



# **IX SIMPOSIO · SYMPOSIUM | 2024**

## **BIOPSIA LÍQUIDA · LIQUID BIOPSY**

EL CAMINO A LA ONCOLOGÍA DE PRECISIÓN · THE WAY TO PRECISION MEDICINE

25, 26 Y 27 DE ENERO · JANUARY 25<sup>th</sup>, 26<sup>th</sup> and 27<sup>th</sup>

### **Deciphering Tumor Evolution using longitudinal ctDNA.**

**ACT-Discover: identifying karyotype heterogeneity in cancer evolution using ctDNA**

Rodrigo Toledo

Biomarkers and Clonal Dynamics Group

Vall d'Hebron Institute of Oncology (VHIO)

Barcelona

#SimposioBiopsiaLiquida

[www.simposiobiopsialiquida.com](http://www.simposiobiopsialiquida.com)

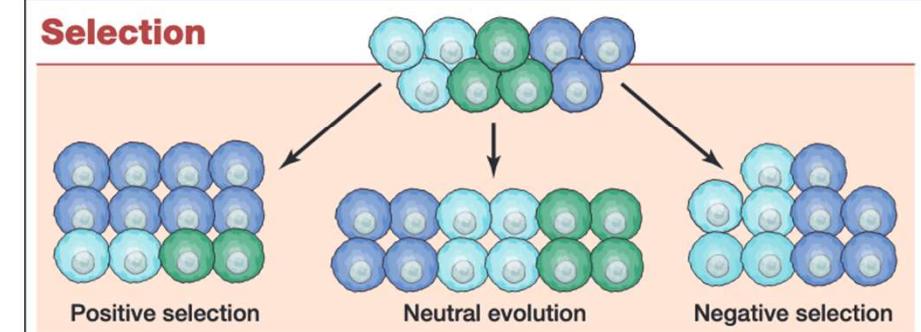
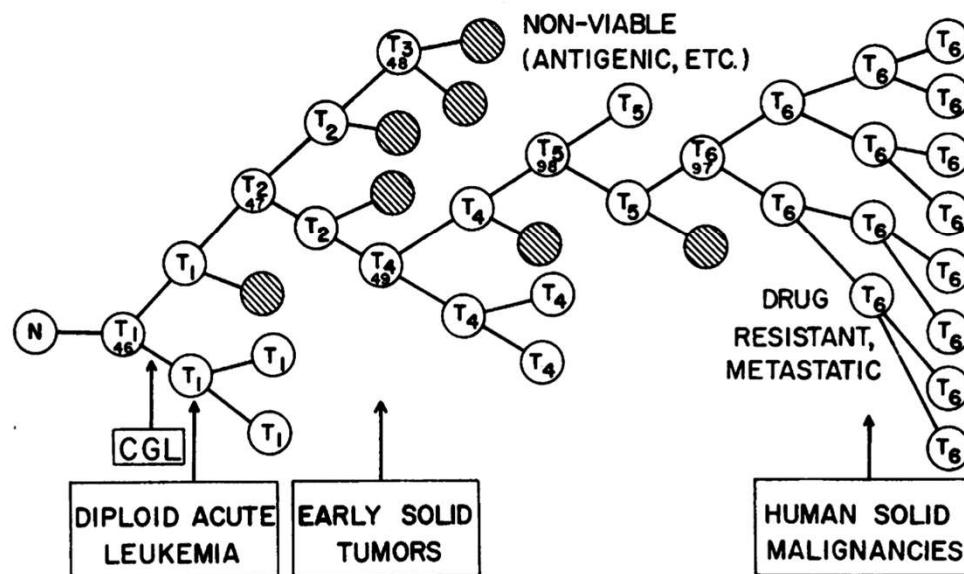
**Organizado por:**  
*Organized by:*



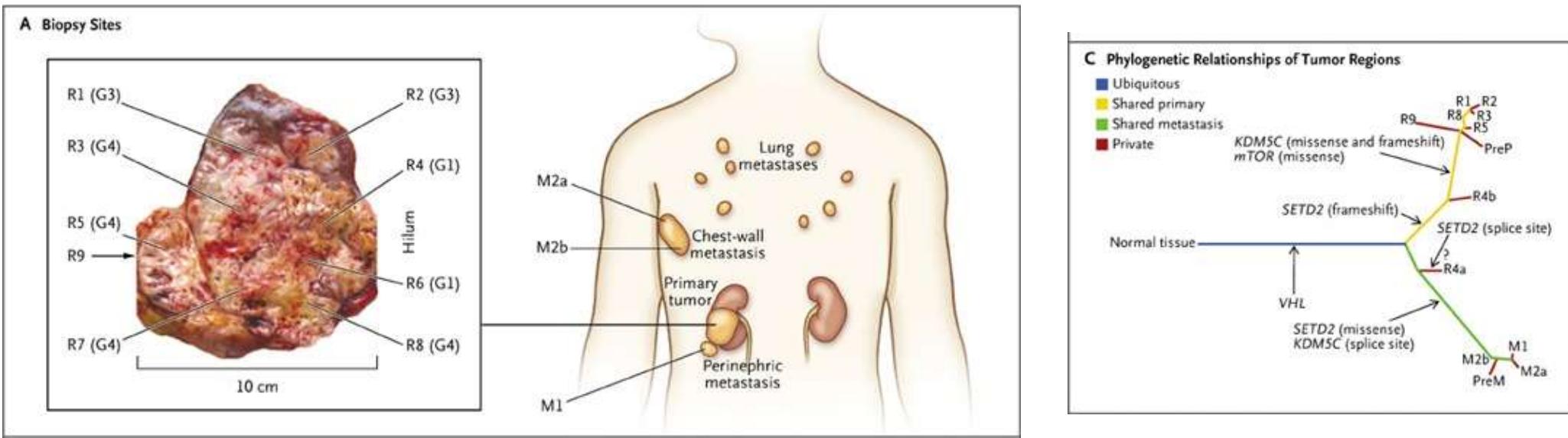
# The Clonal Evolution of Tumor Cell Populations

Acquired genetic lability permits stepwise selection of variant sublines and underlies tumor progression.

Peter C. Nowell  
Science, 1976

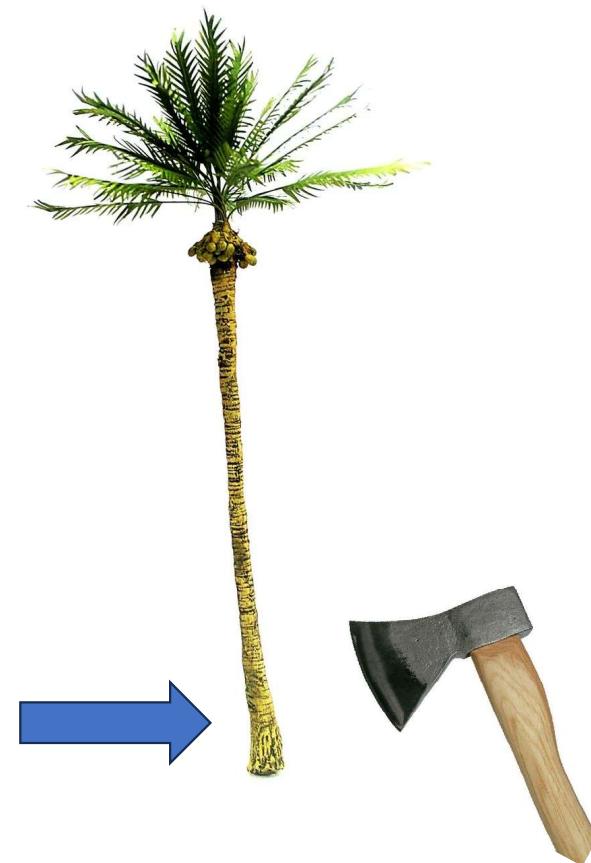
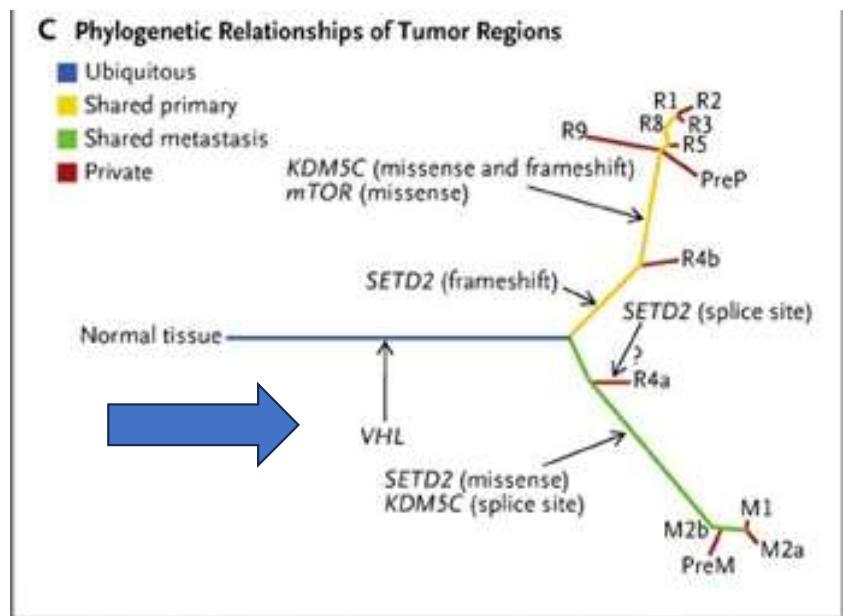


