Genomics and precision medicine

Outline

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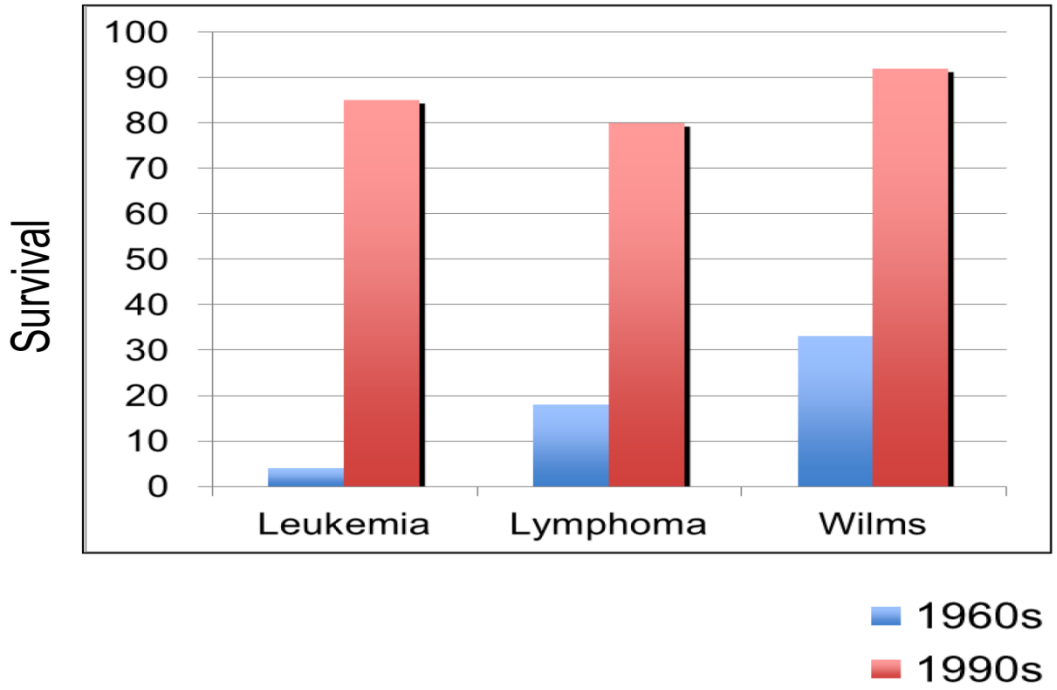
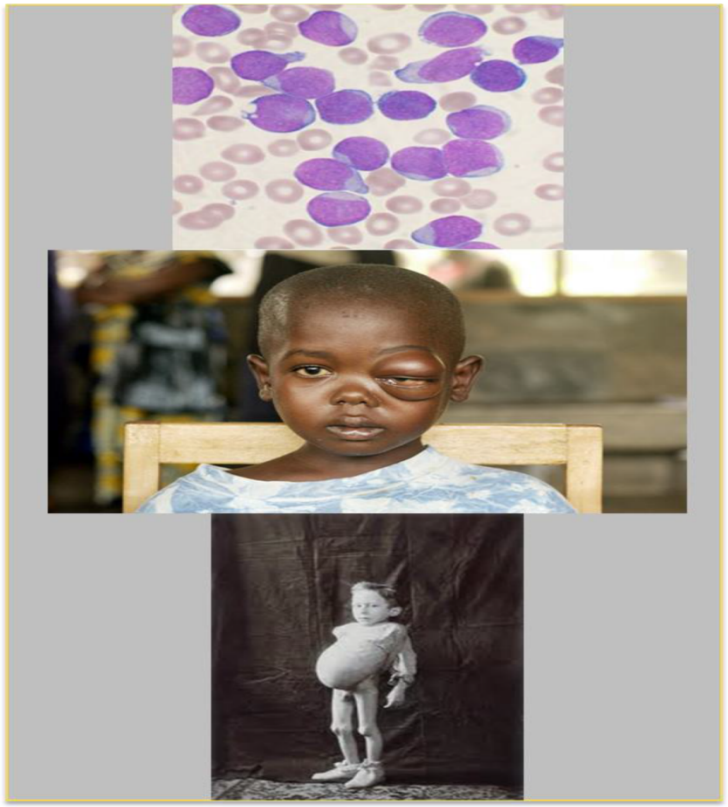
- Success and Challenges of Treating Pediatric Cancers
- Genomics
- Next-generation Sequencing
- Application of next-generation sequencing:
  - Diagnosis
  - Identification of molecular target
- Precision Therapy

Childhood cancer

National Cancer Institute

Childhood cancer: The beginning of a modern medical success story



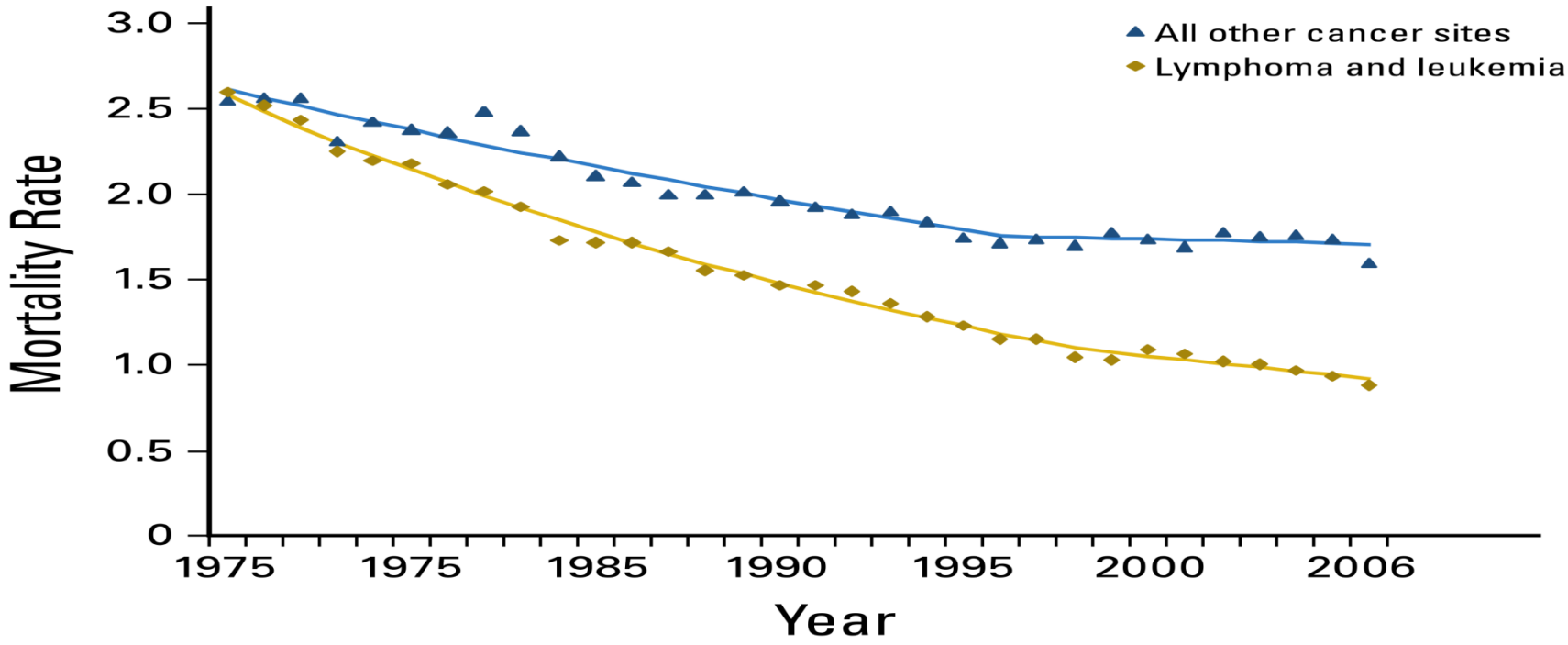


Courtesy: John Maris

Mortality rates

National Cancer Institute

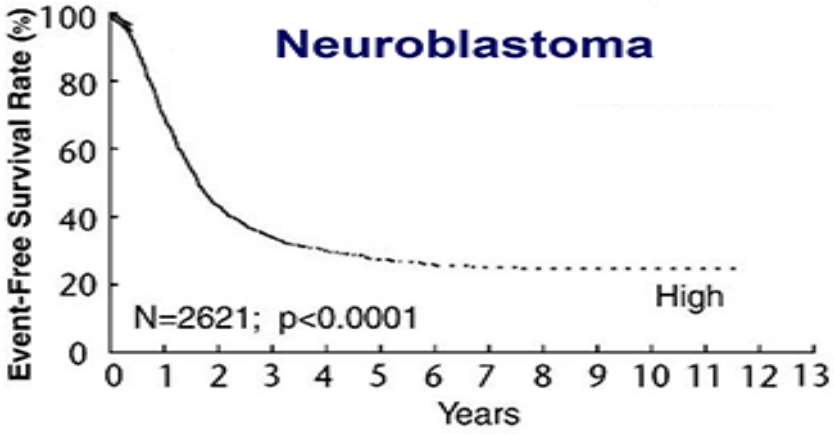
However in the past 16 years no improvement in mortality rates despite increased intensity of treatment

