

Ricerca traslazionale: la biopsia liquida nel tumore dell'ovaio



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FARMACOLOGICHE
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The liquid biopsy

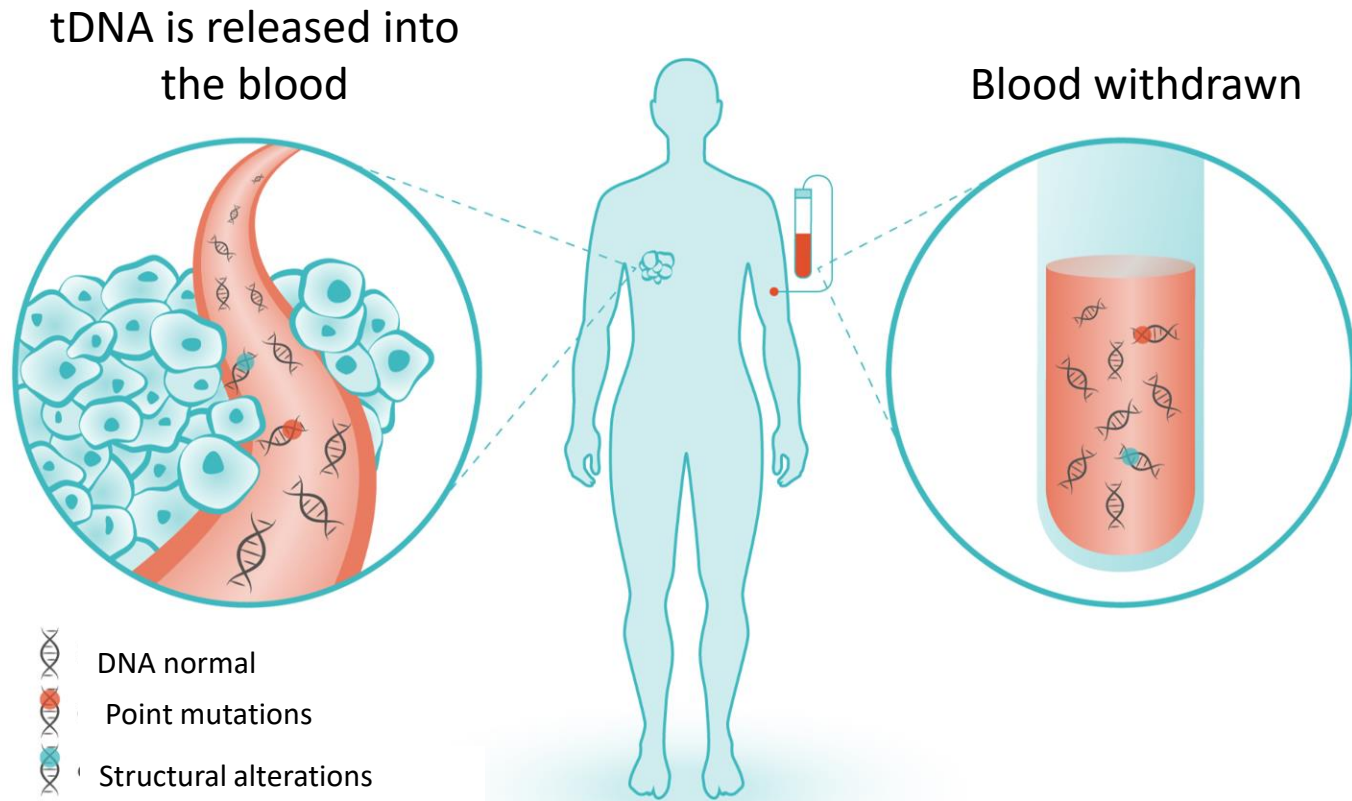
A test done on a sample of blood to look for cancer cells from a tumor that are circulating in the blood or for pieces of DNA from tumor cells that are in the blood. A liquid biopsy may be used to help find cancer at an **early stage**. It may also be used to help **plan treatment** or to find out **how well treatment is working** or if cancer **has come back**. Being able to take multiple samples of blood over time may also help doctors understand **what kind of molecular changes** are taking place in a tumor.

From the “NCI's Dictionary of Cancer Terms”



Liquid biopsy

A new path

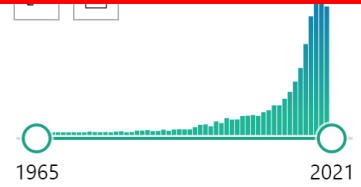


SNV as well as SCNA can be identified in the plasma to discriminate cfDNA from ctDNA



