Inter-laboratory evaluation of somatic *BRCA* mutations in clinical practice: a ring trial of the Spanish Group of Research in Ovarian Cancer (GEICO).

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Background & objectives

Given the proven effectiveness of PARP inhibitor treatment in germline or somatic BRCA mutation-associated ovarian cancer (OC), the aim of this inter-laboratory ring trial is to assess next generation-sequencing (NGS)-based BRCA mutation detection and interpretation approaches in formalin-fixed paraffin embedded (FFPE) tissue.

Methods

Five independent clinical diagnostic and two reference laboratories tested 9 specimens, including commercial synthetic human FFPE (n=3) and OC tumor tissue DNA (n=3) and FFPE (n=3) samples (figure 1). Each center performed their routine next-generation-sequencing (NGS) workflow and report. To estimate the concordance rate 17 variants were evaluated: 10 pathogenic (P), 1 likely pathogenic (LP), 3 variants of unknown significance (VUS) and 3 wild-type.

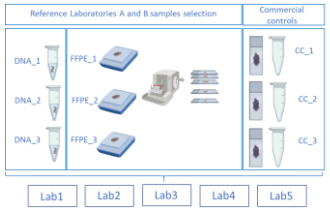

Figure1. *Ring trial sample design.*

Results

Hybridization-capture based enrichment followed by MiSeq (Illumina) sequencing was chosen by two of the five laboratories while PCR/amplicon-based target enrichment followed by Ion S5™ System (Thermo Fisher Scientific) sequencing by the remaining 3 (Table 1).

Laboratory	NGS-Techonology	NGS-Panel	NGS-Instrument
Lab1	Amplicons	Oncomine Comprehensive Assay v3 (Thermo Fisher)	Ion S5™ System (Thermo Fisher Scientific)
Lab2	Amplicons	BRCA MASTR Plus Dx (Multiplicom)	MiSeq (Illumina)
Lab3	Capture	Sure Select XT (Agilent)	Ion S5™ System (ThermoFisher Scientific)
Lab4	Capture	MiniHRS (Sophia Genetics)	MiSeq (Illumina)
Lab5	Amplicons	Oncomine BRCA Research Assay (Thermo Fisher)	Ion S5™ System (Thermo Fisher Scientific)

Table 1. *Somatic BRCA1/2 strategies.*

Different NGS bioinformatic pipelines were used to identify and annotate variants (Table 2).

Laboratory	Data Analysis Tools	VAF	Minimal Coverage	Intron Flanking Region	Databases
Lab1	Ion ReporterTM Software Version 5.10	5%	500x	±10bp	ClinVar, Varsome; COSMIC
Lab2	MASTR Reporter 1.3.0	5%	1000x	No	ClinVar; BRCA Exchange
Lab3	novocraft V3.07.01, bamtools-2.4.1, VCFtools (0.1.15), bedtools v2.26.0-40, samtools 1.8, picardtools 2.8.3, ensembl vep release 94, CONTRA.v2.0.8, gatk-3.4.46	10%	20x		NCBI, ClinVar, Ensembl, BRCA Exchange, cBioPortal
Lab4	Sophia DDM v3-Sophia Genetics	5%	500x (1000x)		ClinVar, COSMIC, dbSNP,EXAC, g1000,ESP, EpiCov,GnomAD,
Lab5	Ion Reporter Software Version 5.16	5%	100x	±100	dbSNP, BIC database, BRCA Exchange, BRCA Mutation Database

Table 2. *NGS bioinformatics pipelines.*

The median concordance detection rate was 64.7% (35.3-70.6%). Most of non-reported results correspond to variants within homopolimeric regions, bioinformatic issues, low variant allele frequencies or low coverage. One laboratory reported no results for one commercial specimen due to insufficient DNA; another laboratory reported a false positive variant within a commercial sample (Table 3).

Discrepancies in variant classification affected four alterations, three of them with clinical relevance (VUS vs likely pathogenic) (Table 3).

Sample	Variant	Clinical Classification	Lab1	Lab2	Lab3	Lab4	Lab5	Detection concordance (%)	Interpretation concordance (%)
DNA_1	*BRCA1: c.3334G>T p.(Glu1112*)*	P				*		100%	60%
DNA_2	*BRCA2: c.8802_8828del p.(Met2935_Gln2943del)*	LP				*		80%	75%
DNA_3	*No pathogenic variant*							100%	100%
FFPE_1	*BRCA1: c.80+6T>A*	VUS						40%	100%
FFPE_2	*No pathogenic variant*							100%	100%
FFPE_3	*BRCA2: c.5351dupA p.(Asn1784Lysfs)*	P						40%	100%
CC_1	*BRCA2:c.5351del p.(Asn1784fs)*	P						20%	100%
CC_1	*BRCA1:c.4327C>T p.(Arg1443Ter)*	P						100%	100%
CC_1	*BRCA2:c.5073del p.(Lys1691fs)*	P						60%	100%
CC_1	*BRCA2:c.8021dup p.(Ile2675fs)*	P						20%	100%
CC_1	*BRCA1:c.1303G>T p.(Asp435Tyr)*	VUS						20%	100%
CC_2	*BRCA2:c.5351del p.(Asn1784fs)*	P					*	50%	50%
CC_2	*BRCA1:c.4327C>T p.(Arg1443Ter)*	P						50%	100%
CC_2	*BRCA2:c.5073delAp.(Lys1691AsnfsTer15)*	P						25%	100%
CC_2	*BRCA2:c.8021dup p.(Ile2675fs)*	P						0%	100%
CC_2	*BRCA1:c.1303G>T p.(Asp435Tyr)*	VUS						25%	100%
CC_3	*No pathogenic variant*							100%	100%

Table 3. *Summary of BRCA1/2 variants and results obtained in the ring trial. P=pathogenic; LP=likely pathogenic; VUS=variant of unknown significance; green=concordance in detection and interpretation; red=no detection; orange=concordance in detection but not in interpretation; grey=no results; *=discrepancy with clinical relevance.*

Conclusion

This ring trial showed a wide range of concordance rates in the identification and interpretation of BRCA somatic analysis. It highlights the relevance of establishing standard criteria for detecting, interpreting and reporting BRCA somatic variants. Validation of both NGS methodology and bioinformatic pipelines are required. Standardization in analytical criteria is also mandatory. Regarding interpretation, discrepancies affecting non-reported variants in databases remain a challenge with relevant clinical implications.

We wish to acknowledge Astra Zeneca for financial support

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