Pediatric Cancers

The application of genomics to identify diagnostic biomarkers, drivers and therapeutic targets for pediatric cancers

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TRACO
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Outline

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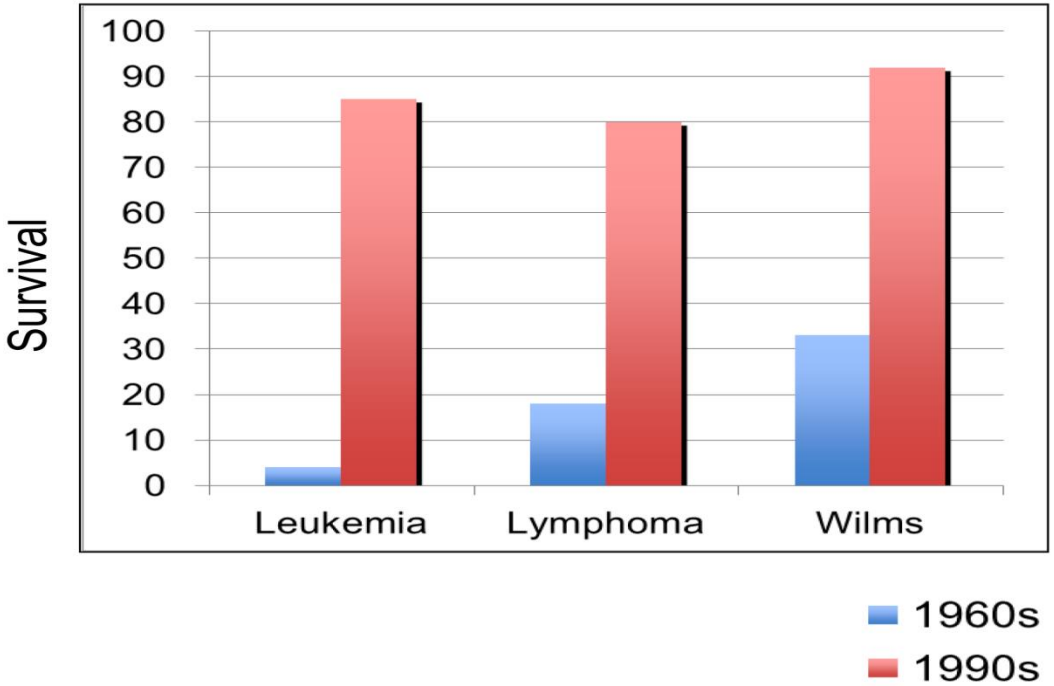
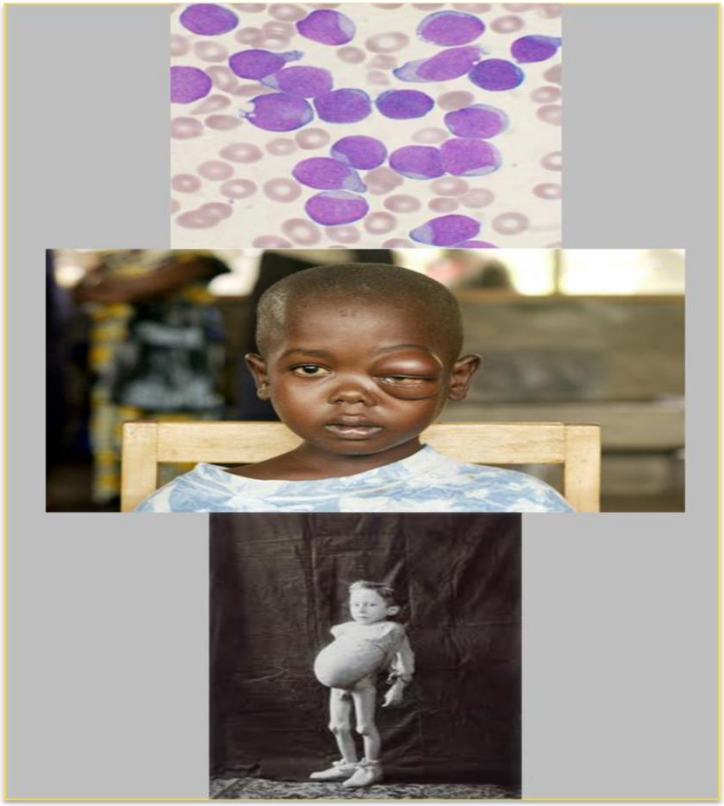
- Success and Challenges of Treating Pediatric Cancers
- Genomics
- Next-generation Sequencing
- Application of next-generation sequencing:
  - Diagnosis
  - Identification of molecular target
- Precision Therapy

Childhood cancer

National Cancer Institute

Childhood cancer: The beginning of a modern medical success story



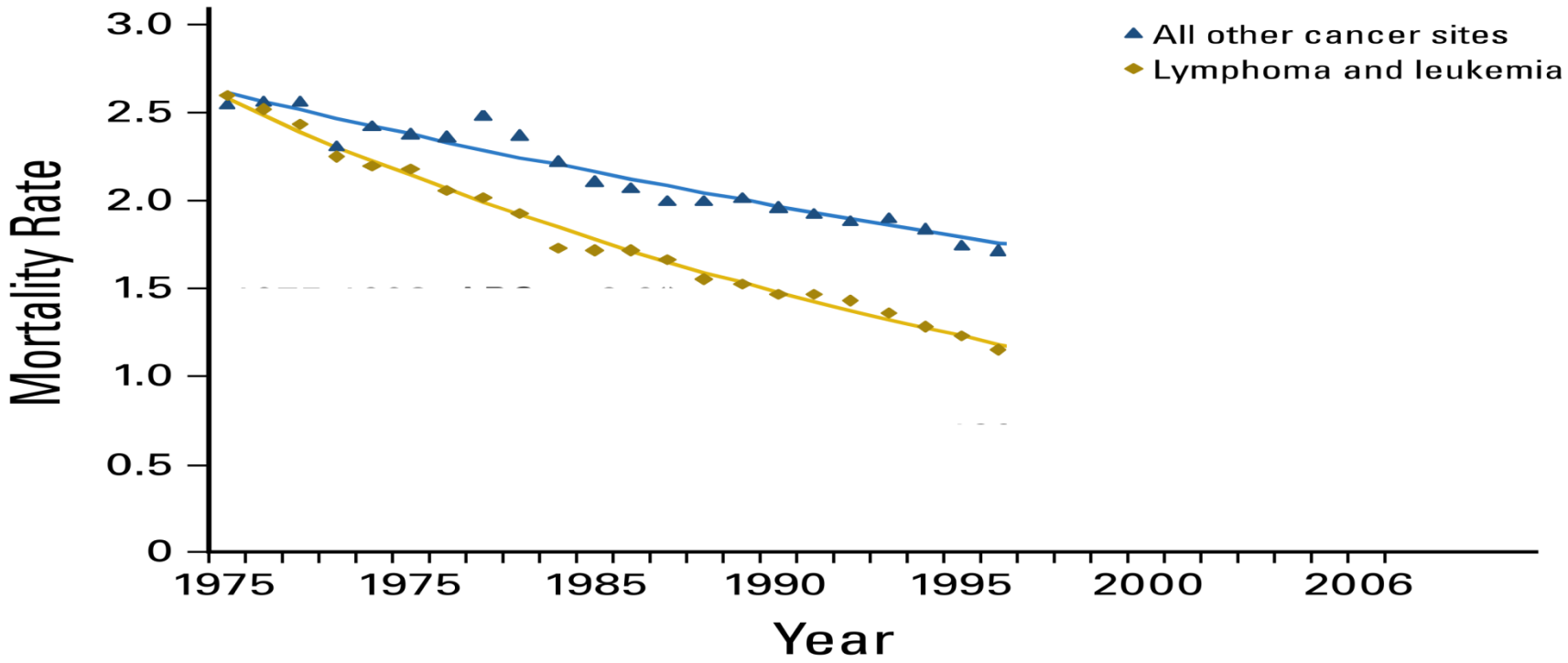


Courtesy: John Maris

Success Rate

National Cancer Institute

Success rate attributed to careful NCI funded empirical (observational) clinical efficacy trials

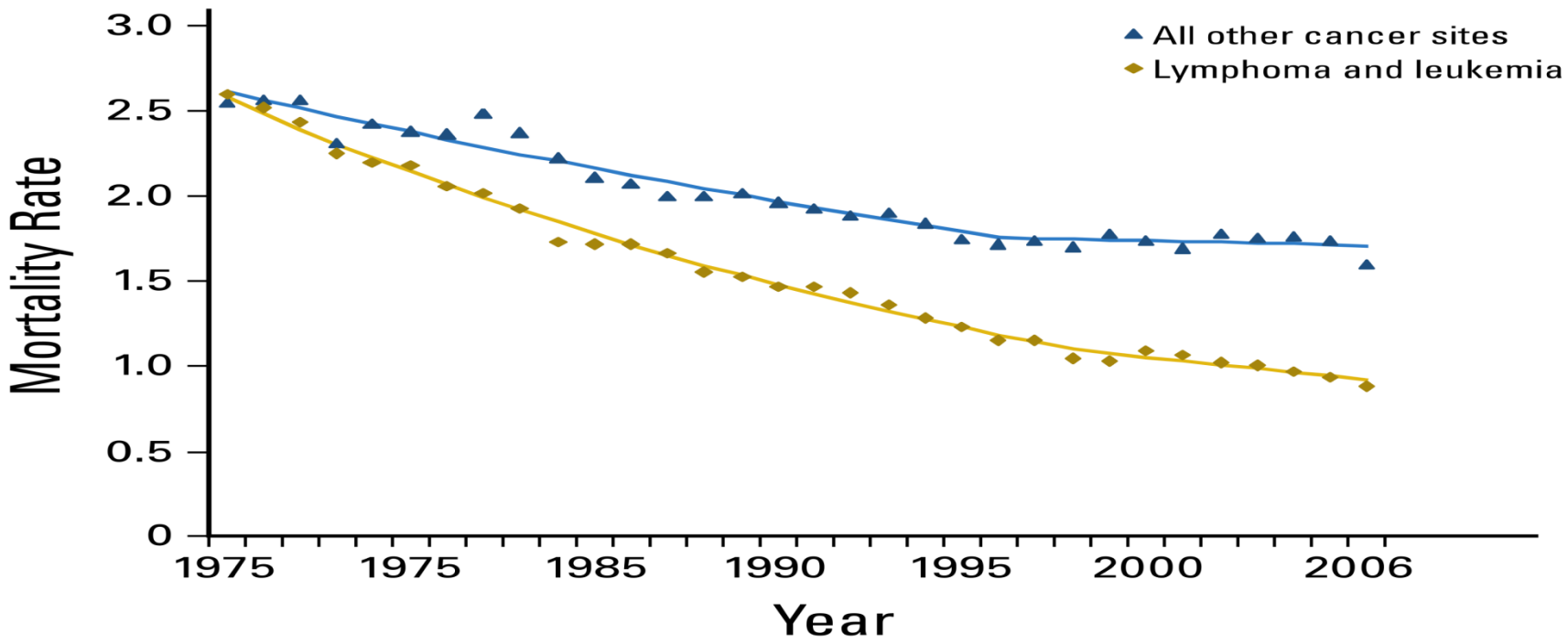


Courtesy: Malcolm Smith

Mortality rates

National Cancer Institute

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

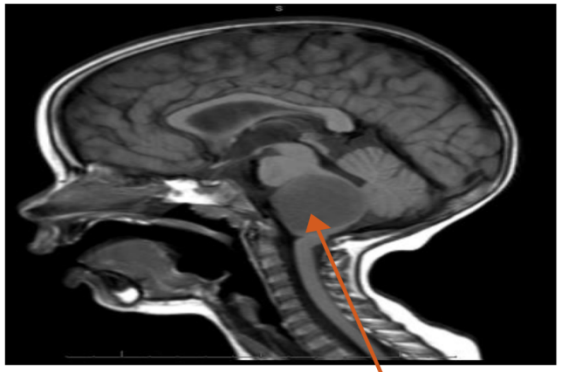


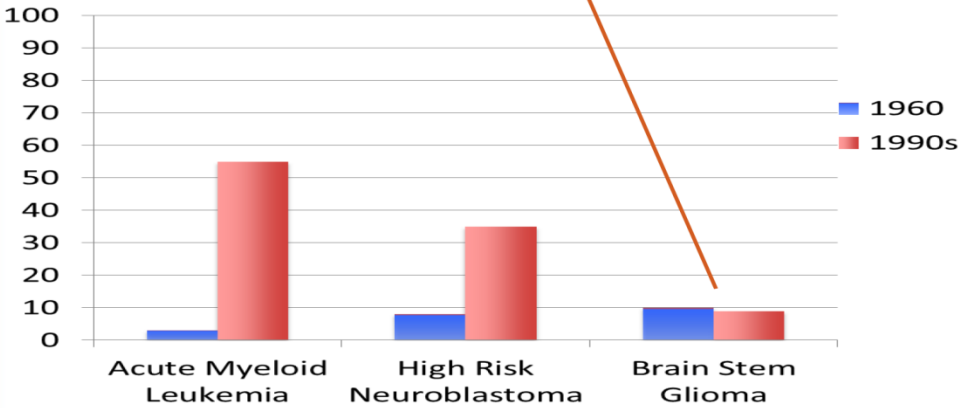
Courtesy: Malcolm Smith

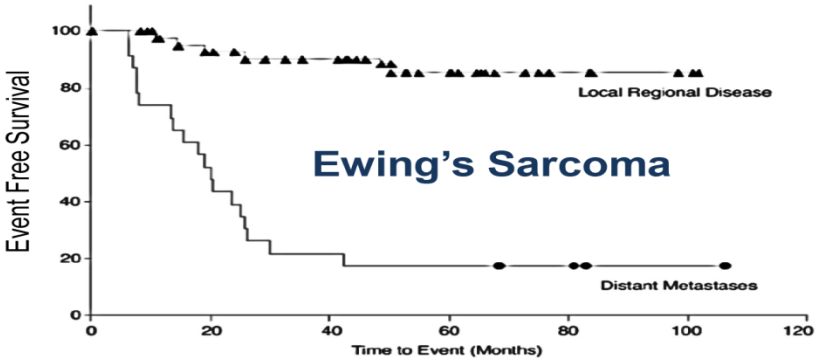
Brain stem glioma cancer

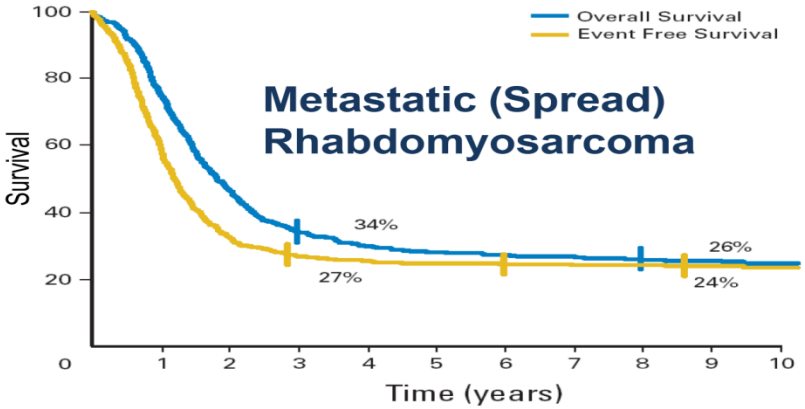
National Cancer Institute

Certain cancers remain incurable- brain stem glioma Cancer when spread remains incurable (<30% survive)









