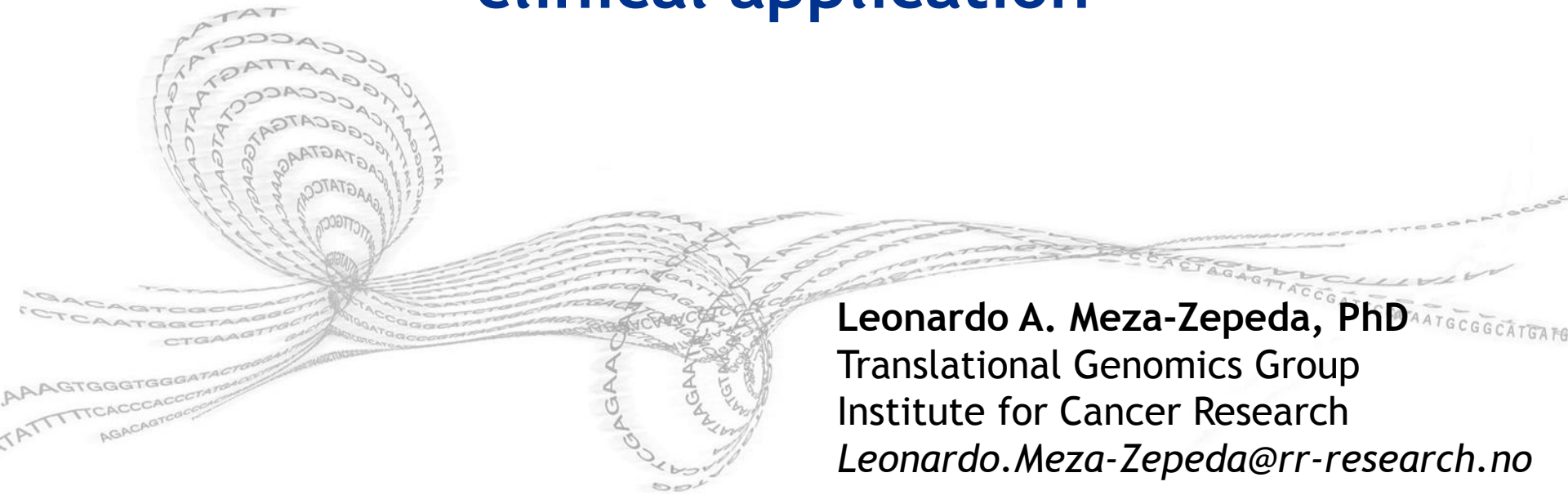
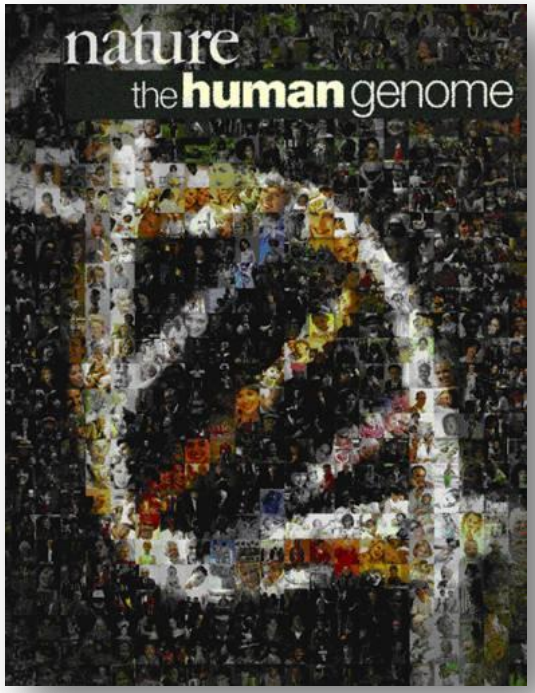
Next generation diagnostics
Bringing high-throughput sequencing into clinical application