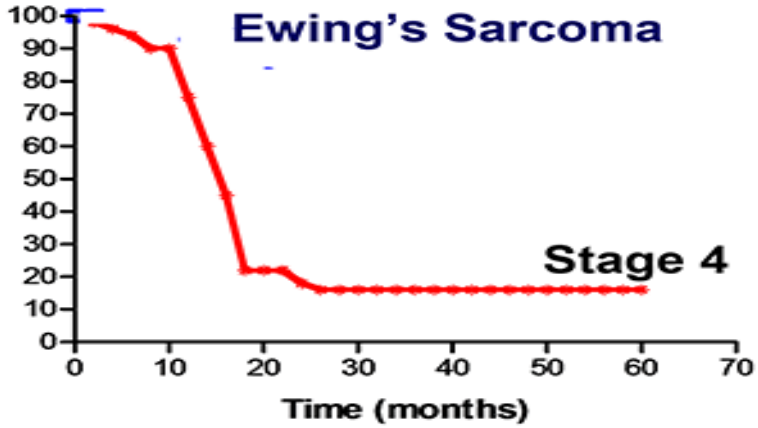


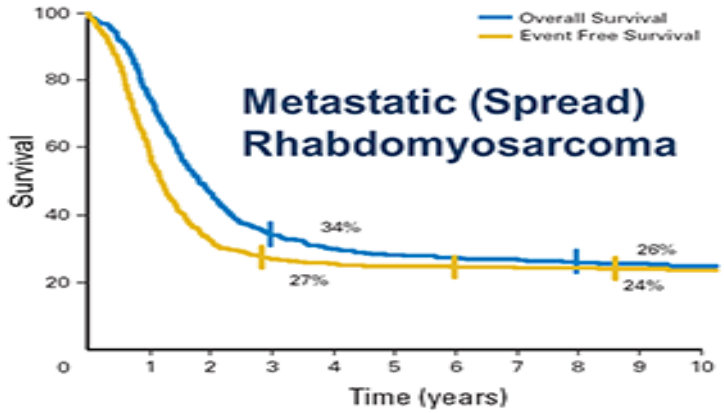
Courtesy: Malcolm Smith

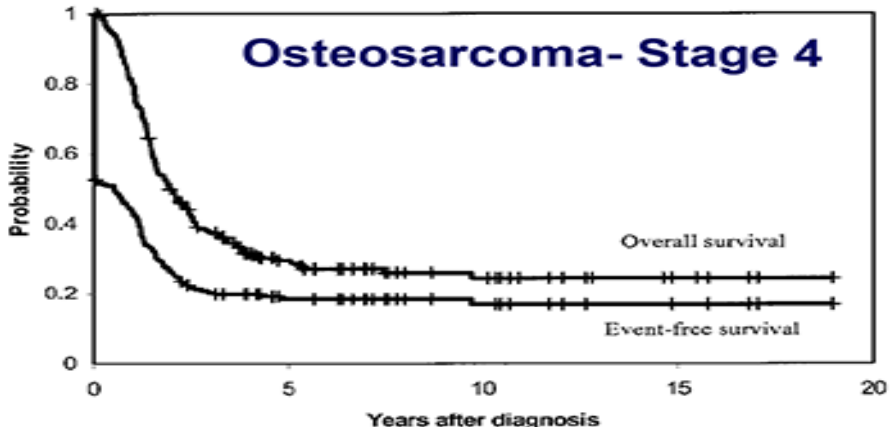
Pediatric cancers

Metastatic, Recurrent, & Refractory Disease Remains Incurable









Gene expression

The dramatic consequences of gene expression in biology

Anise swallowtail, *Papilio zelicaon*

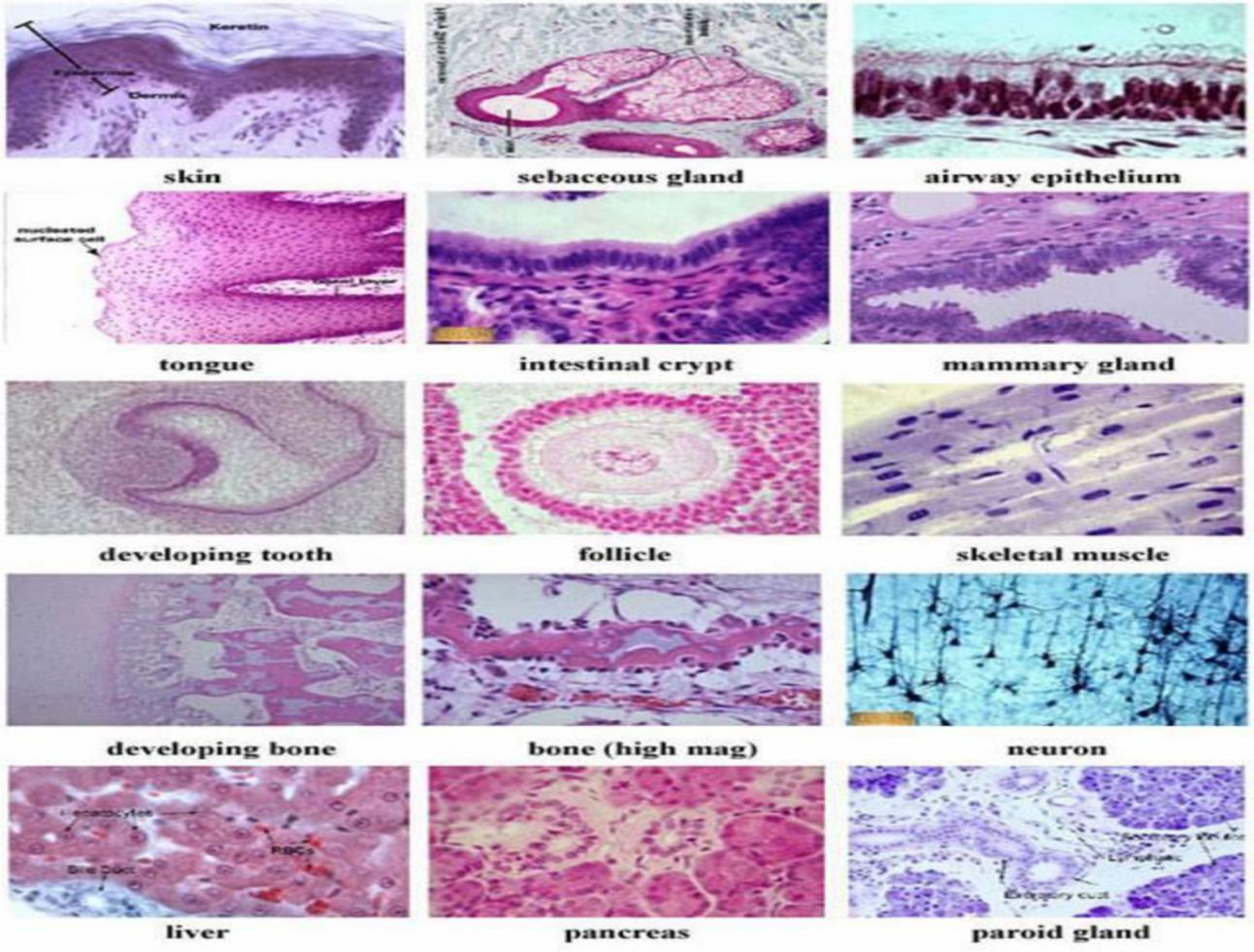
Same genome →
Different expression pattern
Different proteome
Different tissues
Different physiology