Gene expression

The dramatic consequences of gene expression in biology

Anise swallowtail, *Papilio zelicaon*

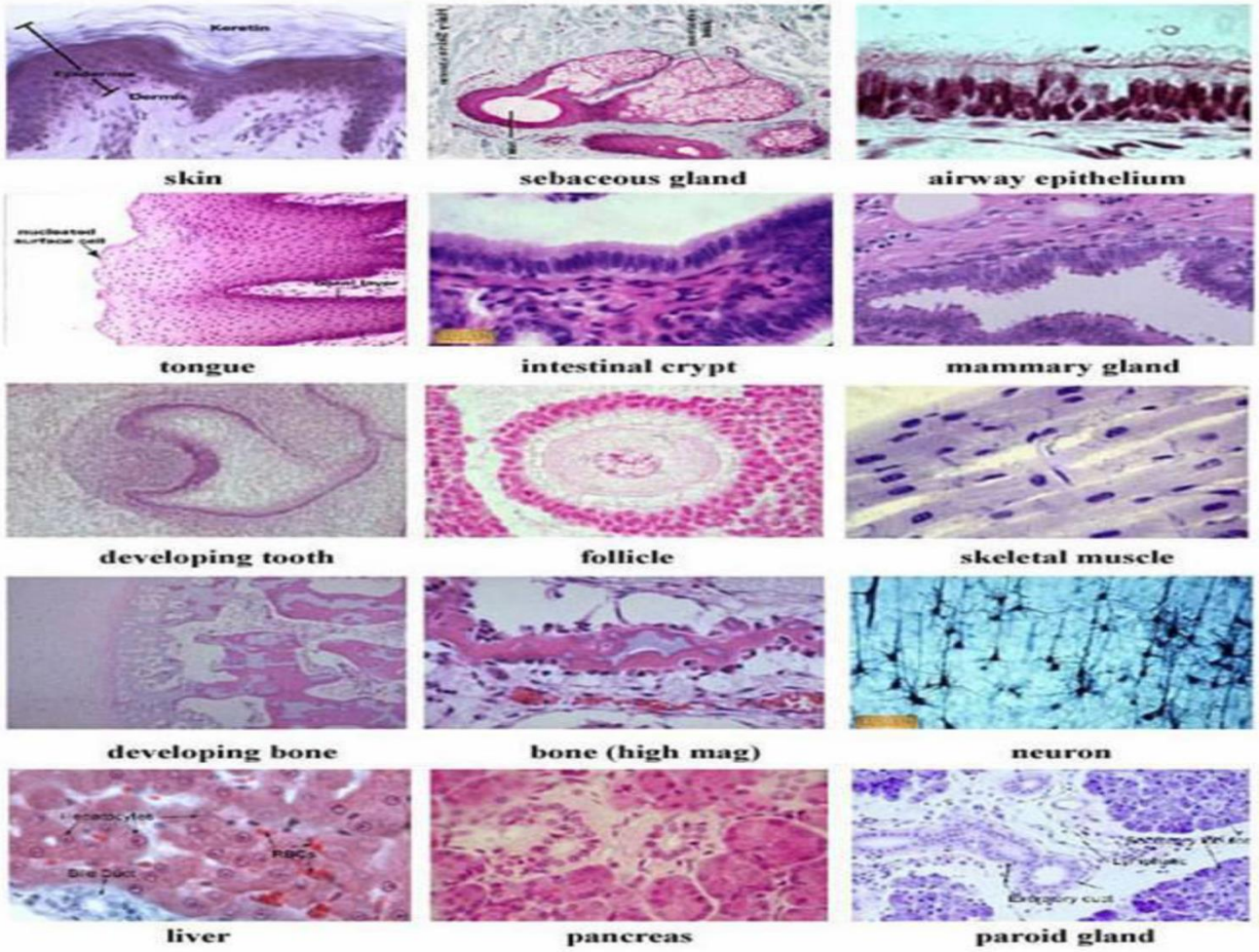
Same genome →
Different expression pattern
Different proteome
Different tissues
Different physiology

