Diagnosi precoce del carcinoma ovarico in donne sane portatrici di varianti patogenetiche in BRCA1 e BRCA2

Dalle linee guida ai biomarcatori cervicovaginali

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Cancer Pharmacology

HUMANITAS
RESEARCH HOSPITAL

Verona, 7 marzo 2025

Pathogenic germline variants in BRCA1 or BRCA2 genes

75.000 Italian women carry PVs in BRCA1 (chr 17)or BRCA2 (chr 13) genes

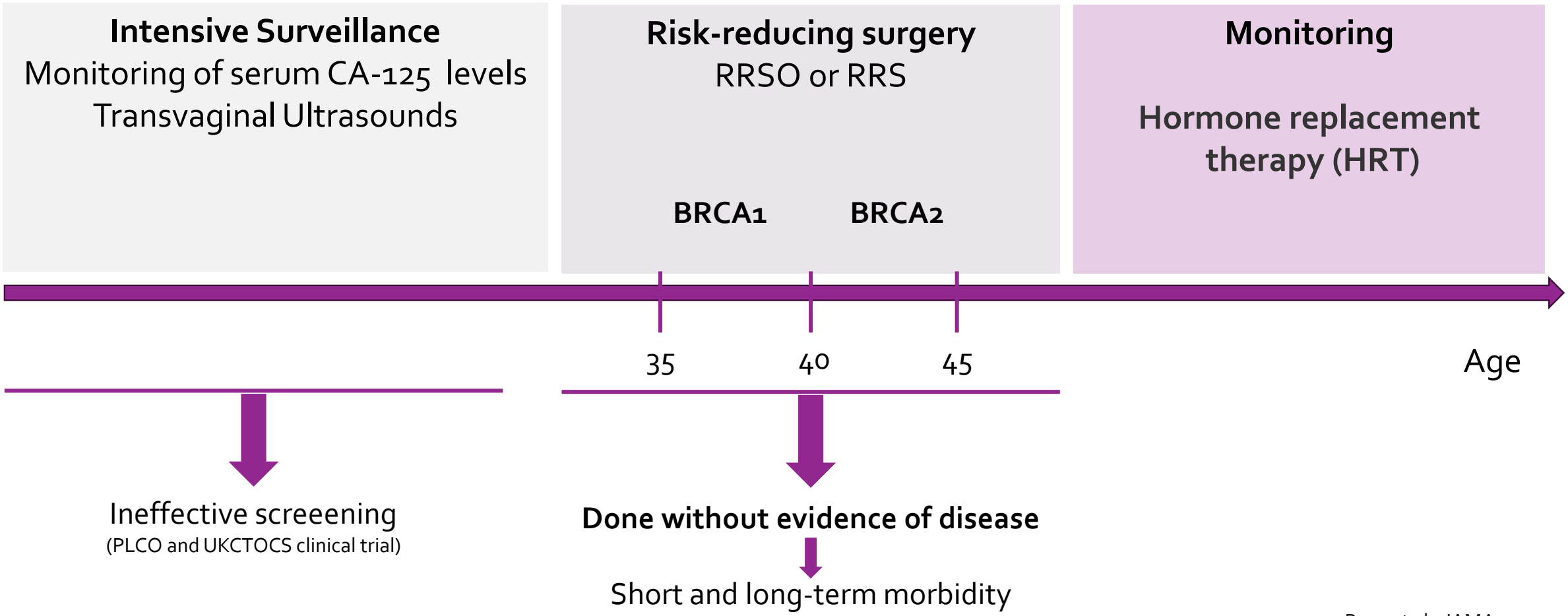
Cancer risk

	BRCA1	BRCA2	General Population
Female breast cancer	50-65%	40-55%	10-13%
Ovarian cancer	**39-58%**	**13-29%**	**1.2-2%**
Pancreatic cancer	5%	5-10%	1.7%

Source: NIH: BRCA Gene Changes: Cancer Risk and Genetic Testing

Clinical Guideline

Management of Increased Cancer Risk in Healthy Women with BRCA1 BRCA2 PV

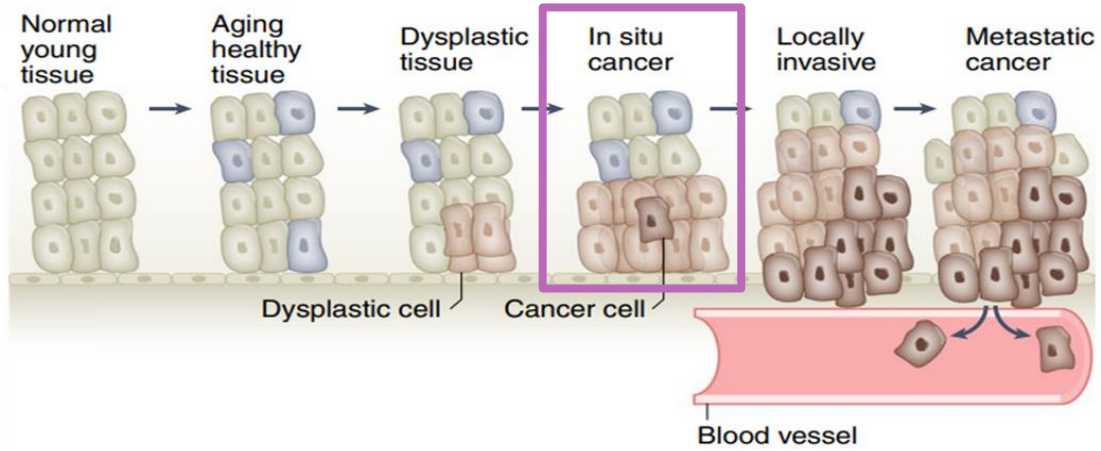


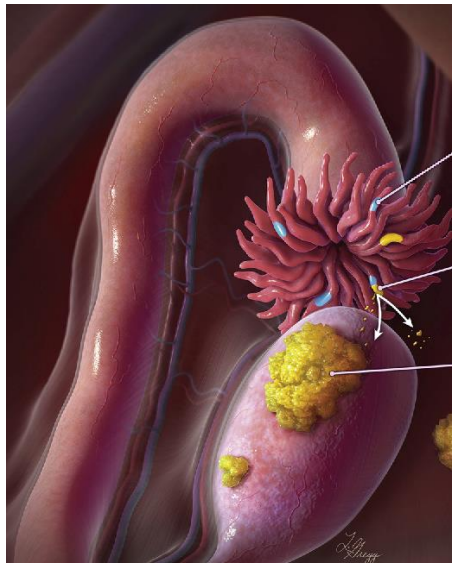
Buys et al., JAMA 2011
Menon et al., The Lancet 2021

