

Circulating tumor DNA: Disruptive Technology in Medicine

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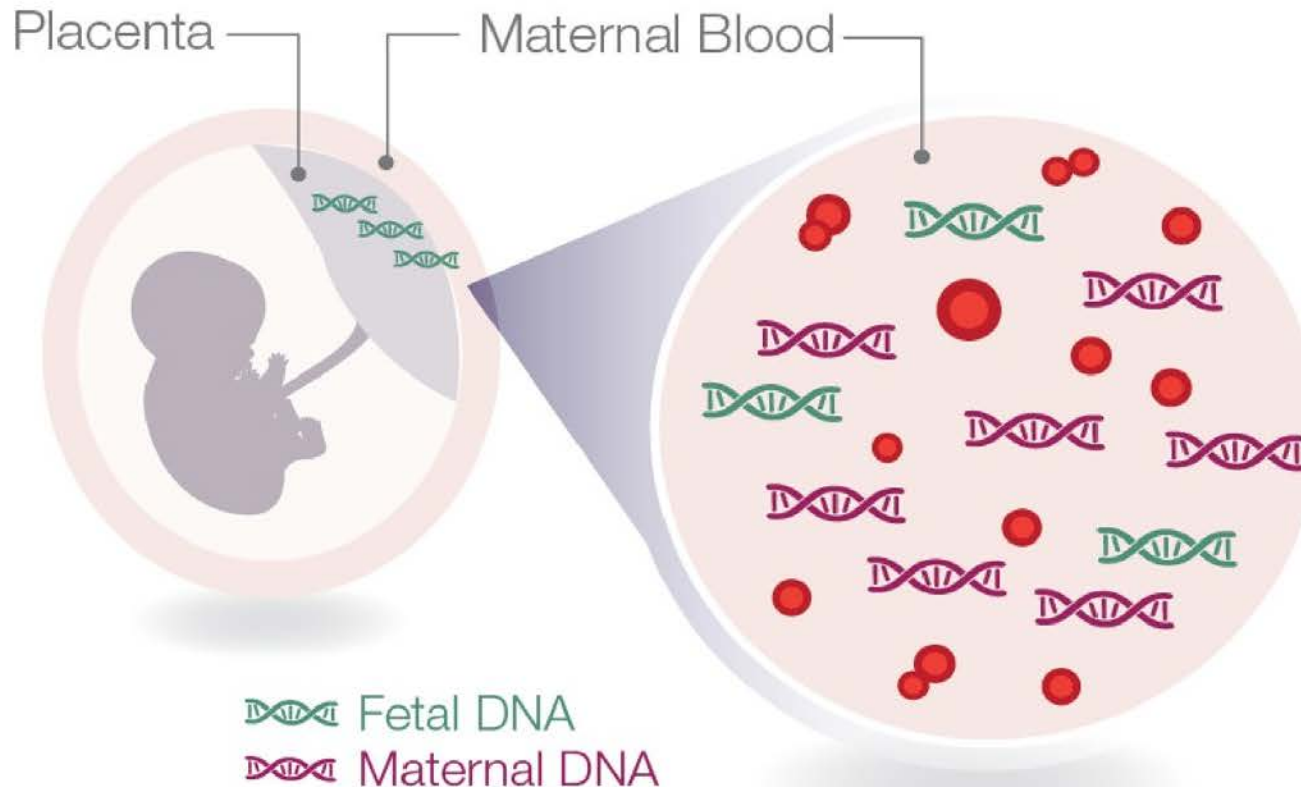
ARUP Laboratories and Department of Pathology
University of Utah Health Sciences Center

A revolution in cancer:

ctDNA: liquid biopsy

Cancer sheds mutated DNA into
blood

Cell-Free Fetal DNA in Maternal Circulation



Discovery of cell-free fetal DNA in the maternal circulation in 1997*

2008 publications drove implementation of this new technology into clinical practice

* Lo YM, et al., Lancet 1997; 350:485-7

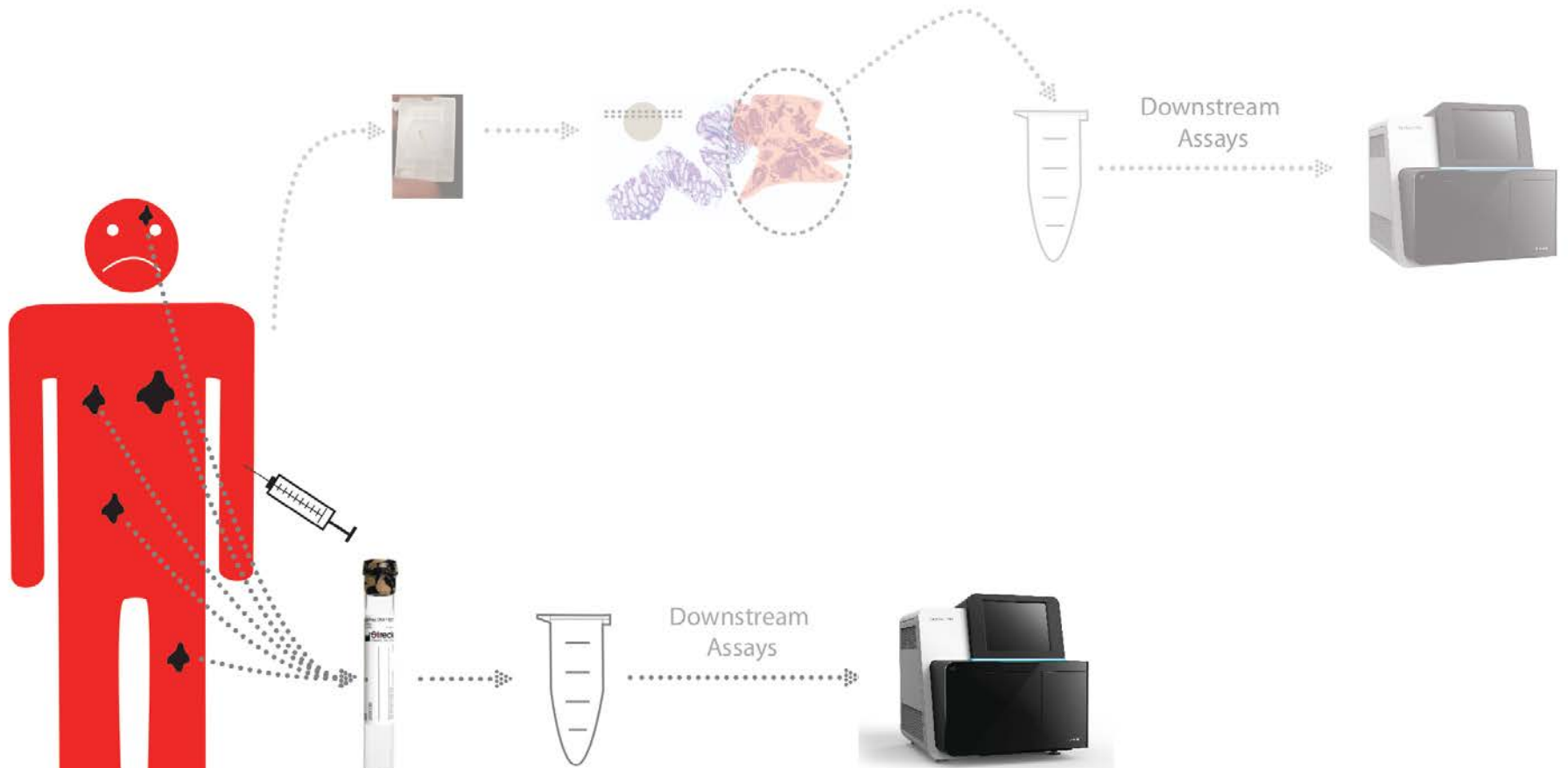
Paternal SNPs

- unique to fetus

Tumor genomic change

- unique from germline

Circulating free DNA (cfDNA) workflow



- Pros

- Minimally invasive
- Faster turnaround time
- Reduced sampling error
- Serial monitoring

