Detection of *TP53* clonal mutations in PAP test collected up to six years prior to high-grade serous epithelial ovarian cancer diagnosis

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AACR American Association for Cancer Research

Background

- The low five-years survival rate of High Grade Serous Epithelial Ovarian Cancer (HGS-EOC) is mainly related to late diagnosis. The anticipation of diagnosis constitutes a crucial step to increase the curability of this disease.
- It has been demonstrated that the same *TP53* clonal mutation detected in primary tumor site is also present in pre-cancerous lesions in the Fallopian tubes (STIC)[1].
- A mathematical model based on lesion-specific proliferation rate suggests that STIC progression to carcinoma takes approximately six years[2].
- Several studies showed the feasibility of detecting somatic mutations of endometrial and ovarian cancers in DNA retrieved from various types of vaginal samples collected at the time of diagnosis (such as Pap tests)[3,4,5].

Aim

This study explores an innovative potential HGS-EOC screening approach exploiting PAP tests routinely executed for cervical cancer surveillance and the presence of *TP53* clonal alterations in almost all HGS-EOCs. Indeed, it investigates the possibility to detect in DNA purified from PAP tests of 17 HGS-EOC patients, collected years before diagnosis, the clonal pathogenic *TP53* variant identified in the matched primary tumor biopsy (**Figure 1**).

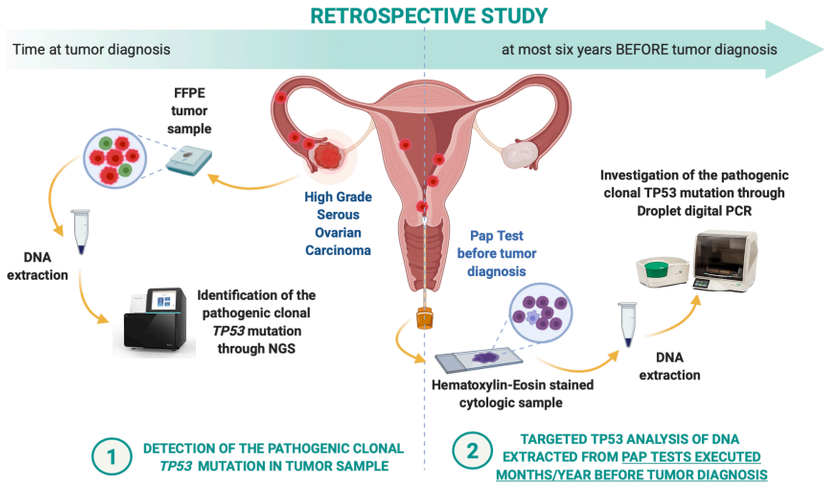

Figure 1: Overview of the experimental plan.

Methods

- 17 HGS-EOC patients (FIGO stage II-IV) who underwent surgery from 2015 to 2019 at San Gerardo Hospital (Monza, Italy) were retrospectively selected. The median age at diagnosis was 60 years (I-III quartiles, 53-69 years; Table 1). The prevalence of *BRCA1* and *BRCA2* germline mutations was 58.8% (n = 10).
- Next Generation Sequencing (NGS) was used to identify clonal *TP53* mutations in tumor tissue (**Figure 2**).
- Droplet digital PCR (ddPCR) was performed on DNA purified from all available patients' PAP tests executed up to almost six years before diagnosis (**Figure 2**).
- Serial dilutions of DNA derived from patients' tumor and blood samples and DNA extracted from healthy women's PAP tests were analyzed to define respectively the sensitivity and specificity of ddPCR (**Figure 2**).

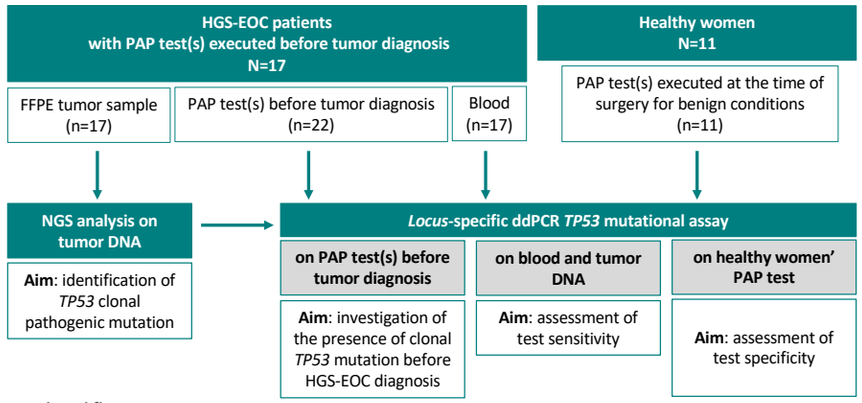

Figure 2: Experimental workflow.

Results

- A clonal *TP53* variant was identified by NGS in each tumor sample (**Table 1**).
- In eleven out of 17 (64%) patients the clonal *TP53* variant was detected by ddPCR in DNA from the matched PAP tests collected within six months before diagnosis (T1) or earlier (T2, T3 and T4). For patients 21561 and 21521 the *TP53* mutations were identified in all available PAP tests (**Table 1**).
- Serial dilutions of tumor and blood DNA verified that all *TP53* ddPCR assays were confidently able to detect until 0.01% of mutated allele (Relative Abundance, RA).
- None of the healthy women' Pap tests resulted mutated.

Patient ID	Tumor *TP53* mutation	PAP tests Time before tumor diagnosis			
		T1 (0-6 months)	T2 (7-24 months)	T3 (25-48 months)	T4 (≥49 months)
21561	c.818G>A p.R273H	0.2 months 0.24 %	-	25 months 0.21 %	49 months 0.26 %
21585	c.817C>T p.R273C	-	11.3 months 0.21 %	-	-
21567	c.281C>A p.S94*	3 months 0.7 %	-	-	-
21587	c.469G>T p.V157F	2 months Not Detected	-	-	-
21586	c.818G>A p.R273H	-	19.3 months 0.15 %	-	-
21569	c.574C>T p.Q192*	5.2 months 1.18 %	-	-	-
21624	c.820G>T p.V274F	-	-	37.5 months 0.04 %	65.3 months Not Detected
21570	c.844C>T p.R282W	0.3 months 2.62 %	-	-	-
21627	c.425_427del p.P142_V143del_insL	0.7 months 2.4 %	-	-	-
21640	c.993+2T>G	-	8 months Not Detected	-	-
21507	c.1025G>C p.R342P	-	9.2 months 0.9 %	-	-
21635	c.844C>T p.R282W	1.3 months Not Detected	-	-	-
21549	c.393_395del p.N131del	-	-	31.2 months Not Detected	65.3 months Not Detected
21521	c. 722 C>G p.S241C	-	-	26.7 months 0.05 %	67.3 months 0.07 %
21654	c.586 C>T p.R196*	4.7 months 0.09 %	-	-	-
21665	c.393_395del p.N131del	-	-	37.6 months Not Detected	-
21683	c.602 T>A p.L201*	-	18.5 months 0.06 %	-	-

Table 1: ddPCR results generated on patients' PAP Tests DNA. For each Pap test sample, the time at which it was executed and the relative abundance (RA) percentage of the tumor *TP53* variant identified is reported.

Conclusions

- ✓ The study shows that **the clonal *TP53* mutations** found in the ovarian cancer **are detectable in PAP tests of the same patients executed up to six years before tumor diagnosis.**
- ✓ The results hint at a promising prospect to significantly improve the future diagnosis of HGS-EOC.
- ✓ Large longitudinal prospective clinical studies are required to validate these findings.

References

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