Gene expression

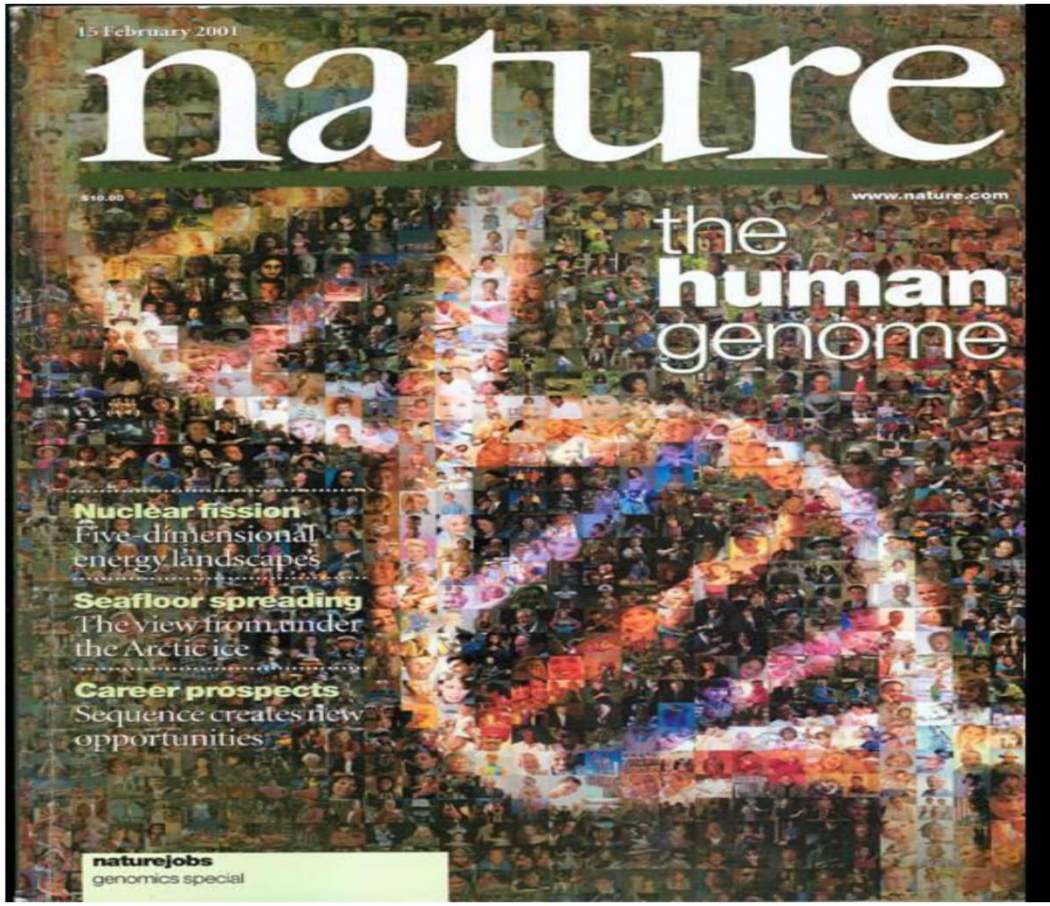
...but the complexity and diversi

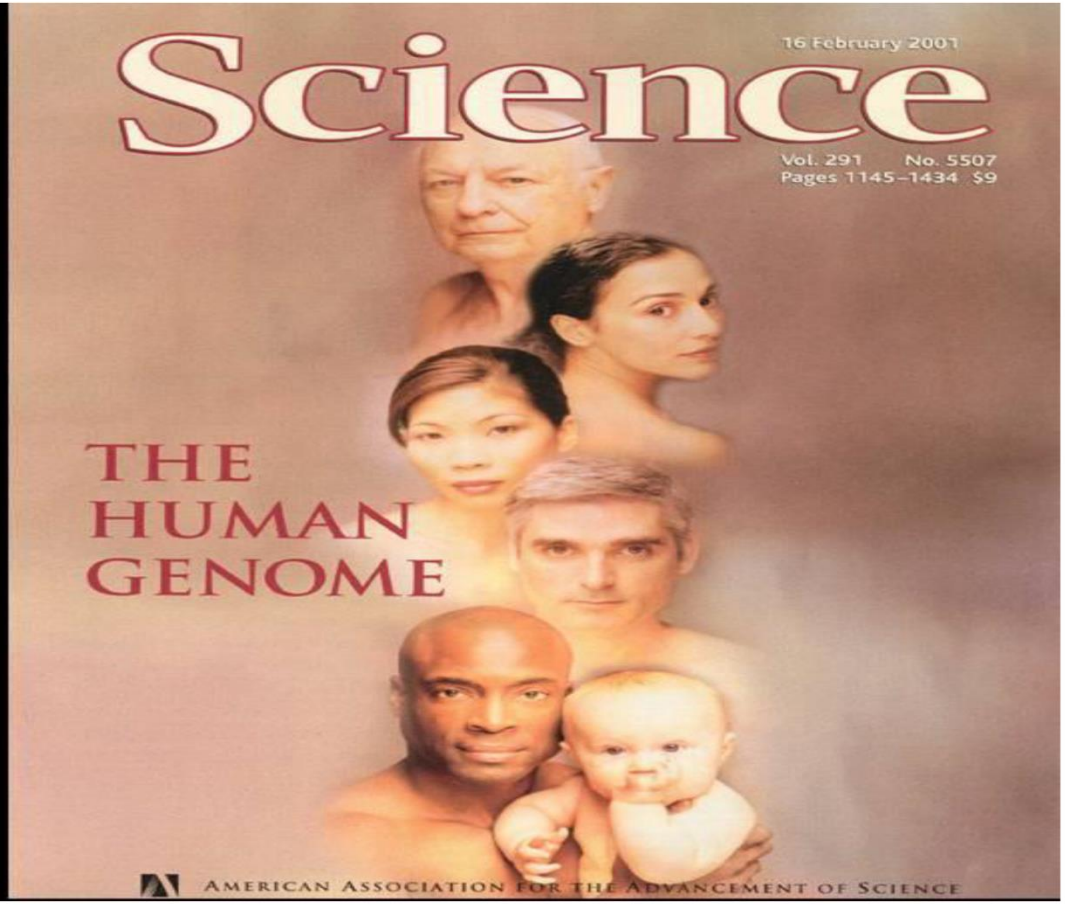
Same genome or DNA →

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

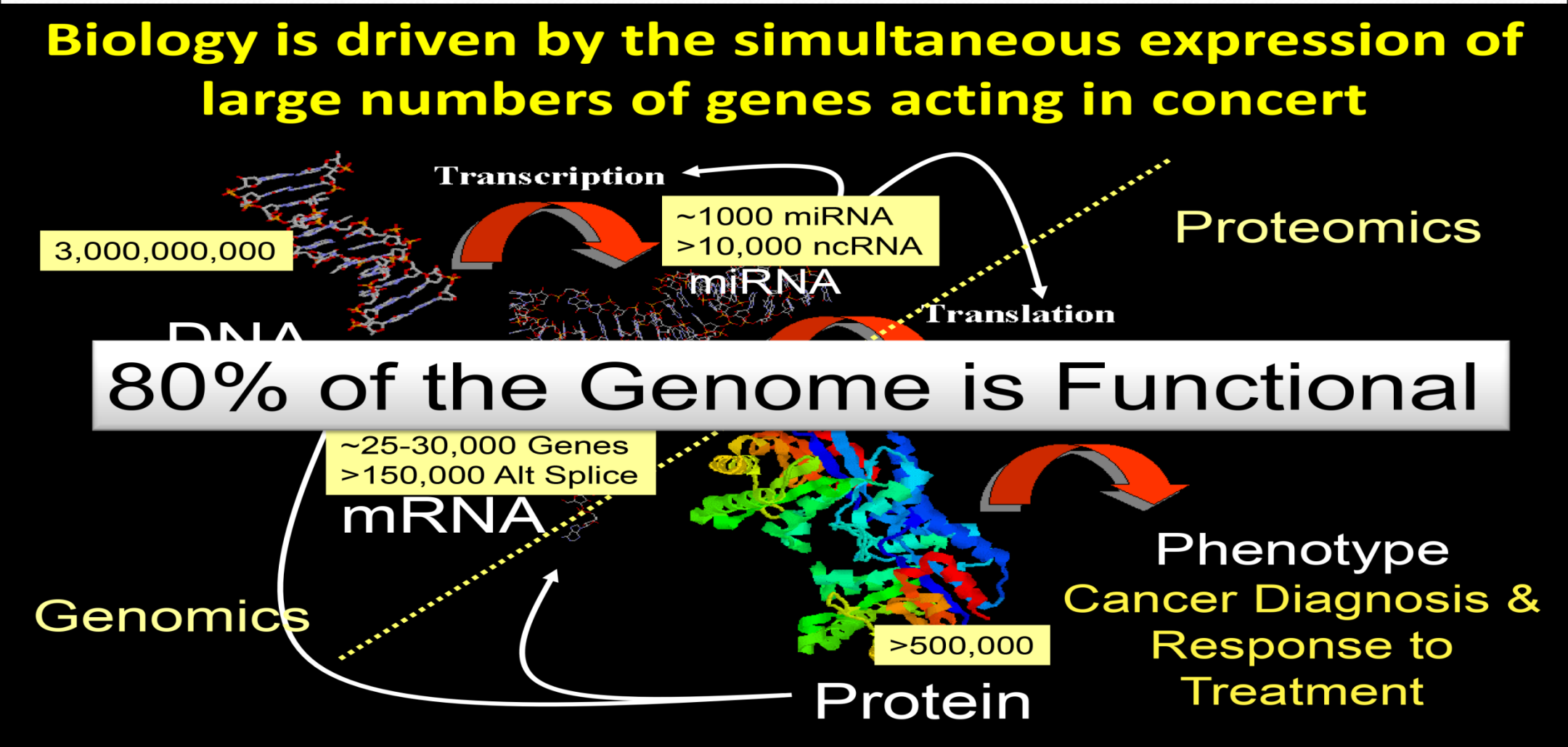


Human genome



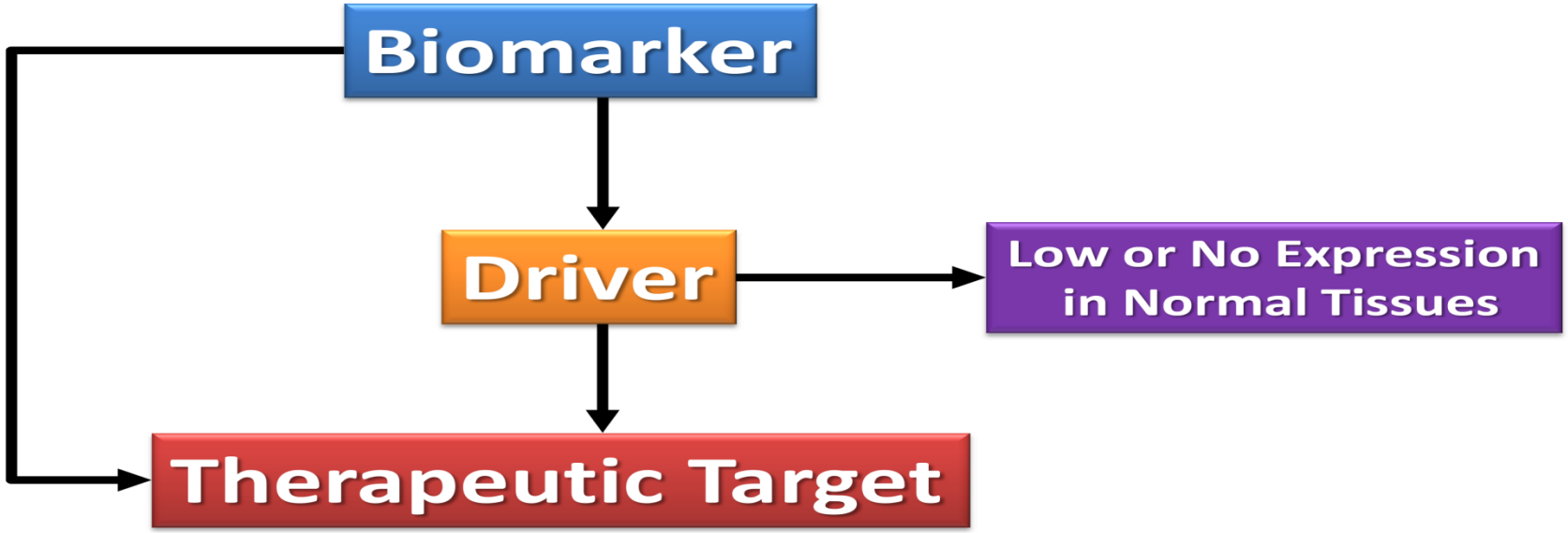


Gene expression



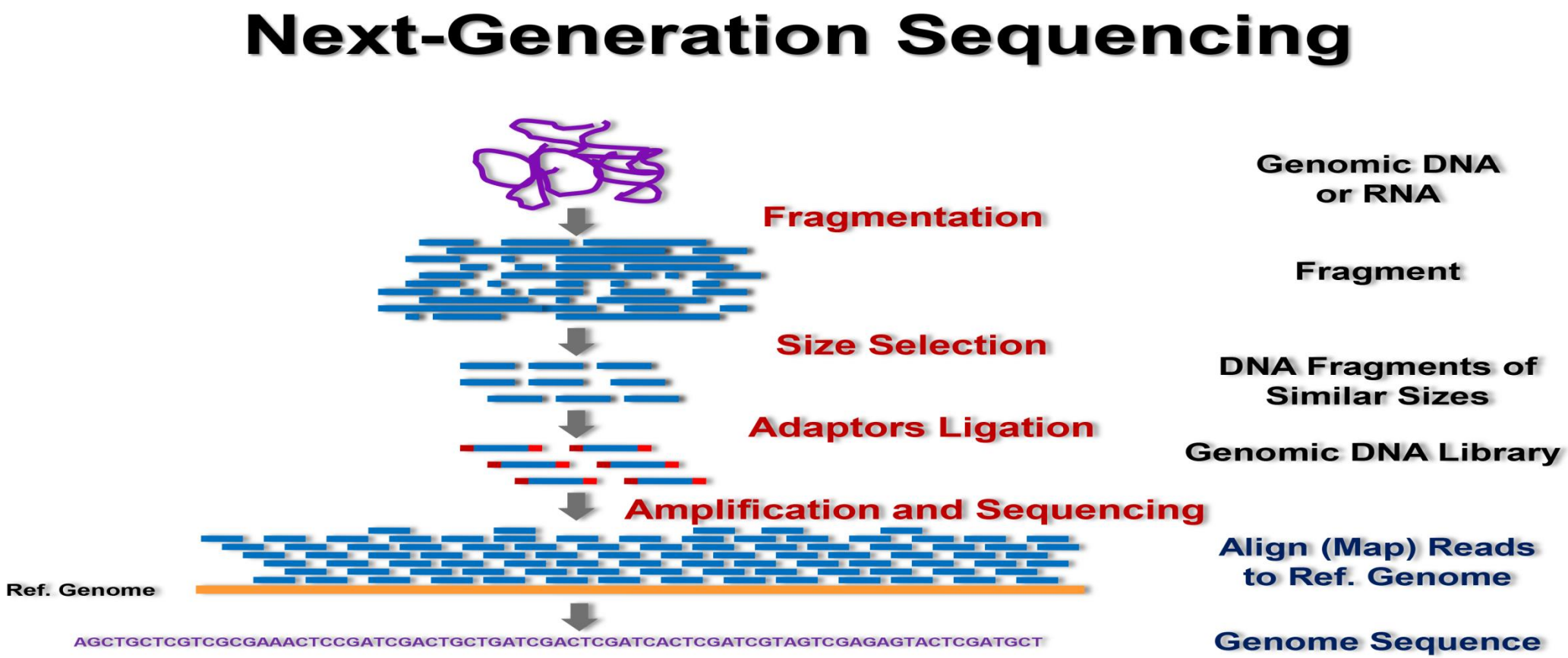
Genomic research

Genomic Research – identification of biomarker, driver, and target



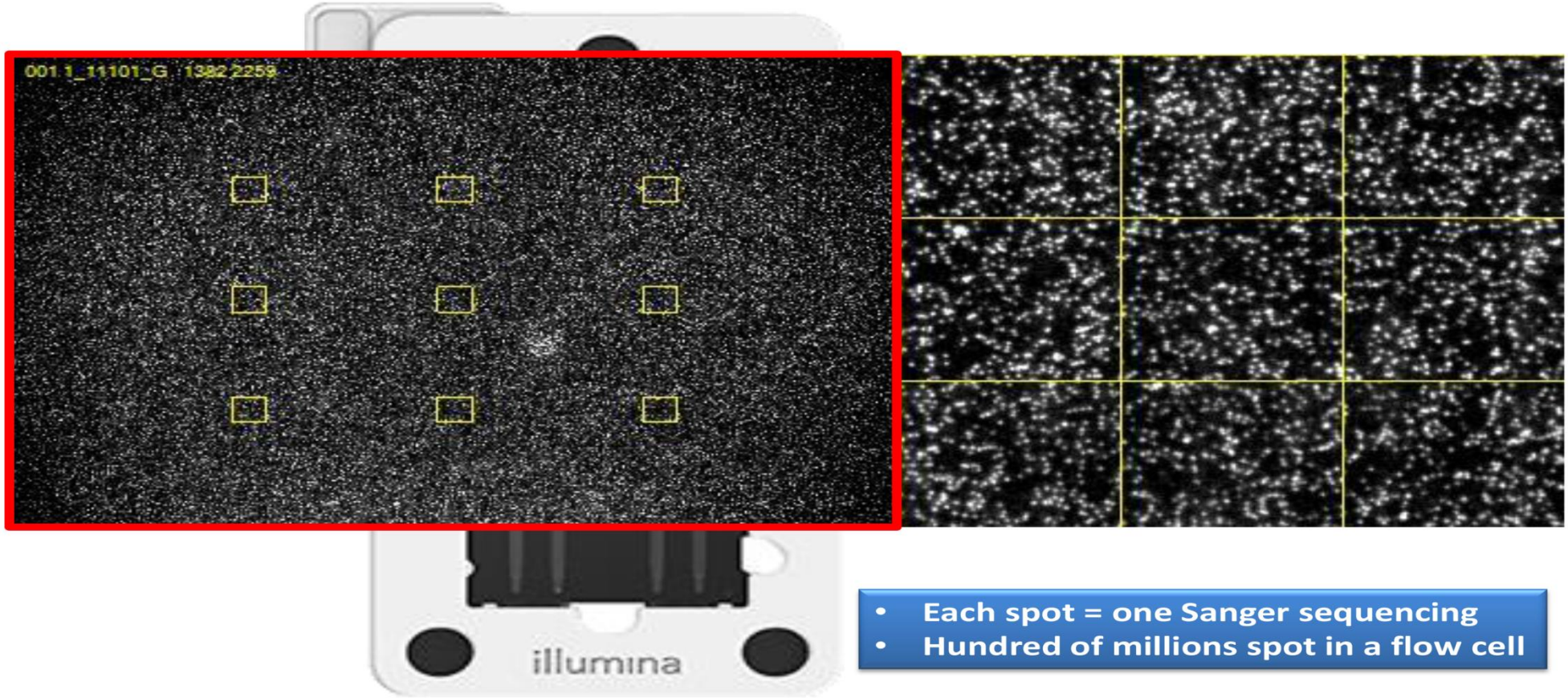
Next-Generation Sequencing (Massively Parallel Sequencing)

Next-generation sequencing



Massively Parallel Sequencing

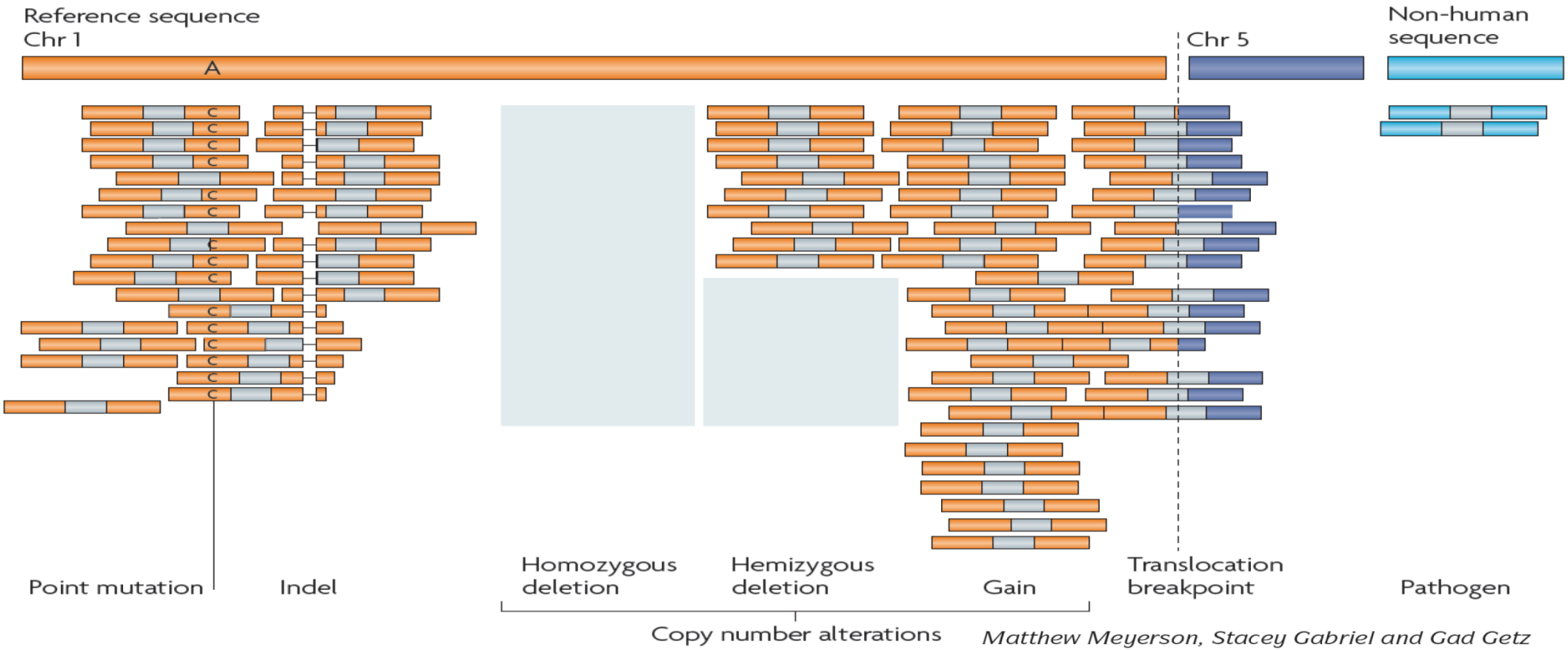
Massively Parallel Sequencing



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

Genomic Alterations

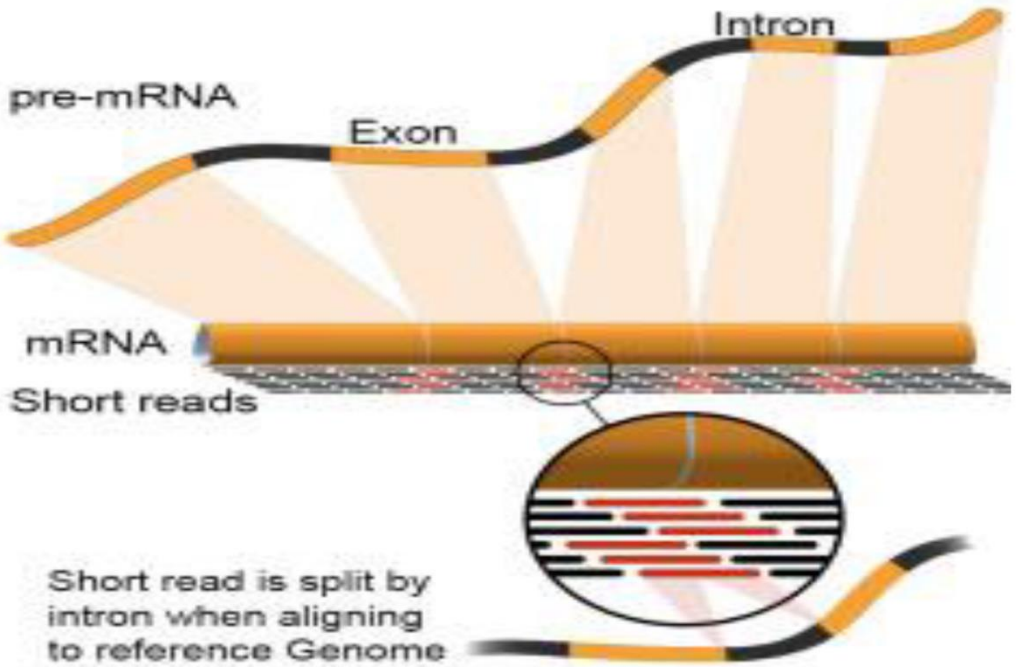
Genomic alterations detected by DNA sequencing



Matthew Meyerson, Stacey Gabriel and Gad Getz
NATURE REVIEWS | GENETICS VOLUME 11 | OCTOBER 2010