- Cons

- Greater sensitivity needed
- Clinically unproven
- Unknown limitations

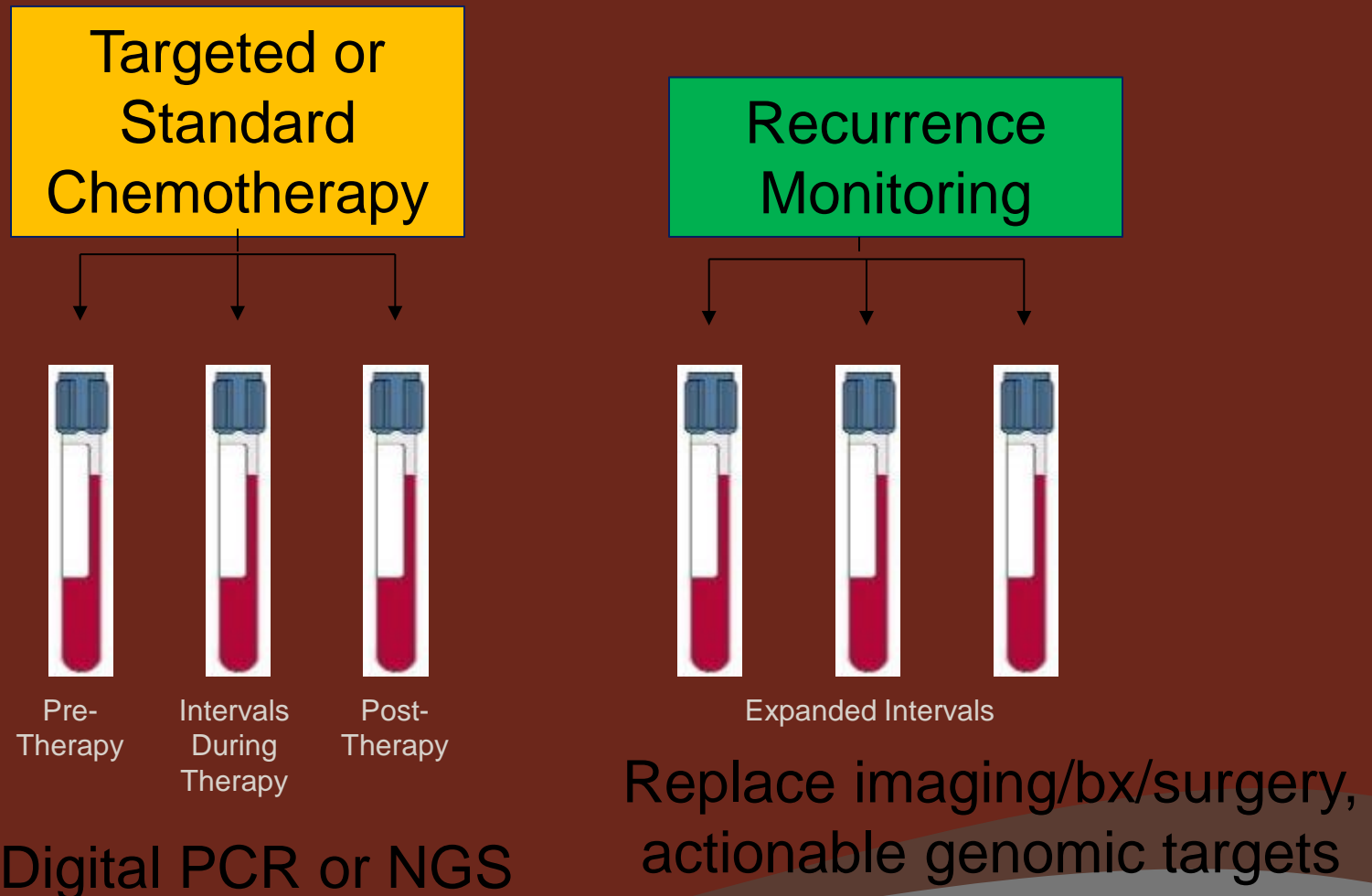
ctDNA: Financial World

- NYT front page business section articles
- Guardant Health: Stanford spin off, \$10M Sequoia venture capital investment
- Personal Genome Dx: JHU spin off
- Natera: NIPT to ctDNA
- Roche \$1 Billion purchase of 56% FM

ctDNA: Disrupting Oncology

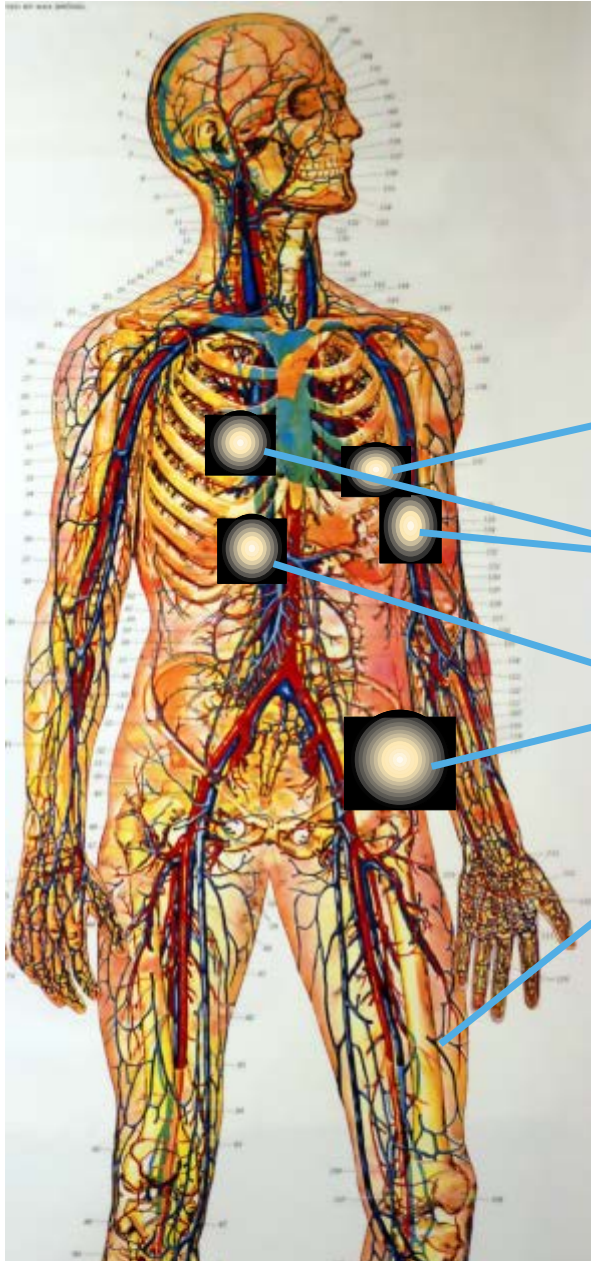
- Replace \$\$ biopsy, surgery, imaging
- Targeted real-time resistance and recurrence detection
- Perfusion sampling of entire tumor and body
- Targeted therapy

Treatment & Recurrence Monitoring



ctDNA reduces tumor sampling error by analyzing the entire tumor burden via perfusion sampling: primary, mets, heterogeneous clones

Blood is the “window to the body”



"Tumor5"



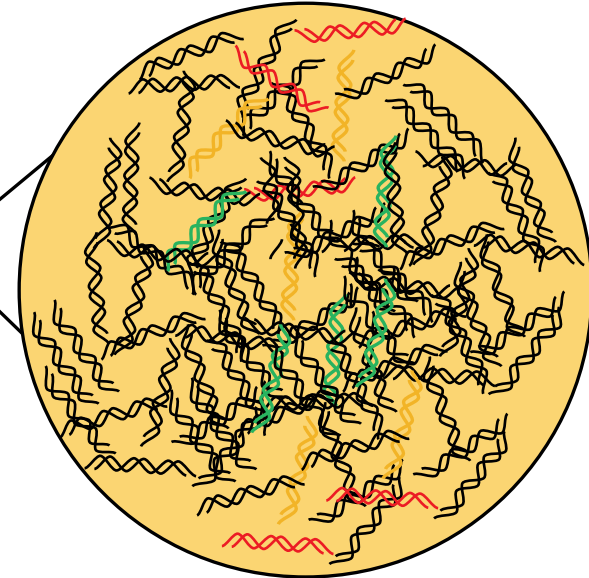
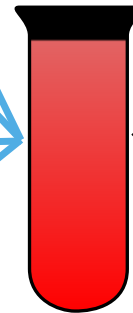
"Tumor3+4"



