Detection of homologous recombination deficiency using cell-free DNA whole-genome sequencing profile in ovarian cancer

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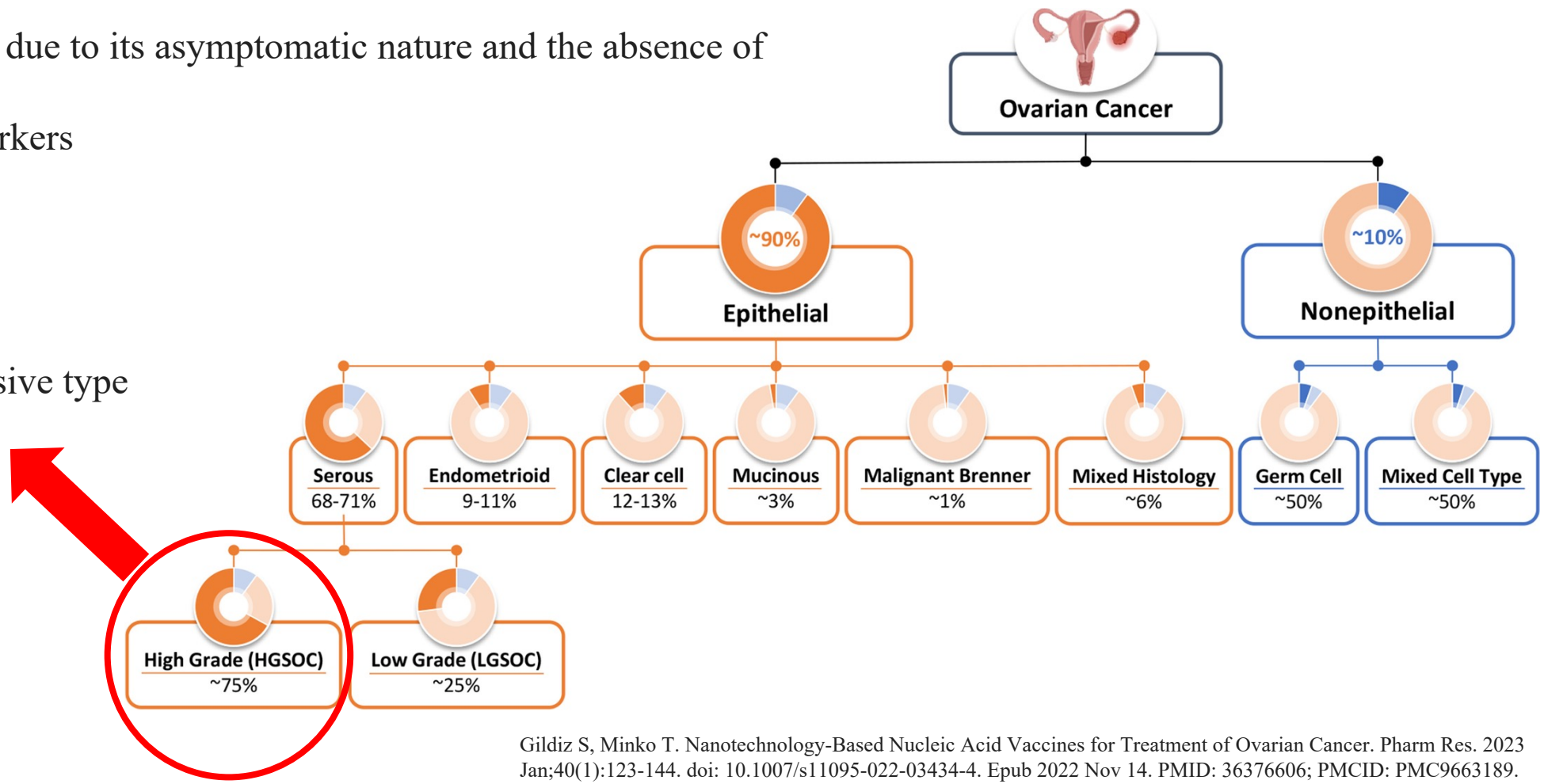
Background: Ovarian cancer

