La biopsia liquida

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Obstacles to precision oncology

- Genomic heterogeneity of tumors
- Emergence of drug resistance
- Insufficient means for monitoring responses and predict tumor recurrence

Collins FS & Varmus H. *N Engl J Med.* 2015

Obstacles to precision oncology

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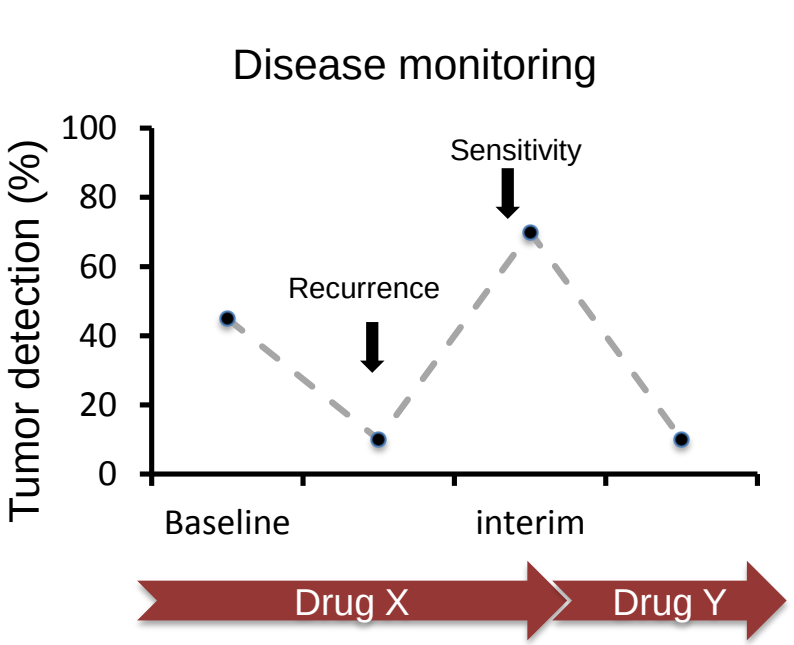
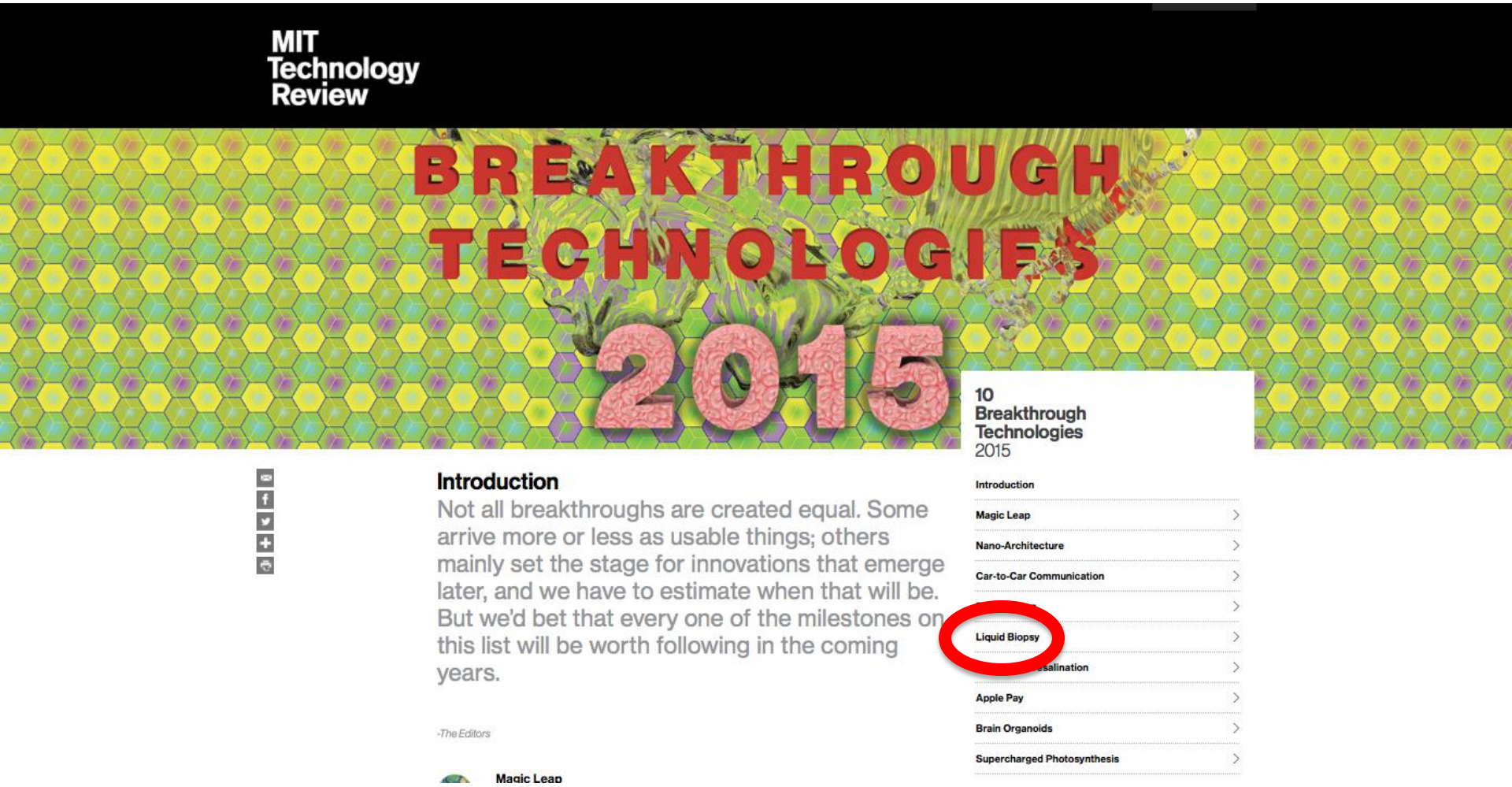
LONGITUDINAL MONITORING OF DISEASE DYNAMICS



One of the Top 10



One of the Top 10

MIT Technology Review

EmTech MIT

10 Breakthrough Technologies 2015

Introduction

Magic Leap

Nano-Architecture

Car-to-Car Communication

Project Loon

Liquid Biopsy

Megascale Desalination

Apple Pay

Brain Organoids

Supercharged Photosynthesis

Internet of DNA

Liquid Biopsy

sequencing machines are leading to simple blood tests for cancer.

Availability: now

Breakthrough

A blood test to catch cancer early.

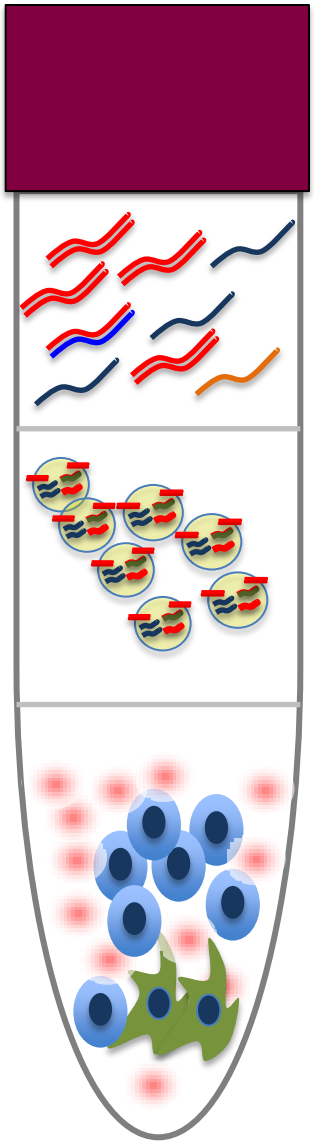
Why It Matters

Cancer kills some eight million people a year around the world.

Key Players

- Dennis Lo, Chinese University of Hong Kong
- Illumina
- Bert Vogelstein, Johns Hopkins

Liquid biopsy: what is it?

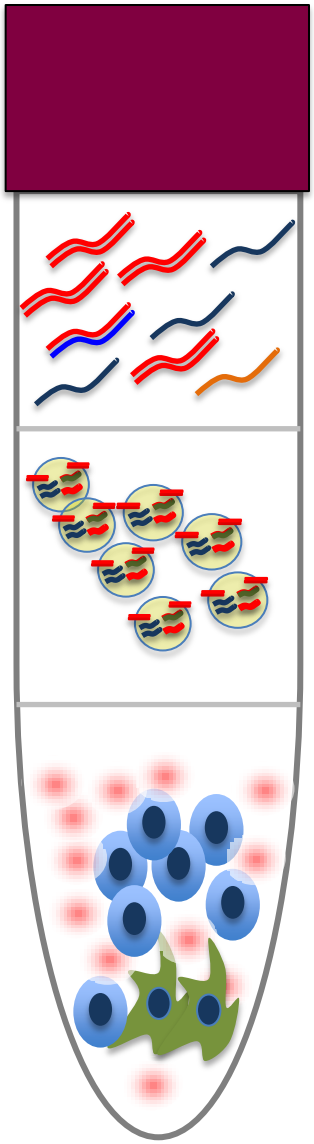


cfNA

Exosomes

CTCs

Liquid biopsy: what is it?



cfNA → • **DNA** • mRNA • miRNA

Exosomes → • DNA • mRNA • miRNA • Protein

CTCs → • DNA • mRNA • miRNA • Protein

Liquid biopsy: what is it for?

Non-invasive access to information through genetics

- early diagnosis
- correlation with the burden load
- minimal residual disease - relapse
- emergence of drug resistance

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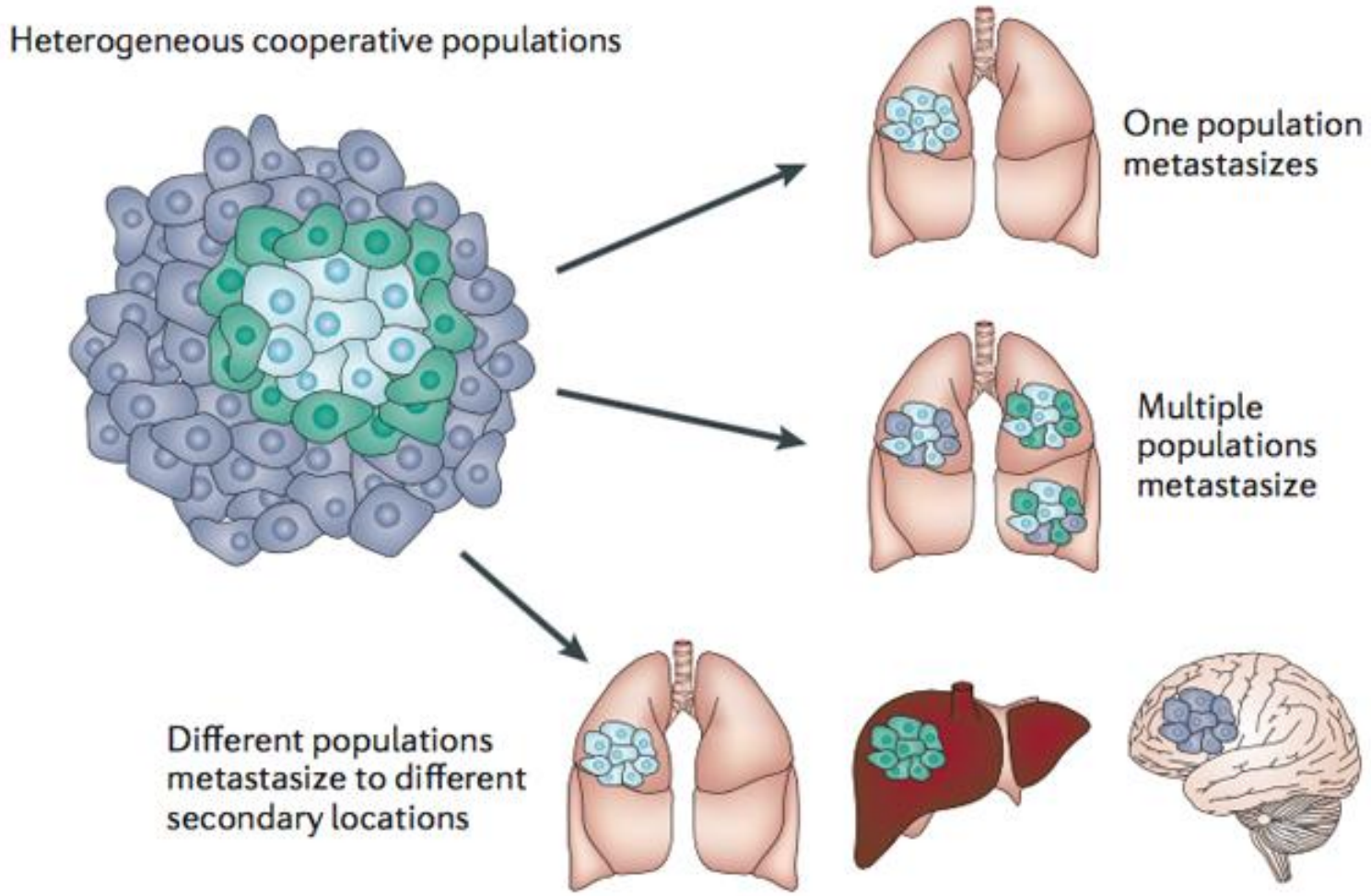
It represents a summary of all the different cancer lesions in a patient

“clonal evolution”

Liquid biopsy: what is it for?



