cfDNA in liquid biopsy

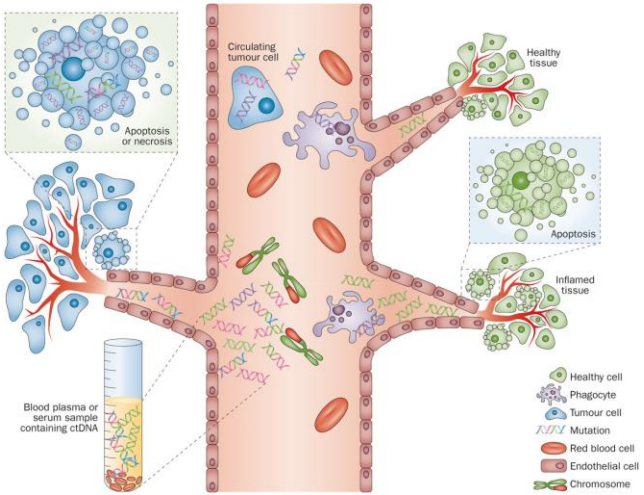
Dra. Ana Vivancos

What is liquid biopsy

- ***Circulating-free DNA (cfDNA)*** **is a naturally occuring DNA that is present in the bloodstream. We can isolate it from the plasmatic fraction of blood.**

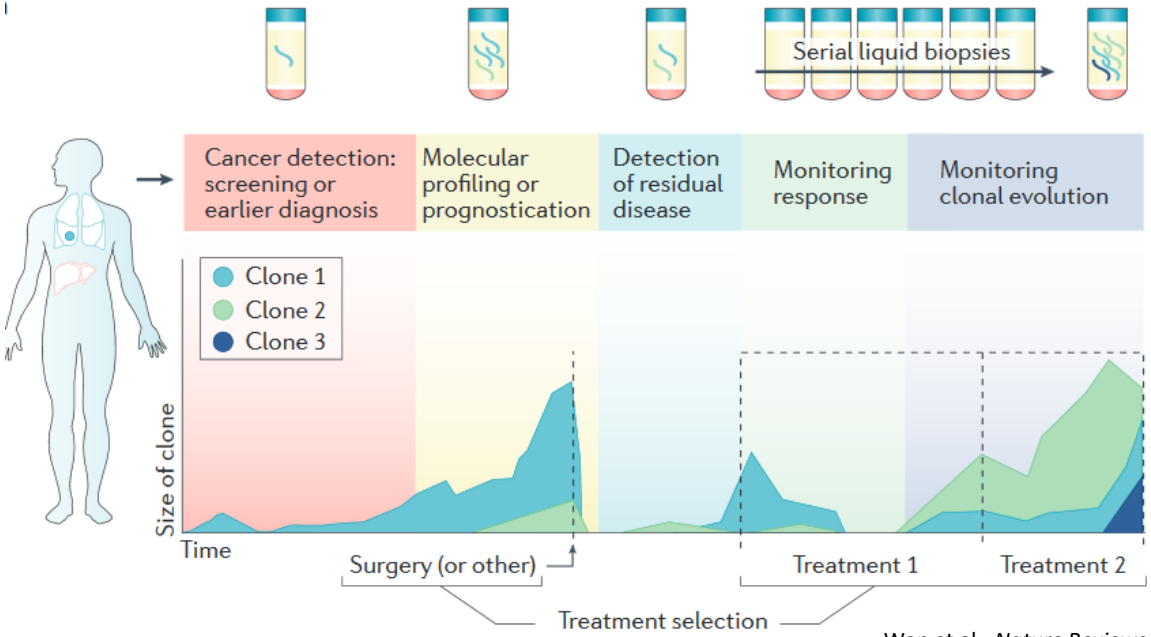
- ***Circulating-free tumor DNA (ctDNA)*** **is a part of cfDNA, cell-free DNA released from a solid tumor and, therefore, carries <u>mutations or other genomic alterations</u>.**

- ***Liquid biopsy*** **refers to the ability to detect mutations / alterations present in a patient's tumor from a blood sample.**



Crowley, E. *et al.* (2013) Liquid biopsy: monitoring cancer-genetics in the blood
Nat. Rev. Clin. Oncol. doi:10.1038/nrclinonc.2013.110

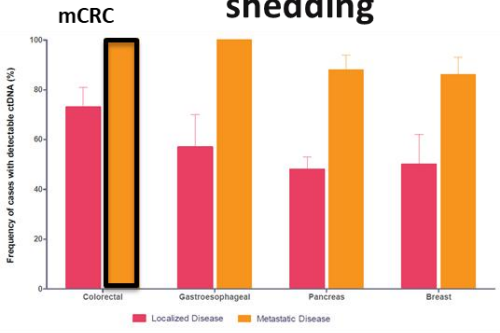
Just a blood draw, multiple applications