Gene expression

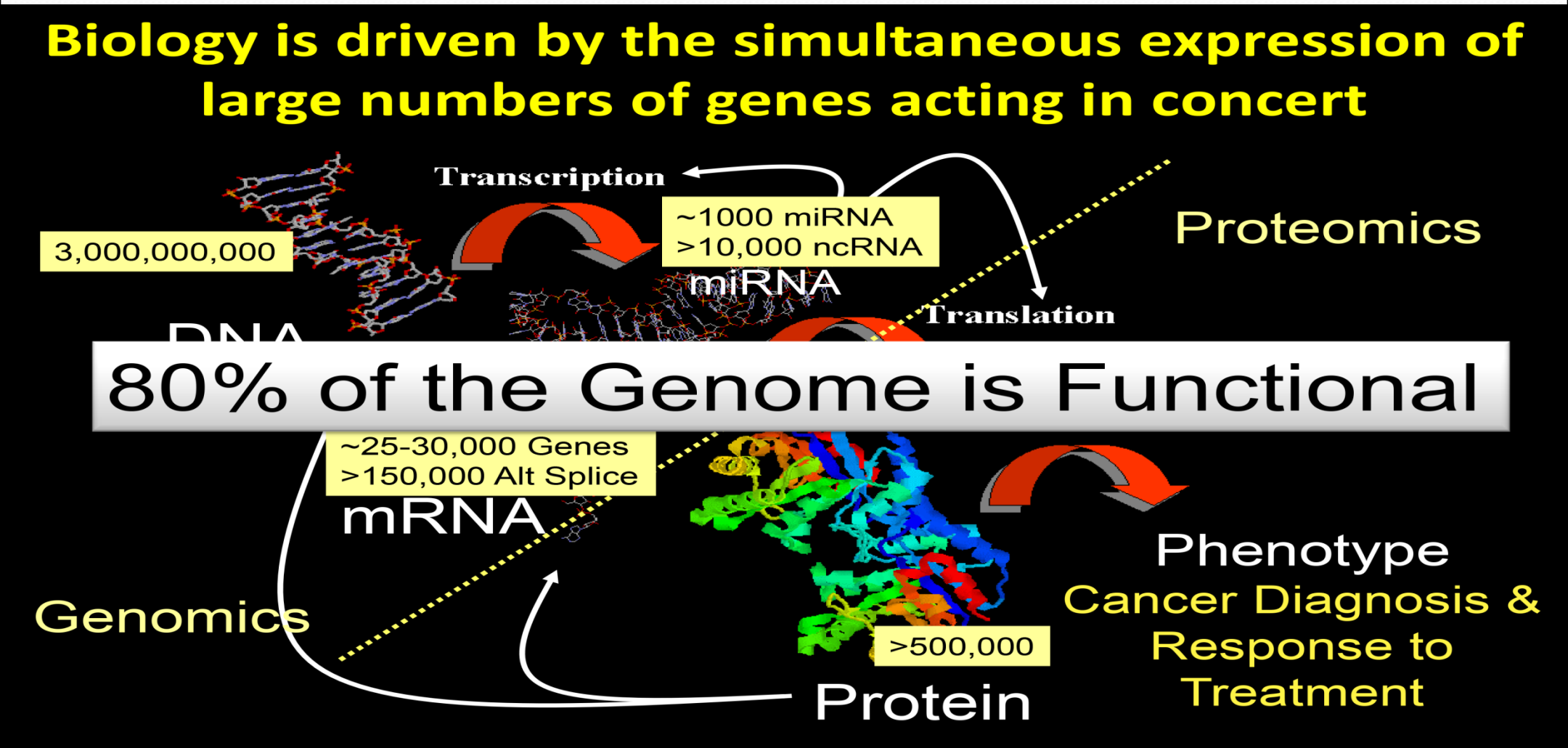
...but the complexity and diversi

Same genome or DNA →

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



Gene expression

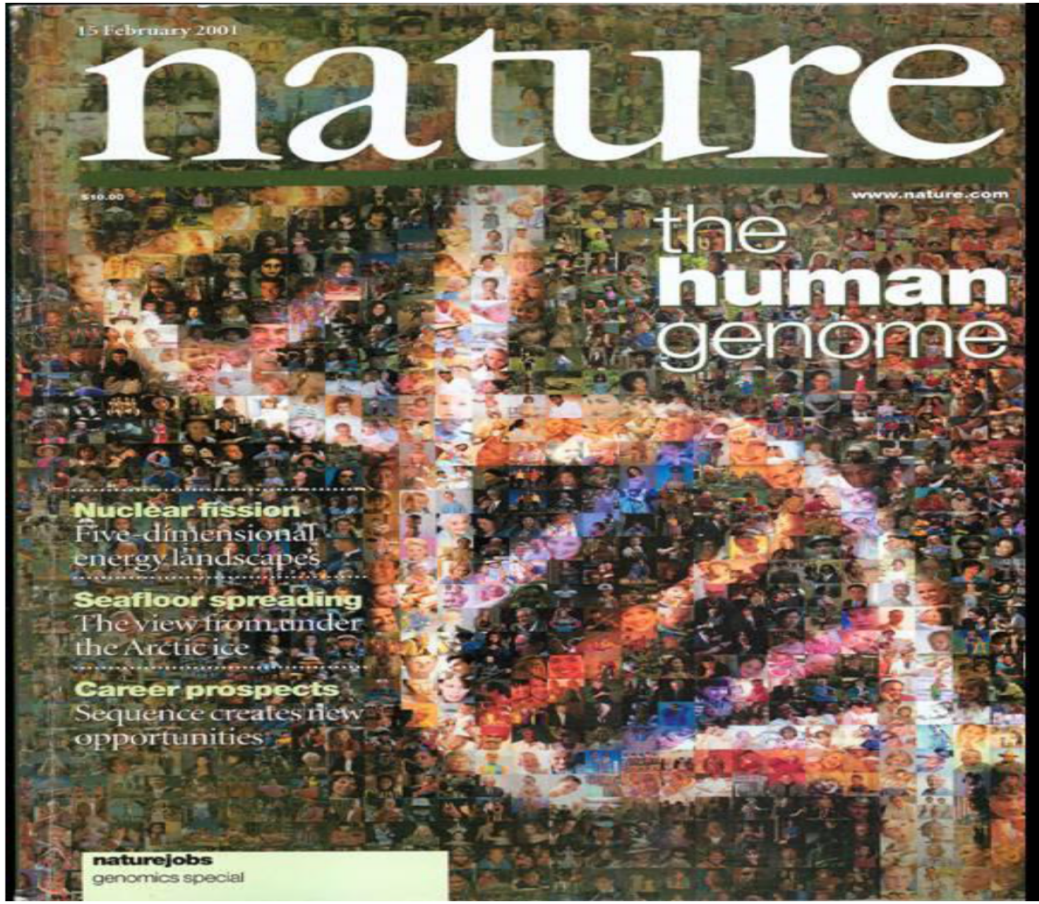


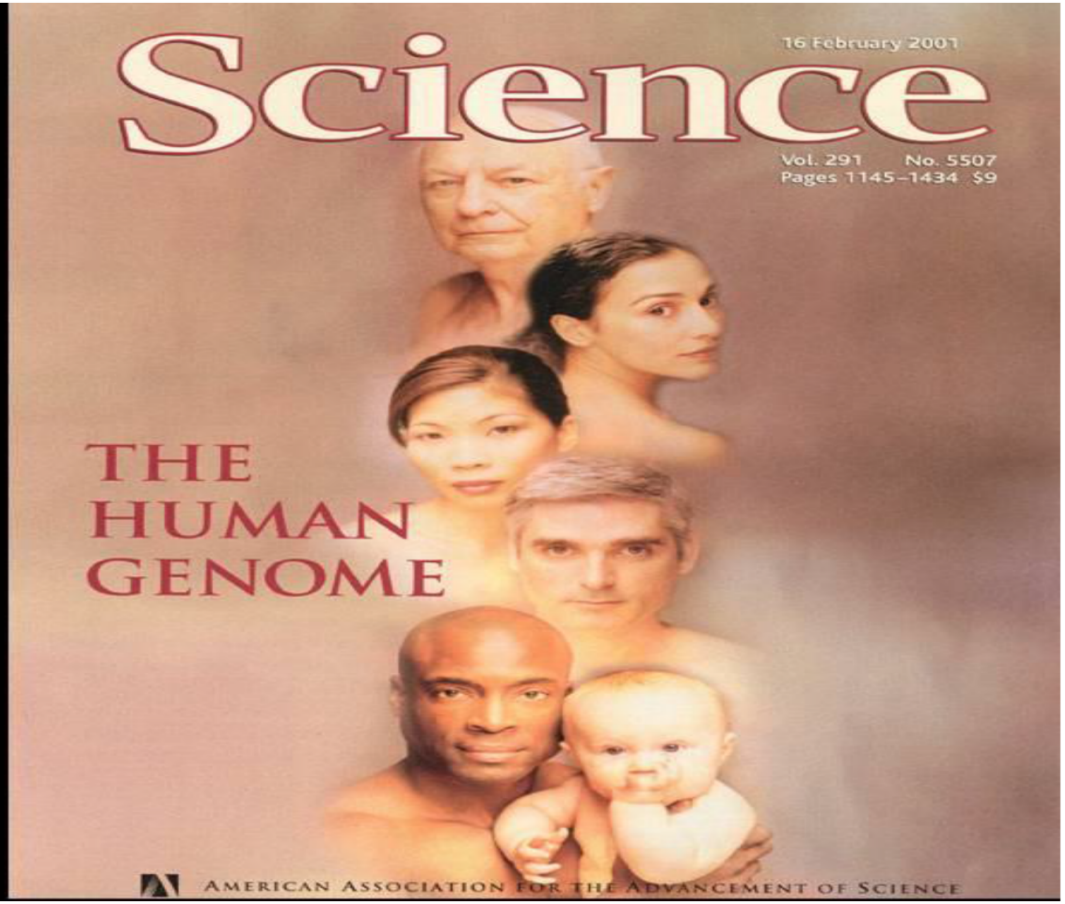
Gene measurement

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

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

Human genome





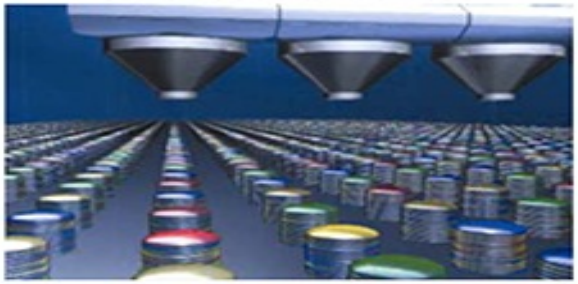
First generation tools

1st generation genomic tool: microarrays

Printing microarrays

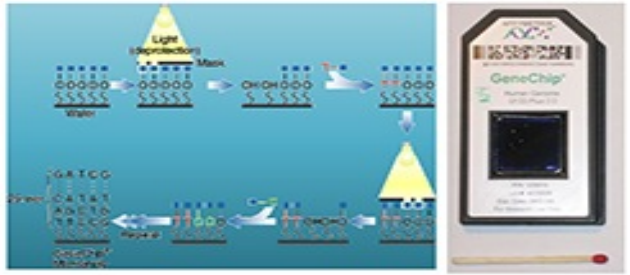
Mechanical

Electronic Piezo

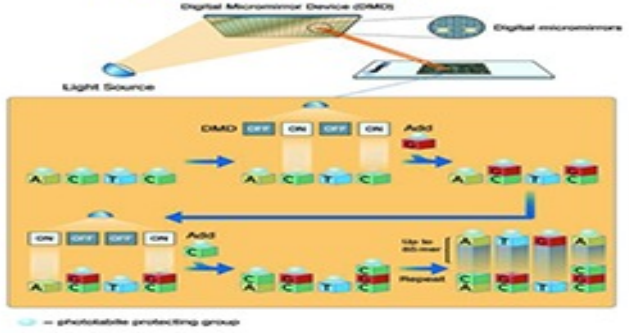


In-situ synthesis microarrays

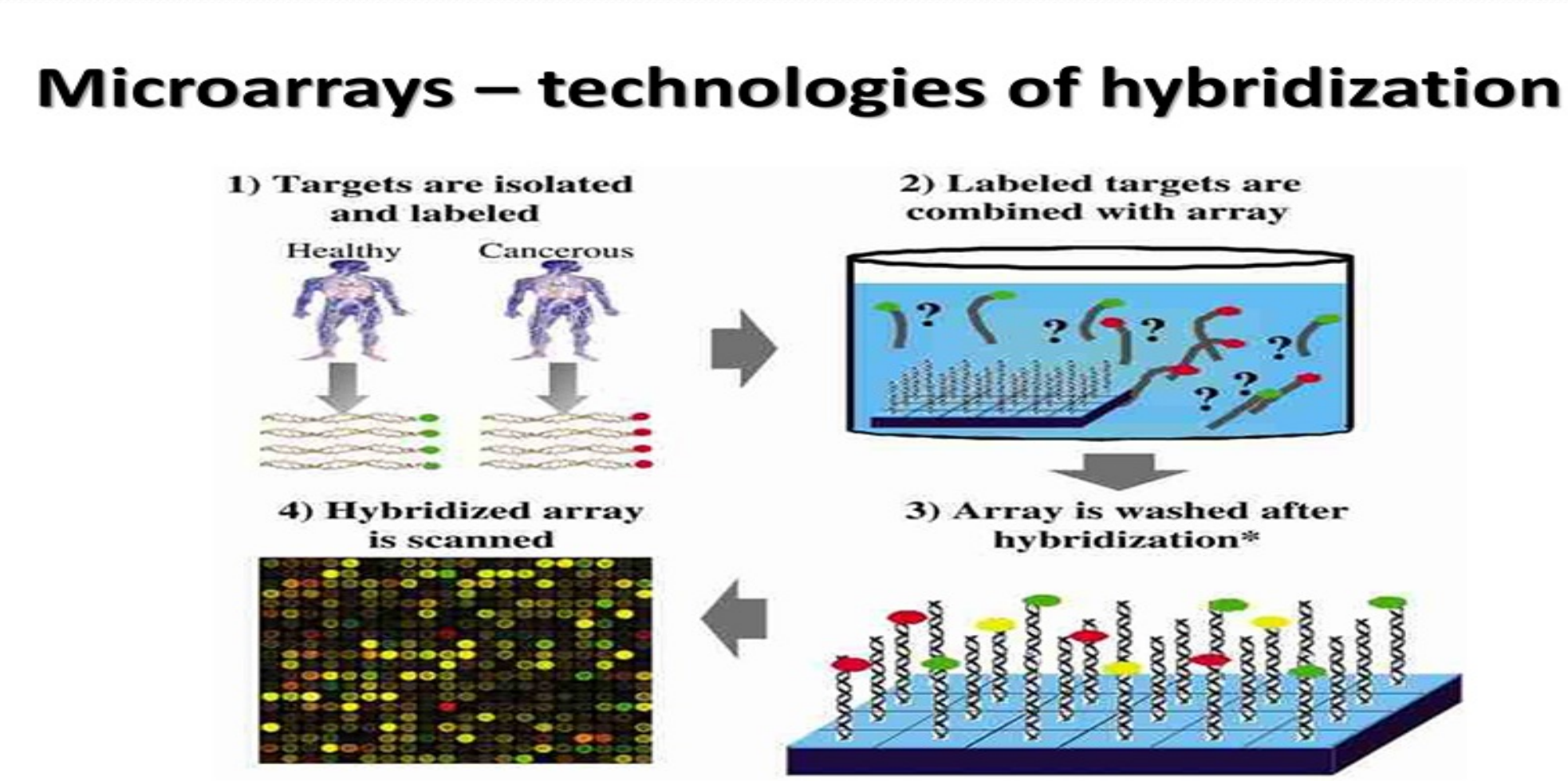
Lithographic masks and de-protection through illumination



Digital micromirrow device (DMD)