ctDNA in the clinical practice

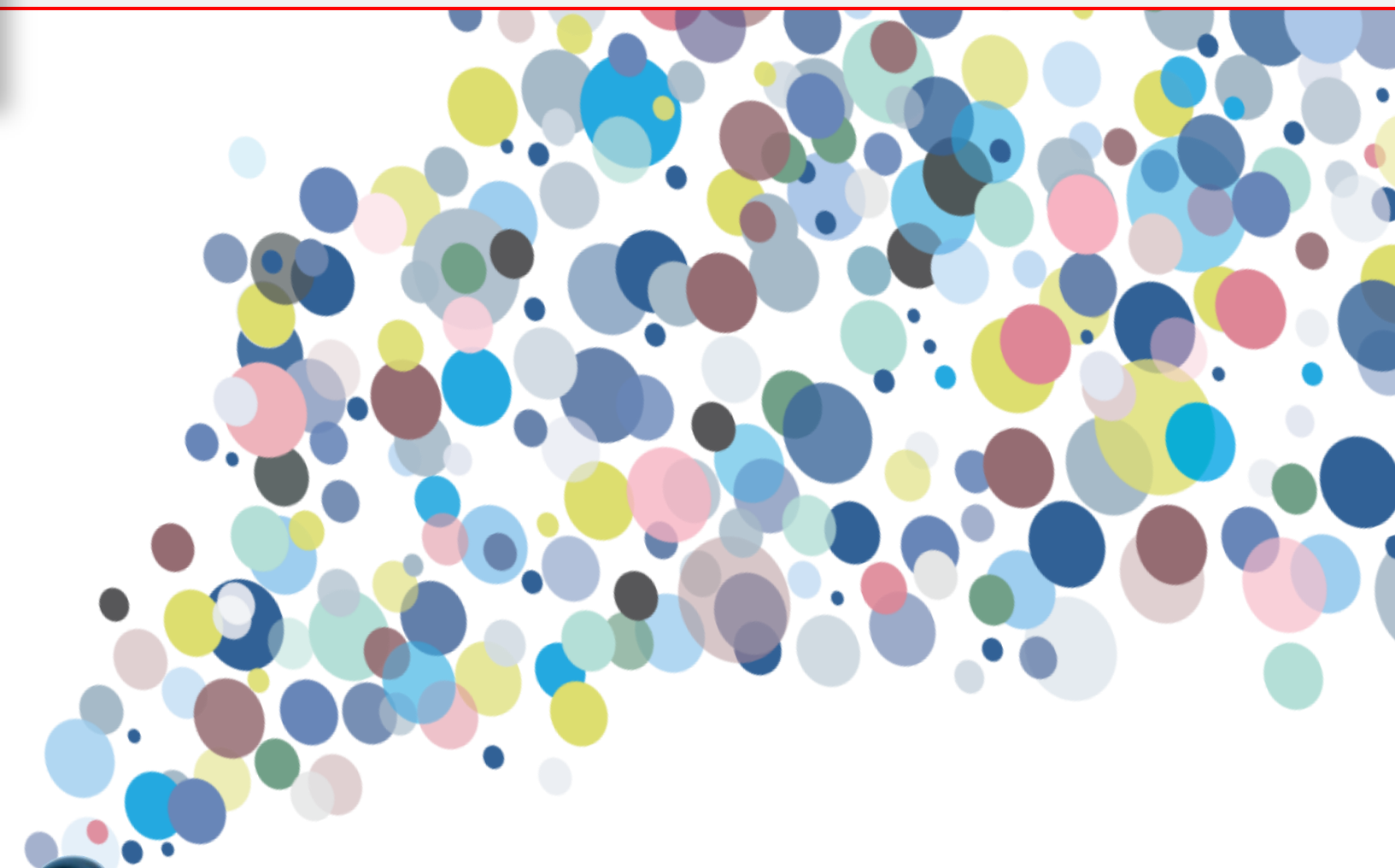
Introduction



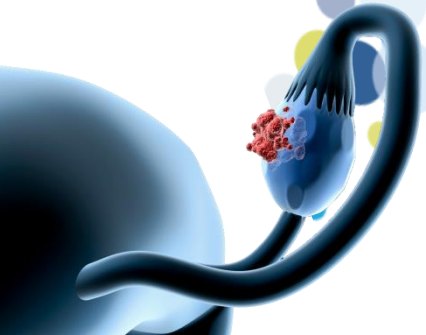


Epithelial Ovarian Cancer

Introduction

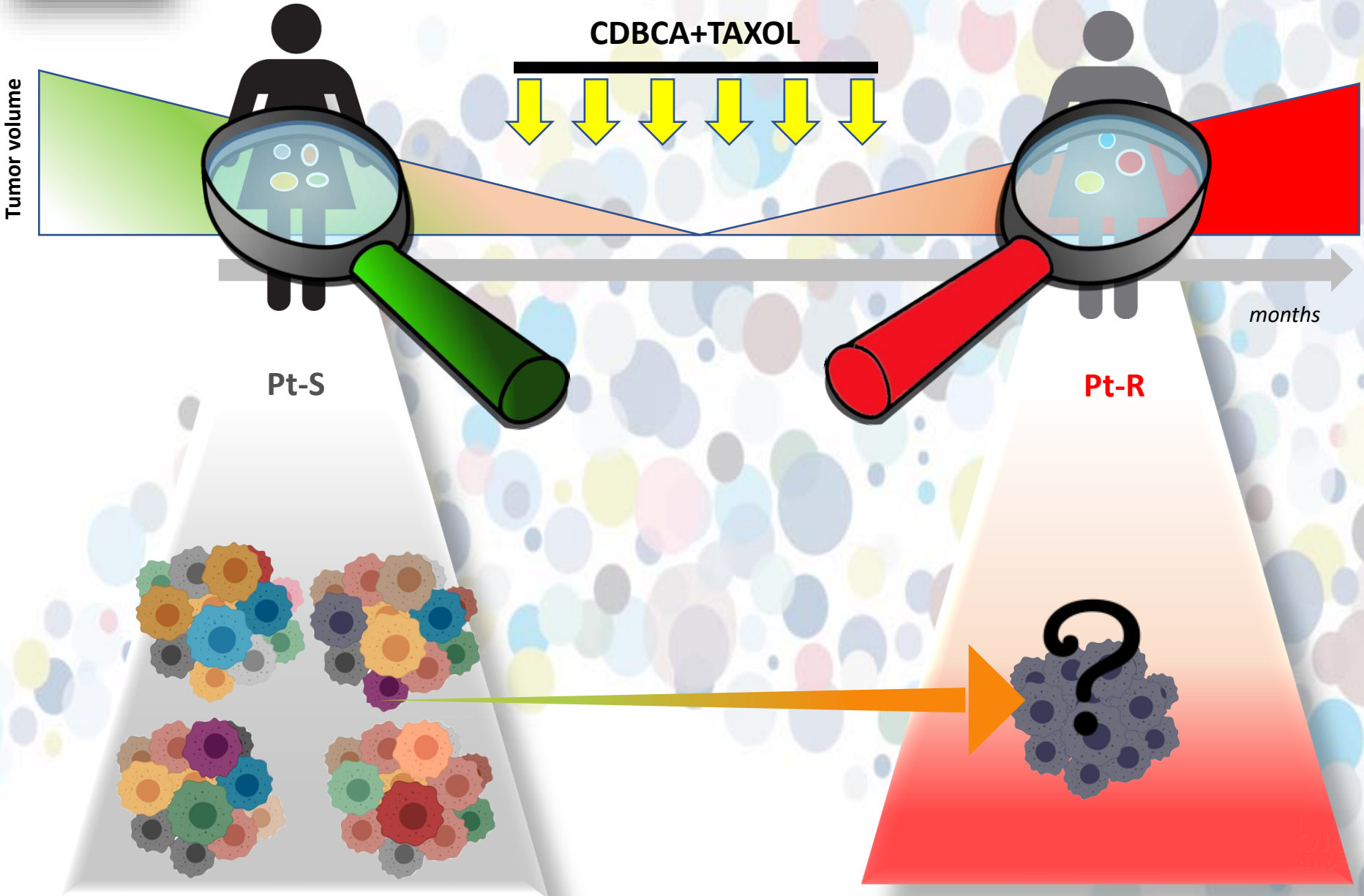


***Heterogeneity
is the hallmark of EOC***



Epithelial Ovarian Cancer

Clinical consequences



Epithelial Ovarian Cancer

Clinical issue



Tracking tumor evolution over time
is mandatory to improve clinical outcome of HGS-EOC patients

Strategy

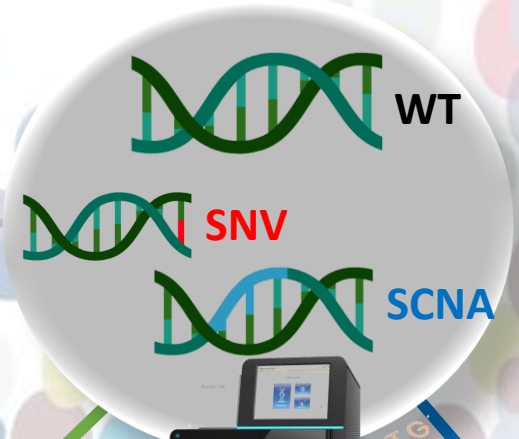
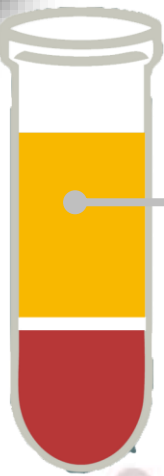
The Trojan horse



Turn that “strength” into a “weakness”.