# Multi-region tumor whole-exome sequencing



Gerlinger et al, NEJM 2012

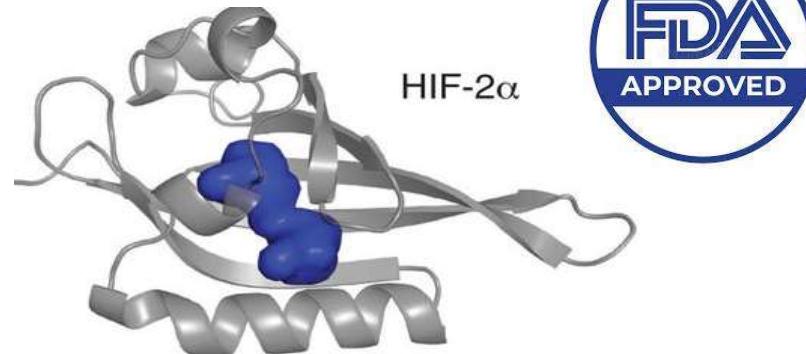
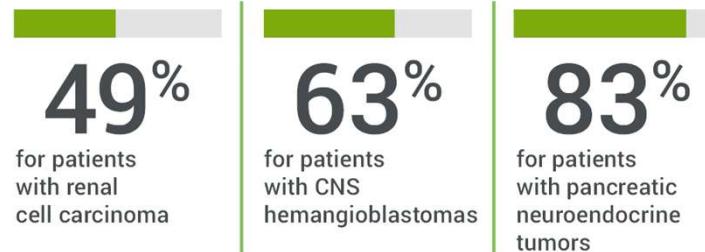
# Evolutionary history of tumors unveils therapeutic susceptibility



Gerlinger et al, NEJM 2012

# Evolutionary history of tumors unveils therapeutic susceptibility

## ORRs with belzutifan for von Hippel-Lindau disease-associated cancers

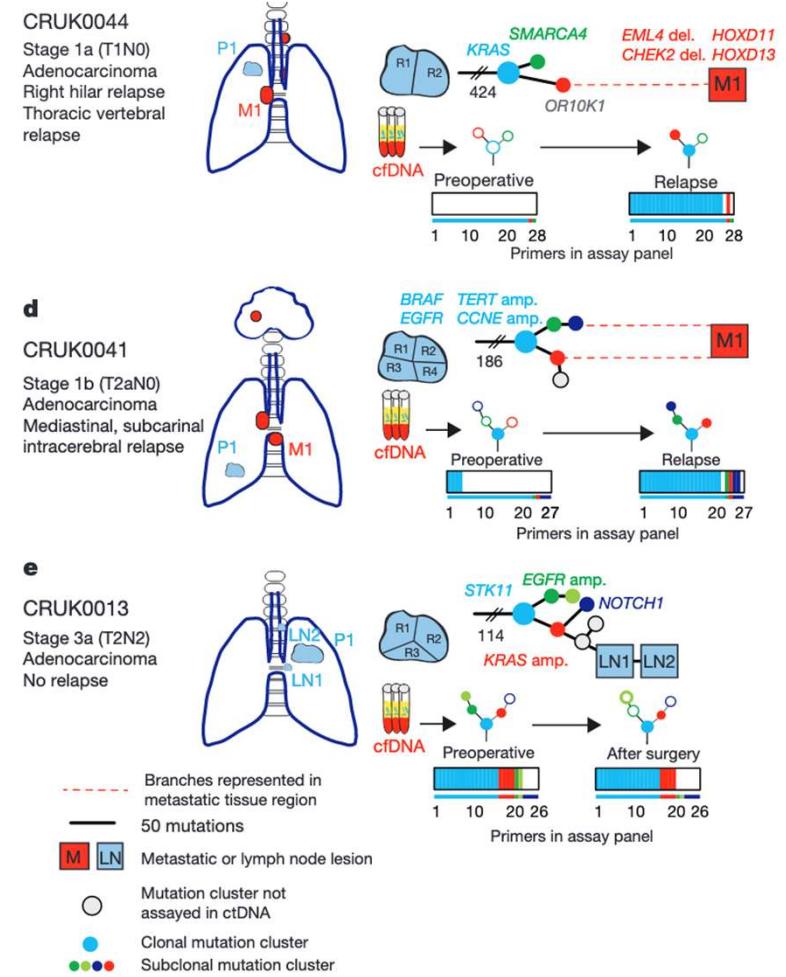
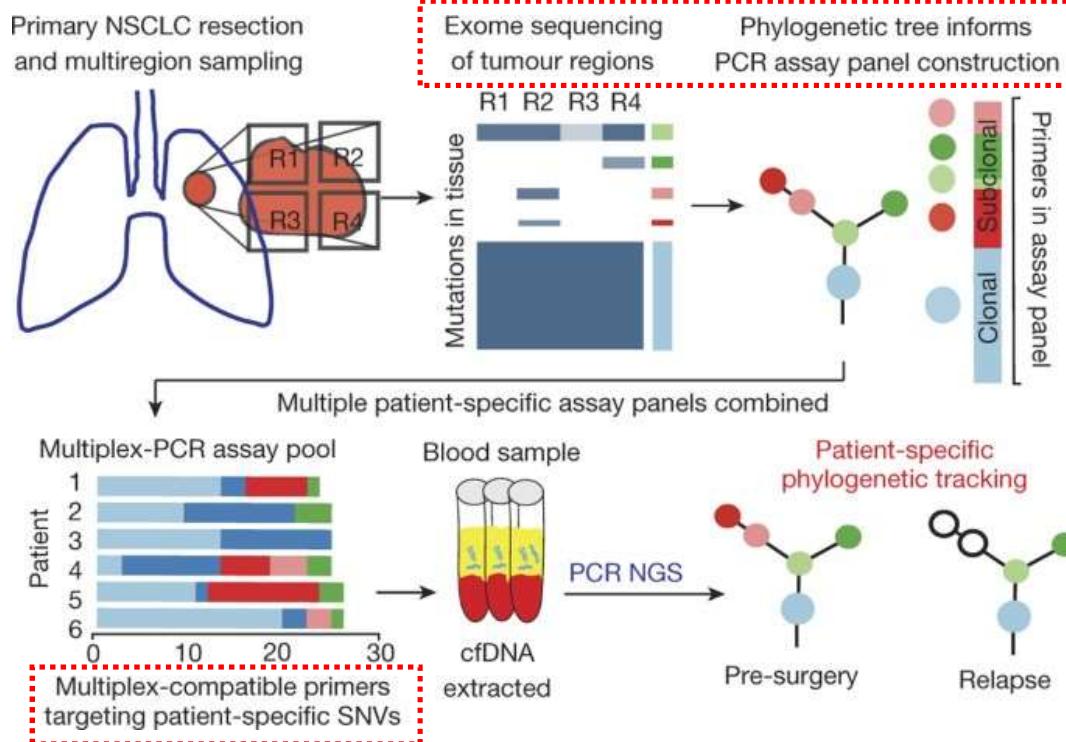