Clinical Need

We need to find a new approach based on molecular analysis
to intercept the early phases of disease

Optimal Timing for Intervention





Benign lesion

Serous Tubal Intraepithelial Carcinoma (STIC)

HGS-EOC

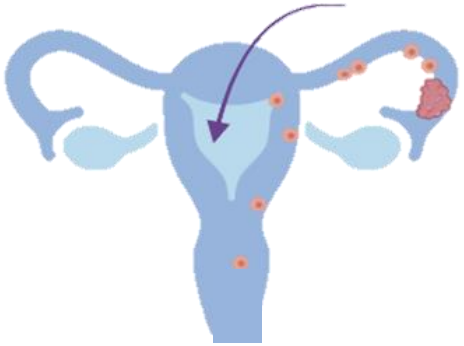
Molecular features

- Clonal pathogenic *TP53* mutation
- Genomic Instability
- Methylation Changes
- …

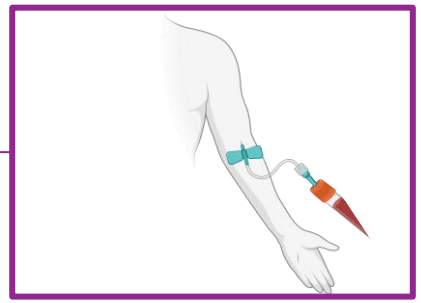
Modified from Shih et al., American Journal of Pathology 2021

Biological Sources for STIC Detection

Backword Flow

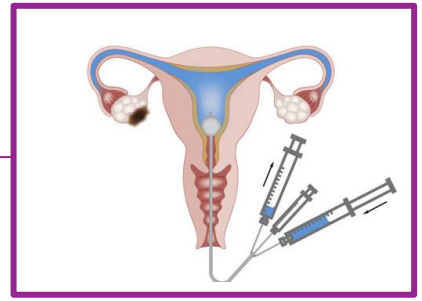


STIC



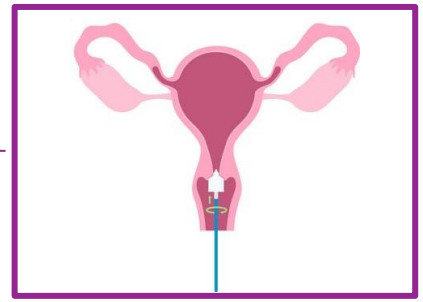
Circulating-tumor DNA detection (Medina et al., Cancer Discovery 2025)

- Fragmentomics + Protein Biomarkers (CA-125 and HE4)
- 94 Ovarian cancer (stage I, II, III, IV), 203 benign masses, 182 healthy
- Specificity >99%, Sensitivity 72-100%
- Women who already have ovarian cancer ➡ **Late diagnosis**



Uterine Lavage (Bahar-Shany et al., IJC 2023)

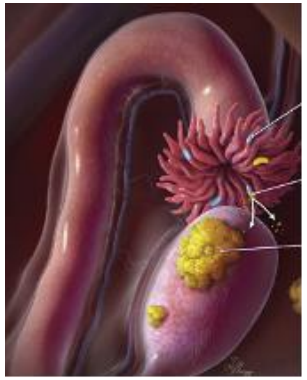
- 7-protein diagnostic signature
- Ovarian cancer (stage II/II e III/IV), healty gBRCA mut and WT
- AUC >0.97, NPV >99%
- Uterine Lavage ➡ **Invasive procedure**



Pap test

- Is the pap test a suitable source for STIC-DNA detection?
- What is the best strategy to intercept the molecular aberrancies associated with STIC?

Pap test: TP53 mutational analysis



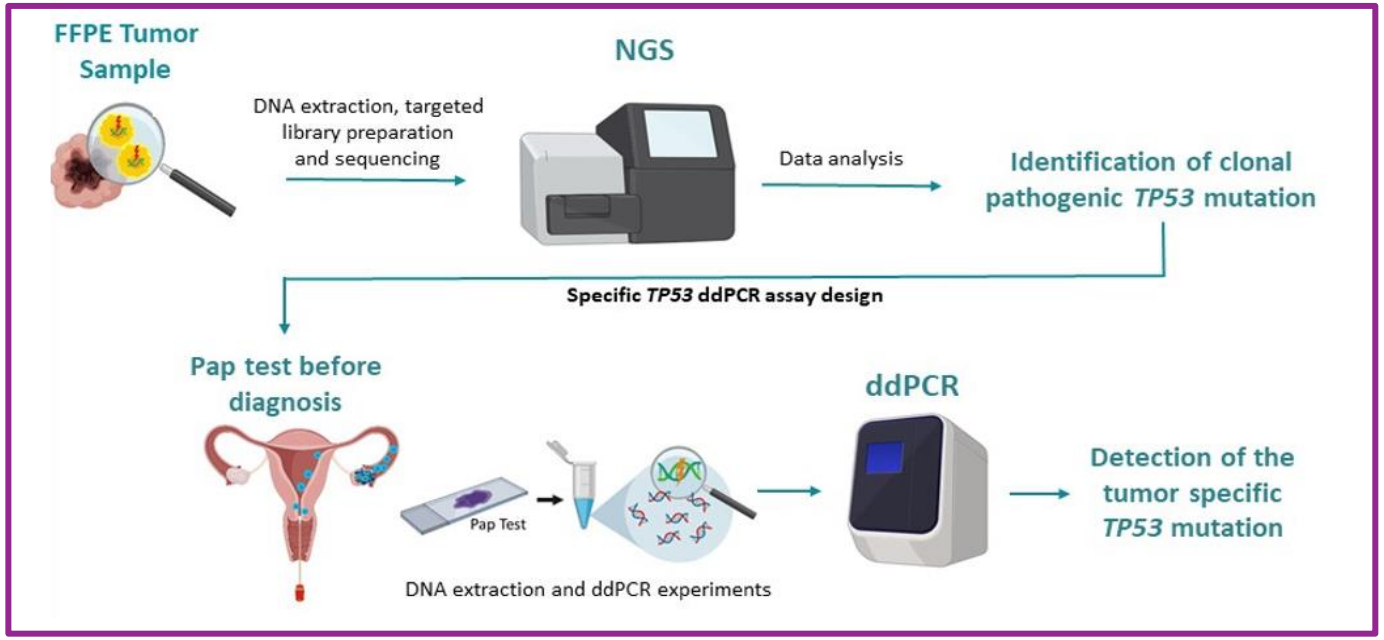
Benign lesion

Serous Tubal Intraepithelial Carcinoma (STIC)