"Tumor1+2"



"Normal"



ctDNA Methodologies

Cost/Extent of Testing

Digital Droplet PCR
1-5 "hotspot" mutations



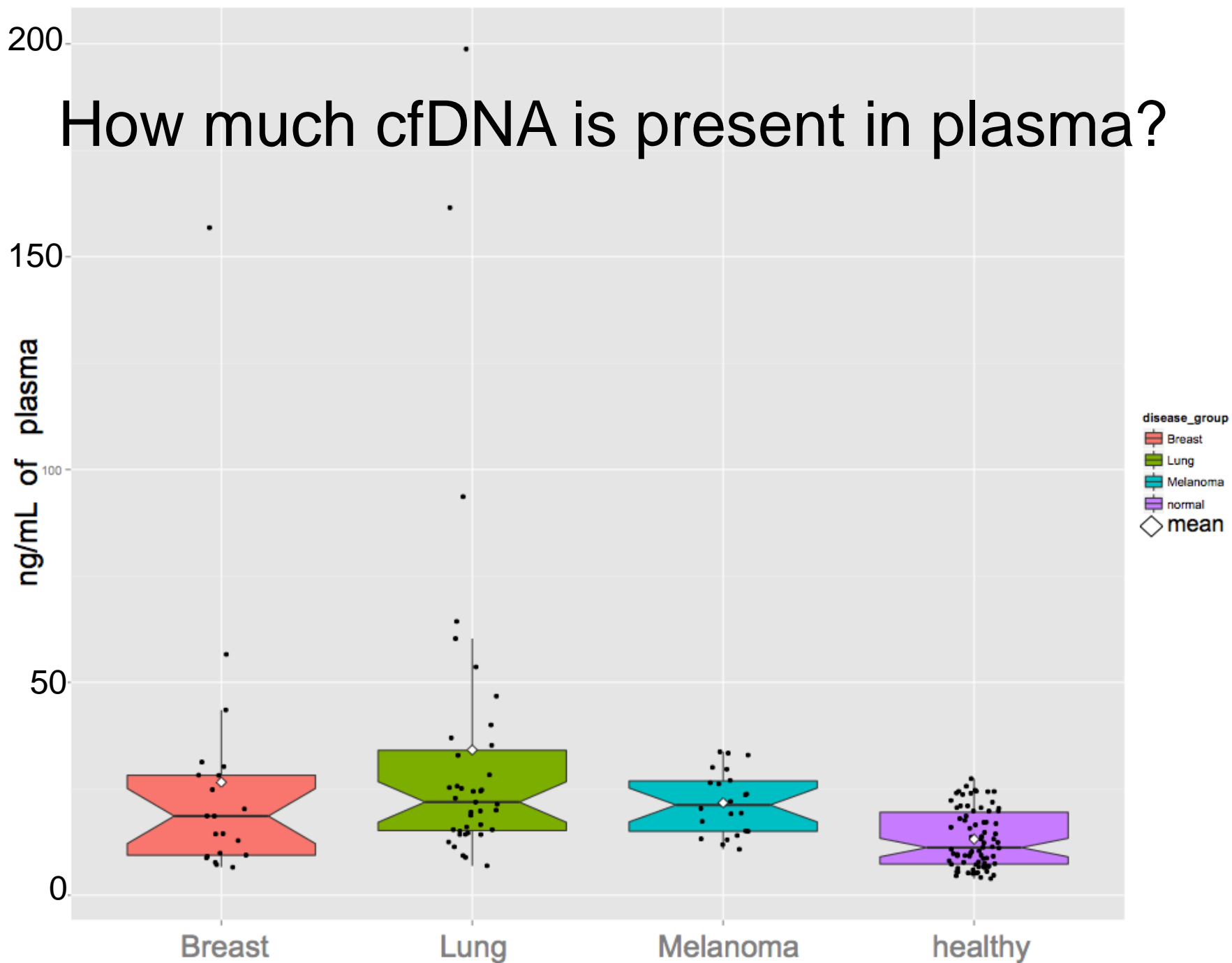
BMF_amplicon
parts of 10-20 genes



BMF_capture
Full exonic coverage of 25-50 genes

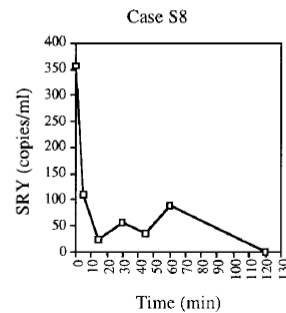
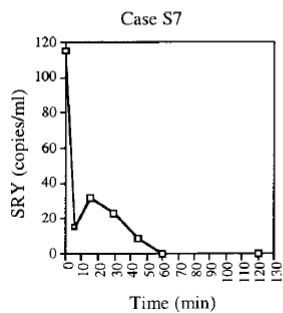
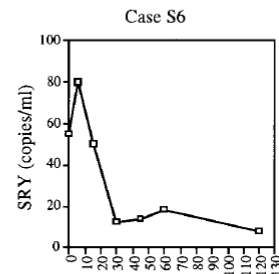
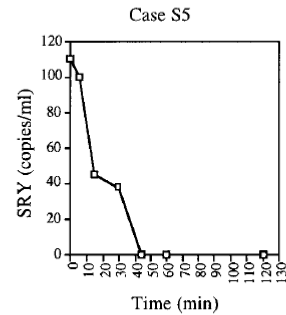
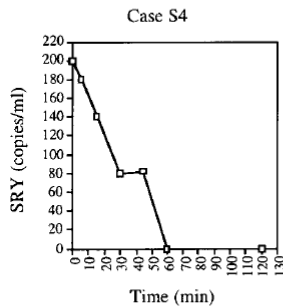
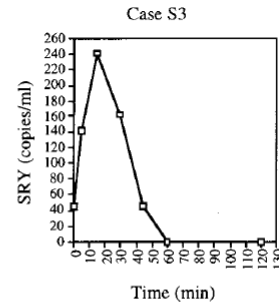
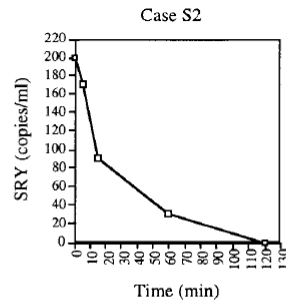
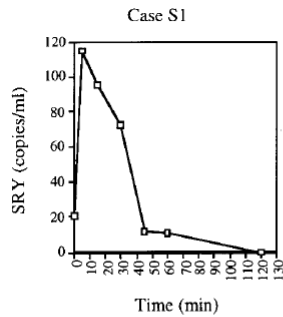


How much cfDNA is present in plasma?



cfDNA kinetics

- Fetal cfDNA from maternal plasma with male fetus
- Clearance of SRY (chrY) gene: **1-2 hours!**



Am. J. Hum. Genet. 64:218-224, 1999

Rapid Clearance of Fetal DNA from Maternal Plasma

Y. M. Dennis Lo,¹ Jun Zhang,¹ Tse N. Leung,² Tze K. Lau,² Allan M. Z. Chang,² and N. Magnus Hjelm¹

Departments of ¹Chemical Pathology and ²Obstetrics and Gynecology, Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong

ctDNA by BMF Capture NGS

Goal: ctDNA gene panel with $<1\%$ sensitivity for low-level cancer variants & translocations diluted by circulation

Keith Gligorich, Daniel Baker, Brett Kennedy

ctDNA Mutation and Translocation Detection by BMF Capture NGS

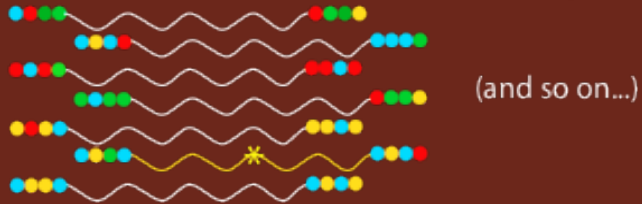
- 5-20 ng of DNA input (1 BCT blood tube)
- Flexible capture method
- Barcodes
- High depth sequencing
- Analysis & variant calling by ARUP's BMF software