Leonardo A. Meza-Zepeda, PhD
Translational Genomics Group
Institute for Cancer Research
Leonardo.Meza-Zepeda@rr-research.no

The Human Genome



First draft of
“the human genome” 2001

Faster sequencing instruments

Can sequence genes to genomes

Million reactions in parallel

Low sequencing cost

Targetable Mutations in NSCLC

Gene	Alteration	Frequency
AKT1	Mutation	1%
ALK	Rearrangement	3-7%
BRAF	Mutation	1-3%
DDR2	Mutation	4%
EGFR	Mutation	10-35%
FGFR1	Amplification	20%
HER2	Mutation	2-4%
KRAS	Mutation	15-25%
MEK1	Mutation	1%
MET	Amplification	2-4%
NRAS	Mutation	1%
PIK3CA	Mutation	1-3%
PTEN	Mutation	4-8%
RET	Rearrangement	1%
ROS1	Rearrangement	1%

		PCR			IHC	FISH
		QIAGEN therascreen	Roche cobas	bioMériux THxID™	Dako PharmDx	Abbott Vysis
KRAS	Cetuximab					
	Panitumumab					
EGFR	Erlotinib					
	Afatinib					
BRAF	Vemurafinib					
	Tramatenib					
	Dabrafenib					
c-Kit	Imatinib Mesylate					
Alk	Crizotinib					

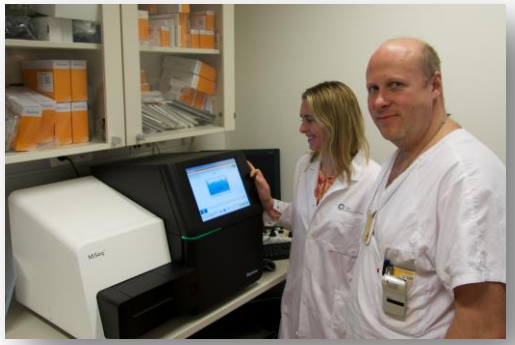
O'Brien et al, Lung Cancer, 2014

A Multi-Gene Molecular Testing Platform

Illumina TruSight Tumour

AKT1	EGFR	GNAS	NRAS	STK11
ALK	ERBB2	KIT	PDGFRA	TP53
APC	FBXW7	KRAS	PIK3CA	
BRAF	FGFR2	MAP2K1	PTEN	
CDH1	FOXL2	MET	SMAD4	
CTNNB1	GNAQ	MSH6	SRC	

- Includes druggable genes
- Compatible with FFPE
- Sequencing both DNA strands
- High coverage