Gerlinger et al, NEJM 2012

# ARTICLE

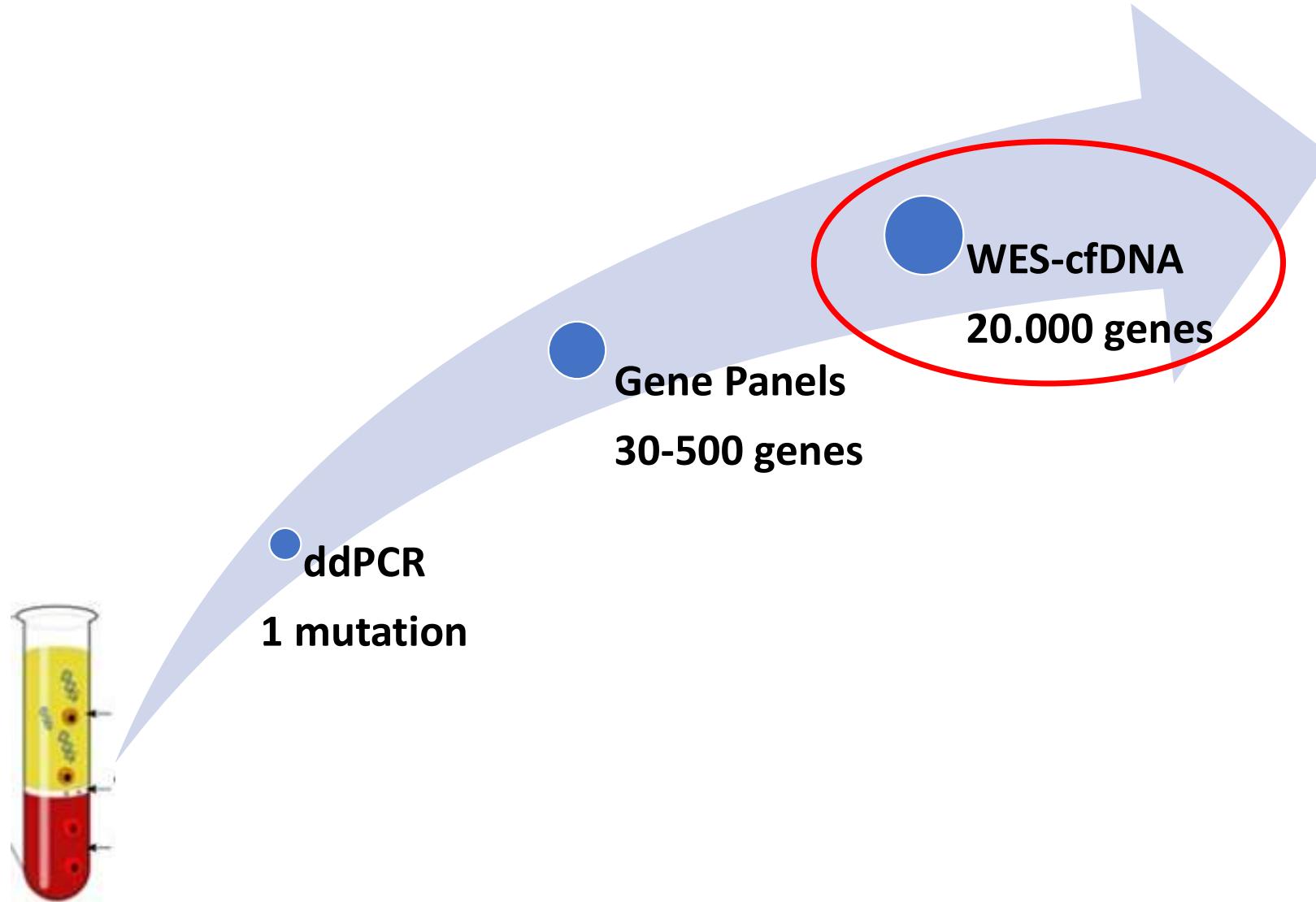
doi:10.1038/nature22364

## Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution



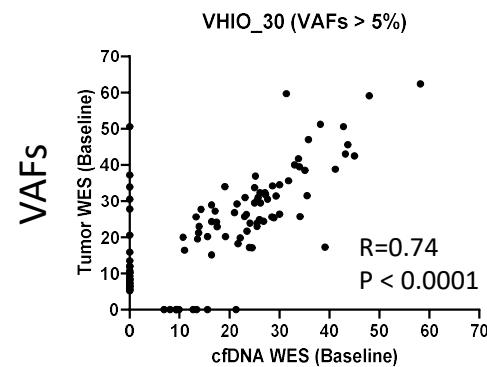
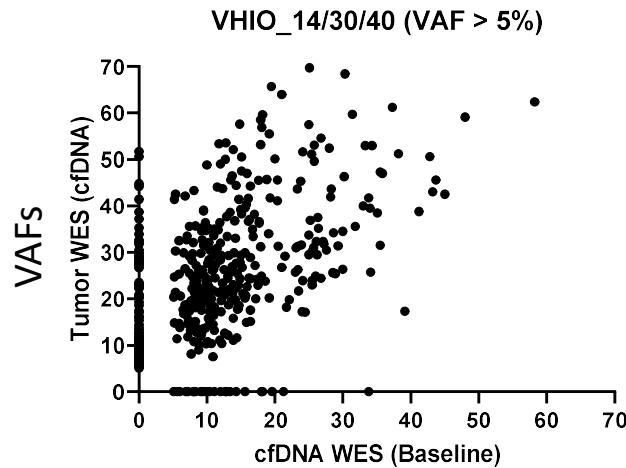
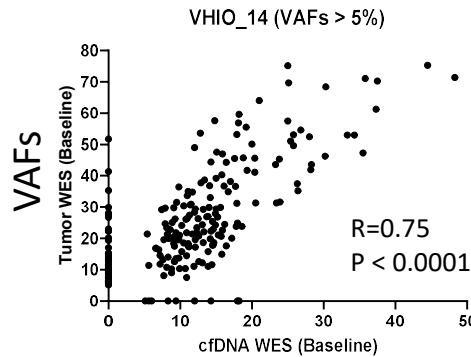
Abbosh C et al, Nature 2017

# Can whole-exome sequencing be applied to ctDNA ?

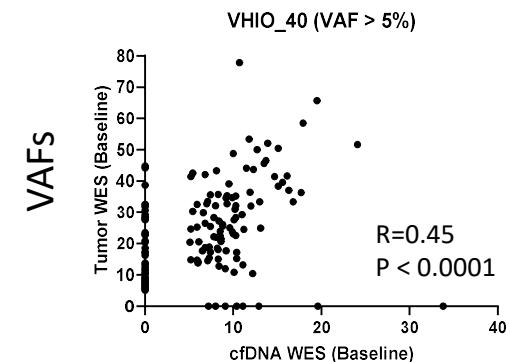


# Plasma WES-cfDNA portrays a comparable genetic profile to tumor-WES at baseline

metastatic CRC<sup>BRAF</sup>



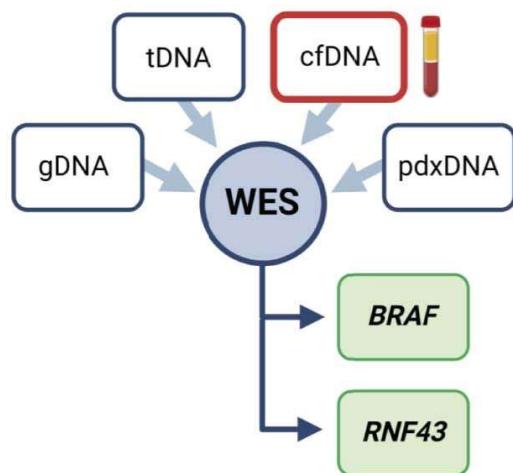
cfDNA-Tumor Concordance:  
63,8% (315/494)