HGS-EOC

Molecular features

- Clonal pathogenic *TP53* mutation
- Altered copy number profile (genomic instability)



OPEN

Detecting *TP53* mutations in diagnostic and archival liquid-based Pap samples from ovarian cancer patients using an ultra-sensitive ddPCR method

Nicolai Skovbjerg Arildsen[1], Laura Martin de la Fuente[1], Anna Måsbäck[2], Susanne Malander[1], Ola Forslund[3], Päivi Kannisto[4] & Ingrid Hedenfalk[1*]

JAMA Network Open

Original Investigation | Oncology

Detection of *TP53* Clonal Variants in Papanicolaou Test Samples Collected up to 6 Years Prior to High-Grade Serous Epithelial Ovarian Cancer Diagnosis

Lara Paracchini, MSc; Chiara Pesenti, MSc; Martina Delle Marchette, MD; Luca Beltrame, PhD; Tommaso Bianchi, MD; Tommaso Grassi, MD; Alessandro Buda, MD; Fabio Landoni, MD; Lorenzo Ceppi, MD; Cristina Bosetti, PhD; Mariachiara Paderno, MD; Marco Adorni, MD; Debora Vicini, MD; Patrizia Perego, MD; Biagio Eugenio Leone, MD; Maurizio D'Incalci, MD; Sergio Marchini, PhD; Robert Fruscio, MD, PhD

Pap test: TP53 mutational analysis

Cohort 1

Retrospective and Monocentric Cohort

N. HGS-EOC patients analyzed : 17

N. Pap test: 22

Δ time before diagnosis (y): 0-6

TP53 Detection Rate in Pap test: **64%**

Cohort 2

Retrospective and Multicentric Cohort

N. HGS-EOC patients analyzed: 51

N. Pap test: 74

Δ time before diagnosis (y): 0-10

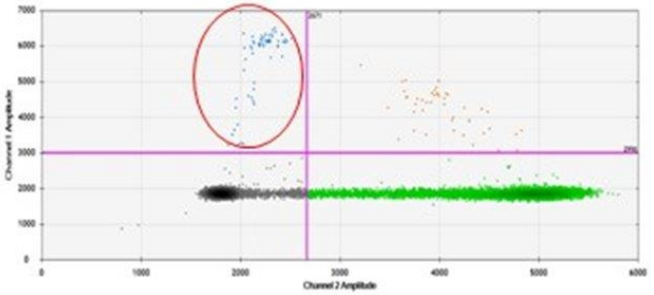
TP53 Detection Rate in Pap test: **63%**

Paracchini et al., JAMA Network Open 2020

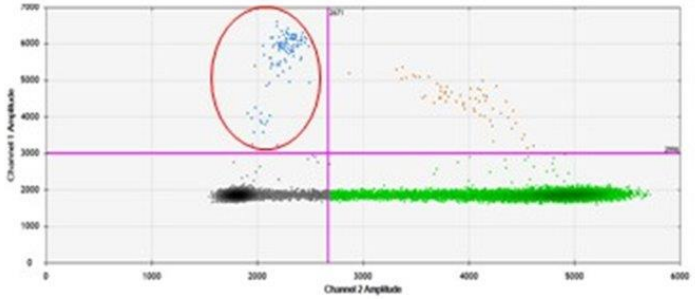
Paracchini, Mannarino et al., Science Translational Medicine 2023

Pap test: TP53 mutational analysis

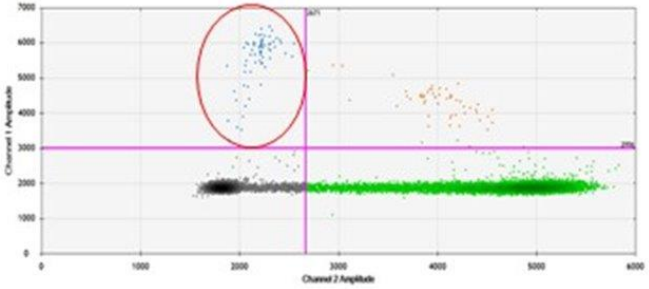
Pap test: C2010-012149 (9 years pre-diagnosis)



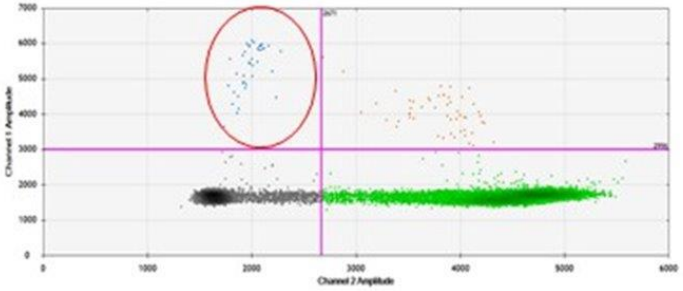
Pap test: C2010-015801 (8.5 years pre-diagnosis)



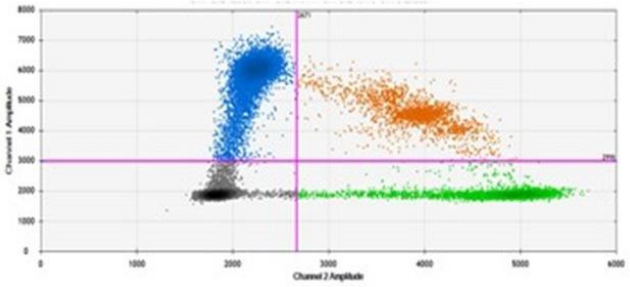
Pap test: C2013-011083 (5.5 years pre-diagnosis)



Pap test: C2016-002303 (3 years pre-diagnosis)



Tumor sample (positive control)