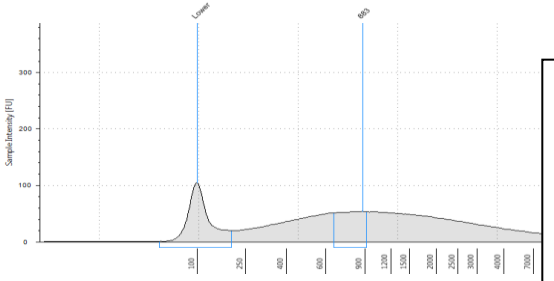


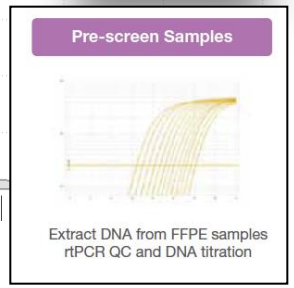
Drs. Namløs and Lund-Iversen

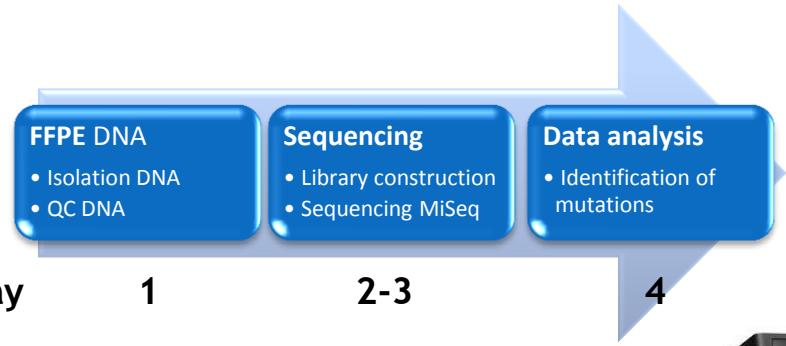
illumina

DNA degradation and chemical damage

FFPE DNA







- Input 30-300 ng DNA
- Library construction 48 samples (96-well format)
- Sequencing 32 samples on MiSeq (22 hours)

NSCLC FFPE Samples

70-80% of samples with good enough DNA quality

Patient ID	Sample	Previous finding	Actionable cancer panel, TruSight Tumor	
1	Normal lymph node		No mutations	✔
1	Normal lung		No mutations	✔
2	Adenocarcinoma		%)	✔
2	Normal lung			✔
3	Normal lung			✔
4	Adenocarcinoma		(5%)	?
4	Normal lymph node			✔
4	Normal lung			✔
5A & 5B	Adenocarcinoma		sense (18%) for A & B 31%) for A & B	✔
5	Normal lung			✔
6	Adenocarcinoma		nse (44%)	✔
7A & 7B	Adenocarcinoma		nse (A 35%, B 29%) *shift (16%)*	✔
8	Adenocarcinoma		nse (10%)	✔
9A &9B	Adenocarcinoma		nse (A 33%, B 18%) ***STK11***, A347T, Ins, Missense (A 19%, B 38%)	✔
10	Adenocarcinoma	***EGFR***	Mutation not targeted by the panel	✔

