VII Jornada EN Cáncer DE Mama Hereditario

Suspected of genetic alterations of germinal origin in liquid biopsy

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Organizado por:

GEICAM investigación en cáncer de mama
www.geicam.org

SOLTI INNOVATIVE BREAST CANCER RESEARCH
www.gruposolti.org

Sección SEOM Cáncer Familiar y Hereditario
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- Grant support: ISCIII, AECC
- Other:

Outline

- General concepts of liquid biopsy
- Detection of germline/somatic variants on cfDNA
  - -How to discriminate both
  - -Their clinical value
- Detection of BRCA1/2 germline/somatic variants on cfDNA
- Take-home message

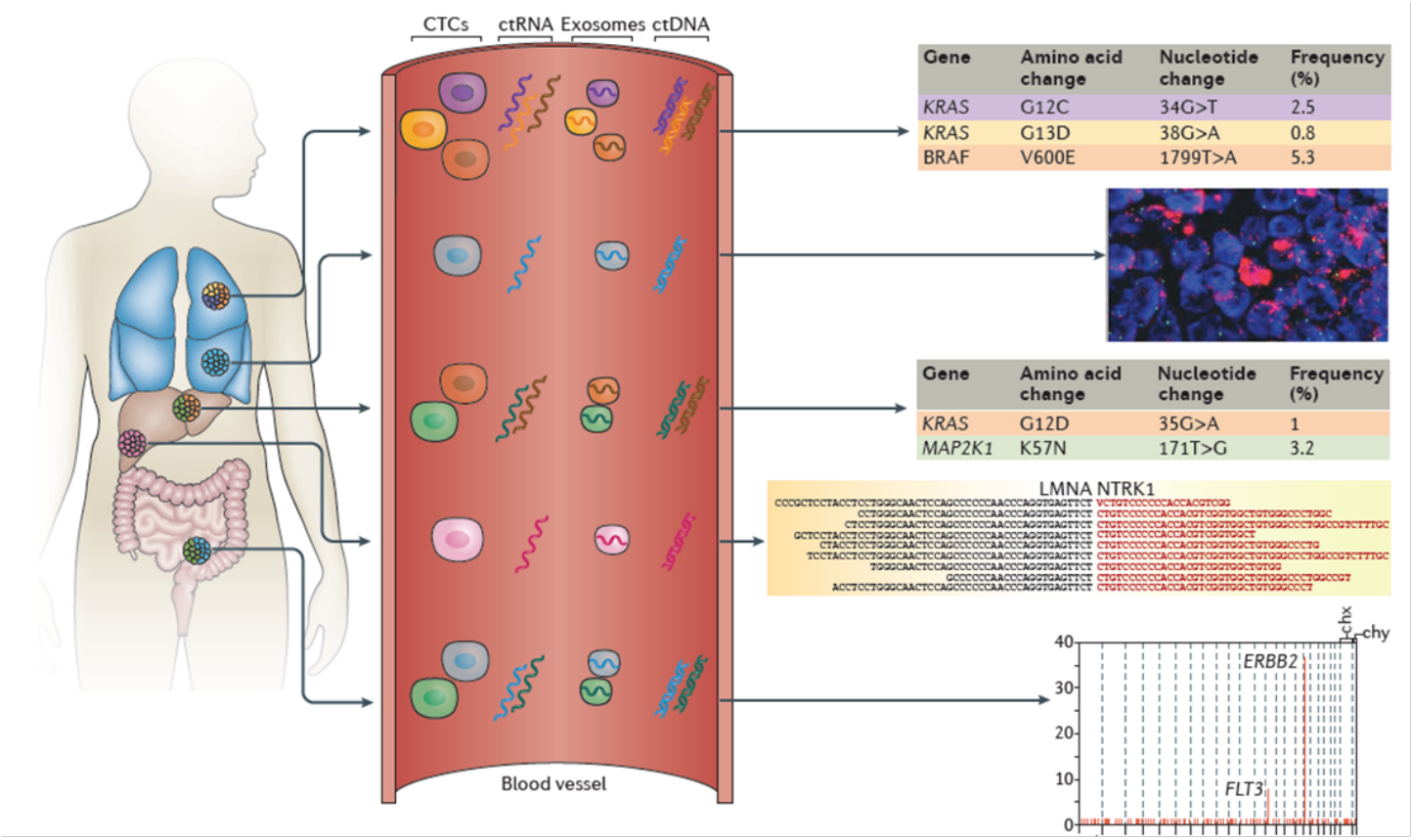
General concepts of liquid biopsy

More than blood……..

Samples: blood, serum/plasma, urine, CSF, saliva

General concepts of liquid biopsy

What are we looking for?

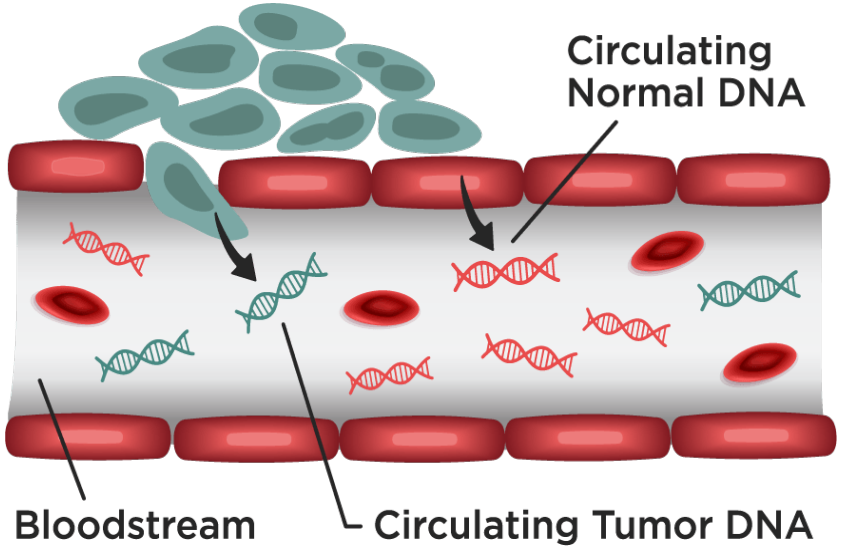


Siravenga et al., Nature Rev 2017

General concepts of liquid biopsy

MAIN CHARACTERISTICS OF CFDNA???

- Is normally released into the circulation **during cell death (necrosis and apoptosis)**
- cfDNA in the circulation is typically **fragmented to 160 to 180 bp in length**
- The **fraction of ctDNA** represents normally **less than 1% of the total cfDNA**
- ctDNA may come from **primary tumors**, **metastatic lesions** or **CTCs**