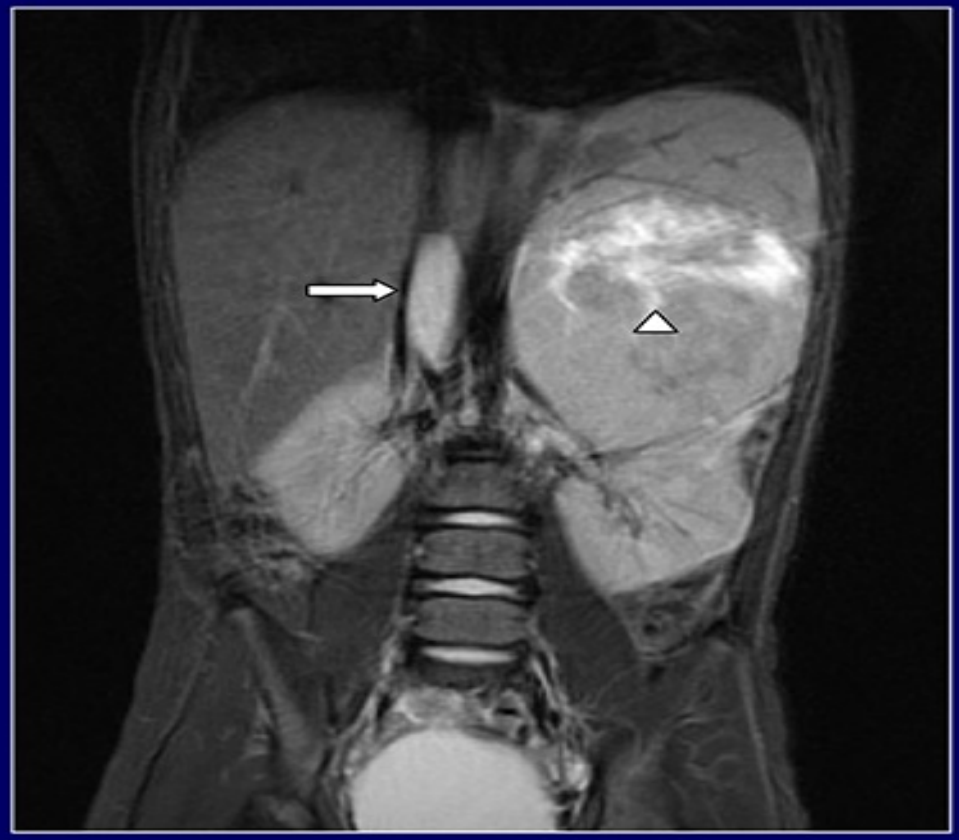


Microarrays

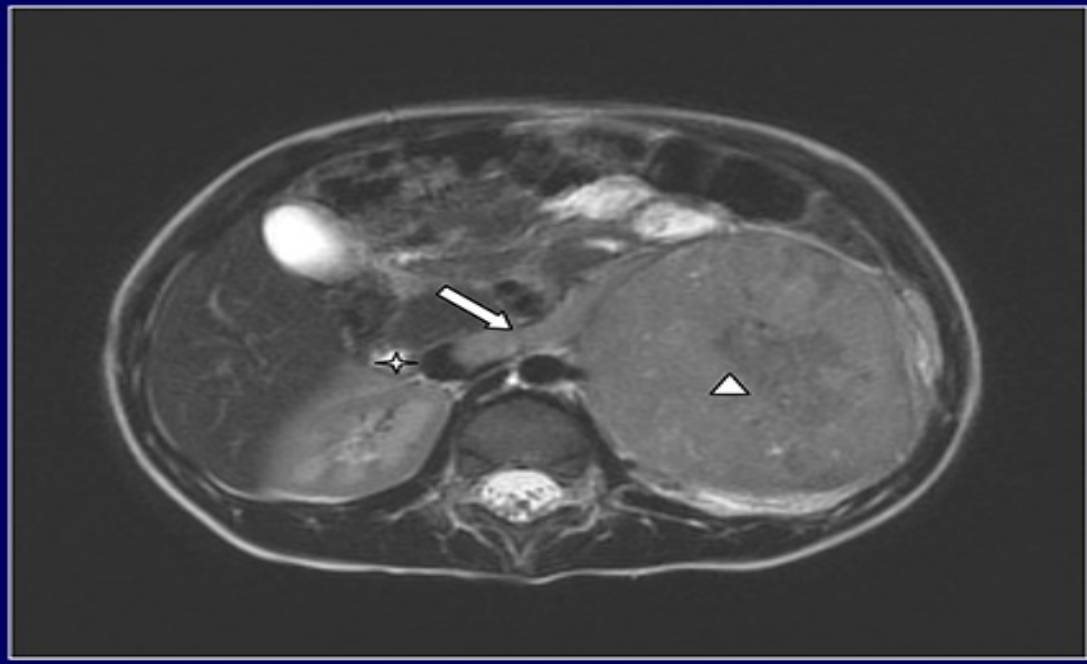


Wilms tumor

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

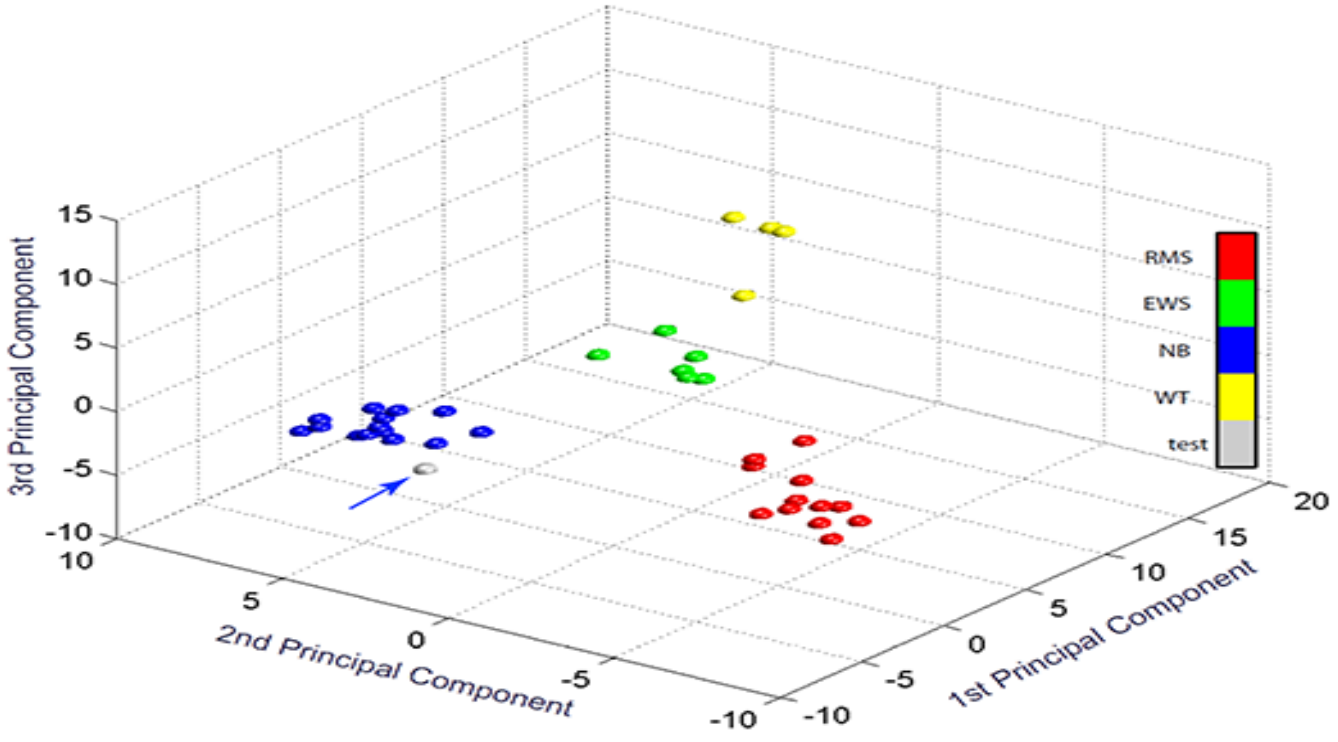


Initial diagnosis: Wilm's tumor



Cancer diagnosis

Diagnosis of cancers using gene expression profiles



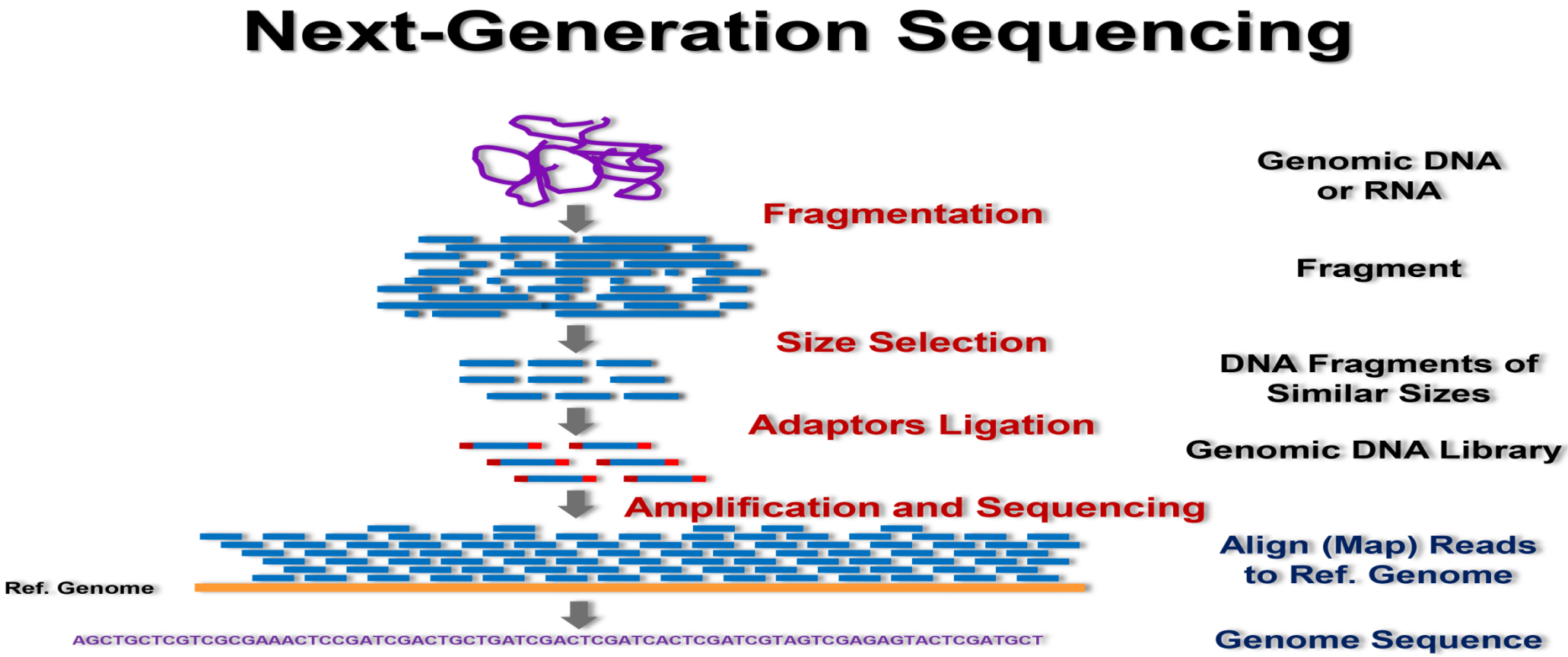
Wilm's tumor

↓

Neuroblastoma

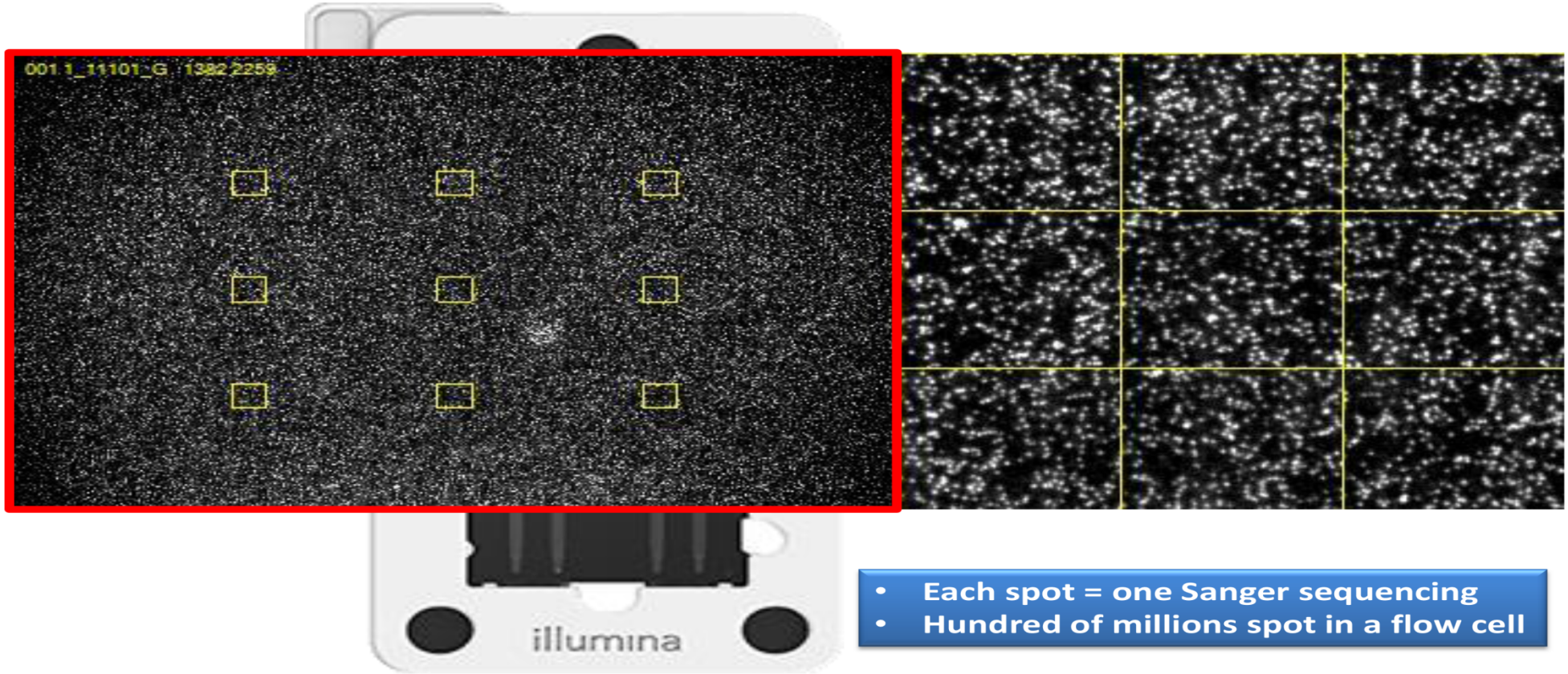
- **Patient was switched to high risk neuroblastoma treatment included stem cell transplant**
- **Doing well 1 yr after diagnosis**

Next-generation sequencing



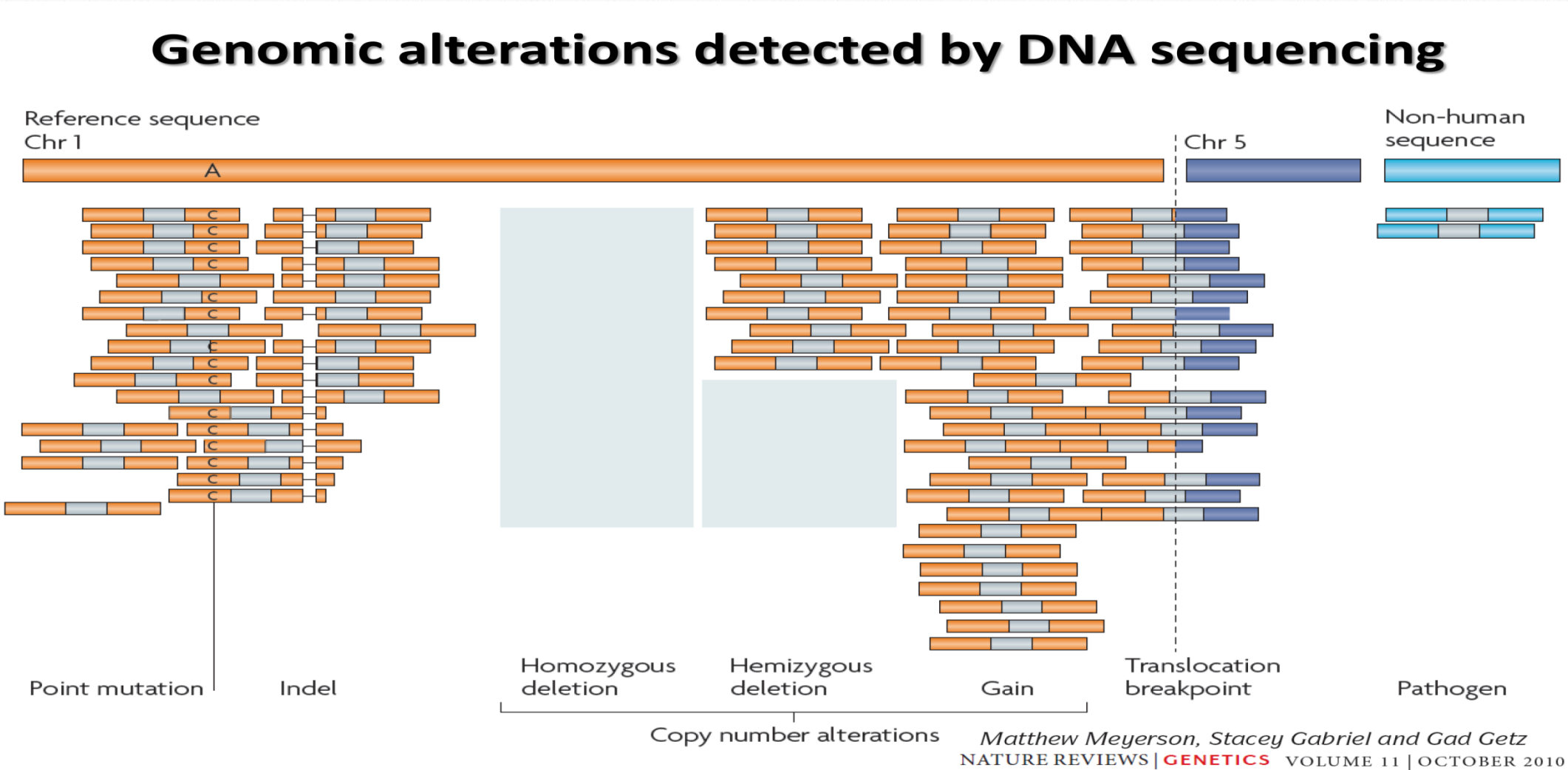
Massively Parallel Sequencing

Massively Parallel Sequencing



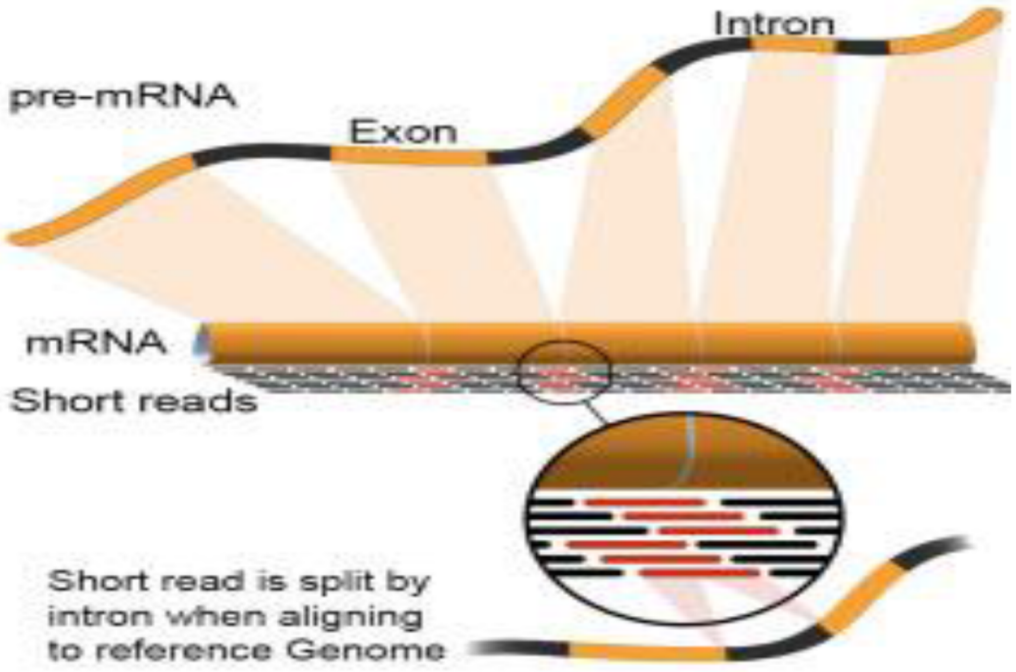
- Each spot = one Sanger sequencing
- Hundred of millions spot in a flow cell