Liquid biopsy

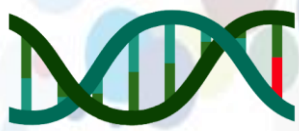
General strategy



ctDNA
identification

Targeted Approach

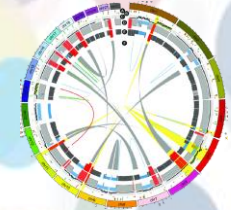
Untargeted Approach



Panel



WES



WGS

Increased sensitivity

Increased complexity

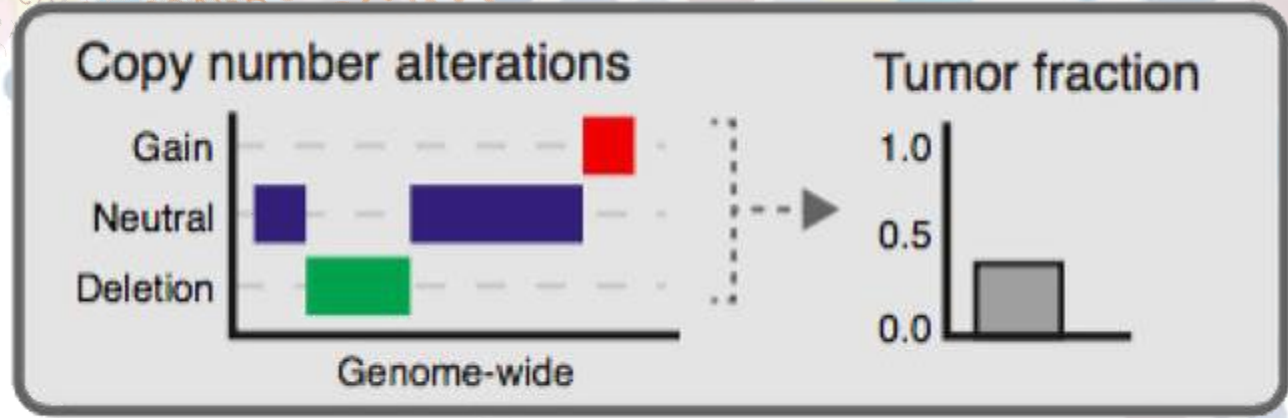
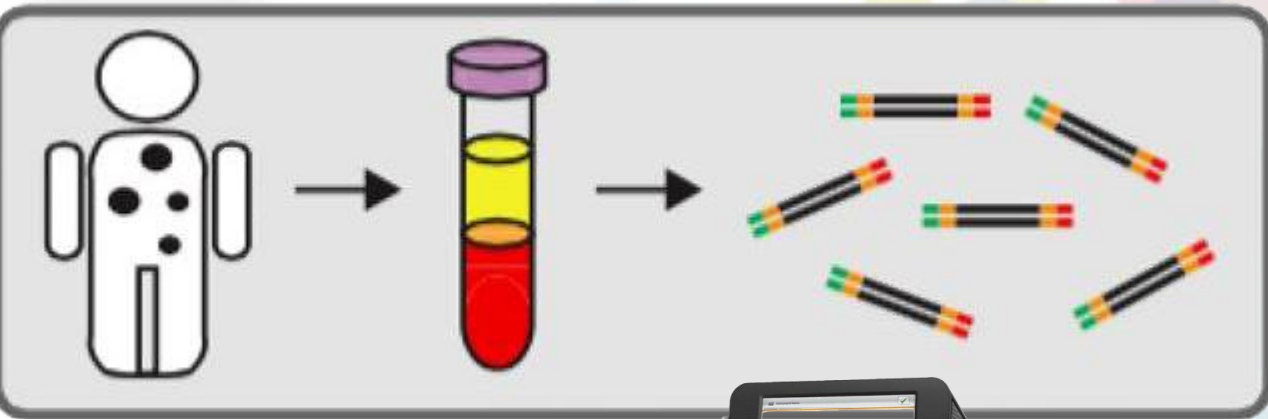
High coverage

Low coverage



Low pass WGS

General strategy





Cohort Selection

Pandora selection

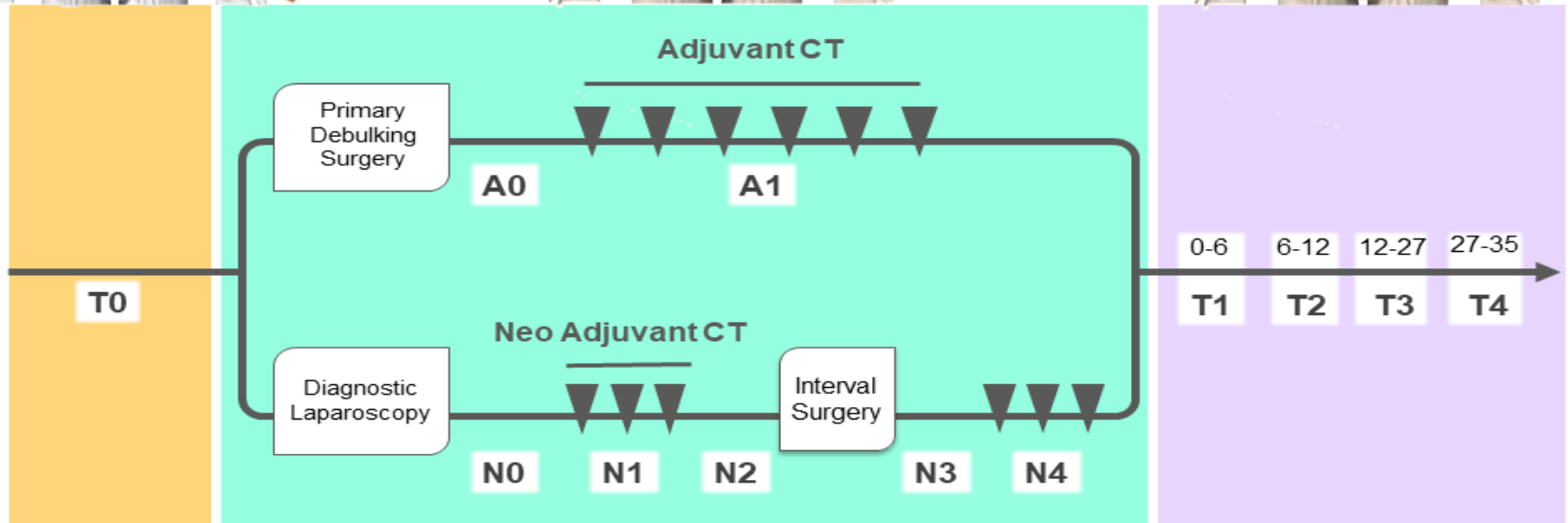
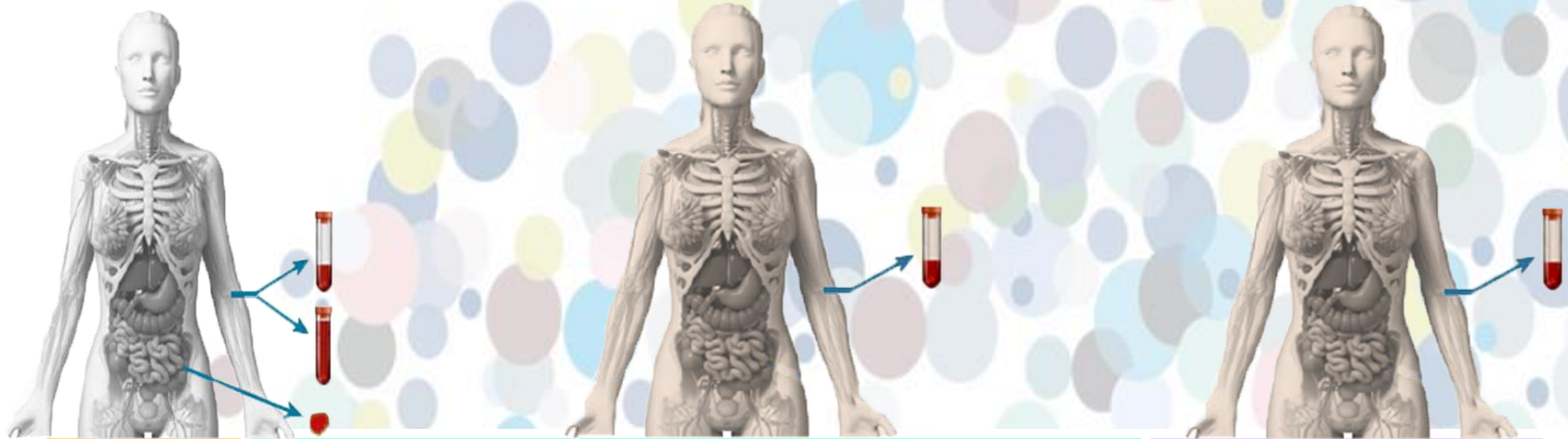
Retrospective study