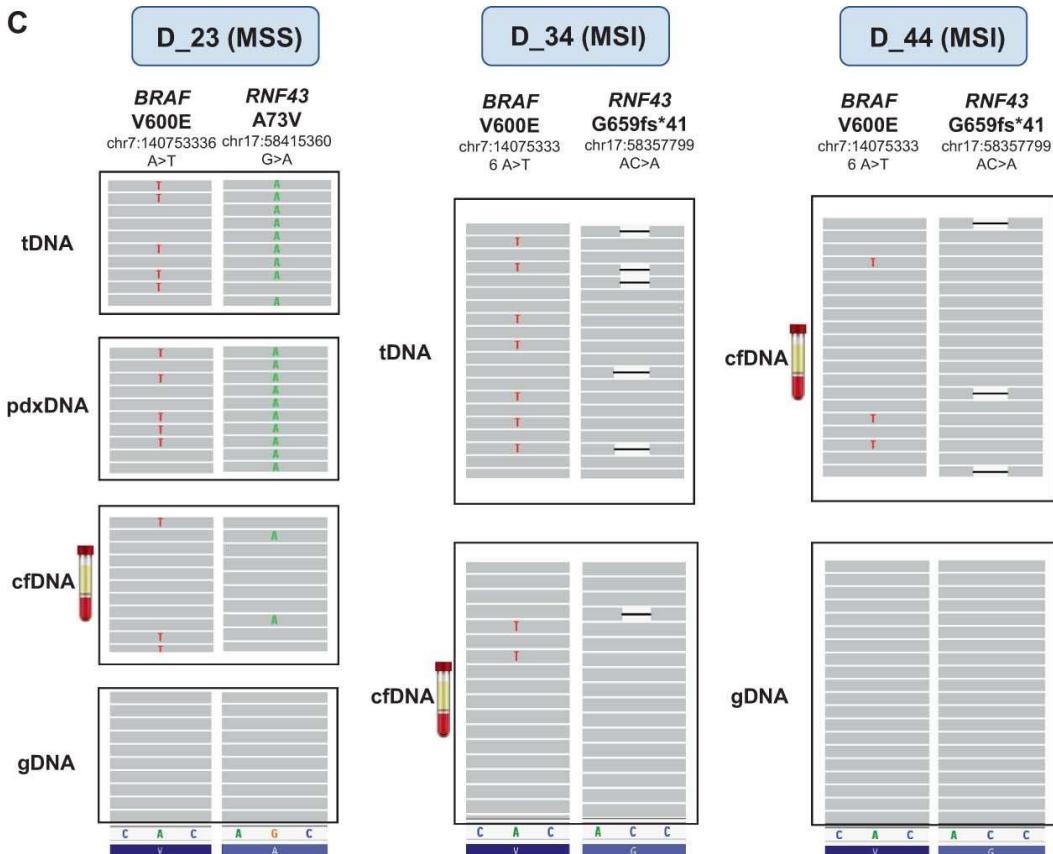
Unpublished, do no post

# Whole-exome sequencing of cell-free DNA to predictive biomarker analysis

**A**



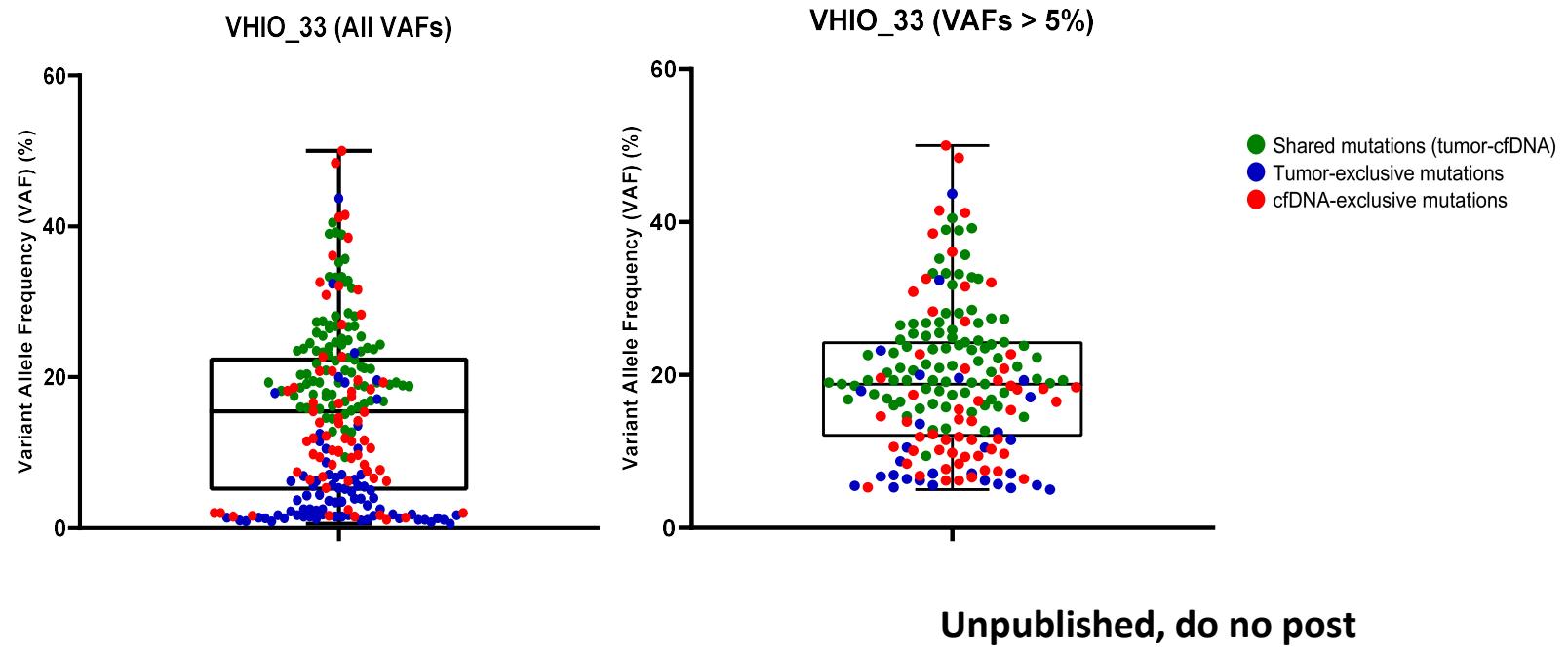
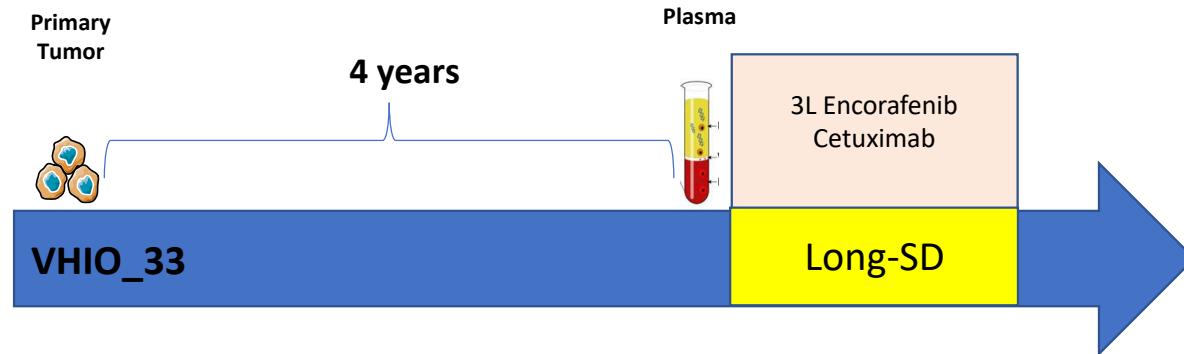
**C**



