Abstract 406674: BRCA1 and RAD51 Methylation impact on advanced ovarian cancer patient outcome: a PAOLA-1 ancillary study

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INTRODUCTION / BACKGROUND

- The bevacizumab(bev)/olaparib(ola) maintenance regimen was approved for women affected with ***BRCA*-mutated (BRCAm) and homologous recombination deficiency (HRD) high-grade ovarian cancer (HGOC)**, based on a greater progression-free survival (PFS) compared to bevacizumab alone in the PAOLA-1/ENGOT-ov25 trial (NCT02477644).
- **Homologous recombination DNA repair** (HRR) is an important mechanism by which double-stranded DNA breaks can be corrected. It depends upon properly functioning **BRCA1-2 proteins** and many others including **RAD51C.**
- **HRD is observed in 50% of HGOC and** identifies patients likely to respond to PARP inhibitors such as olaparib and is **scored by various genomic instability scores** as Myriad myChoice® or other tests developed in the Engot program
- **HRD testing is challenging** due to an important rate of non informative results, its dependence on high throughput sequencing devices and costs.
- **Here, we evaluated the validity of scoring *BRCA1* and *RAD51C* methylation to identify patients that would benefit from ola + bev versus bev alone as maintenance therapy .**

METHODOLOGY

- **519 tumor DNA samples** were available for methylation analysis (348 randomized to ola+bev and 171 to bev+placebo)
- *BRCA1* and *RAD51C* methylation was analyzed **from bisulfite converted DNAs** using **MS-ddPCR** (droplet digital PCR, Bio-Rad) (Fig1) and **methylation specific PCR (MS-PCR)** (3730xl Genetic Analyzer, Thermofischer Scientific) (Fig2)
- 3 CpG regions analyzed for *BRCA1*, 2 CpG for *RAD51C* (detailed methodology available at request)
- Methylation status was correlated to clinical data, BRCA1/2 mutations, GIS and HRD scores, PFS and OS. R (r-project.org/) and JMP software version 10.0 were used for statistical analysis. A p-value < 0.05 was considered significant.

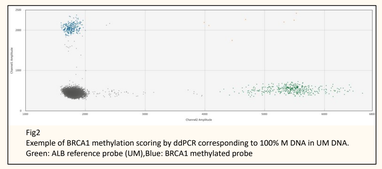


Fig2
Exemple of BRCA1 methylation scoring by ddPCR corresponding to 100% M DNA in UM DNA.
Green: ALB reference probe (UM),Blue: BRCA1 methylated probe

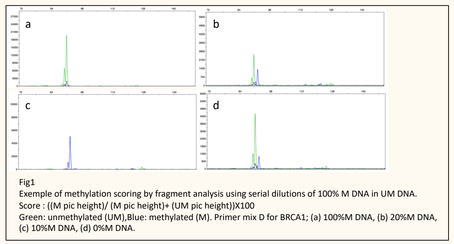
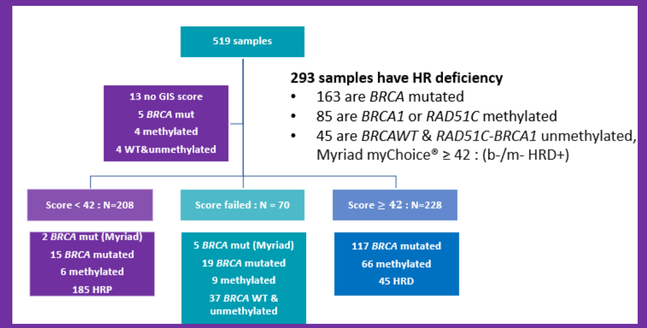


Fig1
Exemple of methylation scoring by fragment analysis using serial dilutions of 100% M DNA in UM DNA.
Score : ((M pic height)/ (M pic height)+ (UM pic height))X100
Green: unmethylated (UM),Blue: methylated (M). Primer mix D for BRCA1; (a) 100%M DNA, (b) 20%M DNA, (c) 10%M DNA, (d) 0%M DNA.