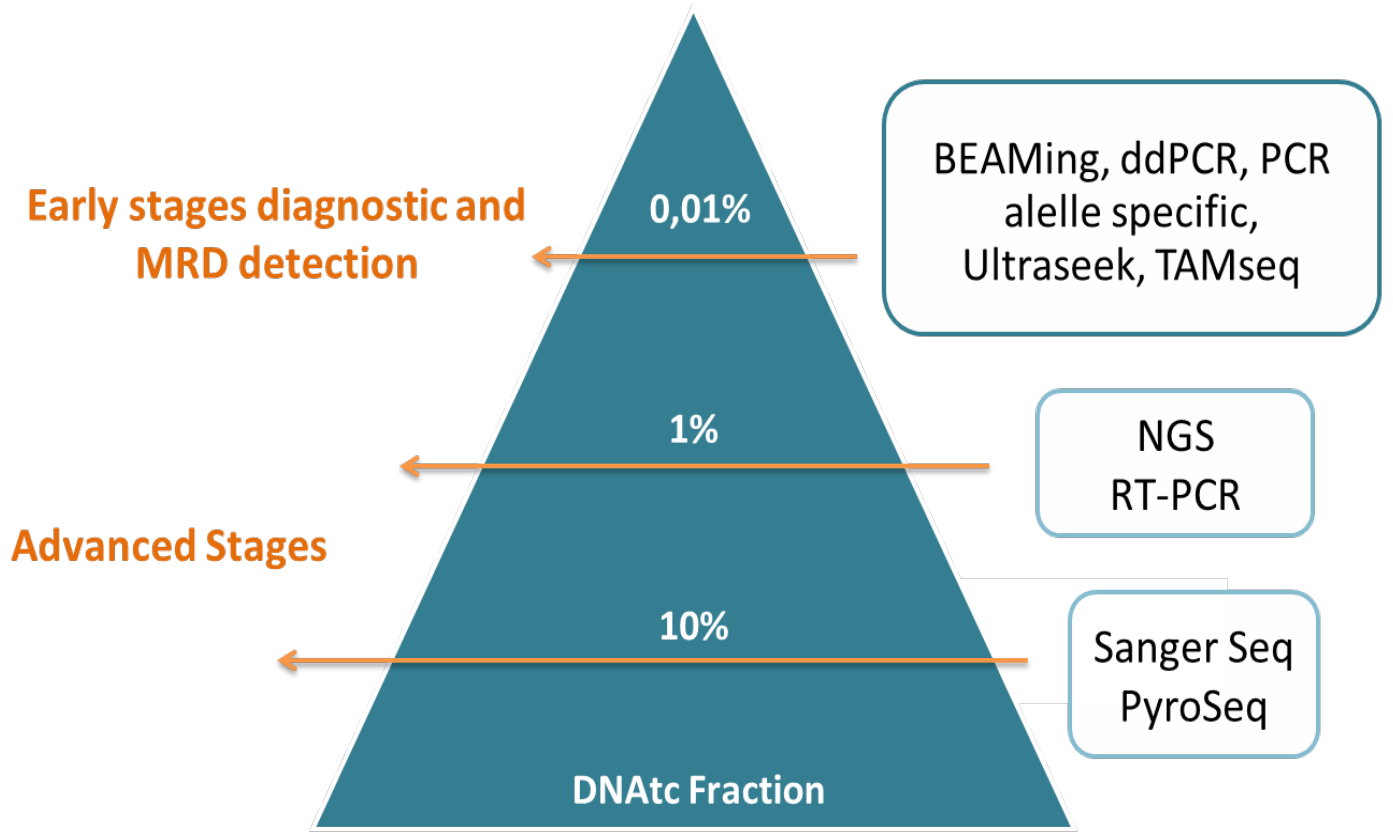


General concepts of liquid biopsy

Main characteristics of ctDNA???

ctDNA analysis strategies/ clinical application

Point mutations, CNV, gene fusions, short Indels, etc



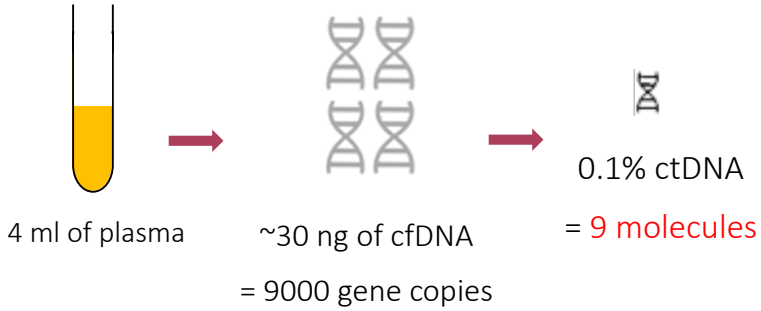
General concepts of liquid biopsy

The limit of detection matters

•**The limit of detection (LoD):** The limit of detection (LoD) is the minimum concentration of the mutant (rare) sequence that can be reliably differentiated from a negative control (100% wild type).

•LoD is typically quoted as a ratio or a percentage: for example, **1 mutant copies in 1000 wt = 0.1%.**

•Consider a sample at a mutant or rare sequence concentration of 1 mutant or sequence in 1000 wild type. To guarantee with 95% confidence that at least 1 mutant molecule will be screened in this sample, statistics dictates that at least 3,000 wild-type molecules must be screened.



General concepts of liquid biopsy

FDA approved technologies for ctDNA analyses

Table 1. Food and Drug Administration (FDA)-approved liquid biopsies assays.

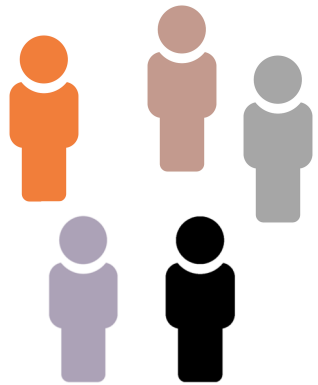
Liquid Biopsy Assay	Clinical Application	Genes Analyzed
FoundationOne ® Liquid CDx assay	NSCLC, mCRPC	70 genes + MSI-H (BRCA 1, 2, EGFR)
Guardant360 ® CDx assay	NSCLC, pan-cancer	70 genes using NGS
Therascreen ® (Qiagen) PI3KCA	Breast cancer	11 mutation in PIK3CA gene
EpiproColon ®	Colorectal cancer	PCR, methylation
Cobas ® EGFR mutation test (Roche)	NSCLC	EGFR variants
In Vision First-Lung ®	NSCLC	37 genes NSCLC
Oncobeam Lung-1 ®	NSCLC	EGFR
Oncobeam Lung-2 ®	NSCLC	EGFR, KRAS, BRAF
Oncomine ® (Thermo Fisher Scientific)	Breast, lung, colon cancer, pan-cancer	52 genes cancer assay
TS0500 ctDNA ® (Illumina)	Pan-cancer	500+ genes
Avenio ctDNA ® (Roche)	Breast, lung, colorectal, gastric, melanoma, pancreatic, ovarian, glioma, thyroid cancers	17 genes

Non-small cell lung cancer (NSCLC); metastatic castration resistant prostate cancer (mCRPC); microsatellite instability (MSI-H).

Mesquita et al., Cancers, 2020

Detection of germline/somatic mutations on cfDNA

Identification of Incidental Germline Mutations in Patients With Advanced Solid Tumors Who Underwent Cell-Free Circulating Tumor DNA Sequencing



10,888 patients with
> 50 types of advanced cancer

Guardant360
(70 genes)
average reads depth
15000X

The main outcome was prevalence of putative germline mutations identified among 16 actionable hereditary cancer predisposition genes

Slavin et al. JCO, 2018

Detection of germline/somatic mutations on cfDNA

Identification of Incidental Germline Mutations in Patients With Advanced Solid Tumors Who Underwent Cell-Free Circulating Tumor DNA Sequencing

Guardant360
(single nucleotide variants, small indels, copy number amplifications, and fusion events) average reads depth 15000X

16 clinically actionable hereditary cancer genes, (APC, ATM, BRCA1, BRCA2, CDKN2A, KIT, MLH1, NF1, PTEN, RB1, RET, SMAD4, STK11, TP53, TSC1, and VHL)

- **All variants with VAFs from 40% to 60% were included in the screen for pathogenic germline alterations.**
- **Variants with VAFs <40% are more likely to represent somatic alterations related to ctDNA or clonal hematopoiesis**
- **Variants with VAFs >60% were included if a concomitant copy number change was identified.**

Slavin et al. JCO, 2018

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- **Variants with VAFs <40% are more likely to represent somatic alterations related to ctDNA or clonal hematopoiesis**
- **Variants with VAFs >60% were included if a concomitant copy number change was identified,**

Using Ingenuity Variant Analysis, variants observed with an allele frequency of 2.0% or greater were excluded.