Paracchini et al., JAMA Network Open 2020

Pap test: TP53 mutational analysis

Conclusion

In two independent cohorts (tot n= 68) of patients we confirmed that Pap test smear is a suitable source of material to longitudinally monitor molecular feature (*TP53* mutations) characterizing early phase of malignancy up to **10 years before diagnosis**

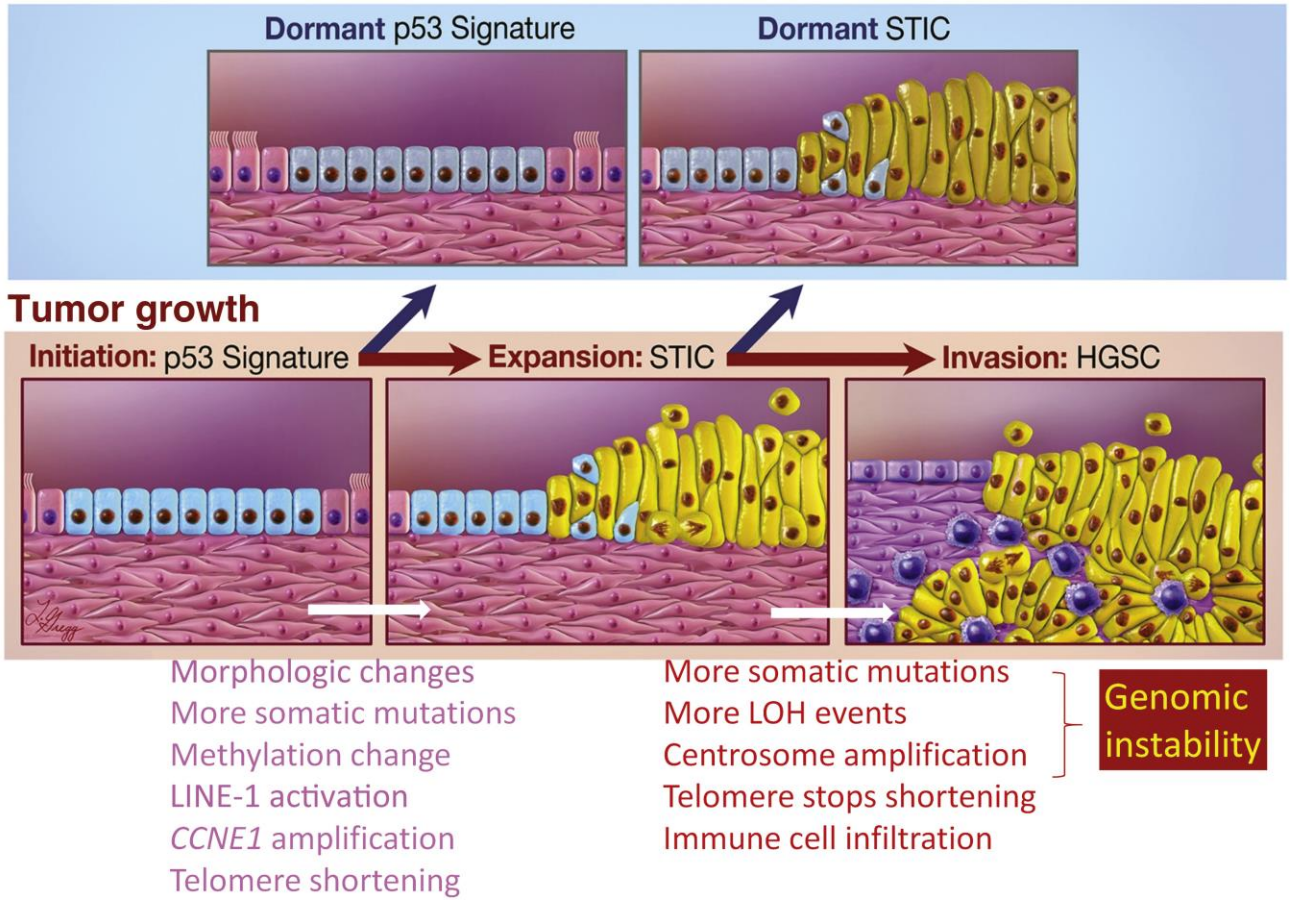
Limits

Previous knowledge about tumor-related pathogenic *TP53* mutation

Normal tissue could have somatic *TP53* mutations that do not trigger neoplastic transformation:

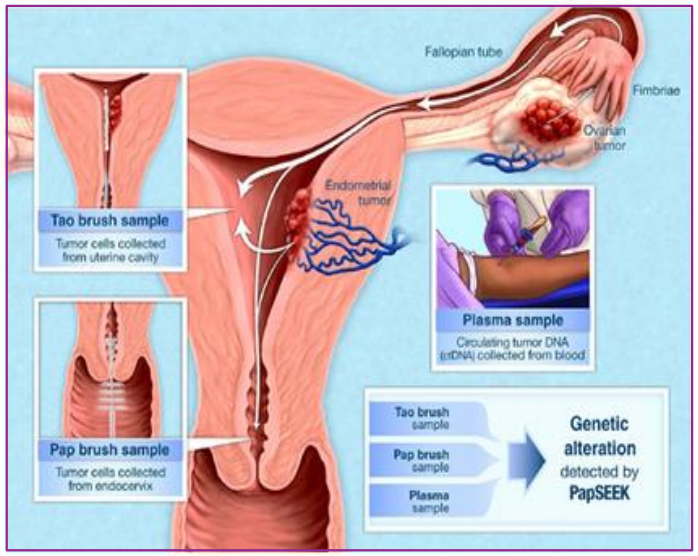
- Age
- Tissue-specific cell proliferation rate
- Benign conditions (i.e. p53 signature)

