral nervous system not CNS

Incidence:

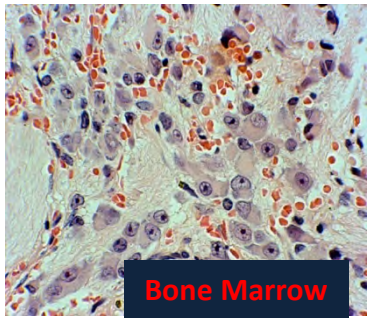
- 1 per 100,000 in children < 15 yrs in US (650 cases per year)
- The most common extra cranial solid tumor for children
- 7-10% of cancers of childhood

Survival Rates:

- 95-70% for low stage tumors (1,2,3)
- ~50% patients present with advanced disease
- < 30% of children over 1 year old with advanced disease and/or MYCN amplification despite aggressive therapy

Patient (19yr) with High-risk Neuroblastoma

Diagnosis

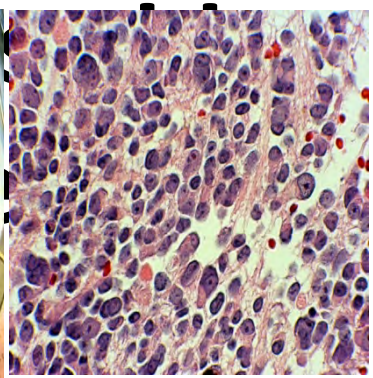


Met1-BM:
bone marrow biopsy at diagnosis.
>80% tumor

~4 Months

- 4 cycles Induction
- A3973

Surgery

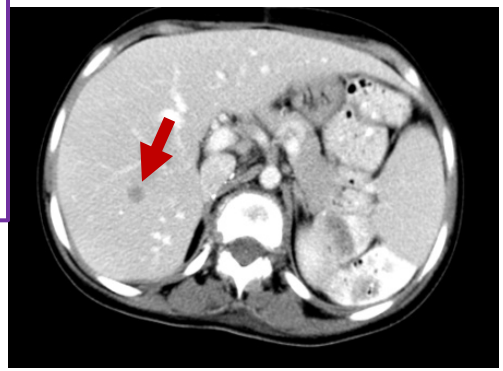


Primary: tumor removed by surgery
viable margin

3 years

- 8 cycles of Salvage Therapy
- 7 cycles of RA
- Radiation multiple sites
- Low dose mIBG