Verified mutations

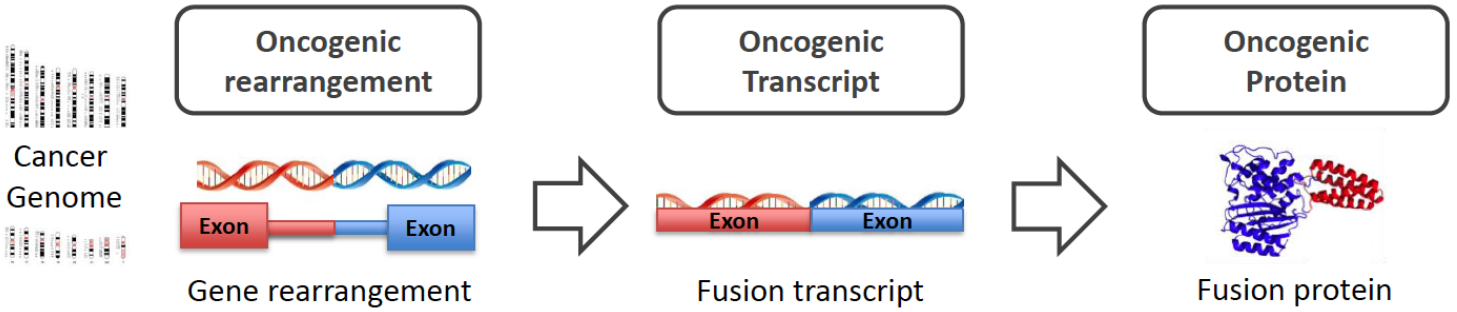
EGFR
KRAS
BRAF

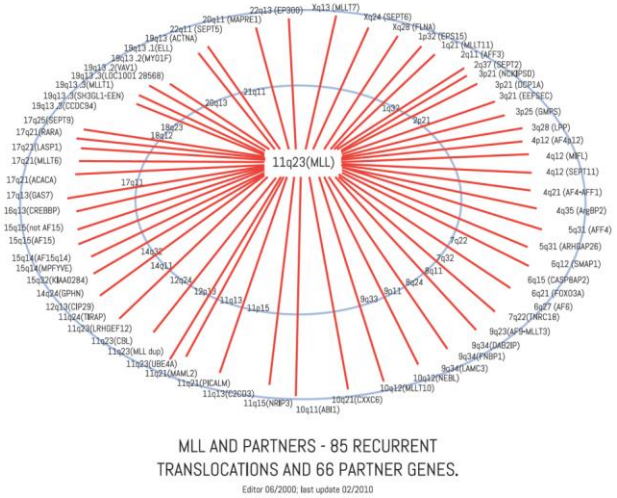
New mutations

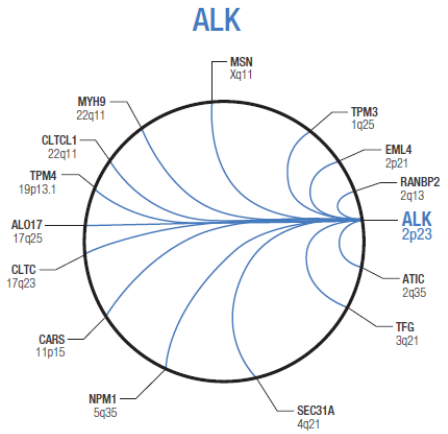
TP53
STK11

Challenges with InDels

Oncogenic Rearrangements