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

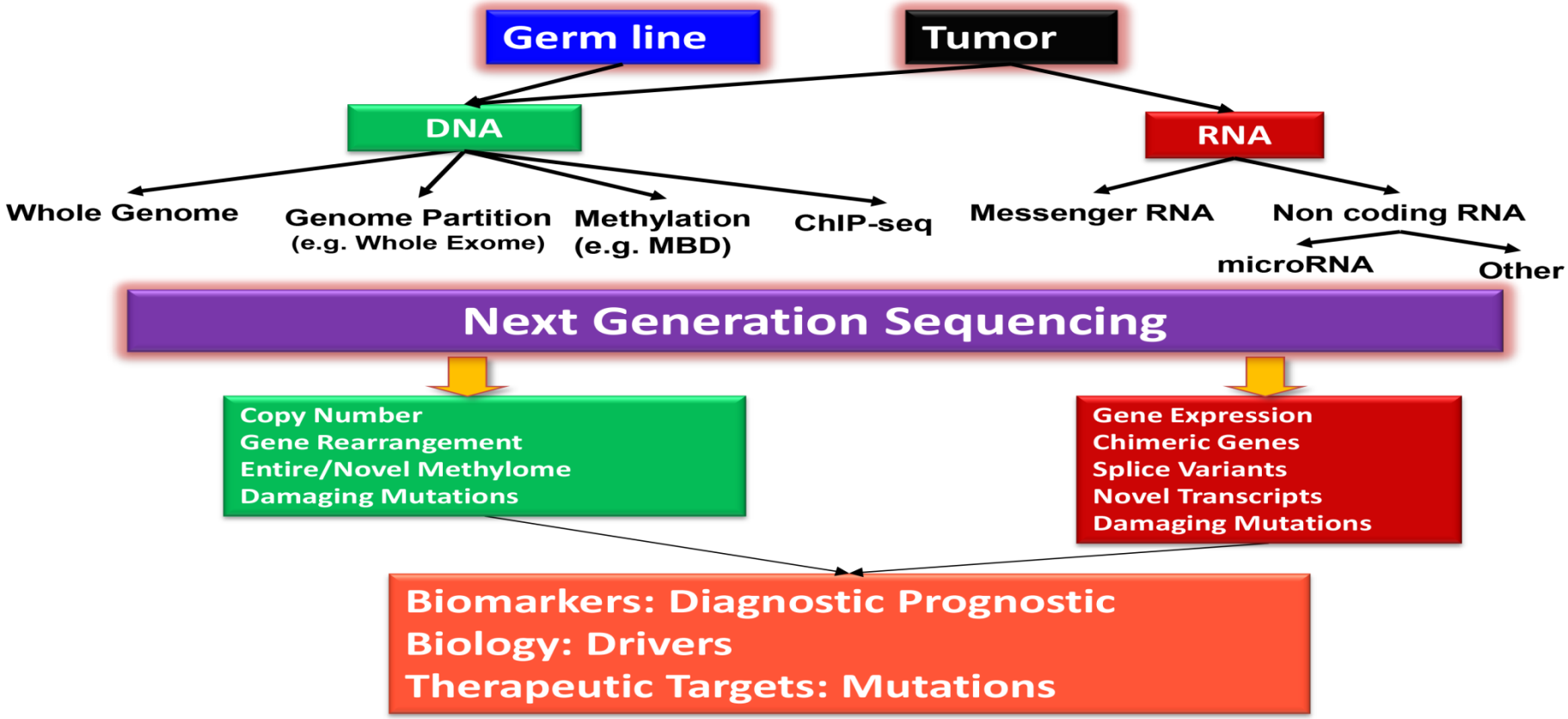
Properties

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

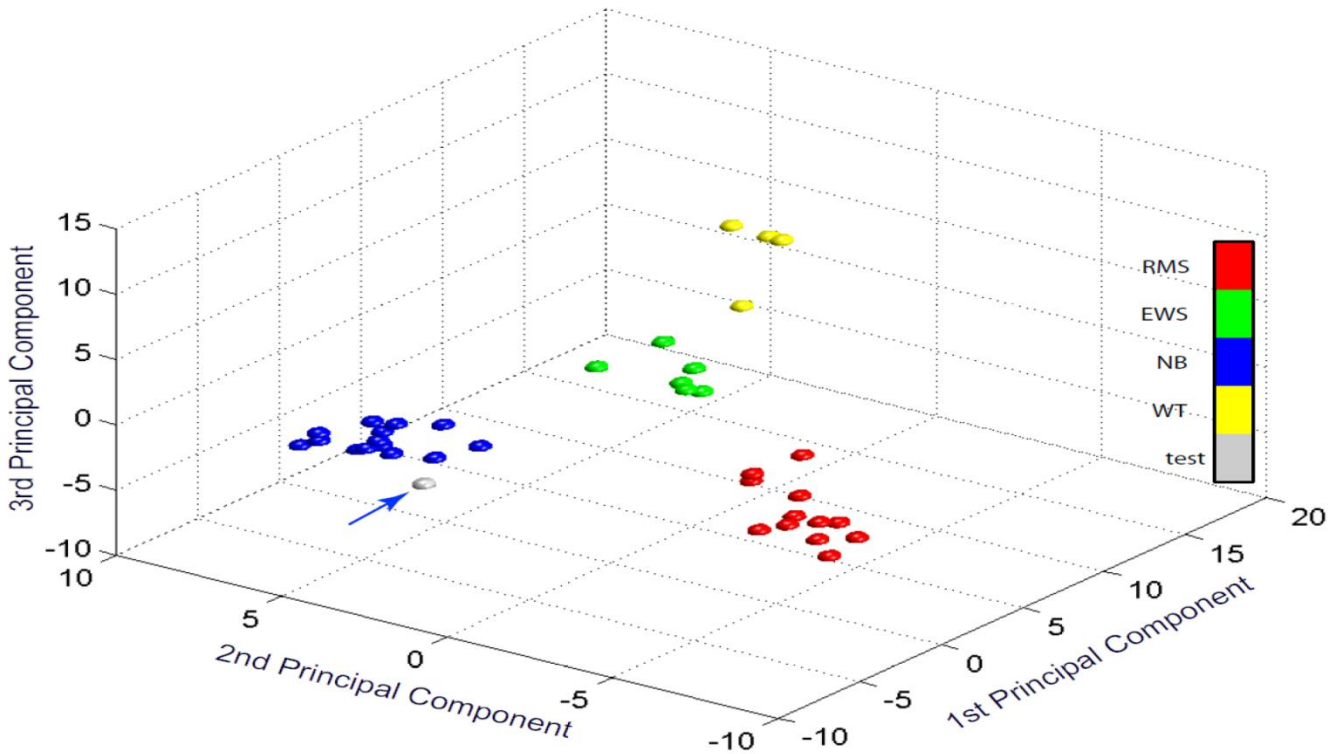
Cancer Genomes

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



Diagnosis

Diagnosis of cancers using gene expression profiles



Wilm's tumor

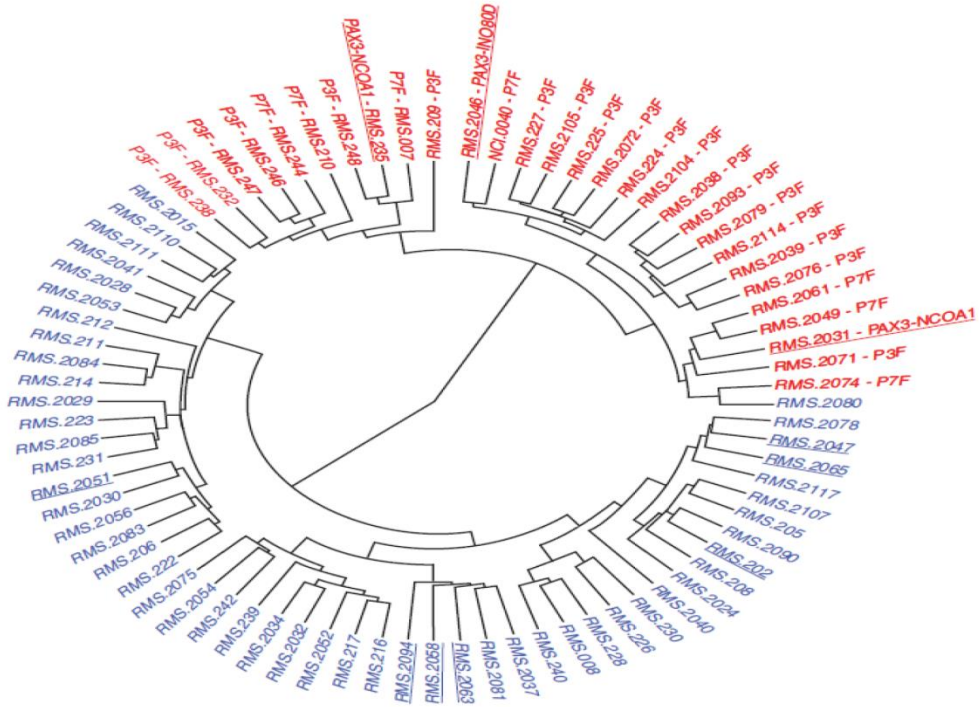
↓

Neuroblastoma

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

RMS subtypes

Distinguish RMS sub-types using unsupervised clustering of transcriptome data



Fusion-positive RMS

Fusion-negative RMS

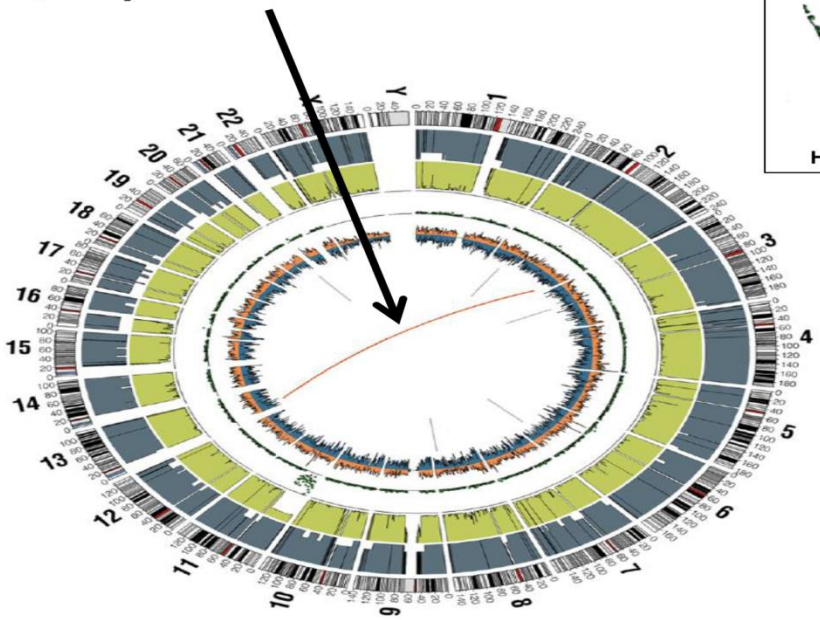
Shern et al., Cancer Discovery 2014, 4(2):216-31

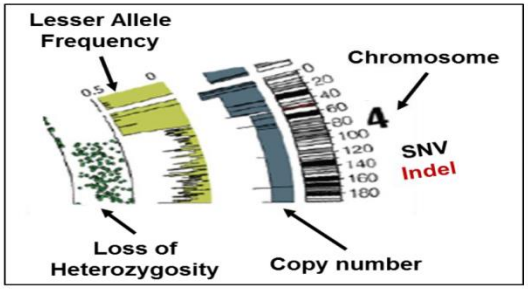
Diagnosis

Diagnosis of fusion positive pediatric tumors using whole genome sequencing

Rhabdomyosarcoma

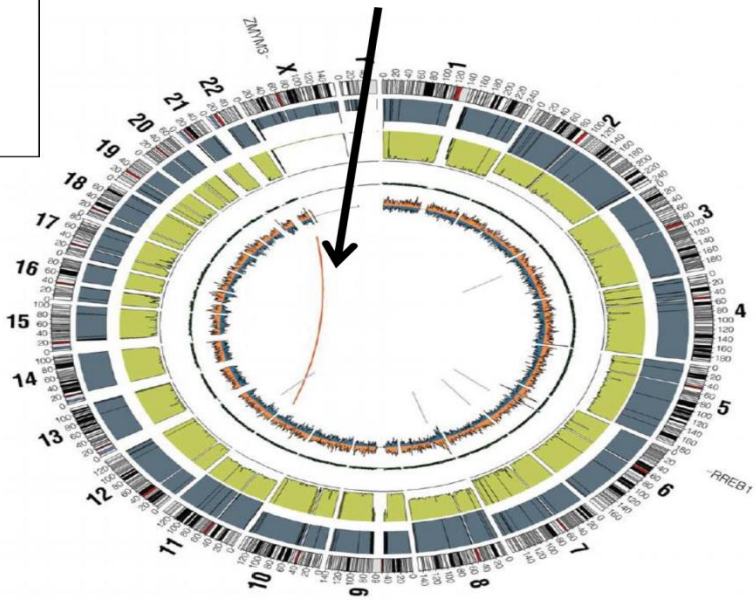
t(2;13) PAX3-FOXO1





Ewing's Sarcoma

t(11;22) EWS-FLI1



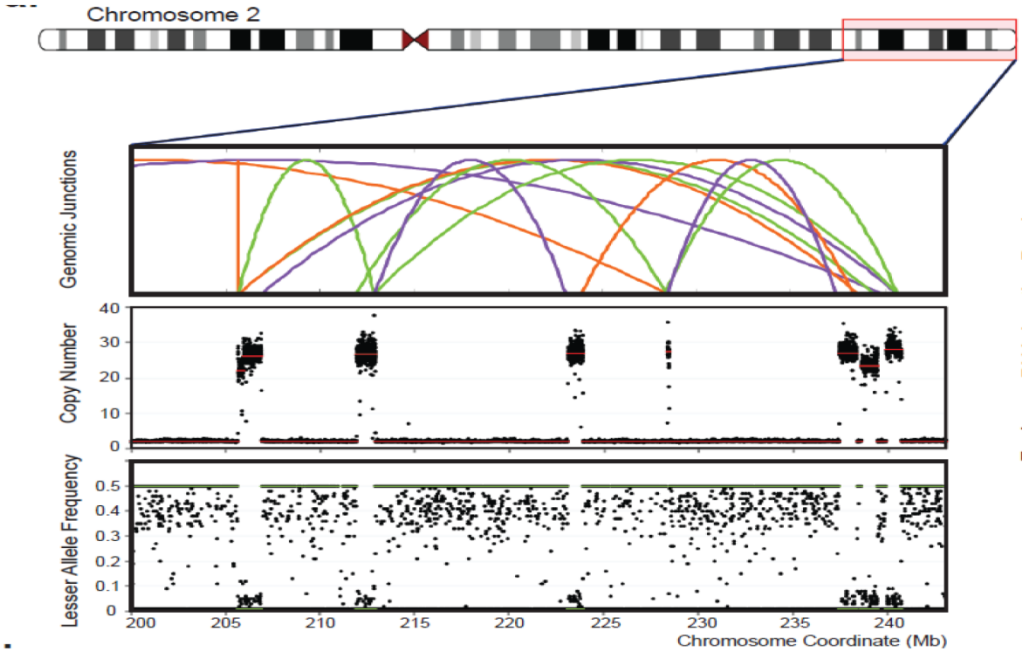
Shern et al., Cancer Discovery 2014, 4(2):216-31

Brohl et al., PLOS Genetics 2014, 10(7):e1004629

Rearrangement

Novel in-frame *PAX3-INO80D* fusion with massive 2q rearrangement in RMS,
Expression fusion gene verified by RNAseq

DNA



RNA

Correct Diagnosis

Correct Diagnosis using whole genome sequencing

Case 1

- Original Histology: Sarcoma, undifferentiated RMS, not otherwise specified.
- Had *ALK-NPM1* fusion by whole genome sequencing;
- Review of Histology = Hematological malignancy- most likely misdiagnosed ALCL

Case 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 the source

Correct Diagnosis

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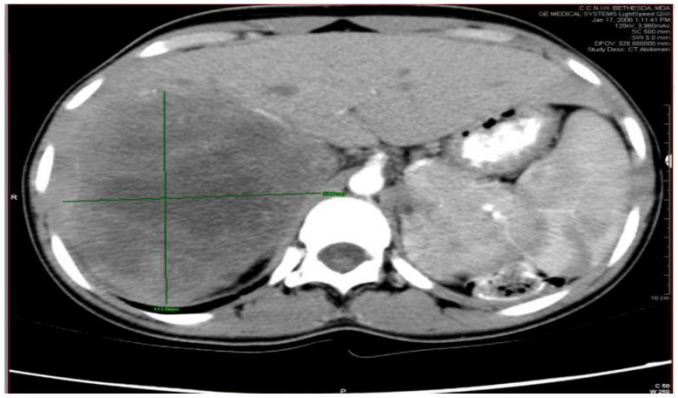
Neuroblastoma

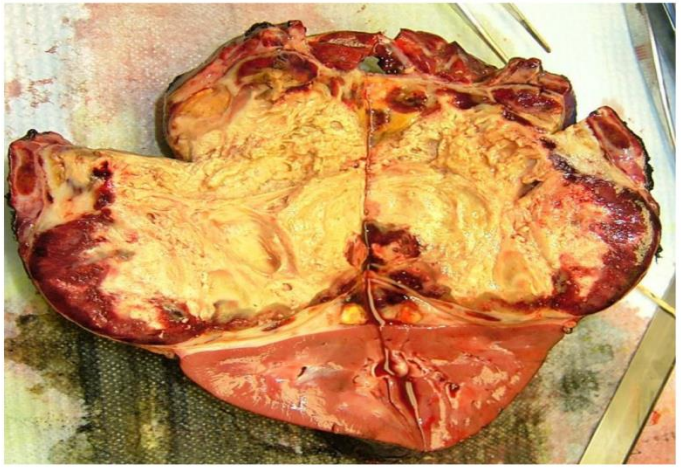
Neuroblastoma: an enigmatic cancer of childhood

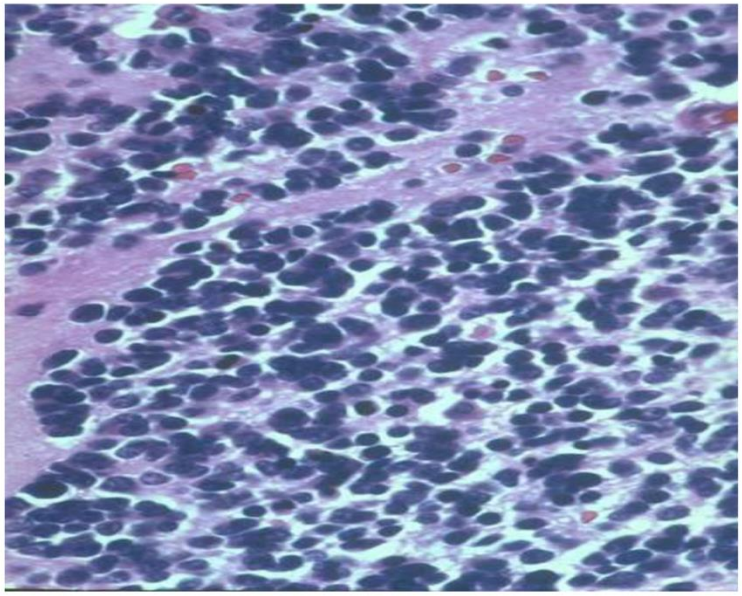
- Very diverse outcomes
- Survival rate for patients with high-risk neuroblastoma is <50% despite of intensive multimodal therapies
- Current cytotoxic therapies result in high morbidity rate
- High mortality and morbidity is substantial for patients with metastatic pediatric tumors
- Novel targeted therapies are highly desirable

Neuroblastoma stage 4

Neuroblastoma Stage 4



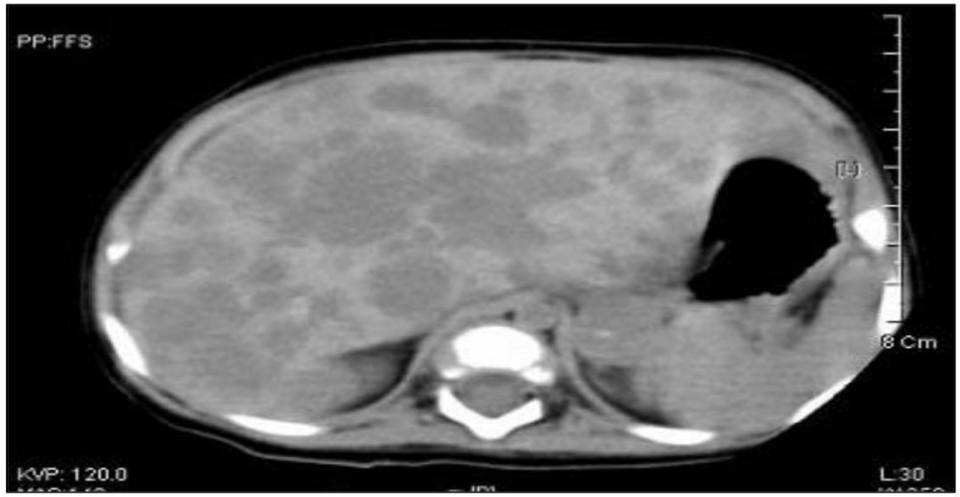


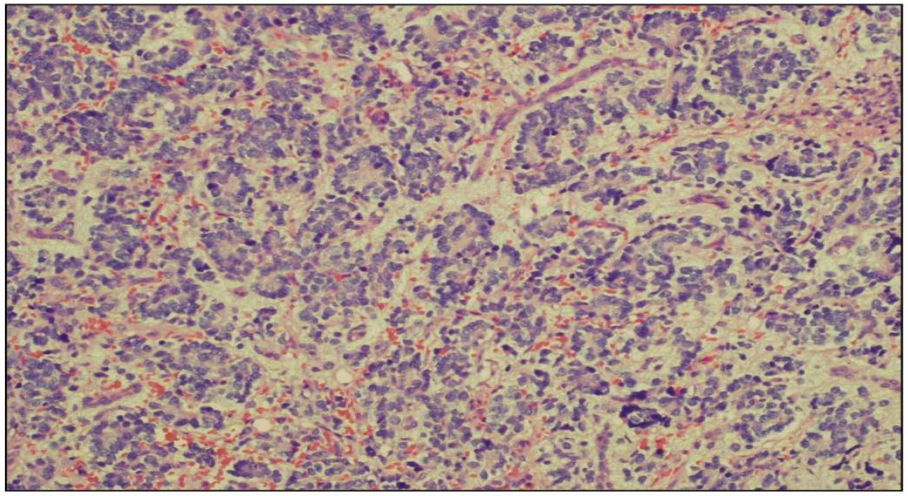


Survival <50%

Neuroblastoma Stage 4S

Neuroblastoma Stage 4S

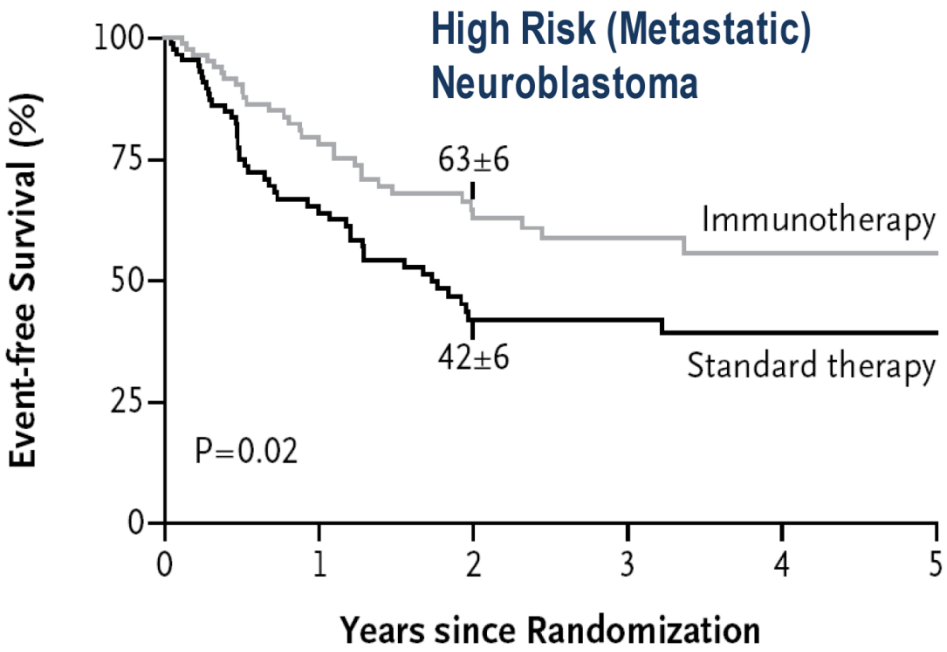




Survival >90%

Cure Rate

Cure rate for metastatic neuroblastoma remains poor despite of aggressive treatment