Pap test: Beyond TP53 Mutational Analysis

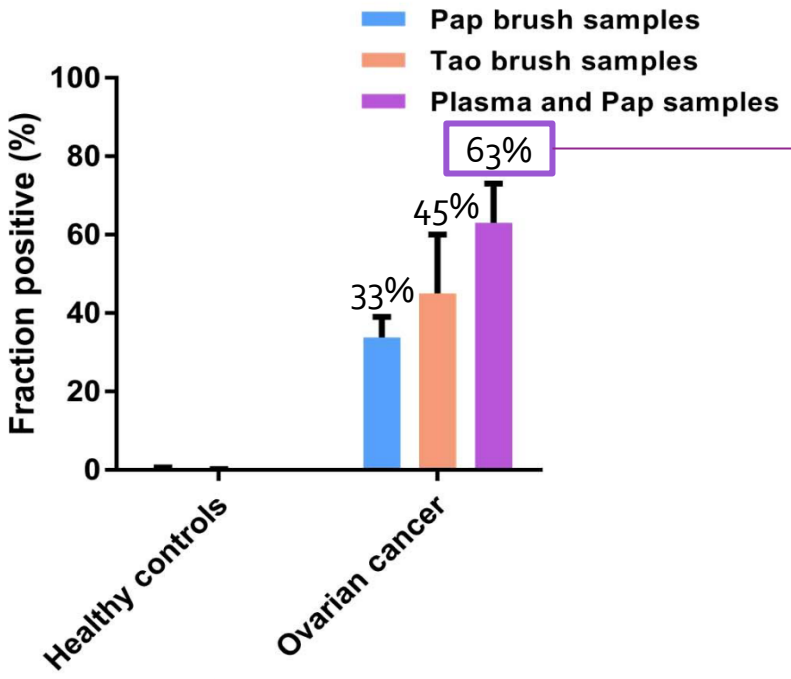


Modified from Shih et al., American Journal of Pathology 2021

PapSEEK



- **Biological features**: Mutations in 18 disease-related genes + aneuploidity
- **Biological source**: Pap brush, Tao brush, cfDNA in plasma
- **Cohort population**: 245 ovarian cancer patients (early and late stages) + 1002 cntr

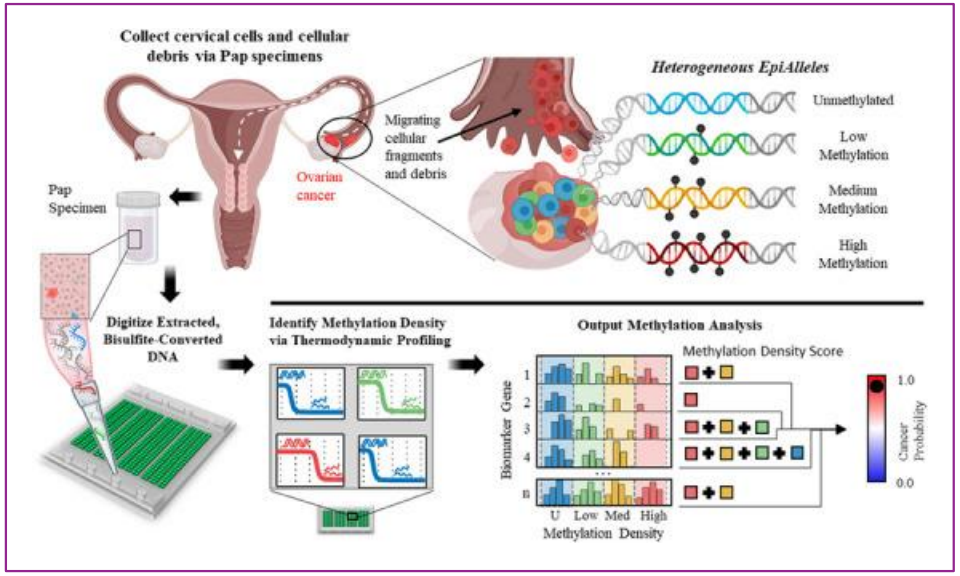


Sensitivity decreases to 54 % for stage I and II

These women already have cancer!

Wang et al., Sci Transl Med 2018

PapDREAM



- **Biological features**: methylation pattern in 9 genomic loci
  (based on Pisanic et al., Clin Canc Res 2018)
- **Biological source**: Pap test sample
- **Cohort population**: 18 ovarian cancer patients + 25 cntr

- Technical consideration:
  - Bisulfite-conversion approach
  - Based on microfluidic HYPER-Melt platform → methylation patterns of multi-loci panel → Methylation density score → cancer probability

Sensitivity: 50%
Specificity: 99%

→ Low sensitivity
17 FIGO stage III-IV and 1 FIGO stage I patients

These women already have cancer!