Digital Next Generation Sequencing (NGS)

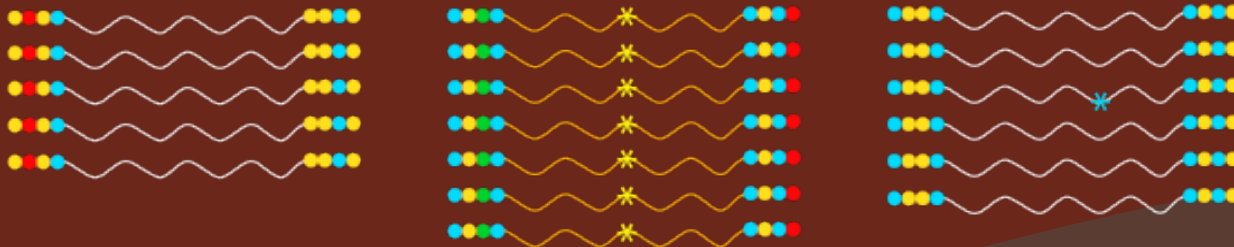
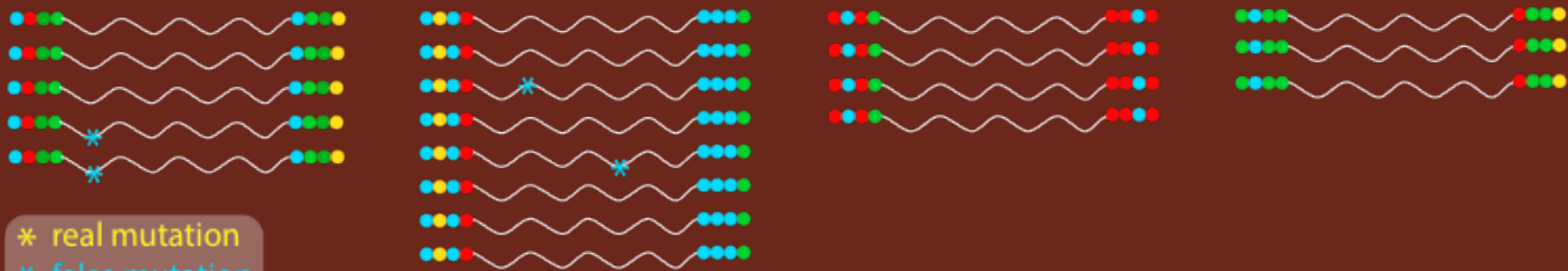
1. Sample with low (<0.1%) frequency mutation (*); a needle in a haystack



2. "Barcode" each molecule with a unique "Nmer" tag during library generation



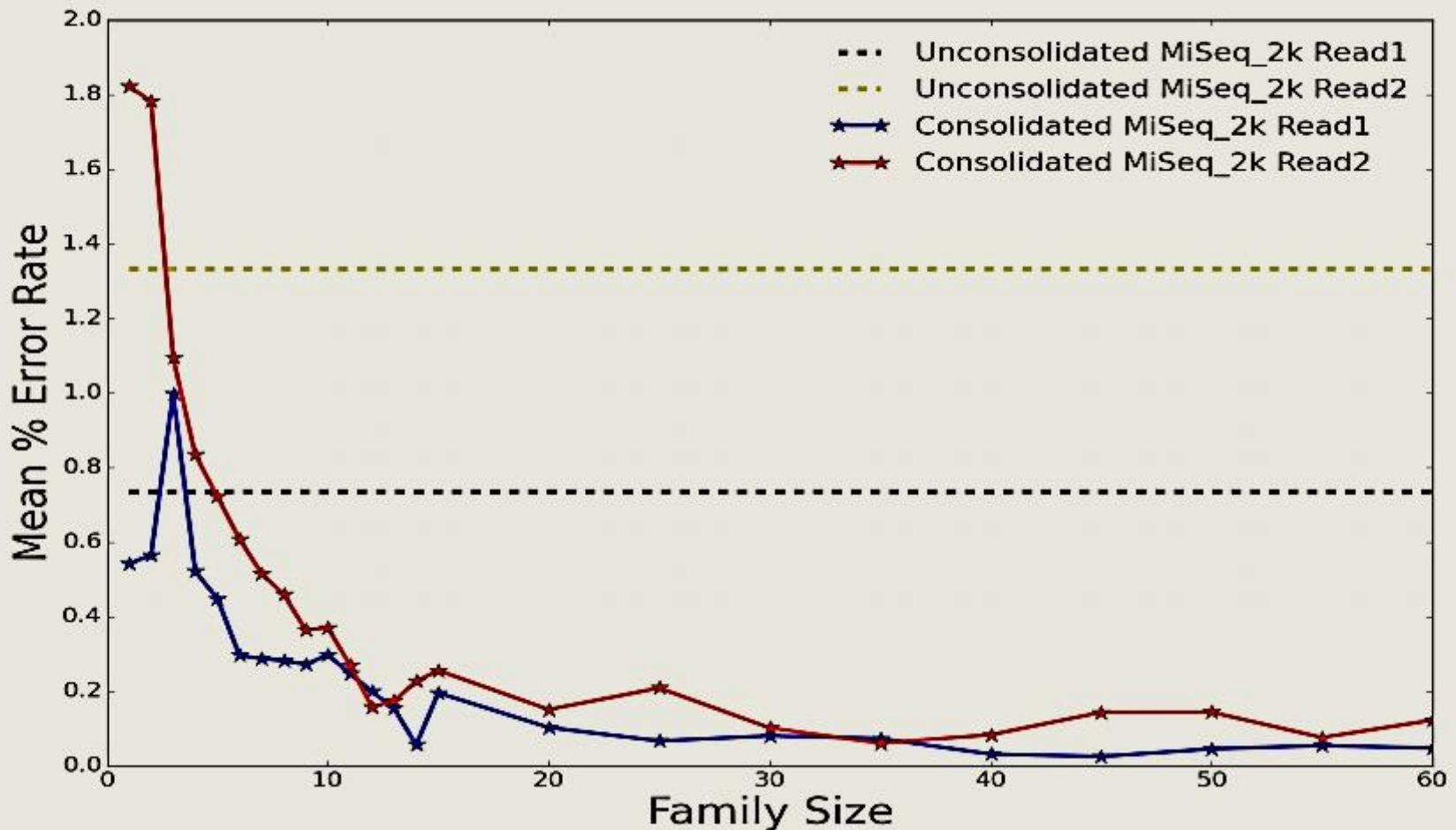
3. PCR amplify molecules, capture regions of interest, redundant sequencing (50million reads)