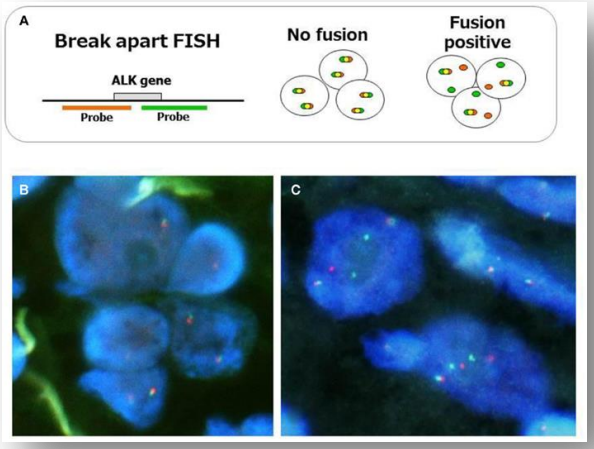




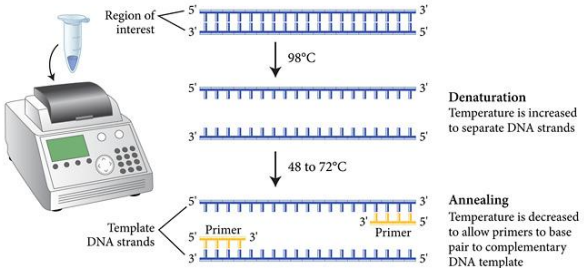
One gene, many partners

Identification of Genomic Rearrangements

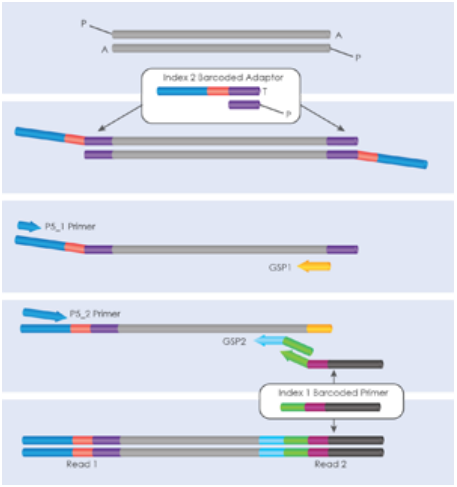
Today



or



Must know breakpoint and fusion partner