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

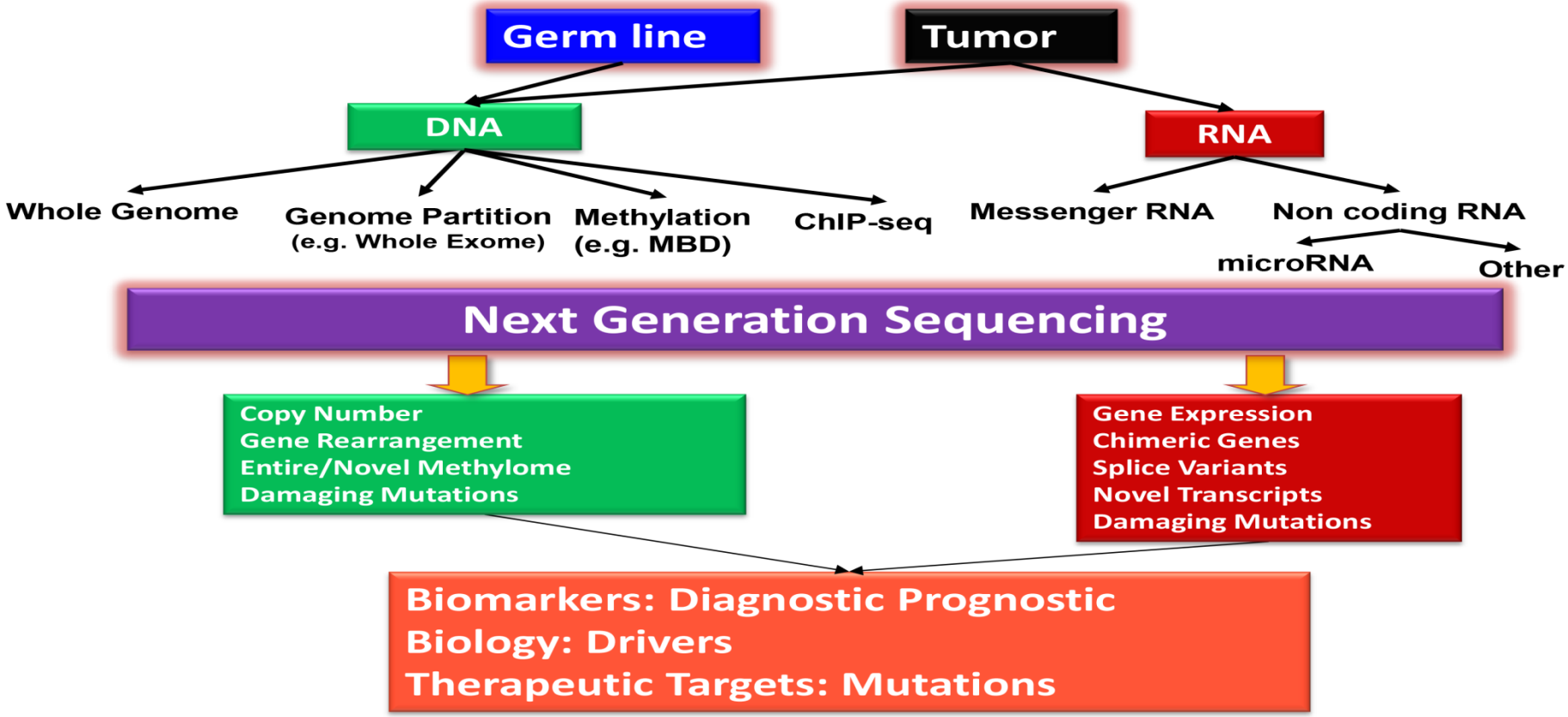
Properties

Properties of the next-generation sequencing technologies

- No need to prepare clones for DNA fragments
- No need of prior knowledge for probe design
- Able to detect balanced genome structure changes
- Parallel sequencing at basepair resolution– massive-throughput (up to 100s Gb/run)
- Cheaper (per nucleotide) and faster per genome

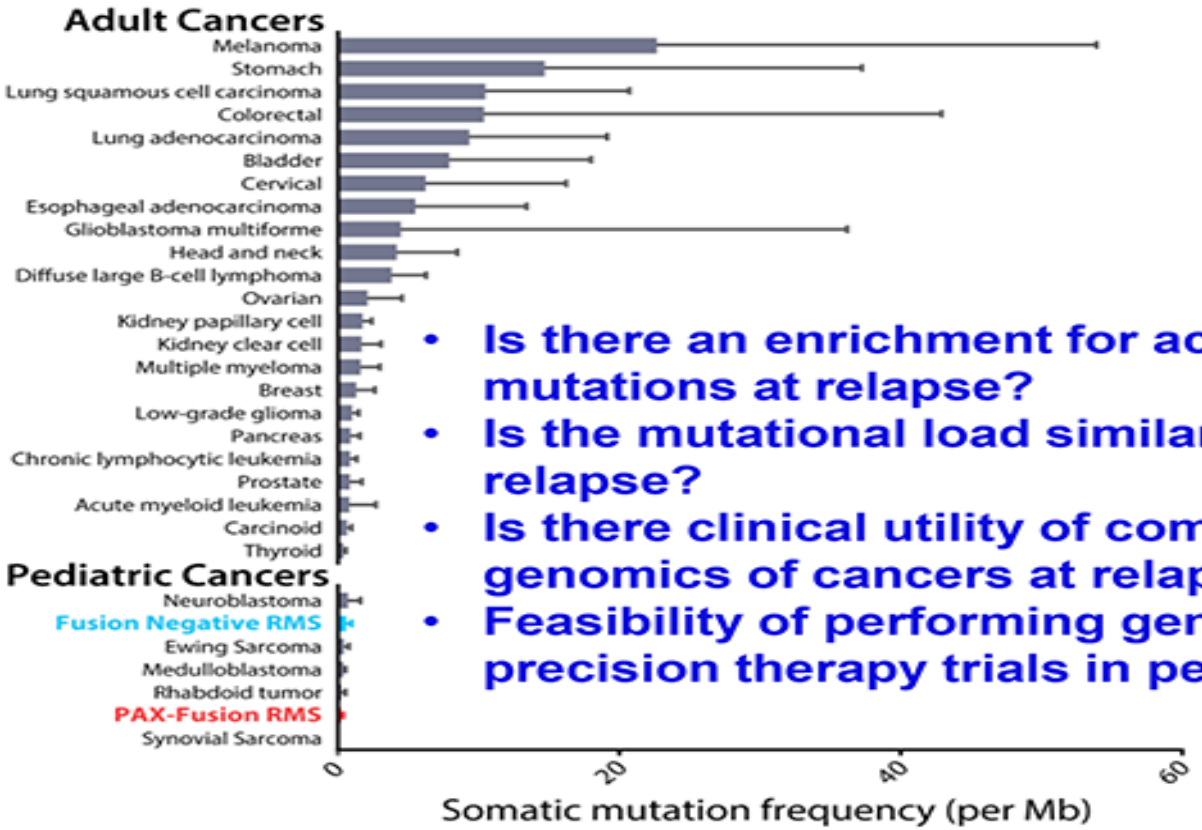
Cancer Genomes

Next Generation Sequencing Allows for Comprehensive Analysis of Cancer Genomes on the Same Platform



Pediatric cancer mutations

Pediatric cancers have a low number of somatic and actionable mutations at initial diagnosis



- Is there an enrichment for actionable mutations at relapse?
- Is the mutational load similarly low at relapse?
- Is there clinical utility of comprehensive genomics of cancers at relapse?
- Feasibility of performing genome guided precision therapy trials in pediatrics?

2014, *Cancer Discovery*, Shern, J. F. *et al.*, Comprehensive Genomic Analysis of Rhabdomyosarcoma **2013**, *Nature*, Lawrence, M. S. *et al.*, Mutational heterogeneity in cancer

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

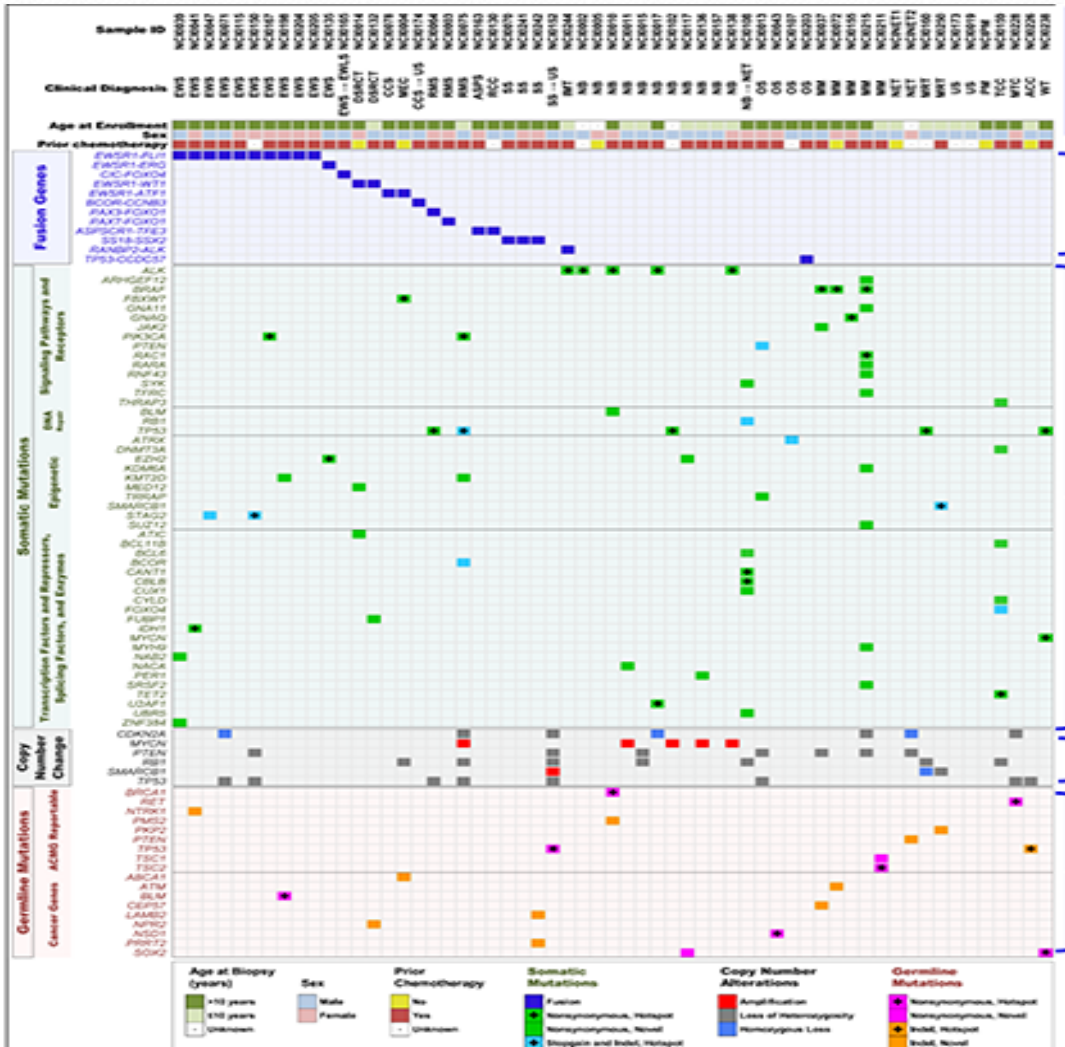
- Pilot study to determine the utility and feasibility of performing comprehensive genomic analyses to identify clinically actionable mutations in pediatric and young adult patients with metastatic, refractory or relapsed solid tumors
- 59 patients enrolled to the pediatric oncology branch, Center for 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

Mutations

Definitions: Actionable

- **Actionable germline mutation:** loss of function mutation or known hotspot activating mutation of a cancer consensus gene or pathogenic or likely pathogenic mutation of an American College of Medical Genetics (ACMG) Gene
- **Actionable somatic mutation:** genomic alterations that changes the patient's diagnosis, or may be targeted with FDA approved drugs or in the context of existing clinical trials according to the NCI-adult MATCH-Criteria

