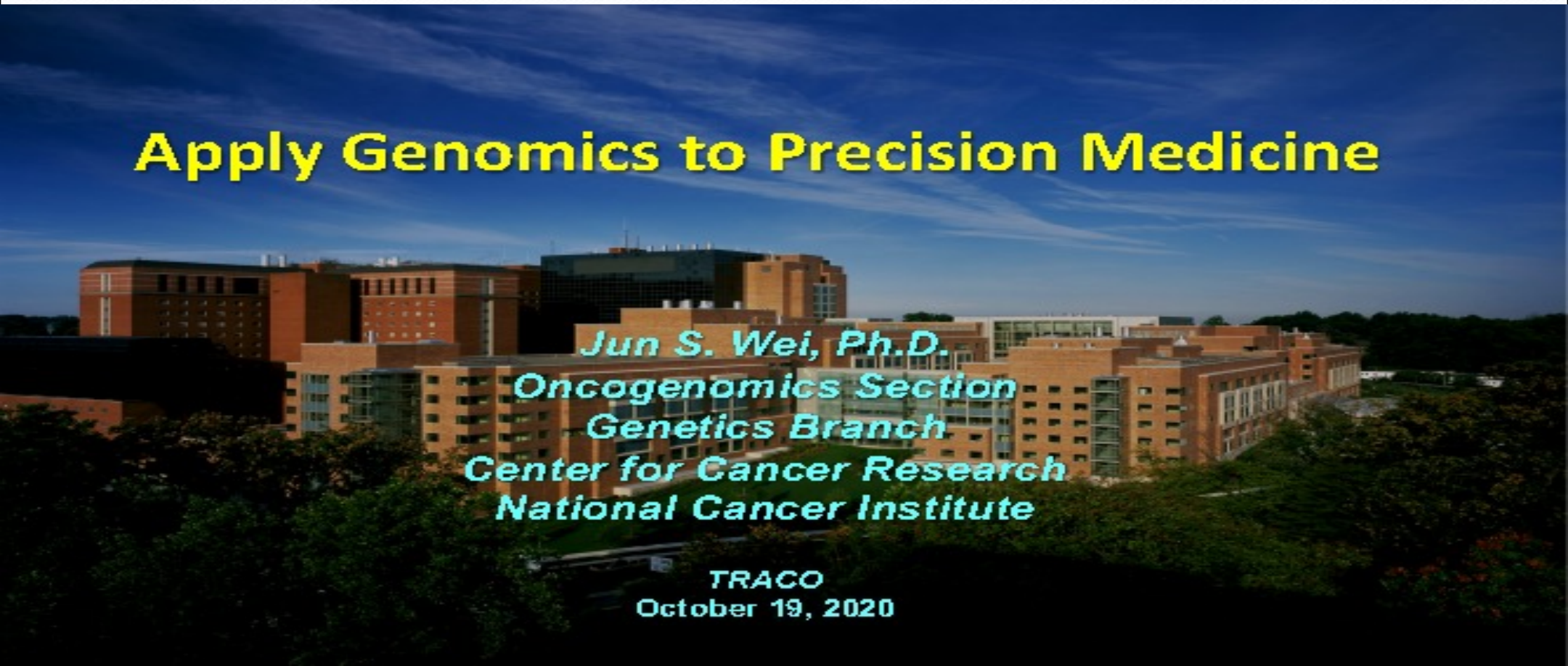
# Genomics


Apply Genomics to Precision Medicine
Jun S. Wei, Ph.D.
Oncogenomics Section
Genetics Branch
Center for Cancer Research
National Cancer Institute
TRACO
October 19, 2020

# Outline

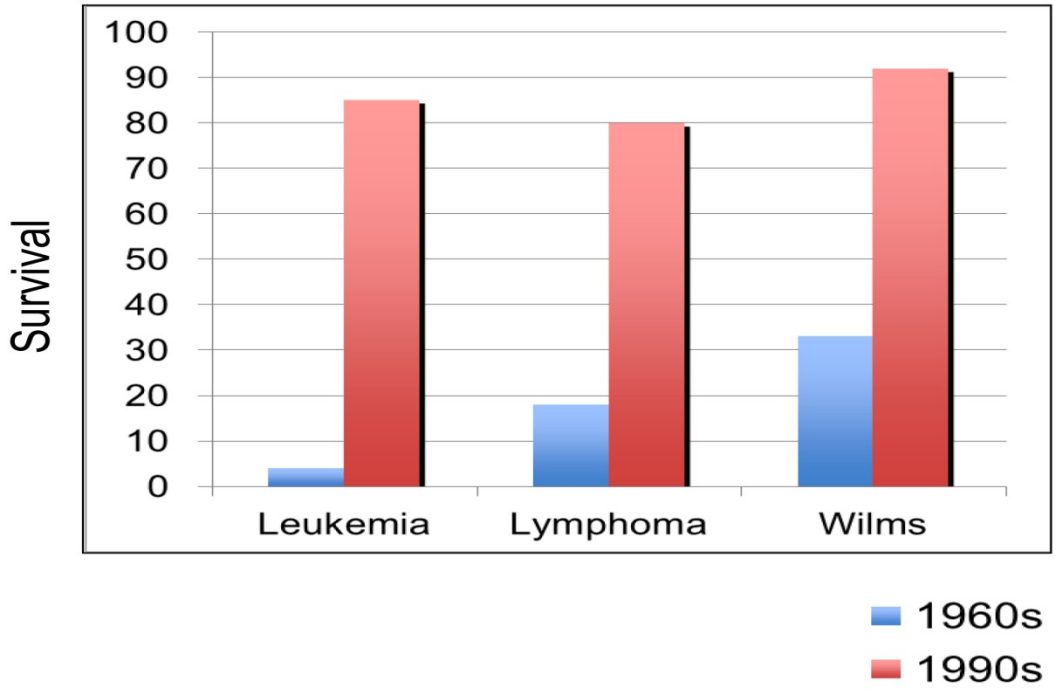
Outline

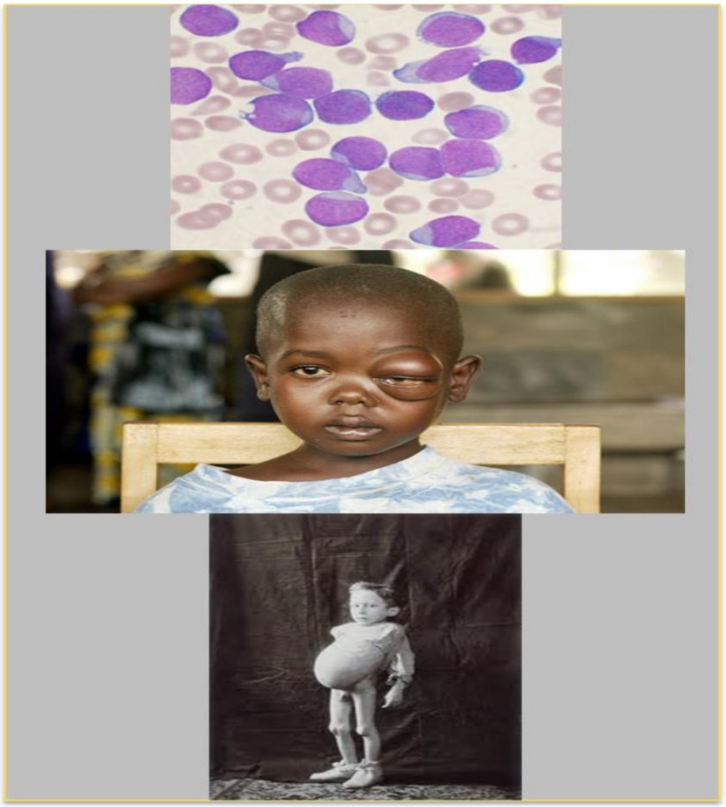
- Success and Challenges of Treating Pediatric Cancers
- Genomics
- Next-generation Sequencing
- Application of next-generation sequencing:
  - Diagnosis
  - Identification of molecular target
- Precision Therapy

# Childhood cancer

National Cancer Institute

## Childhood cancer: The beginning of a modern medical success story



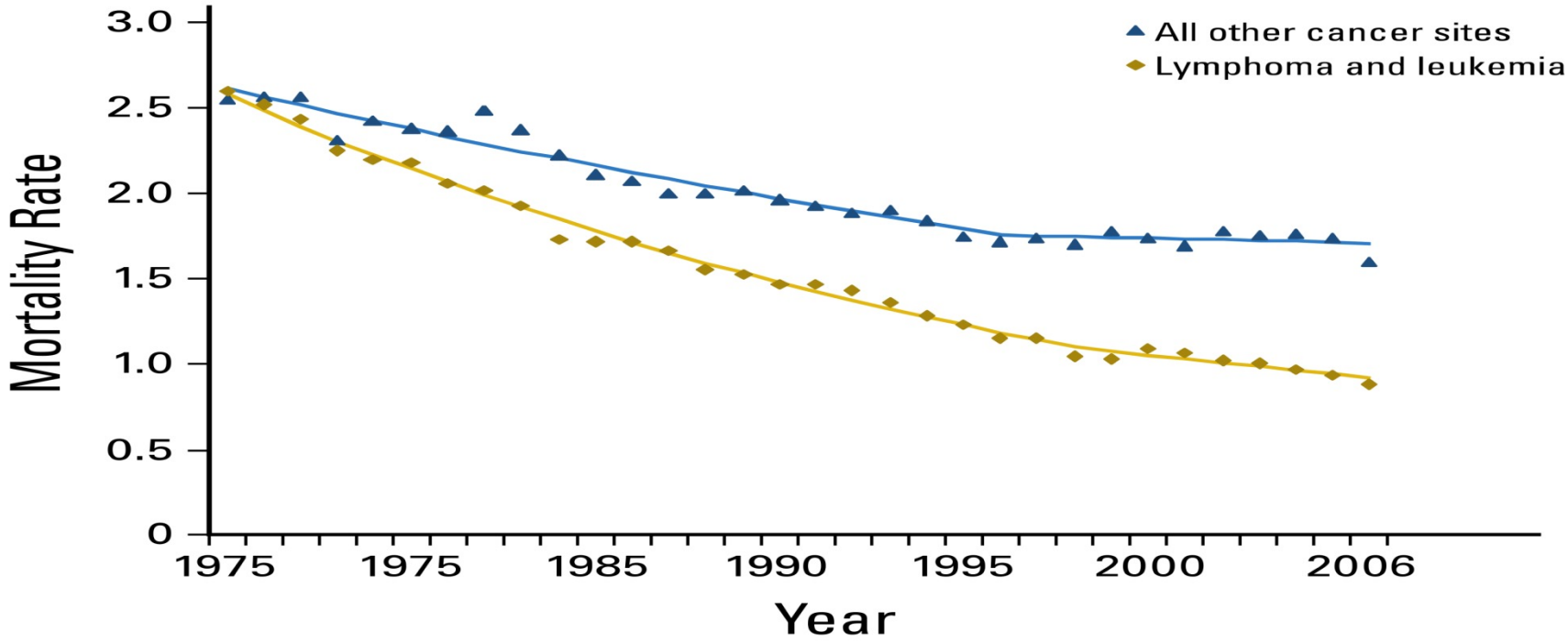



Courtesy: John Maris

# Mortality rates

National Cancer Institute

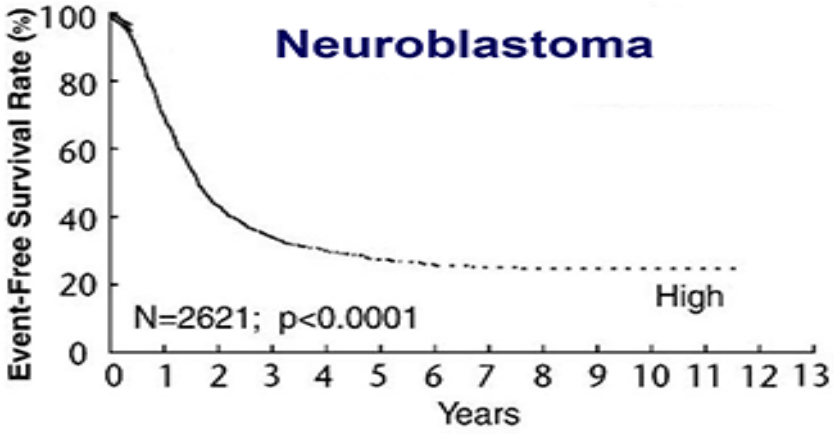
**However in the past 16 years no improvement in mortality rates despite increased intensity of treatment**