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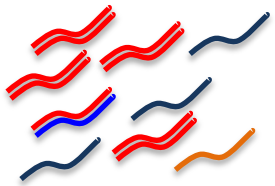
"clonal evolution"

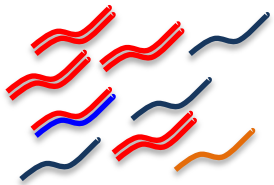
Tabassum & Polliack Nat Rev Cancer 2015

Liquid biopsy: cfDNA



- small fragments from apoptotic or necrotic cells
- has short half-life
- highly variable level (<0.1% to >50% of total cfDNA)

Liquid biopsy: cfDNA



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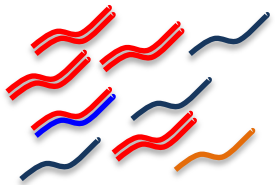
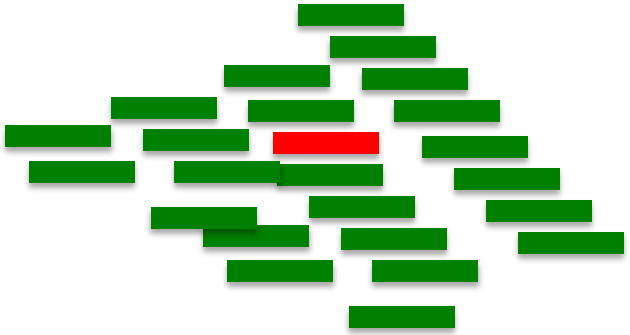
Technical challenges: **sensitivity** and **dynamic range**

Liquid biopsy: cfDNA



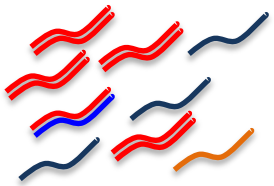
- **BEAMing** (**B**eads, **E**mulsions, **A**mplification, and **M**agnetics)
- **Droplet digital PCR** (ddPCR)
- **Targeted-NGS**

Sensitivity < 0.01%
Dynamic range > 10.000

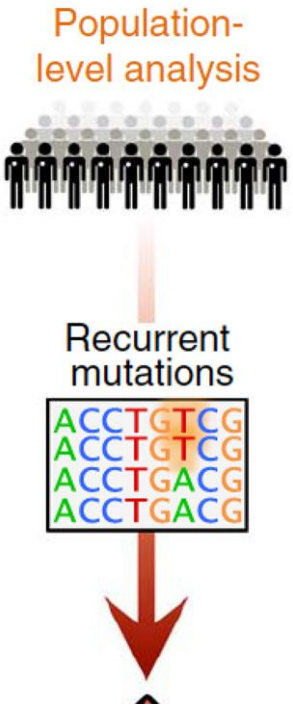


Conventional qPCR Sensitivity: up to 0.1%

Liquid biopsy: cfDNA applications



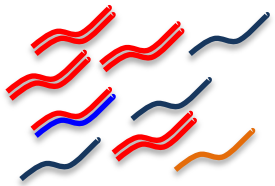
- ***Early diagnosis***



lack of specificity

Newman AM et al. Nature Medicine. 2013

Liquid biopsy: cfDNA applications



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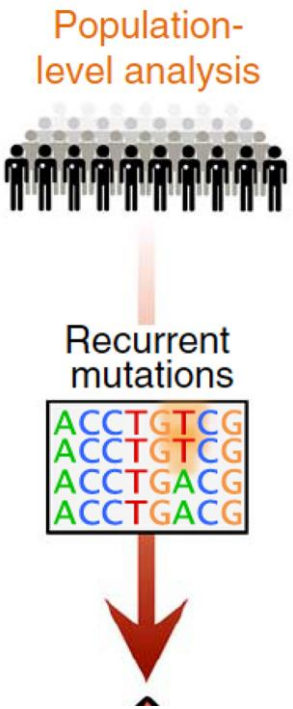
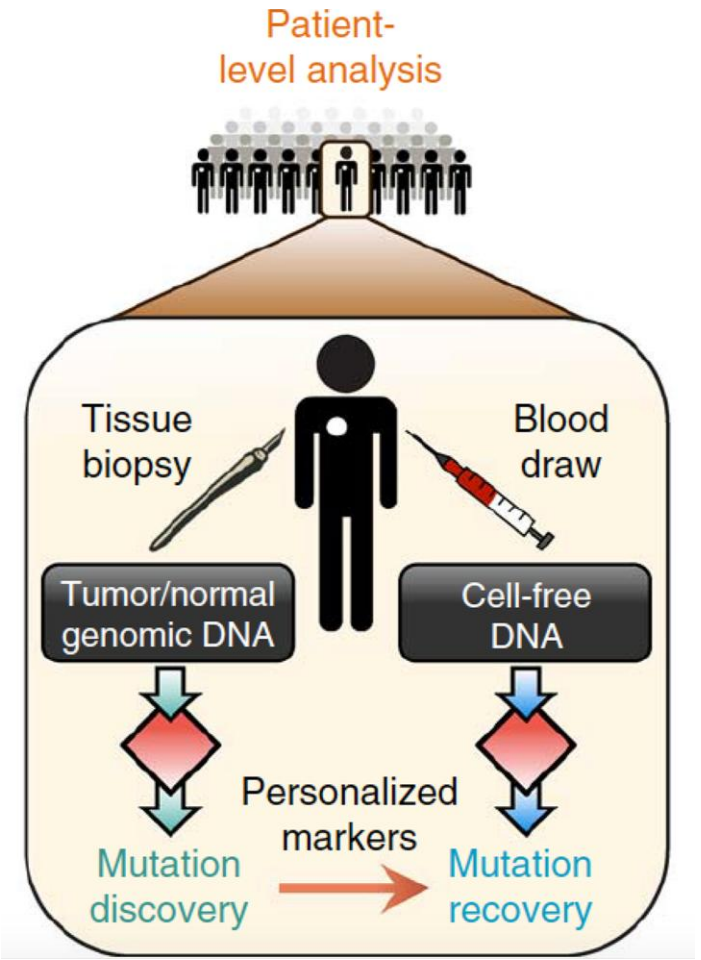


lack of specificity

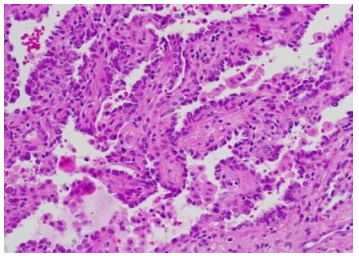
- ***Disease monitoring***



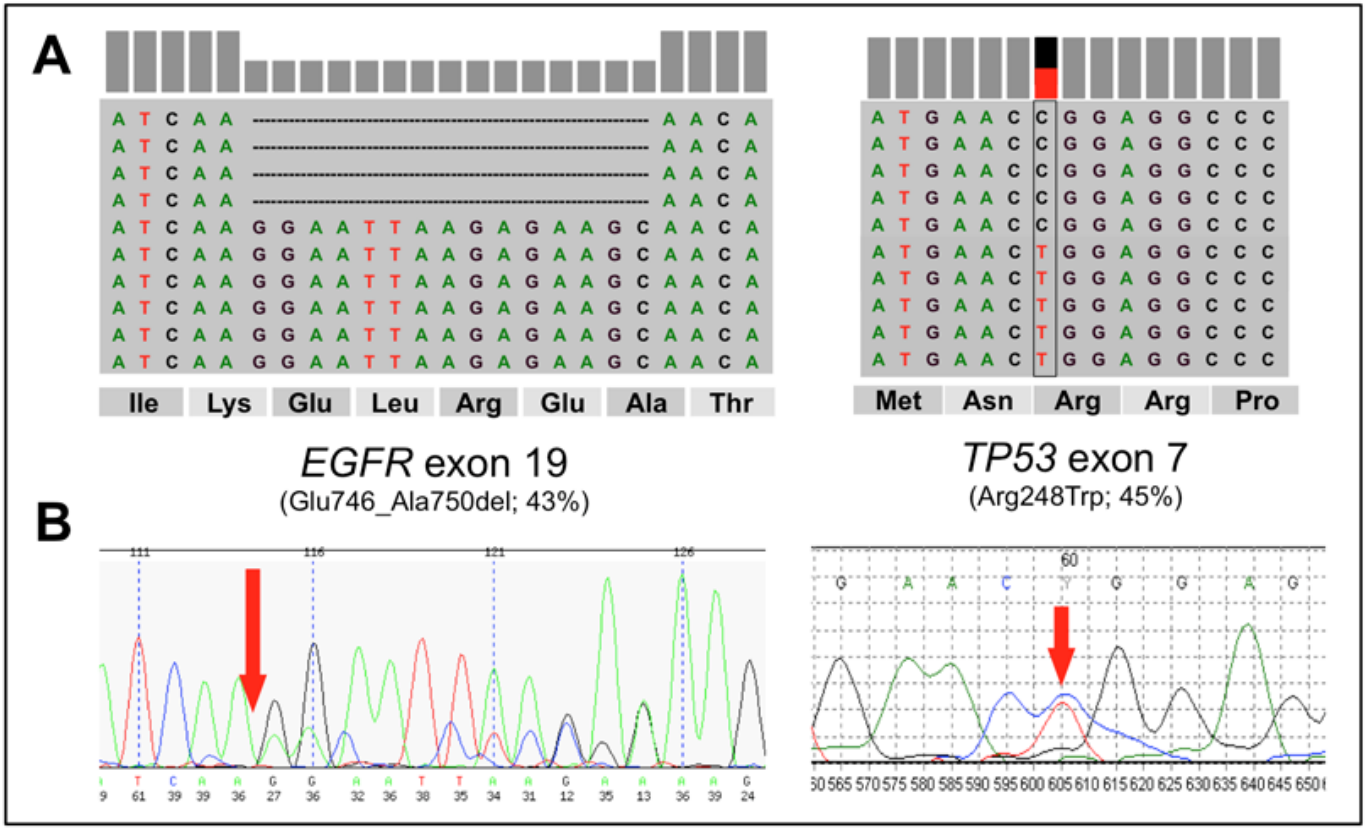
Newman AM et al. Nature Medicine. 2013

Molecular Heterogeneity

Lung adenocarcinoma



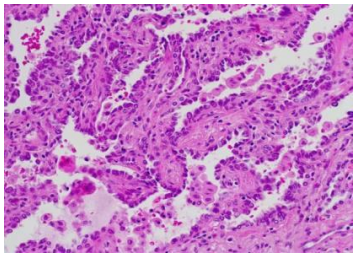
Coexistent *EGFR* and *TP53* mutations



A) NGS aligned to reference genome by Integrative Genomics Viewer v.2.1 (Broad Institute) software
B) Validation of NGS results by Sanger sequencing

Bria et al., Oncotarget 2015

Molecular Heterogeneity



Lung adenocarcinoma

Response to gefitinib of 17 patients with *EGFR* mutant cancers

Patients' groups according to resistance to Gefitinib and Progression-Free-Survival; 17 evaluable patients (Log-Rank $p<0.0001$).