Remaining SNVs and indels were classified by Ingenuity Variant Analysis as pathogenic, likely pathogenic, unknown significance, likely benign, or benign according to American College of Medical Genetics and Genomics guidelines.

Slavin et al. JCO, 2018

Detection of germline/somatic mutations on cfDNA

Identification of Incidental Germline Mutations in Patients With Advanced Solid Tumors Who Underwent Cell-Free Circulating Tumor DNA Sequencing

Table 1. Putative Germline Mutation Occurrences by Cancer Type

Patient Characteristic	Cancer Type, No. (%)							
	All	Ovarian	Prostate	Pancreatic	Breast	Lung	Colorectal*	Other†
No. of patients	10,888	210	617	332	2,064	4,459	878	2,328
Sex								
Female	6,242 (57.3)	210 (100)	0 (0)	143 (43.1)	2,037 (98.7)	2,449 (54.9)	397 (45.2)	1,006 (43.2)
Male	4,646 (42.7)	0 (0)	617 (100)	189 (56.9)	27 (1.3)	2010 (45.1)	481 (54.8)	1,322 (56.8)
Mean age, years (range)	63.6 (18-95)	62.3 (25-89)	68.7 (35-89)	64.5 (34-89)	58.6 (25-95)	66.4 (20-89)	59.5 (21-89)	62.4 (18-89)
Patients with putative germline mutation	156 (1.4)	17 (8.1)	21 (3.4)	11 (3.3)	45 (2.2)	33 (0.7)	5 (0.6)	24 (1)
Gene								
BRCA2	81	3	20	7	27	17	1	6
BRCA1	41	14		1	10	11	1	4
CDKN2A	10			1	3	2		4
ATM	5			1	2	2		
TP53	5				3			2
APC	4						1	3
NF1	4		1			1		2
RB1	2							2
RET	2			1				1
MLH1	1						1	
SMAD4	1						1	

NOTE. No putative germline mutations were identified in *PTEN*, *STK11*, *TSC1*, *KIT*, or *VHL*. Full coding sequence data were available for *BRCA1*, *BRCA2*, *CDKN2A*, *KIT*, *NF1*, *PTEN*, *RB1*, *TP53*, and *VHL*.

*Lynch syndrome genes not sequenced except for *MLH1* exon 12.

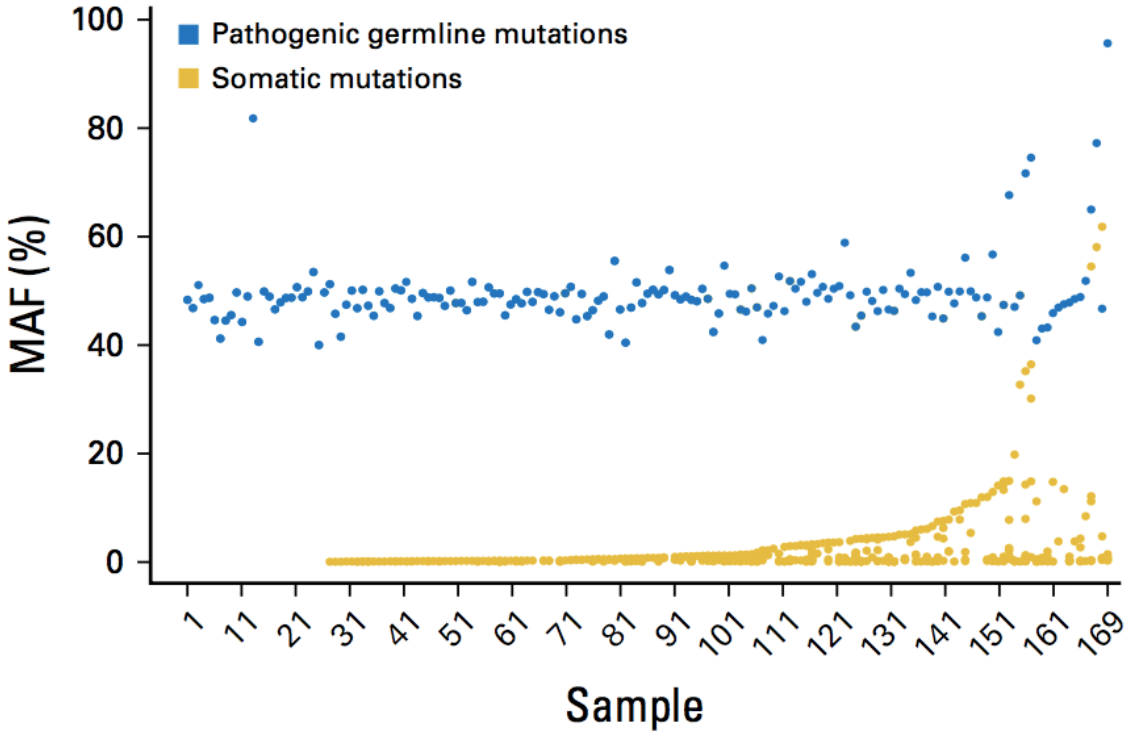
†Other types include carcinoma of unknown primary, endometrial carcinoma, melanoma, thyroid carcinoma, gastric adenocarcinoma, cholangiocarcinoma, renal pelvic urothelial carcinoma, renal cell carcinoma, neuroendocrine carcinoma, and sarcoma.

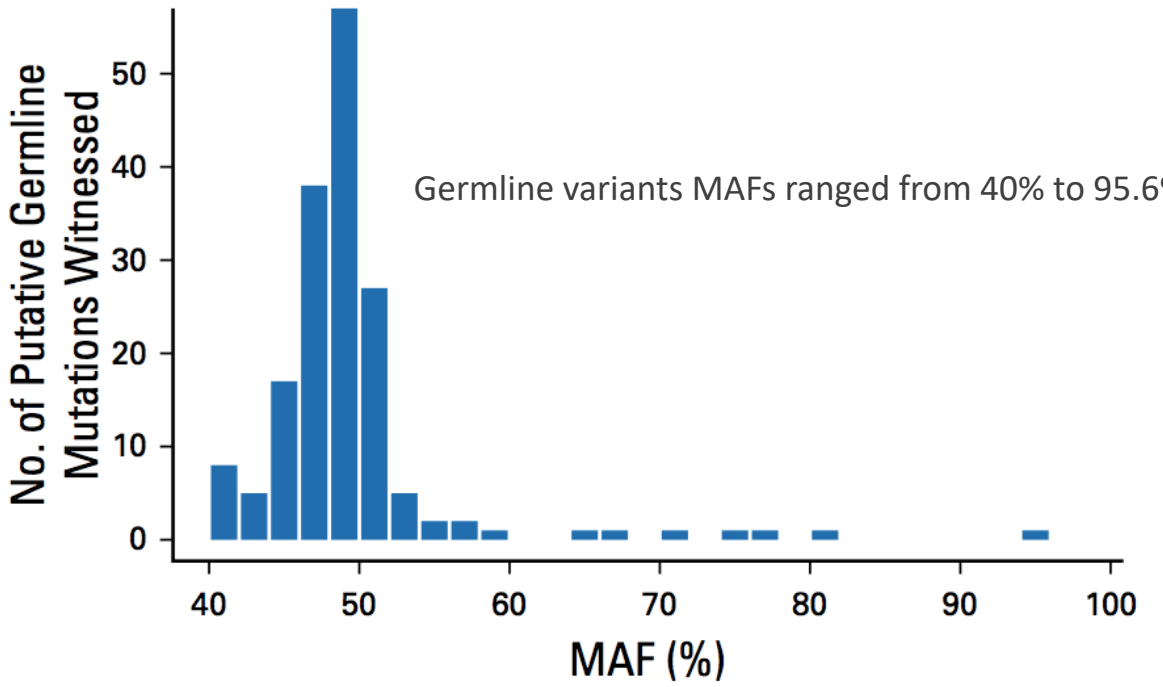
Slavin et al
JCO, 2018

Detection of germline/somatic mutations on cfDNA

Identification of Incidental Germline Mutations in Patients With Advanced Solid Tumors Who Underwent Cell-Free Circulating Tumor DNA Sequencing