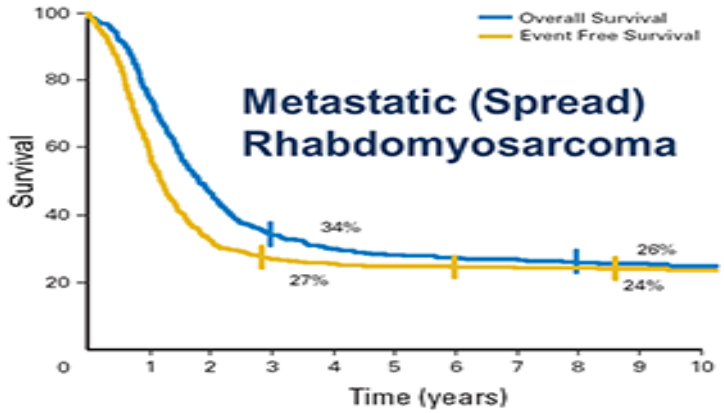

Courtesy: Malcolm Smith

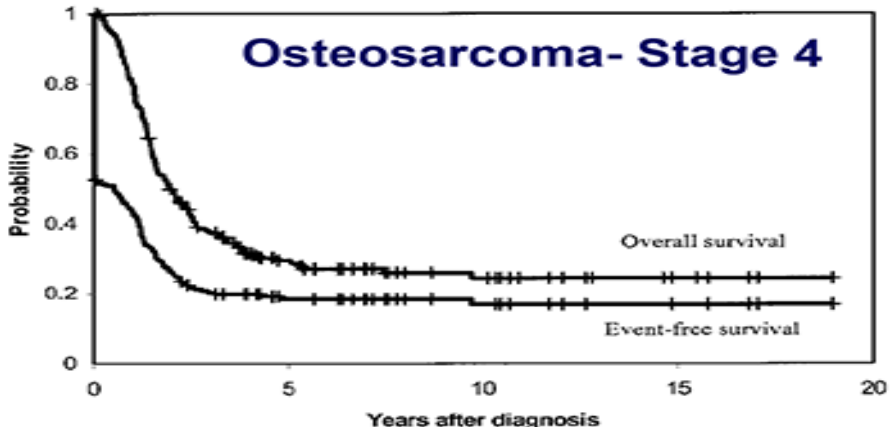
# Pediatric cancers

**Metastatic, Recurrent, & Refractory Disease Remains Incurable**







# Gene expression

## The dramatic consequences of gene expression in biology

Anise swallowtail, *Papilio zelicaon*

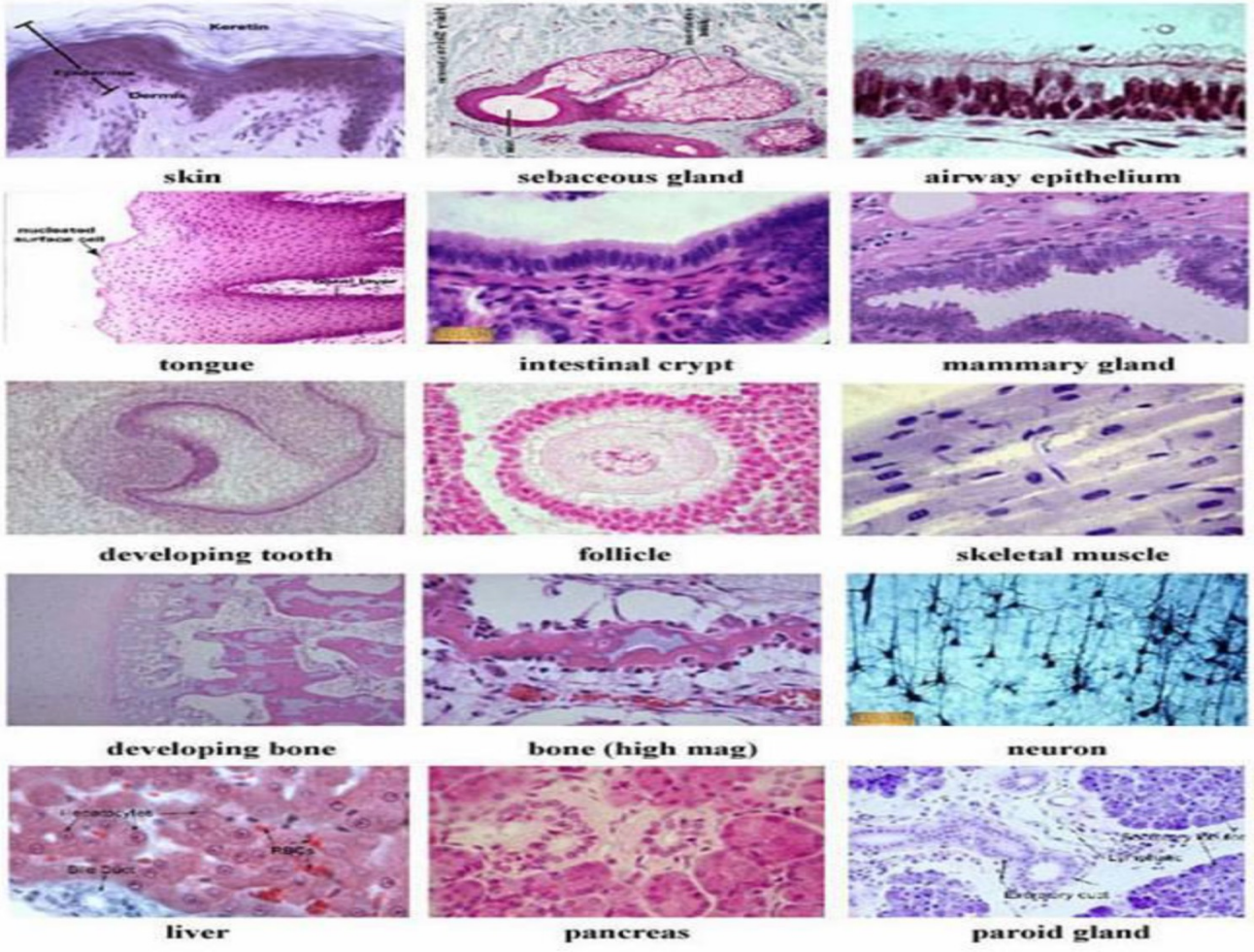
**Same genome →**
Different expression pattern
Different proteome
Different tissues
Different physiology

# Gene expression

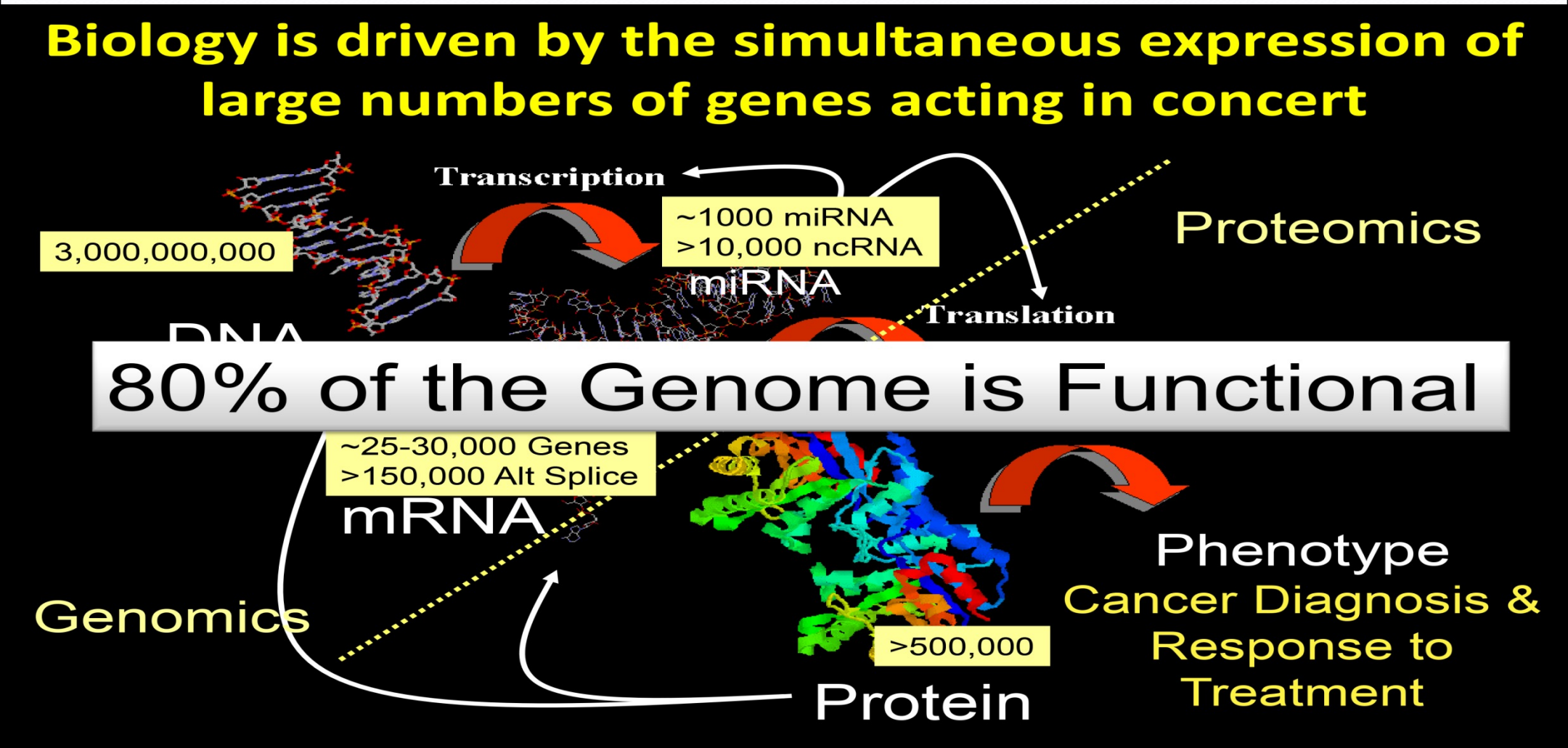
...but the complexity and diversi

**Same genome or DNA →**

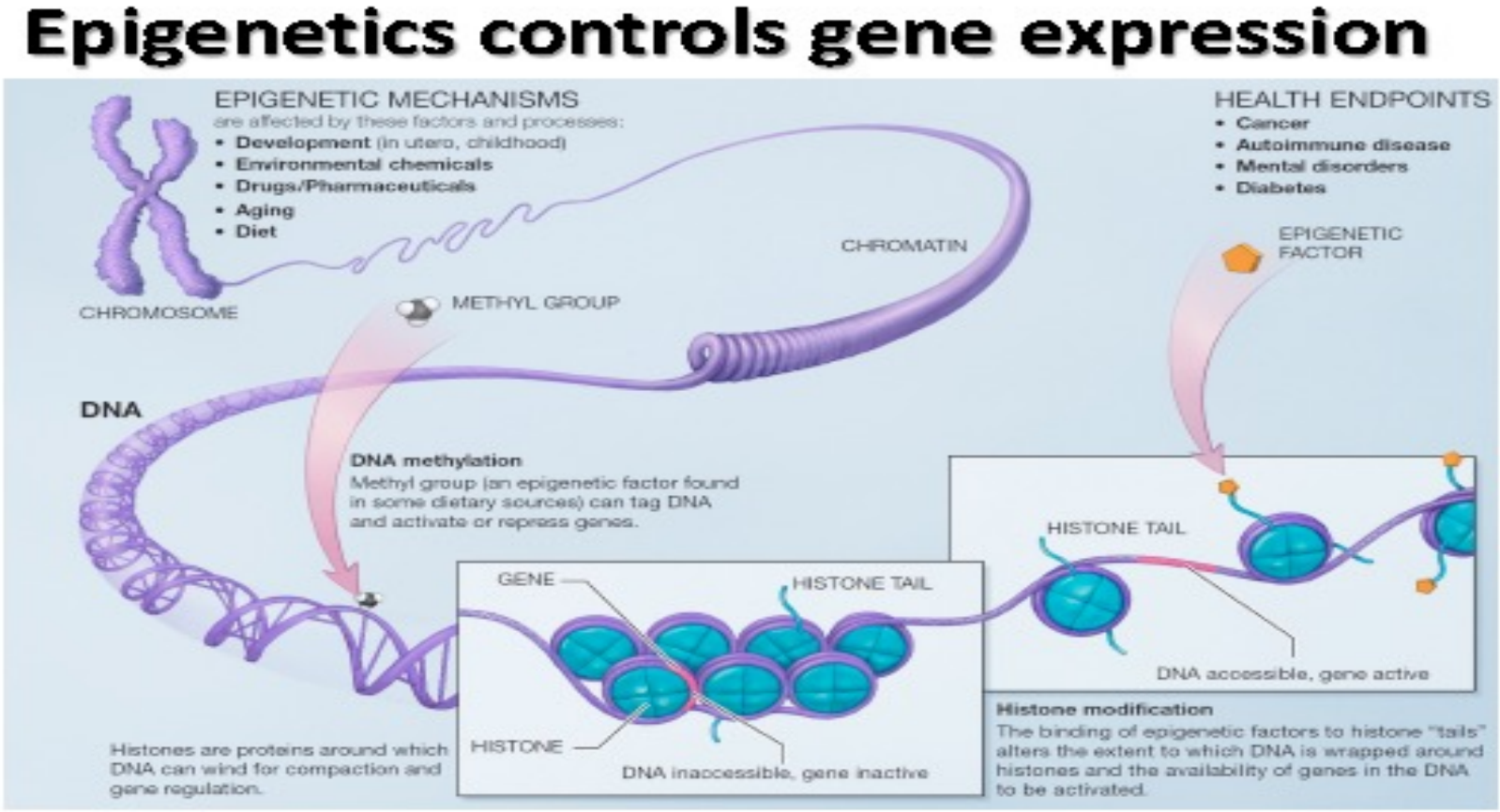
- Different expression pattern
- Different proteome
- Different tissues
- Different physiology