KEYS / CONCLUSIONS

- **Met-*BRCA1* & Met-*RAD51C* are not *BRCA* mutated (2 co-alteration)**
- **Met-*BRCA1* & Met-*RAD51C* are exclusive (4 co-alteration)**



- **Met-*BRCA1* and Meth -*RAD51C* benefit from ola+bev maintenance**
- **Methylation assessment coupled with *BRCA1-2* somatic testing allows the identification of 85% (248/293) of HRD+ high grade AOC.**

	BRCAmut		met		b-/m- HRD+		HRP	
	Olaparib	**placebo**	**olaparib**	**placebo**	**olaparib**	**placebo**	**olaparib**	**placebo**
N	106	50	59	26	31	20	152	75
Median PFS1	66.3	22.0	29.8	17.4	57.1	16.6	16.7	15.1
	[42.6-75.2]	[16.6-26.3]	[22.0-42.1]	[11.1-27.7]	[18.7-NR]	[11.8-24.9]	[15.3-18.8]	[14.0-18.7]
HR	**0.42 [0.27-0.66]**		**0.49 [0.29-0.84]**		**0.34 [0.17-0.67]**		0.9 [0.73-1.34]	
Median OS	NR	66.8	64.3	65.4	NR	54.4	36.6	42.1
		[55.6-NR]	[53.3-NR]	[32.3-NR]	[38.5-NR]	[39.9-NR]	[30.5-44.9]	[28.7-54.2]
5-years survival	75%	54%	56%	52%	54%	44%	28%	35%
HR	**0.52 [0.30-0.92]**		0.76 [0.42-1.50]		0.78 [0.35-1.76]		1.2 [0.86-1.71]	

- **Methylation testing is a cost effective method to identify HGOC patients that benefit from ola+bev maintenance**

RESULTS

Fig3: Met-*BRCA1* (13%) or met-*RAD51C* (4.8%) tumors are HRD

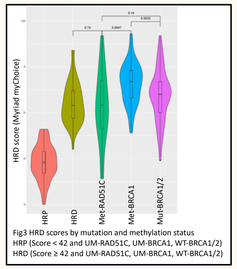


Fig3 HRD scores by mutation and methylation status
HRP (Score < 42 and UM-RAD51C, UM-BRCA1, WT-BRCA1/2)
HRD (Score ≥ 42 and UM-RAD51C, UM-BRCA1, WT-BRCA1/2)

Table 1: *BRCA1*/*RAD51C* methylation testing rescue inconclusive Myriad myChoice®results

	Myriad myCHOICE® score			
Tumor genotype	**FAILED & (not done)**	**NEGATIVE**	**POSITIVE**	**Total**
***BRCA* MUT**	19 (5)	15	117	156
***BRCA* WT-No Methylation(NM)-score inconclusive**	42 (4)			48
HRD: *BRCA* WTNM score POS			45	45
HRP: *BRCA* WTNM score NEG		187		185
METHYL	9 (4)	6	66	85
Total	70 (13)	208	228	519

Fig4: PFS1 according to tumor genotypes as defined in Table 1 and treatment arms

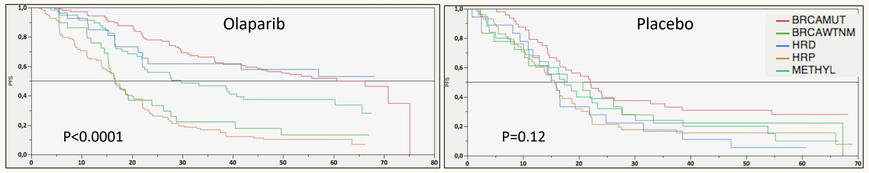
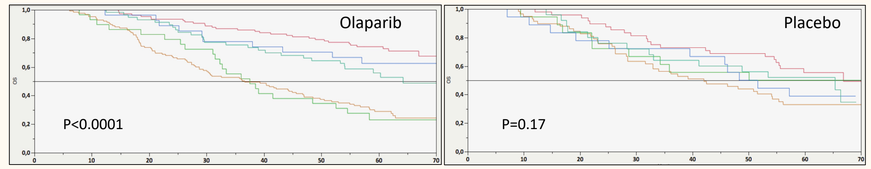


Fig5: OS according to tumor genotypes as defined in Table 1 and treatment arms



- **Benefit of adding ola maintenance to bev was similar between patients with *met*-HGOC and those with *unmethylated BRCA1/2 WT HRD+* tumors**

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