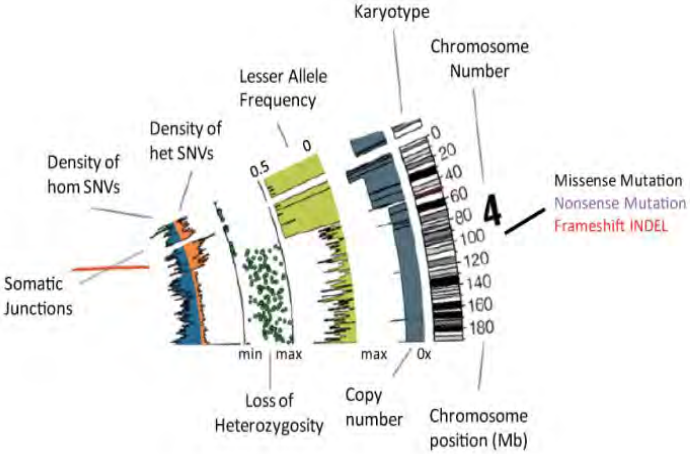
Death



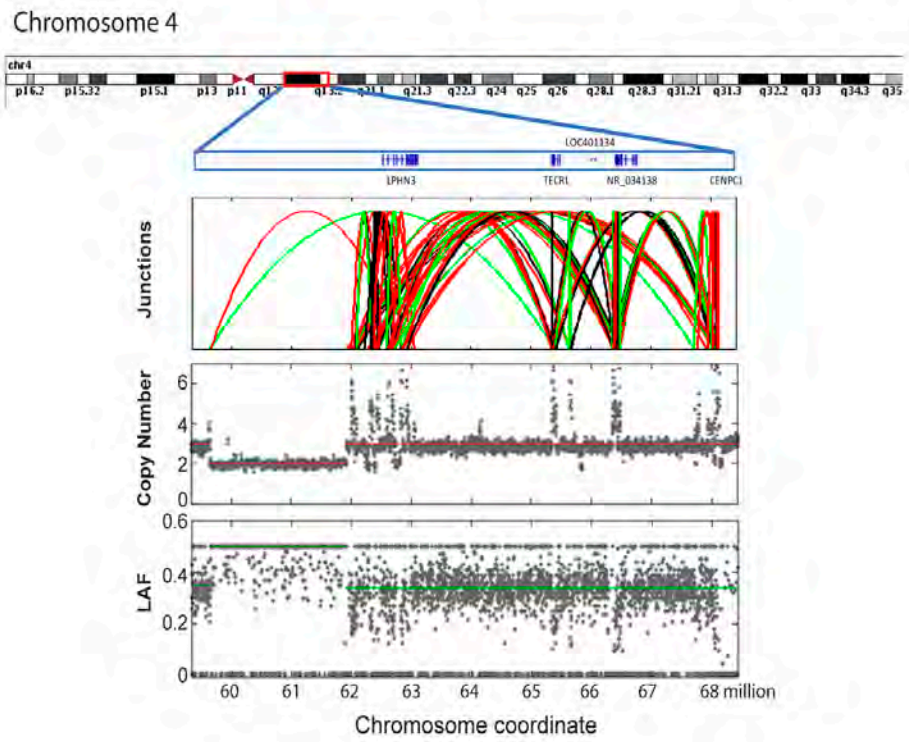
Met2-Liver:

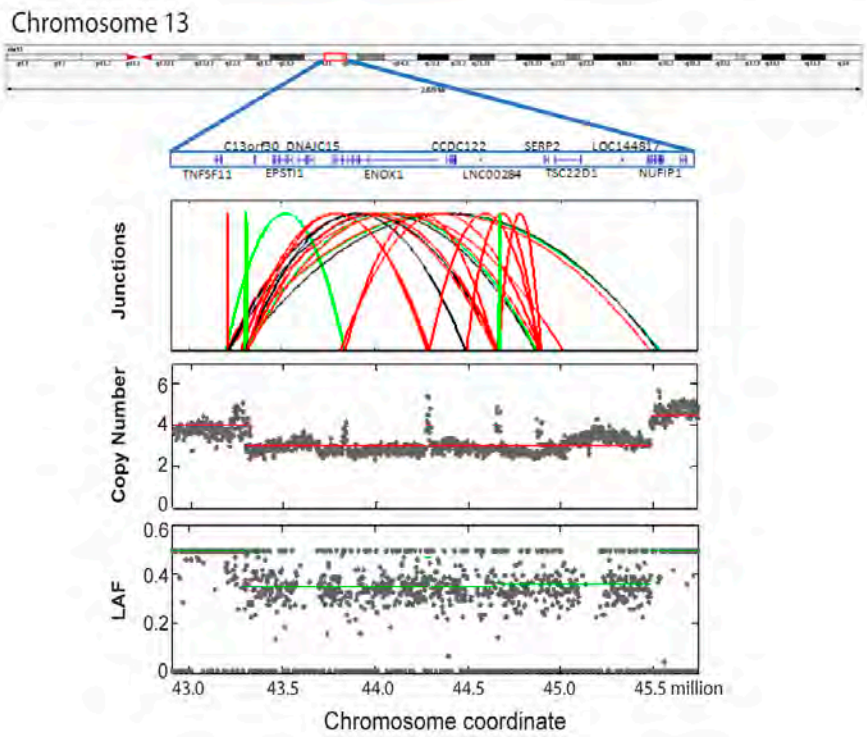
Whole genome seq of liver Met2 & RNAseq of Met1, primary and Met2

Forty-four (44) non-synonymous mutations found in index sample (Liver Met)