Yu *et al.* 2010 NEJM

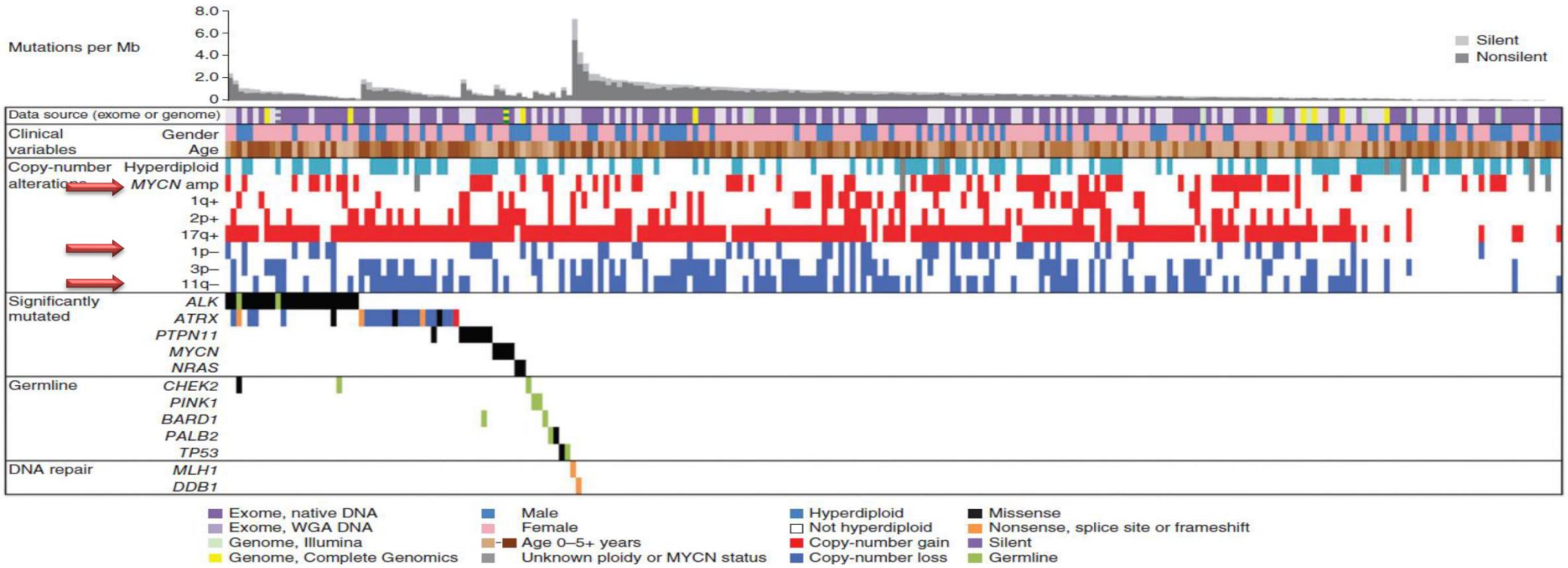
Mission of Oncogenomics Section

Apply high throughput genomic and proteomic methods to investigate these cancers

- Decipher biology
- Identify and validate novel
  - diagnostic and prognostic biomarkers
  - therapeutic targets
- Translation of our discoveries to the clinic

Neuroblastoma mutations

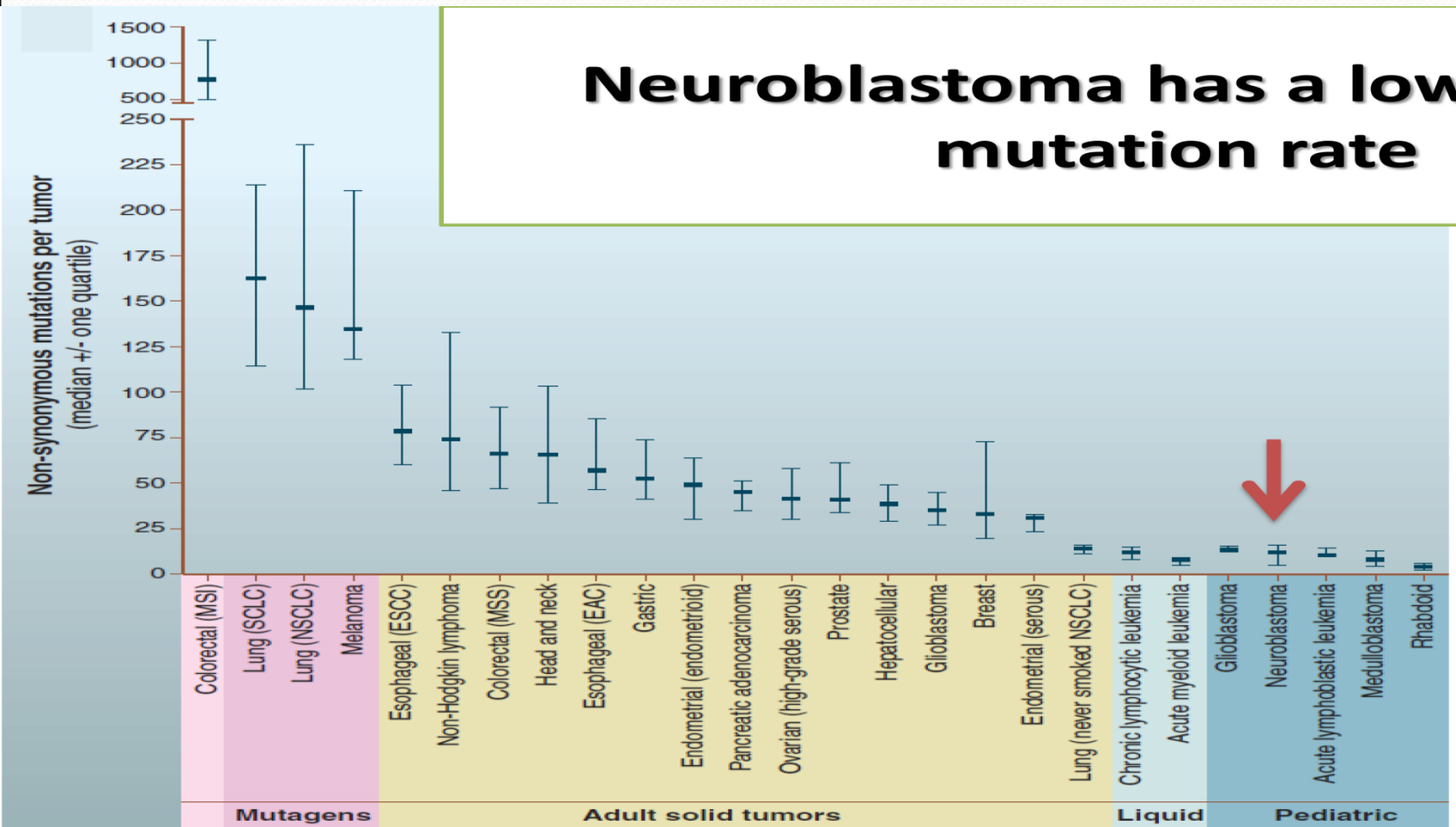
Relative paucity of recurrent somatic mutations in neuroblastoma



Pugh *et al.* Nature Genetics, 2012

Neuroblastoma

Neuroblastoma has a low somatic mutation rate

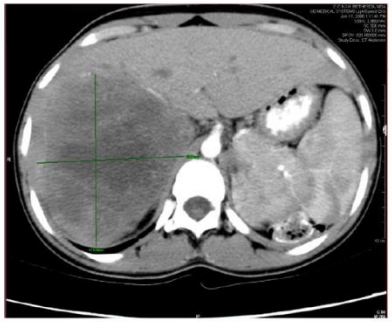


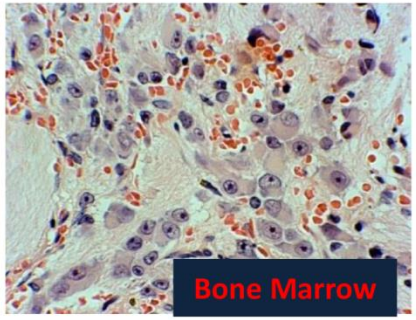
Bert Vogelstein *et al.*
Science **339**, 1546 (2013)

High-risk neuroblastoma

Patient (19yr) with High-Risk Neuroblastoma

Diagnosis



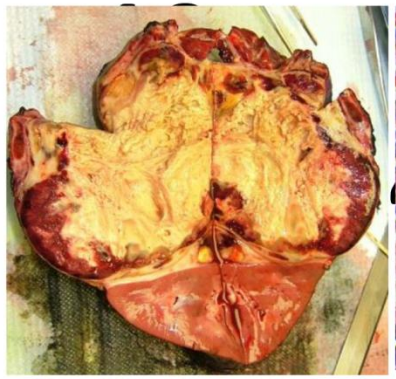


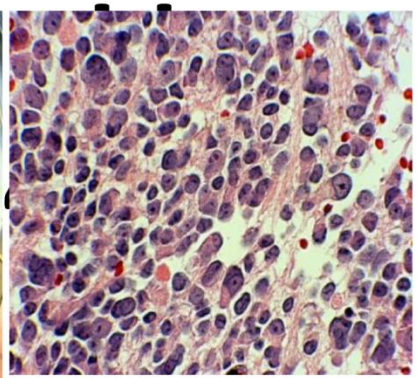
Met1-BM:
bone marrow biopsy at diagnosis.
>80% tumor

~4 Months

- 4 cycles Induction
- A3973

Surgery

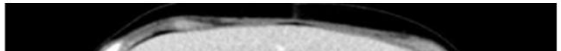




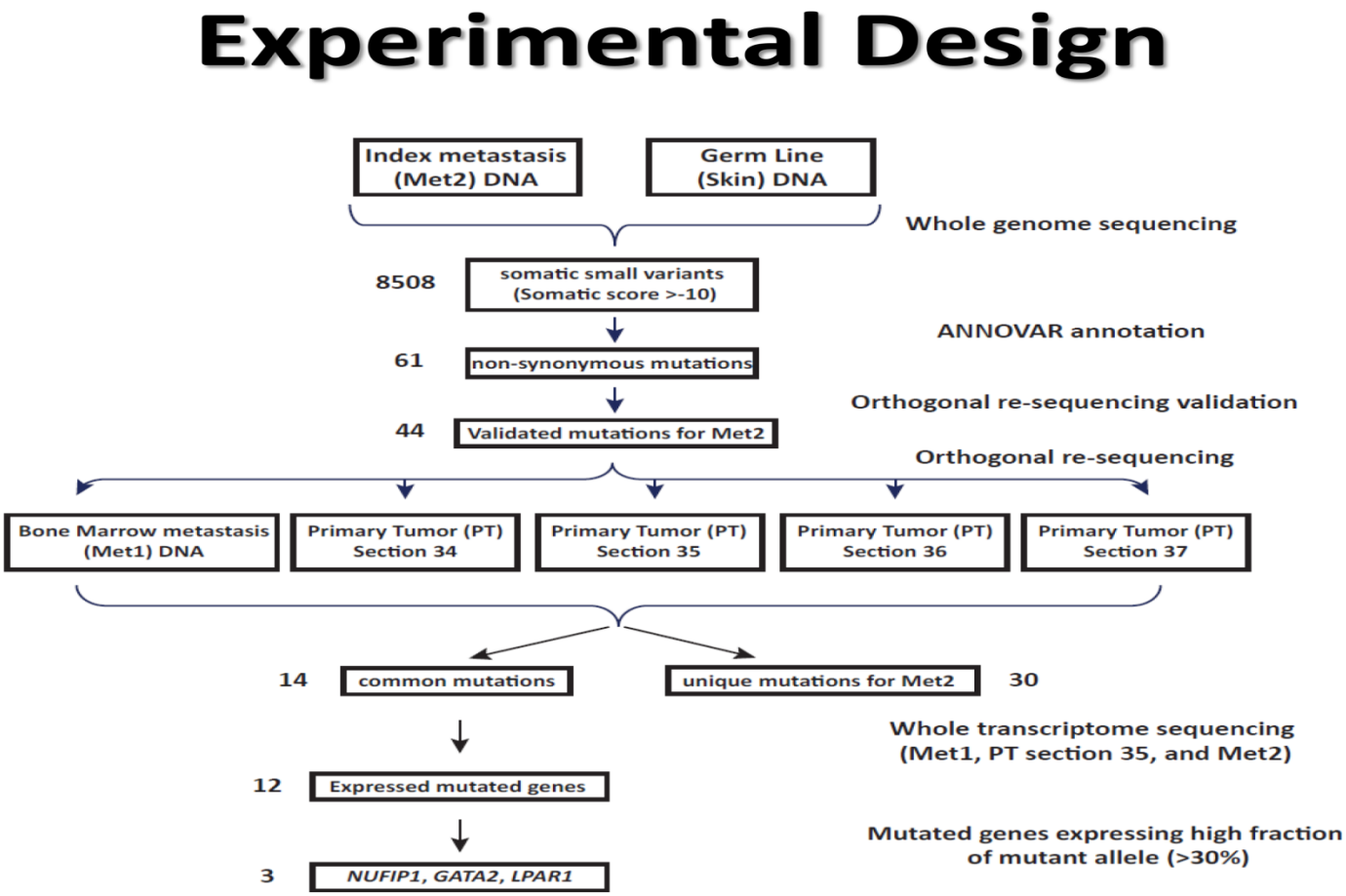
Primary: tumor removed by surgery viable margin

3 years

- 8 cycles of Salvage Therapy
- 7 cycles of RA
- Radiation

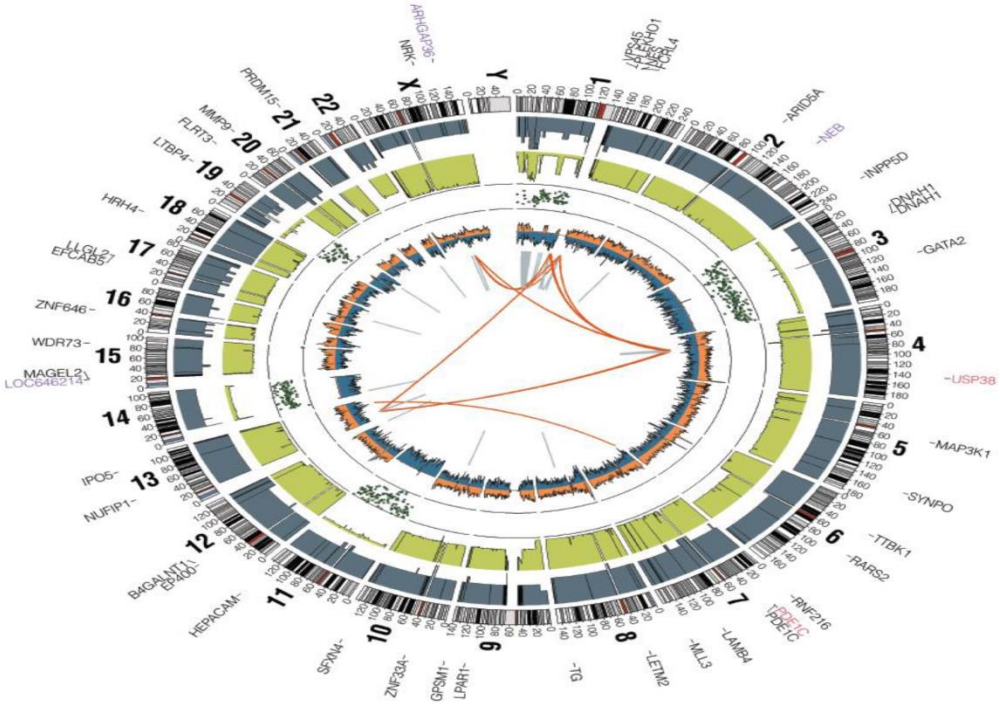


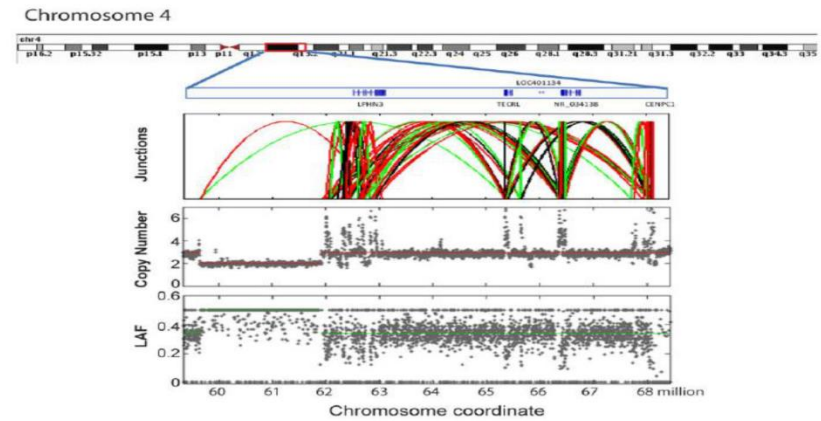
Experimental design

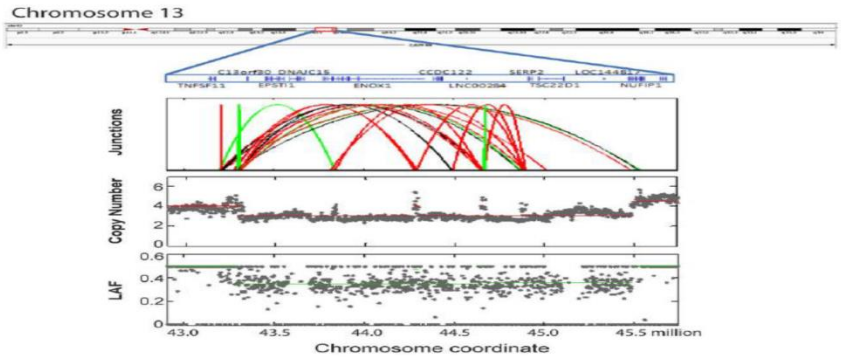


Mutations

Whole genome sequencing identified 44 non-synonomous mutations with many structural alterations