Rates of putative germline mutations were elevated in individuals younger than 50 years





Germline variants MAFs ranged from 40% to 95.6%

Slavin et al JCO, 2018

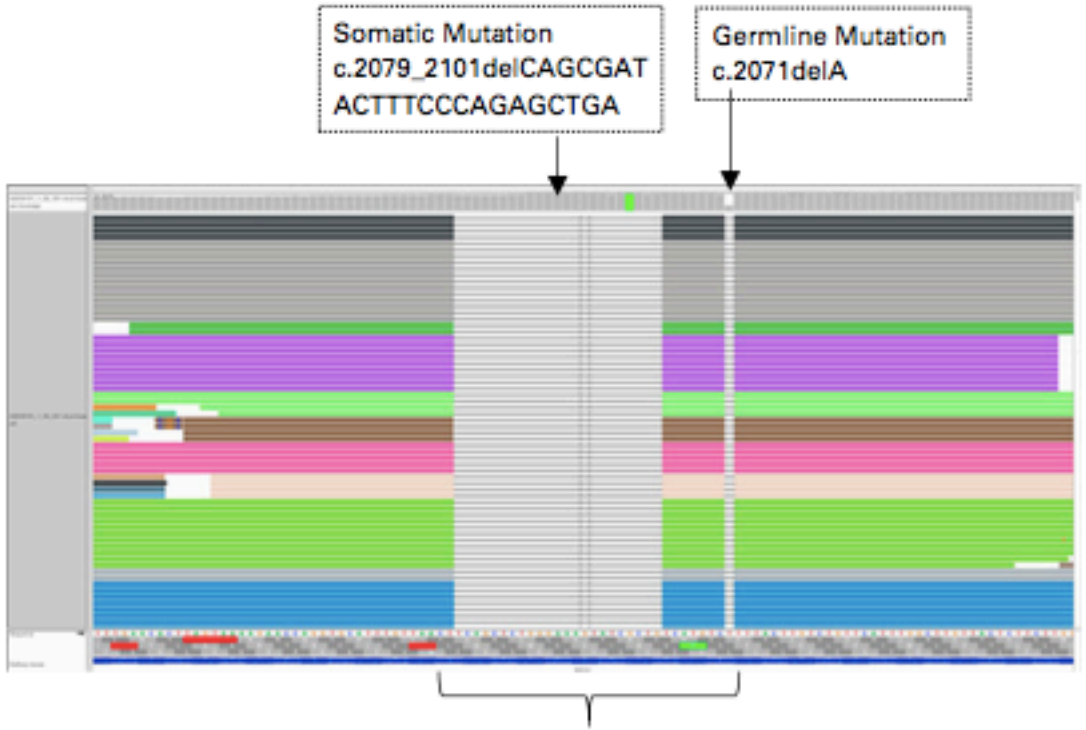
Detection of germline mutations/somatic on cfDNA

Identification of Incidental Germline Mutations in Patients With Advanced Solid Tumors Who Underwent Cell-Free Circulating Tumor DNA Sequencing

Patient with an ovarian cancer

(restores reading frame)

Mutation	Allele Frequency or Copy Number, %	Interpretation
BRCA1 c.2071delA (p.Arg691fs)	74.5	Germline mutation
BRCA1 gene deletion	1.66	Somatic mutation (second hit)
BRCA1 c.2079_2101del CAGCGATACTTTCCCAGA GCTGA (p.Asp693fs)	2.15	Somatic mutation (reversion mutation)

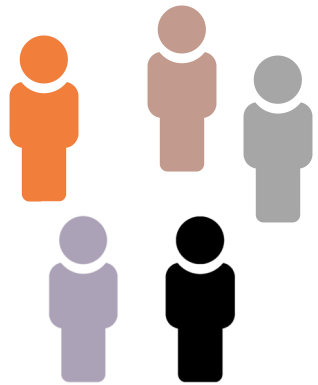


Taken individually, each mutation results in a frameshift (loss of function). Combined, the net result is a 24-nucleotide deletion that puts *BRCA1* back in frame (ie, reverts to pseudo–wild type).

Slavin et al JCO, 2018

Detection of germline/somatic mutations on cfDNA

Routine Plasma-Based Genotyping to Comprehensively Detect Germline, Somatic, and Reversion BRCA Mutations among Patients with Advanced Solid Tumors



828 patients with advanced breast, ovarian, prostate and pancreatic cancer

Guardant360 (73 genes) intron/exon of BRCA1/2 fully sequenced

COSMIC, cBioPortal for Cancer Genomics, UniProt, Integrative Genomics Viewer, PubMed

- **The bioinformatics algorithm considers the VAF of the mutation of interest relative to that of other somatic mutations in the sample and nearby germline single- nucleotide polymorphisms (SNP)**
- **Germline variants VAFs were close to 50%**
- **The median VAF of somatic mutations was 0.46%**

Vidula et al., Clin Cancer Res. 2020

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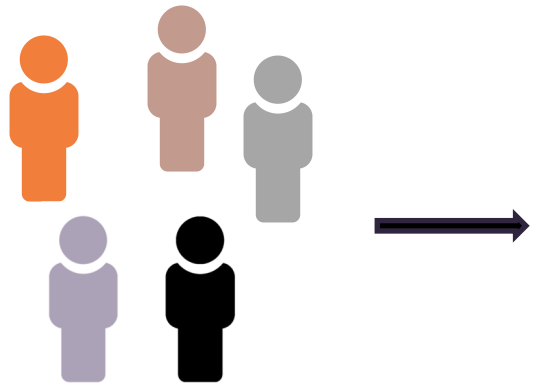

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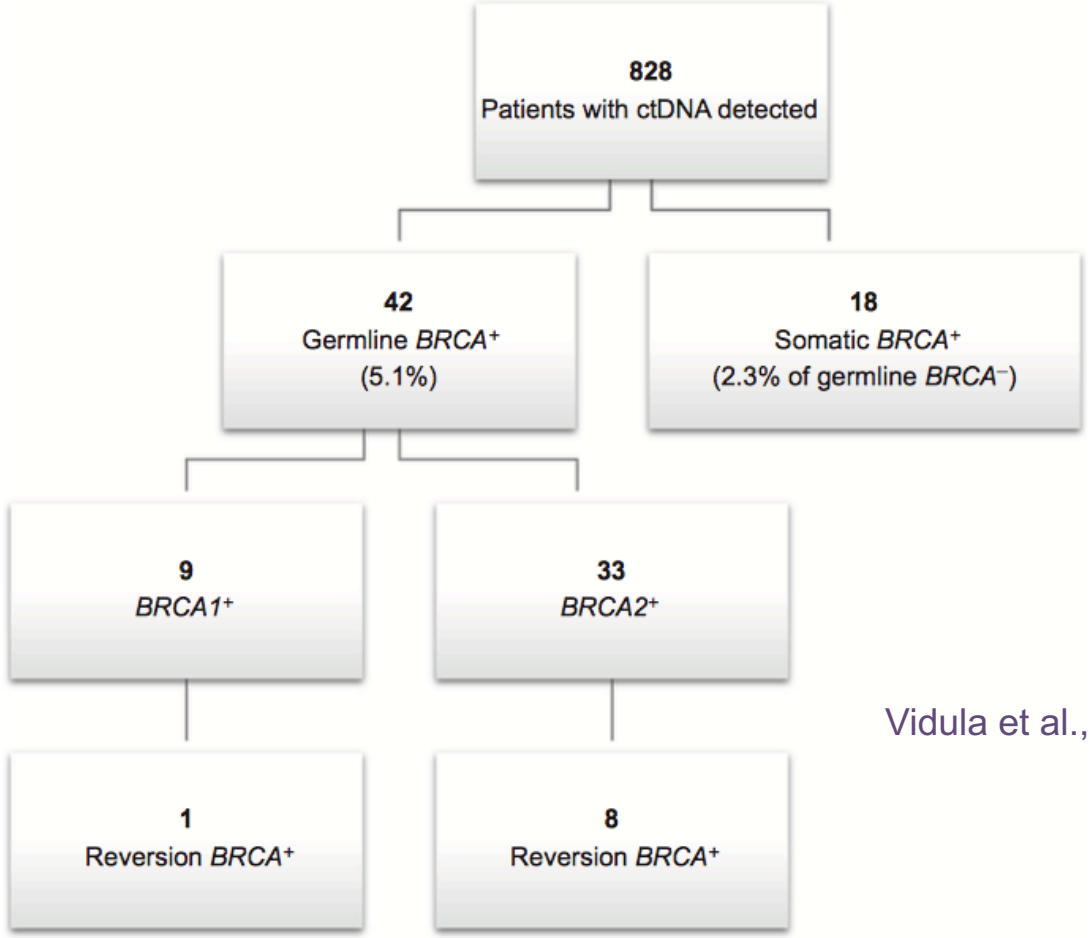