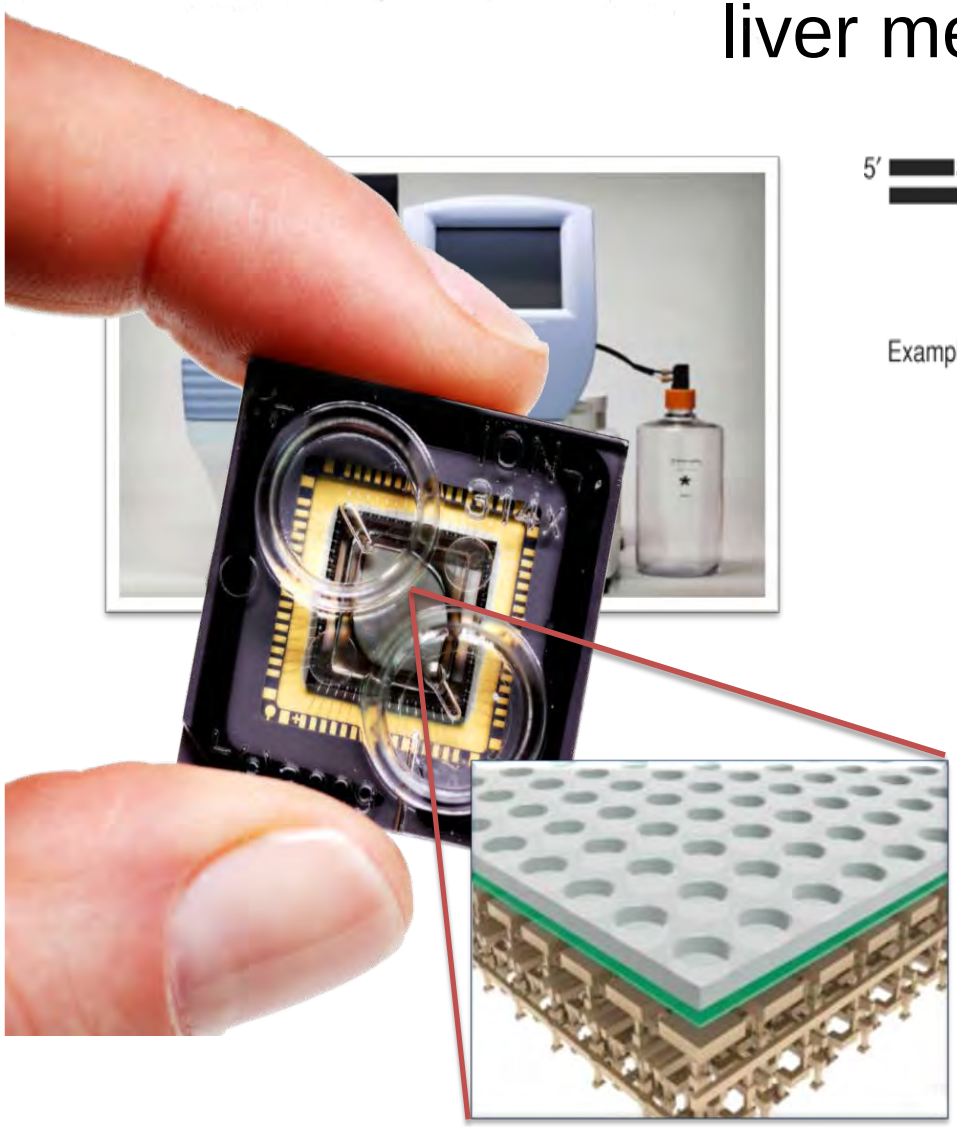


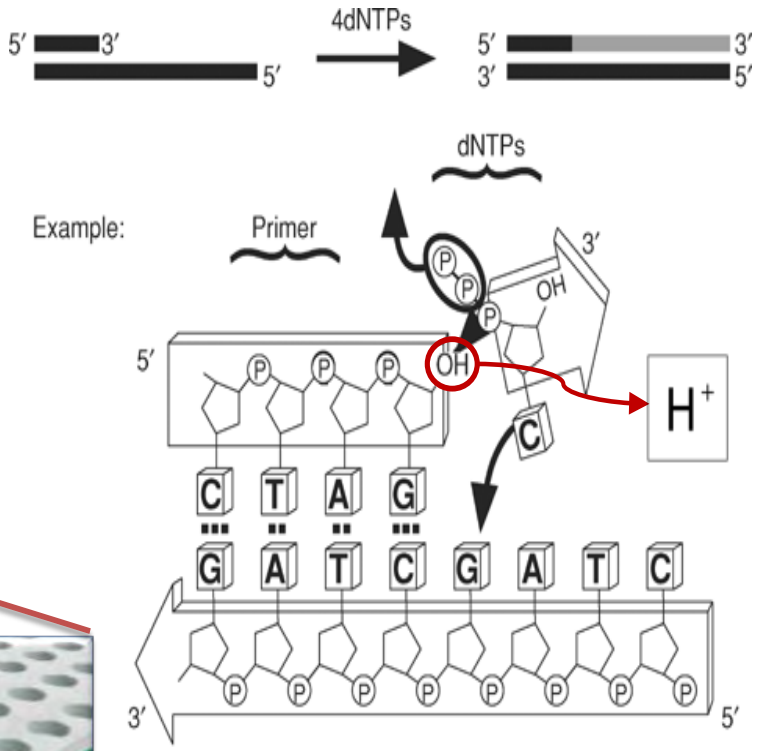
Chromothripsis was evident by massive complex rearrangements detected at chromosomes 4q and 13p

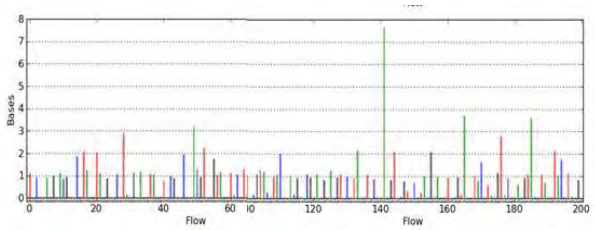




Ion Torrent: Deep Re-sequencing (1000x) of primary (bone marrow) and 4 primaries (adrenal): 14/44 (32%) small variants were present in all samples, 30 unique to liver met