# Gene expression



# Epigenetics

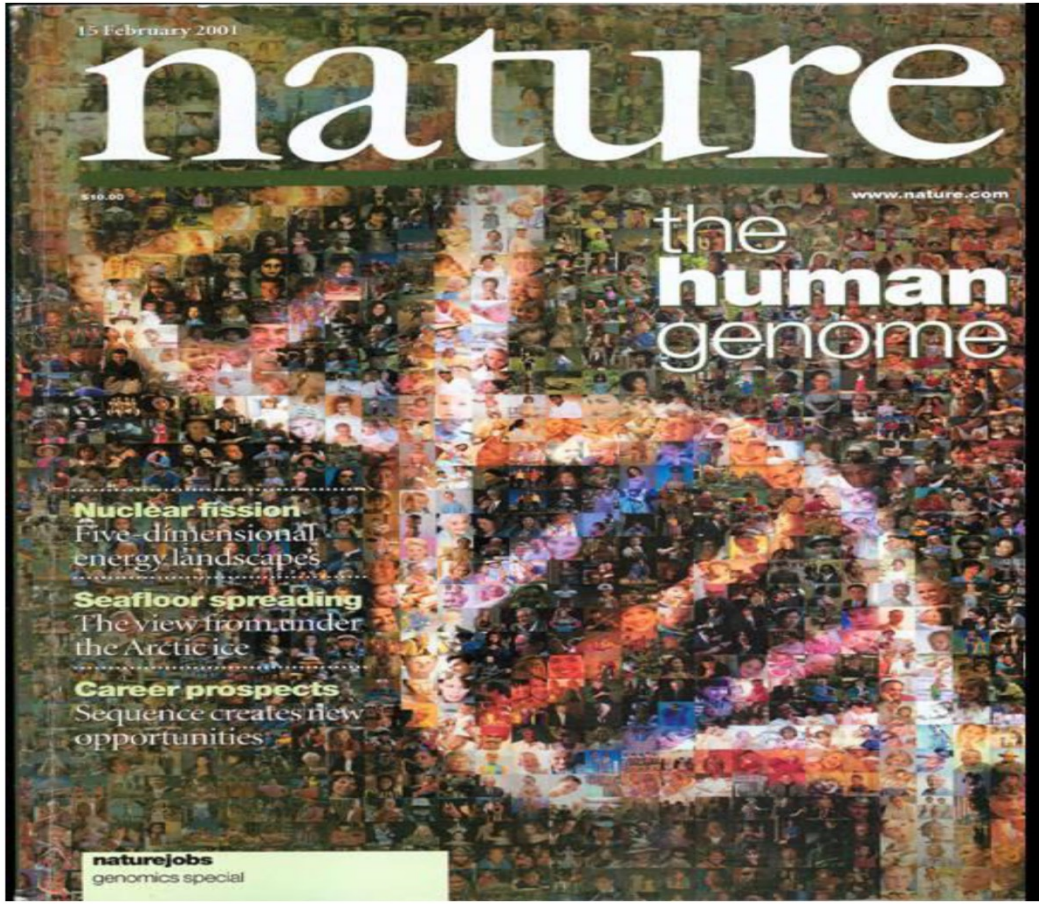


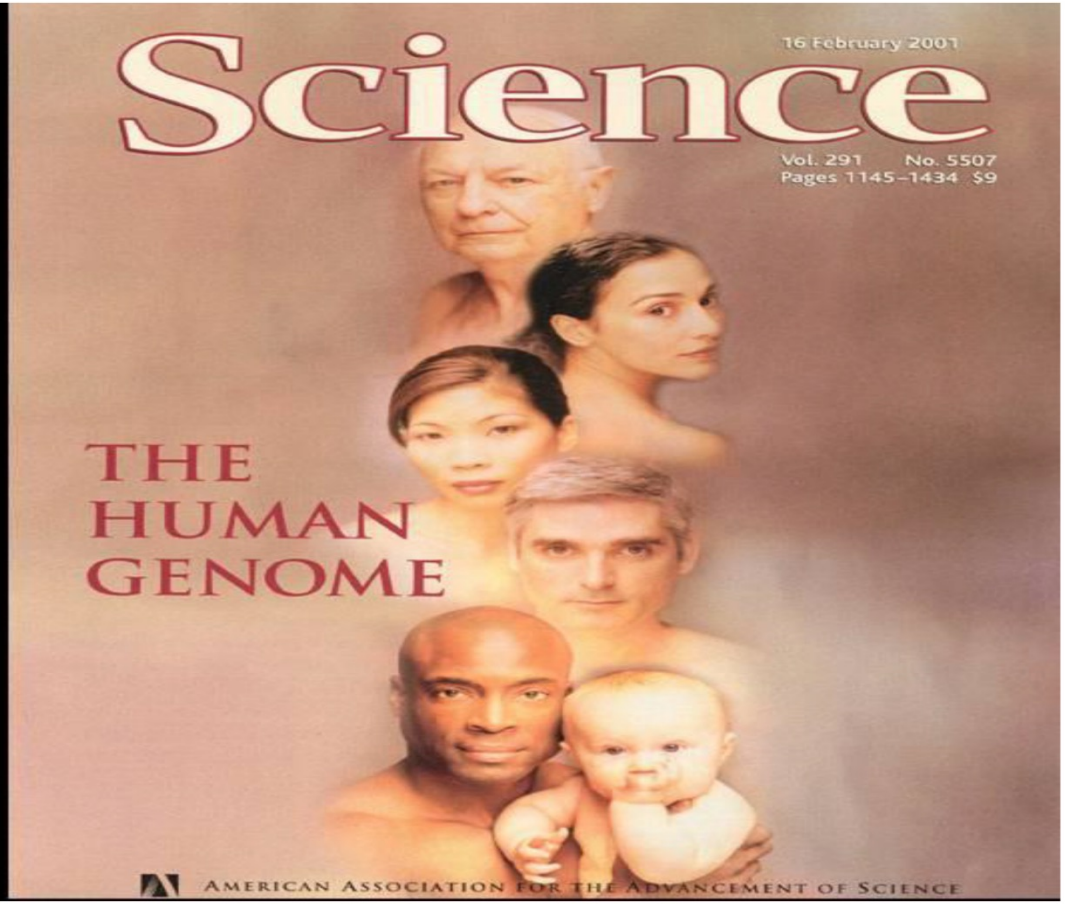
# Gene measurement

**Challenge: how to measure/detect genes and their products in a massively parallel way?**

- **High-throughput technologies**
- **Computational power**

# Human genome



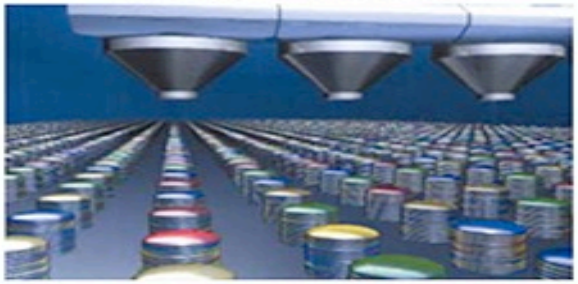
# First generation tools

## 1st generation genomic tool: microarrays

**Printing microarrays**

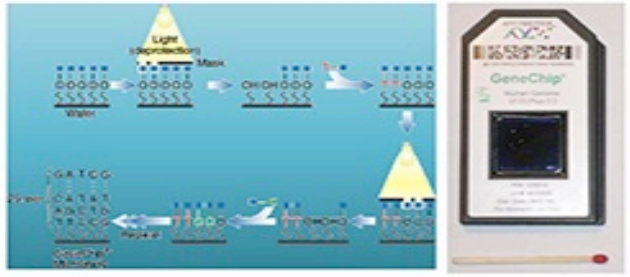
**Mechanical**

**Electronic Piezo**

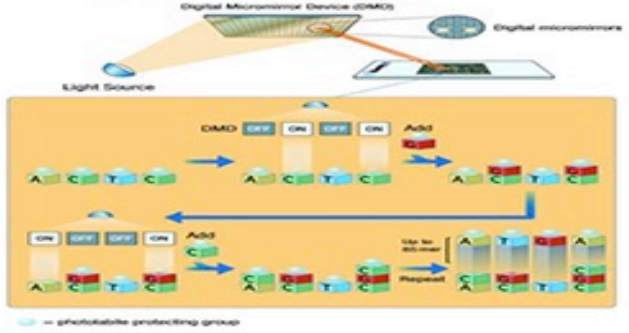


**In-situ synthesis microarrays**

**Lithographic masks and de-protection through illumination**

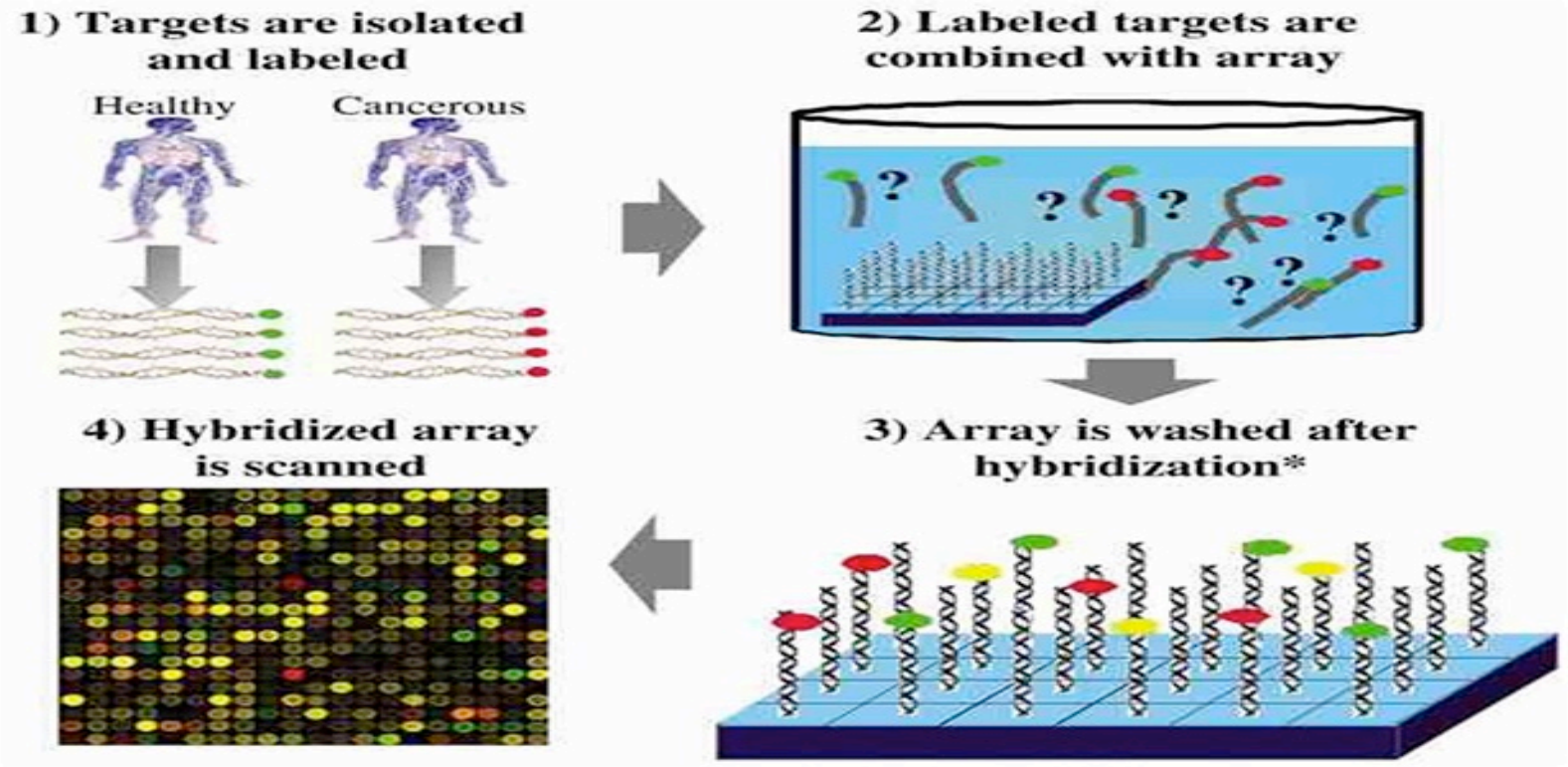



**Digital micromirrow device (DMD)**



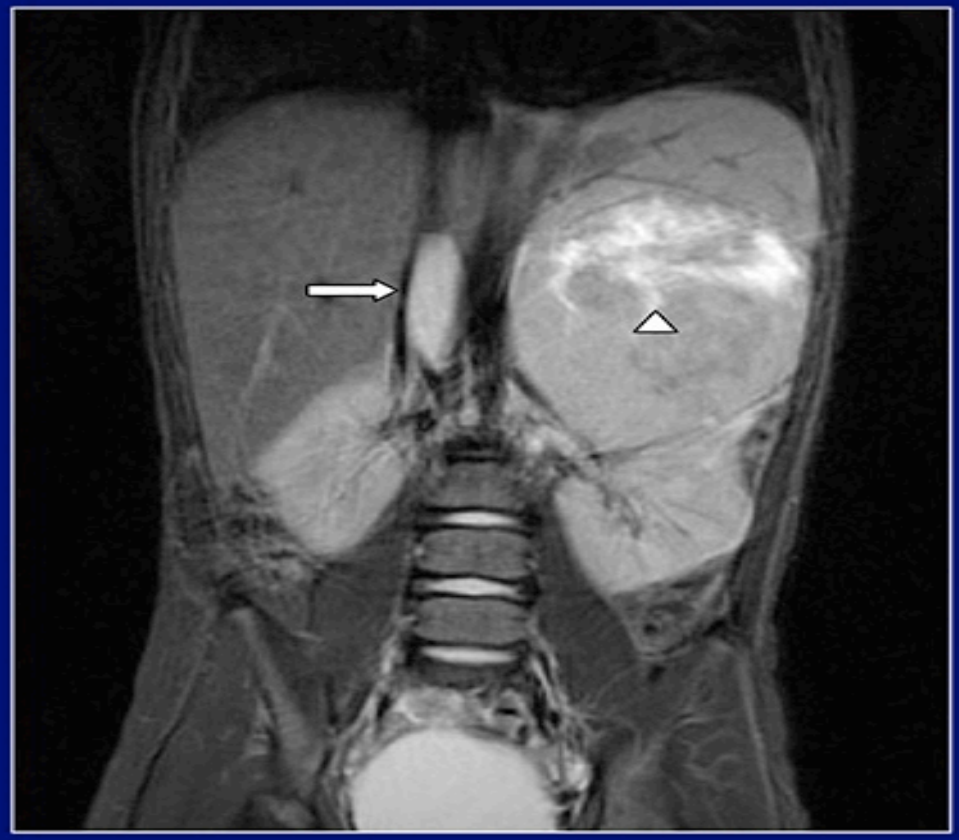
# Microarrays

## Microarrays – technologies of hybridization

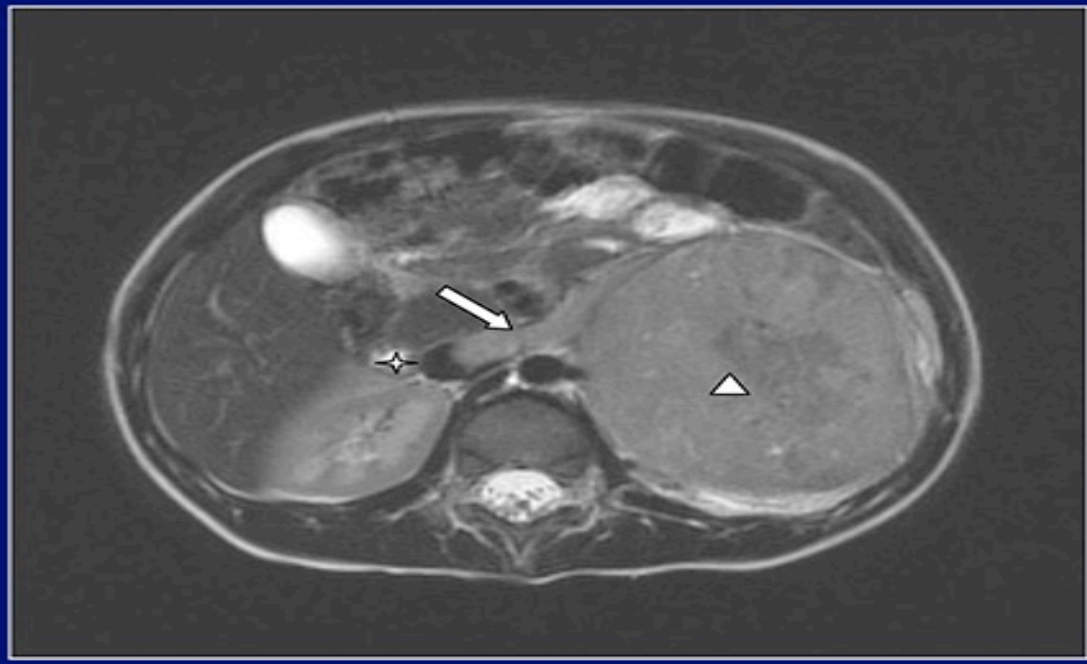


# Wilms tumor

MRI: 9 x 8 x 9 cm mass in upper pole left kidney, tumor in Left renal vein and inferior vena cava