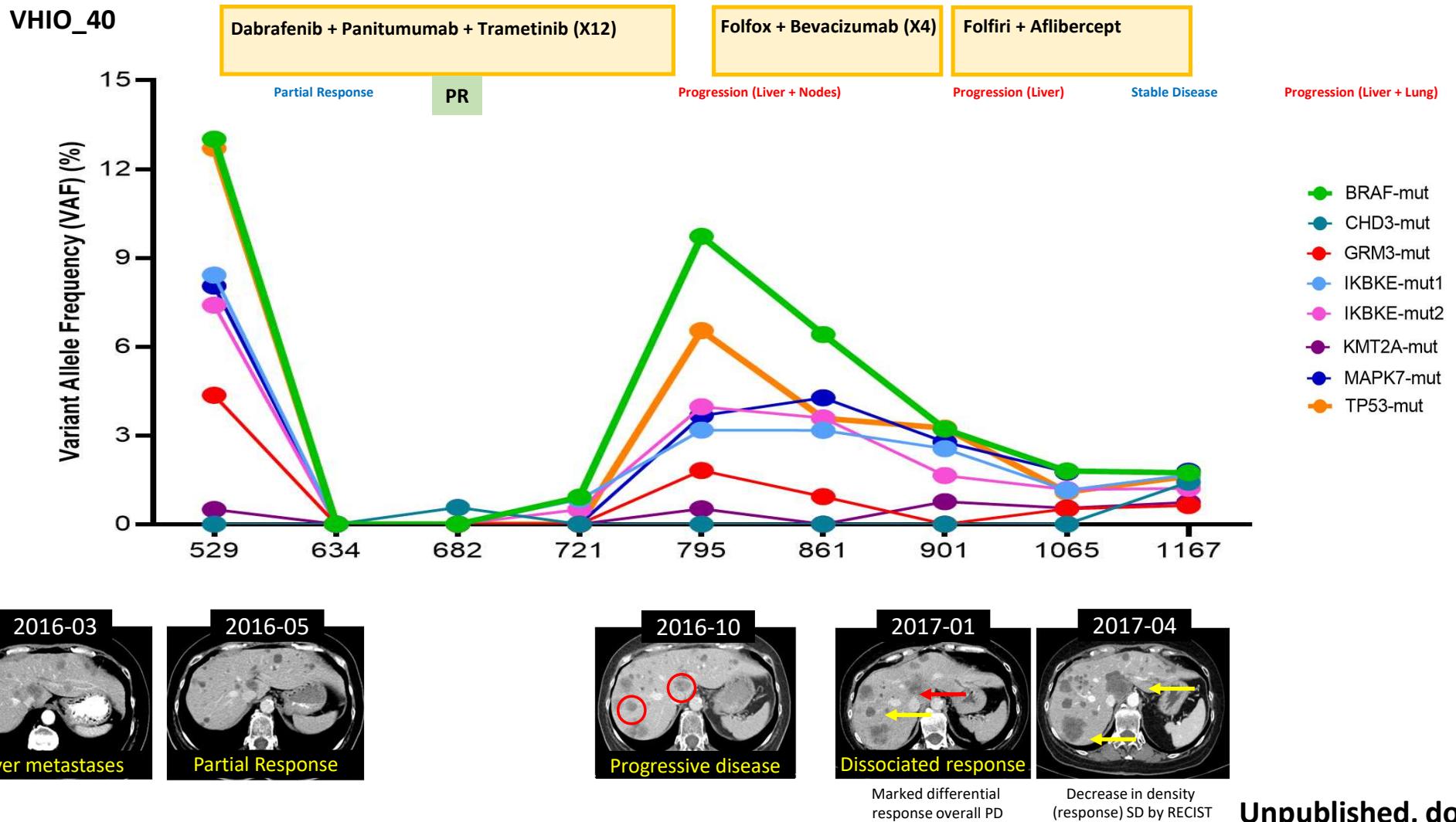
**B**

Patient ID	Mutations	Mutation Allele Frequency (MAF) (%)			
		tDNA	pdxDNA	cfDNA	gDNA
D_23	BRAF V600E	33/79 (41.7%)	48/86 (55.8%)	15/120 (12.5%)	0/112 (0.0%)
	RNF43 A73V	215/281 (76.5%)	149/149 (100%)	15/147 (10.2%)	0/420 (0.0%)
D_34	BRAF V600E	47/253 (18.6%)	NA	15/171 (8.8%)	NA
	RNF43 G659fs*41	20/73 (27.4%)	NA	8/185 (4.3%)	NA
D_44	BRAF V600E	NA	NA	10/95 (10.5%)	0/110 (0.0%)
	RNF43 G659fs*41	NA	NA	18/79 (22.8%)	0/177 (0.0%)

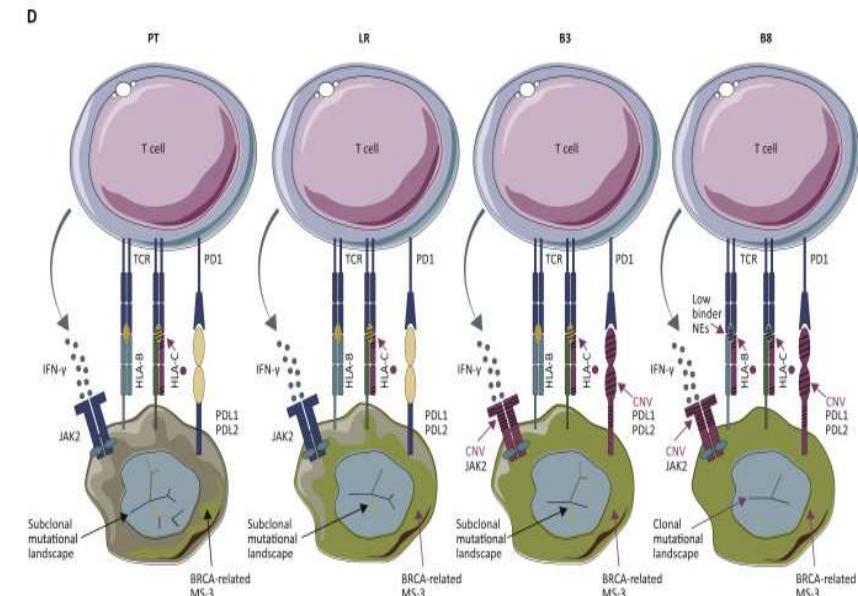
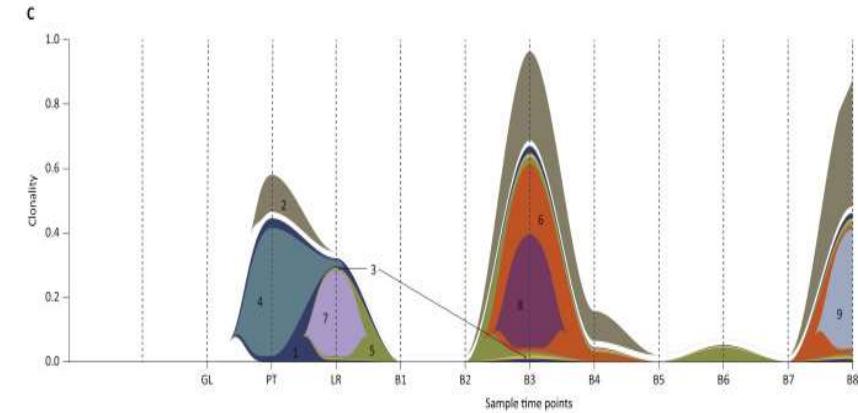
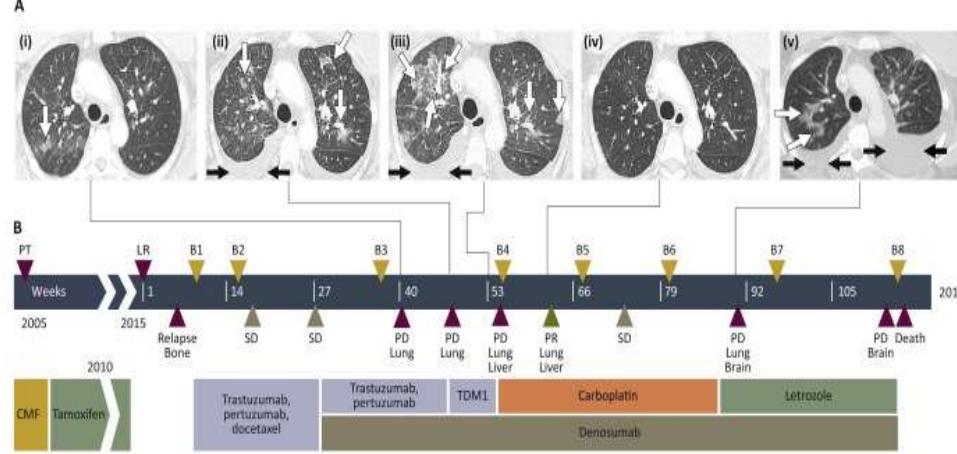
## Signs of tumor evolution in baseline plasma cfDNA and diagnostic sample (4 yrs gap)