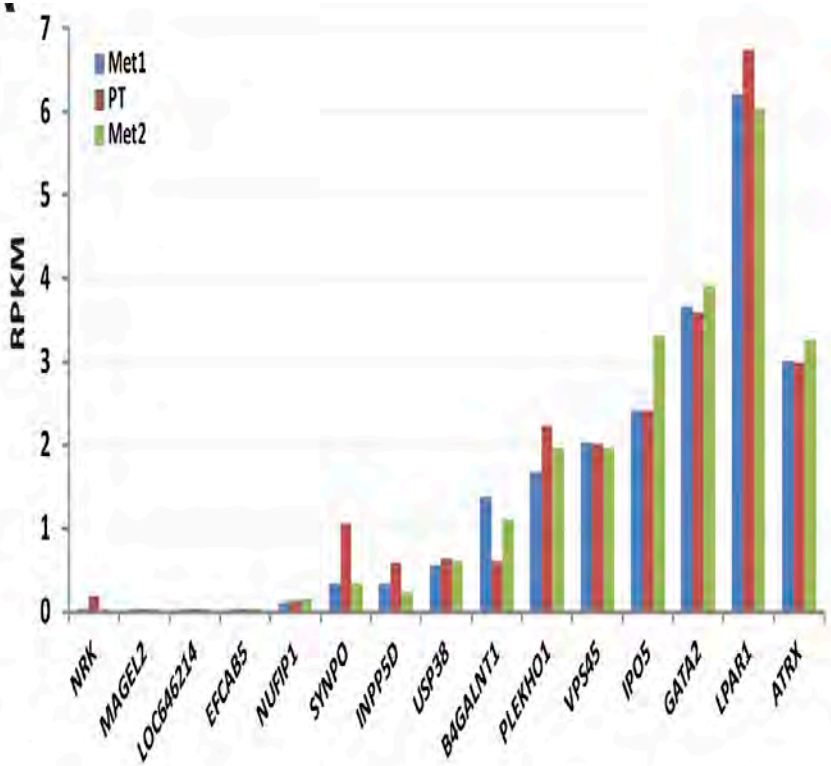


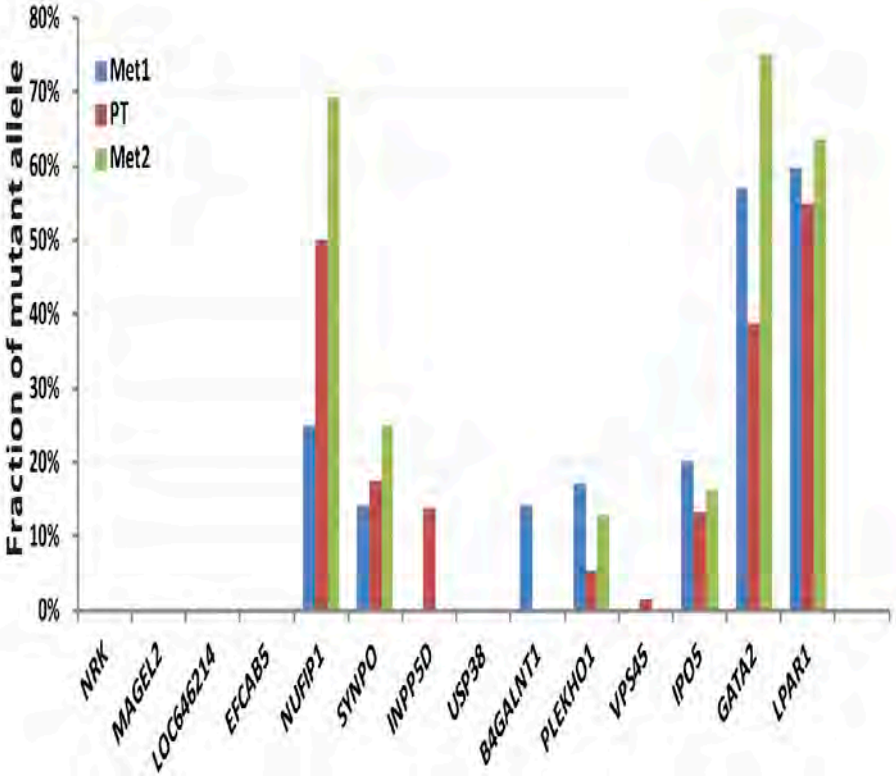




RNAseq of Met1, Primary, Liver Met2 to identify expressed driver mutations:

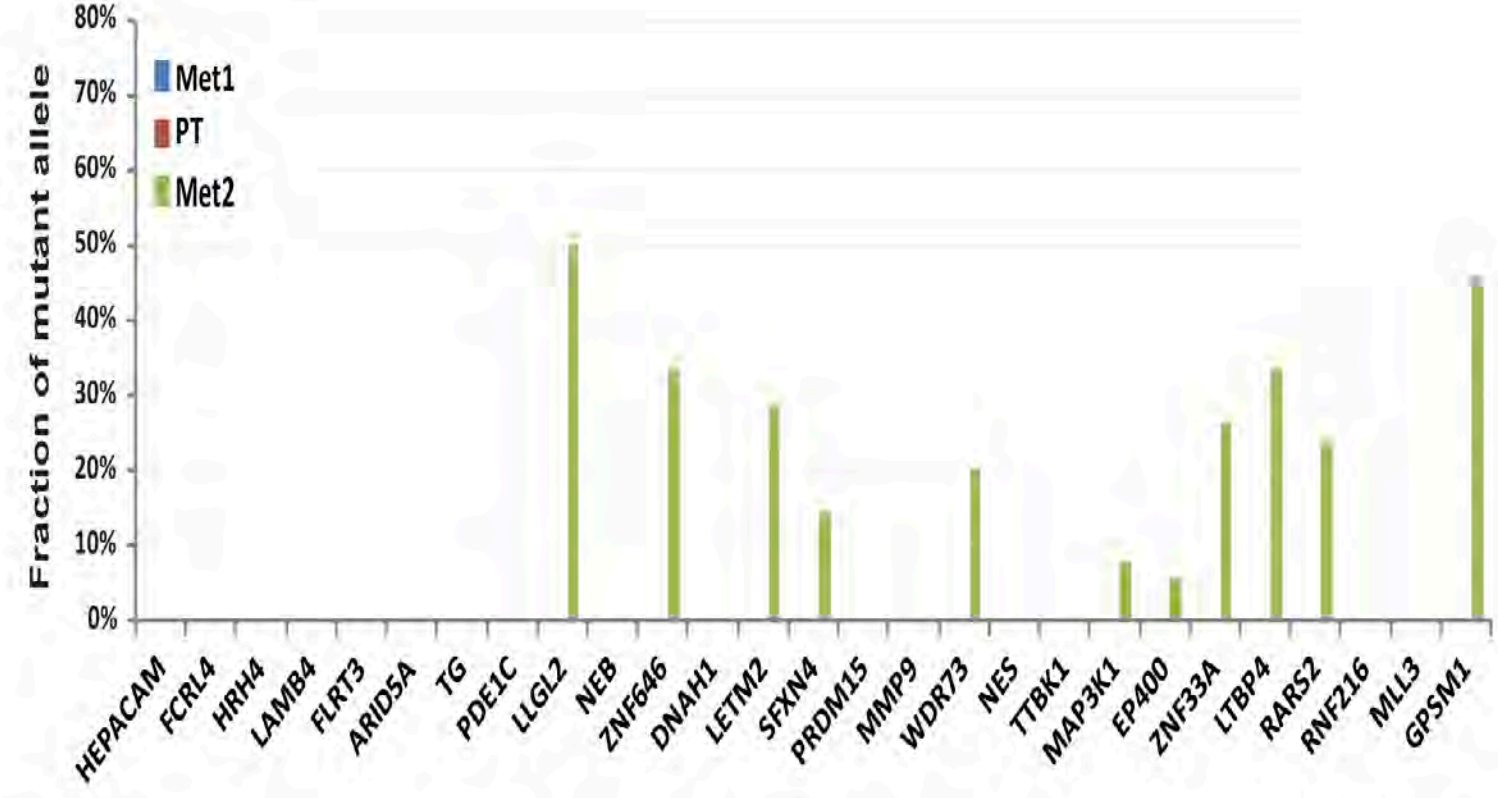
- 14/44 commonly mutated in all 3 tumors
- 12 the gene is expressed
- 9 variant allele expressed
- 3 genes (*NUFIP1*, *GATA2*, and ***LPAR1***) high variant allele fraction >30%





30/44 Somatic Mutations Unique to Liver Met2

- 11/30 the variant was expressed in Liver Met2
- *De-Novo* mutations in liver arising during the course of disease but absent in primary (4 regions) and bone marrow met



Summary

1. Neuroblastoma is marked by aneuploidy in recurrent regions but lack frequently recurring mutations
2. Possible that classic mutations may not drive tumorigenesis
3. Possible that each individual tumor has its own set of driver mutations
4. Ongoing efforts including RNAseq underway and will identify key onocogenic drivers and targets for therapy

Many Novel Drivers Epigenetic (red) Many Not Currently Druggable

PBRM1 – Renal cell carcinomas
EZH2, MEF2B – Lymphomas
KCNJ5 – Adrenal adenomas
DNMT3A, *SF3B1, SRSF2*, *U2AF35* – Leukemias
MLL2, MLL3, *DDX3X* – Medulloblastomas
ARID1A, PPP2R1A – Ovarian cancers
DAXX, ATRX – Pancreatic endocrine tumors
BAP1, *TTRAP, PREX2* –Melanomas
IDH1, 2 – Gliomas
CIC, FUBP1 – Oligodendrogliomas
MED12 - Leiomyomas
H3F3A, HIST1H3B- Diffuse intrinsic pontine glioma
ATRX, ARID1A, ARID1B, *PTPN11*- Neuroblastoma