4. Bioinformatic processing to separate "signal (*)" from "noise (*)" (sequencing errors, PCR errors)

NGS error reduction via BMF bioinformatics

2000 Copy Read Depth

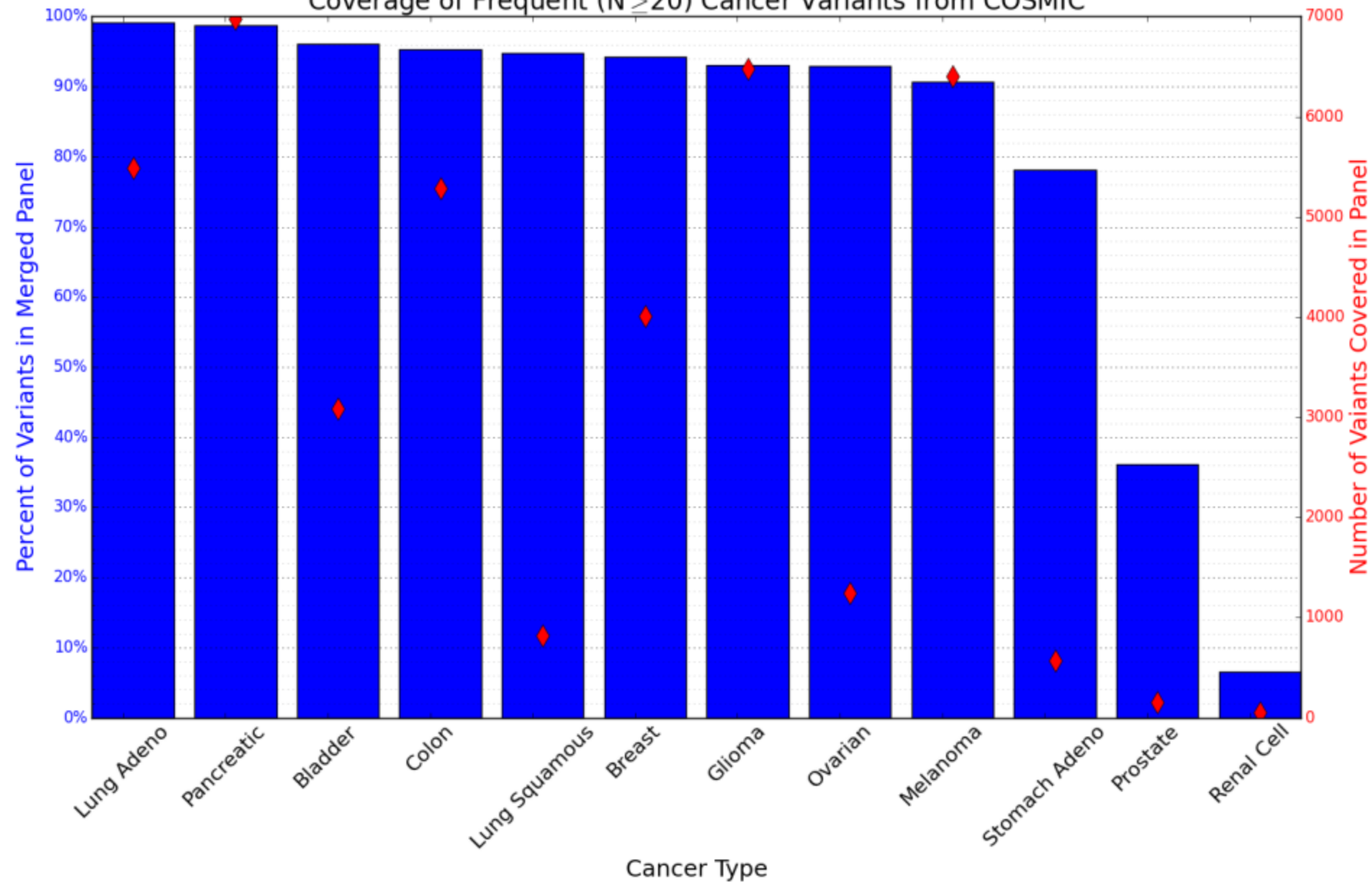


Targeted Hot Spot ctDNA Panel:

ABL1	ERBB2/4	JAK2/3	PIK3CA
AKT1	FBXW7	KDR	PTEN**
ALK*	FGFR1	KIT	RB1
APC	FGFR2	KRAS	RET
ATM	FGFR3	MAP2K1	ROS1
BRAF	GNA11	MET	SMAD4
CDH1	GNAQ	MTOR	SMO
CDKN2A	GNAS	NOTCH1	STK11
CTNNB1	HRAS	NRAS	TERT
DDR2	IDH1	NTRK1	TP53**
EGFR**	IDH2	PDGFRA	VHL

*Intron 19 coverage for translocations, **Full exon coverage

Coverage of Frequent ($N \geq 20$) Cancer Variants from COSMIC



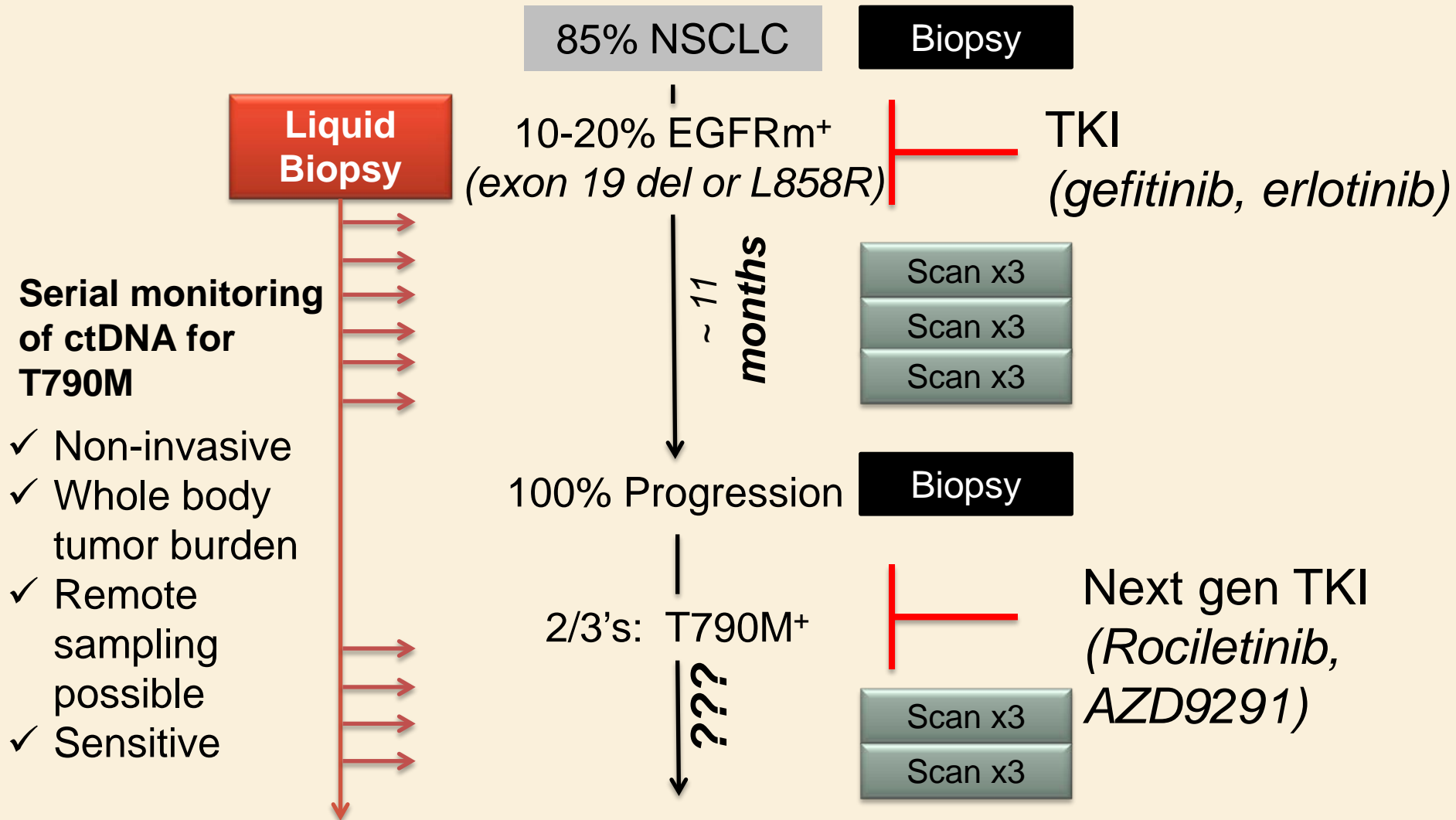
ctDNA testing by
digital droplet PCR (ddPCR):
EGFR T790M mutation

Sabine Hellwig, PhD

EGFR targeted therapy in lung cancer

Estimated new US lung CA's 2015: 220,000

Estimated US lung CA deaths 2015: 158,000 (27% all CA)