- ✓ **46** HGS-EOC patients
- ✓ **109** solid tumor biopsies (average 2 per patient)
- ✓ **185** plasma samples (average 4 per patient)

Clinical and demographic characteristics	
Clinical annotations	N. of patients (%)
Number of patients	46
Median Age (years)	62
Age Range (years)	21-81
Follow up time (range) years	2 (0-4)
Hystological type	
Serous	46 (100)
FIGO classification	
III NA	-
III A	-
III B	1 (2)
III C	29 (62)
IV	16 (36)
Platinum Status	
Sensitive	24 (52)
Partially sensitive	10 (22)
Resistant	5 (11)
Refractory	5 (11)
NA	2 (4)
Chemotherapy	
Neo-adjuvant chemotherapy (NACT)	24 (52)
Adjuvant chemotherapy (CT)	22 (48)
Line of chemotherapy	
I	46 (100)
II	33 (71)
III	20 (43%)
BRCA status	
gBRCA1 mut	7 (15)
gBRCA2 mut	3 (7)
BRCA wt	13 (28)
NA	23 (50)
CA125 levels at time of diagnosis	
CA125 < 35 U/mL	2
CA125 > 35 U/mL	38
Interquartile range	264-2581
Total number of biopsies	109
Average biopsies per patient (range)	2 (0-6)
Total number of plasma samples	185
Average plasma samples per patient (range)	4 (2-8)

Study Workflow

Liquid biopsy



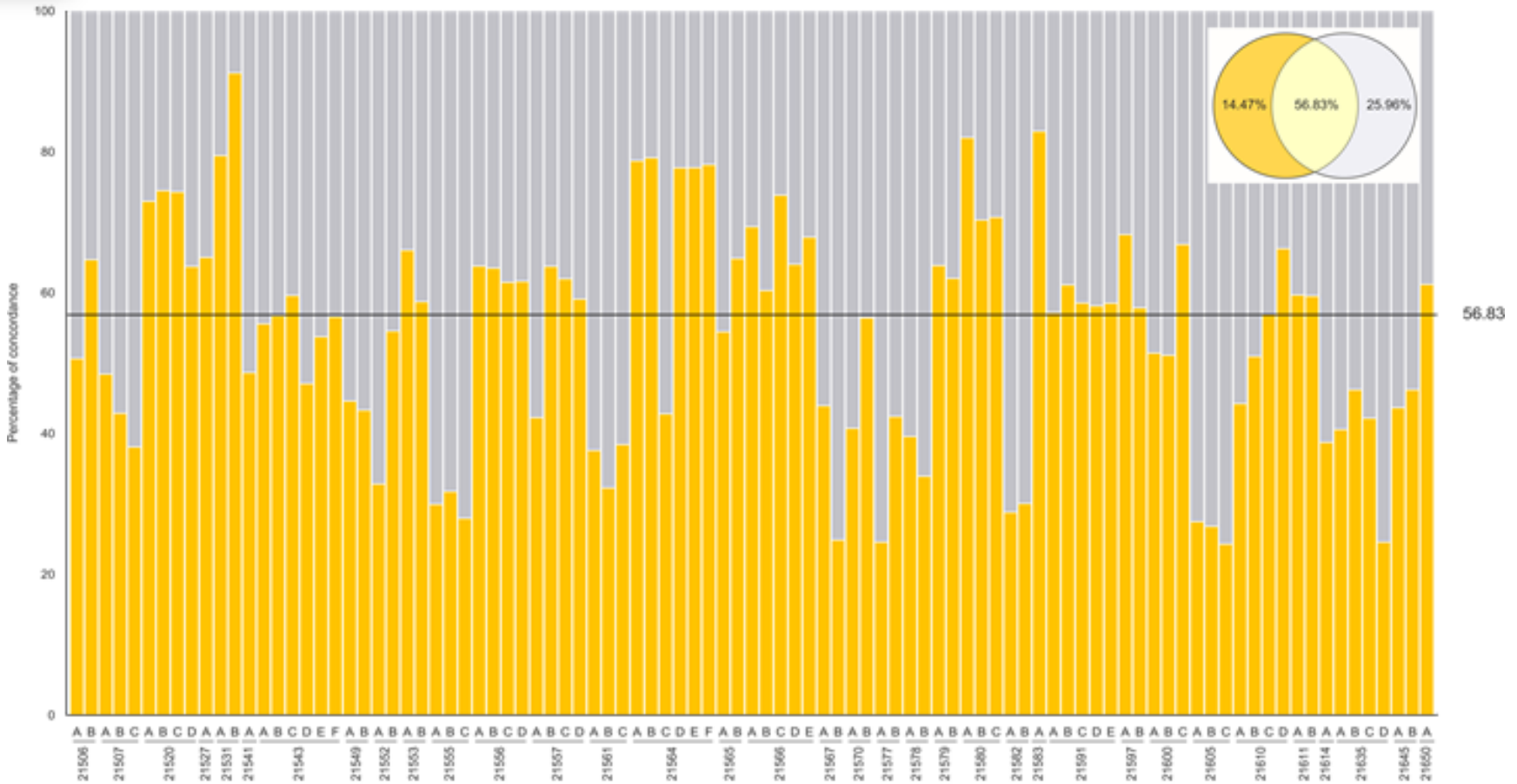
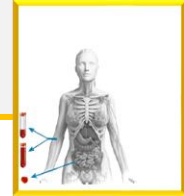
Basal