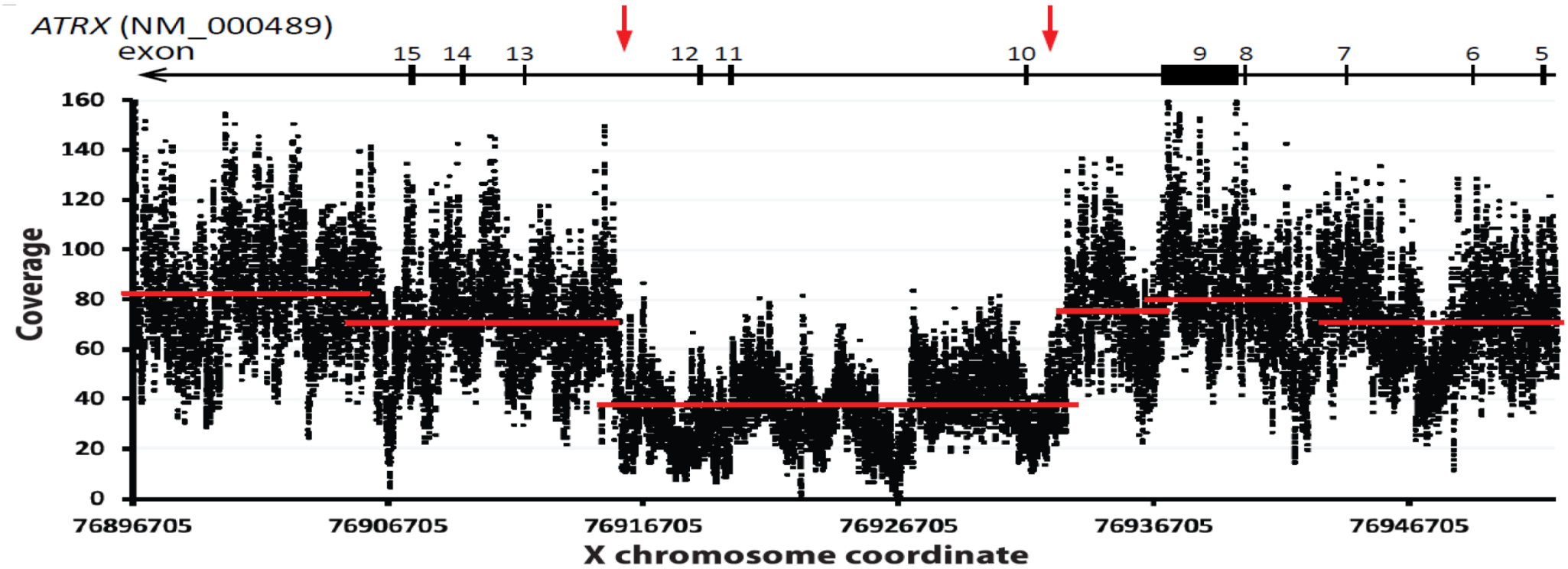






ATRX gene

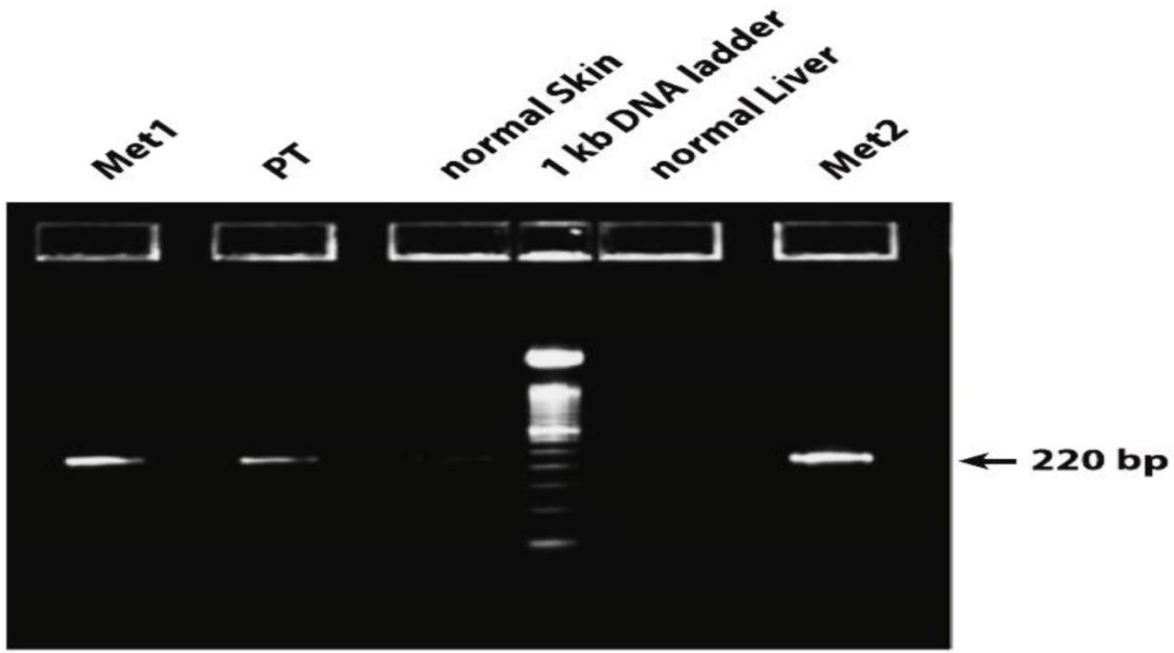
ATRX gene is hemizyously deleted in Met2

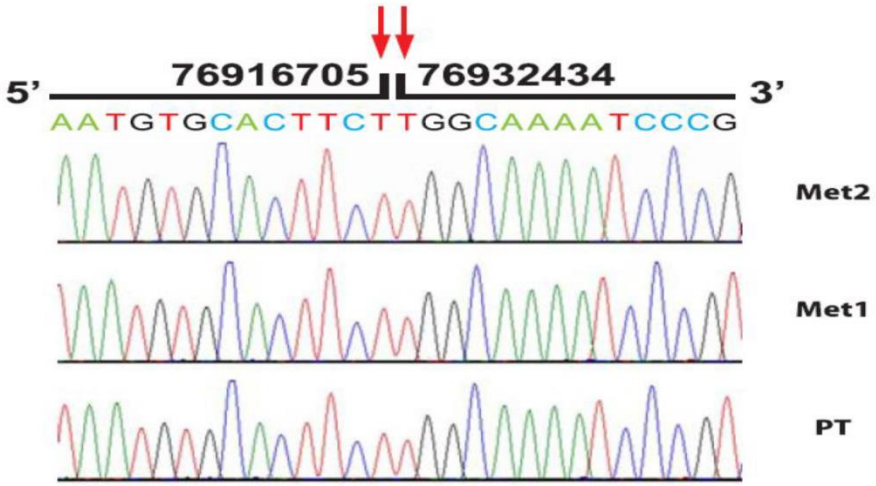


ATRX **gene is mutated in ~40% adolescent and young adult neuroblastoma patients (Cheung *et. al.* 2012, JAMA)**

ATRX deletion

ATRX deletion was detected in both diagnostic sample and primary tumor





Somatic mutations

Accumulation of *De Novo* somatic mutations in Met2

Table S2. Validated 44 somatic non-synonymous mutations in Met2

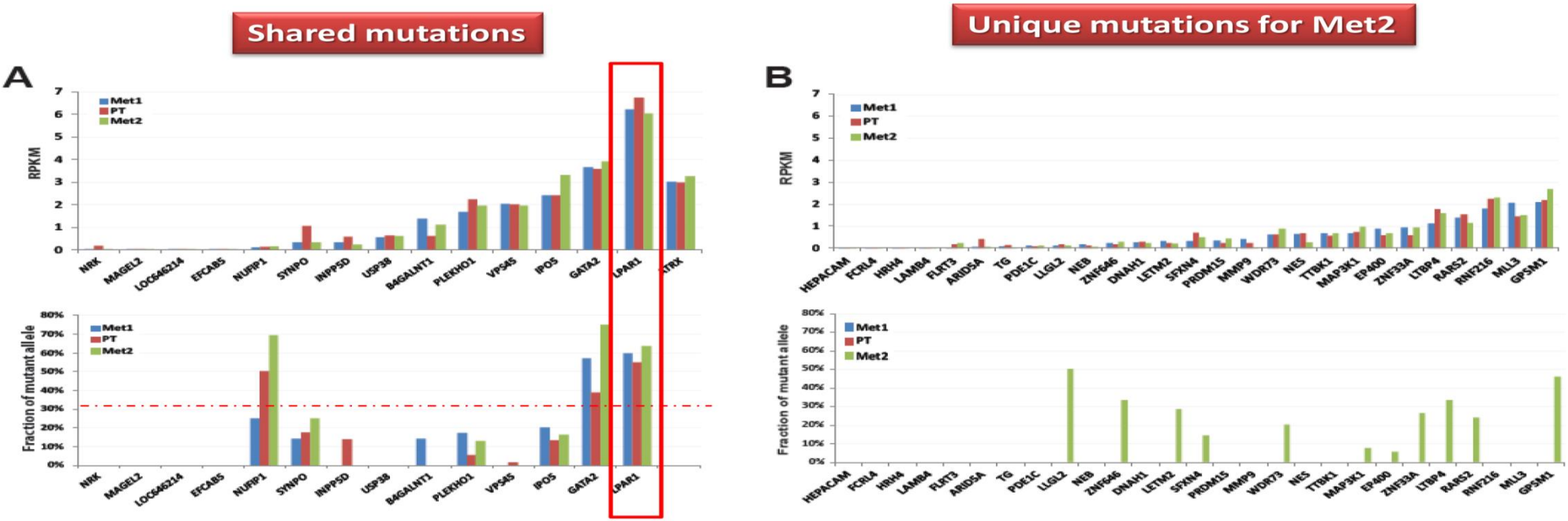
Gene	chr	begin	end	cytoBand	zygosity	somaticCategory	reference	variant	a.a. change	variant type	SIFT_Prediction	SIFT_Score	pph2_prediction	pph2_class	pph2_prob	pph2_FPR	pph2_TPR	pph2_FDR	Variant allele frq_Met1	Variant allele frq_PT_34	Variant allele frq_PT_35	Variant allele frq_PT_36	Variant allele frq_PT_37	Variant allele frq_Met2	Variant allele frq_Normal Skin	Variant allele frq_Normal Liver	Coverage at the variant	Sequencing Method:	Shared [PT&Met2&Met1
VPS45	1	150064139	150064140	1q21.2	het-ref	snp	G	C	G405A	nonsynonymous	TOLERATED	0.7	benign	neutral	0	1	1	0.575	15%	15%	16%	14%	15%	19%	0%	0%	1000s	Ion Torrent	Yes
PLEKHO1	1	150131220	150131221	1q21.2	het-ref	snp	G	C	E245Q	nonsynonymous	TOLERATED	0.31	benign	neutral	0.361	0.108	0.898	0.14	G/C	G/C	G/C	G/C	G/C	G/C	G	G	1 or 2	Sanger	Yes
INPPSD	2	234070455	234070456	2q37.1	het-ref	snp	C	T	S251L	nonsynonymous	TOLERATED	1							29%	24%	25%	16%	19%	35%	1%	0%	100s	Ion Torrent	Yes
GATA2	3	128199937	128199938	3q21.3	het-ref	snp	G	T	P456Q	nonsynonymous	TOLERATED	1							9%	74%	88%	63%	74%	90%	0%	0%	10s	Ion Torrent	Yes
USP38	4	144135650	144135654	4q31.21	het-ref	del	TATT		f.s.	frameshift_deletion									80%	18%	24%	10%	12%	37%	0%	0%	100s	Ion Torrent	Yes
SYNPO	5	150028224	150028225	5q33.1	het-ref	snp	C	T	P374S	nonsynonymous	TOLERATED	0.16	benign	neutral	0.002	0.704	0.987	0.452	C/T	C/T	C/T	C/T	C/T	C/T	C	C	1 or 2	Sanger	Yes
LPAR1	9	113704006	113704007	9q31.3	het-ref	snp	G	A	R163W	nonsynonymous	DAMAGING	0	probably damaging	deleterious	1	0.00026	0.00018	0.0109	G/A	G/A	G/A	G/A	G/A	G/A	G	G	1 or 2	Sanger	Yes
B4GALNT1	12	58024081	58024082	12q14.1	het-ref	snp	C	T	V189M	nonsynonymous	TOLERATED	0.06	probably damaging	deleterious	1	0.00026	0.00018	0.0109	C/T	C/T	C/T	C/T	C/T	C/T	C	C	1 or 2	Sanger	Yes
NUFIP1	13	45533548	45533549	13q14.12	het-ref	snp	C	G	D330H	nonsynonymous	TOLERATED	1							15%	18%	18%	17%	15%	19%	0%	0%	1000s	Ion Torrent	Yes
IPO5	13	98645247	98645248	13q32.2	het-ref	snp	A	T	T276S	nonsynonymous	TOLERATED	0.73							A/T	A/T	A/T	A/T	A/T	A/T	A	A	1 or 2	Sanger	Yes
LOC646214	15	21935022	21935023	15q11.2	het-ref	snp	G	T	S190X	stopgain	Not scored	NA							16%	8%	11%	8%	8%	10%	1%	1%	1000s	Ion Torrent	Yes
MAGEL2	15	23892045	23892046	15q11.2	het-ref	snp	G	T	P282T	nonsynonymous	Not scored	NA							51%	29%	29%	24%	27%	28%	0%	0%	1000s	Ion Torrent	Yes
EFCAB5	17	28407946	28407947	17q11.2	het-ref	snp	G	A	R1125K	nonsynonymous	DAMAGING	0	probably damaging	deleterious	1	0.00026	0.00018	0.0109	17%	3%	4%	1%	4%	10%	0%	0%	100s	Ion Torrent	Yes
NRK	X	105137828	105137829	Xq22.3	het-ref	snp	T	A	V128E	nonsynonymous	DAMAGING	0	probably damaging	deleterious	1	0.00026	0.00018	0.0109	T/A	T/A	T/A	T/A	T/A	T/A	T	T	1 or 2	Sanger	Yes
NES	1	156646973	156646974	1q23.1	het-ref	snp	G	T	A28E	nonsynonymous	TOLERATED	0.06	probably damaging	deleterious	0.997	0.0167	0.409	0.0357	G	G	G	G	G	G/T	G	G	1 or 2	Sanger	No
FCRL4	1	157557143	157557144	1q23.1	het-ref	snp	C	T	Q257R	nonsynonymous	DAMAGING	0	probably damaging	deleterious	0.995	0.0277	0.681	0.0521	0%	0%	0%	0%	0%	27%	0%	0%	1000s	Ion Torrent	No
ARID5A	2	97215082	97215083	2q11.2	het-ref	snp	G	A	R49Q	nonsynonymous	TOLERATED	0.6	benign	neutral	0.005	0.26	0.968	0.267	1%	0%	0%	0%	0%	60%	0%	0%	1000s	Ion Torrent	No
NEB	2	152551122	152551123	2q23.3	het-ref	snp	C	T	W565X	stopgain	DAMAGING	0.01	probably damaging	deleterious	1	0.00026	0.00018	0.0109	0%	0%	0%	0%	0%	32%	0%	0%	1000s	Ion Torrent	No
DNAH1	3	52380685	52380686	3p21.1	het-ref	snp	C	T	L619F	nonsynonymous	TOLERATED	0.15	benign	neutral	0.054	0.164	0.94	0.191	0%	0%	0%	0%	0%	45%	0%	0%	1000s	Ion Torrent	No
DNAH1	3	52380692	52380693	3p21.1	het-ref	snp	G	T	S621I	nonsynonymous	TOLERATED	0.51	benign	neutral	0.002	0.704	0.987	0.452	0%	0%	0%	0%	0%	45%	0%	0%	1000s	Ion Torrent	No
MAP3K1	5	56177866	56177867	5q11.2	het-ref	snp	C	A	T947K	nonsynonymous	) *Warning! Low con	0.05	benign	neutral	0.002	0.704	0.987	0.452	0%	0%	0%	0%	0%	32%	0%	0%	1000s	Ion Torrent	No
TTBK1	6	43226894	43226895	6p21.1	het-ref	snp	C	G	S379C	nonsynonymous	TOLERATED	0.06	possibydamaging	deleterious	0.761	0.0768	0.852	0.109	0%	0%	0%	0%	0%	29%	0%	0%	100s	Ion Torrent	No
RARS2	6	88240580	88240581	6q15	het-ref	snp	T	G	E231A	nonsynonymous	TOLERATED	1							0%	0%	0%	0%	0%	27%	0%	0%	1000s	Ion Torrent	No
RNF216	7	5778927	5778928	7p22.1	het-ref	snp	T	A	K367M	nonsynonymous	TOLERATED	1							0%	0%	0%	0%	0%	28%	0%	0%	1000s	Ion Torrent	No
PDE1C	7	31848692	31848693	7p14.3	het-ref	del	C		f.s.	frameshift_deletion									0%	0%	0%	0%	0%	28%	0%	0%	100s	Ion Torrent	No
PDE1C	7	32209549	32209550	7p14.3	het-ref	snp	C	T	C52Y	nonsynonymous	) *Warning! Low con	0							0%	0%	0%	0%	0%	26%	0%	0%	1000s	Ion Torrent	No
LAMB4	7	107735684	107735685	7q31.1	het-ref	snp	G	C	C486W	nonsynonymous	N/A	N/A							0%	0%	0%	0%	0%	14%	0%	0%	1000s	Ion Torrent	No
MLL3	7	151845517	151845518	7q36.1	het-ref	snp	G	T	D4498E	nonsynonymous	DAMAGING	0.02	probably damaging	deleterious	1	0.00026	0.00018	0.0109	0%	0%	0%	0%	0%	22%	0%	0%	1000s	Ion Torrent	No
LETM2	8	38250397	38250398	8p12	het-ref	snp	A	T	E82V	nonsynonymous	TOLERATED	1							0%	0%	0%	0%	0%	19%	0%	0%	100s	Ion Torrent	No
TG	8	134024237	134024238	8q24.22	het-ref	snp	G	T	A2119S	nonsynonymous	DAMAGING	0.02	probably damaging	deleterious	0.974	0.0438	0.763	0.0722	0%	0%	0%	0%	0%	28%	0%	0%	1000s	Ion Torrent	No
GPSM1	9	139252574	139252575	9q34.3	het-ref	snp	G	A	R135H	nonsynonymous	DAMAGING	0	probably damaging	deleterious	1	0.00026	0.00018	0.0109	G	G	G	G	G	G/A	G	G	1 or 2	Sanger	No
ZNF33A	10	38344246	38344247	10p11.21	het-ref	snp	G	T	A399S	nonsynonymous	TOLERATED	0.77	probably damaging	deleterious	0.967	0.046	0.773	0.0746	G	G	G	G	G	G/T	G	G	1 or 2	Sanger	No
SFXN4	10	120917237	120917238	10q26.11	het-ref	snp	T	G	K160T	nonsynonymous	TOLERATED	0.61							0%	0%	0%	0%	0%	39%	0%	0%	1000s	Ion Torrent	No
HEPACAM	11	124791305	124791306	11q24.2	het-ref	snp	G	A	P327S	nonsynonymous	TOLERATED	0.47	benign	neutral	0	1	1	0.575	no coverage	0%	0%	0%	0%	91%	0%	0%	10s	Ion Torrent	No
EP400	12	132466066	132466067	12q24.33	het-ref	snp	A	T	H490L	nonsynonymous	TOLERATED	1	benign	neutral	0.001	0.852	0.994	0.514	0%	0%	0%	0%	0%	16%	0%	0%	1000s	Ion Torrent	No
WDR73	15	85188896	85188897	15q25.2	het-ref	snp	C	G	V230L	nonsynonymous	DAMAGING	0.01	probably damaging	deleterious	0.979	0.0411	0.755	0.0687	0%	0%	0%	0%	0%	72%	0%	0%	1000s	Ion Torrent	No
ZNF646	16	31091627	31091628	16p11.2	het-ref	snp	G	T	C1328F	nonsynonymous	DAMAGING	0	probably damaging	deleterious	0.998	0.0112	0.273	0.0274	G	G	G	G	G	G/T	G	G	1 or 2	Sanger	No
LLGL2	17	73564892	73564893	17q25.1	het-ref	snp	C	A	P432Q	nonsynonymous	DAMAGING	0.02	probably damaging	deleterious	0.992	0.0314	0.704	0.0568	0%	0%	1%	1%	1%	76%	1%	0%	100s	Ion Torrent	No
HRH4	18	22040745	22040746	18q11.2	het-ref	snp	A	T	L18F	nonsynonymous	TOLERATED	1							0%	0%	0%	0%	0%	21%	0%	0%	1000s	Ion Torrent	No
LTBP4	19	41105140	41105141	19q13.2	hom	snp	G	C	G19R	nonsynonymous	N/A	N/A	possibydamaging	deleterious	0.651	0.0877	0.866	0.121	0%	0%	0%	1%	0%	93%	0%	0%	1000s	Ion Torrent	No
FLRT3	20	14307153	14307154	20p12.1	het-ref	snp	C	A	M333I	nonsynonymous	TOLERATED	0.51	benign	neutral	0.409	0.104	0.894	0.136	0%	0%	0%	0%	0%	21%	0%	0%	1000s	Ion Torrent	No
MMP9	20	44640897	44640898	20q13.12	het-ref	snp	G	A	A374T	nonsynonymous	DAMAGING	0.05	probably damaging	deleterious	0.998	0.0112	0.273	0.0274	0%	0%	0%	0%	0%	24%	0%	0%	1000s	Ion Torrent	No
PRDM15	21	43267231	43267232	21q22.3	het-ref	snp	A	C	S240A	nonsynonymous	TOLERATED	0.09	possibydamaging	deleterious	0.816	0.0707	0.84	0.102	0%	0%	0%	0%	0%	21%	0%	0%	100s	Ion Torrent	No
ARHGAP36	X	130218251	130218252	Xq26.1	het-ref	snp	C	T	Q207X	stopgain	N/A	N/A							0%	0%	0%	0%	0%	40%	0%	0%	1000s	Ion Torrent	No