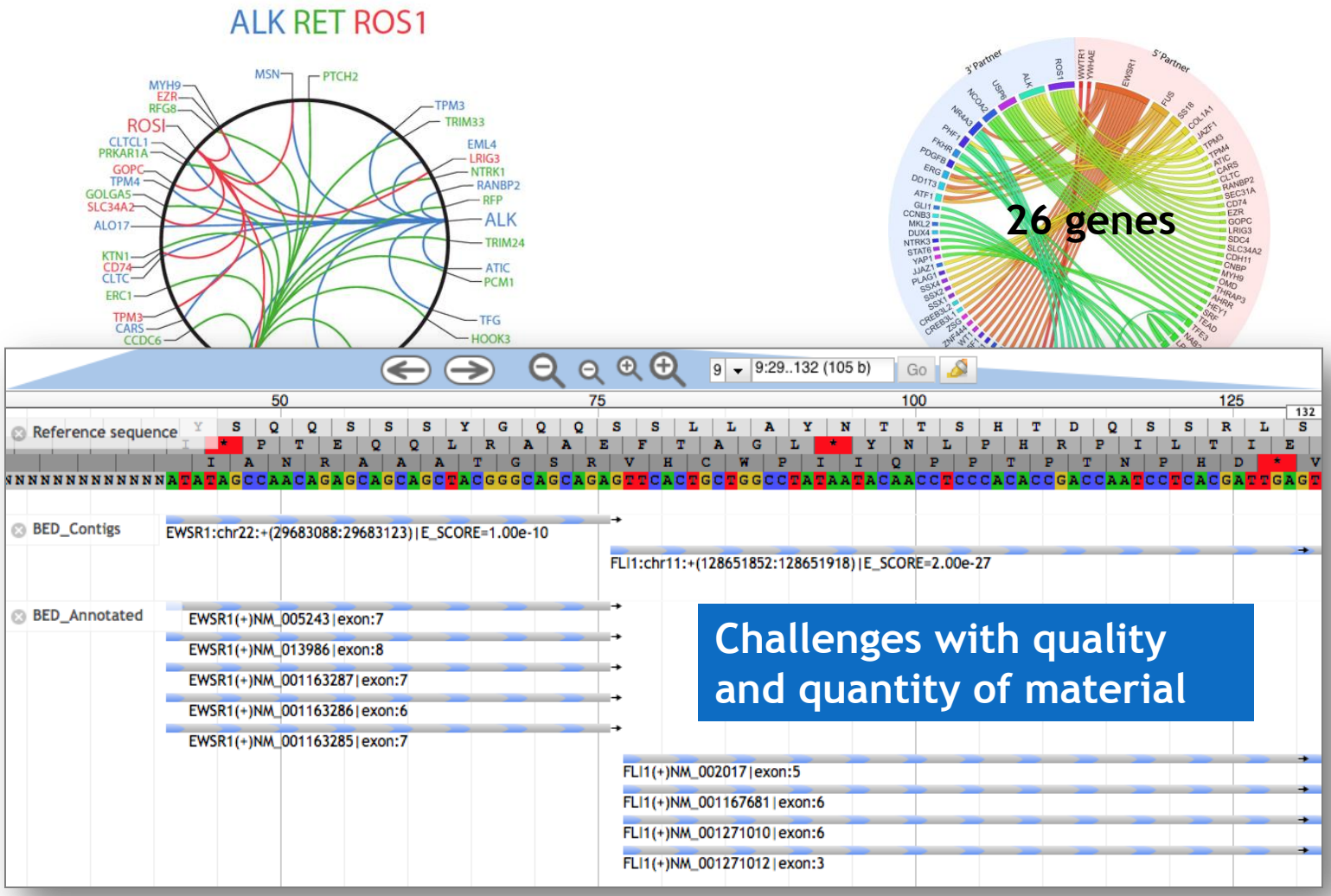


ARCHER by enzymatics

enzymatics catalyzing the genomic healthcare revolution™

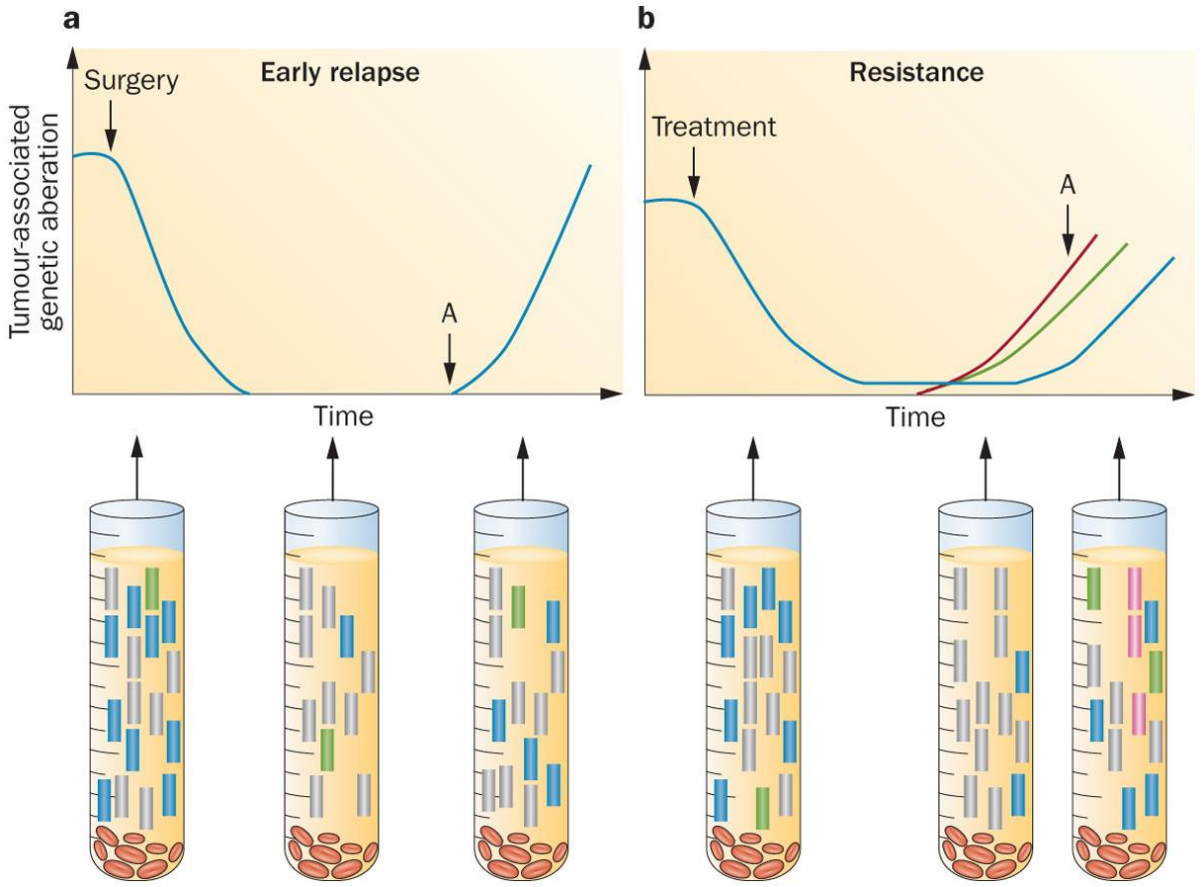
- Targeted RNA-Sequencing
- No previous knowledge of fusion partner
- Multiple genes in one reaction