Vidula et al., Clin Cancer Res. 2020

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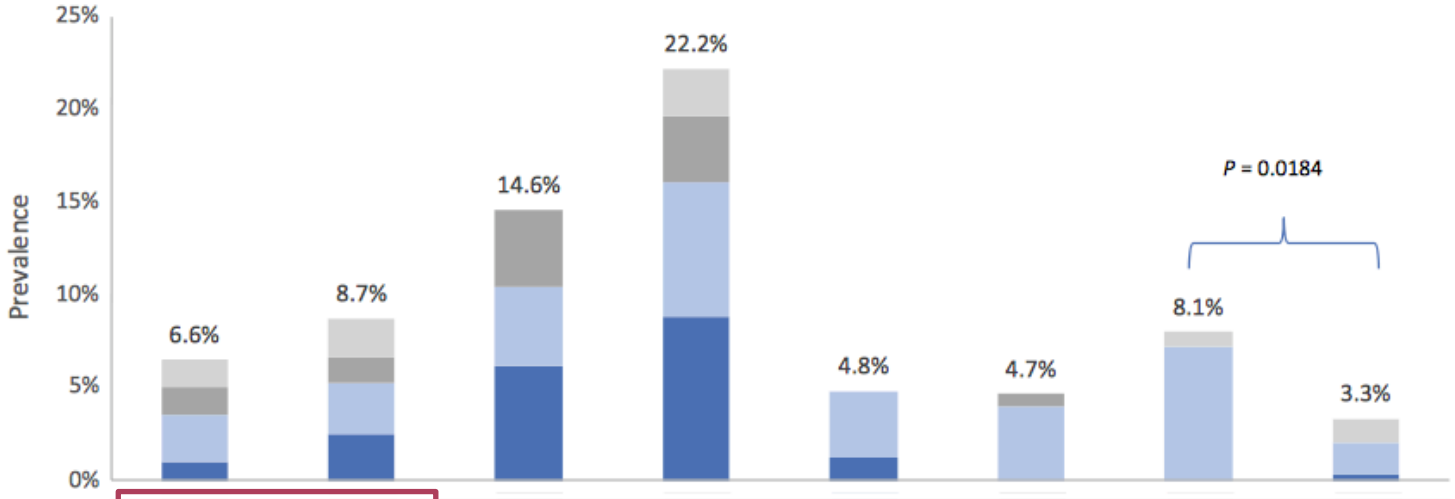

	cfDNA		TCGA	cfDNA	TCGA	cfDNA	TCGA	cfDNA	TCGA
	Breast			Ovarian		Pancreatic		Prostate	
Total tested	472		507	48	316	84	150	223	333
BRCA1 Germline	5	1%	13	3	28	1	0	0	1
BRCA2 Germline	12	2,5%	14	2	23	3	6	16	6
BRCA1 Somatic	7	1,5%	7	2	11	0	1	0	0
BRCA2 Somatic	7	1,5%	10	0	8	0	0	2	4

Figure 2.
Prevalence of germline and somatic deleterious *BRCA1/2* mutations (*gBRCA* and *sBRCA*, respectively) by cancer type and comparison to TCGA data.

Detection of BRCA germline/somatic mutations on cfDNA

Routine Plasma-Based Genotyping to Comprehensively Detect Germline, Somatic, and Reversion BRCA Mutations among Patients with Advanced Solid Tumors

high prevalence (9 of 42, 21.4%) of reversions among patients with a germline BRCA1/2 mutation.

Table 3. Reversions detected in the cfDNA of 9 germline *BRCA1/2*-positive patients.

Pt	Sample	Gene	Mutation	Origin	Exon	Reversions, *n*	Reversions
A	1 of 1	*BRCA2*	c.5576_5579delTTAA	Germline	11	1	c.5550_5577del28insC
B	1 of 1	*BRCA2*	c.4876_4877delAA	Germline	11	1	c.4876_4887del12
C	1 of 1	*BRCA2*	c.2385_2386insAA	Germline	11	16	c.2401_2420del20 c.2404_2429del29 c.2415_2455del41 c.2370_2387del18 c.2362_2369del8 c.2397dupA c.2354_2364del11 c.2392dupC c.2366_2373del8 c.2395_2414del20 c.2352_2374del23 c.2379_2390del12 c.2380_2397del18 c.2385_2386insAAA c.2414_2436del23 c.2401_2419dup19
D[a]	1 of 1	*BRCA2*	c.5946delT	Germline	11	11	c.5959_5966del8 c.5994_5999del6ins4 c.5992_6005del14 c.5998_6008del11ins3 c.5946_5990del45 c.5944_5952del c.5949_5952dup4 c.5964_5998del35 c.5941_5956del16ins1
		BRCA2	c.5754_5755delAT	Somatic	11		c.5736delA c.5748_5754del7insC

Vidula et al., Clin Cancer Res. 2020

Detection of BRCA germline/somatic mutations on cfDNA

Routine Plasma-Based Genotyping to Comprehensively Detect Germline, Somatic, and Reversion BRCA Mutations among Patients with Advanced Solid Tumors

Table 2. Treatments received prior to first detection of a *BRCA* reversion mutation.

Patient	Cancer type	Origin of deleterious *BRCA* mutation(s)	Duration of PARPi (mo)	Other systemic cancer therapies
A	Breast	gBRCA2	n/a	ET, capecitabine, everolimus, paclitaxel, eribulin, gemcitabine, mitoxantrone, vinorelbine
B	Prostate	gBRCA2	n/a	ADT, taxotere, enzalutamide, abiraterone acetate, cabazitaxel, mitoxantrone
C	Breast	gBRCA2	11	Adriamycin/cytoxan, paclitaxel, eribulin, letrozole/palbociclib, carboplatin
D	Prostate	gBRCA2 + sBRCA2	12	ADT, docetaxel, cabazitaxel,
E	Breast	gBRCA2	8	Adriamycin/cytoxan, capecitabine, eribulin, gemcitabine, vinorelbine, paclitaxel, Wee1 inhibitor
F	Breast	gBRCA2	3	Cisplatin, etoposide, paclitaxel, carboplatin, capecitabine
G	Breast	gBRCA1	12	Gemcitabine, carboplatin, PD-1 inhibitor
H	Prostate	gBRCA2	10	ADT, radium-223, abiraterone acetate, PD-1 inhibitor
I	Breast	gBRCA2	5	Carboplatin

Abbreviations: ADT, androgen deprivation therapy; ET, endocrine therapy; gBRCA, germline BRCA; sBRCA, somatic BRCA.