Wan et al.; *Nature Reviews Cancer*, 2017

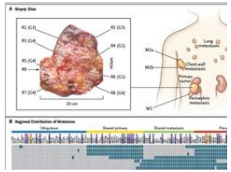
Critical aspects in tissue vs plasma

ctDNA shedding



Tumor load
Met locations
Necrosis, other biological processes

Intratumor heterogeneity

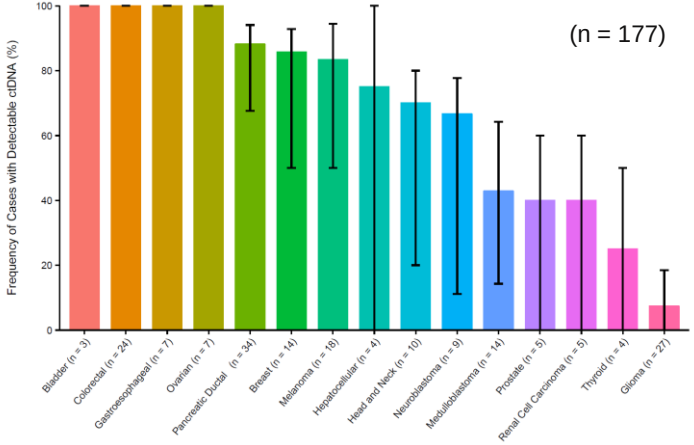


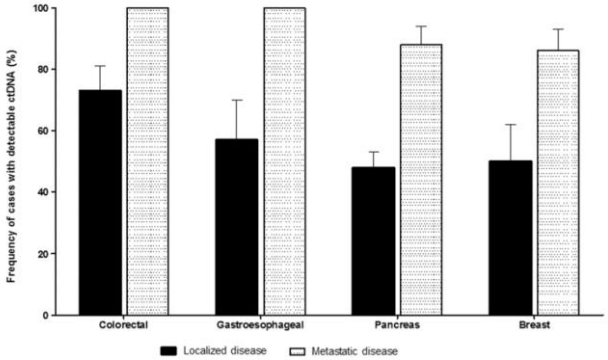
Clonality
mCRC: APC, KRAS vs BRAF, PIK3CA
Lung: EGFR indel 19 vs T790M vs C797S

Targeted therapies

Selection
mCRC: anti-EGFR, RAS clones
Lung: EGFR T790M

ctDNA prevalence: to shed or not to shed





Bettegowda et al Sci Transl Med 2014

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Intratumor heterogeneity

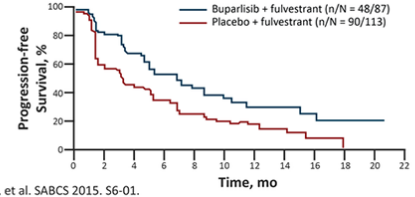
BELLE-2, ctDNA better predictor than tissue

BELLE-2

Buparlisib + Fulvestrant

PFS Improvement in Patients With ctDNA *PIK3CA* Mutations

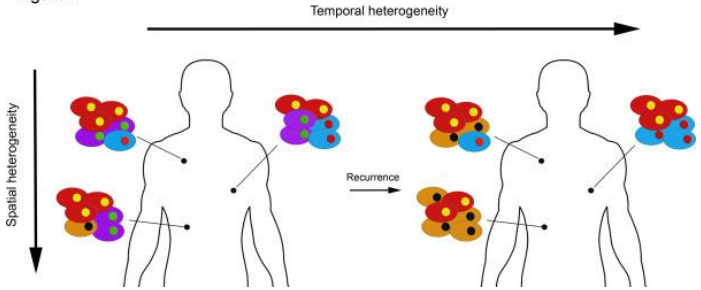
ctDNA *PIK3cA* Mutant (n = 200)	Buparlisib + Fulvestrant (n = 87)	Placebo + Fulvestrant (n = 113)
Median PFS, mo (95% CI)	7.0 (5.0-10.0)	3.2 (2.0-5.1)
HR (95% CI)	0.56 (0.39-0.80)	
One-sided nominal *P* value	< .001	



Baselga J, et al. SABCS 2015. S6-01.

Baselga et al., 2017 Lancet Oncology

Figure 1



It happens in both ways: gain and loss of mutations!!!

Discrepancy in PIK3CA mutation:

Primary tumor vs Metastases 18%

Archival tumor vs Plasma 15%

González-Angulo et al., Mol Can Ther.,2011

Higgins et al CCR 2012

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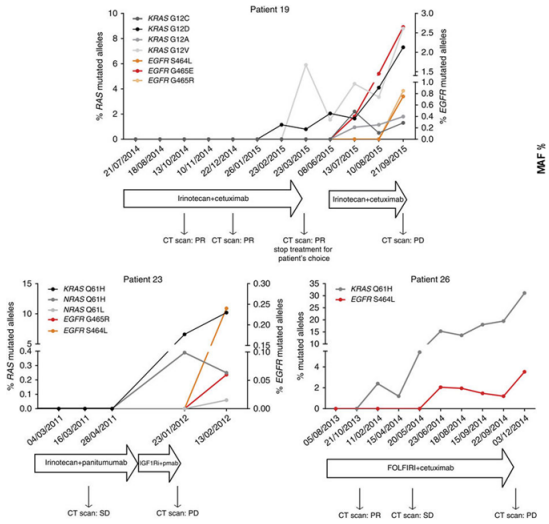
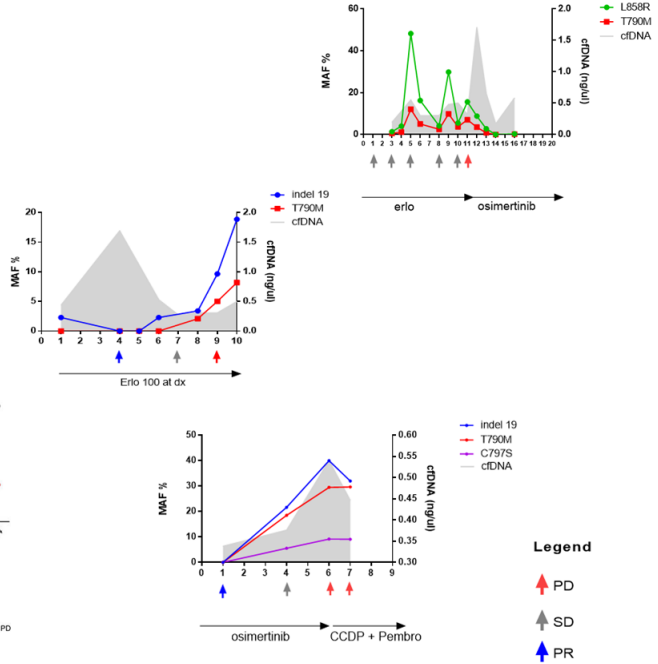
Targeted therapies