O'Keefe et al., Clinical and Translational Medicine 2024

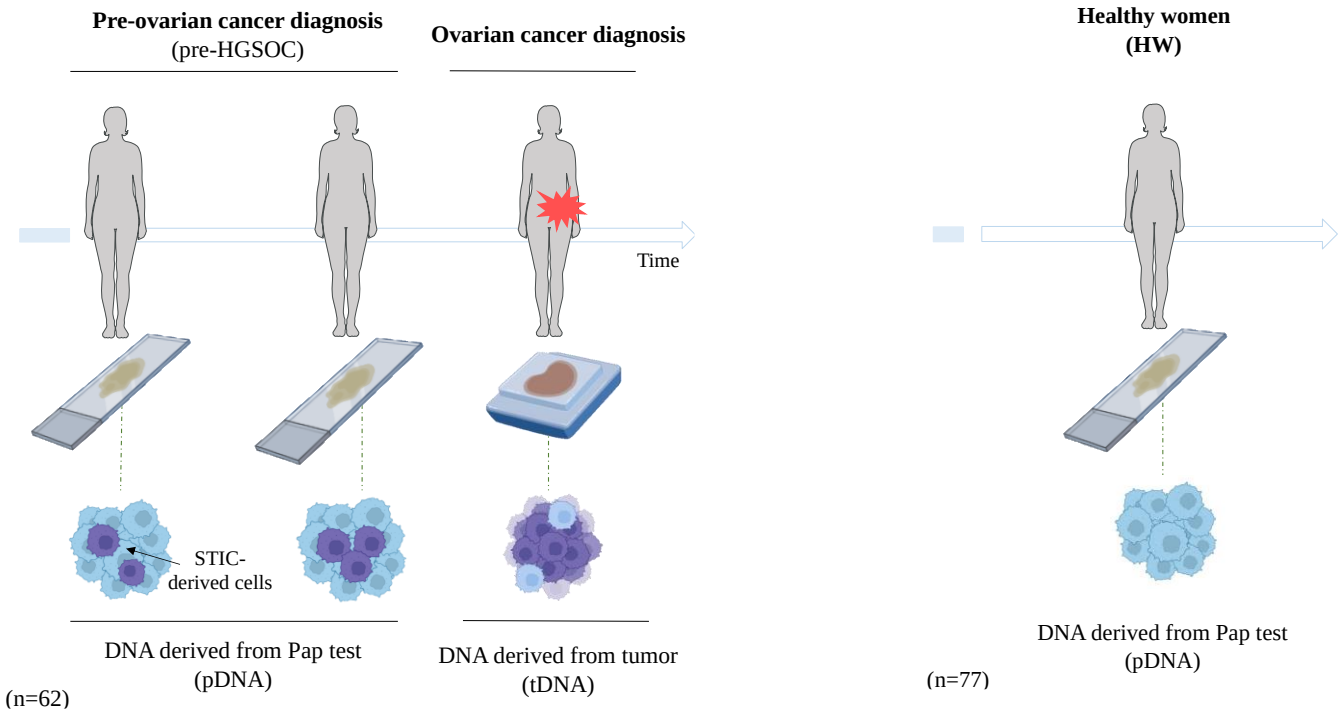
EVA (Early oVArian cancer) test

- **Biological features**: Genomic Instability
- **Biological source**: archival Pap test samples
- **Cohort Population**: 62 ovarian cancer patients + 77 cntr

Cohort

- Restrospective and Multicentric
- N. of HGS-EOC patients analyzed: 62
- N. of Pap test sample: 99
- Δ temp before diagnosis (y): 0-13
- N. of Healthy Donor: 77

Shallow-whole genome sequencing (0.5X coverage)

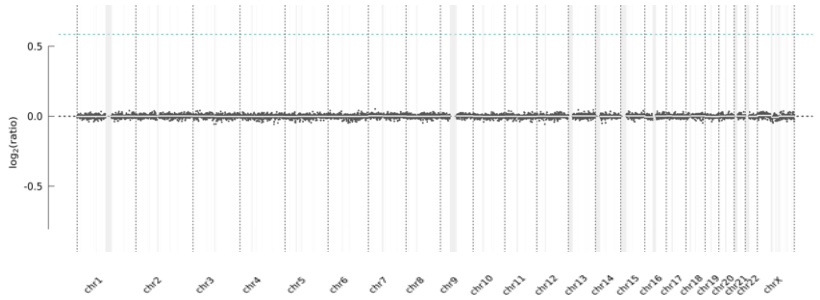


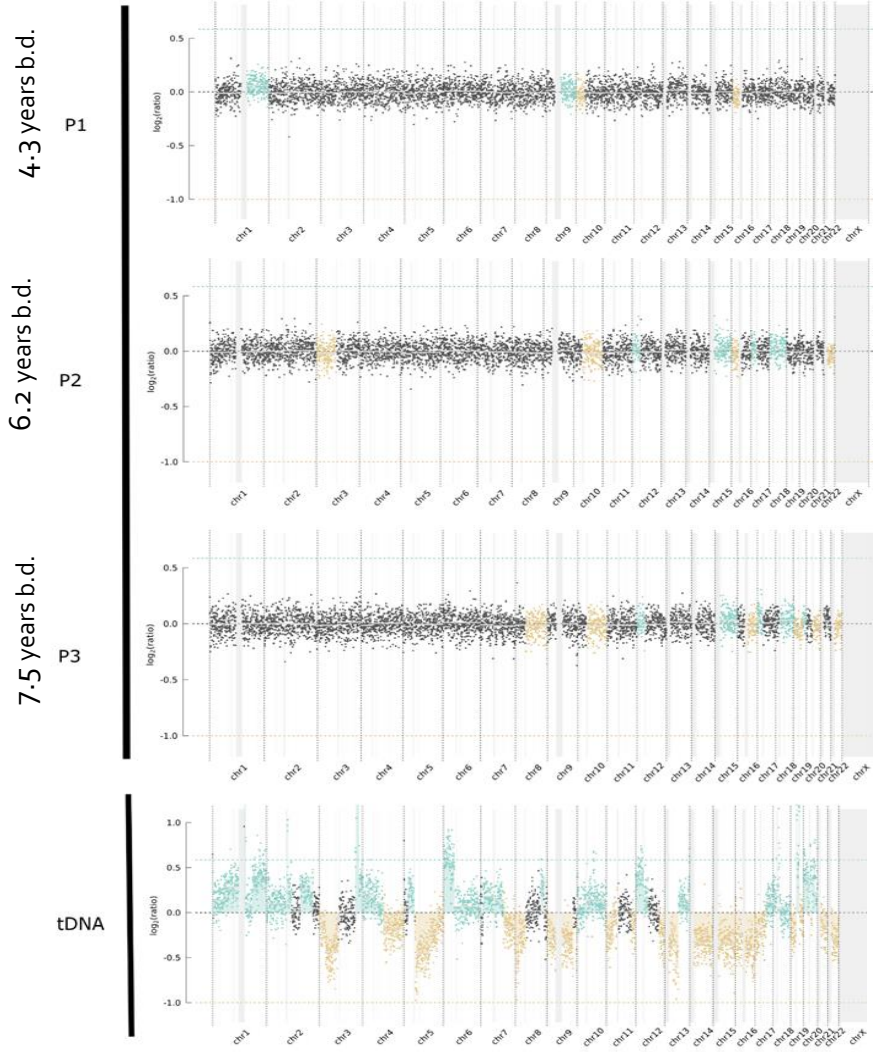
Paracchini, Mannarino et al., Sci Transl Med 2023

EVA (Early oVArian cancer) test

Graphical representation:

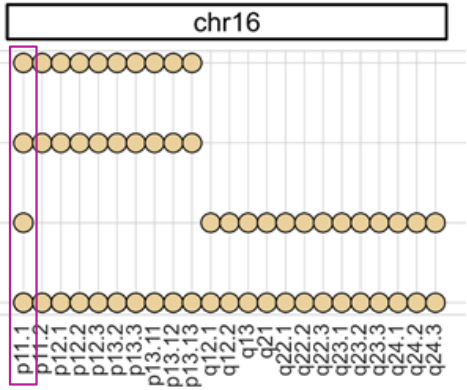
HW





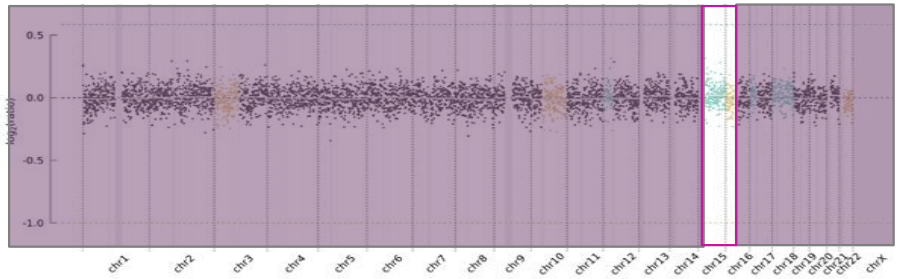
As an example: patient ID: 1240-11

Consistent alteration:

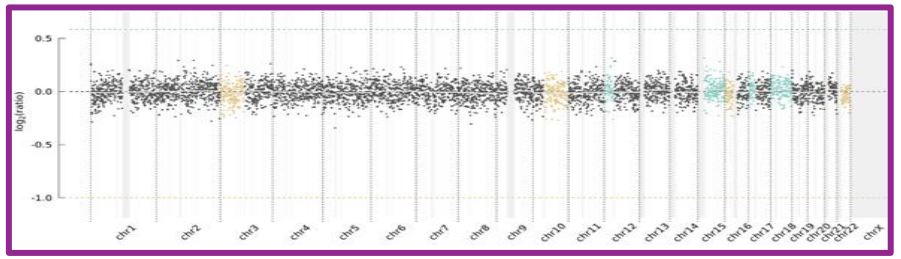


Paracchini, Mannarino et al., Sci Transl Med 2023

EVA (Early oVArian cancer) test



From individual SCNAs analysis



To analyse and quantify the overall genomic complexity

copy number profile abnormality score
CPA score

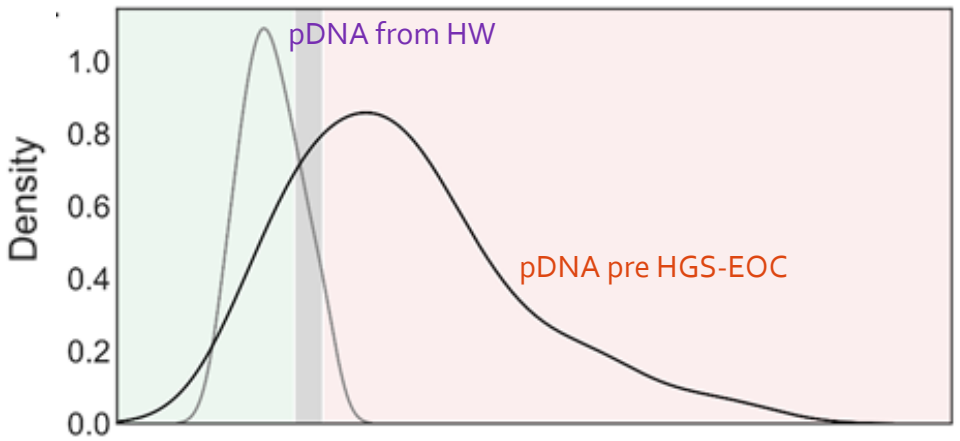
Wisecondorx
(Raman et al., Genome Med 2020)

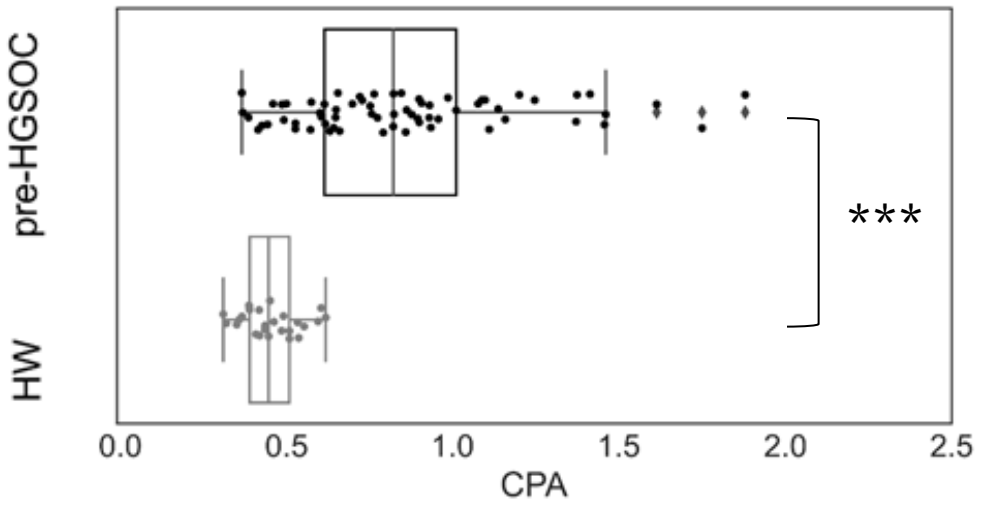
Constitutive SCNAs (SCNAs frequently occurring in healthy population) were excluded (black-list)

The higher the CPA value, the greater the genomic instability

Paracchini, Mannarino et al., Sci Transl Med 2023

EVA (Early oVArian cancer) test





- Statistically different CPA distribution
- CPA distribution defines 3 different interval

Green Zone	**negative for genomic alterations** 0<CPA<0.52372
Gray Zone	**uncertain** 0.52372<CPA<0.61
Red Zone	**positive for genomic alteration** CPA>0.61