EGFR T790M ddPCR Assay

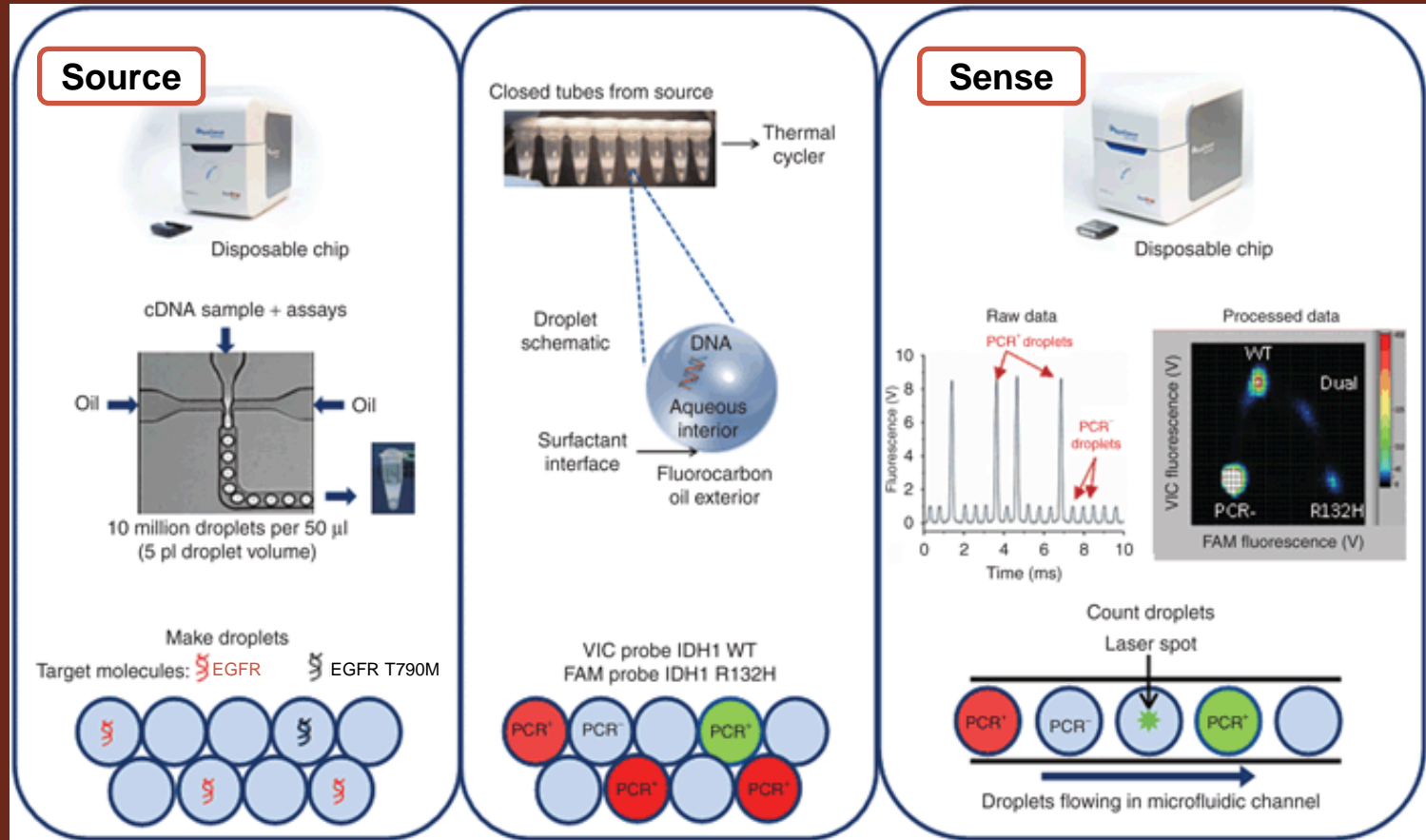
1. PCR reaction setup

- 8-well strip tubes
- Mix (25µL reaction):
 - cfDNA
 - 25X primer/probe cocktail
 - 2X PCR master mix
 - 25X droplet stabilizer
- Include positive control

2. Droplet generation

3. PCR (Amplification + fluorescent dye release)

4. Droplet count



15 min



30 min



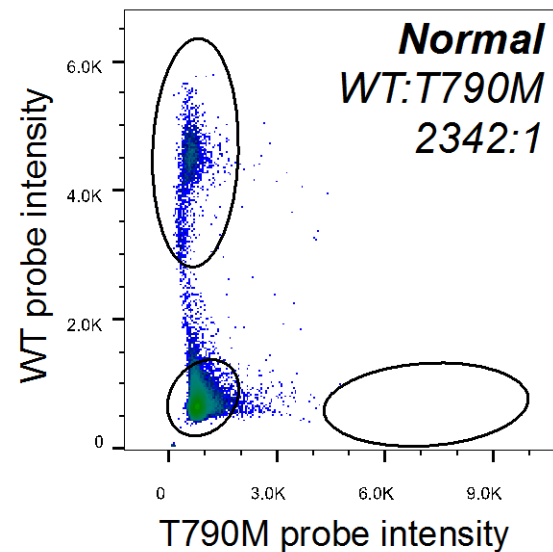
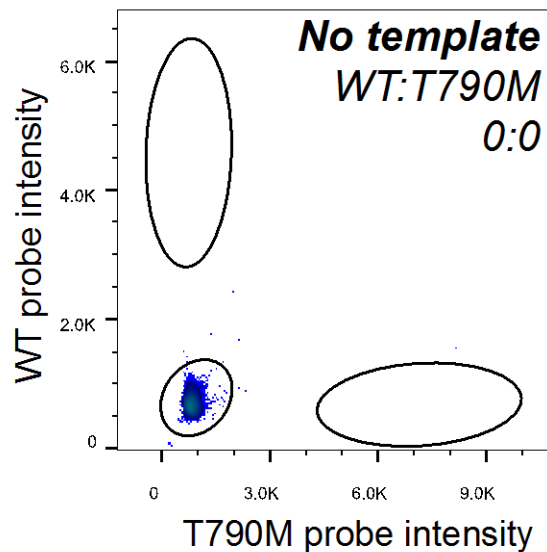
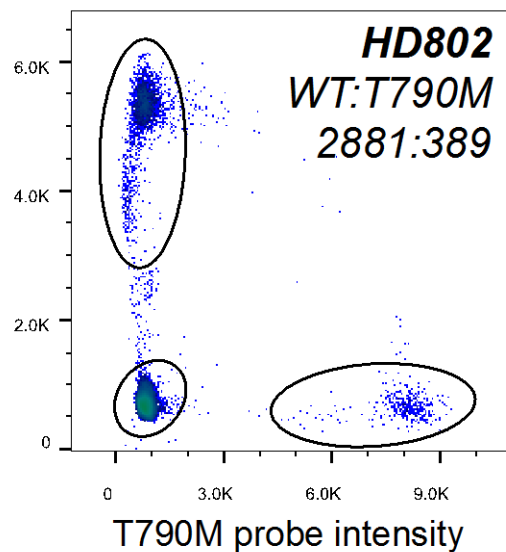
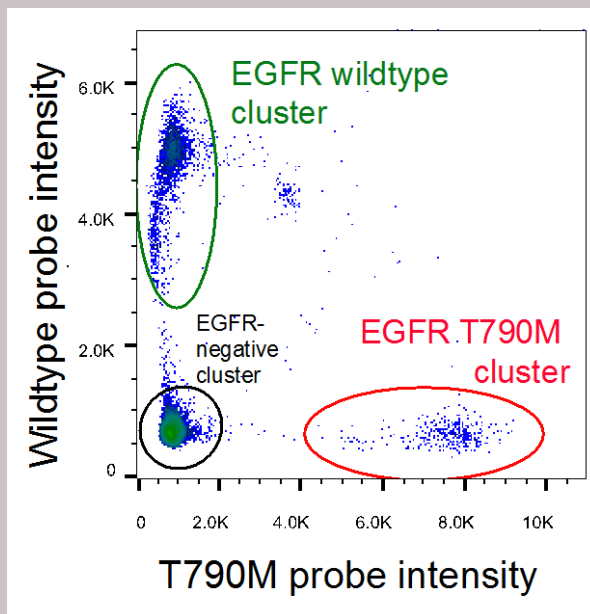
3 – hours