## Evidence of different clonal profiles in cases with dissociated response to BRAFi



# Longitudinal whole-exome sequencing of cell-free DNA for tracking the co-evolutionary tumor and immune evasion dynamics

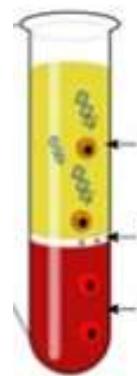


Hastins et al, Annals of Oncology 2021  
Collaboration with Jackie Shaw (UK)

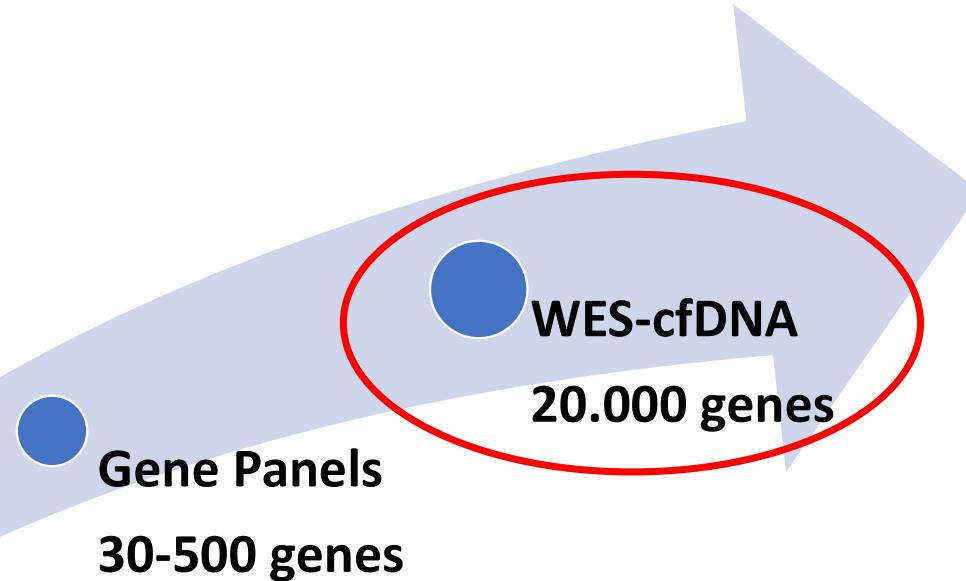
# Can whole-exome sequencing be applied to ctDNA ?

Yes ! (high shedders)

- Mutation profile
- Clonal dynamics
- **Copy Number profile**



ddPCR  
1 mutation





CANCER  
RESEARCH  
UK

CITY OF  
LONDON  
CENTRE

UCL • King's • Barts • Crick



Nicholas  
McGranahan



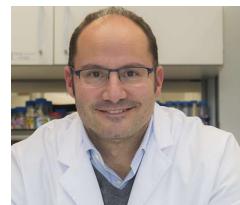
Ariana  
Huebner



James R M  
Black



Manuel Hidalgo



Rodrigo Toledo

Weill Cornell  
Medicine

VHIO  
VALL D'HEBRON  
Institute  
of Oncology

## RESEARCH

Open Access



# ACT-Discover: identifying karyotype heterogeneity in pancreatic cancer evolution using ctDNA

Ariana Huebner<sup>1,2,3†</sup>, James R. M. Black<sup>1,2†</sup>, Francesca Sarno<sup>4</sup>, Roberto Pazo<sup>5</sup>, Ignacio Juez<sup>6</sup>, Laura Medina<sup>7</sup>, Rocio Garcia-Carbonero<sup>8</sup>, Carmen Guillén<sup>9</sup>, Jaime Feliú<sup>10</sup>, Carolina Alonso<sup>4</sup>, Carlota Arenillas<sup>11,12</sup>, Ana Belén Moreno-Cárdenas<sup>11</sup>, Helena Verdaguera<sup>11,13</sup>, Teresa Macarulla<sup>11,13</sup>, Manuel Hidalgo<sup>14\*</sup>, Nicholas McGranahan<sup>1,2\*†</sup> and Rodrigo A. Toledo<sup>11,12\*†</sup>

## Abstract

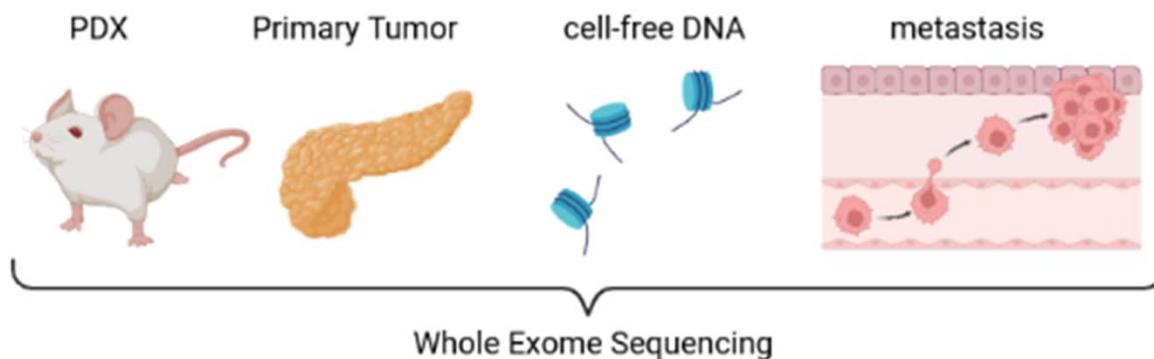
**Background** Liquid biopsies and the dynamic tracking of somatic mutations within circulating tumour DNA (ctDNA) can provide insight into the dynamics of cancer evolution and the intra-tumour heterogeneity that fuels treatment resistance. However, identifying and tracking dynamic changes in somatic copy number alterations (SCNAs), which have been associated with poor outcome and metastasis, using ctDNA is challenging. Pancreatic adenocarcinoma is a disease which has been considered to harbour early punctuated events in its evolution, leading to an early fitness peak, with minimal further subclonal evolution.

**Methods** To interrogate the role of SCNAs in pancreatic adenocarcinoma cancer evolution, we applied whole-exome sequencing of 55 longitudinal cell-free DNA (cfDNA) samples taken from 24 patients (including 8 from whom a patient-derived xenograft (PDX) was derived) with metastatic disease prospectively recruited into a clinical trial. We developed a method, Aneuploidy in Circulating Tumour DNA (ACT-Discover), that leverages haplotype phasing of paired tumour biopsies or PDXs to identify SCNAs in cfDNA with greater sensitivity.

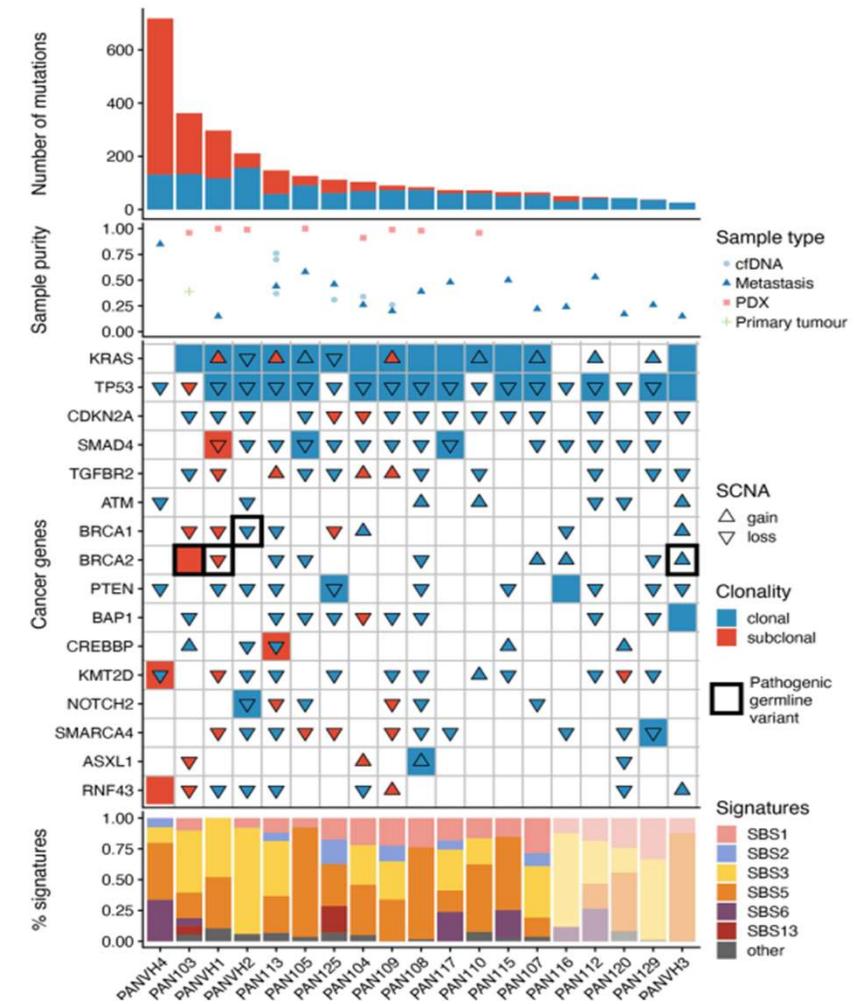
**Results** SCNAs were observed within 28 of 47 evaluable cfDNA samples. Of these events, 30% could only be identified by harnessing the haplotype-aware approach leveraged in ACT-Discover. The exceptional purity of PDX tumours enabled near-complete phasing of genomic regions in allelic imbalance, highlighting an important auxiliary function of PDXs. Finally, although the classical model of pancreatic cancer evolution emphasises the importance of early, homogenous somatic events as a key requirement for cancer development, ACT-Discover identified substantial heterogeneity of SCNAs, including parallel focal and arm-level events, affecting different parental alleles within individual