Red denotes 14 common mutations shared by all three tumors, while black mutations are unique to the Met2 tumor.
Variant allele frequencies were derived from validation experiments using semiconductor or Sanger sequencing.
Met1, bone marrow metastasis taken at diagnosis; PT, primary tumor taken after cytotoxic therapy; met2, a liver metastasis taken at autopsy.

- Ion Torrent sequencing to cover 1000s reads
- 1/3 are commonly shared in other samples
- 2/3 are unique to only Met2 tumor

Tumor mutations

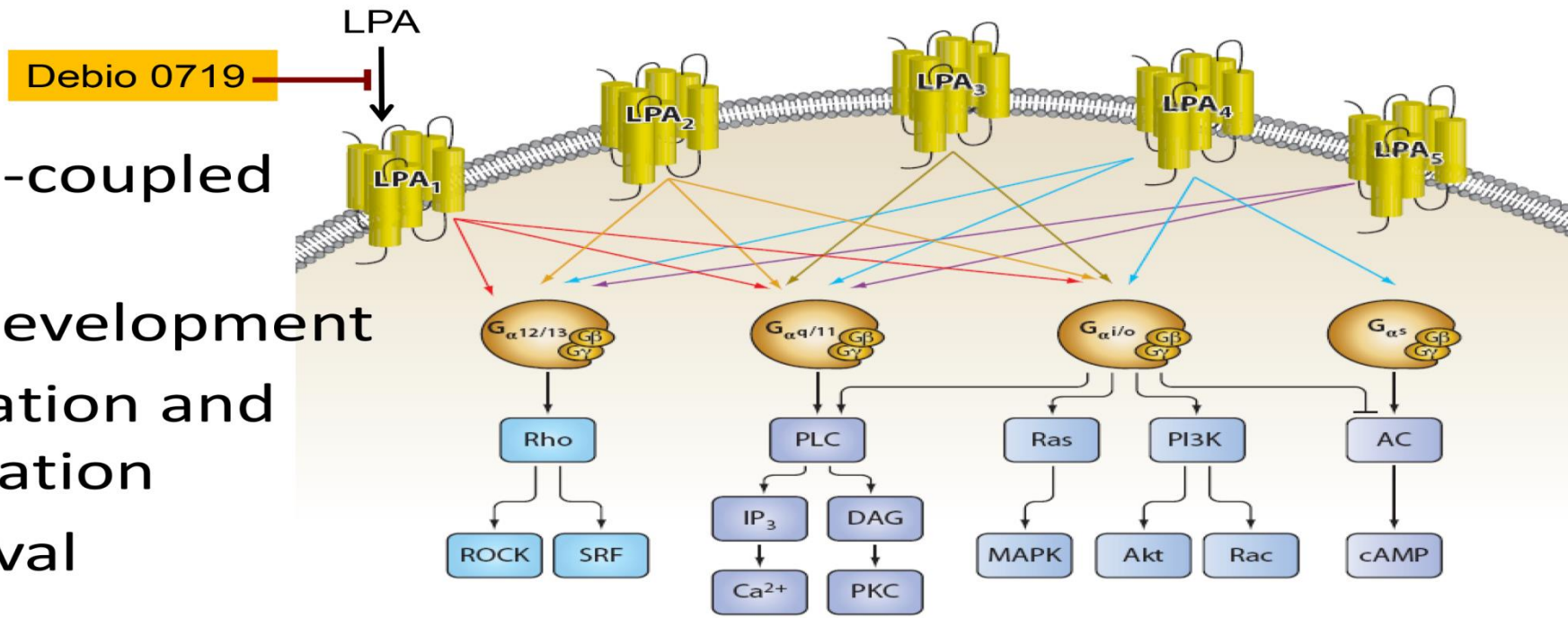
Mutations are expressed in tumors



LPAR1

***LPAR1 (LPA1;* lysophosphatidic acid receptor 1) is involved in key pathways relevant to neuroblastoma biology and inhibitors are available**

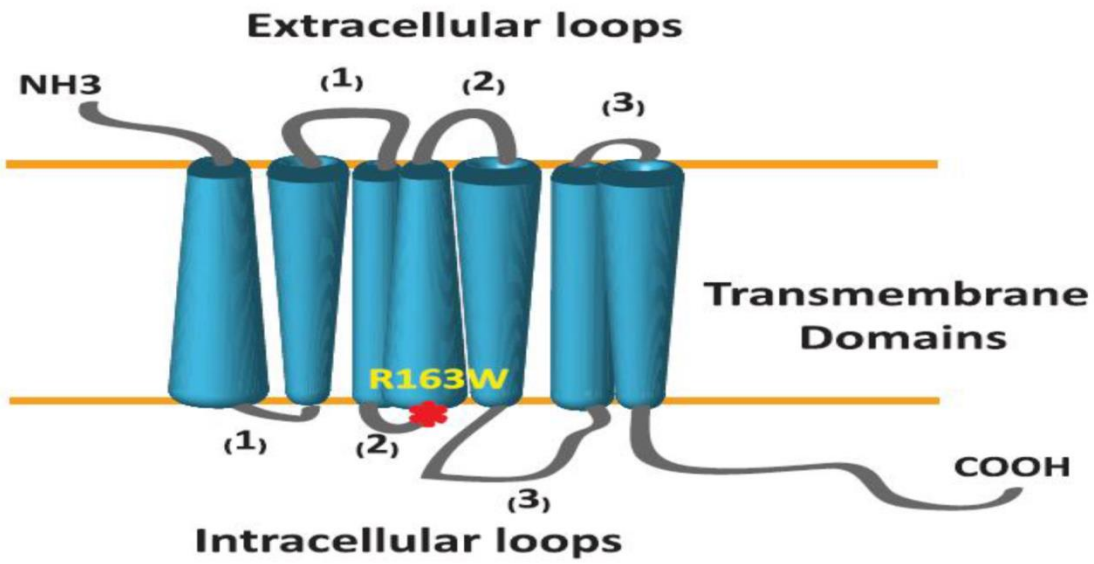
- G protein-coupled receptor
- Neuron development
- Cell migration and differentiation
- Cell survival

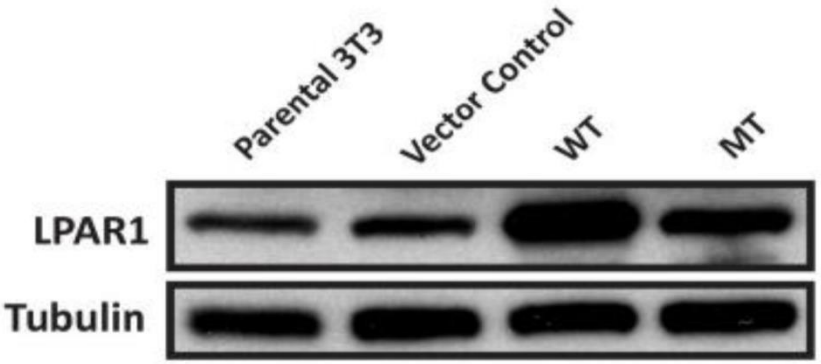


Choi et al, Annu. Rev. Pharmacol. Toxicol. 2010. 50:157-86

LPAR1 pathway

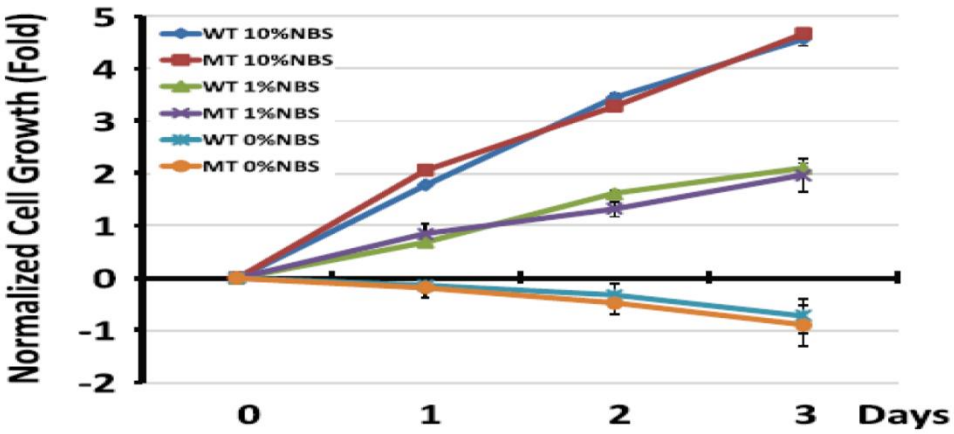
Microarray analysis of cell expressing WT or MT LPAR1 stimulated with LPA shows enrichment directional cell motility genes through Rho-ROCK pathway



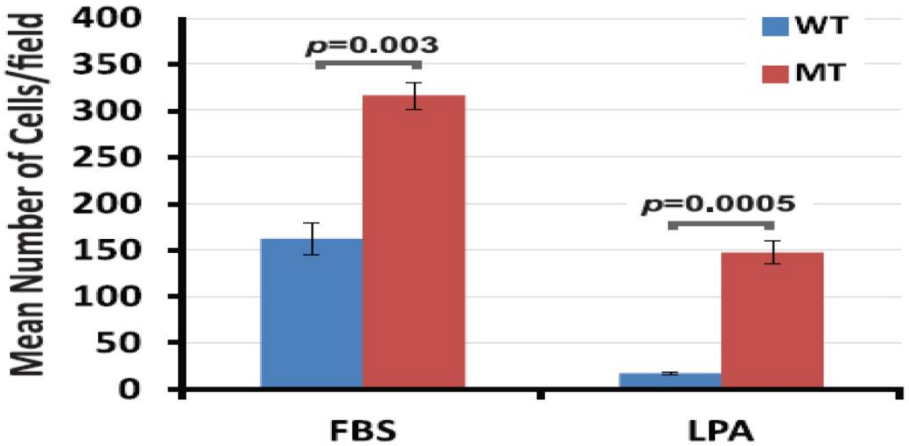


LPAR1 and migration

R163W mutations of *LPAR1* does not alter growth but increases migration in 3T3 cells