- Ovarian cancer - gynecological malignancy with the highest mortality rate
- **5-year survival rate** of approximately 50%
- **Diagnosis**: at later stages - due to its asymptomatic nature and the absence of efficient early-stage biomarkers

most frequent and aggressive type

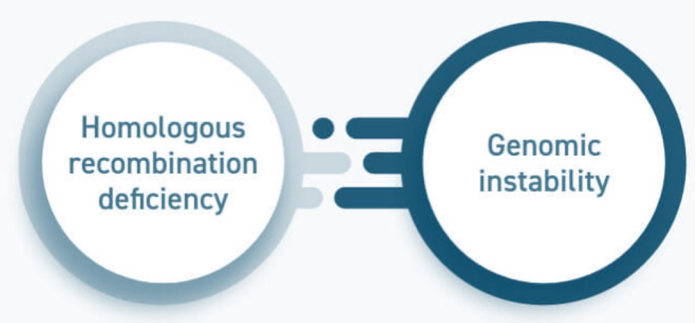


Gildiz S, Minko T. Nanotechnology-Based Nucleic Acid Vaccines for Treatment of Ovarian Cancer. Pharm Res. 2023 Jan;40(1):123-144. doi: 10.1007/s11095-022-03434-4. Epub 2022 Nov 14. PMID: 36376606; PMCID: PMC9663189.

Background: Homologous recombination deficiency (HRD)

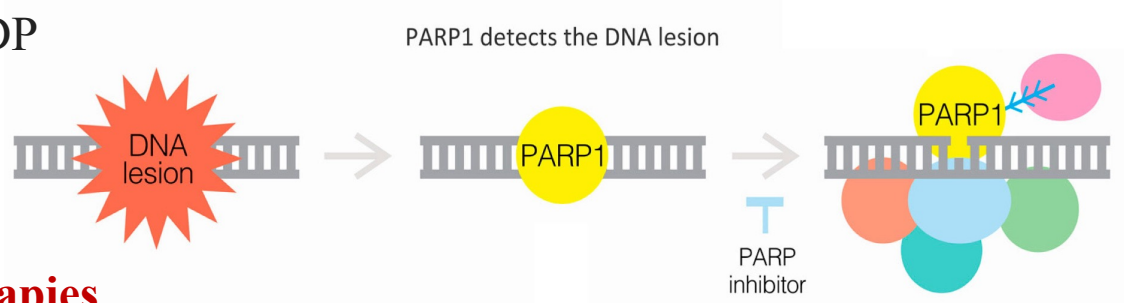
Homologous recombination deficiency:

- Approximately 50% of HGSOC exhibit HRD
- HRD prevents cells from repairing double-stranded DNA damage with high fidelity – leading to the accumulation of DNA damage and **genomic instability** known as homologous recombination deficiency



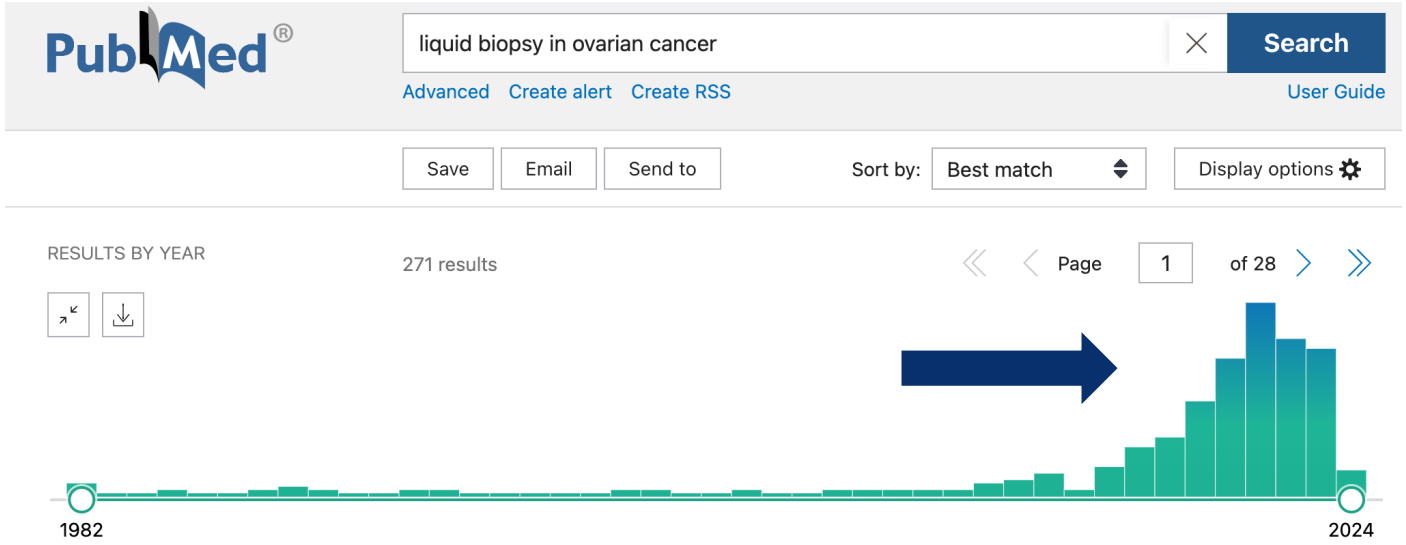
PARP inhibitor therapies:

- Cancer cells with HRD are sensitive to targeted inhibition of poly-ADP ribose polymerase (PARP)
- **Identifying** patients with cancer with **HRD** biomarkers allows the identification of patients likely to **benefit from PARP inhibitor therapies**



https://www.cancer.gov/news-events/cancer-currents-blog/2020/fda-olaparib-rucaparib-prostate-cancer

Background: Liquid biopsy in ovarian cancer



- Currently, determining HRD status is analyzed by **expensive** and **time-consuming genomic profiling** from **tissue** samples
- Unfortunately, HRD testing on formalin-fixed, paraffin-embedded (FFPE) tumor samples yields **non-contributive results** in substantial cases **due to low tumor cellularity or poor-quality DNA**

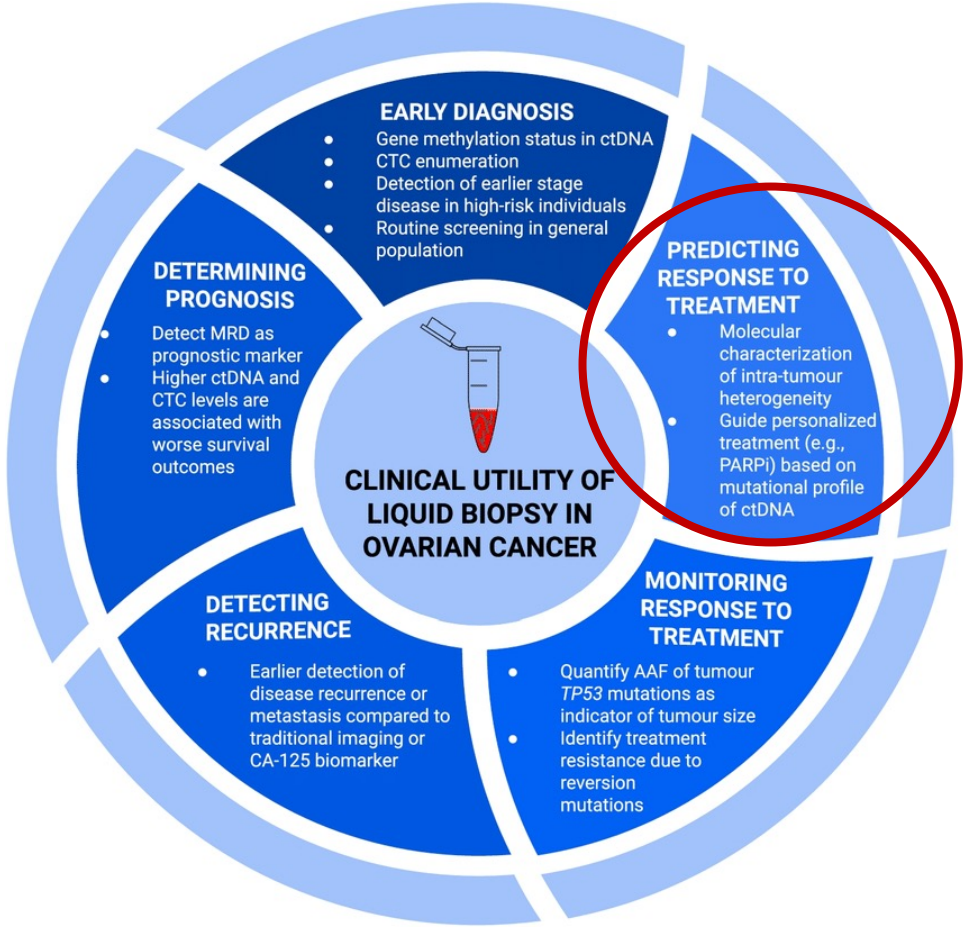
Therefore, the development of new, broadly applicable cost-effective methods is of great importance.

The application of circulating tumor cell and cell-free DNA liquid biopsies in ovarian cancer

Abigél Balla [1], Jong Bhak [2], Orsolya Biró [3]

Affiliations + expand

PMID: 36283501 DOI: 10.1016/j.mcp.2022.101871



Zhu JW, Charkhchi P, Akbari MR. Potential clinical utility of liquid biopsies in ovarian cancer. Mol Cancer. 2022 May 11;21(1):114. doi: 10.1186/s12943-022-01588-8. PMID: 35545786; PMCID: PMC9092780.