Hueber A et al , Genome Medicine 2023

# Genomic profile characterization of Pancreatic Cancer using multi-origin samples

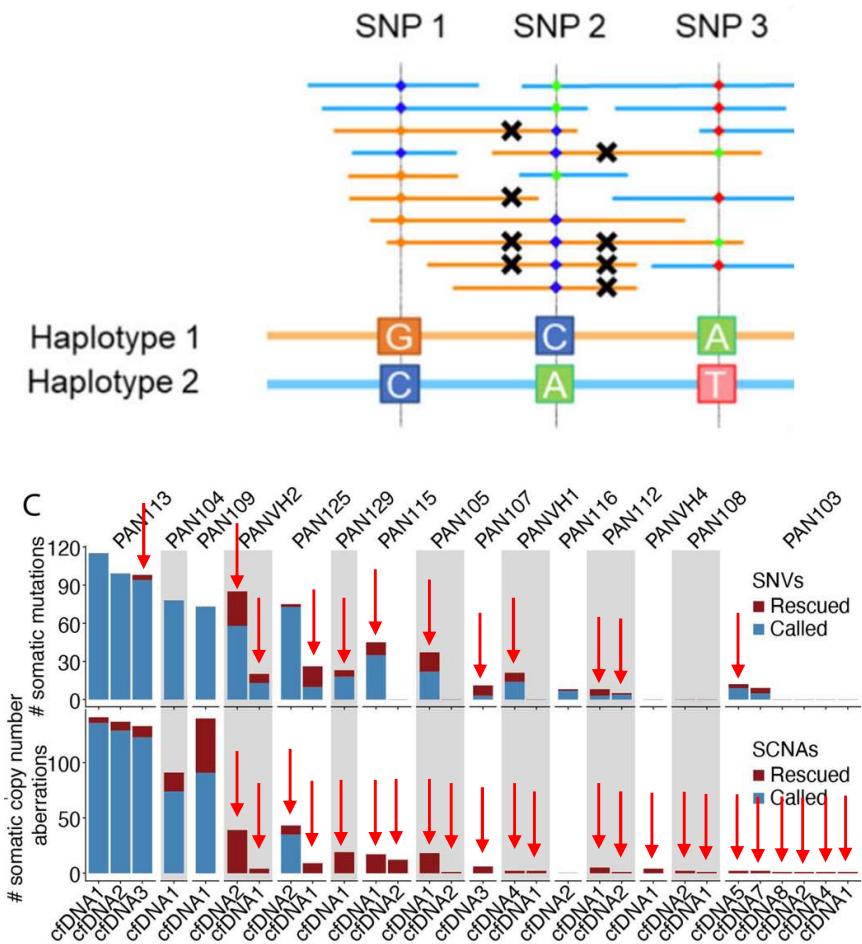
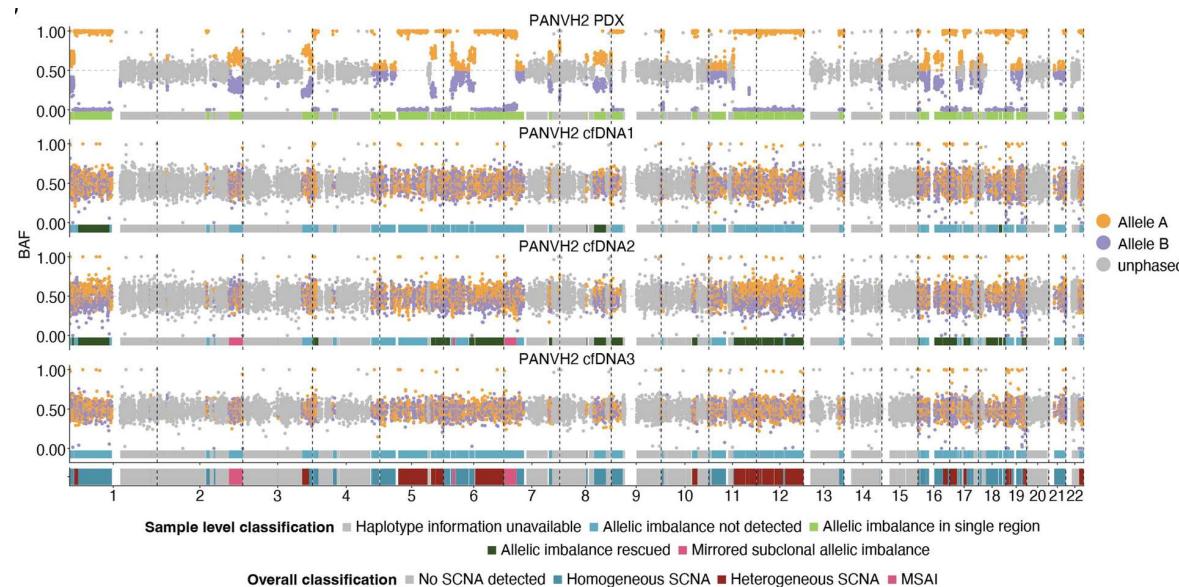


- cfDNA Samples (N=47)
- 6 samples had high tumour purity >=15%
  - 41 samples had low tumour purity <5%