Vidula et al., Clin Cancer Res. 2020

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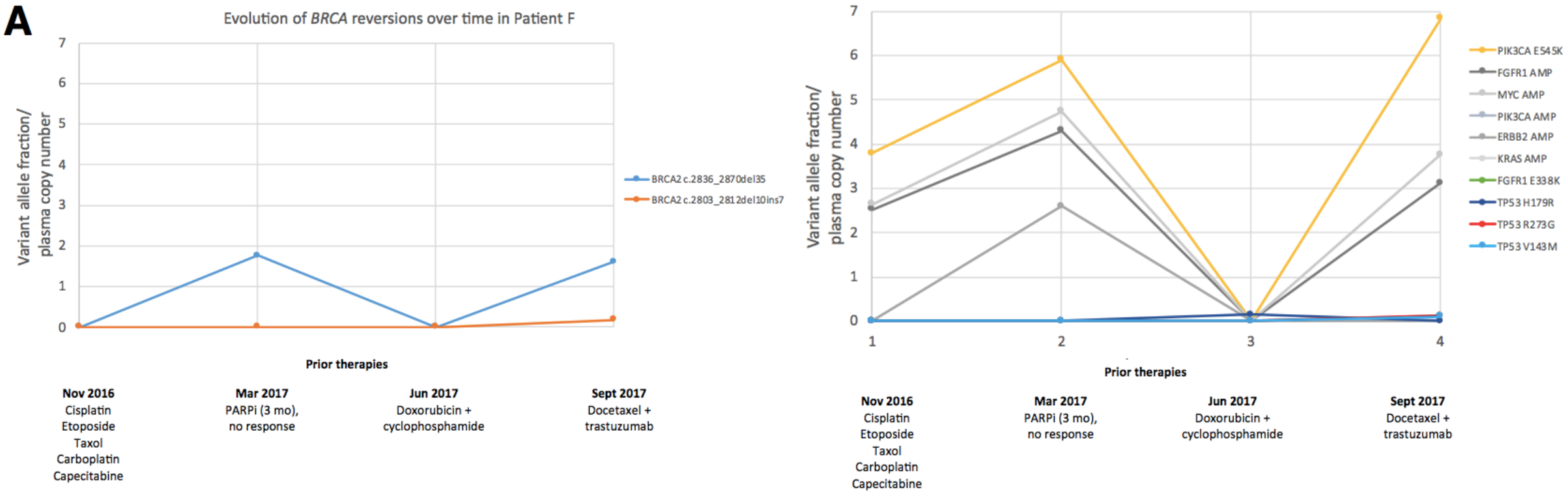
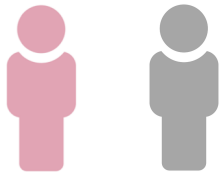


Vidula et al., Clin Cancer Res. 2020

Detection of BRCA germline/somatic mutations on cfDNA

Diverse BRCA1 and BRCA2 Reversion Mutations in Circulating Cell-Free DNA of Therapy-Resistant Breast or Ovarian Cancer



19 ovarian and 5 breast cancers (III/IV) with germline BRCA1/2 mutations and resistant or refractory to platinum-based chemotherapy, (availability of tissue)

Targeted massively parallel sequencing

SAMtools mpileup tool

1) somatic small insertions and deletions (indels) that would restore the reading frame of BRCA1/2 in patients with germline indels

2) somatic single nucleotide variants (SNVs) that restore the BRCA1/2 reference allele in patients with germline point mutations

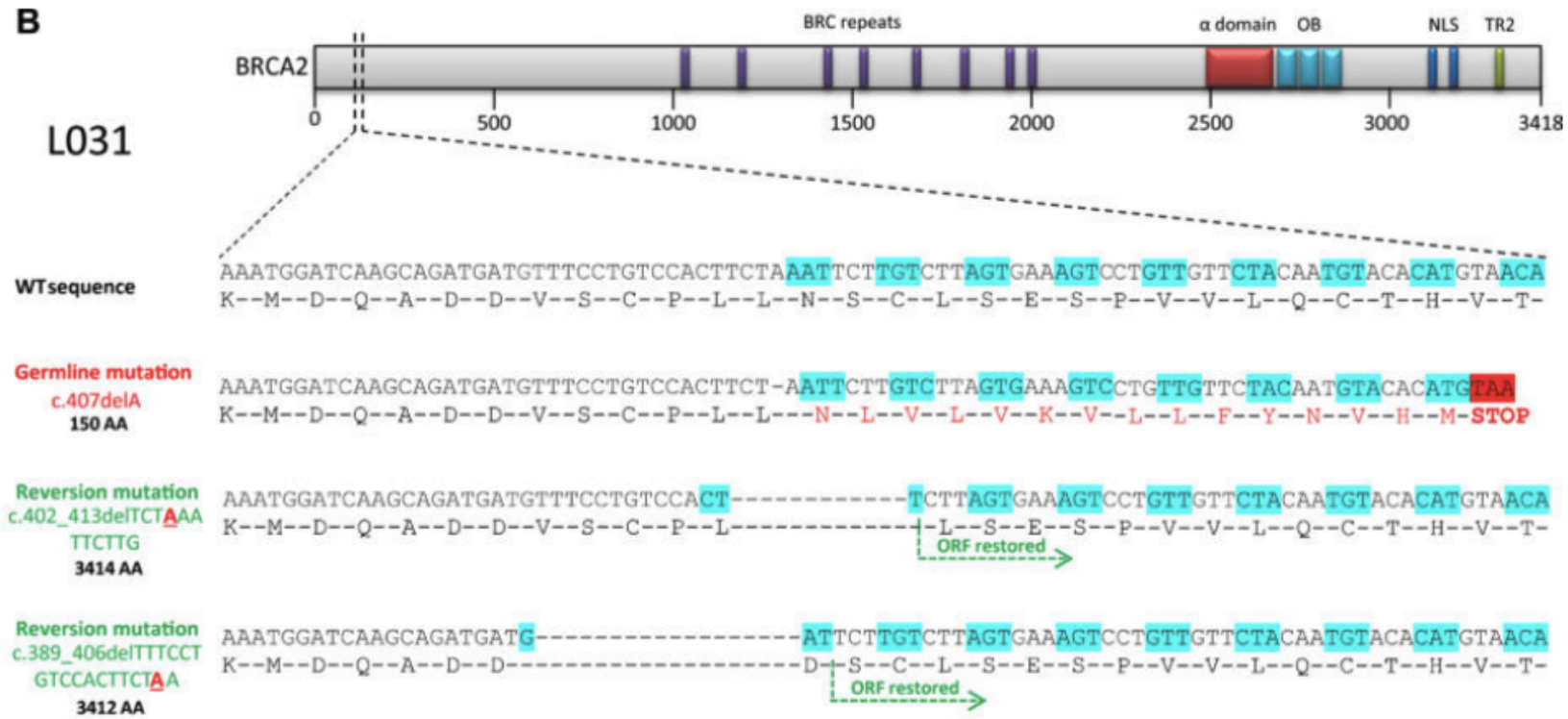
Weigelt et al. Clin Cancer Res. 2018

Detection of BRCA germline/somatic mutations on cfDNA

Diverse BRCA1 and BRCA2 Reversion Mutations in Circulating Cell-Free DNA of Therapy-Resistant Breast or Ovarian Cancer