Objective - determining HRD status

Objective of this study:

To investigate the potential of **shallow whole-genome sequencing** (sWGS)

on **cell-free DNA** (cfDNA)

for **therapy optimization** in ovarian cancer

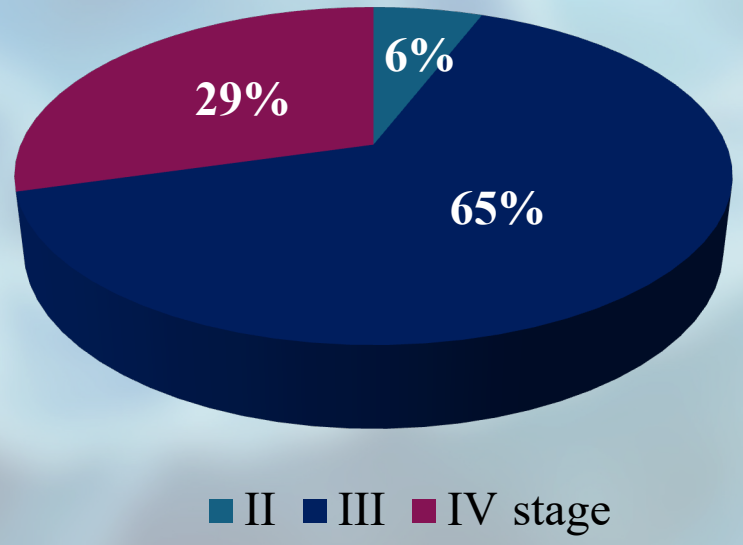
Sample collection, method

- 17 histologically confirmed HGSOC samples + 8 control samples
- K2EDTA whole blood
- Average age: ~60 years

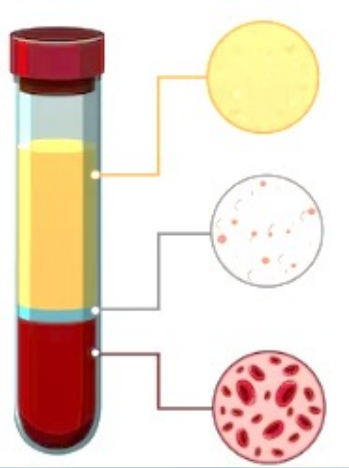
Sample collection

1
2
3
4
5

Distribution of HGSOC samples by FIGO stage



Sample collection, method



cfDNA extraction from plasma samples

Kit: QIAamp Circulating Nucleic Acid Kit (Qiagen)

Sample collection 1

cfDNA extraction 2

3

4

5

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Sample collection, method

Clinomics Europe

Sample collection 1

cfDNA extraction 2

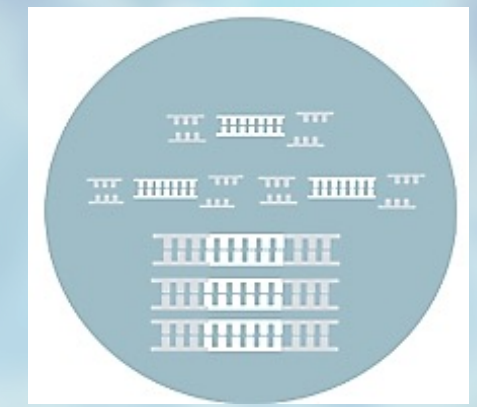
Library preparation 3

4

5

Library preparation

Kit: TruSeq DNA Nano kit (Illumina)



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Sample collection, method

Clinomics Europe

1. Sample collection
2. cfDNA extraction
3. Library preparation
4. **Sequencing**
5.

Shallow whole genome sequencing (sWGS)

Illumina NextSeq 2000 platform

(~1x coverage)

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Sample collection, method

Clinomics Europe

1. Sample collection
2. cfDNA extraction
3. Library preparation
4. Sequencing
5. **Data analysis**

Data analysis:

ichorCNA software

Clinomics Europe

ichorCNA software

Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors

Viktor A. Adalsteinsson, Gavin Ha, Samuel S. Freeman, Atish D. Choudhury, Daniel G. Stover, Heather A. Parsons, Gregory Gydush, Sarah C. Reed, Denisse Rotem, Justin Rhoades, Denis Loginov, Dimitri Livitz, Daniel Rosebrock, Ignaty Leshchiner, Jaegil Kim, Chip Stewart, Mara Rosenberg, Joshua M. Francis, Cheng-Zhong Zhang, Ofir Cohen, Coyin Oh, Huiming Ding, Paz Polak, Max Lloyd, ... Matthew Meyerson