Treatment

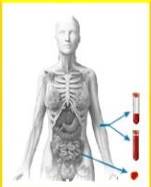
Monitoring

Concordance analysis

ctDNA vs tDNA



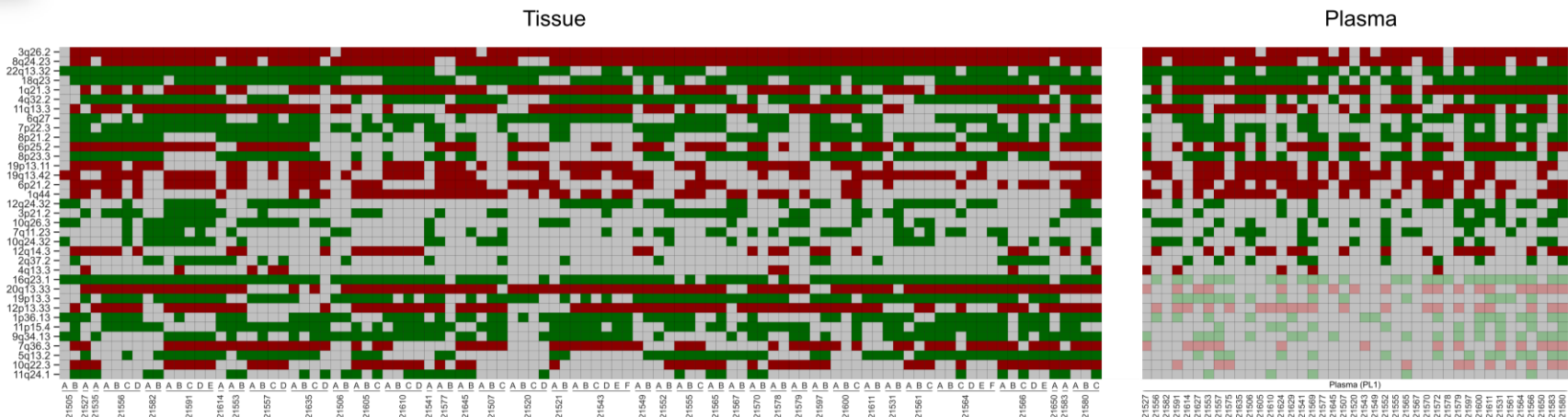
At basal level, 56,8% of identified genomic regions in gain or loss are in common between plasma and matched tumor biopsies



Concordance analysis

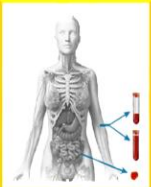
Basal

B

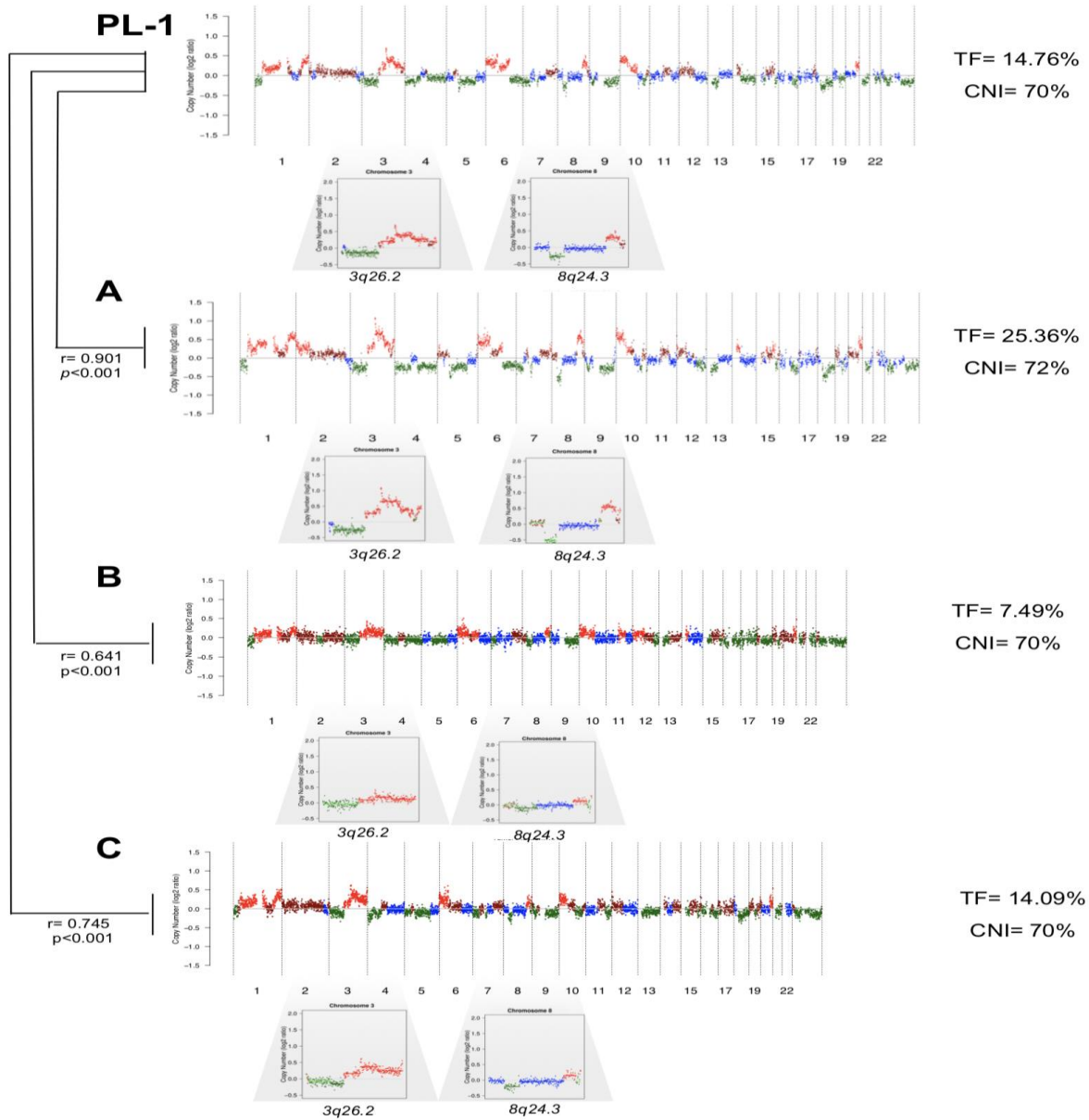


Chromosome	Cytoband	Start	End	Length (Mbp)	Type	Frequency tissue	Frequency plasma	p-value
chr3	3q26.2	162990001	185010000	22.02	Amp	97%	87.8%	0.12
chr8	8q24.23	122490001	145138636	22.65	Amp	89%	78.05%	0.18