2/5 BC patients showed polyclonal reversion variants in cfDNA

All variants were found at low VAFs



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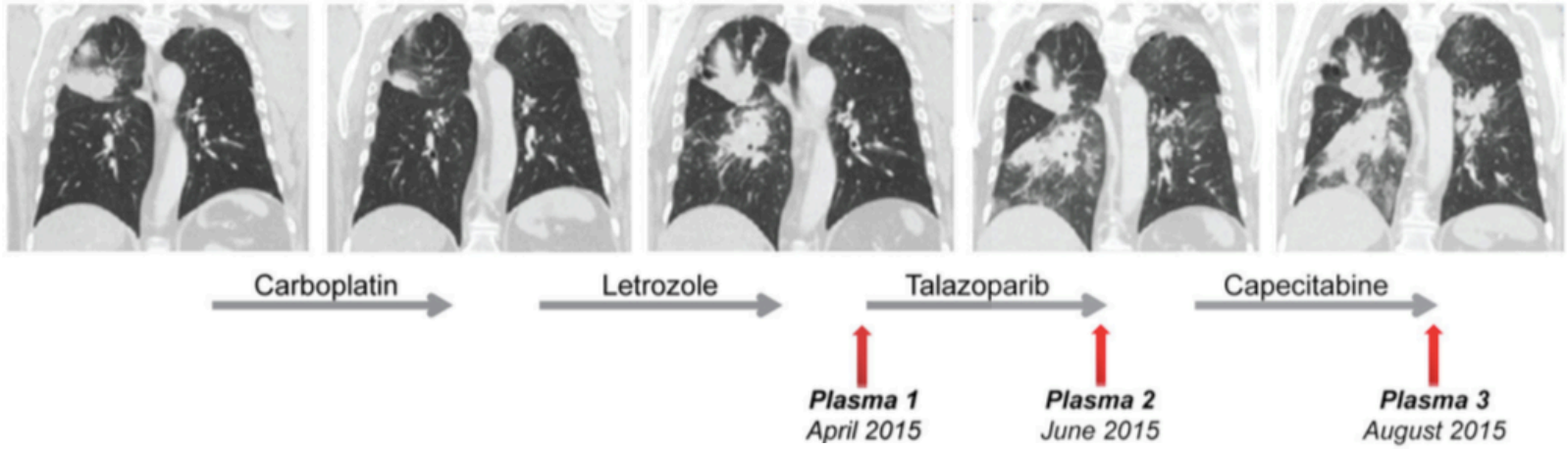
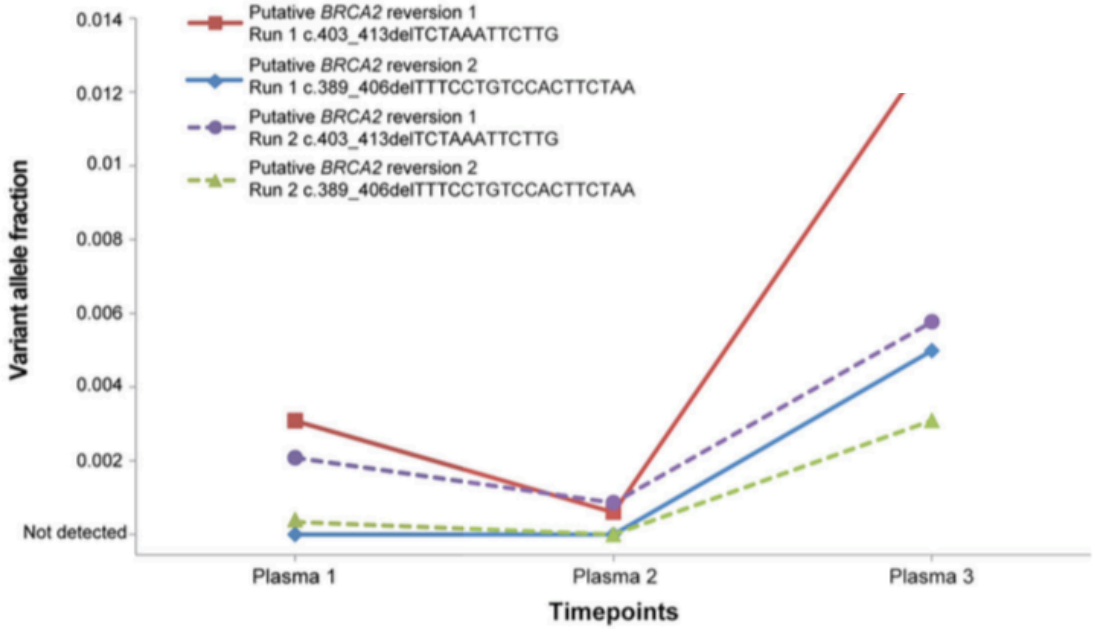
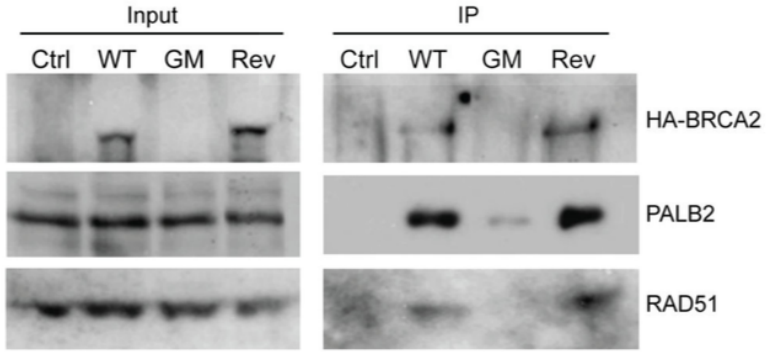






Weigelt et al. Clin Cancer Res. 2018

Detection of BRCA germline/somatic mutations on cfDNA

BRCA Reversion Mutations in Circulating Tumor DNA Predict Primary and Acquired Resistance to the PARP Inhibitor Rucaparib in High-Grade Ovarian Carcinoma

cfDNA from blood collected before and after rucaparib treatment in 112 patients with *BRCA*-mutant ovarian carcinomas. ARIEL2 (NCT01891344).

NGS using the Guardant360 and FoundationACT

(i) a base substitution that changed a nonsense mutation to a missense mutation

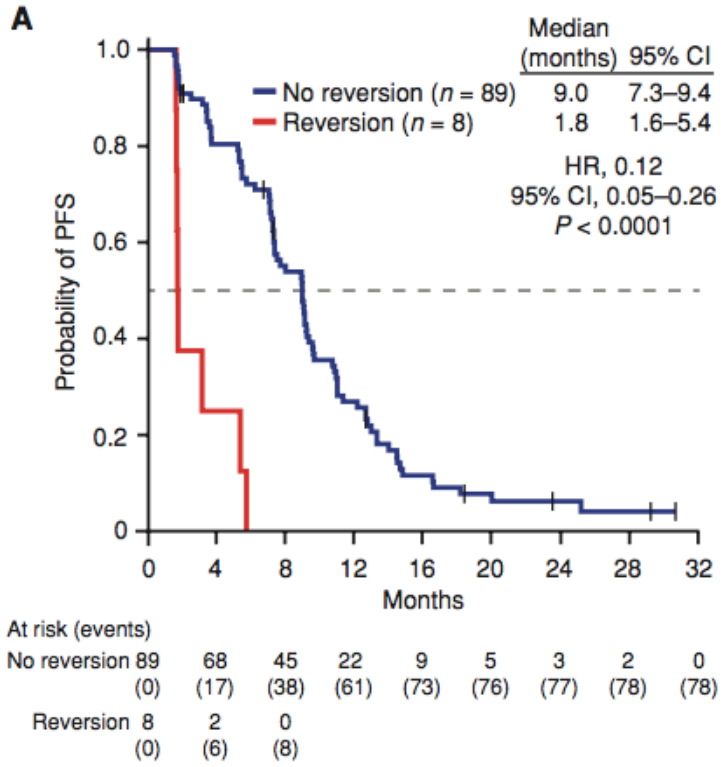
(ii) an insertion/deletion that restored the ORF. Large intragenic deletions that can restore the ORF were visually con rmed using the Integrated Genomics Viewer.