mCRC: anti-EGFR, RAS clones



Van Emburgh et al., 2017 Nature Communications

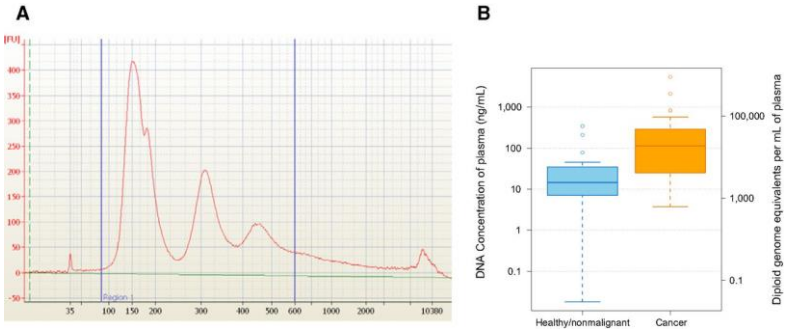
NSCLC: anti-EGFR, anti-EGFR T790M



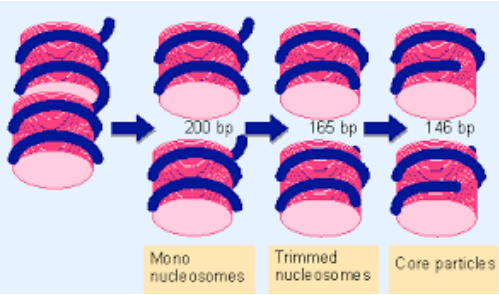
Vivancos Lab, unpublished results

Approaches used in daily practice are hotspot focused

Technical characteristics of cfDNA



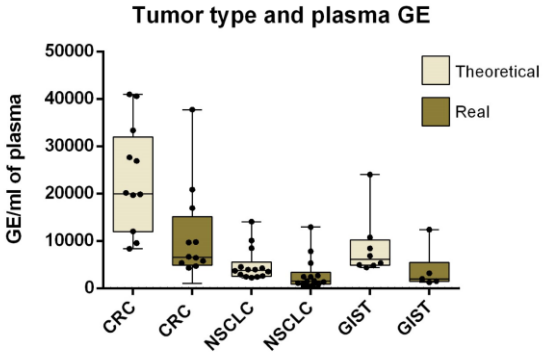
Stanislav Volik et al., MCR, 2016



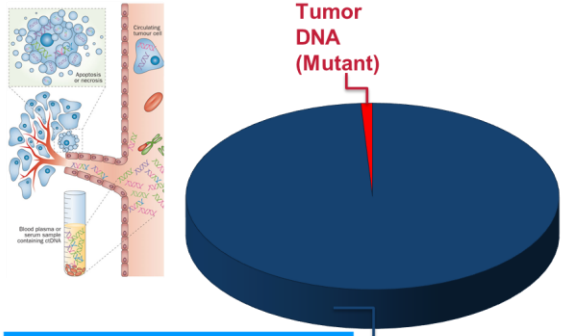
cfDNA vary in terms of yield from patient to patient

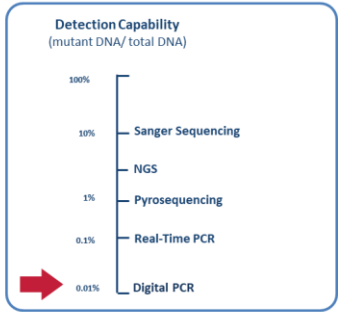
<u>1st line mCRC: up to 100-fold</u>

Vivancos Lab, unpublished data

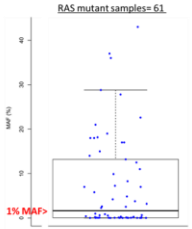


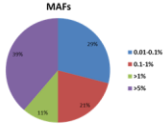
Mutant allele fractions are frequently low in liquid biopsy





MAFs in CONCORDANCE Study: required sensitivity?





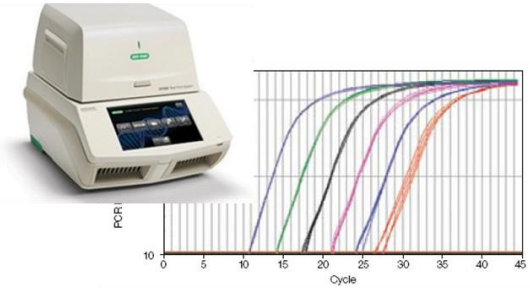
Techniques must show robustness in detection in the 0.01-1% MAFs

Concordance study, VHIO, n=150, retrospective, patients anti-EGFR naïve, testing plasma BEAMing + tissue SOC & BEAMing (RAS panel).