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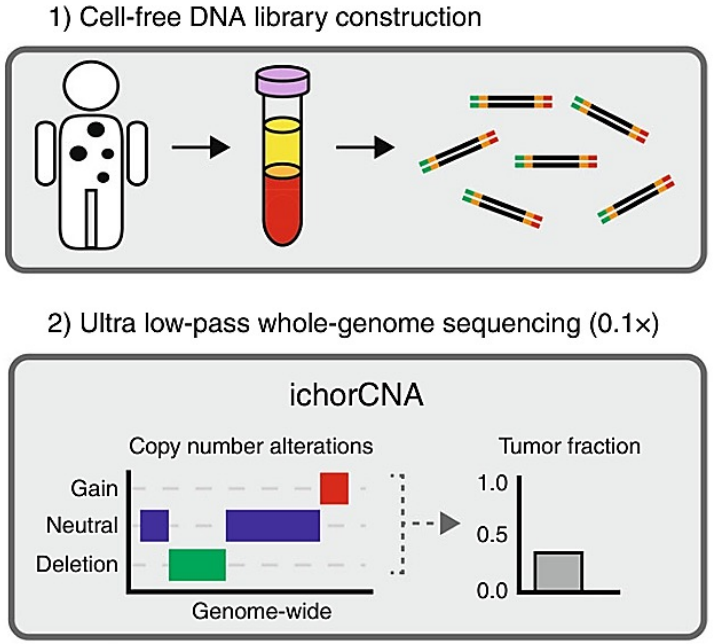
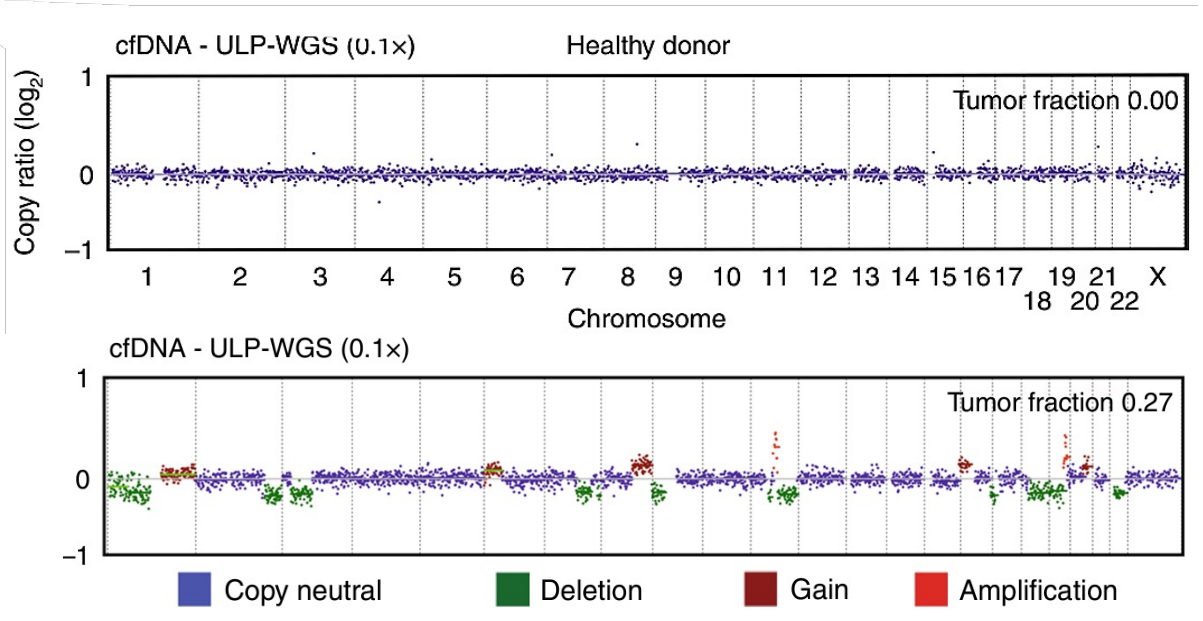
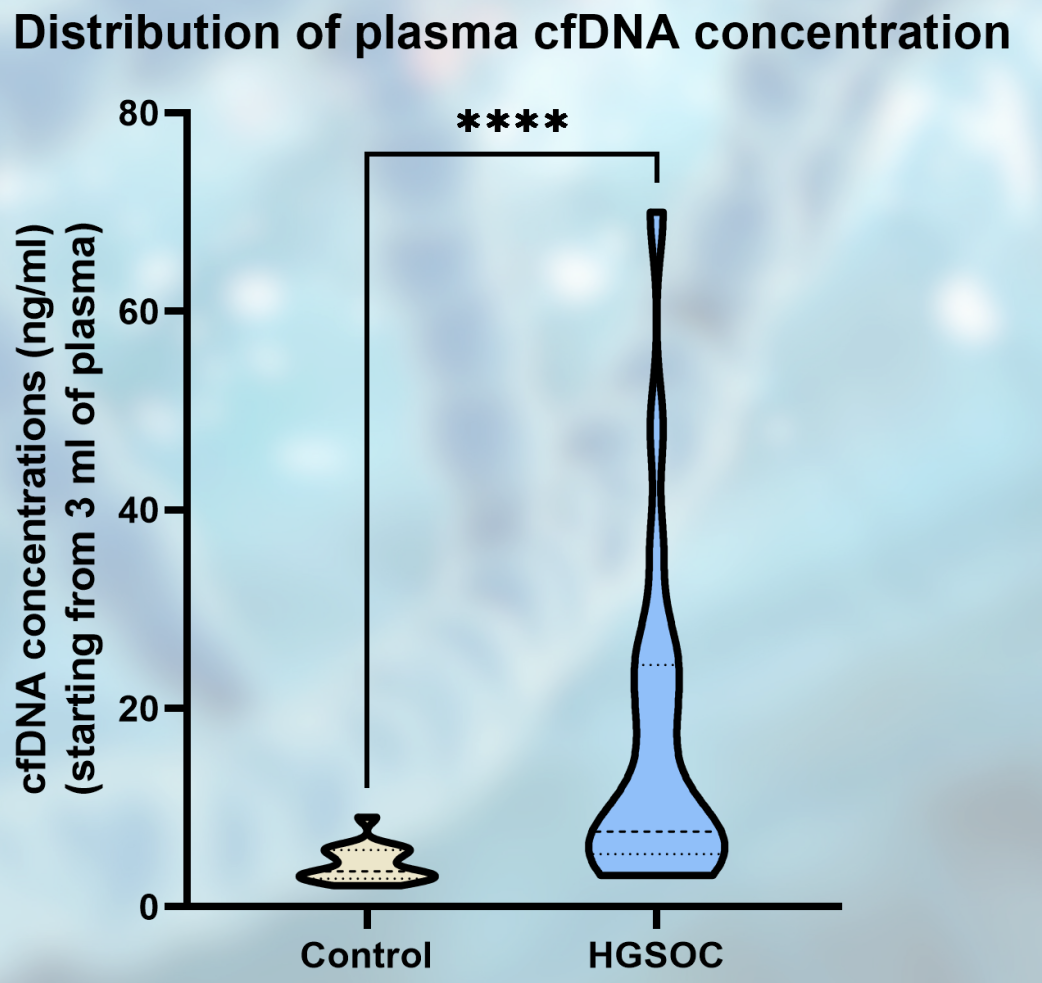
Nature Communications **8**, Article number: 1324 (2017) | Cite this article