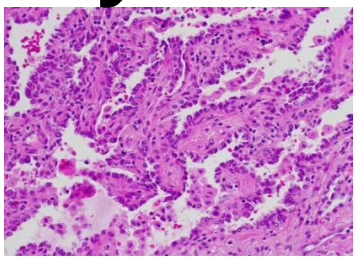
Group	Definition	Pts (%)	Median PFS (months, 95%CI)
Poor	Progression at 1st assessment	6 (35.2)	1.7 (0.1-3.2)
Intermediate	Progression within 12 months	3 (17.7)	6.1 (3.0-9.2)
Good	Progression ≥ 12 months or treatment ongoing	8 (47.1)	17.3 (9.0-25.5)

Pts: patients; PFS: progression-free-survival; CI: confidence intervals.

Analysed for mutations in 20 genes
(oncomine solid tumors – lifetechnologies)

Bria et al., Oncotarget 2015

Molecular Heterogeneity



Lung adenocarcinoma

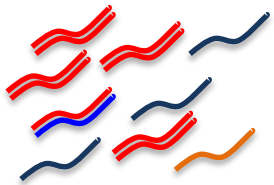
Response to gefitinib of 17 patients with *EGFR* mutant cancers

Patient	Group	Type of Mutation (PMA) *						
		EGFR	*TP53*	*KRAS*	*CTNNB1*	*PIK3CA*	*MET*	*SMAD4*
1	Good	L858R (32%)						
2	Good	p.E746_A750del (31%)						
3	Good	L858R (56%)						
4	Good	L858R (86%)						
5	Good	p.E746_A750del (71%)						
6	Good	p.E746_A750del (88%)						
7	Good	p.L747_T751del (64%)			S33C (20%)			G358E (44%)
8	Good	p.E746_S752delinsA (28%)			S45P (5%)			
9	Intermediate	p.L747QfsTer16 (23%)	R273G (45%)					
10	Intermediate	p.E746_A750del (94%)	R248W (48%)					
11	Intermediate	p.E745_A750del (30%)	R175H (27%)					
12	Poor	L858R (36%)	R273L (47%)					
13	Poor	p.E746_T751delinsIA (11%)		G12C (5%)				
14	Poor	p.E746_A750del (20%)						
15	Poor	L858R (43%)		G12C (22%)		E542K (31%)		
16	Poor	p.E746_A750del (31%)	R248L (40%)					
17	Poor	E746_A750del (94%)	R175L (37%)				N375S (34%)	

* PMA = Proportion of mutated alleles

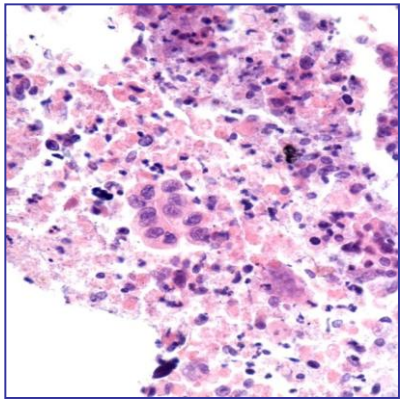
Bria et al., Oncotarget 2015

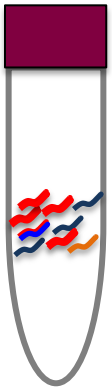
Lung cancer: cfDNA



T-NGS

EGFR status in tissue and matched cfDNA



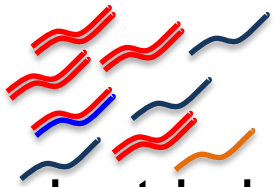


EGFR status in tissues and matched cfDNA

Case	FFPE tissue	cfDNA
1	***KRAS*** G12V, ***TP53*** G298*	***KRAS*** G12V, ***TP53*** G298*
2	***EGFR*** 746-750del ***KRAS*** G12C	***EGFR*** 746-750del ***KRAS*** G12C
3	***KRAS*** G12V, ***TP53*** A337C	***KRAS*** G12V ***TP53*** A337C
4	***EGFR*** 746-750del	***EGFR*** 746-750del
5	***EGFR*** 746-750del ***TP53*** P190L	***EGFR*** 746-750del ***TP53*** P190L

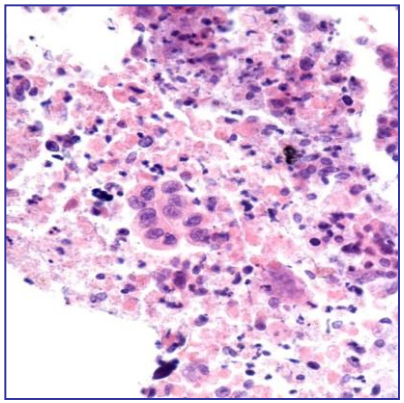
NGS permits simultaneous analysis of multiple genes and **selection of patient specific** gene mutation

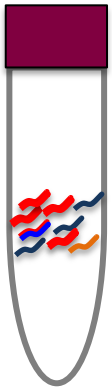
Lung cancer: cfDNA



ddPCR

EGFR status in tissue and matched cfDNA

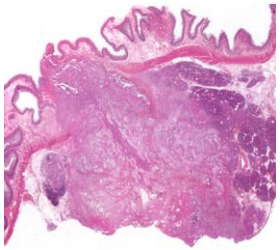


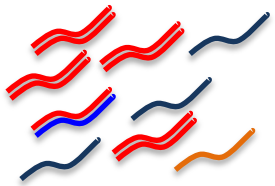


EGFR status in tissues and matched cfDNA

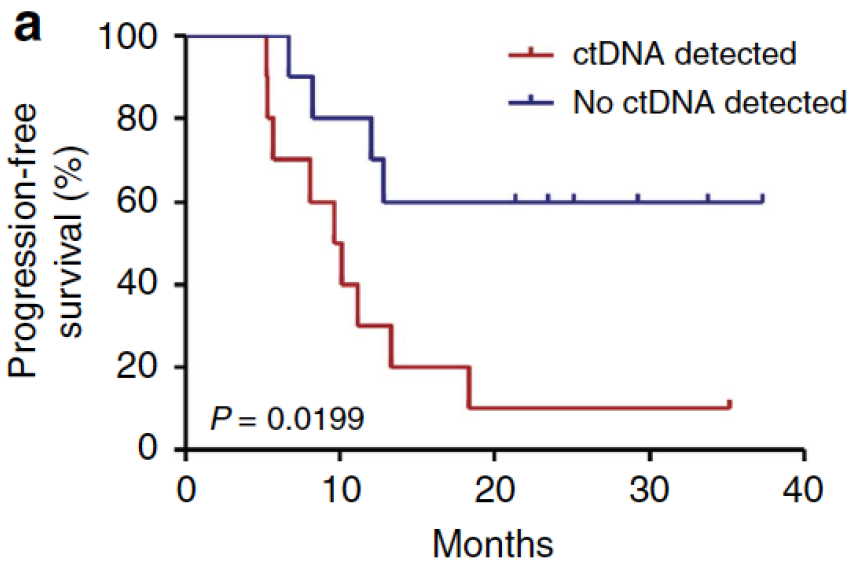
Case	Stage	ALK	FFPE (Pyroseq)	cfDNA (ddPCR)
1	III	-	wt	wt
2	IV	Rearr	wt	T790M
3	IV	-	wt	T790M
4	III	-	L858R	L858R T790M
5	III	-	wt	wt
6	IV	-	746-750del	746-750del
7	IV	-	746-750del	746-750del
8	IV	-	746-750del	746-750del, T790M
9	IV	-	wt	wt
10	III	-	wt	wt
11	III	-	746-750del	746-750del
12	IV	-	wt	T790M
13	IV	-	wt	wt
14	IV	-	wt	T790M
15	IV	-	746-750del	746-750del

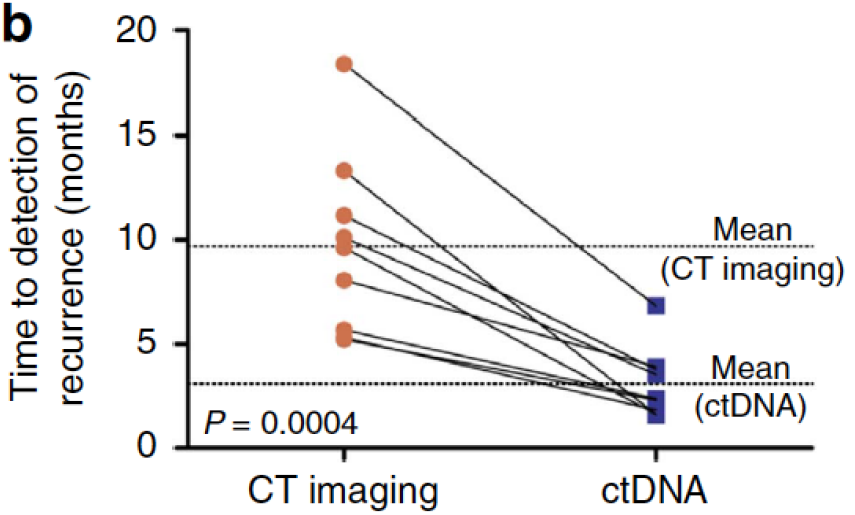
Pancreatic cancer: cfDNA





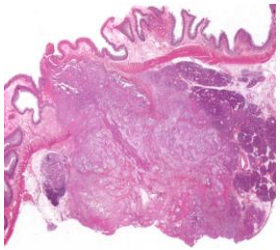
- Stage II resectable
- dPCR detected alterations at diagnosis (specificity 99.9%)
- ctDNA detected recurrence before CT

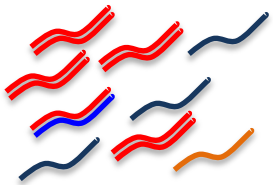




Sausen M et al. *Nature Communications*. 2015

Pancreatic cancer: cfDNA





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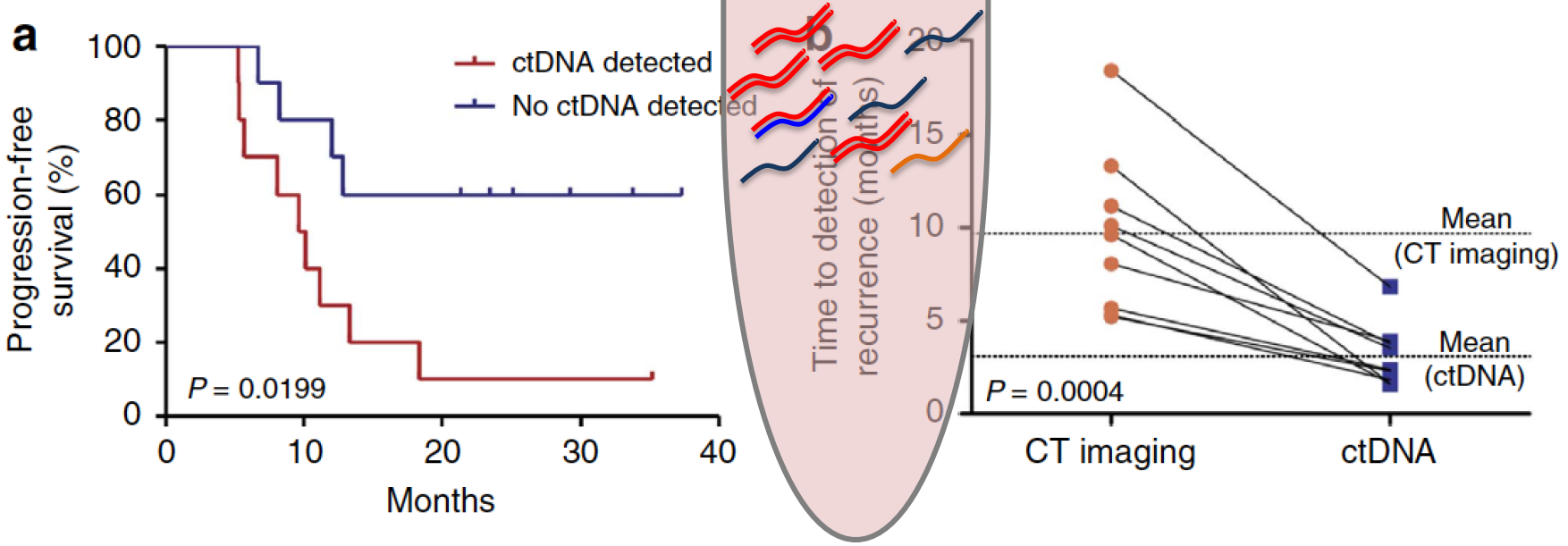
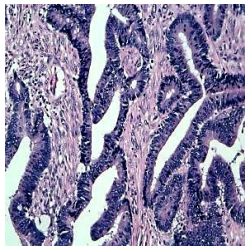
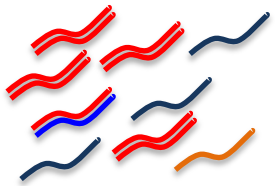