- i. GISTIC analysis identified 35 recurrent genomic regions between ctDNA and tDNA.
- ii. Of these, 33 have been previously annotated in TCGAtlas, as a hallmark of HGS-EOC.
- iii. 3q26.2 and 8q24.3 were the most frequently observed in matched plasma and tumor biopsies



Conclusions

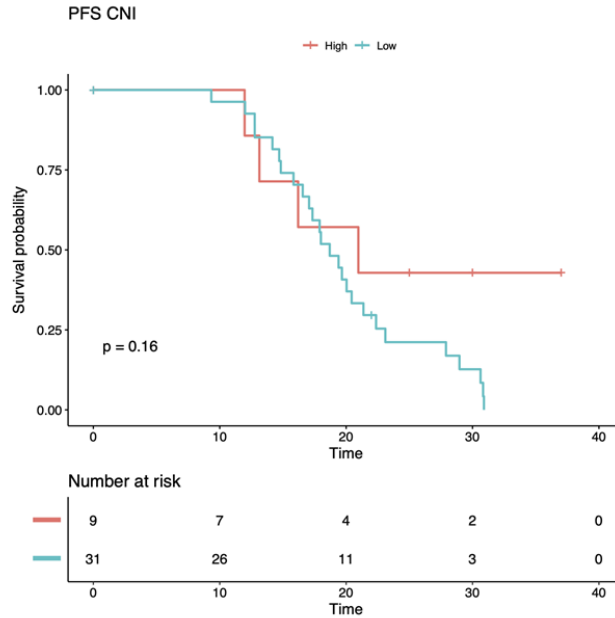


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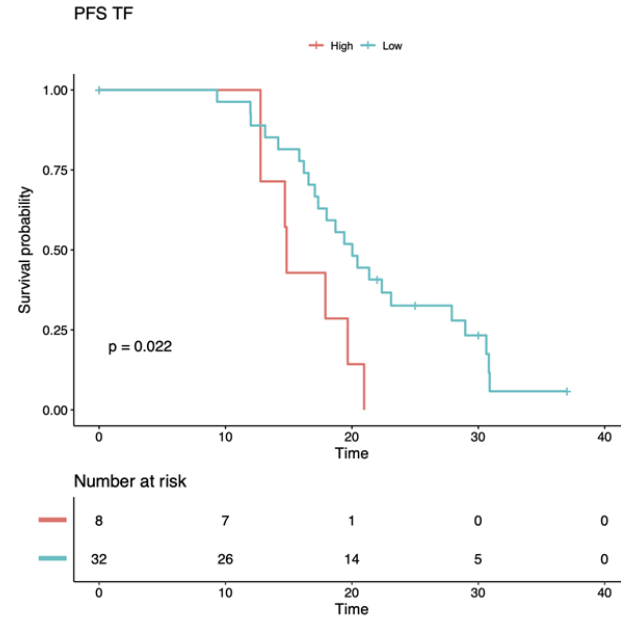
Treatment



A



B



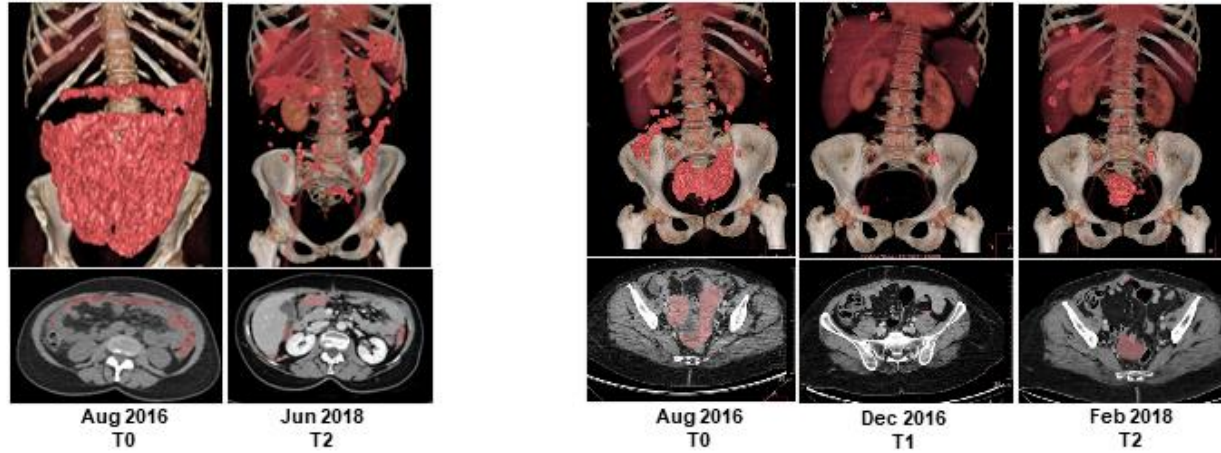
The %TF in the ctDNA at basal is independently associated to PFS



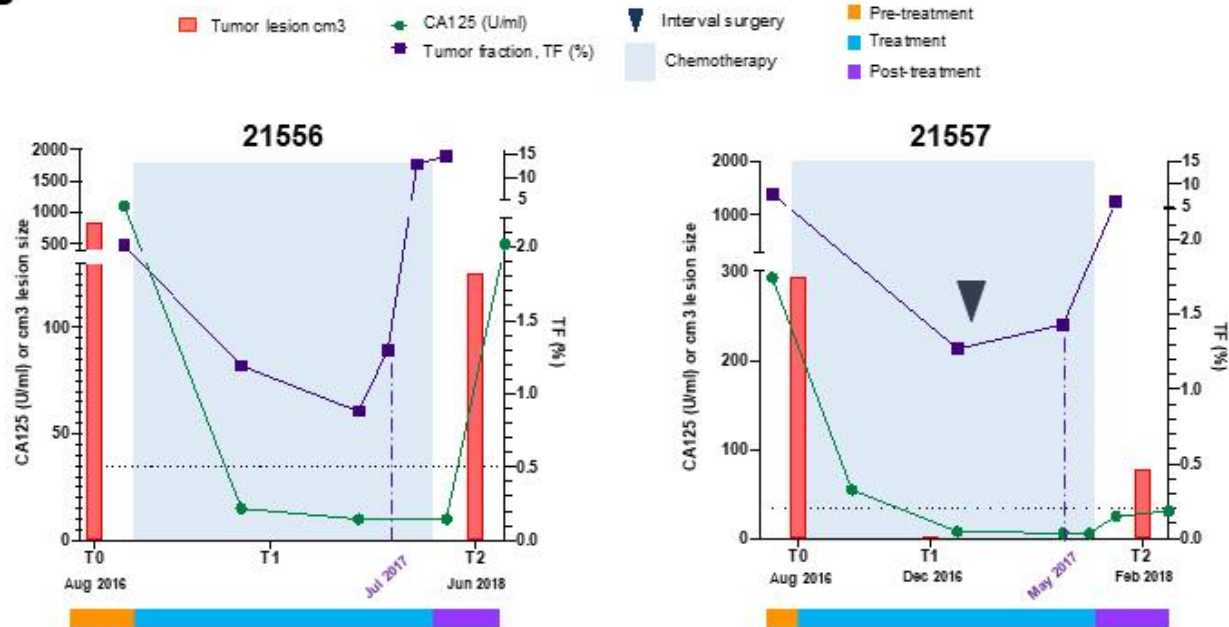
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Post Treatment: NACT