Lin et al., Cancer Discov, 2019

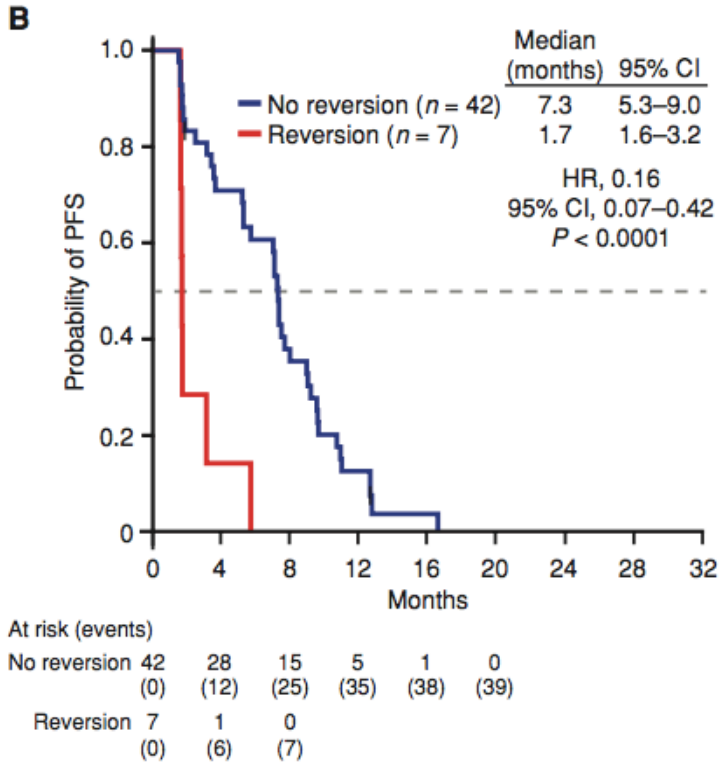
Detection of BRCA germline/somatic mutations on cfDNA

Patients without *BRCA* reversion mutations detected in pretreatment cfDNA have significantly longer rucaparib PFS

All *BRCA*-mutant cases



Platinum-resistant *BRCA*-mutant cases



Lin et al., Cancer Discov, 2019

Take-home message

- **Hereditary cancer predisposition germline mutations can be identified accurately analyzing cfDNA**. We can suspect the presence of a germline variant because **their VAFs will be normally between 40-60%**.

- **Suspicious germline actionable variants detected after cfDNA studies should be validated** based on database information, bibliography and, validation in lymphocytes fraction.

- **cfDNA germline evaluation represents an important clinical tool** to increase the reach of genetic cancer risk assessment, **particularly in cancers without clear hereditary predisposition.**

- **Somatic BRCA1/2 mutations can be identified after NGS studies on cfDNA**. These variants are normally found at **VAFs lower than 5%.**

Take-home message

- **Detection of BRCA1/2 reversion variants in cfDNA have a great potential for guiding the use of PARPi or chemotherapy** since they may not be evident in tissue from the primary tumor and may not be present in tissue from all sites of disease.

- However **some limitation of cfDNA analyses should be taken into account**; a) **detection of large indels is challenging** in plasma and b) some tumors are **poor cfDNA shedders.**

- Practically, **ordering ctDNA testing providers will need to inform patients that ctDNA testing may identify incidental germline information.**

- Laboratories that report **incidental findings will need to distinguish somatic from probable germline findings clearly and suggest confirmatory testing.**

VII Jornada EN Cáncer DE Mama Hereditario

Suspected of genetic alterations of germinal origin in liquid biopsy

Laura Muinelo Romay

Complexo Hospitalario Universitario de Santiago de Compostela

Organizado por:

GEICAM investigación en cáncer de mama
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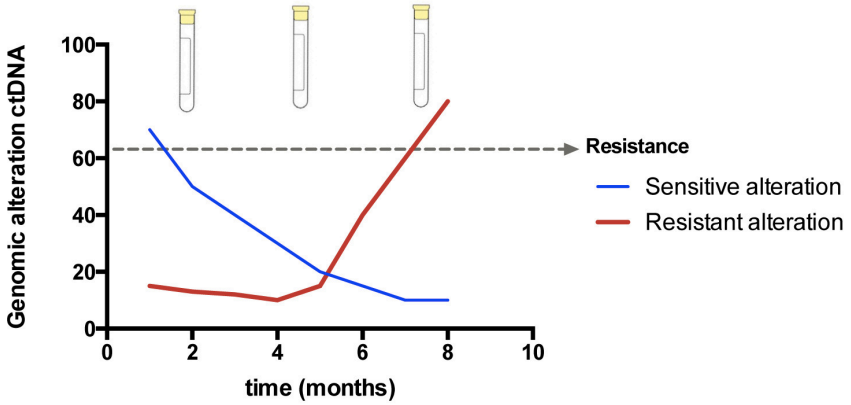
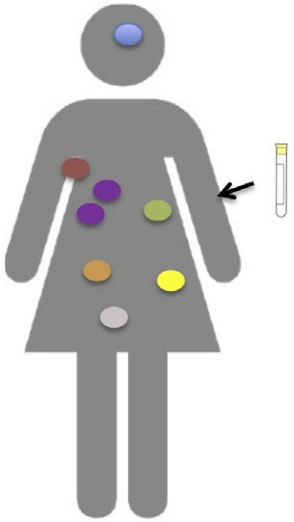
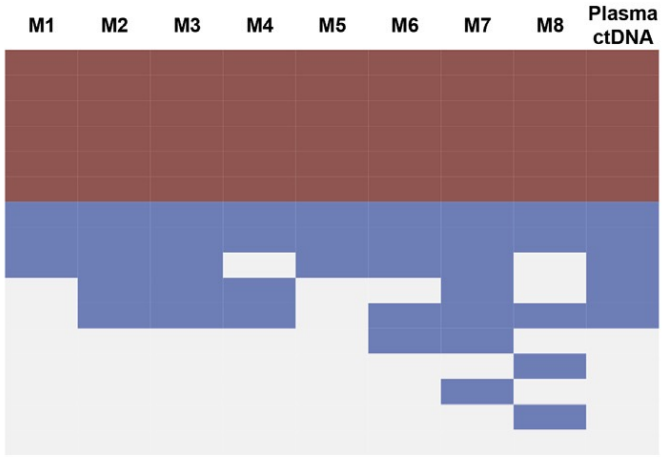
General concepts of liquid biopsy

Advantages of NGS studies on cfDNA

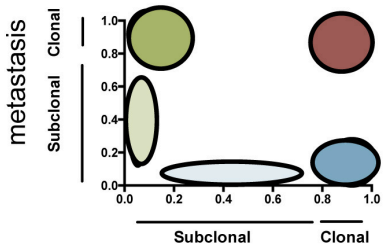
Longitudinal Monitoring

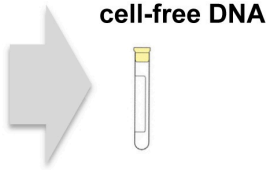


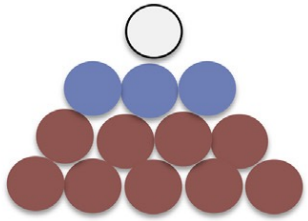




Intra- and inter-tumour heterogeneity







Private mutations

Metastatic clade mutations

Ubiquitous mutations

Plasma ctDNA

De Mattos-Arruda et al. Mol Oncol. 2016