Median MAF	Tumor	
4%	mBC	Dawson et al., 2013
1.5%	mCRPC	Azad et al., 2015
4%	mCRC	Grasselli et al., 2017
6.6%	NSCLC	Fairclough et al., J Clin Oncol 34, 2016 (suppl; abstr e23021)

Technical considerations: Available technologies

Digital PCR
(~0.01% sensitivity)



Real-time PCR
(~0.1% sensitivity)

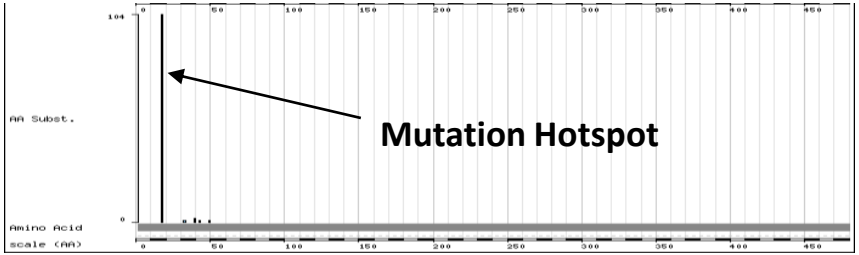
NGS
(~5% sensitivity)

Technical considerations: scope of the panels

Main limitation in the routine application to clinics is that only hotspots in oncogenes can be studied

Oncogenes
ERBB2

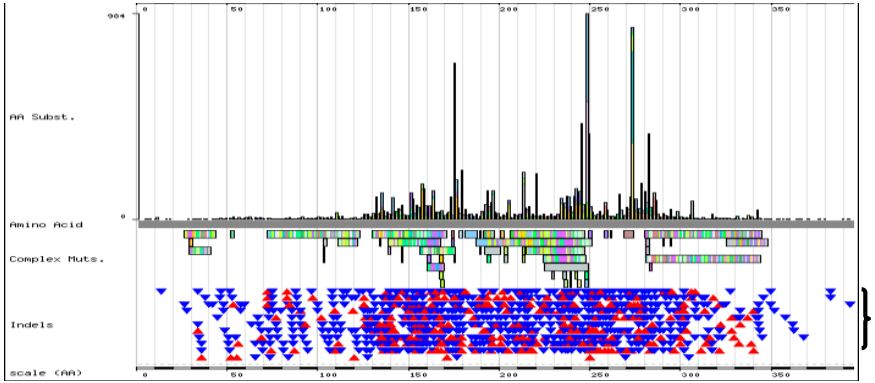
Others: ESR1, PIK3CA…



ddPCR
1 test = 1 mutation

Tumor supressors
TP53

Others: PTEN, NF1, VHL…



NGS
Multiple and unspected mutationns

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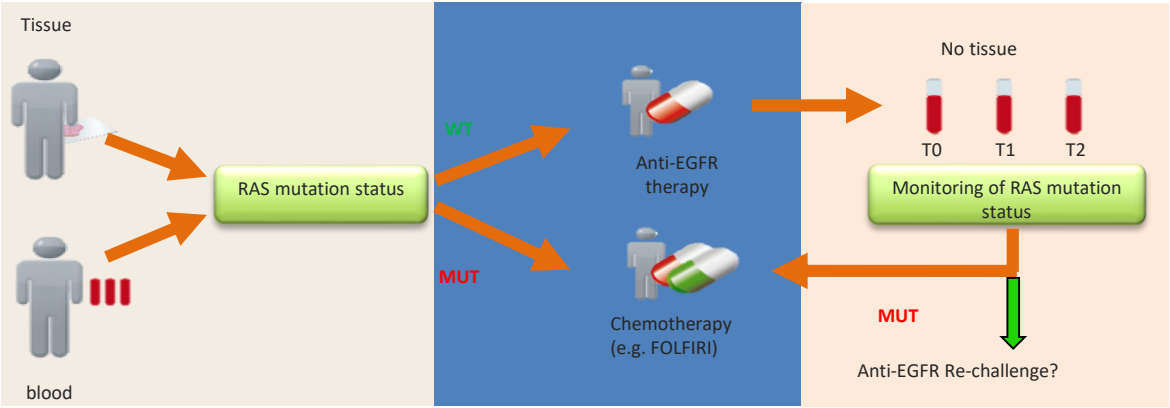
Approaches used in daily practice are currently focused

mCRC

GEN	EXON	MUTATION
KRAS	2	G12S/R/C/D/A/V
	2	G13D
	3	A59T
	3	Q61L/H/R
	4	K117N
	4	A146T/V
NRAS	2	G12S/R/C/D/A/V
	2	G13D/R/V
	3	A59T
	3	Q61K/L/R/H
	4	K117N
	4	A164T



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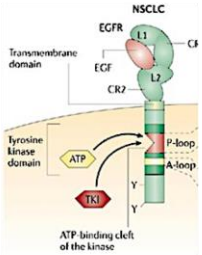
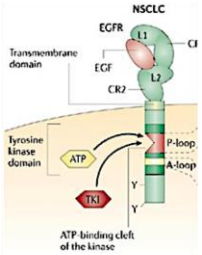
Approaches used in daily practice are currently focused

EGFRmut
NSCLC



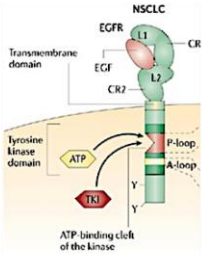
EGFR indel 19 / L858R

Inh. EGFR (Gefitinib)