- first reported the ichorCNA software
- feasibility of shallow whole genome sequencing of cfDNA
- ichorCNA:
  - **Copy number alteration** (CNA) prediction
  - estimation of **tumor fraction** of cfDNA



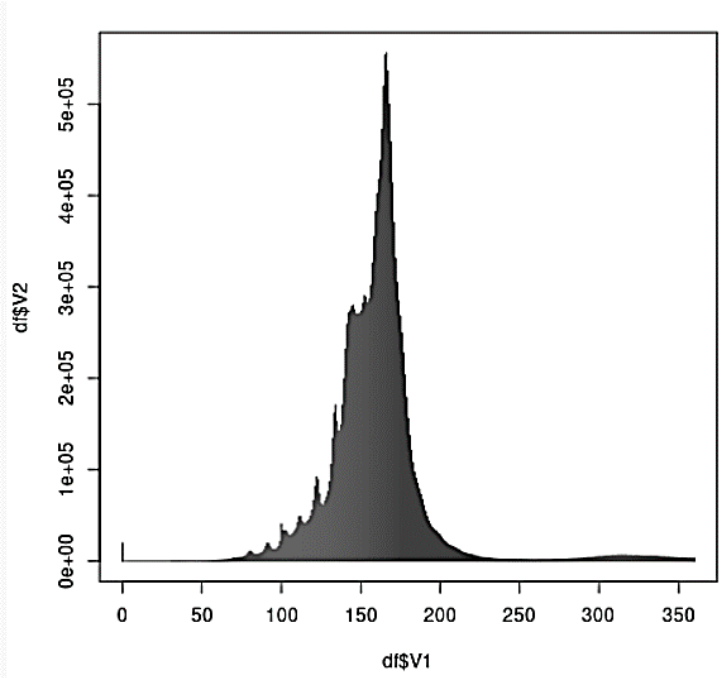
Results: cfDNA concentrations



Based on cfDNA concentrations, the HGSOC patient and control groups are **significantly different**.