Adapted from Vogelste

M-PACT: Molecular Profiling based Assignment of Cancer Therapeutics

Pilot Trial to Assess the Utility of Genetic Sequencing to Determine Therapy and Improve Patient Outcome in Early Phase Trials

NCI-Sponsored Clinical Trial

Objective

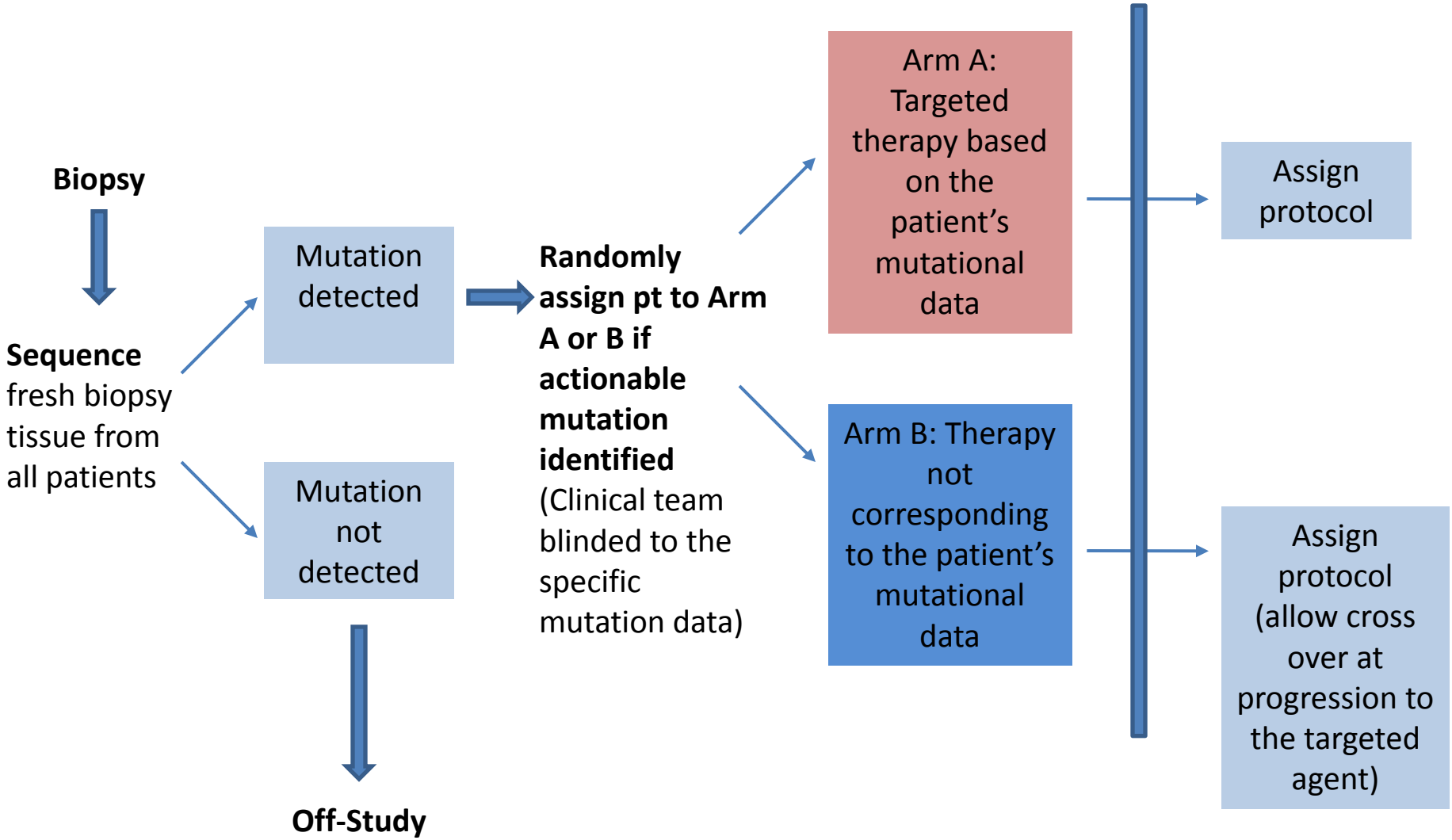
- Assess whether the response rate (CR+PR) and/or 4-month PFS is improved following treatment with agents chosen based on the presence of specific mutations in patient tumors.
  - Only patients with pre-defined mutations of interest will be eligible
  - Study treatments, regardless of cohort, will be chosen from the list of regimens defined in the protocol
  - Arm A: Receive treatment based on an study agent prospectively identified to work on that mutation/pathway
  - Arm B: Receive treatment with one of the study agents in the complementary set (identified to not work on one of the detected mutations/pathways)