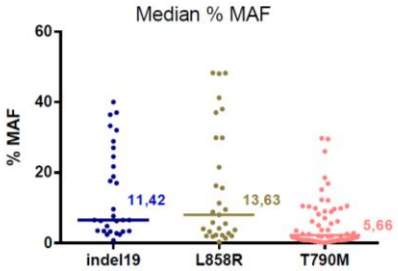
EGFR indel 19 / L858R + T790M

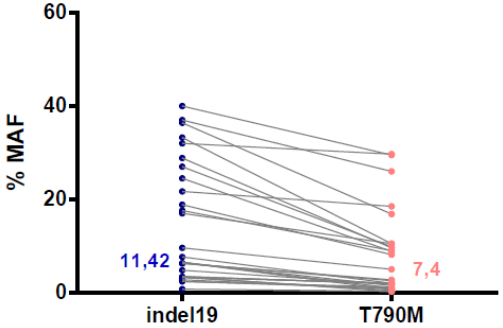
Inh. T790M (AZD 9291)

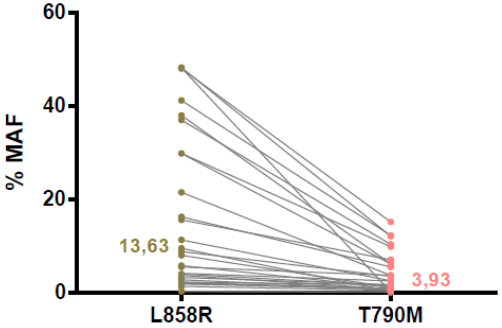


EGFR indel 19 / L858R + T790M + C797S

NSCLC: the importance of sensitivity…







E.Felip & A.Vivancos
Unpublished data

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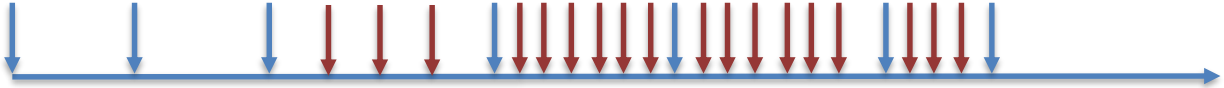
Future in monitoring, minimal residual disease, clonal heterogeneity will require NGS…

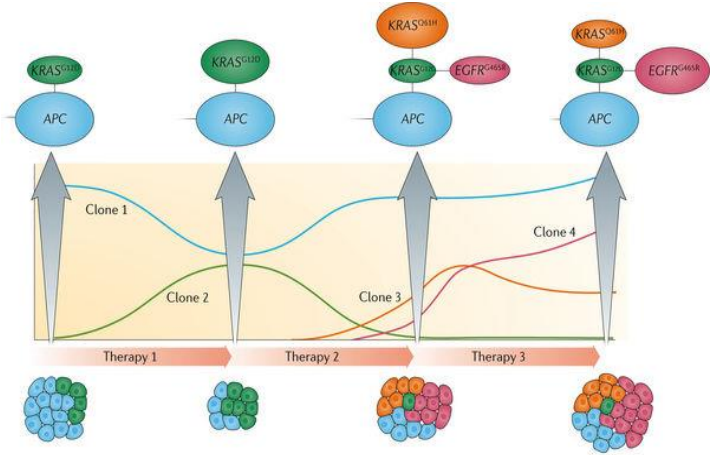
Minimal Residual Disease
Monitoring Tumor Burden

Biomarkers of sensitivity to treatments

Biomarkers of resistance to treatments



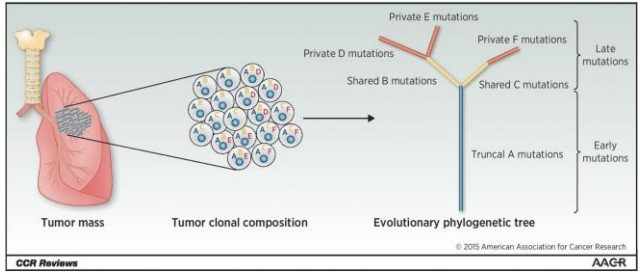
adjuvancy surgery Neo-adjuvancy Baseline Metastatic disease 2nd line 3rd line Etc…



Siravegna et al., Nat. Rev. Can., 2017

Nature Reviews | Clinical Oncology

Clonal mutations (trunk, root)



Mutated genes one can assume as 'clonal'	
CRC	APC, CTNNB1
RCC	VHL
NSCLC	EGFR
GIST	KIT, PDGFR
PDAC	KRAS
BC, GBM, NSCLC…	?

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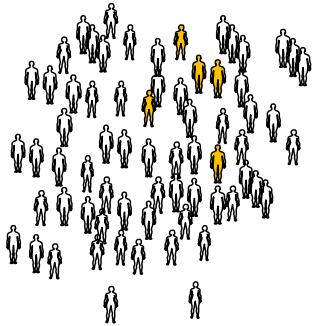
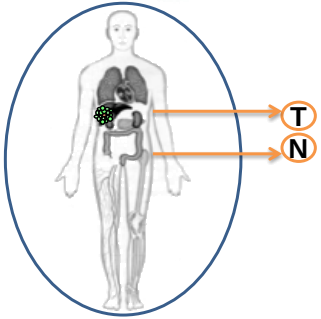
A comprehensive panel required: NGS based

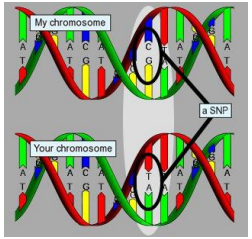
Minimal Residual Disease Monitoring Tumor Burden