Results – Fragment size examination

Fragment length distribution



Mean fragment size comparison:

- Mean of **ovarian cancer** samples: **172,31** bp
- Mean of **controls**: **177,25** bp

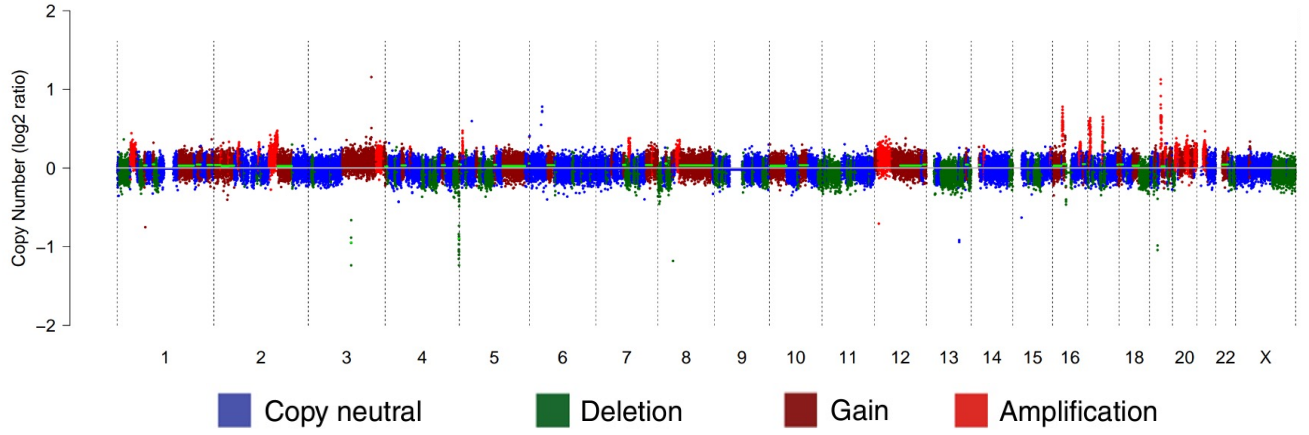
Welch Two sample t-test: t = -2.8197, df = 22.555, p-value = 0.009832

- the **average length of cfDNA fragments** is typically longer than the size of tumor-derived fragments
- **significant differences** between cfDNA fragments from control and HGSOC samples

Results - Identification of copy number variations and HRD status

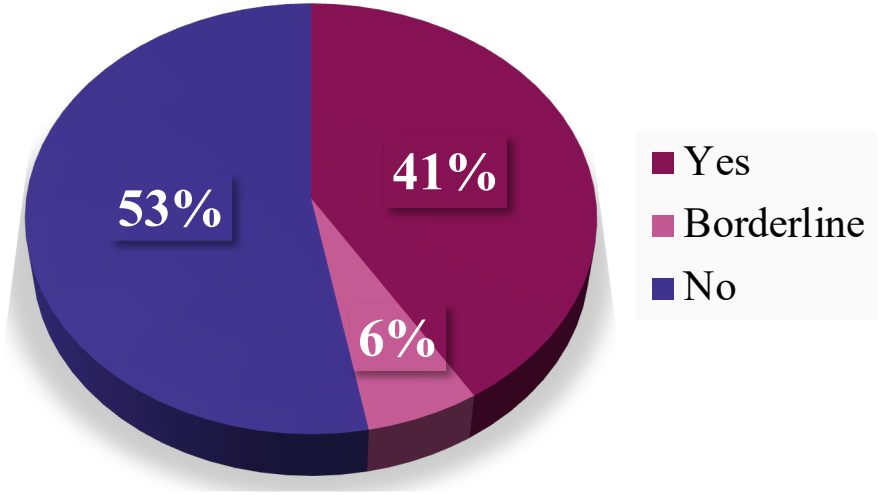
- **Large Genomic Alteration:**
  CNA length ≥ 10 mbp
- **HRD:**
  cut-off value: LGA > 20 variants

Representative figure of genome-wide copy number from sWGS (~1x coverage):



HRD status	Number of samples	LGA variants range
Yes	7	25-48
Borderline	1	19
No	9	0-7

Distribution of HRD status



Conclusion

Shallow whole genome sequencing of cell-free DNA → **cost-effective method** → **selection of personalized therapy for ovarian cancer patients based on their HRD status**

Further plans:

- Comparison of our HRD results with results from tissue samples
- detecting HRD status in follow-up HGSOC plasma samples
- further investigations are planned on a larger patient cohort

Thank you for your attention!