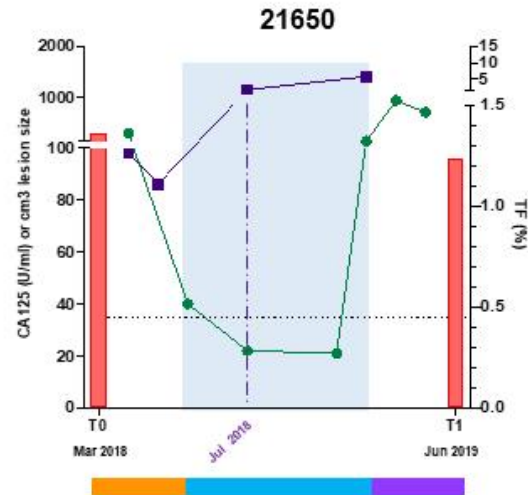
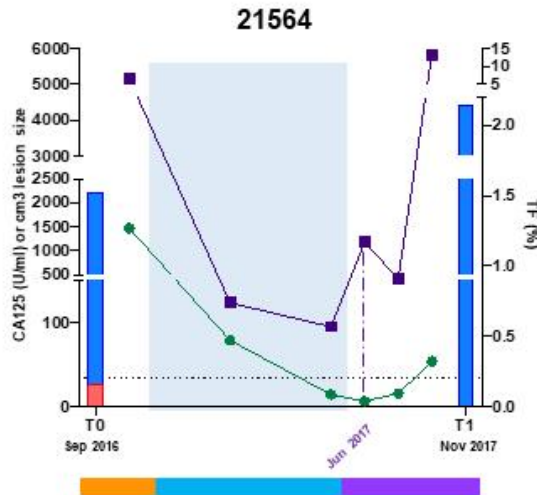
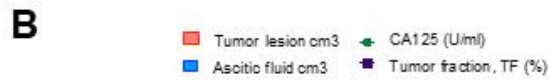
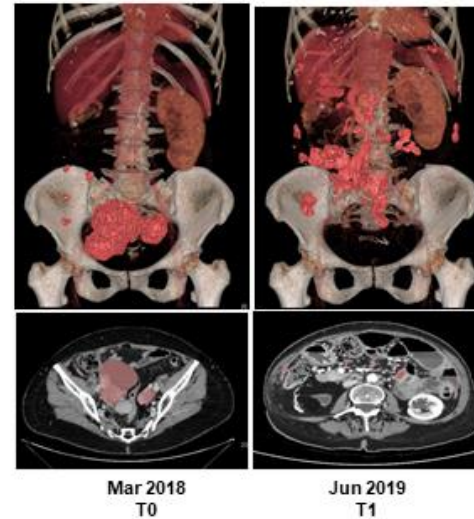
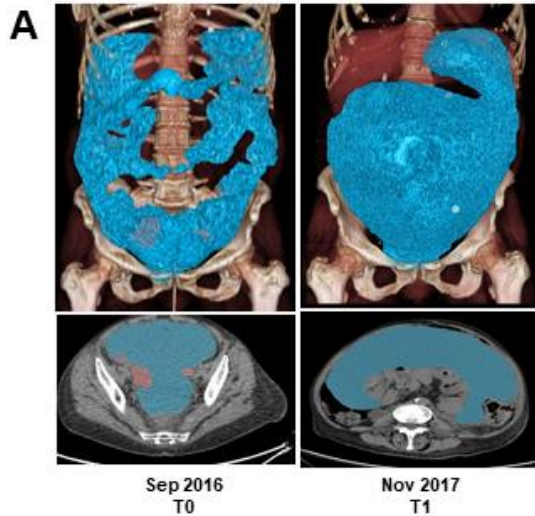
A



B

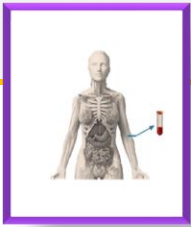


Epithelial Ovarian Cancer



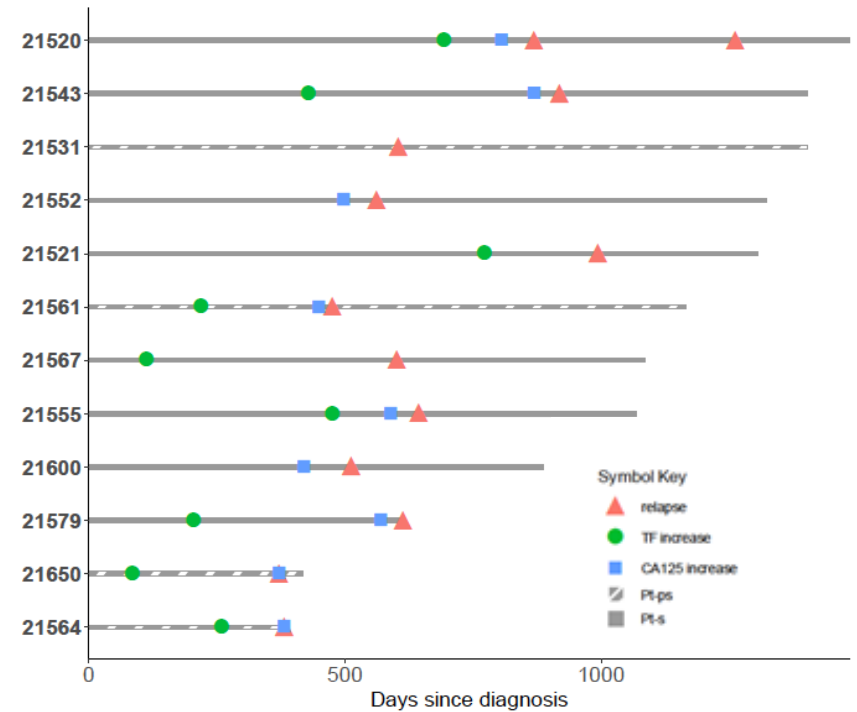
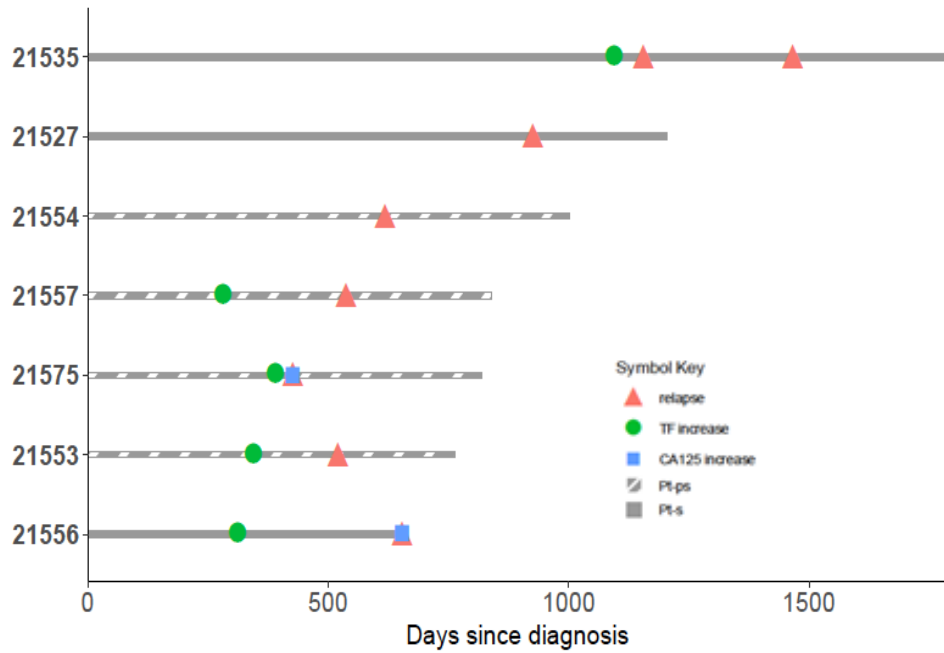
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SWIMMER PLOTS