Biomarkers of sensitivity to treatments

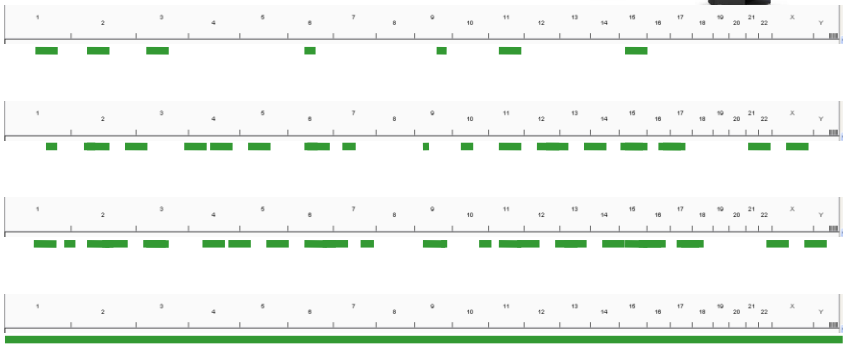
Biomarkers of resistance to treatments







- Amplicon-seq (up to 200 genes)
- Gene-panel capture approaches (up to 24000 genes)
- Exome-seq (24000 genes)
- Whole genome sequencing



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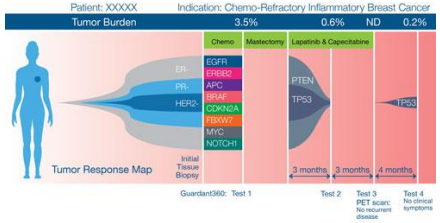
A comprehensive panel required

In order to improve sensitivity issues with NGS, high coverage and UMI-based chemistries are required

Guardant360



Avenio (Roche)

Performance metrics
AVENIO ctDNA Targeted Kit

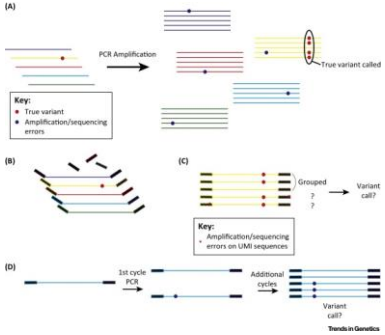
Mutation Classes	SNVs		Indels		Fusions		CNVs	
Mutant Allele Frequency/ Copy Number	0.5%*		1.0%		1.0%		2.3 copies**	
% Sensitivity and PPV	Sensitivity	PPV	Sensitivity	PPV	Sensitivity	PPV	Sensitivity	PPV
	>99%	>99%	>99%	>99%	>96%	>99%	>99%	>99%

Performance samples - cell line mixes, cfDNA 10ng-50ng input

Sensitivity and Positive Predictive Value (PPV) metrics based on typical product performance. Sensitivity and PPV performance reported per variant. SNV performance data based on hotspot calls; CNV performance based on MET variant. Results above were tested at the stated mutant allele frequencies. The AVENIO ctDNA Targeted Kit also achieves >99.99% per base specificity across the panel. Performance data based on 40 million reads per sample. Sequencing performed on an Illumina NextSeq 500.

* Detects variants down to 0.1%
** 10% ctDNA at 5 copies in tumor

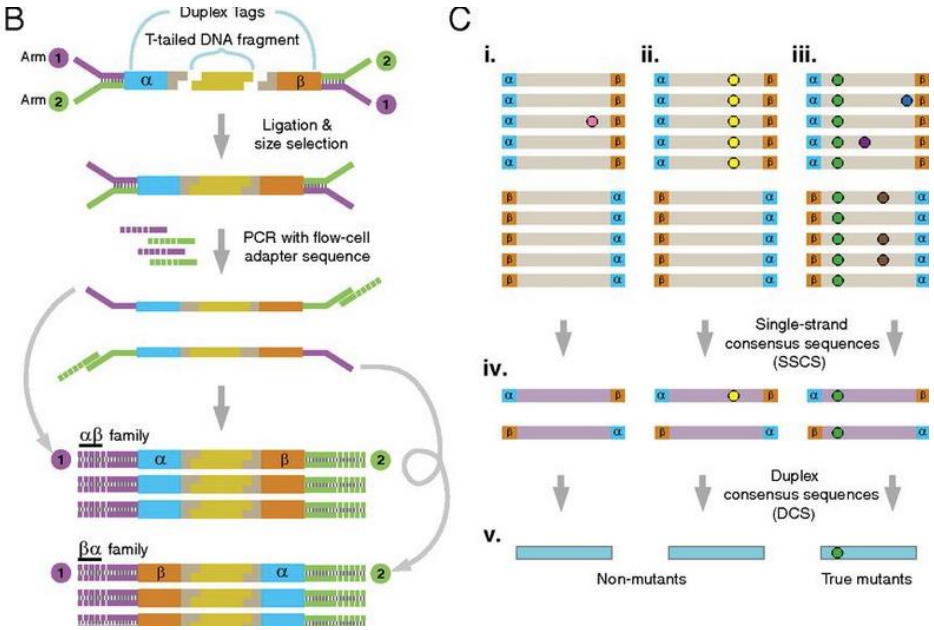
SafeSeq (Sysmex)



Foundation Act (Roche)