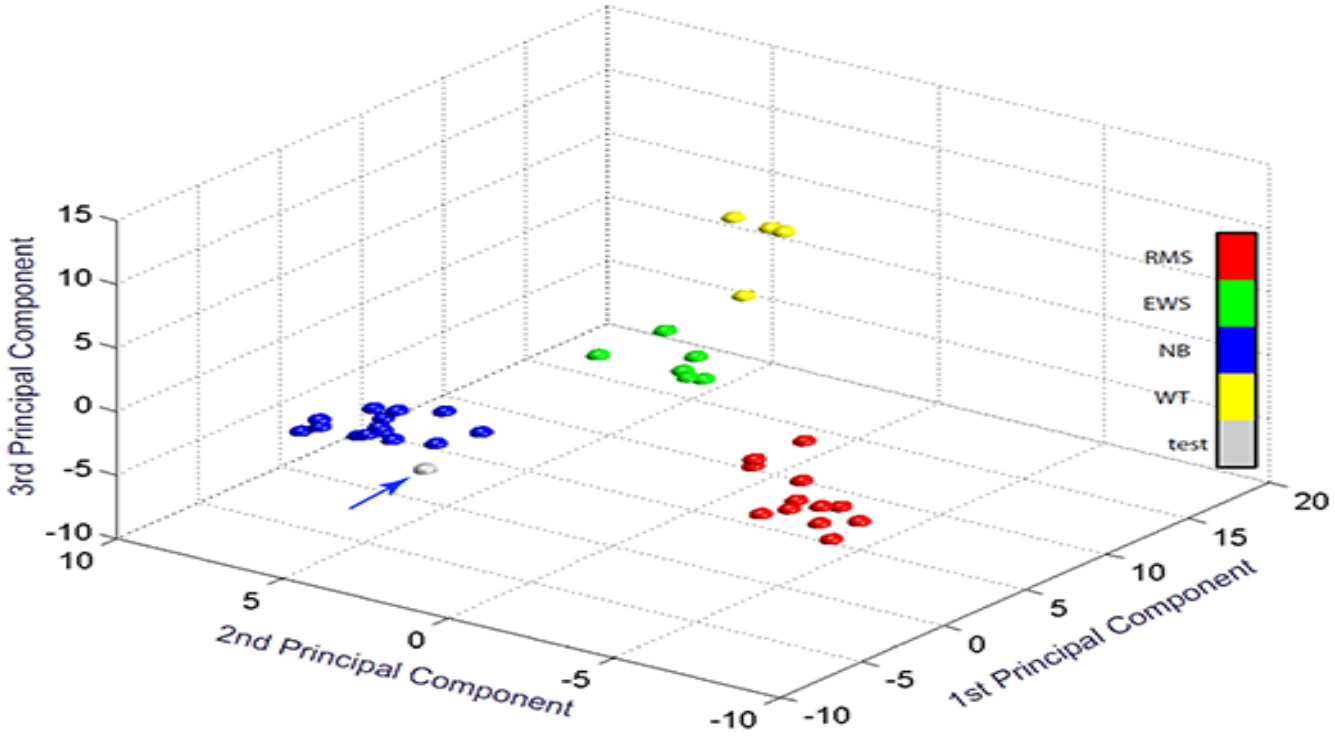


Initial diagnosis: Wilm's tumor



# Cancer diagnosis

**Diagnosis of cancers using gene expression profiles**



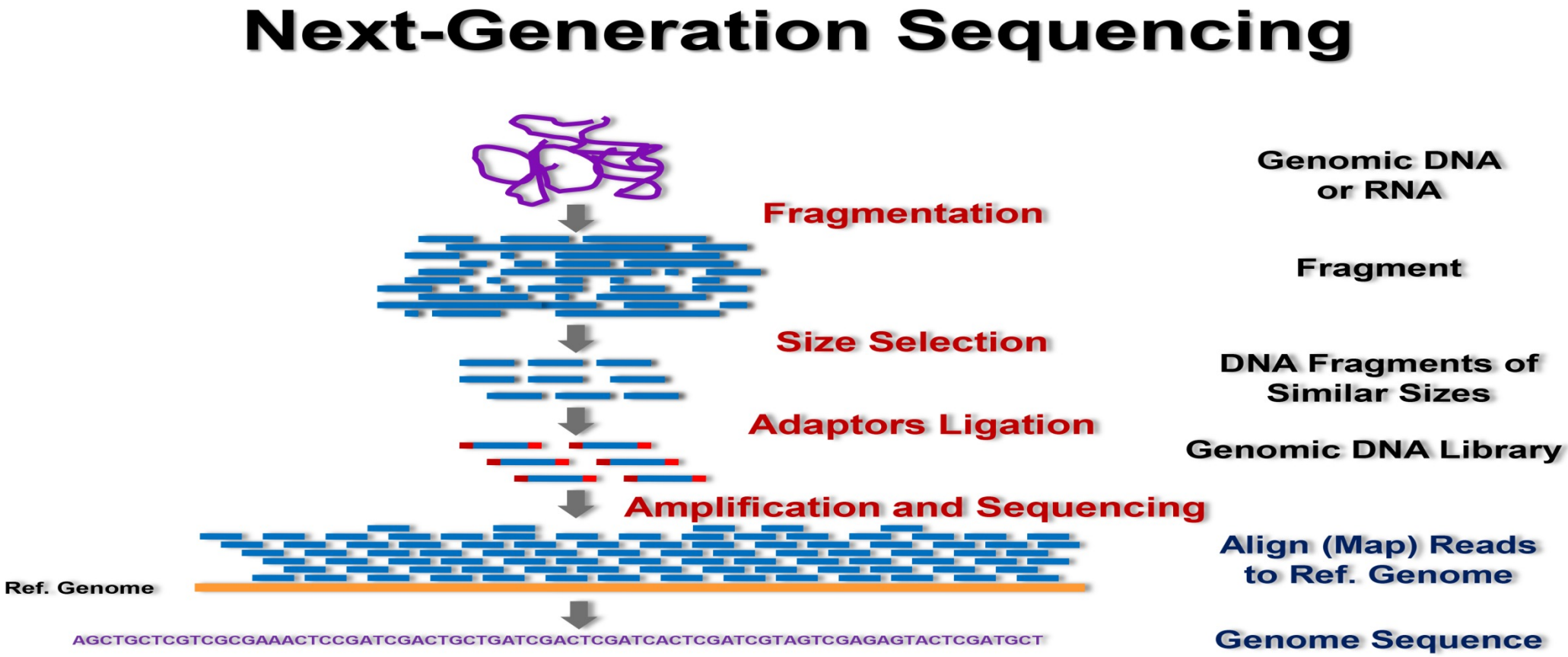

**Wilm's tumor**

↓

**Neuroblastoma**

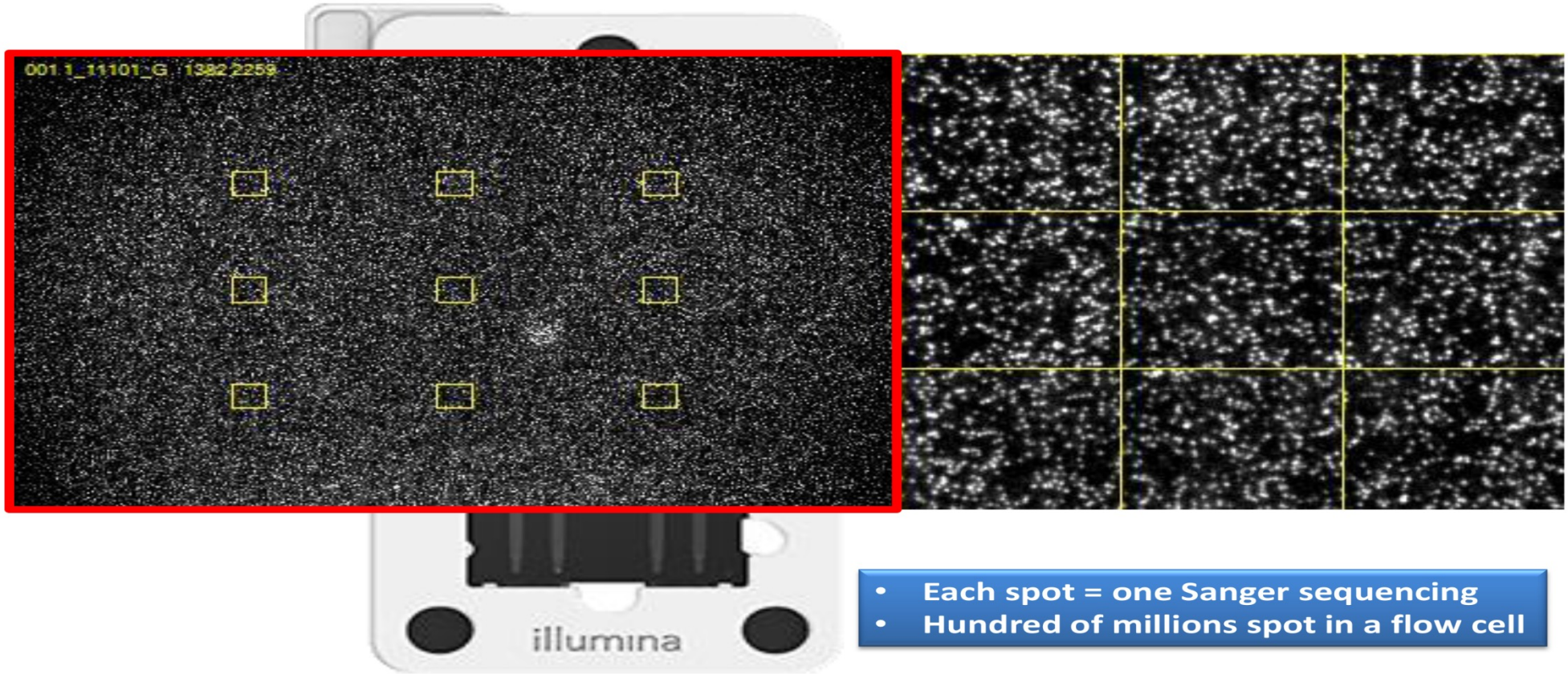
- **Patient was switched to high risk neuroblastoma treatment included stem cell transplant**
- **Doing well 1 yr after diagnosis**

# Next-generation sequencing



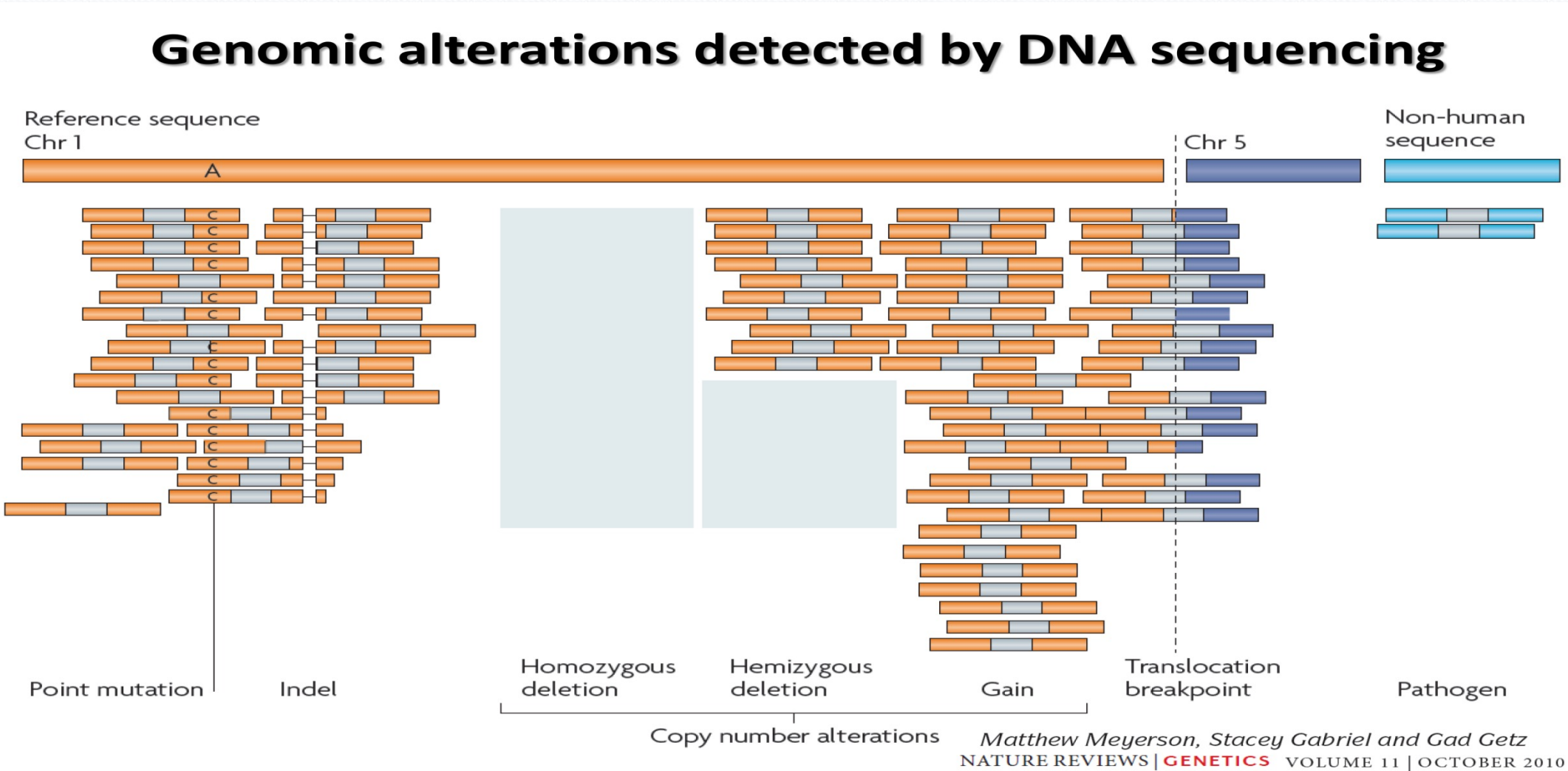
# Massively Parallel Sequencing

Massively Parallel Sequencing



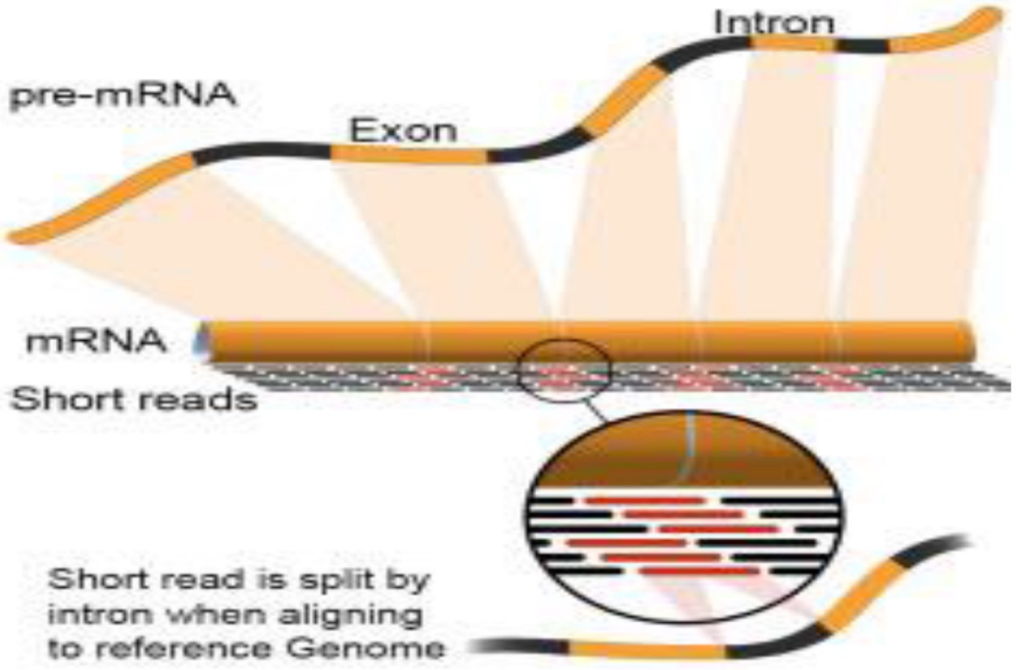

- Each spot = one Sanger sequencing
- Hundred of millions spot in a flow cell

# Genomic Alterations



# Genomic Alterations

## Genomic Alterations Detected by RNA Transcriptome Sequencing




- Digital Gene Expression
- Expressed Mutations
- Alternative Splicing Events
- Expressed Fusion Transcripts
- RNA editing
- Novel Transcripts
- Non-coding RNAs