Multi-omics integrated landscape



Multi-Omics Integrated Landscape

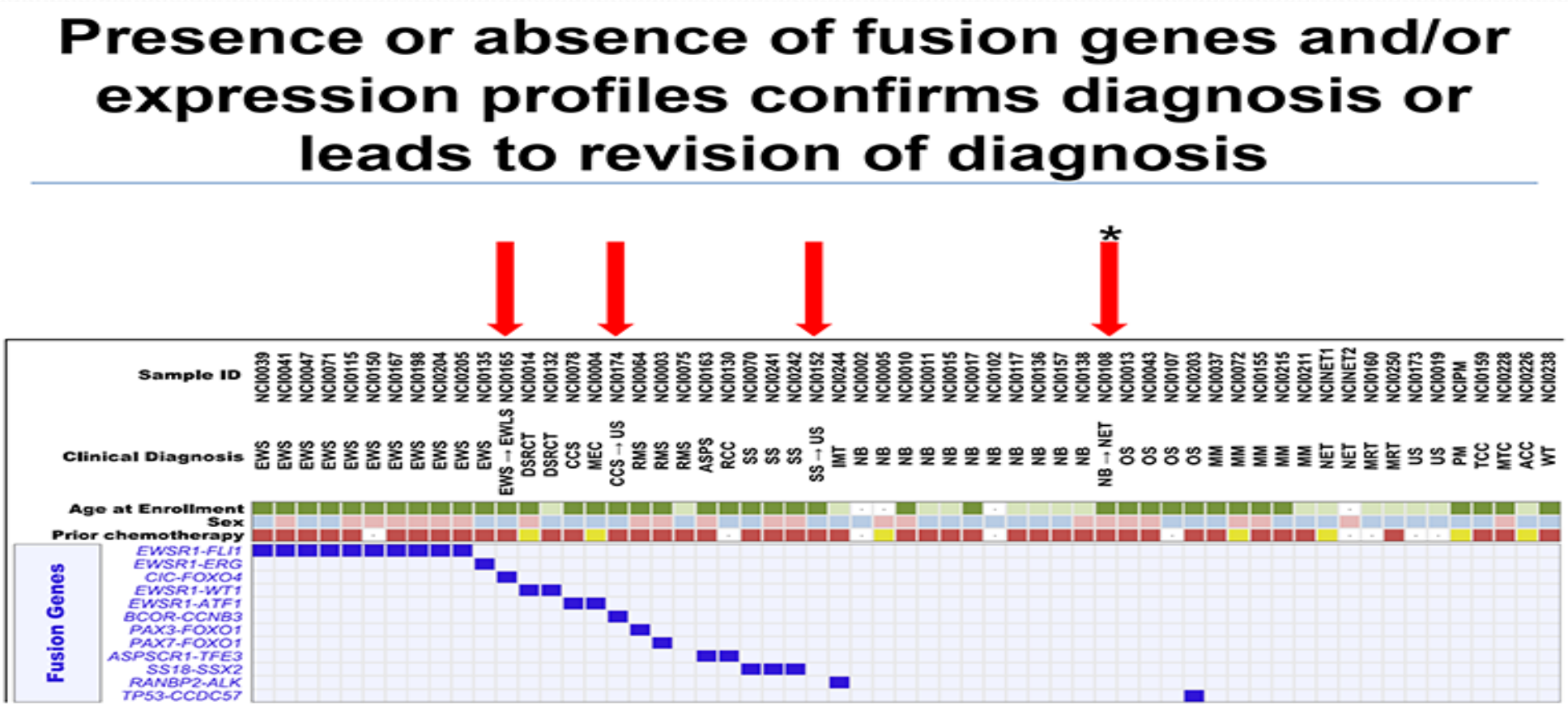
RNAseq
Diagnostic, Driver, Actionable

DNAseq and RNAseq
Somatic: Driver, Actionable

DNA copy number & RNAseq
Somatic: Driver, Actionable

DNAseq
Germ line: Disease causing, Actionable

Fusion genes



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. • Models with variants respond to treatment and models without variant do not respond to treatment • 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) • 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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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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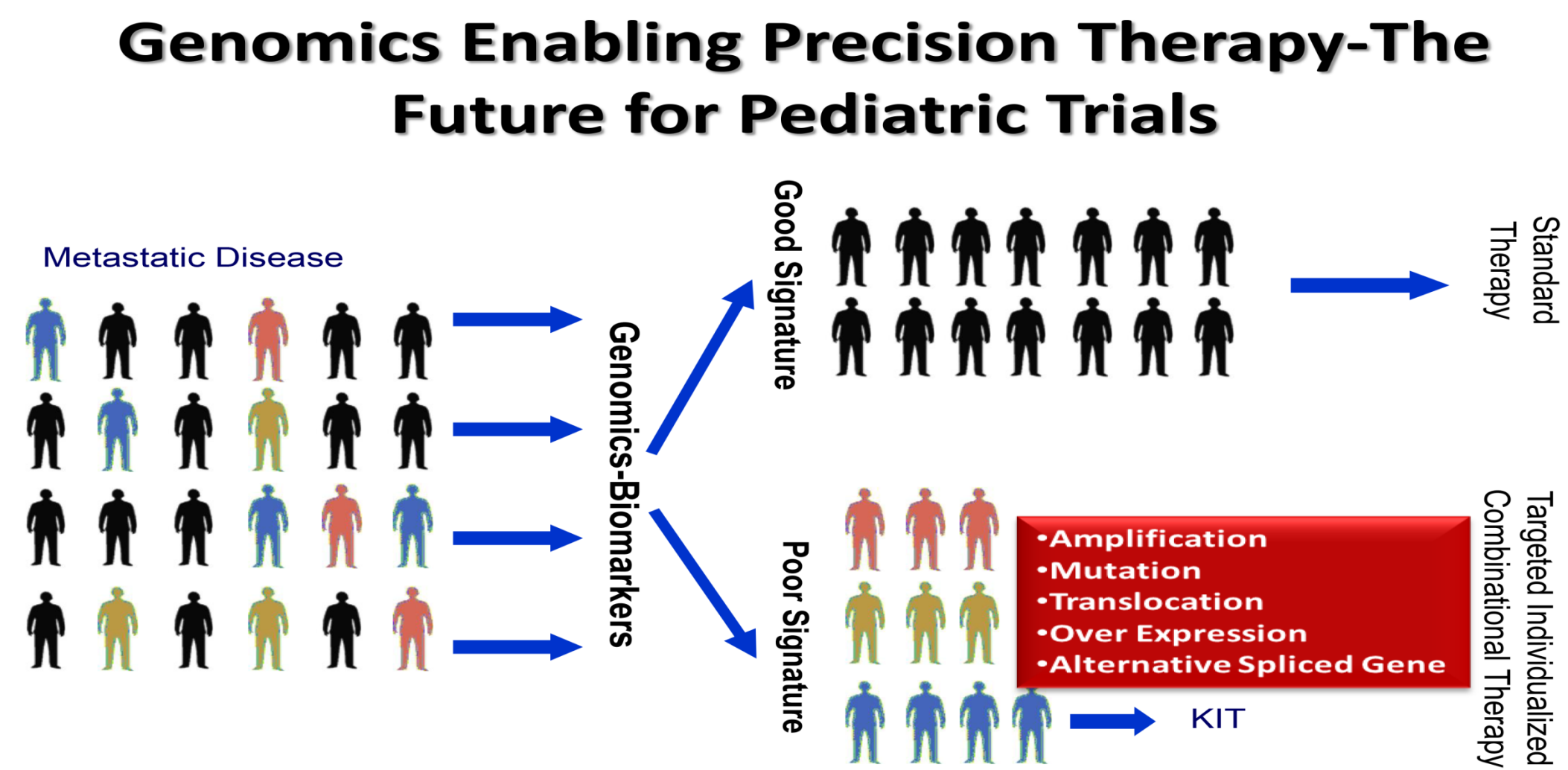
Summary

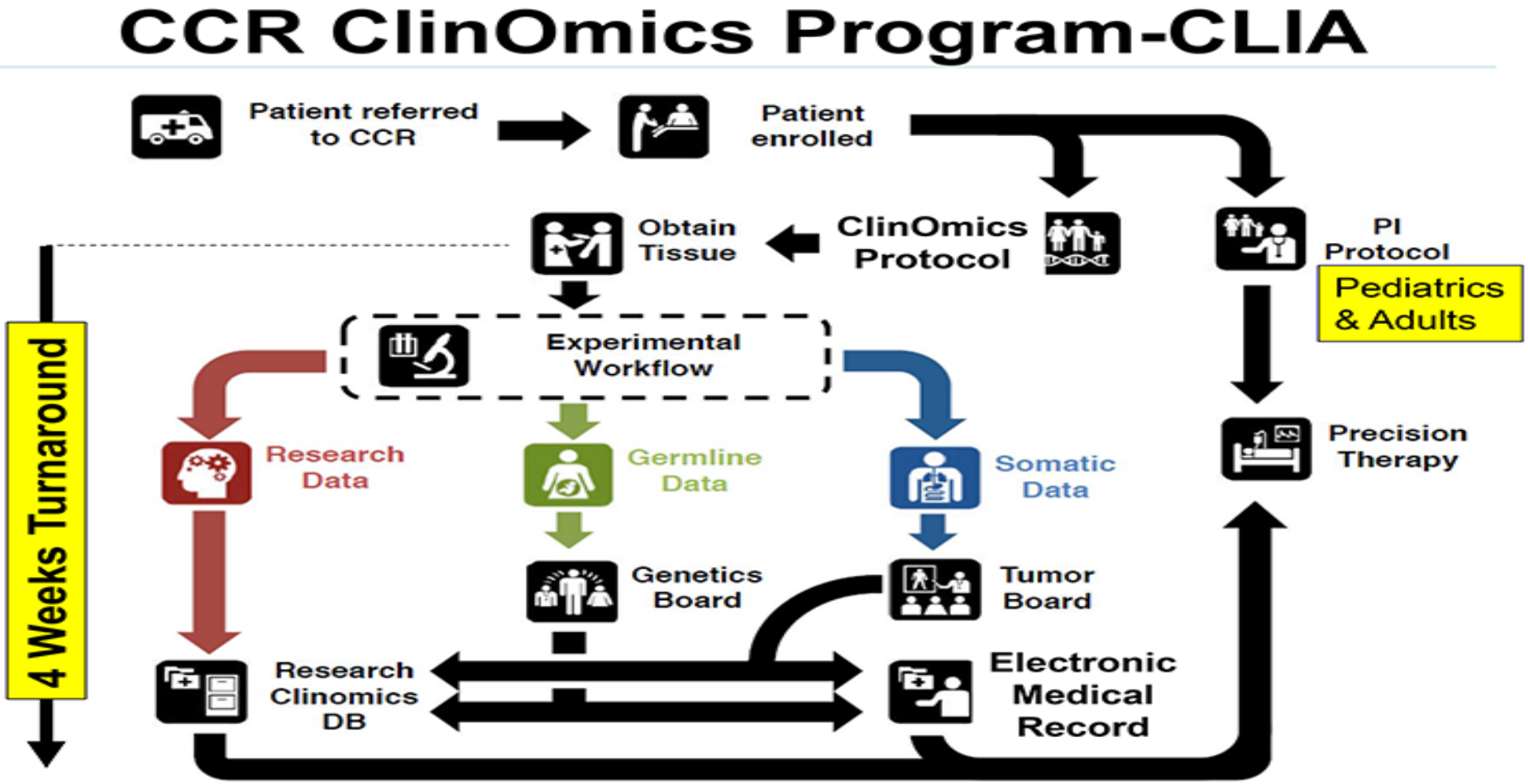
Summary

- 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



ClinOmics program



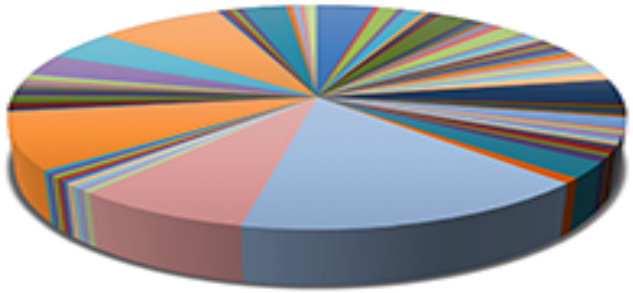
Sequencing equipment

Sequencing Equipment

- Two NextSeq500s for speed and lower throughout
  - 65 Gb/run
  - 14 hours/run
- One HiSeq2500: for high throughput
  - 1000Gb/run
  - 32 exomes or transcriptomes

Patient diagnoses

255 Patients of 81 diagnoses



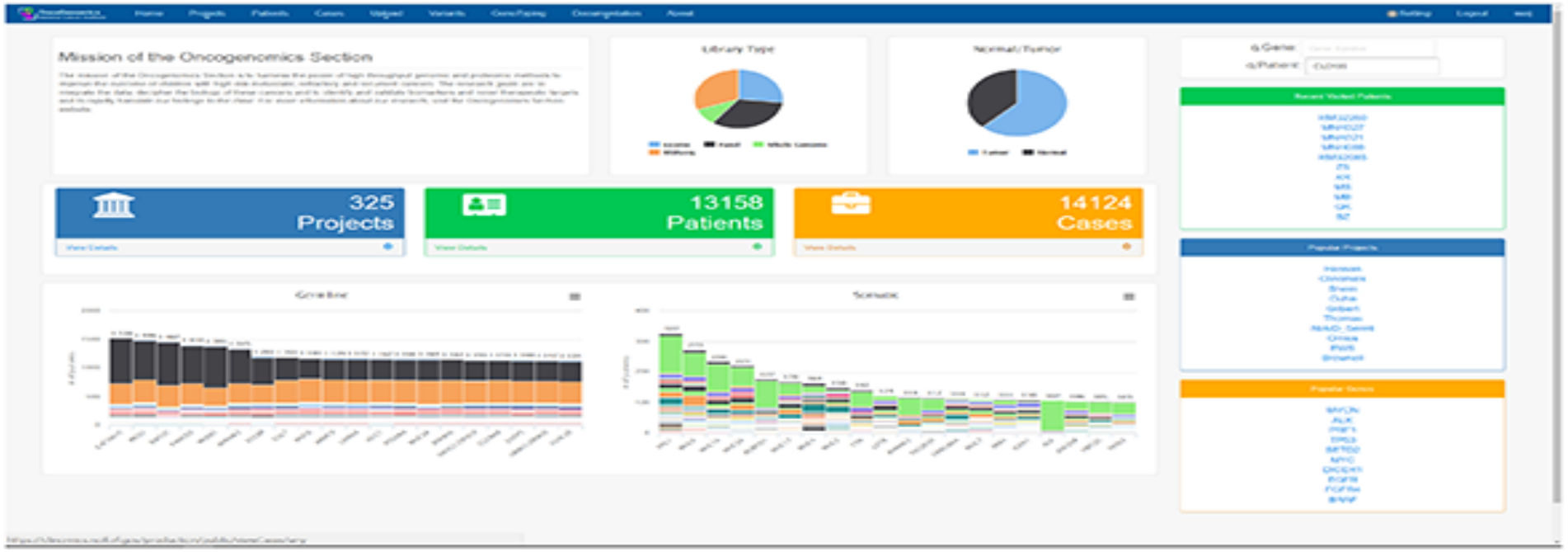
Acute lymphoblastic leukemia
Anaplastic Astrocytoma
Anaplastic Oligodendroglioma
Bladder cancer
Colon cancer
Diffuse intrinsic pontine glioma
Ewing's sarcoma
Glioblastoma
Gliosarcoma
Hepatocellular carcinoma
Lung Adenocarcinoma
Medullary Thyroid Cancer metastatic
Mesothelioma Peritoneal
Multinodular and Vacuolating Neuronal Tumor
Neuroendocrine carcinoma
Non-nans Tenosynovial giant cell tumor
Papillary tumor of the pineal region
Prostate cancer
Small cell bladder
Solid Tumor CAP_Survey
Uveal melanoma

Acute myeloid leukemia
Anaplastic Astrocytoma/Glioblastoma IDH Mutant
Anaplastic PXA
Breast cancer
Desmoid fibromatosis
Endometrial cancer
Extrapulmonary Small Cell Cancer
Glioblastoma IDH Mutant
Hepatic Angiosarcoma
High grade glioma, IDH mutant
Lung adenocarcinoma stage 1A
Melanoma
Mesothelioma Pleural
Multiple
Neurofibromatosis 1
NSCLC
Pilocytic Astrocytoma
Renal cell carcinoma
Small Cell Cancer of rectum
Synovial sarcoma

Adrenocortical carcinoma
Anaplastic Astrocytoma/Glioblastoma IDH WT
Aneurysmal fibrous histiocytoma
Cholangiocarcinoma
Desmoplastic small round cell tumor
Endometrioid Stromal Sarcoma
Gallbladder cancer
Glioblastoma IDH WT
Hepatocellular cancer
Invasive well differentiated squamous cell carcinoma
Lymphocytosis
Mesothelioma
Mesothelioma Tunica Vaginalis
Multiple carcinoma
Neuronal tumor
Osteosarcoma
Pleomorphic Xanthoastrocytoma
Rhabdomyosarcoma
Small Cell Carcinoma of the ovary hypercalcemic type (SCCOHT)
Teratoma