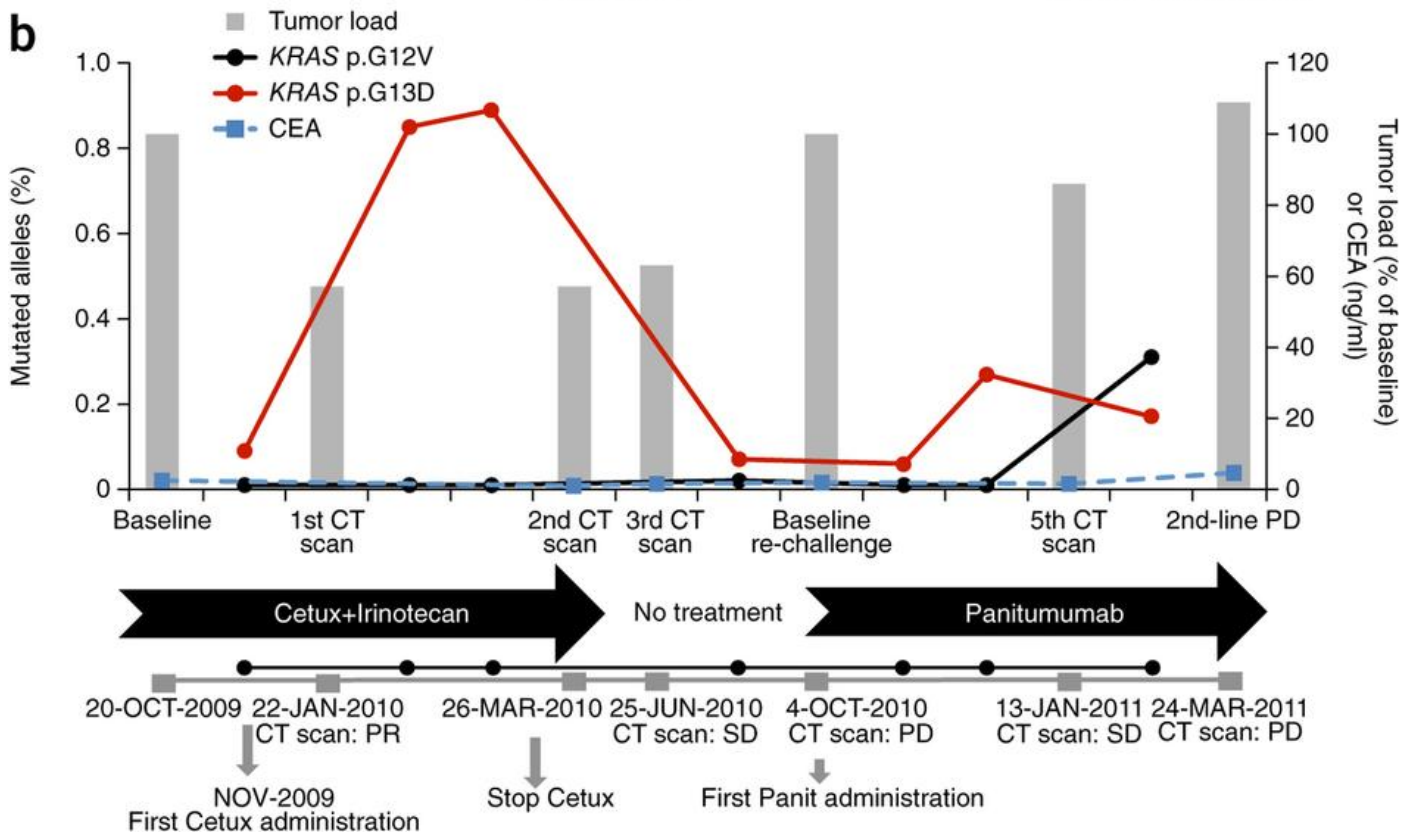
Sausen M et al. *Nature Communications*. 2015

Colorectal cancer: cfDNA



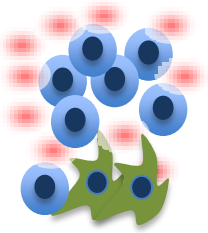


Tracking clonal evolution and resistance in the blood



Siravegna G et al. *Nature Medicine*. 2015

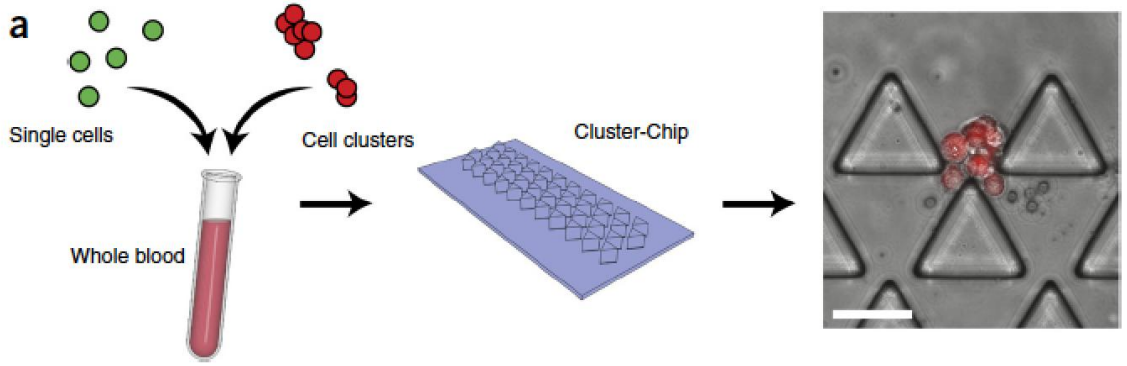
Liquid biopsy: CTCs



- **Enumeration** as prognostic biomarker: ***CellSearch***
  FDA approved (2004) for **prostate** and **breast** cancers

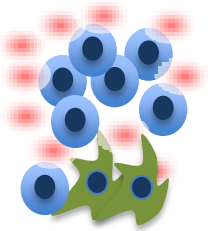
- Typically **<10 cells/mL** of blood from a metastatic patient