Patient Population

- Patients with refractory solid tumors that have progressed on at least one line of standard therapy or for which no standard treatment is available that has been shown to improve survival.
- Adequate organ function (AST/ALT<3xULN, Bil < 1.5 xULN, S. Cr < 1.5 x ULN, platelets > 100K, ANC> 1500)
- Study regimens: As long as the same set of protocols are offered to a given set of patients, the number and actual treatments regimens can vary over time

Mutations in DNA repair pathways	Veliparib+ Temozolomide MK1775 + carboplatin
Mutations in the PI3K pathway; loss of PTEN, Akt amplification	mTOR inhibitor -Everolimus
Mutations in the RAS pathway	GSK 1120212 (MEK inhibitor)

Study Design



Statistical Design

- Patients will be randomized 2:1 to Arm A (experimental) versus Arm B (control)
- Within Arm A, up to 30 patients will be treated within each of the treatment cohorts. Within each treatment cohort of Arm A, discriminate between tumor response rates of 20% vs. 5% and, as a secondary endpoint, 4-month PFS rates of 50% vs. 25%
- The two Arms will be compared with respect to both objective response rate and PFS – this is a randomized comparison.

Gene Name	*Pathways/Function*	*Gain or Loss of Function?*
BRAF	**RAS/RAF/ERK/MEK**	**Gain**
NF1	**RAS**	**Loss**
Kras	**RAS/RAF/ERK/MEK AKT/PI3K**	**Gain**
Nras	**RAS/RAF/ERK/MEK AKT/PI3K**	**Gain**
Hras	**RAS/RAF/ERK/MEKAKT/PI3K**	**Gain**
AKT1	**AKT/PI3K**	**Gain**
AKT2	**AKT/PI3K**	**Gain**
AKT3	**AKT/PI3K**	**Gain**
PIK3CA	**AKT/PI3K/RAS/RAF/ERK/MEK**	**Gain**
PTEN	**AKT/PI3K/RAS/RAF/ERK/MEK**	**Loss**
P53	**DNA Repair**	**Loss**
FBXW7	**DNA Repair**	**Loss**
ATM	**DNA Repair**	**Loss**
PARP1	**DNA repair**	**Loss**
PARP2	**DNA repair**	**Loss**
ERCC1	**DNA repair**	**Loss**
MLH1	**DNA repair**	**Loss**
MSH2	**DNA repair**	**Loss**
NBN	**DNA Repair**	**Loss**
ATR	**DNA repair**	**Loss**
MGMT	**DNA repair**	**Loss**

Conclusions

- **The ability to obtain full genomic data on a given tumor will allow us to make rational choices for therapy**
- **Functional genomics may provide help in choosing combination therapy**
  - Combinations will not be easy due to enhanced toxicities
- **Cancer as a chronic disease is not a bad thing as long as we recognize rapid development of resistance and clonal evolution**