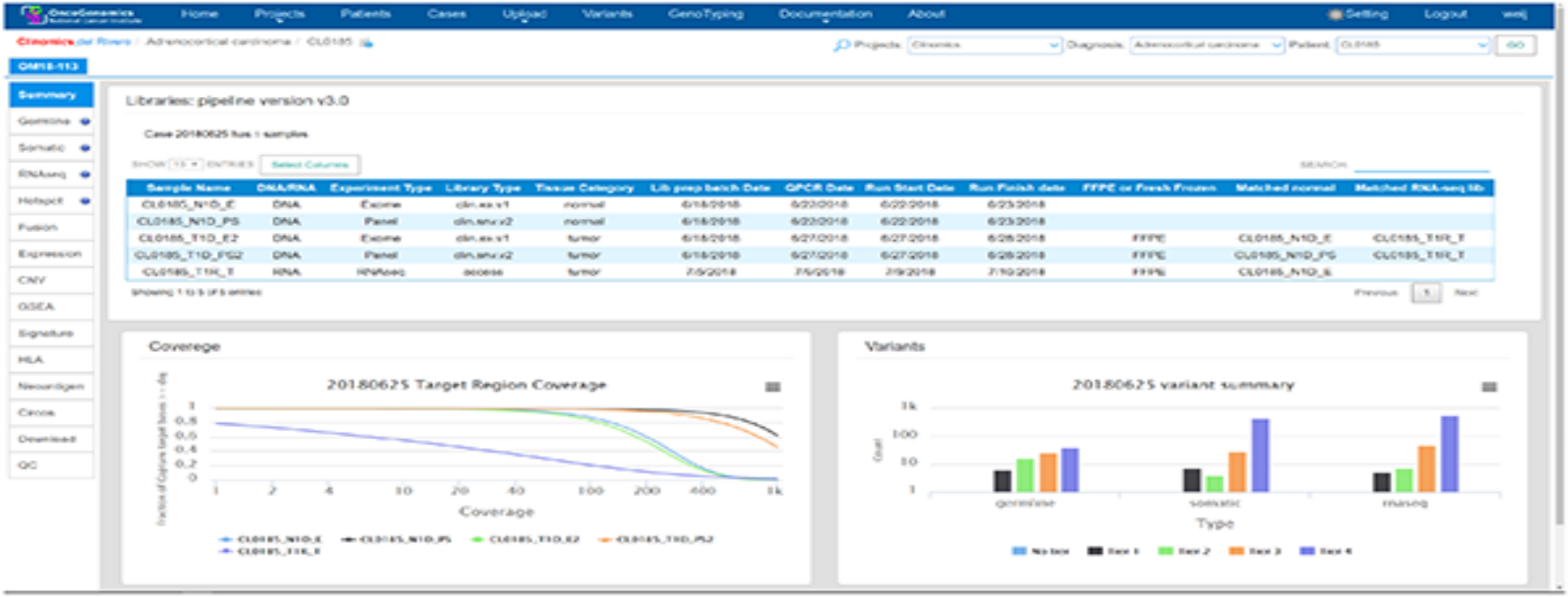
Ampullary cancer
Anaplastic Ependymoma
Astrocytoma
Clear cell sarcoma
Diffuse Astrocytoma, Grade II
Eosinophilia
GBM
Glioblastoma Multiforme
Hepatocellular Carcinoma
Keratoacanthoma
Mastocytosis
Mesothelioma bi compartmental
MPNST
Myxopapillary Ependymoma
Non small cell lung cancer
Pancreatic cancer
Poorly differentiated carcinoma (lung vs. thymic)
SCLC
Small cell endometrium
Undifferentiated sarcoma

ClinOmics Data Portal



Patient Summary

Patient Summary Page



QC report

QC Report: Sequencing Statistics & Genotyping

Run Statistics

Mutations | QC

Circos | Coverage | Transcript Coverage | Hotspot | DNA QC | RNA QC | Genotyping

Show 15 entries | Select Columns | Search:

Sample_ID	Percent bq unique ontarget	Percent bq unique positions at 5x	Percent bq unique positions at 10x	Percent bq unique positions at 15x	Percent bq unique positions at 20x	Percent bq unique positions at 30x	Percent bq unique positions at 50x	Percent bq unique positions at 100x	Percent bq unique positions at 200x	MEAN BAIT COVERAGE	MEAN TARGET COVERAGE	Ontarget reads	Percent ontarget	Unique ontarget reads	Percent unique ontarget	Min mapq	Mean mapq
CL0033_T_P	98.94	97.50	97.08	96.71	96.35	95.58	93.68	87.48	75.91	709	758	33301602	44.54	25701887	77.18	0	59.23
CL0034_T_E	97.24	97.46	97.10	96.73	96.30	95.24	92.20	79.63	50.45	248	263	194065283	63.33	171540148	88.39	0	58.30
CL0034_T_P	98.92	97.30	96.73	96.20	95.64	94.33	91.19	81.83	68.37	464	496	19687910	59.58	16811999	85.39	0	59.21
CL0033_B_E	96.68	97.16	96.43	95.41	94.02	89.98	77.75	42.24	7.27	100	105	71811950	65.27	68733940	95.71	0	57.97
CL0033_B_P	98.67	96.39	95.33	94.12	92.81	89.47	81.07	58.09	19.87	137	143	5141314	58.65	4922738	95.75	0	59.04
CL0033_T3D_E	96.75	97.47	97.18	96.87	96.53	95.72	93.39	82.58	50.80	232	245	190042530	66.15	160140987	84.27	0	58.01
CL0033_T3D_P	98.69	97.38	97.08	96.83	96.62	96.22	95.43	93.21	86.68	732	763	37963486	59.30	26297292	69.27	0	59.07
CL0033_T_E	97.22	97.44	97.07	96.65	96.17	94.97	91.48	77.39	48.03	242	258	195690091	63.51	167866163	85.78	0	58.28

Showing 1 to 8 of 8 entries | Previous | 1

Genotyping

Show 15 entries | Select Columns | Search:

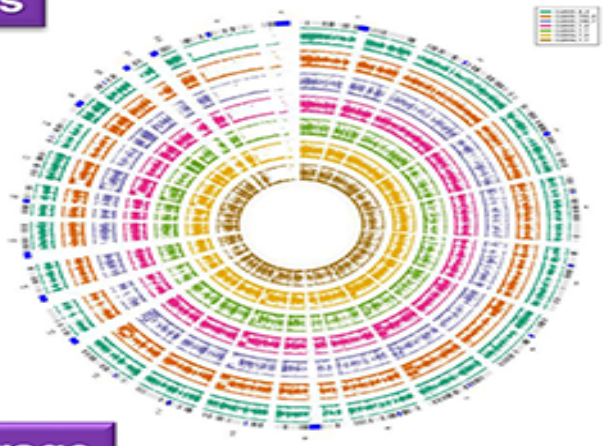
Sample	CL0033_B_E	CL0033_B_P	CL0033_T3D_E	CL0033_T3D_P	CL0033_T3R_T	CL0033_T_E	CL0033_T_P	CL0033_T_T	CL0034_T_E	CL0034_T_P	CL0034_T_T
CL0033_B_E	100%	97%	98%	91%	94%	98%	86%	94%	98%	94%	94%
CL0033_B_P	97%	100%	96%	97%	94%	96%	97%	94%	96%	98%	94%
CL0033_T3D_E	98%	96%	100%	91%	94%	98%	87%	94%	98%	94%	95%
CL0033_T3D_P	91%	97%	91%	100%	89%	90%	96%	90%	91%	97%	89%
CL0033_T3R_T	94%	94%	94%	89%	100%	94%	84%	96%	94%	90%	96%
CL0033_T_E	98%	96%	98%	90%	94%	100%	87%	94%	98%	93%	94%
CL0033_T_P	86%	97%	87%	96%	84%	87%	100%	80%	87%	97%	84%
CL0033_T_T	94%	94%	94%	90%	96%	94%	85%	100%	94%	92%	97%
CL0034_T_E	98%	96%	98%	91%	94%	98%	87%	94%	100%	93%	94%
CL0034_T_P	94%	98%	94%	97%	90%	93%	97%	92%	93%	100%	91%
CL0034_T_T	94%	94%	93%	89%	96%	94%	84%	97%	94%	91%	100%