NACT

CT

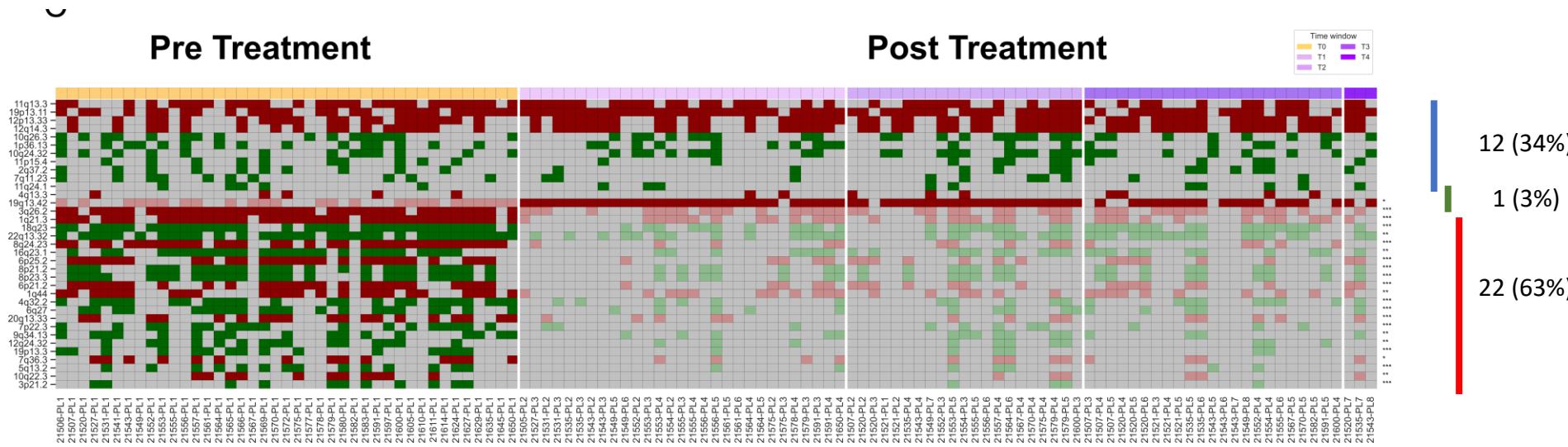


TF outperformed CA-125 in anticipating clinical and radiological progression by 240 days (37-491)



Clonal evolution

Dynamic changes in the clonal heterogeneity composition of HGS-EOC is a critical therapeutic challenge confounding treatment of relapsed disease.

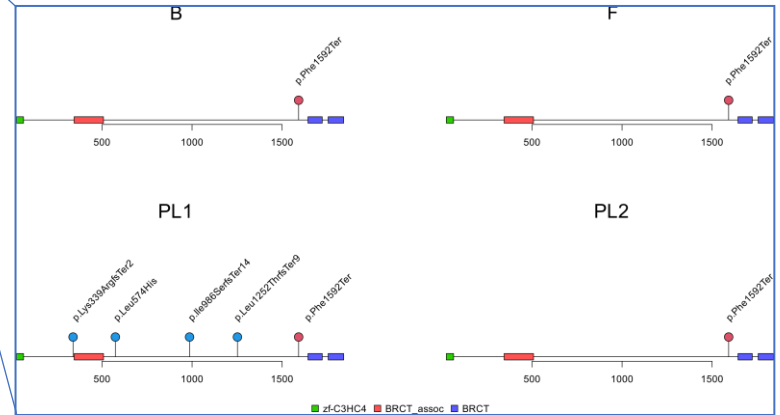


Group	Chromosome	Cytoband	Start	End	Length (Mbp)	Type	Frequency Plasma (T0)	Frequency Plasma (follow up)	pvalue (T0 vs follow up)	pvalue (follow up vs T0)
Blue	chr11	11q13.3	63990001	78510000	14,52	Amp	68.29%	56.58%	0.21	1
	chr19	19p13.11	13490001	23010000	9,52	Amp	65.85%	85.53%	1	0.16
Green	chr19	19q13.42	49490001	58617616	9,13	Amp	60.98%	86.84%	1	0.05

The genomic landscape composition of post-treatment is less heterogeneous than that reported in paired pre-treatment samples

Preliminary Data

SNV analysis



21543-BRCA1



Take Home Message

- ✓ As a proxy for tumor tissue profiling, successful blood biopsy analysis can help to select appropriate patients for clinical trials, provide useful data for treatment monitoring, and discover genomic features of relapsed disease.
- ✓ **TF** and **CNI** parameters can be derived from shallow whole genome sequencing and can be used to:
 - predict time to relapse better than CA-125
 - Dissect the biology of relapsed disease (at both SNV and SCNA level)
 - Independent prognostic biomarker
- ✓ We do strongly believe that , after additional validation experiments, sWGS analysis of ctDNA should be enter into MaNGO in clinical trial as:
 - Cheap
 - Short tourn-around time
 - Informative on the biology and outcome of HGS-EOC patients



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