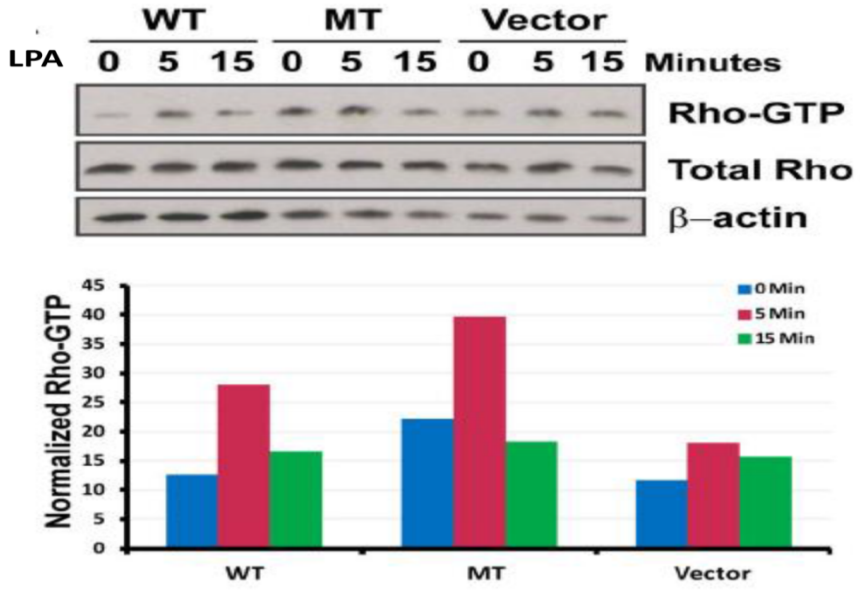
Cell growth assay

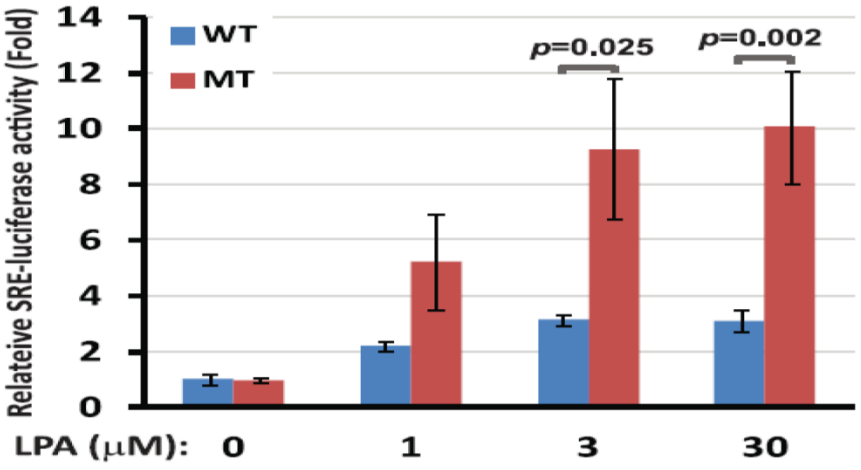


Cell invasion assay

LPAR1 mutations

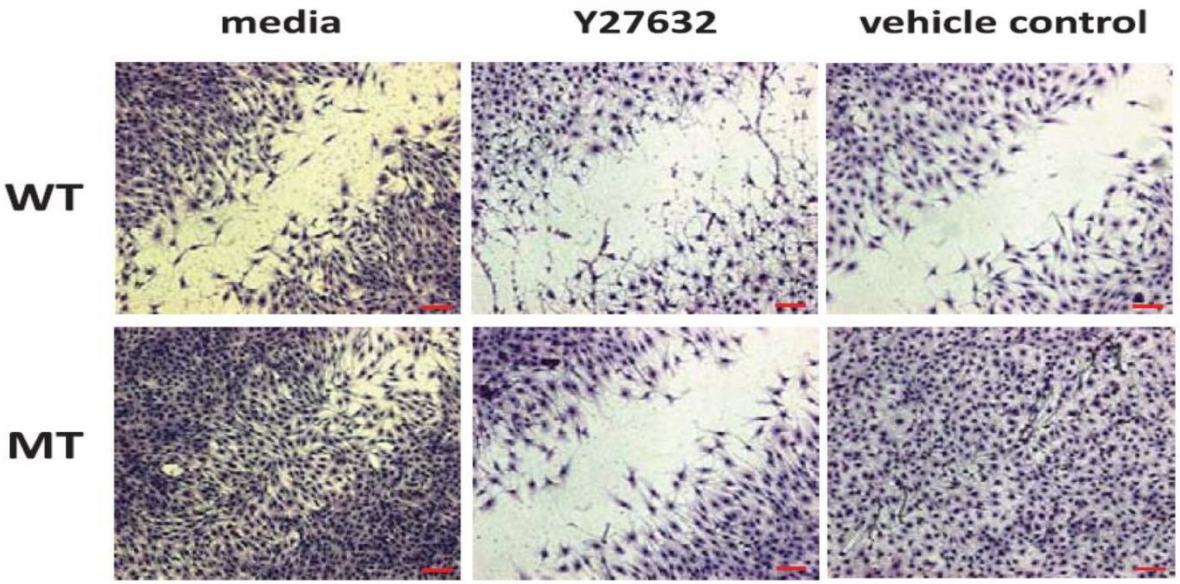
R163W mutations results in enhanced signaling through Rho-ROCK pathway





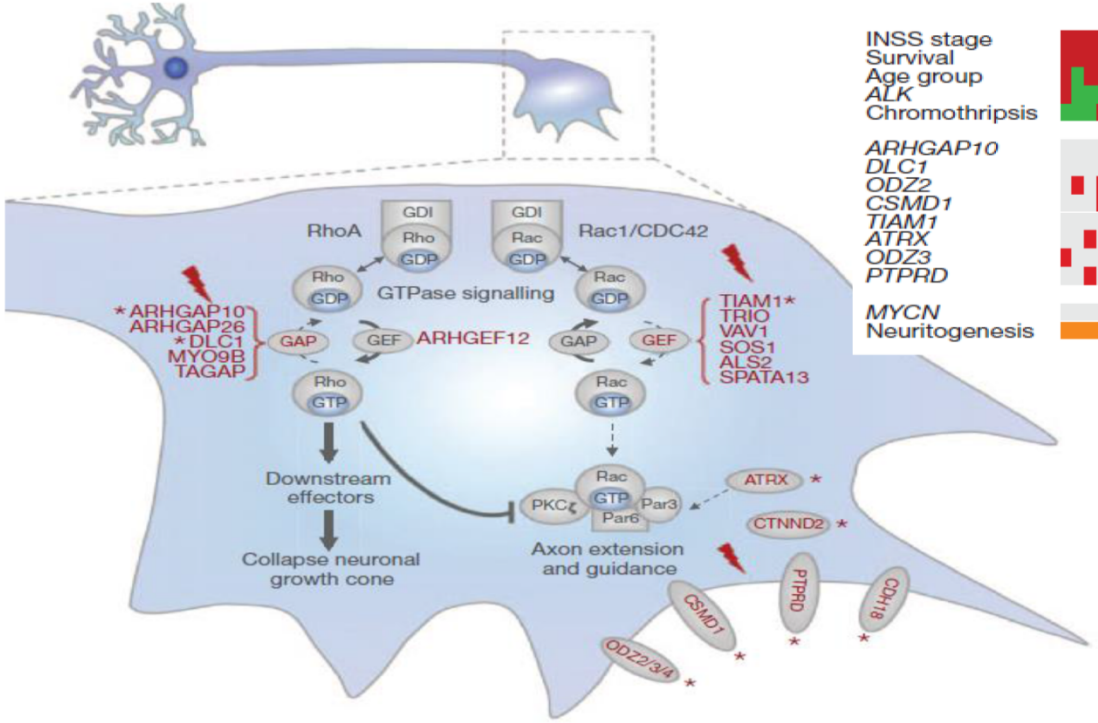
RHO kinase inhibitor

RHO kinase inhibitor Y27632 reduced cell motility mediated by the mutant LPAR1 receptor



RHOA pathway

RHOA pathway activation was associated with defects in neuritogenesis genes in high-risk neuroblastoma



Molenaar et al, Nature, 2012

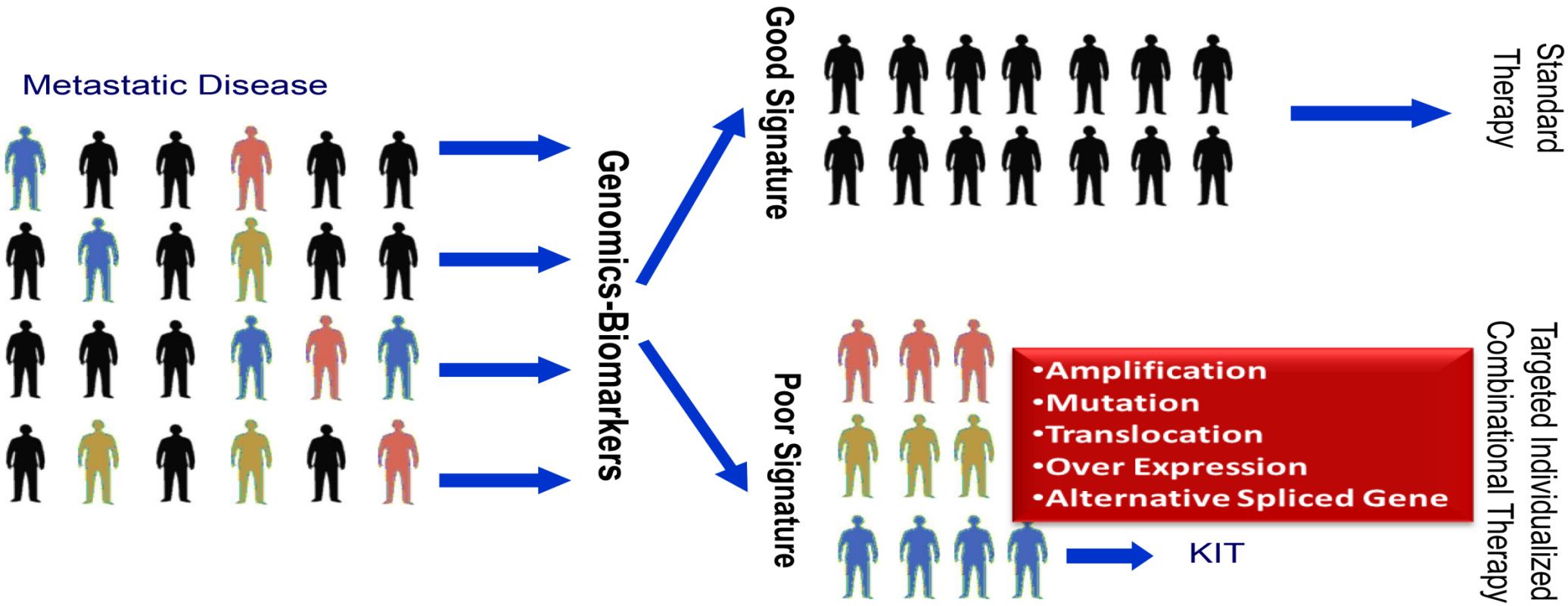
Summary

Summary

- Whole genome sequencing showed massive chromosomal alterations together with a small set of somatically acquired expressed mutations in Met2.
- Examination of somatic mutations in additional tumor samples revealed rapid accumulation of *de novo* mutations during therapy.
- Parallel whole genome and transcriptome sequencing identified a cell motility driver mutation in the *LPAR1* gene, suggesting such combinatorial approach may be leveraged for precision therapy by targeting expressed driver mutations.
- RHO pathway activation may play an important role in high-risk neuroblastoma

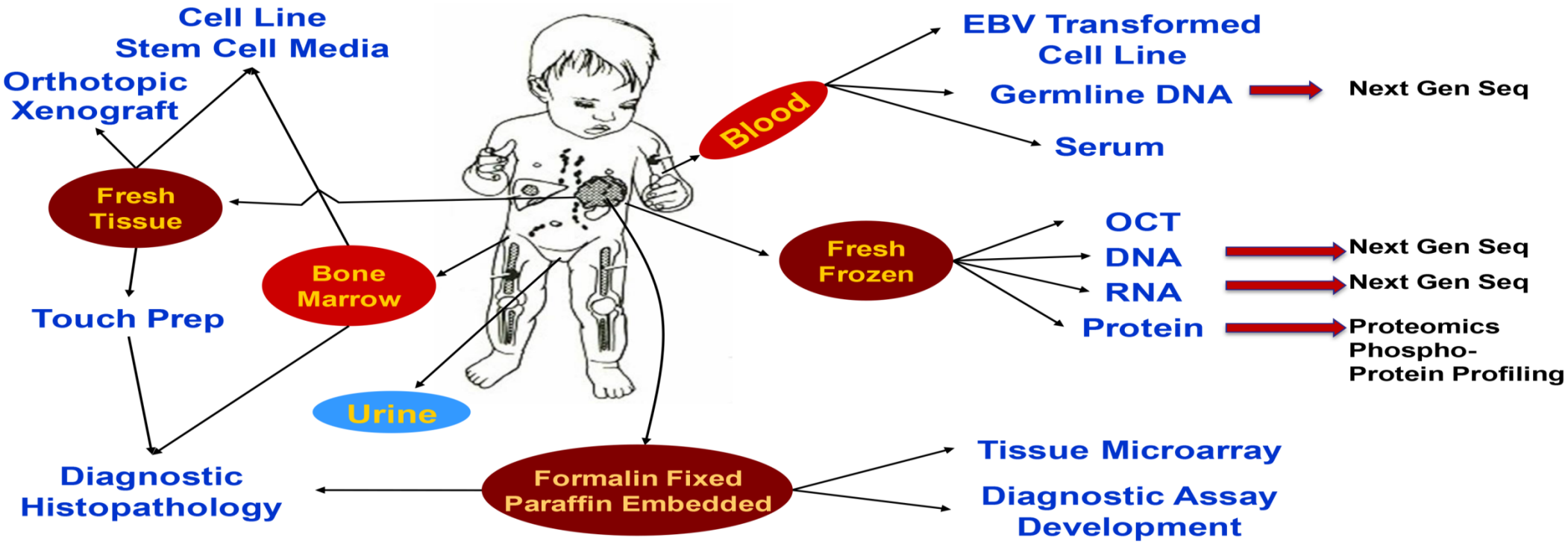
Future Trials

Genomics Enabling Precision Therapy-The Future for Pediatric Trials



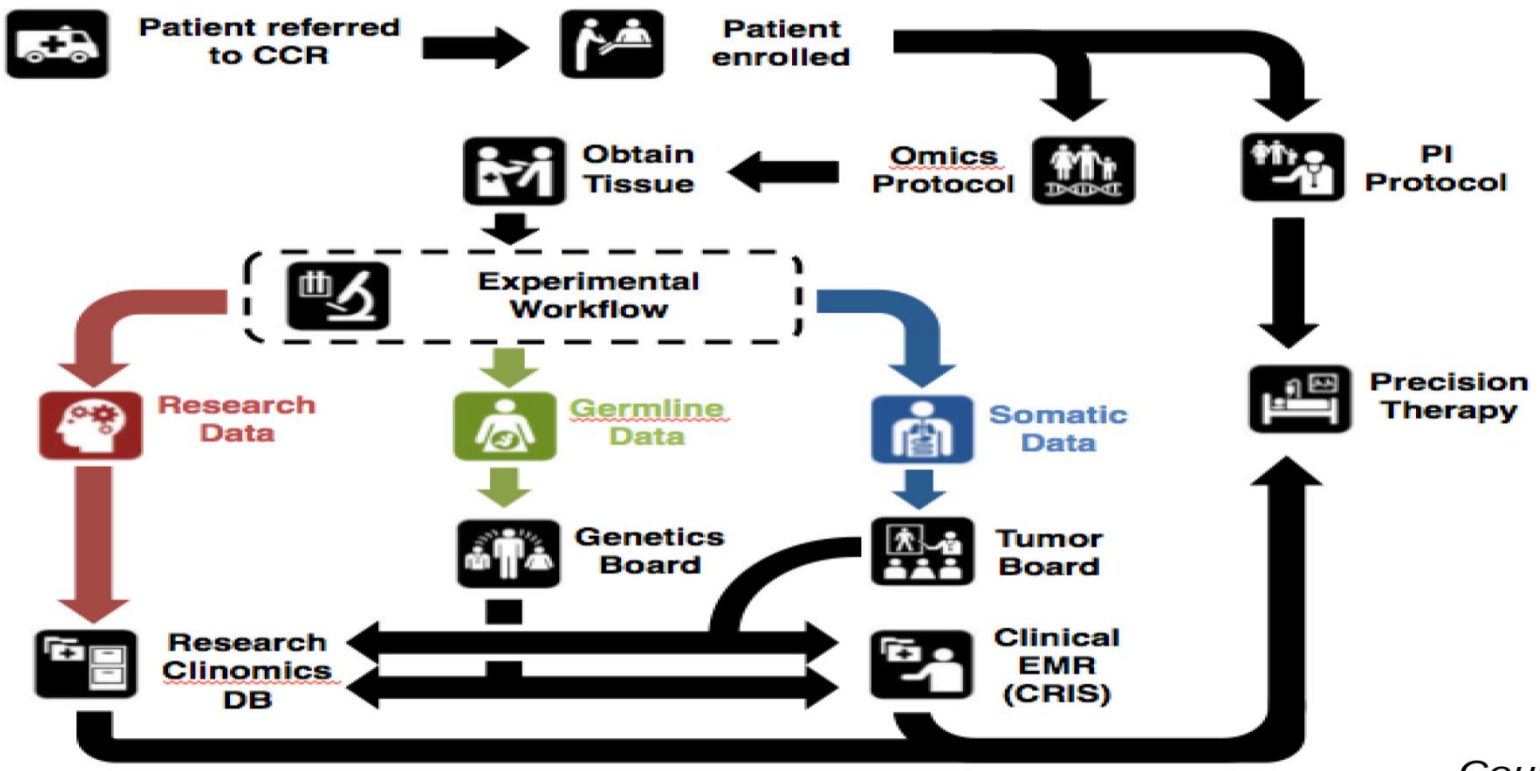
Procurement protocol

Discovery: Comprehensive Omics &Tissue Procurement Protocol- 10-C-0086



CLINOMICS CORE

Use sequencing to inform in clinic: CCR CLINOMICS CORE



Courtesy of Jimmy Lin

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

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