- **Correlates with tumor burden**

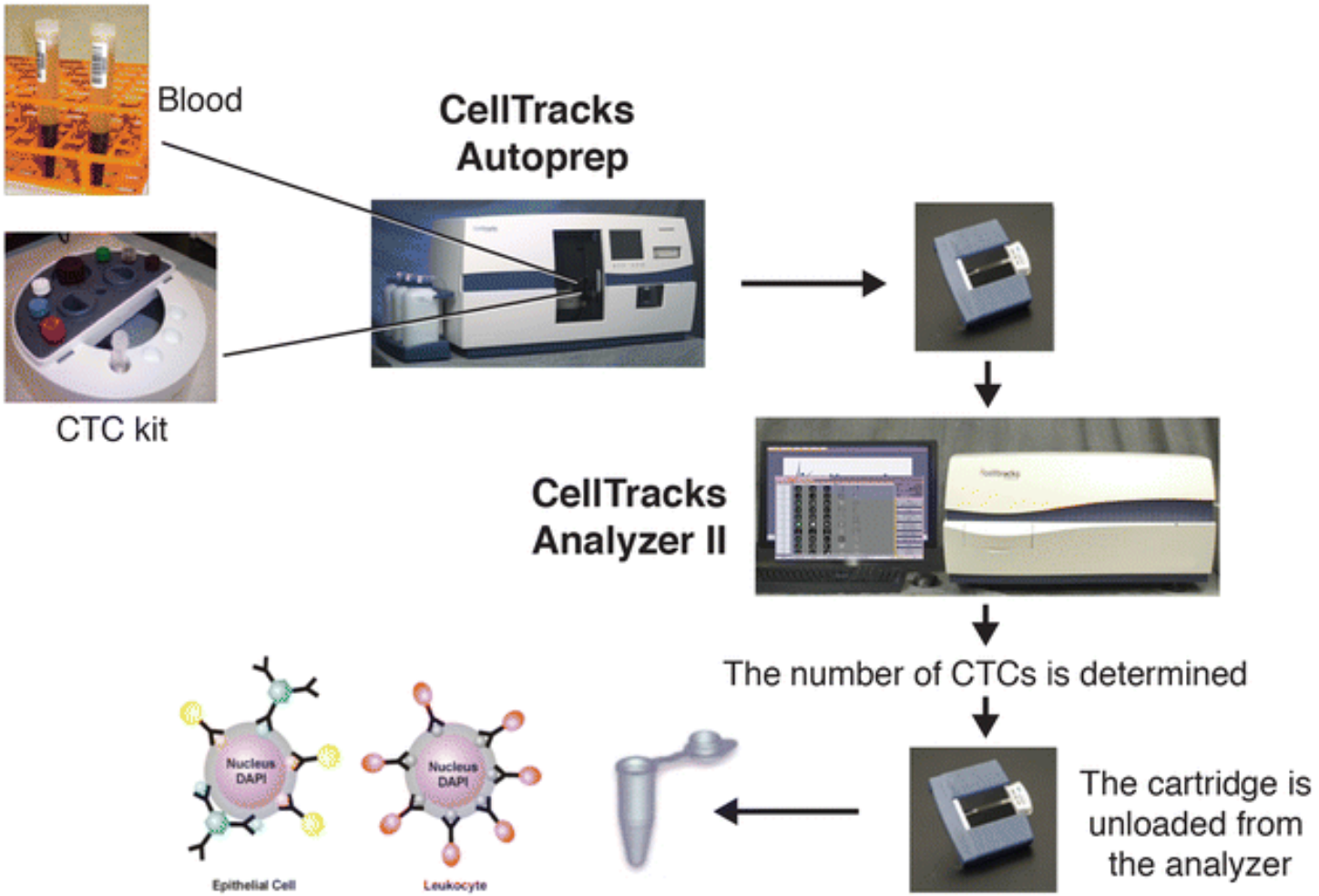


Aceto N et al. Cell. 2014

Liquid biopsy: CTCs

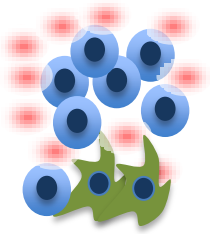


A ***CTCs enrichment with the CellSearch System***

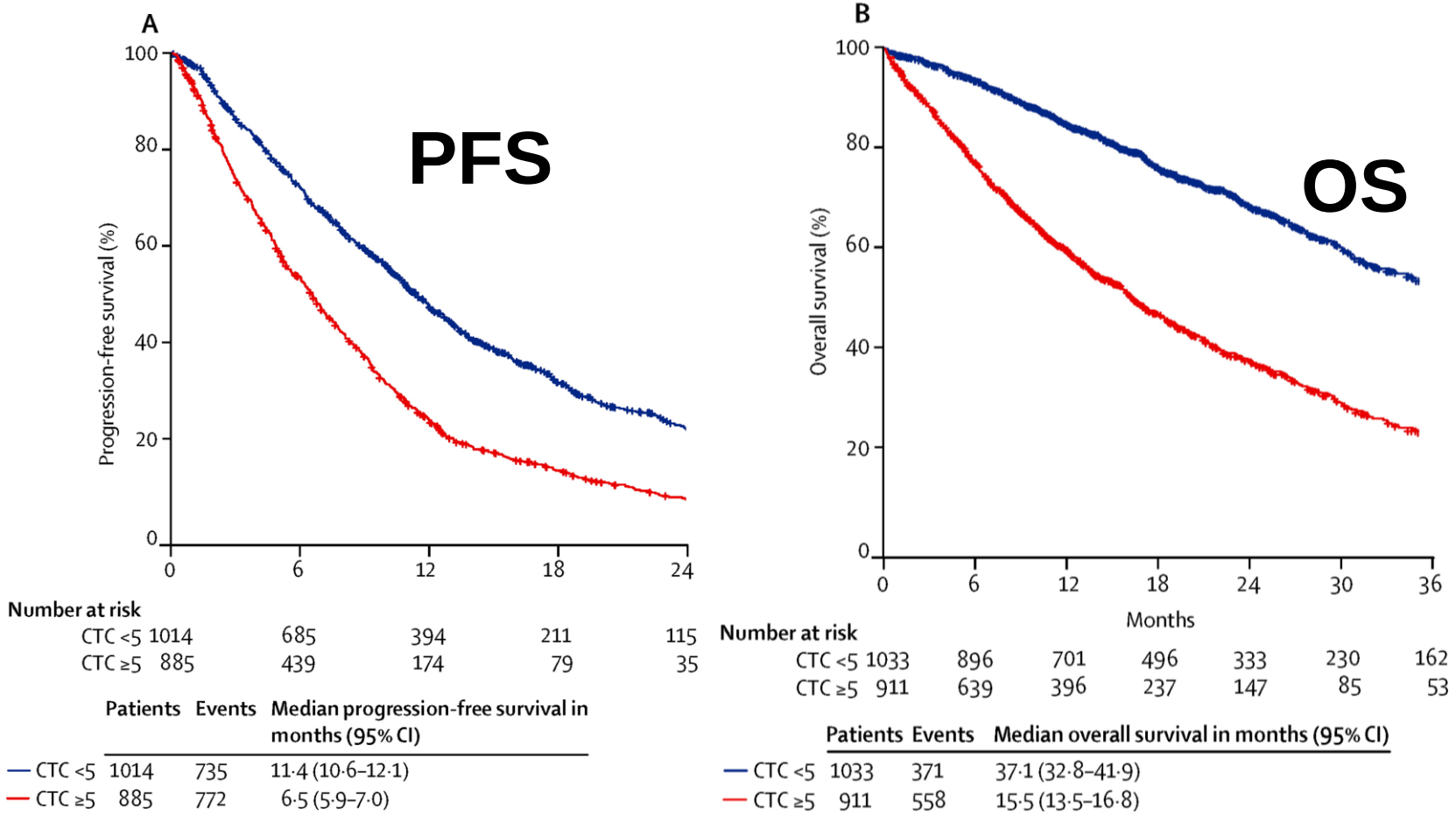


Fernandez *et al. Breast Cancer Research* 2014

Liquid biopsy: CTCs



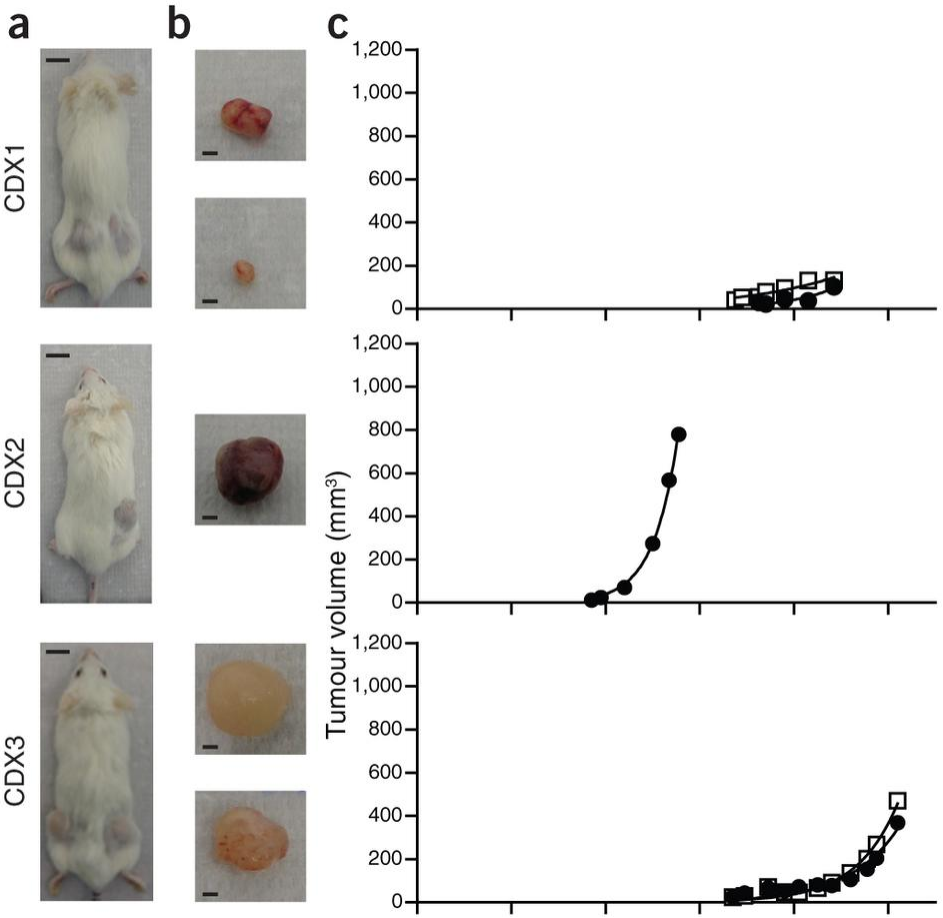
Metastatic breast cancer
pooled analysis of 17 European Centres

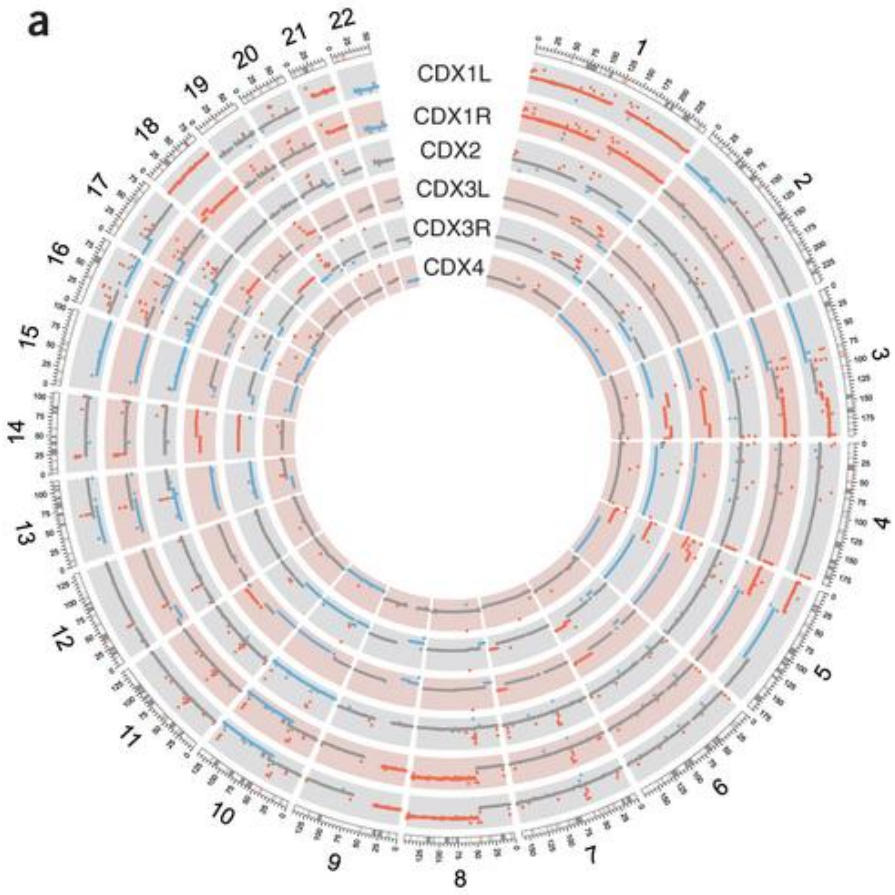


Bidard F-C et al. Lancet Oncology 2014

Liquid biopsy: CTCs

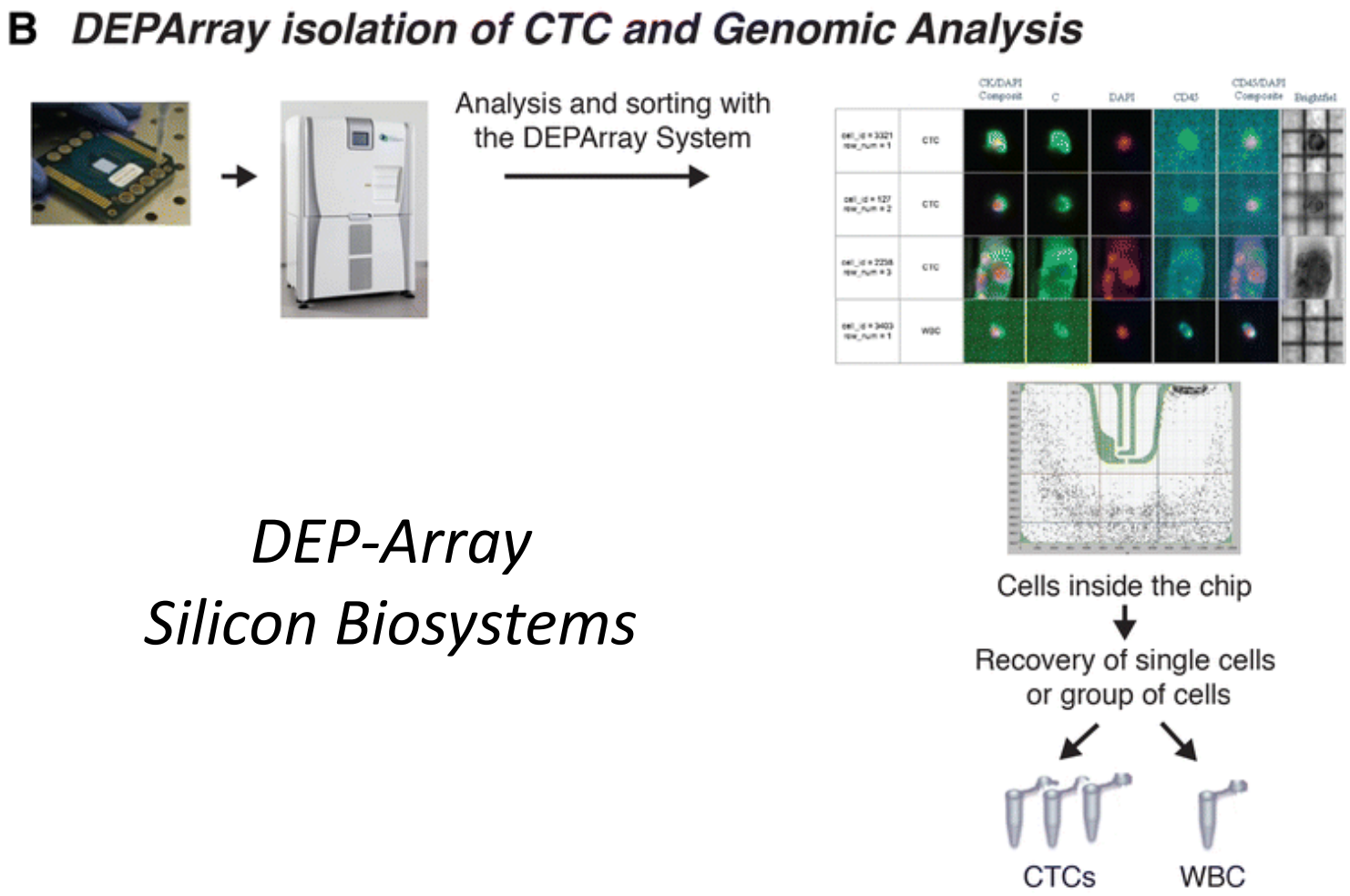
CTCs from patients with SCLC
are tumorigenic and are representative of the primary SCLC