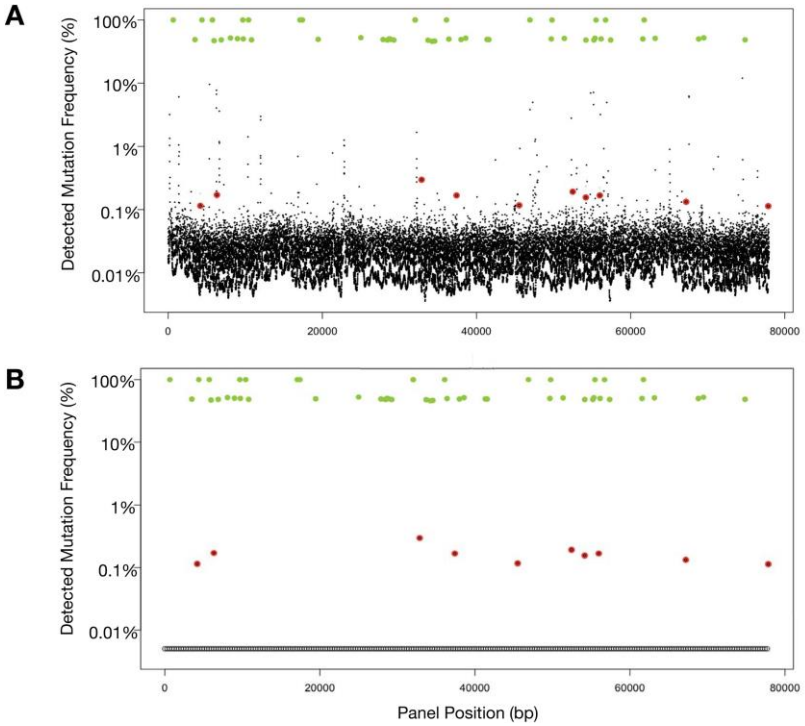
FOUNDATIONONE®LIQUID

Low MAF: UNIQUE MOLECULAR IDENTIFIERS + DUPLEX SEQUENCING



Sensitivity down to 0.5%

Bioinformatics for noise-reduction



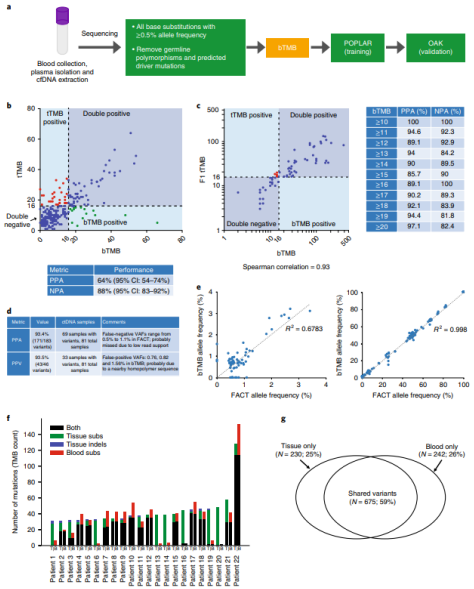
SNPs

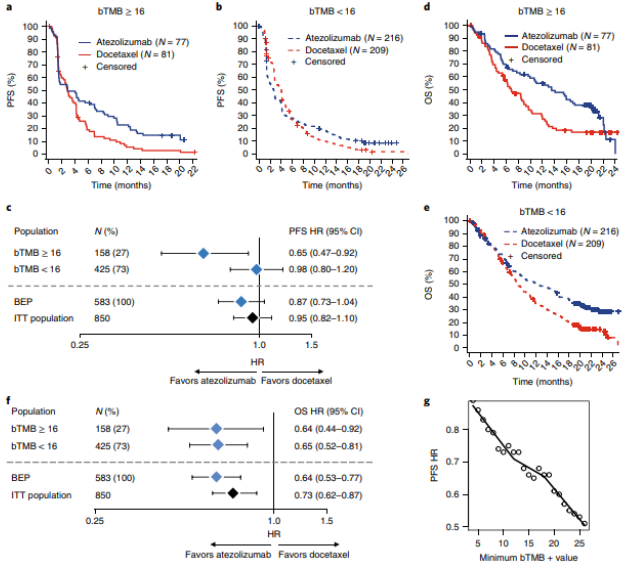
spiked-in cell lines at 0,1-1%

Sensitivity down to 0.02%

Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA. Lanman et al., PLOSone, 2015

TMB in liquid biopsy?



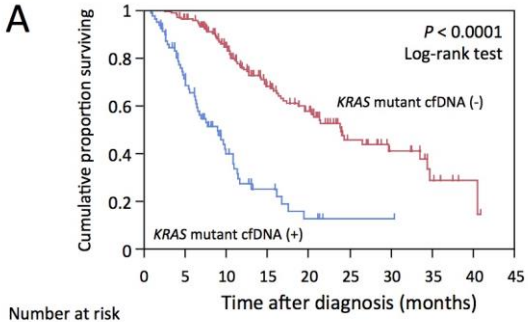


Gandara et al., *Nature Medicine 24, 1441–1448 (2018)*

Beyond classical applications of liquid biopsy…

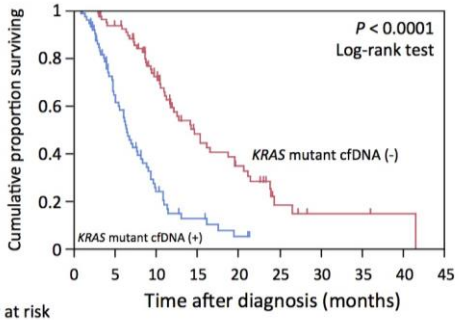
Besides mutations, are there additional layers of data with clinical interest in liquid biopsy?