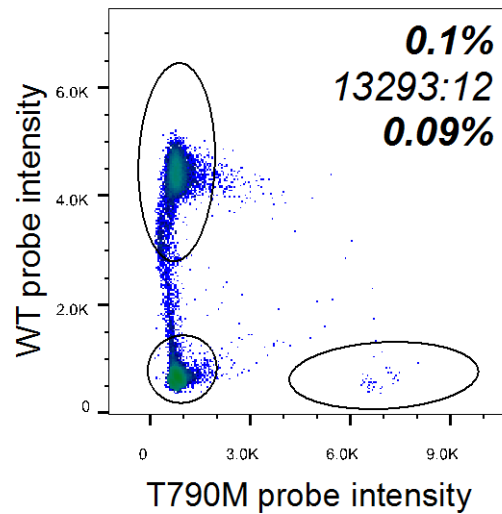
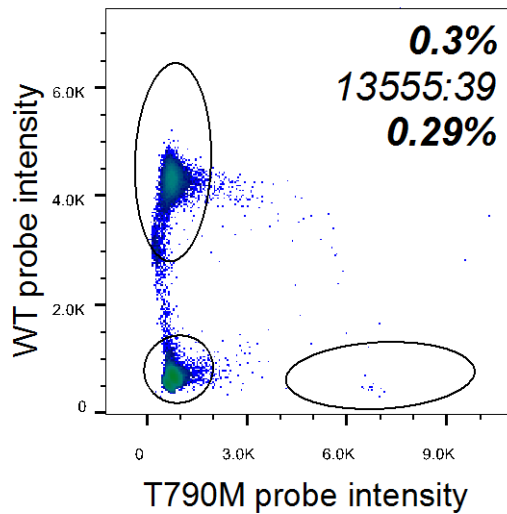
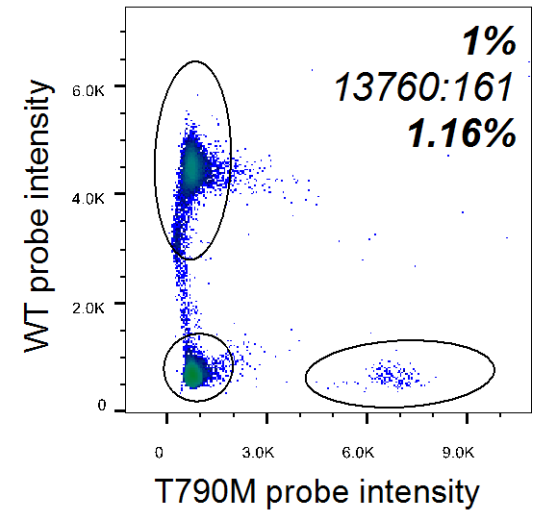
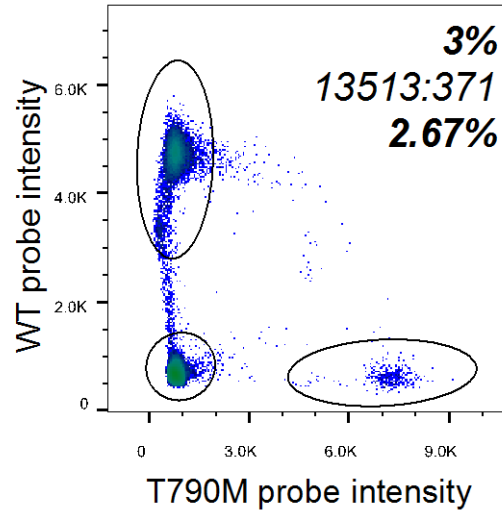
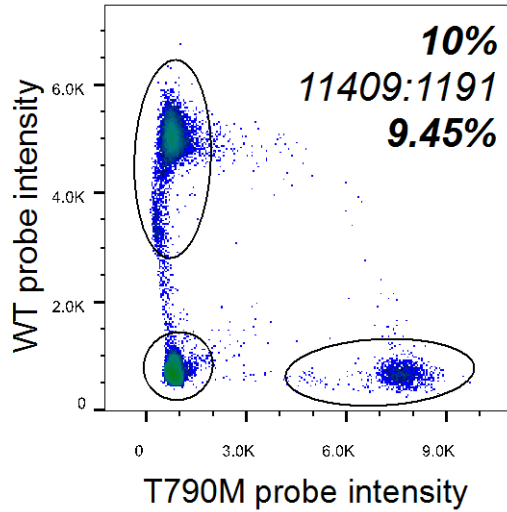
3 – 3 ½ hours



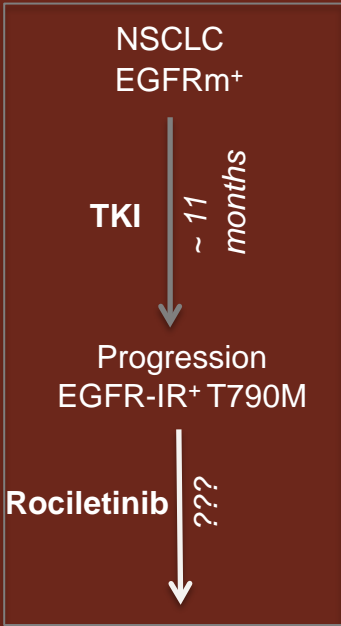
EGFR T790M ddPCR Assay – Data Analysis



Quantitative accuracy: DNA standards

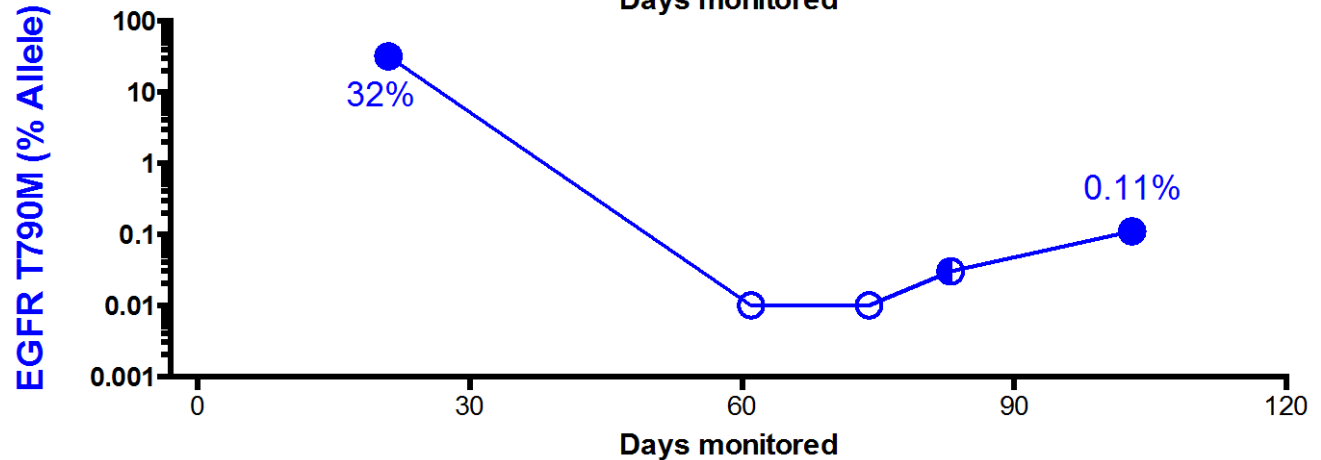
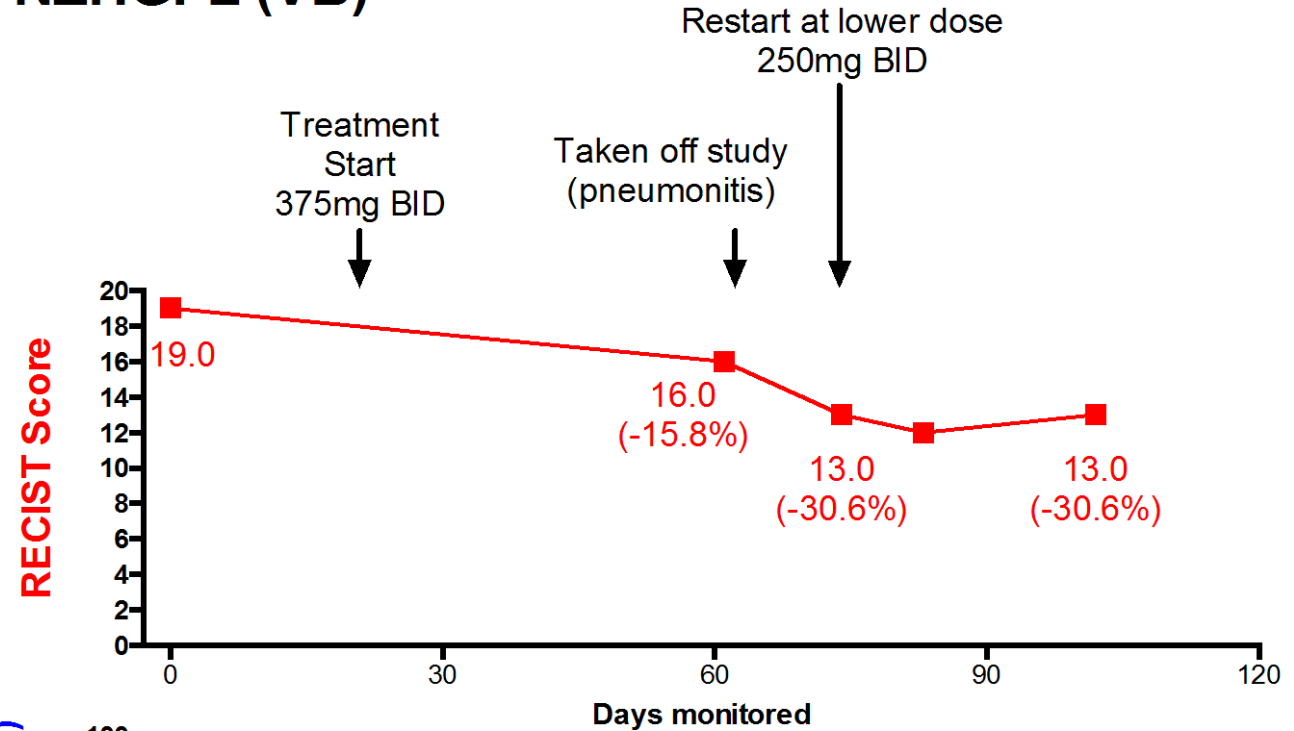