Paracchini, Mannarino et al., Sci Transl Med 2023

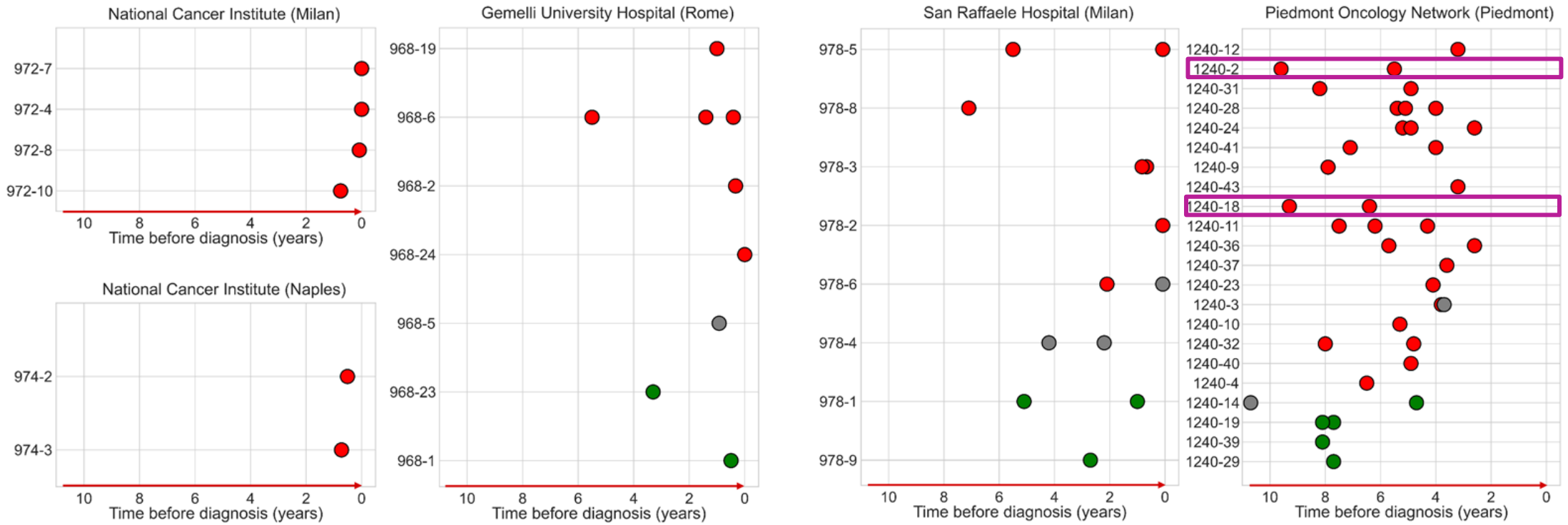
EVA (Early oVArian cancer) test

Illustration by Fisa

NPV: 96%

PPV: 75,38%

EVA (Early oVArian cancer) test



- **aneuploid genome** (red circles) 75.4% (49/65)
- **diploid genome** (green circles) 15.4% (10/65)
- **uncertain** (gray circles) 9.2% (6/65)

Paracchini, Mannarino et al., Sci Transl Med 2023

EVA (Early oVArian cancer) test

Conclusion

Our findings indicate that early detection of HGS-EOC is potentially feasible by examining the genomic instability profile in DNA derived from endocervical swab

Limits and Future Directions

- We have defined the PPV and NPV of the EVA test
- With this study we can only hypothesize that a positive test corresponds to disease in the tubal region
- Need for improvement NPV (specificity) and PPV (sensitivity) of the EVA test

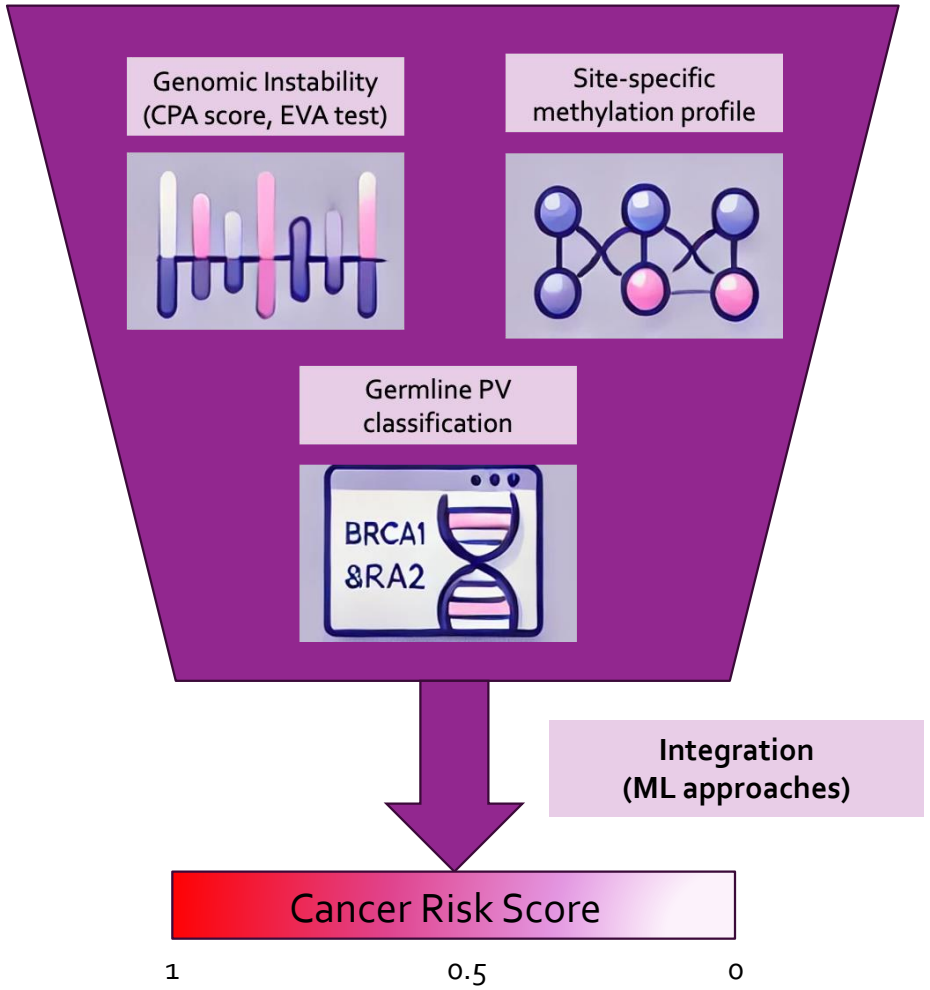
EVA (Early oVArian cancer) test

- Retrospective, multicentric study
- **Cohort population**: women with PV BRCA1 BRCA2 genes
- **Biological source**: Pap test collected at time of risk-reducing surgery
- **Biological Features:**

  1-Genomic instability (CPA score, EVA test)

  2-Site specific methylation profile

  3-PV BRCA1 BRCA2 classification



AIRC

«If you want to go fast go alone, if you want to go far go together»

-African proverb-

All the patients and centers

Cancer Pharmacology group

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Laura Mannarino

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Riccardo Zadro

HUMANITAS RESEARCH HOSPITAL

AIRC

Alessandra Bono FONDAZIONE ONLUS

Ministero della Salute

All of you for your attention