Fusion Genes



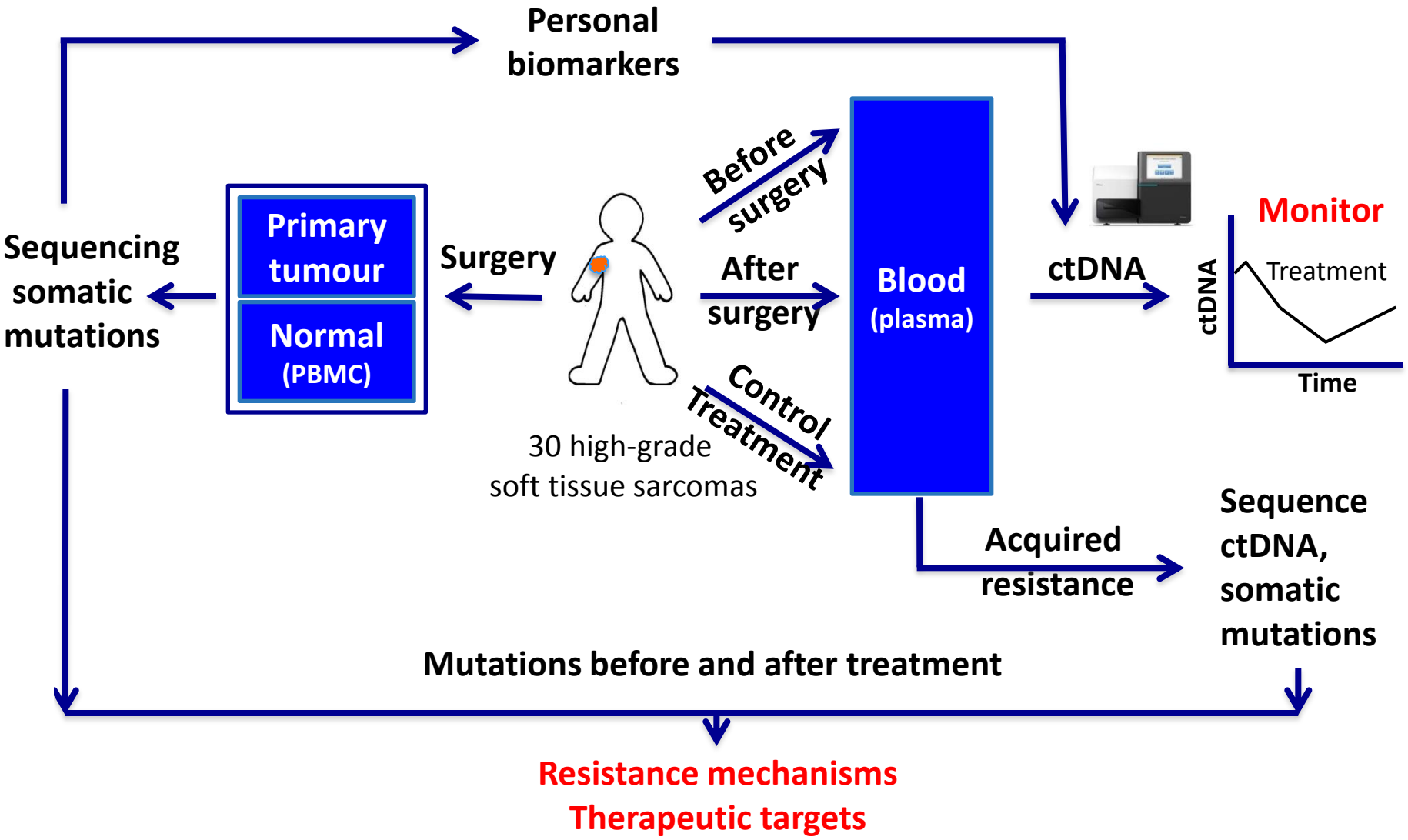
Kresse, Beiske, Bjerkehagen, et al

Liquid Biopsies



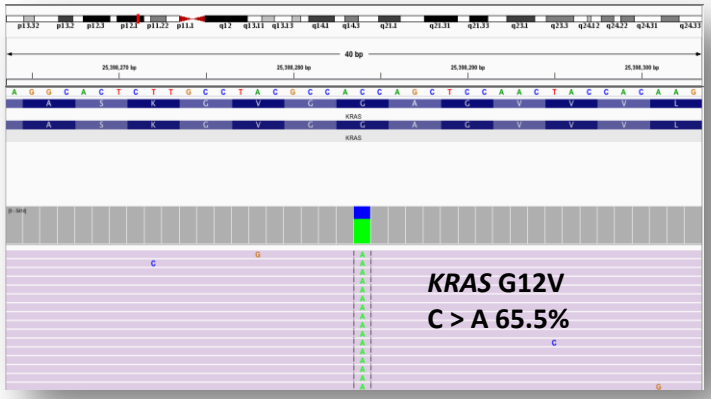
Crowleyet al, Nat Rev Clin Oncol 2013

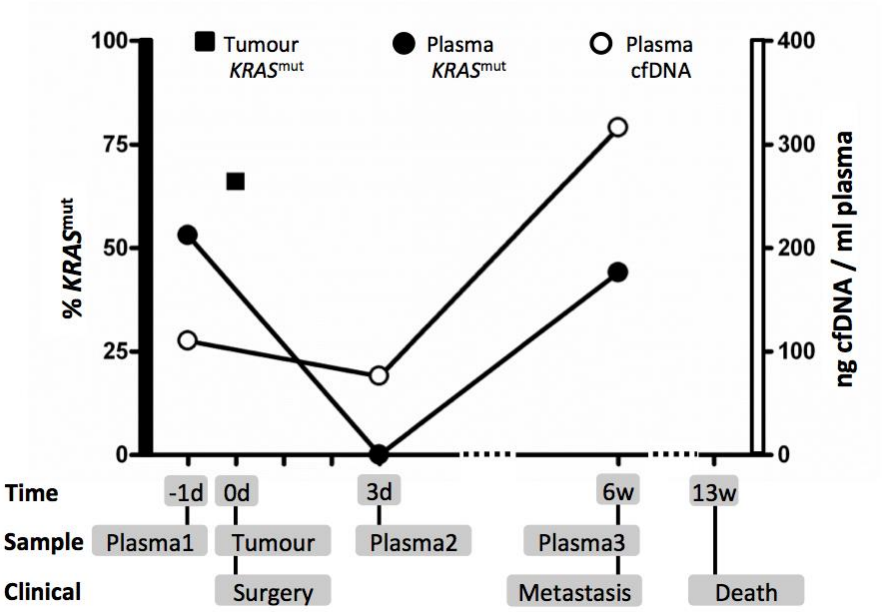
Clinical Protocol ctDNA



Proof of Concept

- 55-year-old male
- Palpable mass upper left thigh
- Large intramuscular tumour
- No metastases by CT
- Biopsy, high-grade malignant spindle cell sarcoma
- Enrolled in CircSarc
- Hemipelvectomy
- Sequencing Tumour and Normal DNA
  - 900 cancer gene panel
- 12 somatic mutations
- Monitor tumour burden by sequencing cell-free DNA in plasma





Summary

- **Advances in sequencing technology allows today the rapid identification of somatic mutations**
- **Sensitive and reproducible for detection of single nucleotide mutations, InDels, fusions**
- **Liquid biopsies may provide a non-invasive insight in to the tumour genome**

Challenges

- Heterogeneity
- Biological interpretation
- Material

Sequencing Workshop for Pathologists

- Theoretical aspects of high-throughput sequencing
- Hands-on targeted resequencing of cancer genes
- Library construction, sequencing and data analysis
- From DNA to mutations

Acknowledgements

Dept. of Tumor Biology, ICR
- Heidi M. Namløs
- Stine H. Kresse
- Ola Myklebost

Dept. of Oncology
- Åslaug Helland
- Odd Terje Brustugun
- Kirsten S. Hall
- Kjetil Boye
- Charlott M Våge
- Synnøve Granlien

Dept. Surgery
- Olga Zaikova

Dept. Pathology
- Bodil Bjerkehagen
- Marius Lund-Iversen
- Klaus Beiske
- Lilach Kleinberg

Genomics Core Facility
- Jinchang Sun
- Ana Lid

Bioinformatics Core Facility
- Daniel Vodak
- Sigve Nakken
- Eivind Hovig

The Research Council of Norway

NORWEGIAN CANCER SOCIETY

HELSE SØR-ØST

Oslo University Hospital

RADIUM HOSPITALETS LEGATER