Hodgkinson C et al. Nature medicine. 2015

Liquid biopsy: CTCs isolation



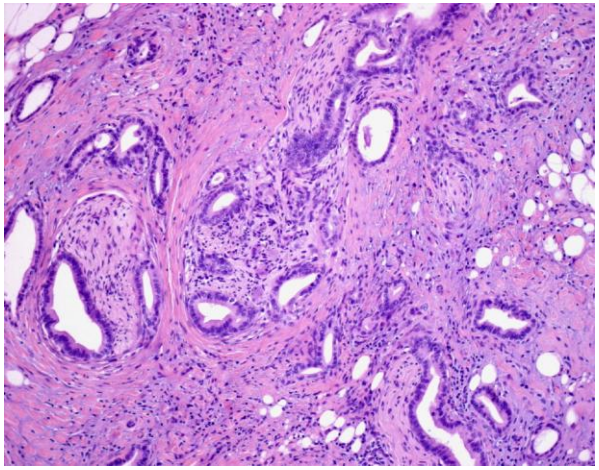
DEP-Array
Silicon Biosystems

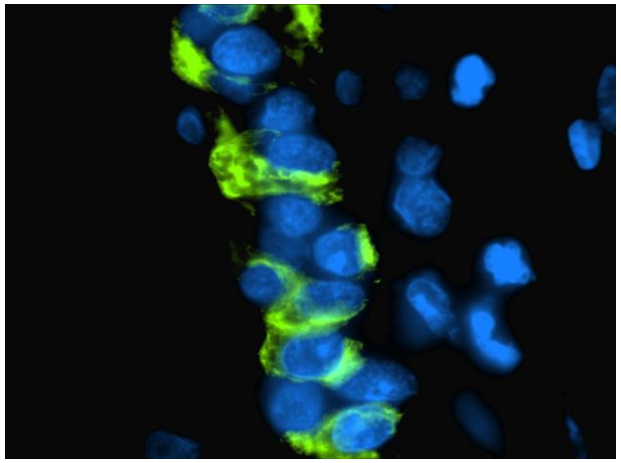
Fernandez *et al. Breast Cancer Research* 2014

Tumor Burden

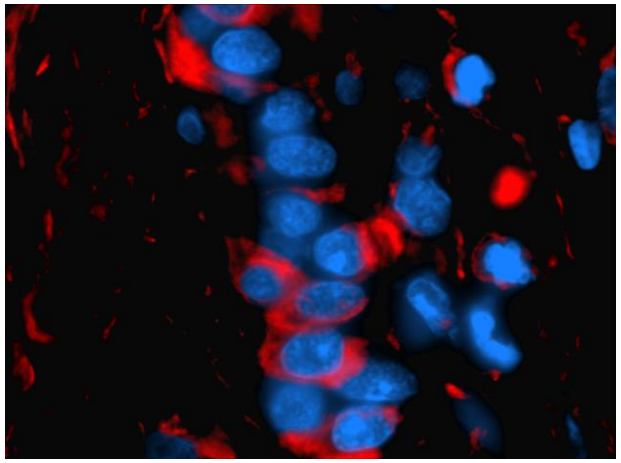
Pancreatic cancer cellularity

Sample	*KRAS*	Cell	KRAS
1	G12R (22%)	**15%**	**60%**

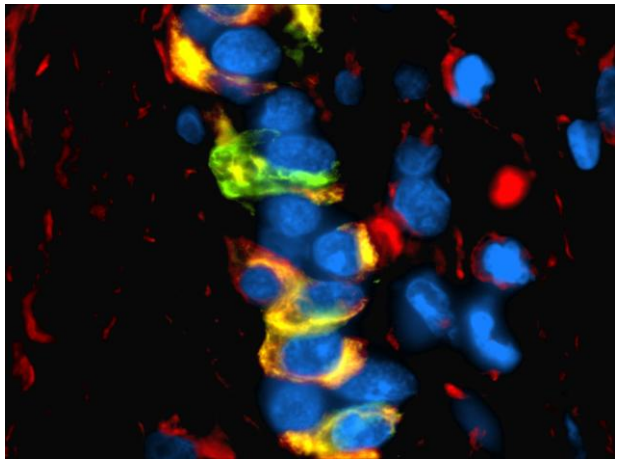




Keratin

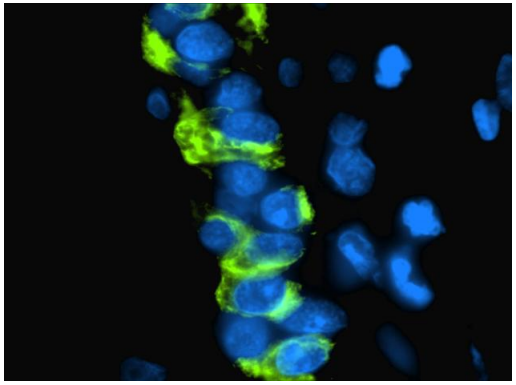


Vimentin

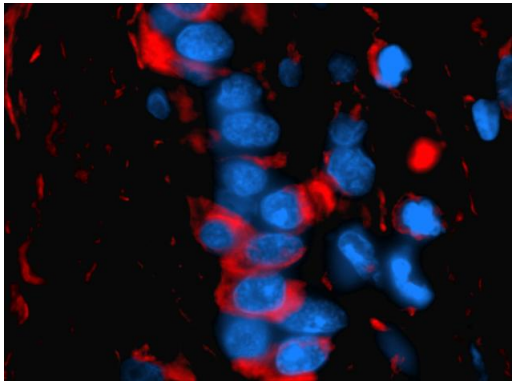


Fusion

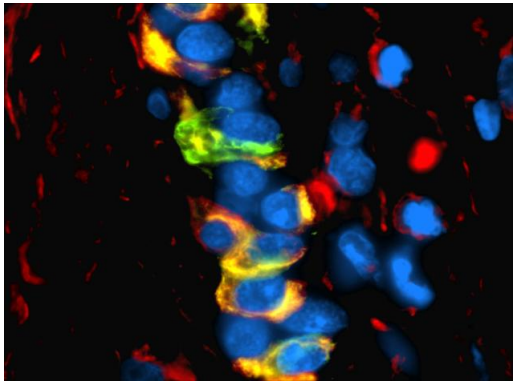
Resolving FFPE Intratumoral Heterogeneity