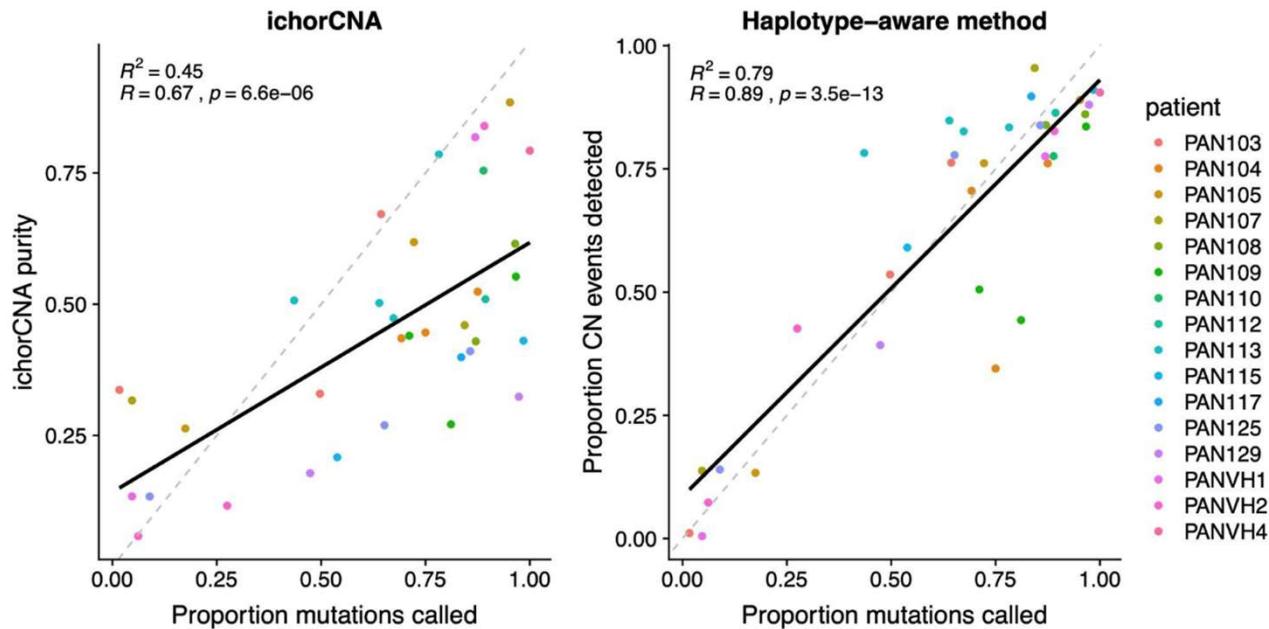


Hueber A et al , Genome Medicine 2023

## **SNP phasing increases sensitivity up to 30% the detection of CNA and mutations in plasma ctDNA of low shedders**



# ACT-Discover haplotype-aware CNA calling outperforms ichorCNA

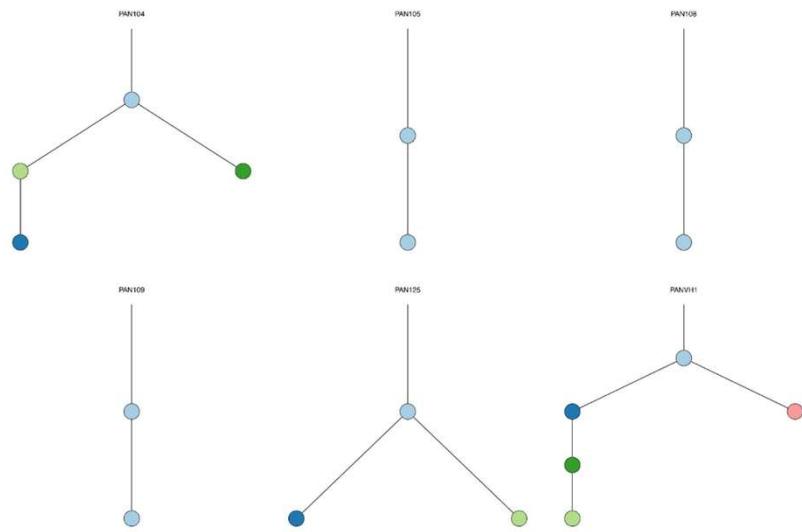


## ACT-Discover

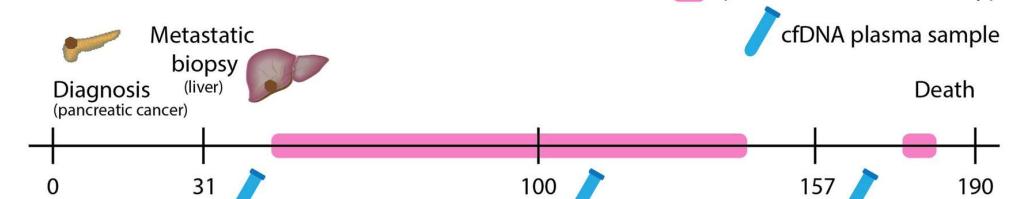
Aneuploidy in Circulating Tumour DNA (ACT-Discover) is an R package to detect somatic copy number aberrations (SCNAs) in circulating tumour DNA (ctDNA) by leveraging haplotype phasing of paired tumour biopsies or patient-derived xenograft (PDX) samples. ACT-Discover can be used to infer karyotype heterogeneity during tumour evolution. For more details on the method and use of ACT-Discover please read our publication [ACT-Discover: identifying karyotype heterogeneity in pancreatic cancer evolution using ctDNA](#). Huebner, Black et al. *Genome Med* (2023)

## Installation guide

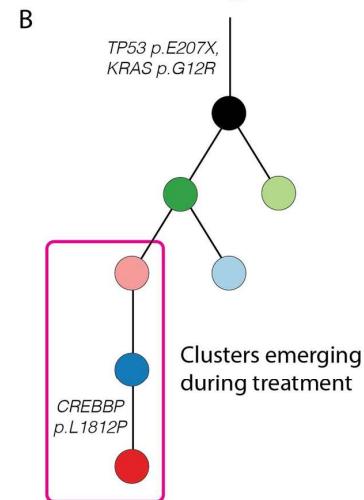
# Detection of acquired somatic events in *CREBBP*, *AKAP9*, *ACSL6*, *ATM* and *KMT2A* in pancreatic cancer during systemic treatment with gemcitabine and paclitaxel



A Clinical overview of PAN113



B



C Pre-treatment

Parallel SCNAs

Gain chr4q

Gain chr18p

Heterogeneous SCNAs

Met cfDNA1

chr2q

chr3q

chr4p

chr7q

chr11

chr12

chr14

chr9q

chr12p

chr14q

Post-treatment

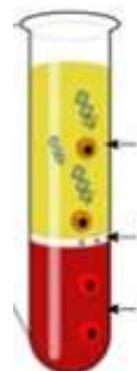
Ongoing aneuploidy

	Met cfDNA1	cfDNA2	cfDNA3
chr3	XX	YY	XX
chr4	XX	YY	YY
chr7	XX	YY	XX
chr11	XX	YY	XX
chr12	XX	YY	XX
chr14	XX	YY	XX
chr18	XX	YY	XX
chr20	XX	YY	XX

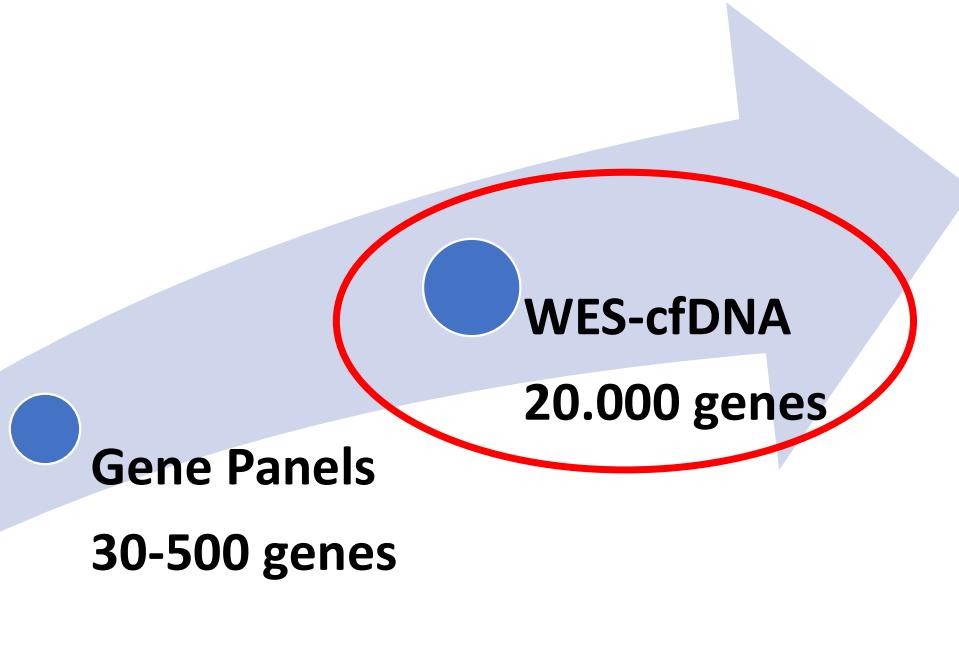
# Can whole-exome sequencing be applied to ctDNA ?

Yes ! (high shedders)

- Mutation profile
- Clonal dynamics
- Copy Number profile
- **bTMB (current ongoing)**
- **Neoantigen detection (A. Gros)**



ddPCR  
1 mutation



# ¡GRACIAS!

**IX SIMPOSIO · SYMPOSIUM | 2024**  
**BIOPSIA LÍQUIDA · LIQUID BIOPSY**  
EL CAMINO A LA ONCOLOGÍA DE PRECISIÓN · THE WAY TO PRECISION MEDICINE