The power of serial monitoring by ctDNA



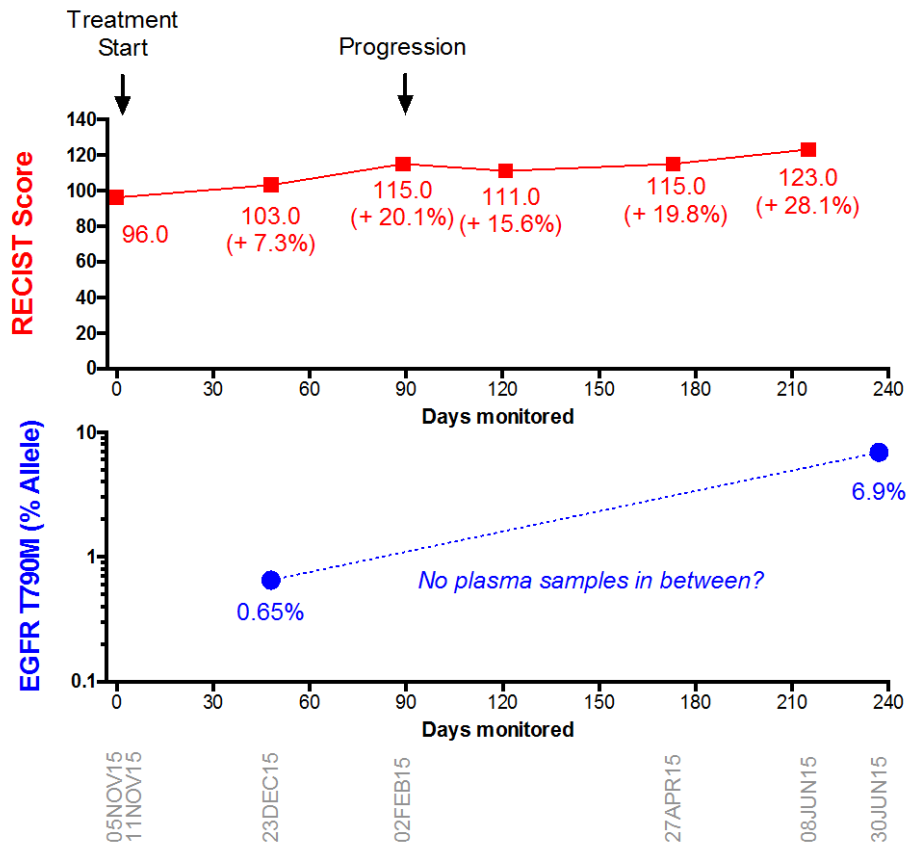
cfDNA
ddPCR
assay:

NZHGFL (VB)

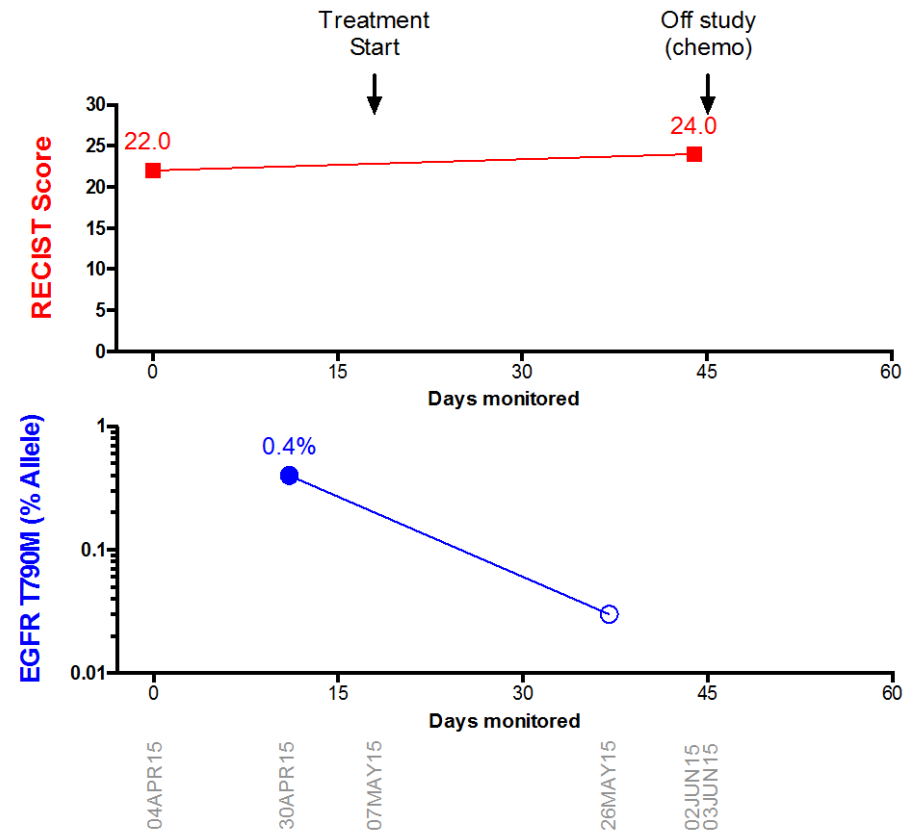


The power of serial monitoring by ctDNA

4SW8WC (MC)



KUD7H9 (CS)



Summary

- Duplex adapters in capture NGS
- BMF tools: new bioinformatic pipeline
 - Powerful NGS error reduction
 - Target sensitivity (0.05-0.1% MAF)

Summary

- ddPCR
 - Cost effective
 - Highly sensitive
 - Focused mutations

Many thanks to ARUP,
D. Baker, K. Gligorich,
S. Hellwig, W. Akerley,
K. Grossman!!