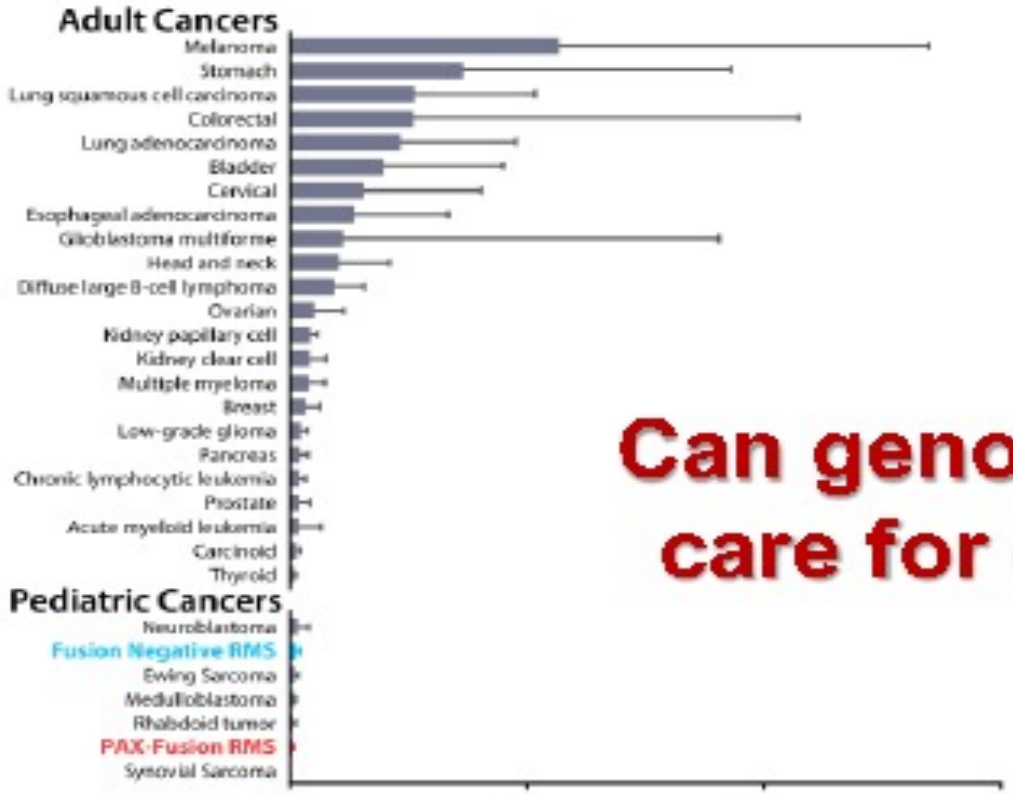
# Next-generation sequencing

## Next-generation sequencing: a platform for many applications to study genome and epigenome

- No need of prior knowledge for probe design as in microarrays
- Parallel sequencing at basepair resolution– massive-throughput
  - Then: *~13 years for the 1st human genome* using Sanger sequencing by *20 centers in 7 countries*
  - Now: *multiple human genomes in 2 days* using a NGS sequencer
- A single platform for different kinds of genomic and epigenomic information
  - DNA and RNA sequencing
  - Genome modification, e.g. methylation
  - Chromatin accessibility, e.g. ATAC-seq
  - Chromatin 3D organization, e.g. Hi-C
  - Protein-DNA interaction, e.g. ChIP-seq

# Pediatric cancer mutations

## Pediatric Cancers Have A Low Number of Somatic and Actionable Mutations At Initial Diagnosis




**Can genomics help clinical care for cancer patients?**

# Clinomics for precision medicine

Personalized Medicine and Imaging

Clinical Cancer Research

## MultiDimensional ClinOmics for Precision Therapy of Children and Adolescent Young Adults with Relapsed and Refractory Cancer: A Report from the Center for Cancer Research

Wendy Chang[1,2,3], Andrew S. Brohl[1,4], Rajesh Patidar[1], Sivasish Sindiri[1], Jack F. Shern[1,2], Jun S. Wei[1], Young K. Song[1], Marielle E. Yohe[1,2], Berkley Gryder[1], Shile Zhang[1], Kathleen A. Calzone[5], Nityashree Shivaprasad[1], Xinyu Wen[1], Thomas C. Badgett[1,6], Markku Miettinen[7], Kip R. Hartman[8,9], James C. League-Pascual[2,8], Toby N. Trahair[10], Brigitte C. Widemann[2], Melinda S. Merchant[2], Rosandra N. Kaplan[2], Jimmy C. Lin[1], and Javed Khan[1]

Clin Cancer Res. May 2016

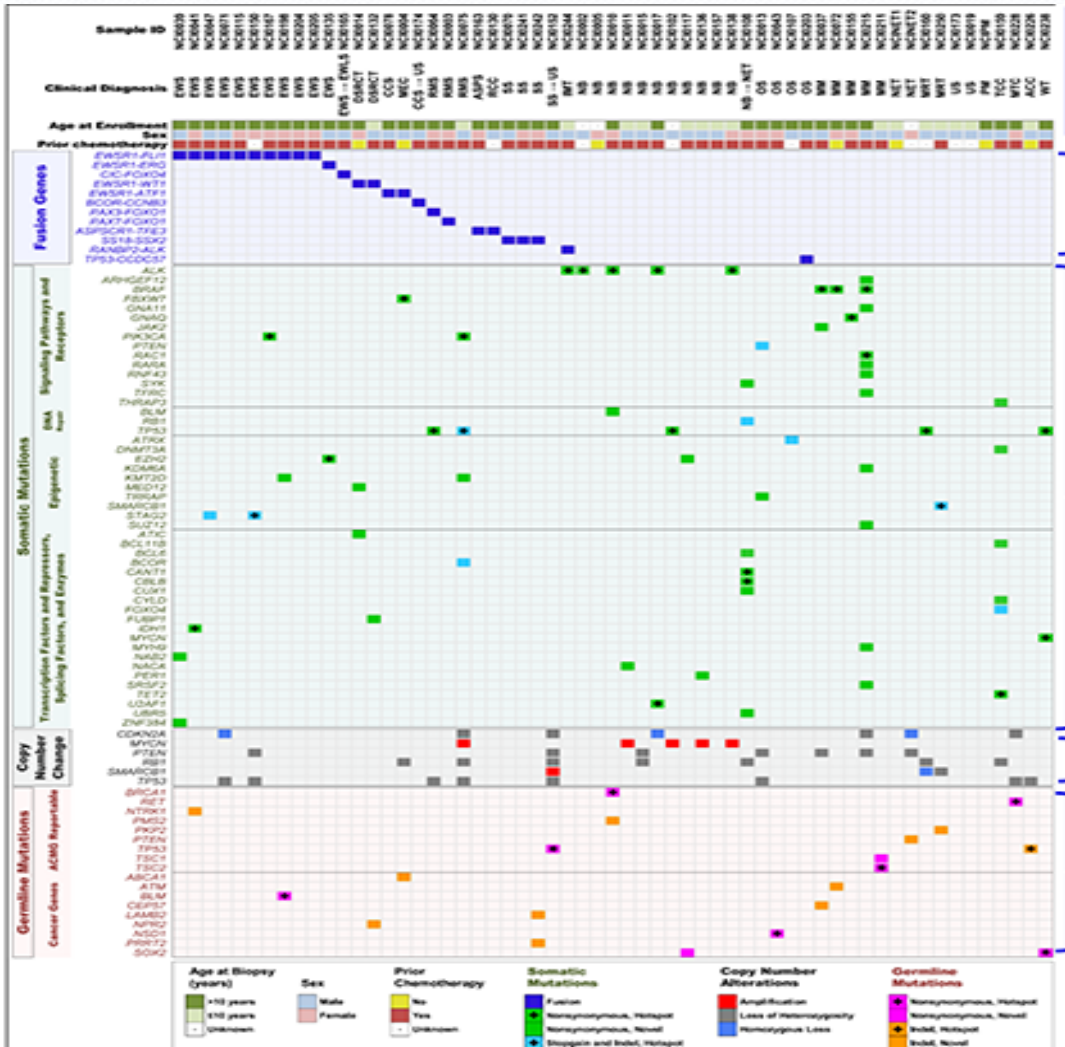
**Protocol Number:** 10-C-0086
**Title:** "Comprehensive Omics Analysis of Pediatric Solid Tumors and Establishment of a Repository for Related Biological Studies" or Omics protocol

# Study design

## Study Design

- Pilot study to determine the utility and feasibility of performing comprehensive genomic analyses to identify clinically actionable mutations in pediatric and young adult patients with metastatic, refractory or relapsed solid tumors
- 59 patients enrolled to the pediatric oncology branch, Center for Cancer Research (CCR), NCI (2010-2014)
- Age 7 months-25 years
- 20 diagnostic categories (non-CNS, solid tumors)
- Comprehensive multi-omics exome germline & tumor, RNAseq tumor & Illumina Omni SNP arrays of tumor

# Multi-omics integrated landscape




**Multi-Omics Integrated Landscape**

**RNAseq**
**Diagnostic, Driver, Actionable**

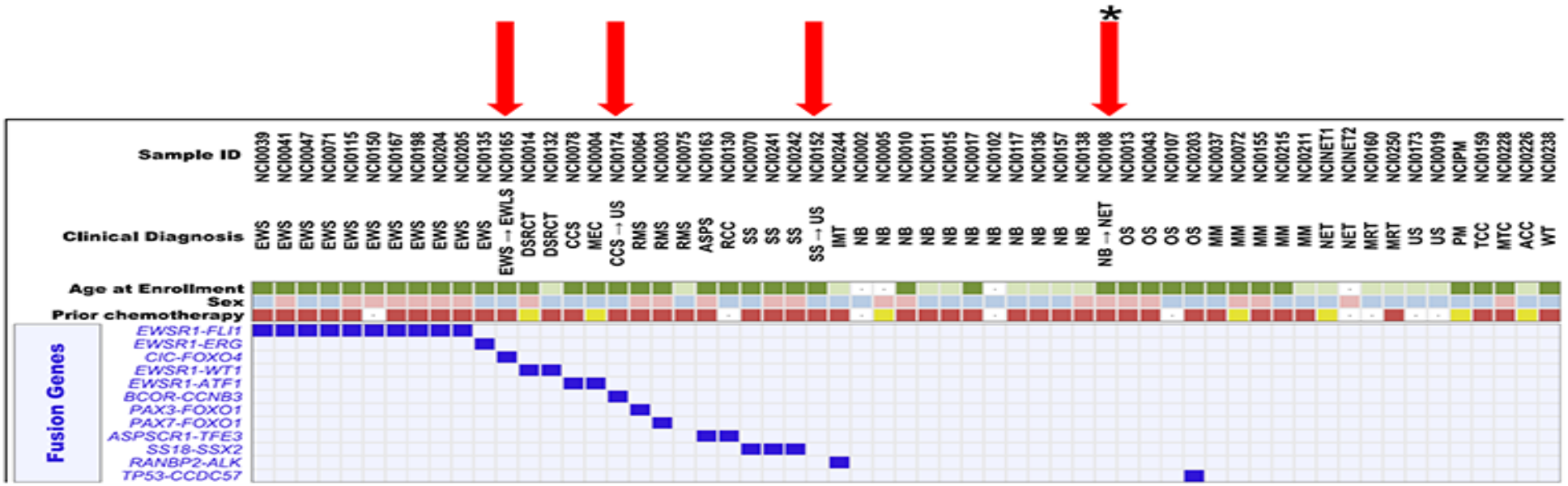
**DNAseq and RNAseq**
**Somatic: Driver, Actionable**

**DNA copy number & RNAseq**
**Somatic: Driver, Actionable**

**DNAseq**
**Germ line: Disease causing, Actionable**

# Fusion genes

## Presence or absence of fusion genes and/or expression profiles confirms diagnosis or leads to revision of diagnosis



# Germline mutations

## ~10% of Pediatric and Adolescent Young Adults with Cancers have Actionable Germline Mutations some Therapeutically

| Sample | Diagnosis | Gene | Mutation | Disease | Hotspot | Notes | ACMG gene |
|---|---|---|---|---|---|---|---|
| NCI0072 | MM | *ATM* | p.Y380fs | Ataxia-Telangiectasia and Cancer Predisposition Syndrome | No | Frameshift Insertion of Tumor Suppressor Gene | Yes |
| NCI0010 | NB | *BRCA1* | Q1313X | Hereditary Breast and Ovarian Cancer Syndrome | Yes | Pathogenic, Reportable | Yes |
| NCI0010 | NB | *PMS2* | p.K356fs | Lynch Syndrome and Mismatch Repair Cancer Syndrome | No | Frameshift Deletion of Tumor Suppressor Gene | Yes |
| [redacted] | NET | *PTEN* | p.R14fs | PTEN Hamartoma Tumor Syndrome | No | Frameshift Deletion of Tumor Suppressor Gene | Yes |
| [redacted] | MTC | *RET* | M918T | Multiple Endocrine Neoplasia 2B | Yes | Pathogenic, Reportable | Yes |
| NCI0152 | SS → US | *TP53* | R175H | Li-Fraumeni Syndrome | Yes | Patient Tumor has LOH of Wild-Type TP53 on Other Allele | No |
| NCI0226 | ACC | *TP53* | A159K | Li-Fraumeni Syndrome | Yes | Tumor has LOH of Wild-Type TP53 on Other Allele, Novel, 2 Base Non-Frameshift Substitution, c.358_359delGCinsTT | No |
| [redacted] | MM | *TSC1* | p.S828R | Tuberous Sclerosis Type 1, Lymphangioleiomyomatosis, Focal Cortical Dysplasia, and Everolimus Sensitivity | No | Nonsynonymous SNV, Autosomal Dominant, Patient also has a Germline TSC2 Mutation | No |
| [redacted] | MM | *TSC2* | p.T246A | Tuberous Sclerosis Type 2, and Lymphangioleiomyomatosis | Yes | Nonsynonymous SNV, Autosomal Dominant, Patient also has a Germline TSC1 Mutation | No |