Showing 1 to 11 of 11 entries | Previous | 1 | Next

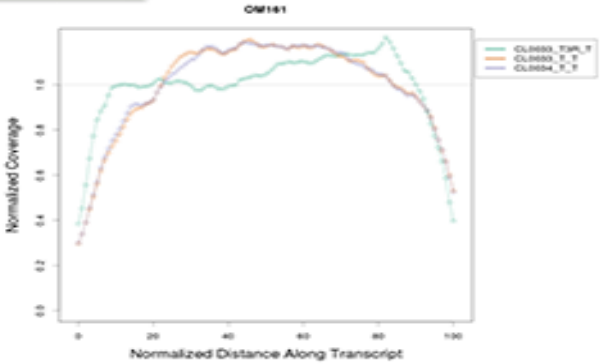
QC report

QC Report: Coverage

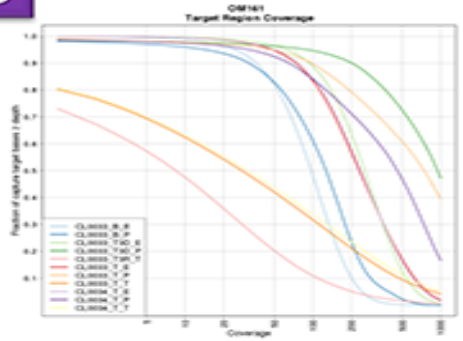
Circos



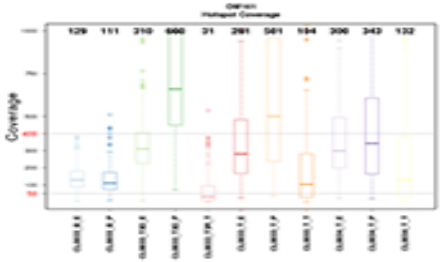
RNA Coverage



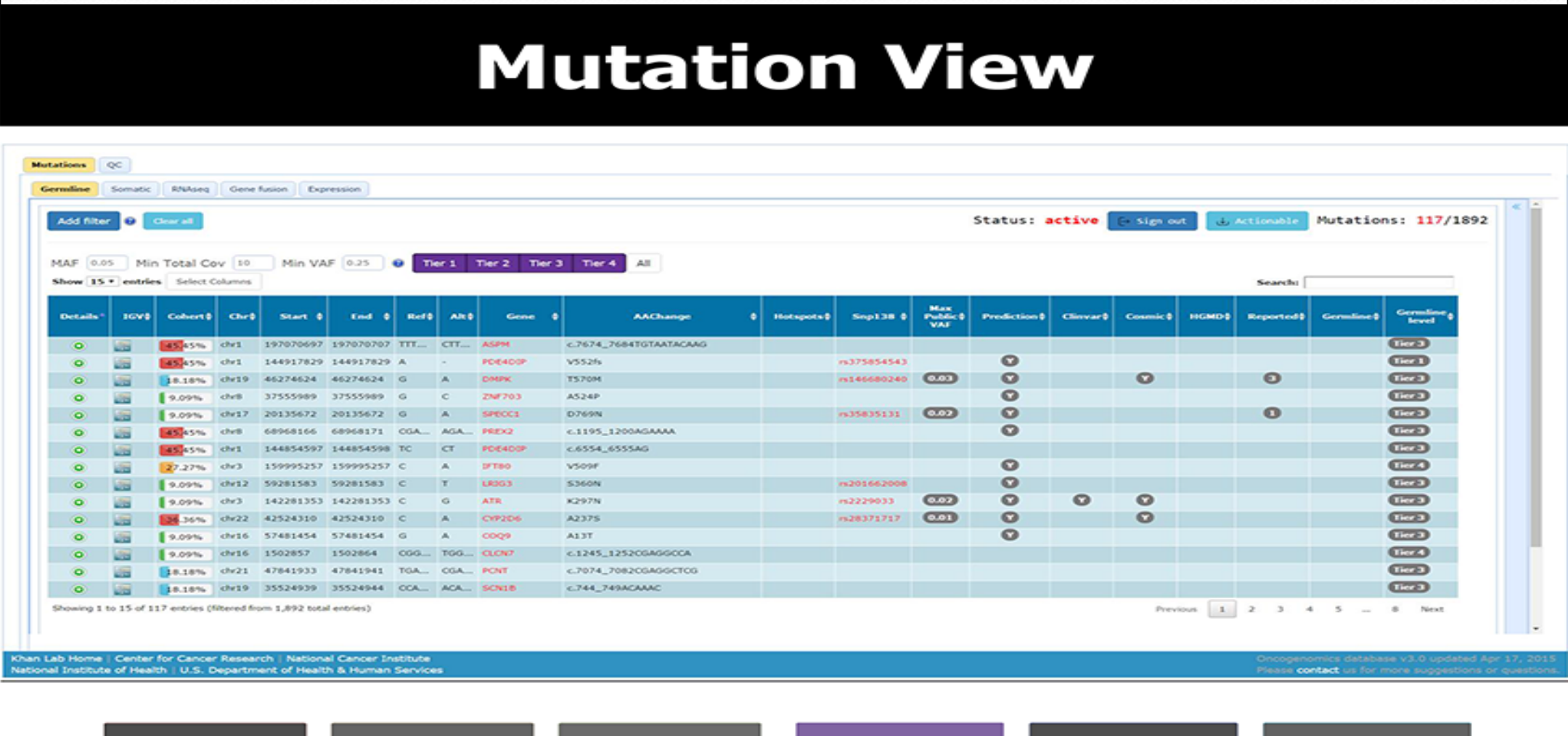
Coverage



Hotspot Coverage

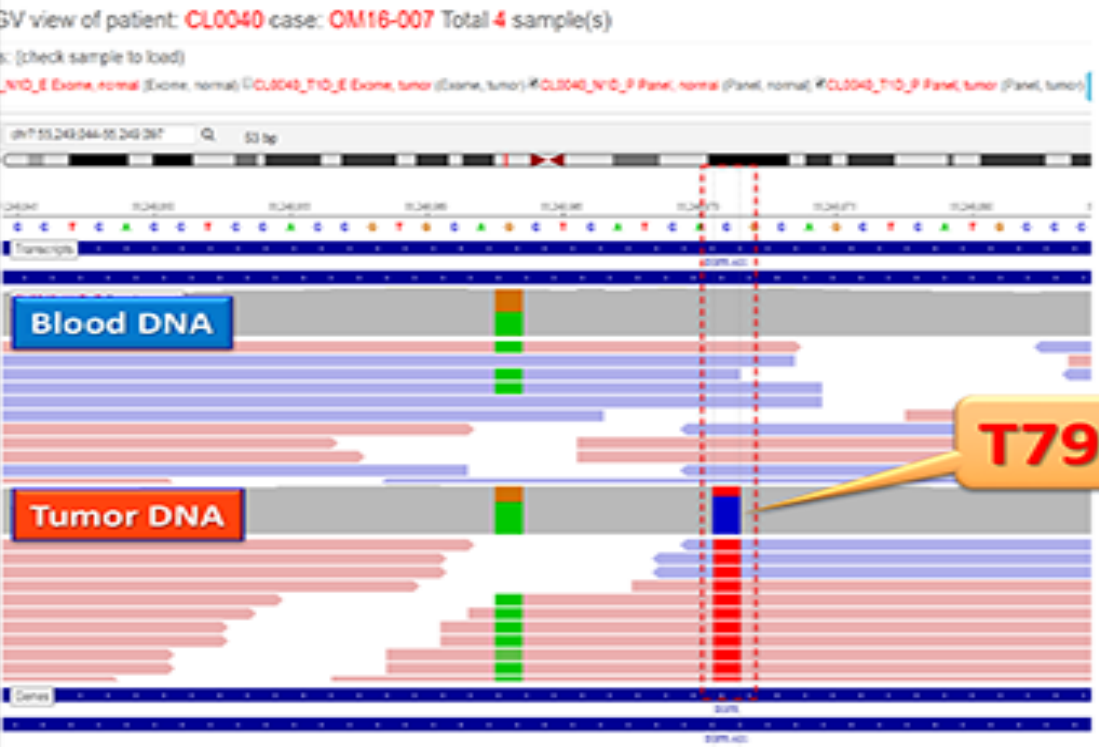


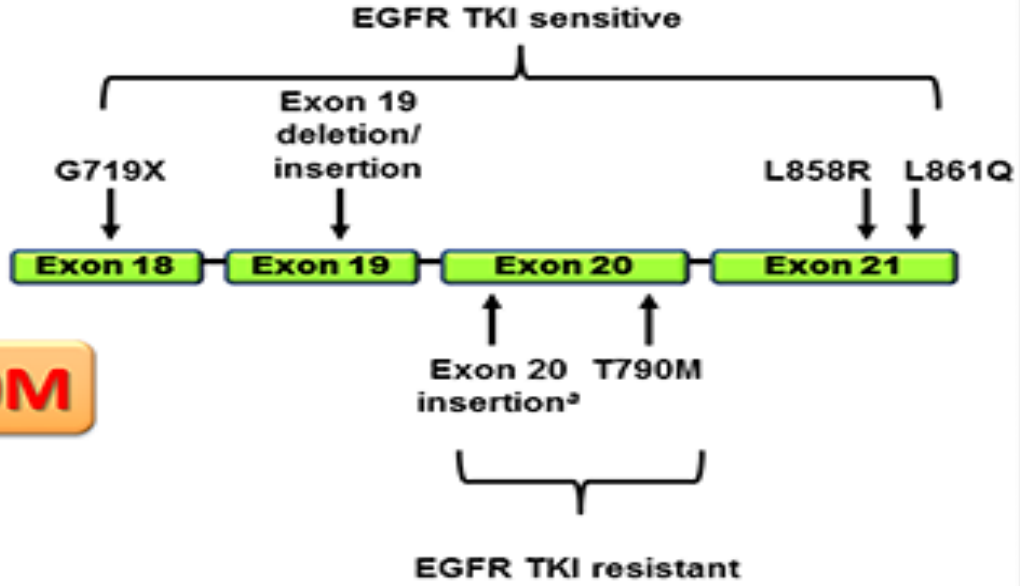
Mutation view



EGFR mutations

EGFR mutations in NSCLC

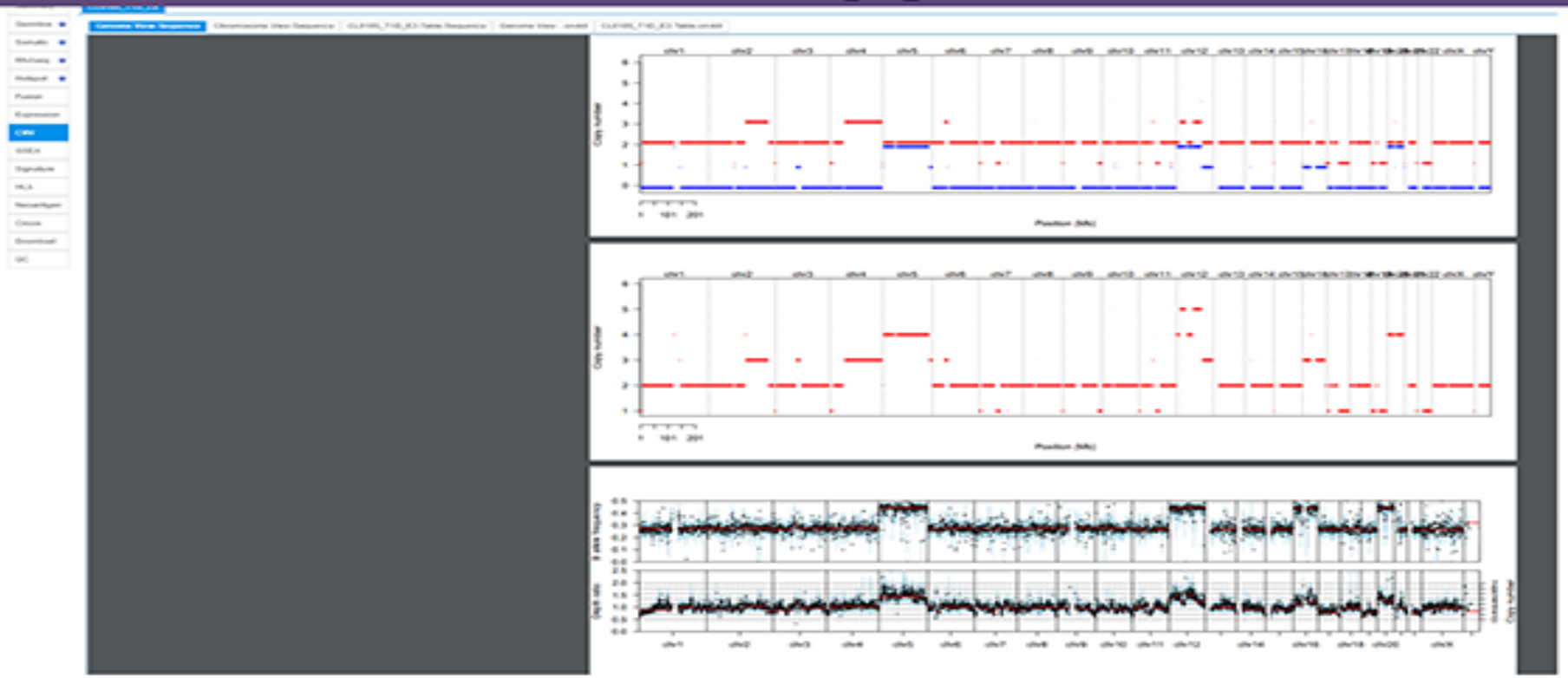




https://www.mycancergenome.org/content/disease/lung-cancer/egfr/

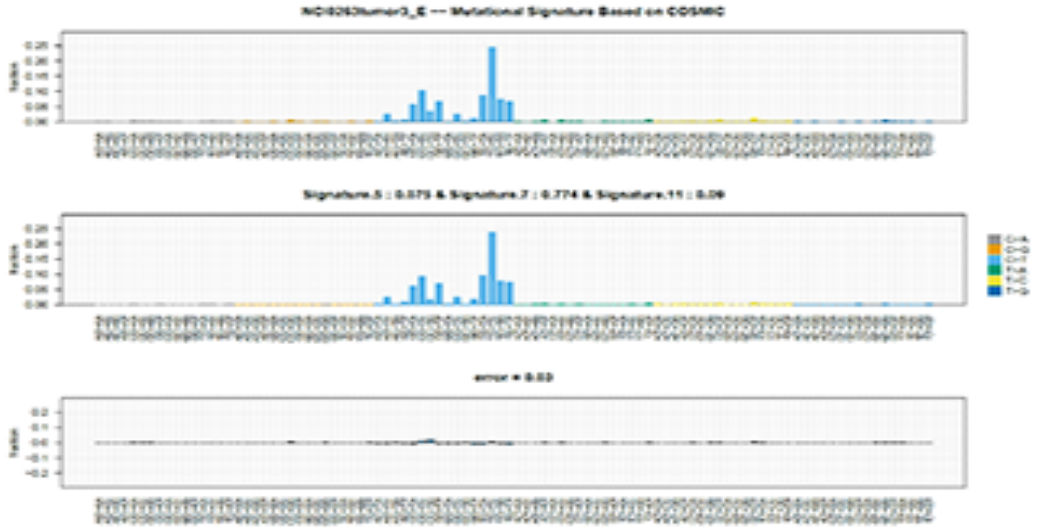
Tumor Copy Number

Tumor Copy Number

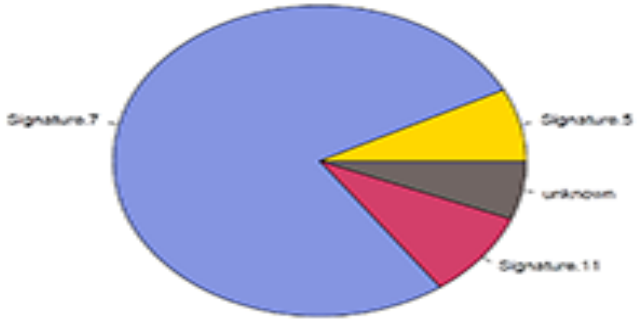


Mutation Signatures

Mutation Signatures for Tumor



NCI0263: Melanoma

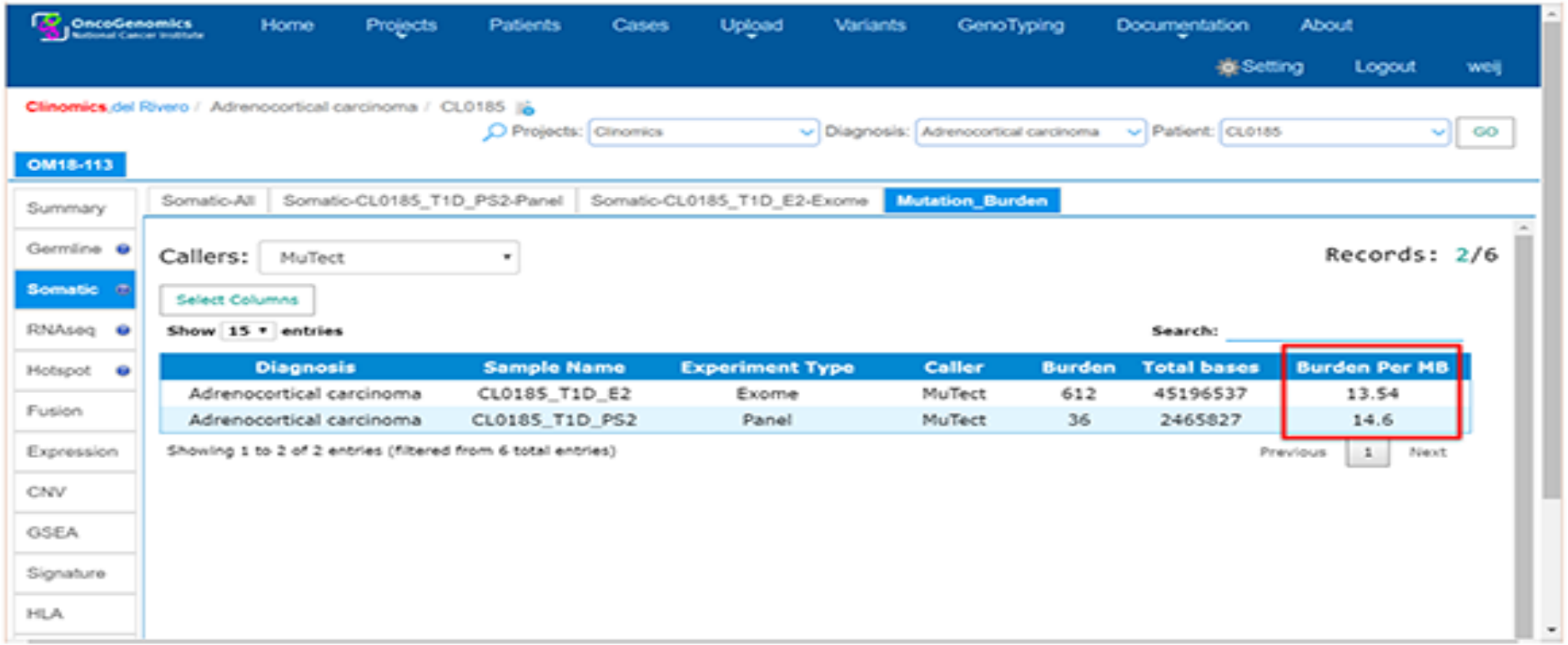


COSMIC (https://cancer.sanger.ac.uk/cosmic/signatures)

Signature 7: UV signature

Mutation Burden

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

Conclusions

1. Integrated analysis of the cancer genome indentifies biologically relevant diagnostic, prognostic biomarkers and novel targets for therapy
2. Powerful emerging tools of next generation sequencing (including whole genome, exome, and transcriptome) will determine the complete genomic portrait of pediatric cancers at the base pair level
3. This will lead to the identification of key drivers and will enable the development of future novel therapies and precision therapy

Acknowledgements