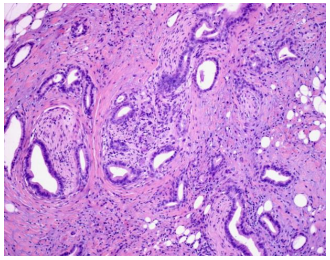
Keratin

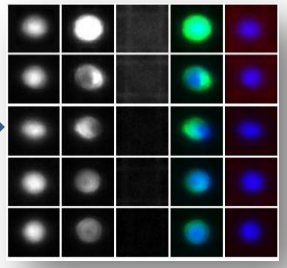


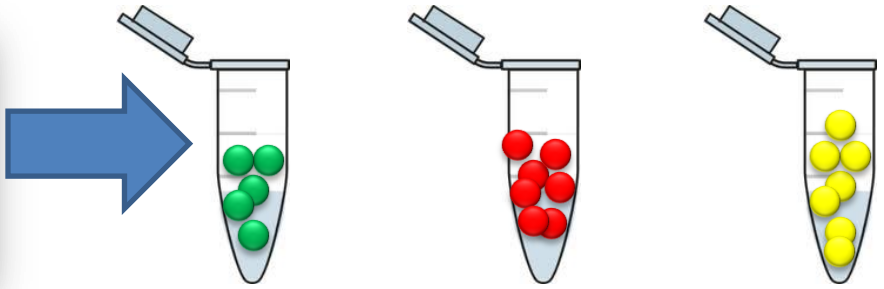
Vimentin



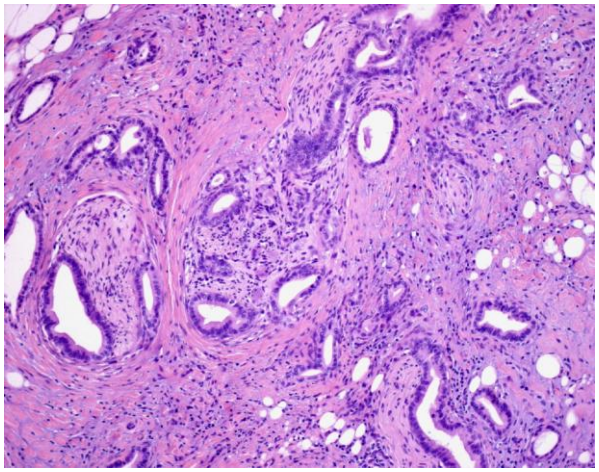
Fusion



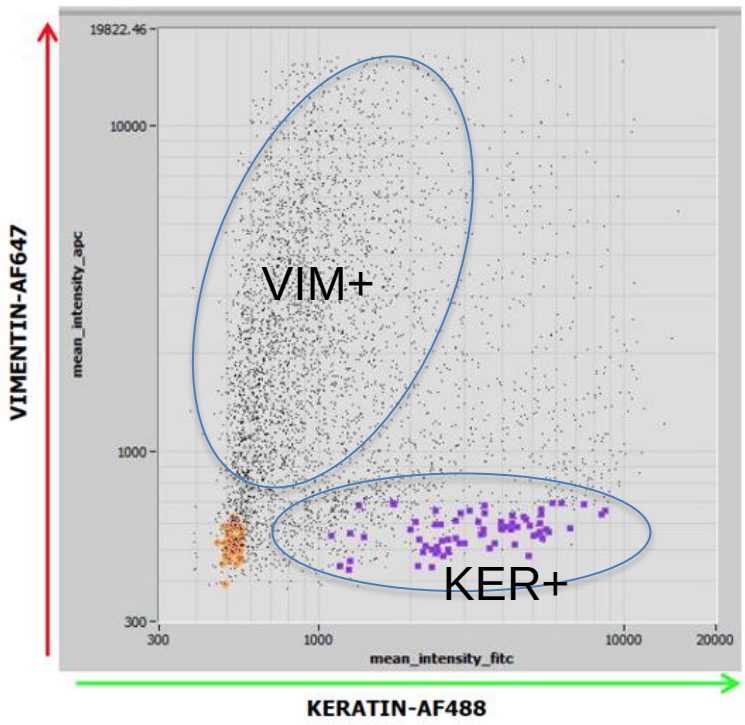


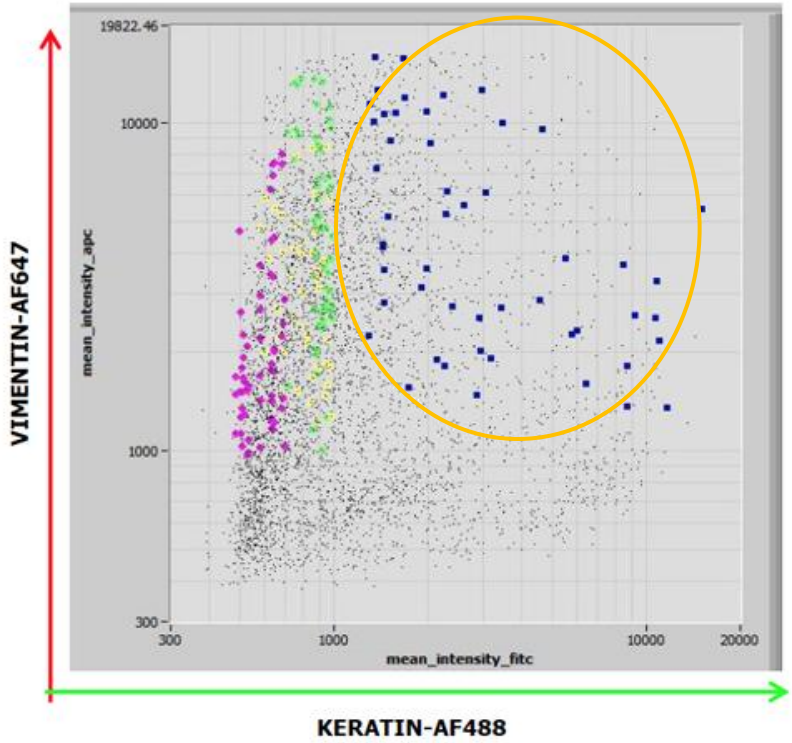


Recovery of homogeneous pools of cells



KRAS	G12R
TP53	R273H
SMAD4	R361H



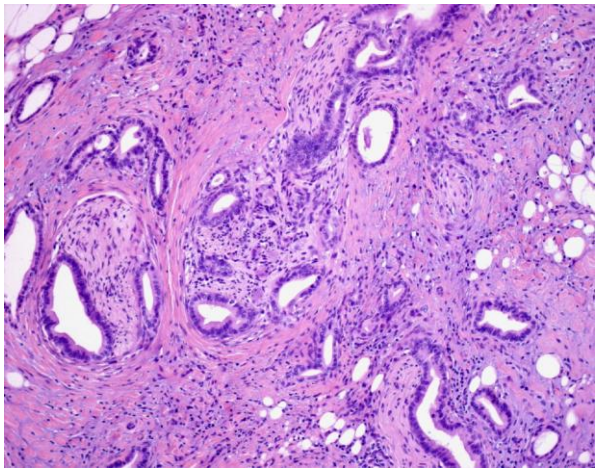


VIM – KER -

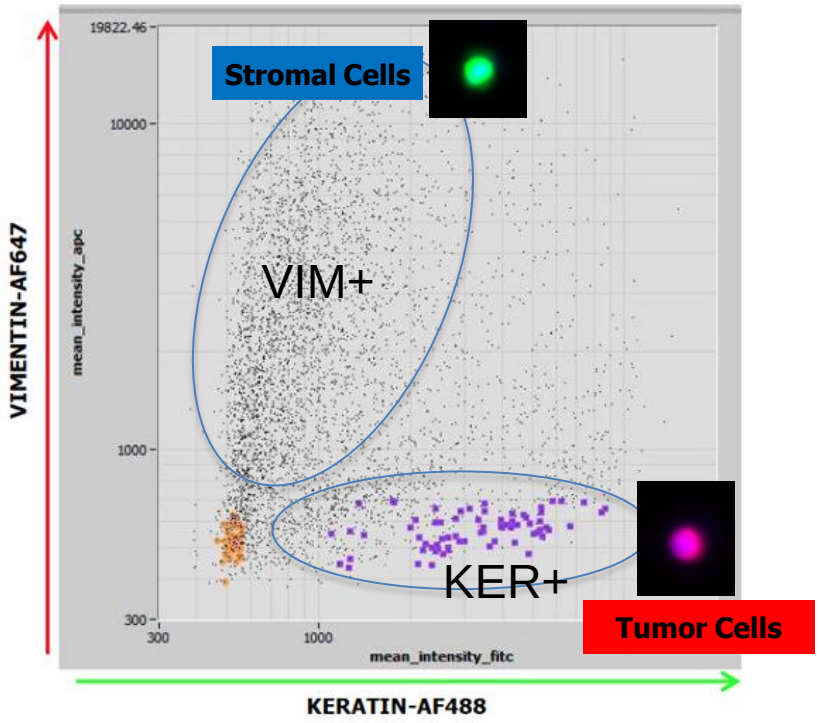
KER +

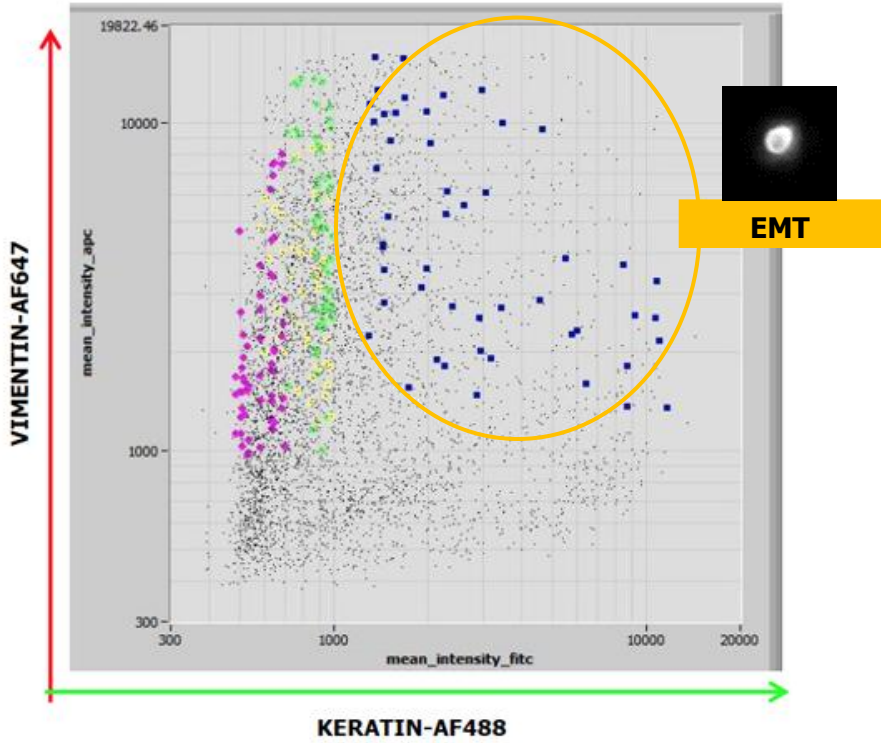
VIM +

VIM + KER +



KRAS	G12R
TP53	R273H
SMAD4	R361H



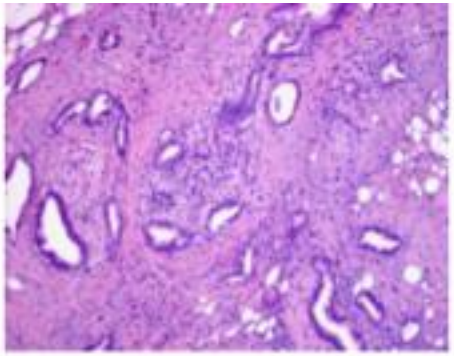


VIM – KER -

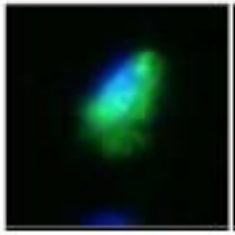
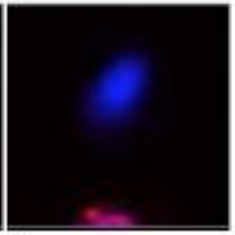
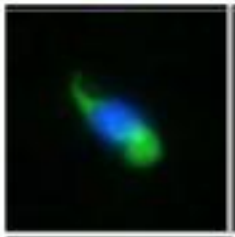
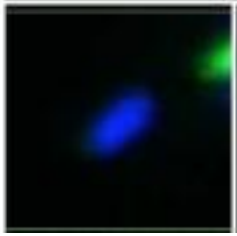
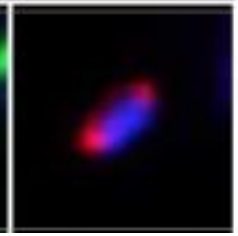
KER +