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# Tumor mutations

## Approximately 50% of Pediatric and Adolescent Young Adults with Cancers have Actionable Tumor Mutations

| Sample | Diagnosis | Gene | Stage | Modality | AA Change | Level | Drug | Clinical Trial: Pediatric | FDA Approval in Adults | Exact Mutation vs. Hotspot |
|---|---|---|---|---|---|---|---|---|---|---|
| NCI0041 | EWS | *IDH1* | Relapsed | *WES/WTS* | p.R132C | 2a | IDH1 inhibitors | No | No | Exact |
| NCI0167 | EWS | *PIK3CA* | Refractory | *WES/WTS* | p.D1017G | 2a | PI3K/AKT/ mTOR inhibitors | Yes | Yes | Exact |
| NCI0071 | EWS | *CDKN2A* | Relapsed | *SNP Array/WTS* | Homozygous loss | 3 | CDK4/6 inhibitor | No | No | - |
| NCI0047 | EWS | *STAG2* | Relapsed | *WES/WTS* | p.E984X | 3 | PARP inhibitors | Yes | No | - |
| NCI0150 | EWS | *STAG2* | - | *WES/WTS* | p.R216X | 3 | PARP inhibitors | Yes | No | Hotspot |
| NCI0244 | IMT | *ALK* | Relapsed | *WTS* | *RANBP2-ALK* fusion | 2a | Crizotinib | No | Yes | Exact |
| NCI0244 | IMT | *ALK* | Relapsed | *WES/WTS* | p.I1171T | 2a | Ceritinib | No | Yes | Exact |
| NCI0037 | MM | *BRAF* | Relapsed | *WES/WTS* | p.V600E | 1 | Vemurafenib, Dabrafenib | Yes | Yes | Exact |
| NCI0072 | MM | *BRAF* | Diagnostic | *WES/WTS* | p.V600E | 1 | Vemurafenib, Dabrafenib | Yes | Yes | Exact |
| NCI0215 | MM | *BRAF* | Relapsed | *WES/WTS* | p.V600E | 1 | Vemurafenib, Dabrafenib | Yes | Yes | Exact |
| NCI0155 | MM | *GNAQ* | Relapsed | *WES/WTS* | p.Q209L | 1 | Temsirolimus, Trametinib, Vorinostat | No | Yes | Exact |
| NCI0215 | MM | *GNA11* | Relapsed | *WES/WTS* | p.S268F | 2a | Trametinib | No | Yes | - |
| NCI0211 | MM | *TSC1* | Relapsed | *WES/WTS* | p.S828R | 3 | Everolimus | No | Yes | - |
| NCI0211 | MM | *TSC2* | Relapsed | *WES/WTS* | p.T246A | 3 | Everolimus | No | Yes | - |
| NCI0160 | MRT | *SMARCB1* | - | *SNP Array/WTS* | Homozygous loss | 3 | EZH2 inhibitors | No | No | - |
| NCI0250 | MRT | *SMARCB1* | Refractory | *WES/WTS* | p.R40X | 3 | EZH2 inhibitors | No | No | - |
| NCI0228 | MTC | *RET* | Relapsed | *WES/WTS* | p.M918T | 2a | Vandetanib | Yes | Yes | Exact |
| NCI0002 | NB | *ALK* | - | *WES/WTS* | p.R1275Q | 2a | Crizotinib | Yes | Yes | Exact |
| NCI0010 | NB | *ALK* | Relapsed | *WES/WTS* | p.F1174V | 2a | Crizotinib | Yes | Yes | Exact |
| NCI0017 | NB | *ALK* | Relapsed | *WES/WTS* | p.F1174L | 2a | Crizotinib | Yes | Yes | Exact |
| NCI0138 | NB | *ALK* | Relapsed | *WES/WTS* | p.Y1278S | 2a | Crizotinib | Yes | Yes | Exact |
| NCI0017 | NB | *CDKN2A* | Relapsed | *SNP Array/WTS* | Homozygous loss | 3 | CDK4/6 inhibitor | No | No | - |

| Sample | Diagnosis | Gene | Stage | Modality | AA Change | Level | Drug | Clinical Trial: Pediatric | FDA Approval in Adults | Exact Mutation vs. Hotspot |
|---|---|---|---|---|---|---|---|---|---|---|
| NCI0011 | NB | *MYCN* | Relapsed | *SNP Array/WTS* | Amplification | 3 | bromodomain inhibitors | No | No | - |
| NCI0102 | NB | *MYCN* | - | *SNP Array/WTS* | Amplification | 3 | bromodomain inhibitors | No | No | - |
| NCI0136 | NB | *MYCN* | Relapsed | *SNP Array/WTS* | Amplification | 3 | bromodomain inhibitors | No | No | - |
| NCI0138 | NB | *MYCN* | Relapsed | *SNP Array/WTS* | Amplification | 3 | bromodomain inhibitors | No | No | - |
| NCINET2 | NET | *PTEN* | - | *WES/WTS* | p.R14fs | 2a | PI3K/AKT/mTOR inhibitors | Yes | No | - |
| NCINET2 | NET | *CDKN2A* | - | *SNP Array/WTS* | Homozygous loss | 3 | CDK4/6 inhibitor | No | No | - |
| NCI0013 | OS | *PTEN* | Relapsed | *WES/WTS* | p.K80fs | 2a | PI3K/AKT/mTOR inhibitors | Yes | No | - |
| NCI0075 | RMS | *PIK3CA* | Relapsed | *WES/WTS* | p.P104Q | 2a | PI3K/AKT/ mTOR inhibitors | Yes | Yes | Exact |
| NCI0075 | RMS | *MYCN* | Relapsed | *SNP Array/WTS* | Amplification | 3 | bromodomain inhibitors | No | No | - |
| NCI0238 | WT | *MYCN* | Relapsed | *WES/WTS* | p.P44L | 3 | bromodomain inhibitors | No | No | - |

### NCI-Adult MATCH
### Criteria for Matching Mutation to Drug

| | |
|---|---|
| **Level 1** | Gene variant approved for selection of an approved drug (BRAF V600E and vemurafenib). The variant will be Level 1 in all tissues open to treatment with the approved drug. |
| **Level 2a** | Gene variant is an eligibility criteria for an ongoing clinical trial for that treatment. |
| **Level 2b** | Gene variant has been identified in an N of 1 responses (TSC1 and everolimus) for that treatment |
| **Level 3** | Preclinical inferential data *(in vivo* and *in vitro* models*)* that provide biological evidence sufficient to support the use of a variant for treatment selection, e.g.<br>• Models with variants respond to treatment and models without variant do not respond to treatment<br>• Gain of function mutations demonstrated in pre-clinical model, e.g. D769H variant of ERBB2 results in increased tyrosine kinase-specific activity and up regulates pathway signaling (does not require treatment evidence)<br>• Loss of function genes, tumor suppressor or pathway inhibitor (e.g. NF1) any variant that produces a stop codon including frameshift or demonstrated loss of function in pre-clinical model (does not require treatment evidence |

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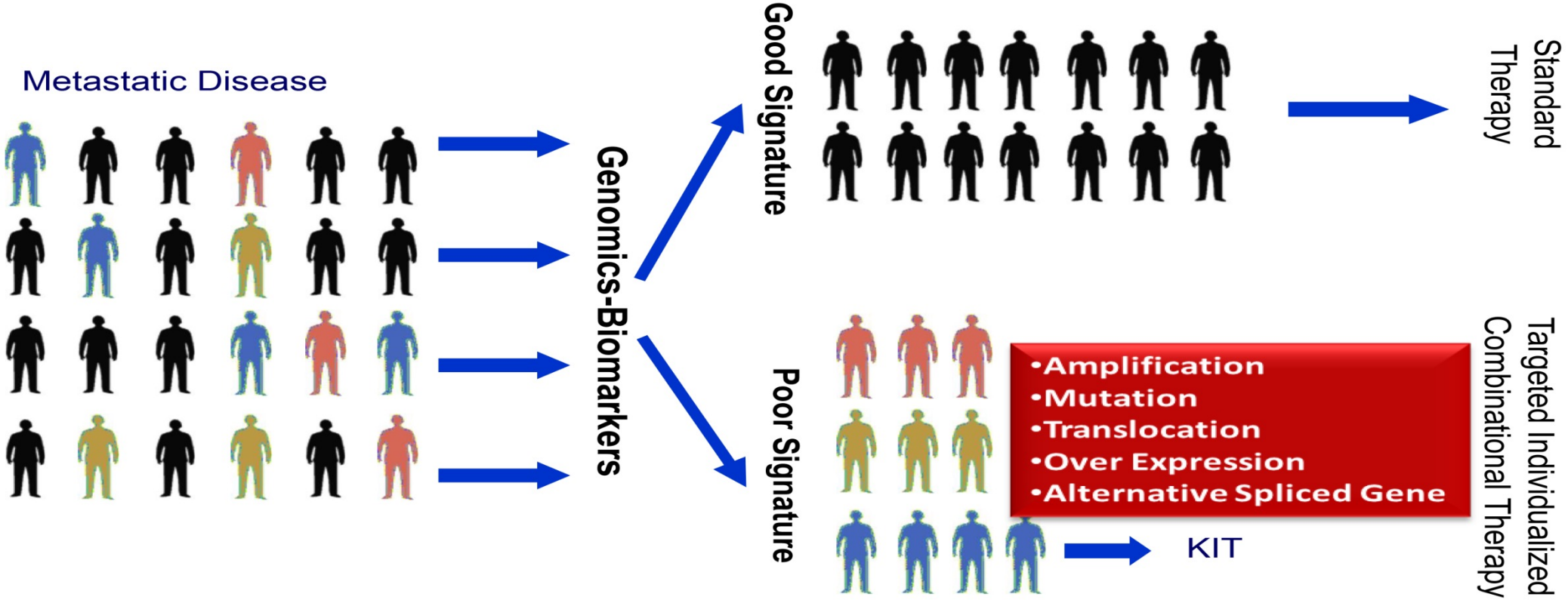
# Summary

Summary

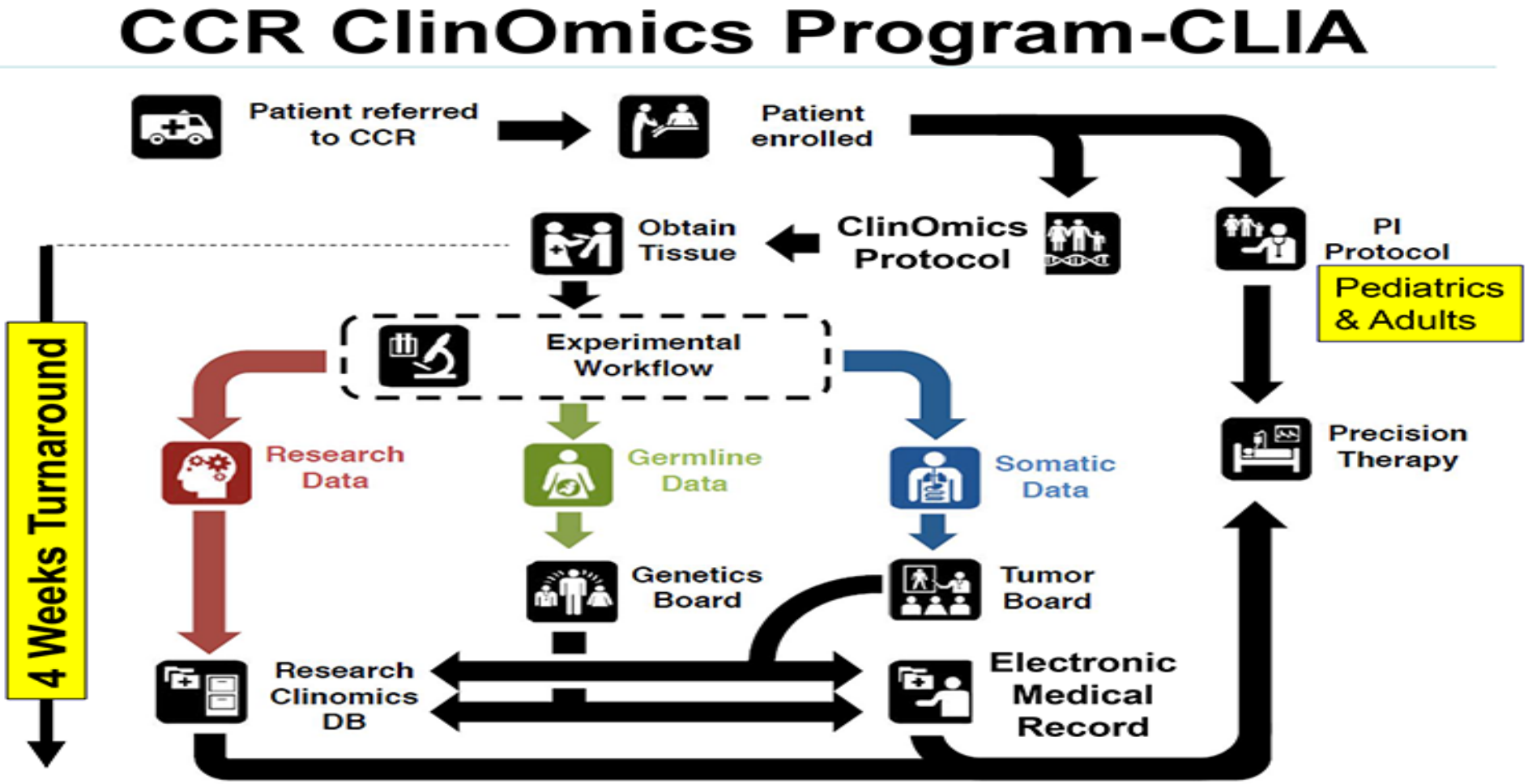
- Demonstrated the importance and feasibility of performing multi-dimensional ClinOmics in the clinical setting in real time
- ~50% of children with pediatric or AYA patients with relapsed or refractory cancers have actionable somatic mutations
- ~ 10% have actionable germline mutations
- Importance of performing parallel germline sequencing; some therapeutically actionable (e.g. DNA repair, PTEN, TSC1, TSC2, HRAS, RET, ALK)
- Increased tumor burden in relapsed tumors; implications for immunotherapy
- Single agent pediatric MATCH like trials are planned by COG-NCI