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Contacts

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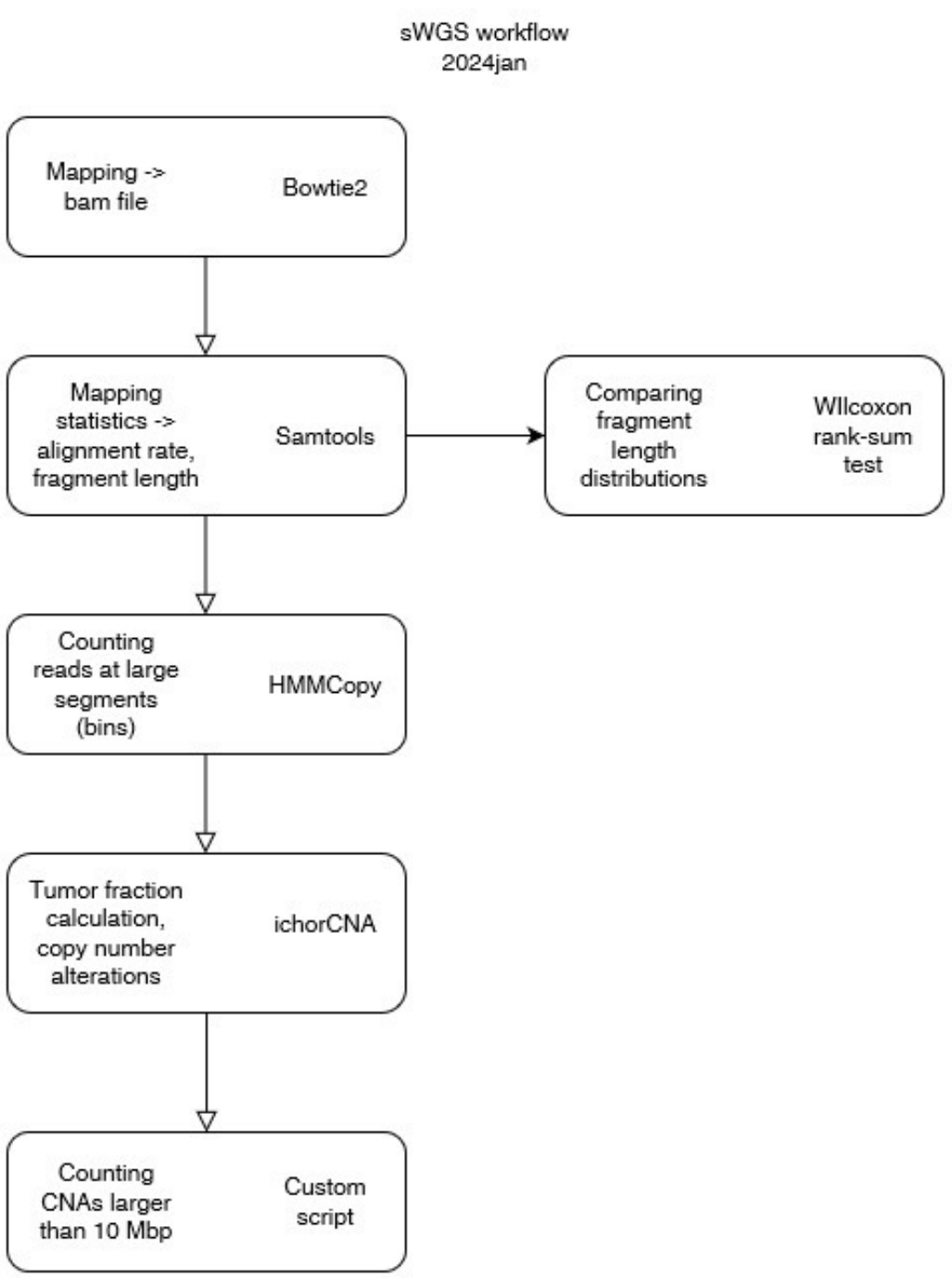
https://clinomicsdiag.hu/en/



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