Presence / absence of ctDNA shedding in pancreatic cancer as prognostic factor in OS



Number at risk									
KRAS mutant cfDNA (-)	176	150	114	73	52	25	15	7	3
KRAS mutant cfDNA (+)	83	50	21	10	5	2	2		

Stages I-IV



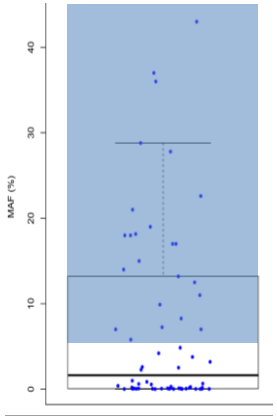
Number at risk									
KRAS mutant cfDNA (-)	86	71	49	26	18	6	3	3	2
KRAS mutant cfDNA (+)	76	42	15	7	3				

Stage IV

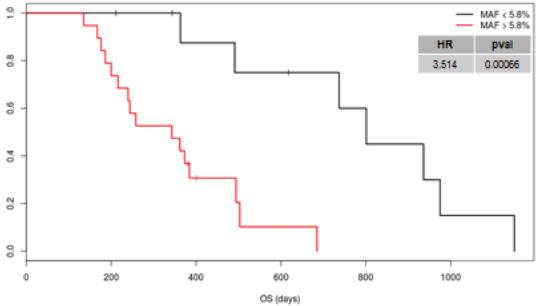
Takai et al., *Scientific Reports* **5**, Article number: 18425 (2015)

Besides mutations, are there additional layers of data with clinical interest in liquid biopsy?

Presence of high or low MAFs in KRASmut mCRC is prognostic factor in PFS and OS

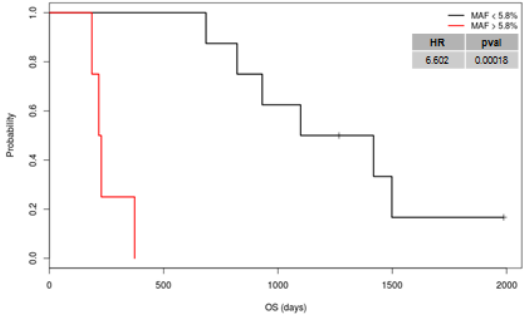


1st line



	n	events	median
MAF$ KRA$ < 5.8%	10	7	801
MAF$ KRA$ > 5.8%	19	16	343

2nd line



	n	events	median
MAF$ KRA$ < 5.8%	8	6	1258
MAF$ KRA$ > 5.8%	4	4	222

Élez et al., Unpublished data

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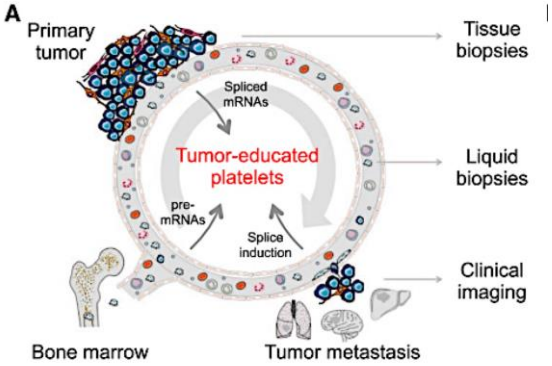
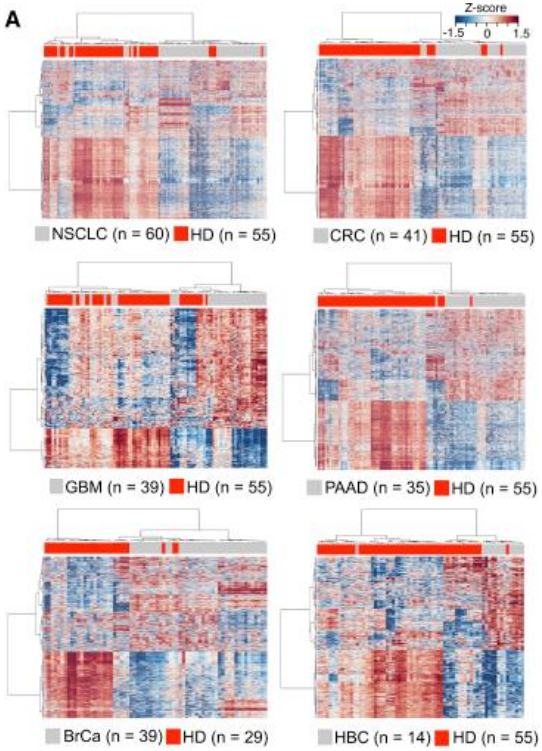
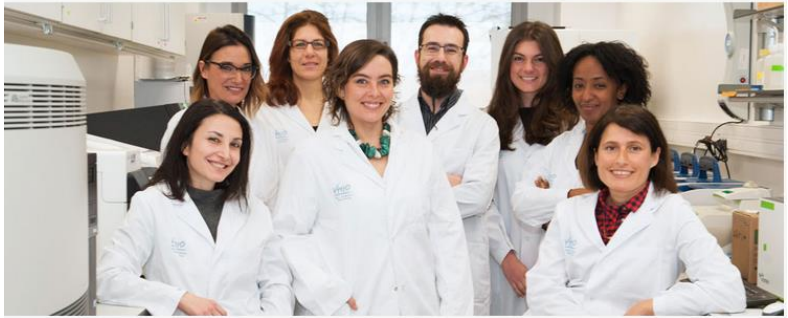
‘Other’ liquid biopsies





Best et al., Cancer Cell, 2015



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