# Future Trials

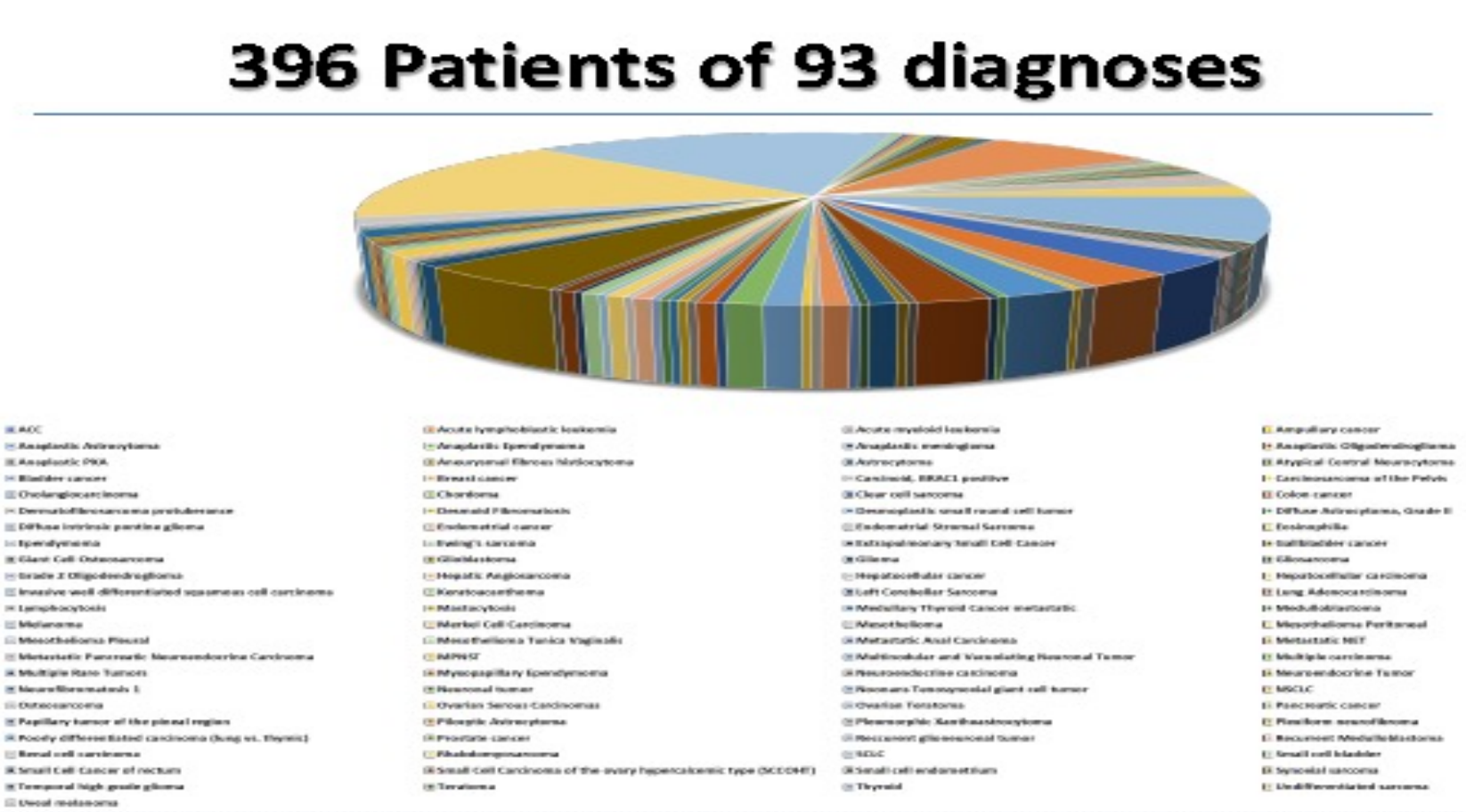
## Genomics Enabling Precision Therapy-The Future for Pediatric Trials



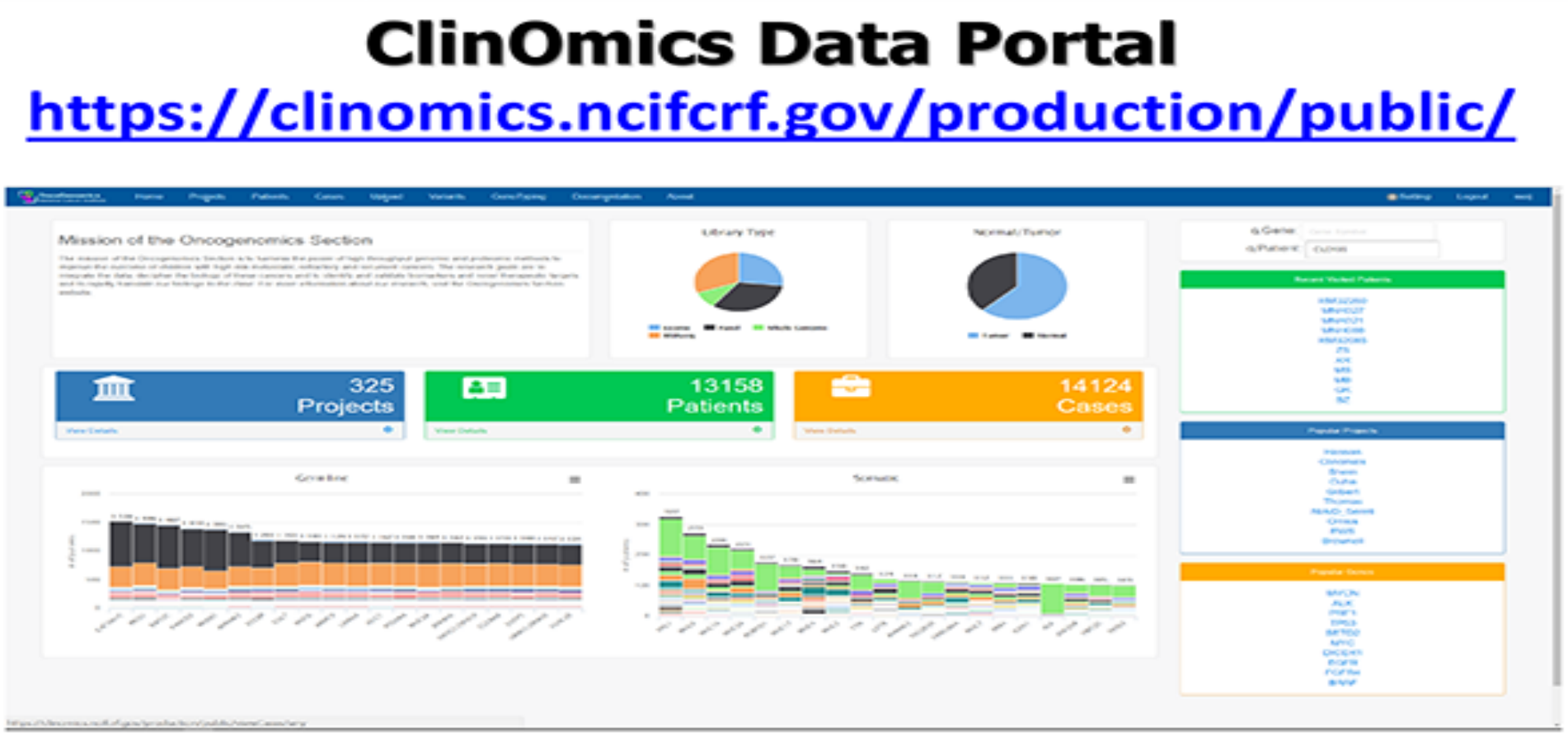
# ClinOmics program



# Patient diagnoses

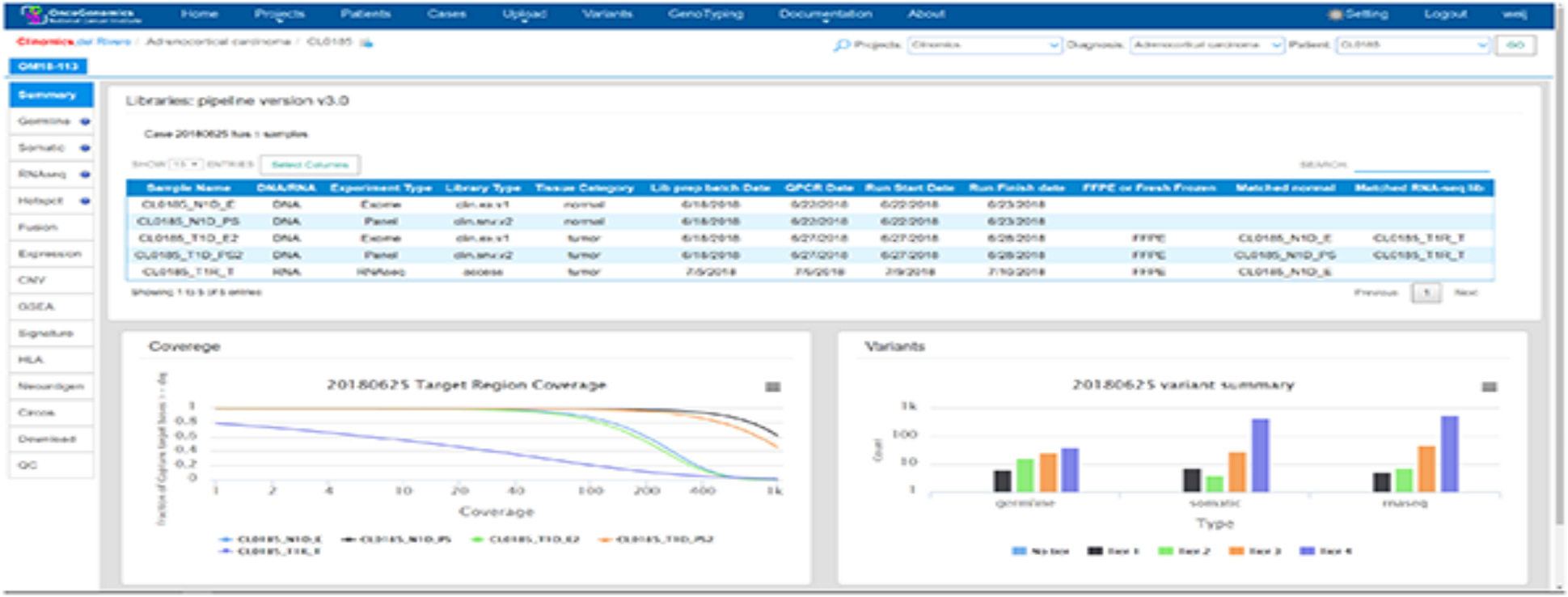


# ClinOmics Data Portal



# Patient Summary

## Patient Summary Page



# QC report

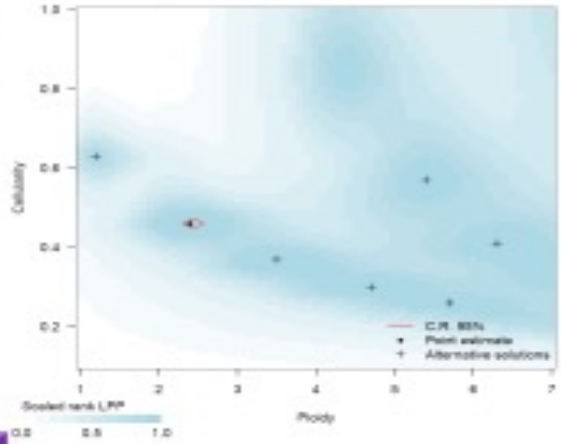
## QC Report:
## Sequencing Statistics & Genotyping