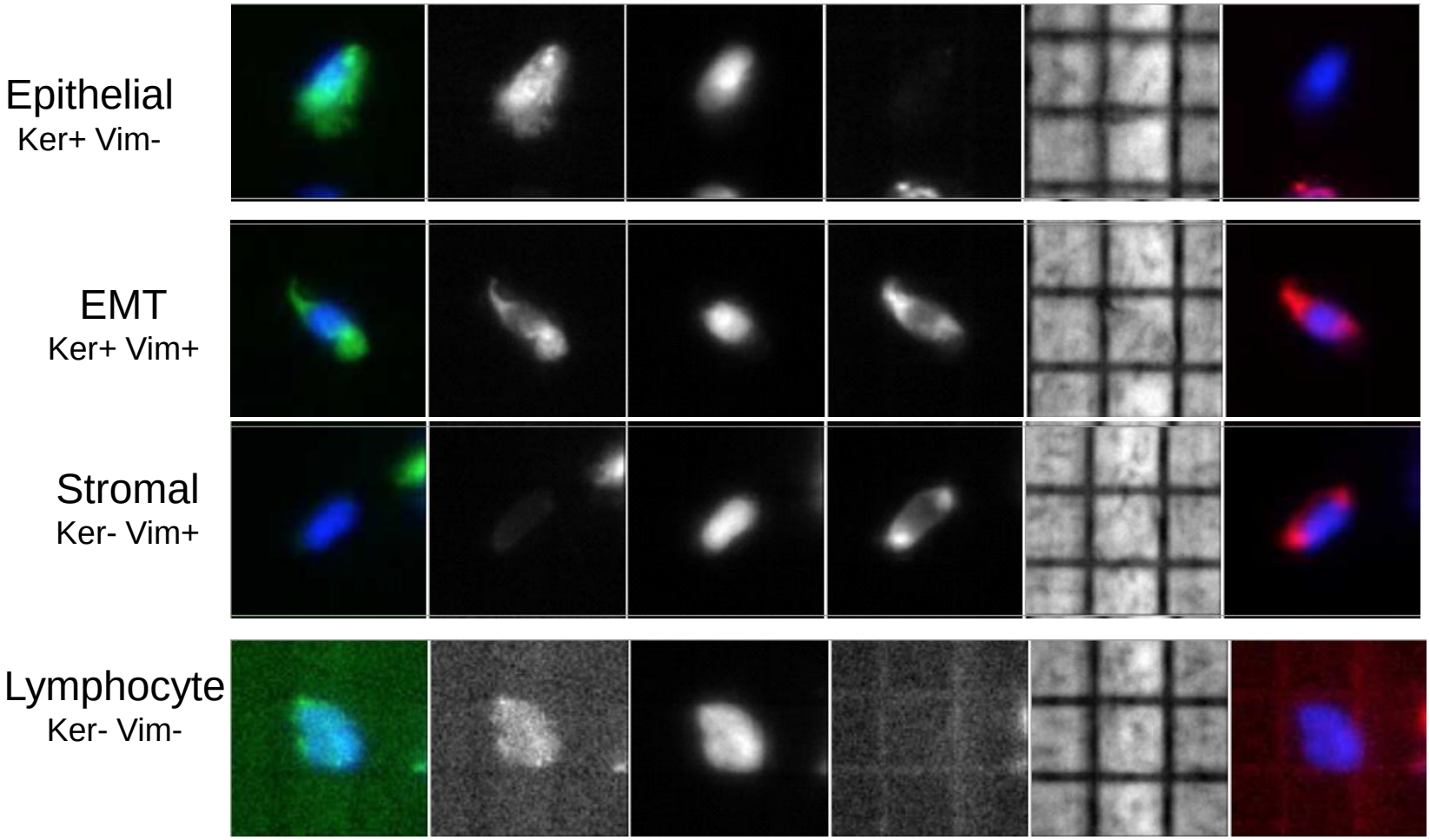
VIM +

VIM + KER +

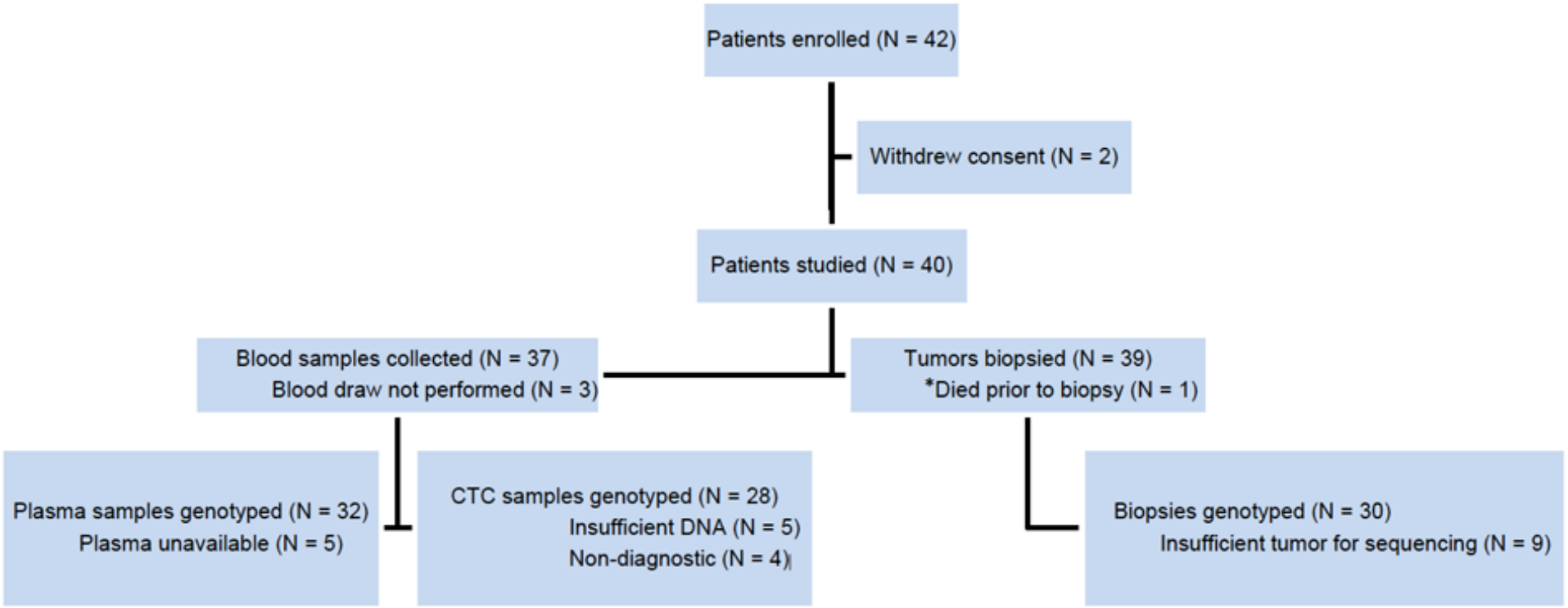


KRAS	G12R
TP53	R273H
SMAD4	R361H

	Keratin	Vimentin	*KRAS*	*TP53*	*SMAD4*
Epithelial Ker+ Vim-			**G12R**	**R273H**	**R361H**
EMT Ker+ Vim+			**G12R**	**R273H**	**R361H**
Stromal Ker- Vim+			None	None	None

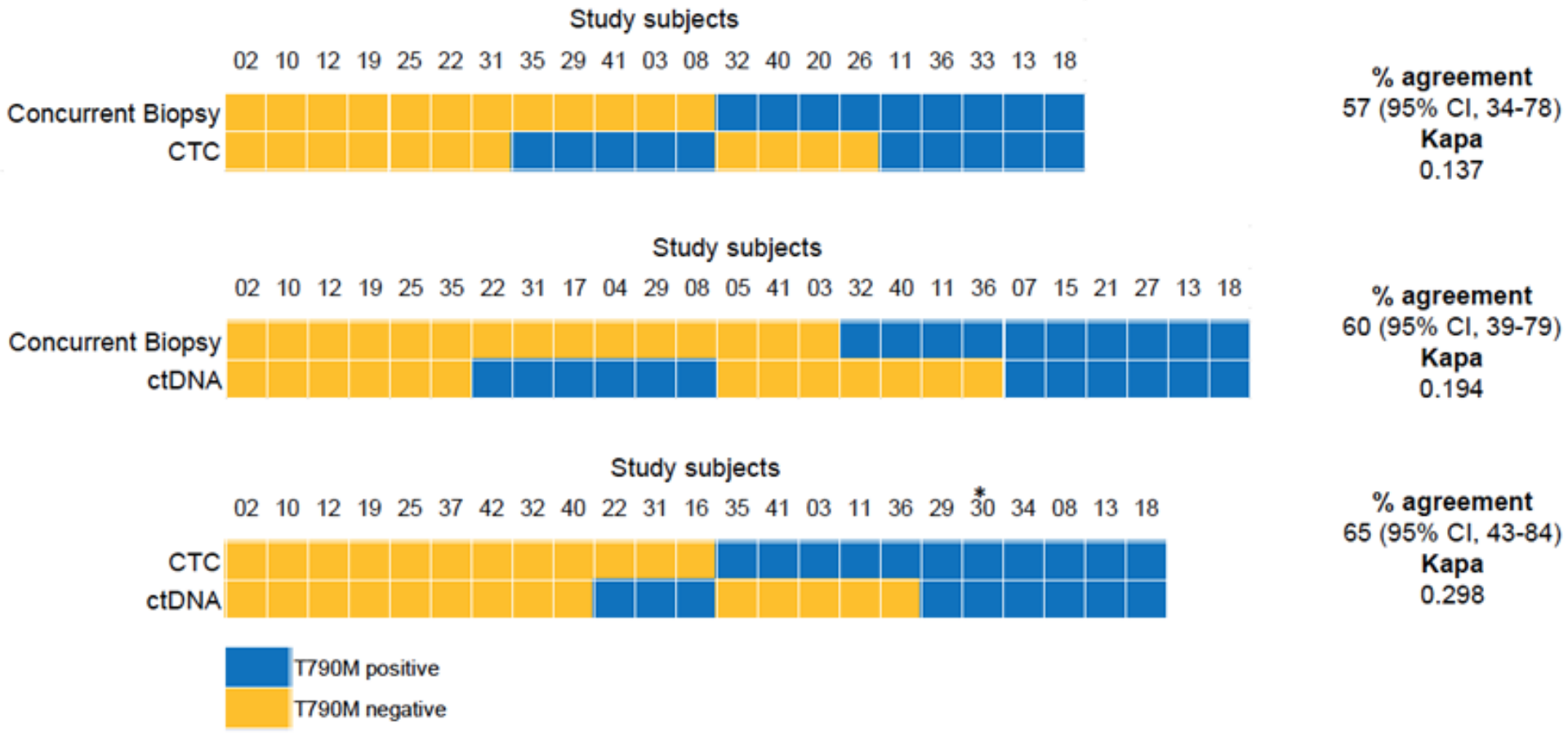

Epithelial
Ker+ Vim-
EMT
Ker+ Vim+
Stromal
Ker- Vim+
Lymphocyte
Ker- Vim-

Detection of T790M, the acquired resistance *EGFR* mutation, by tumor biopsy versus noninvasive blood-based analyses



Sundaresan et al. Clin Cancer Res 2015

Detection of T790M, the acquired resistance *EGFR* mutation, by tumor biopsy versus noninvasive blood-based analyses



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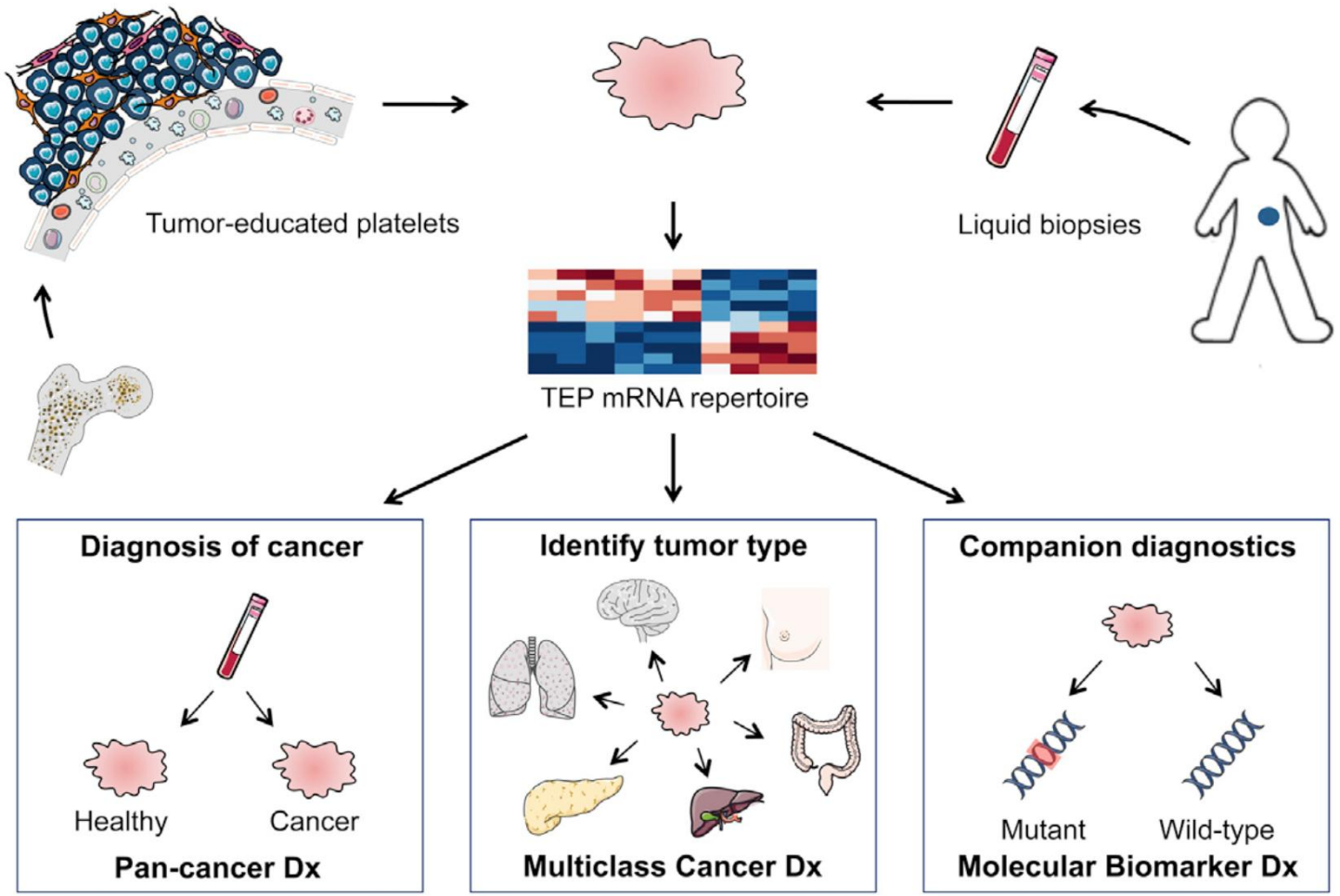
Article

Cancer Cell

RNA-Seq of Tumor-Educated Platelets Enables Blood-Based Pan-Cancer, Multiclass, and Molecular Pathway Cancer Diagnostics


Tumor-educated platelets
Liquid biopsies
TEP mRNA repertoire
Diagnosis of cancer
Healthy
Cancer
Pan-cancer Dx
Identify tumor type
Multiclass Cancer Dx
Companion diagnostics
Mutant
Wild-type
Molecular Biomarker Dx

Introduction in Routine Diagnostics

ACCE criteria

Analytical validity. degree of accuracy with which a test detects the presence or absence of a mutation

Clinical validity. Are the variants the test is intended to identify associated with disease?

Clinical utility. clinically useful, or how that risk might be managed

ELSI = ethical, legal, and social issues.