# QC Report: Coverage

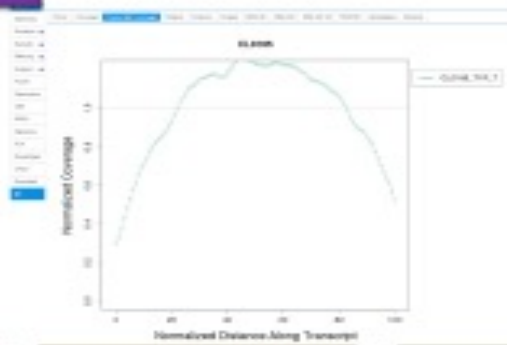
QC Report: Coverage

Circos

Tumor Content



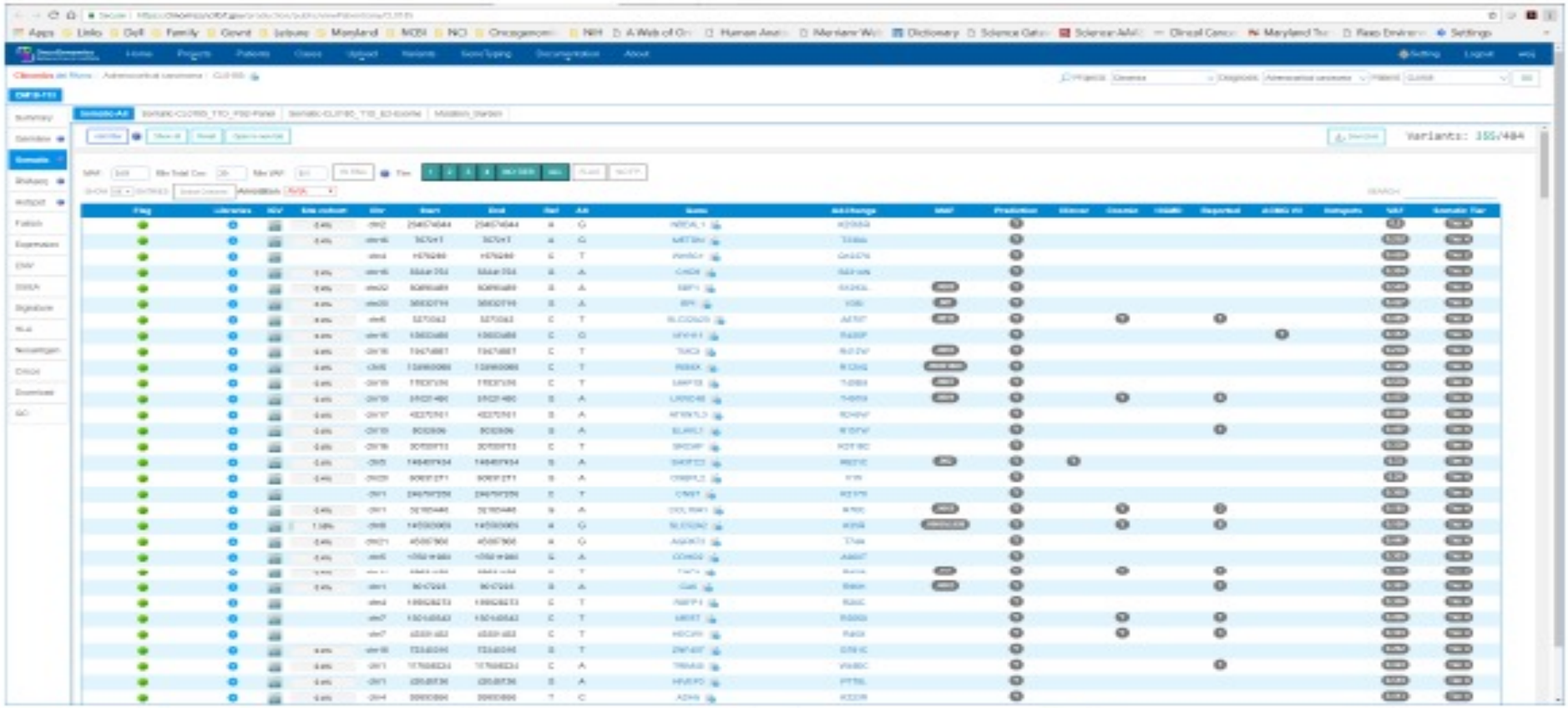

RNA Coverage




Hotspot Coverage

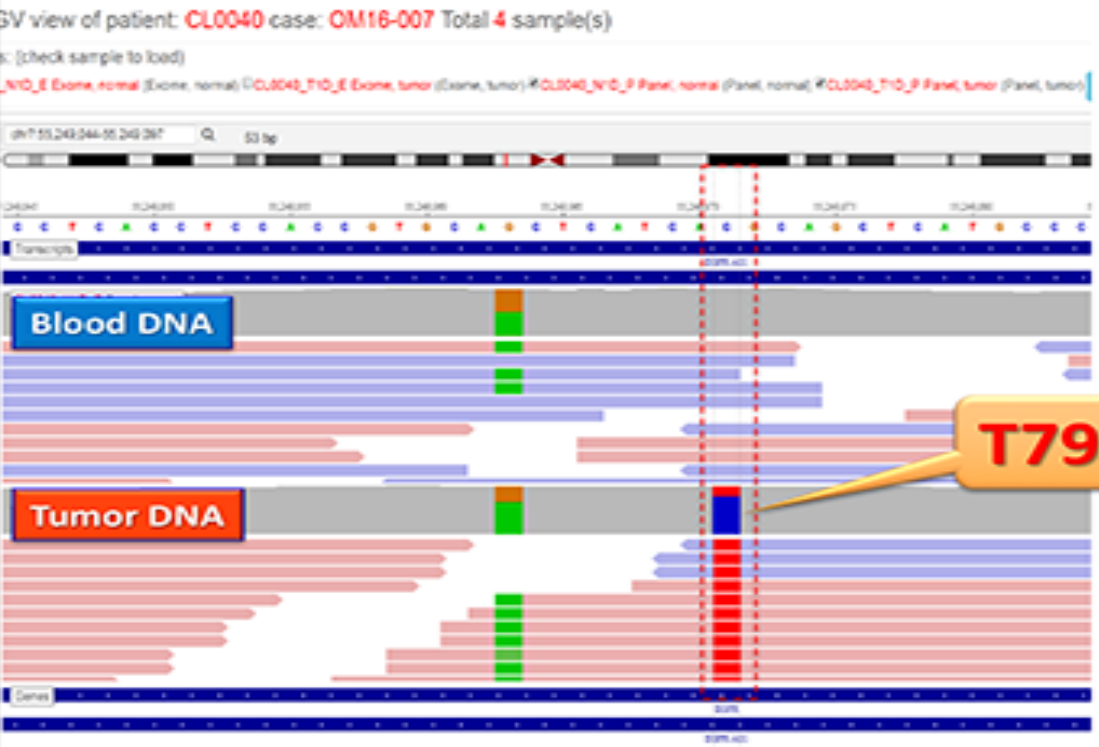
# Germline and somatic mutations

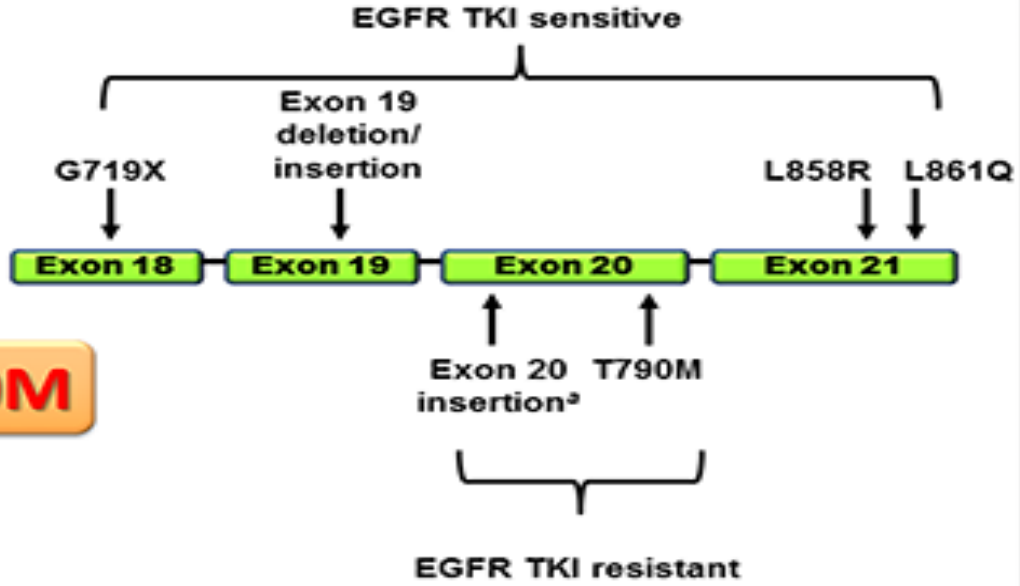
## Germline and Somatic Mutations



# EGFR mutations

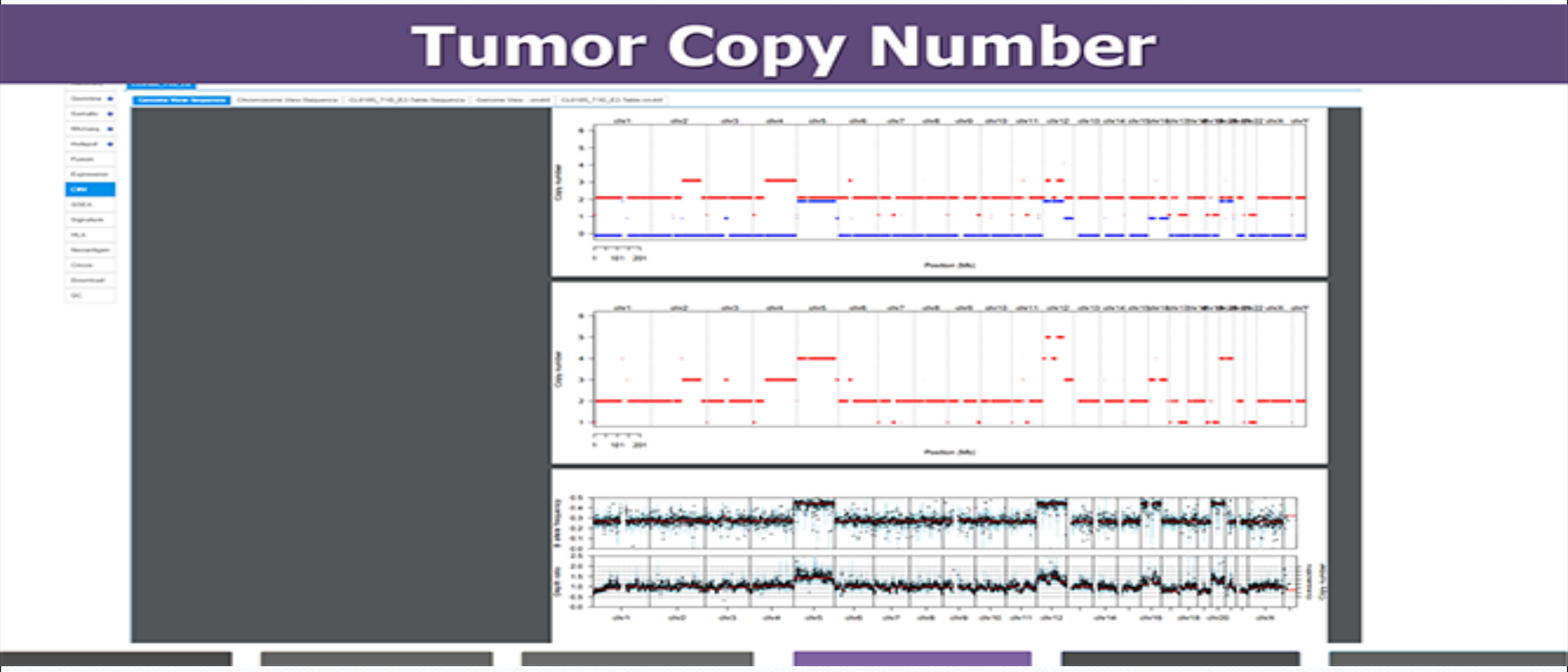
## EGFR mutations in NSCLC



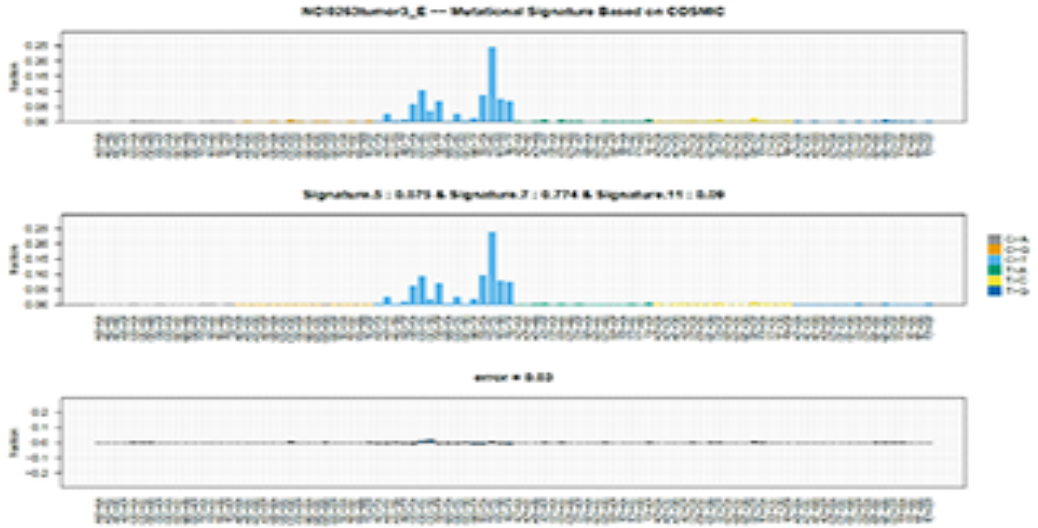

https://www.mycancergenome.org/content/disease/lung-cancer/egfr/

# Tumor Copy Number

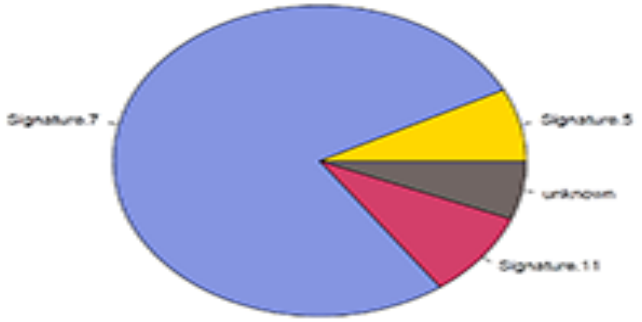


# Mutation Signatures

## Mutation Signatures for Tumor



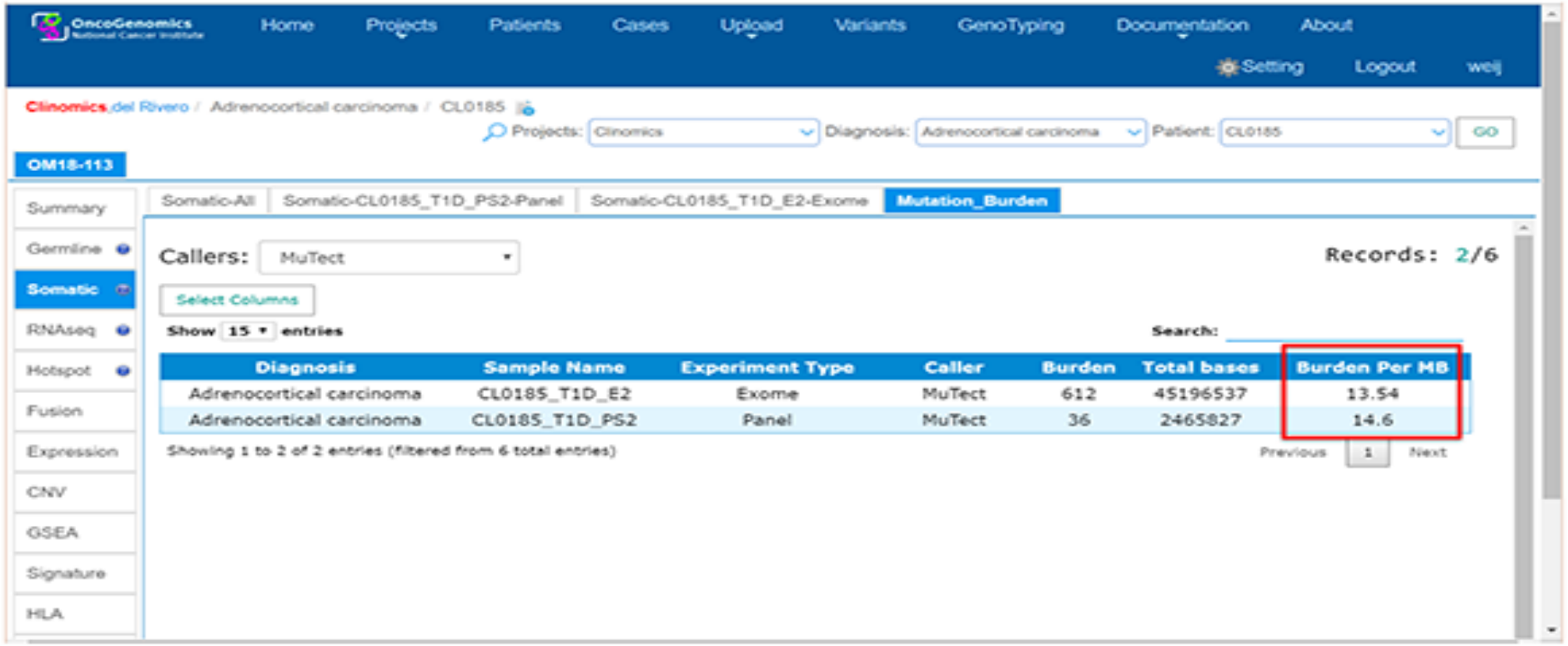
NCI0263: Melanoma




COSMIC (https://cancer.sanger.ac.uk/cosmic/signatures)

Signature 7: UV signature

# Mutation Burden



# Fusion Gene Detection

## Fusion Gene Detection from RNA-seq experiments

# Useful Genomic Information

## Other Useful Genomic Information

- HLA typing (Tissue typing)
- Neoantigen prediction
- Gene expression
- Gene Set Enrichment Analysis (GSEA)
- Survival analysis if outcome data is available

# Conclusions:

Next generation sequencing (including whole genome, exome and transcriptome) determines the complete genomic and epigenetic portrait of cancers at the base pair level.

Integrated analyses of the cancer can identify biologically relevant diagnostic, prognostic biomarkers and novel targets for precision medicine.

# Acknowledgements