Evaluation of Homologous Repair Deficiency Status in Ovarian Carcinoma using In House Next Generation Sequencing Panels

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Introduction

- Homologous repair pathways are frequently aberrant in cancer, leading to the accumulation of DNA damage and genomic instability known as homologous recombination deficiency (HRD).
- Assessment of HRD status is important for ovarian cancer management as it has been shown that Poly(ADP-ribose) polymerase (PARP) inhibitors are effective in treating HR-deficient tubo-ovarian high-grade serous carcinomas (HGSC).
- Pillar's oncoReveal™ HRDv2 assay was evaluated in our laboratory for detection of causal single nucleotide variants (SNVs), indels and exon level copy number variants for analysis of HRD.
- The oncoReveal Methylation Assay was also evaluated for detection of methylation in promoter regions of *BRCA1, BRCA2, RAD51C* and *XRCC3*.
- Retrospective clinical samples of ovarian carcinoma from the past 10 years at Temple University Hospital were used to evaluate 33 genes using the SLIMamp NGS technology.

Methods

- A total of 31 formalin-fixed paraffin embedded (FFPE) ovarian cancer specimens were used for both mutation and methylation analysis.
- The oncoReveal HRD genetic and epigenetic assays were performed on histologically classified 20 high grade serous, 4 clear cell, 3 mucinous, 3 endometrioid and 1 mixed tumor specimens.
- FFPE specimens were microdissected and DNA was extracted using standard laboratory protocols.
- The oncoReveal NGS assay was performed for detection of mutations on the Illumina MiSeq.
- The methylation assay involved bisulfite conversion using the EpiTect Fast DNA Bisulfite kit followed by NGS analysis using the methylation panel on the Illumina MiSeq.
- Bioinformatic analysis was performed using the PIVAT analysis software.

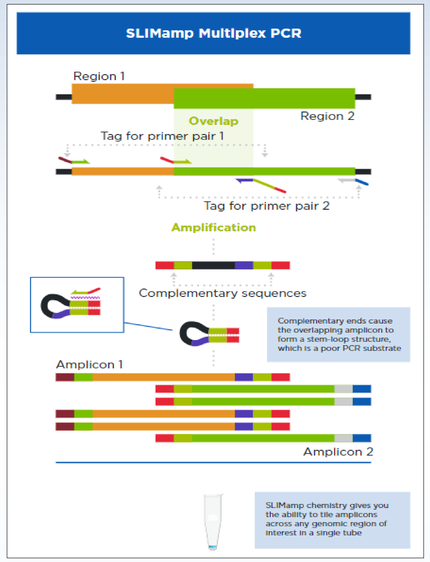

Fig 1:Workflow process for SLIMamp tumor panel

SAMPLE NO	TUMOR TYPE	Overall SNV/Indel Calls^		
		P	VUS	Benign
1	ENDOMETROID	ARID1A D1850Tfs*33 16.2% PTEN R130Q 29.3%	ATM L612F 50.3%	
2	SEROUS	TP53 L289Pfs*56 4.7% BRCA1 Q1756Pfs*74 50.4%	ATR R1451W 47.7%	
3	SEROUS	TP53 P58Qfs*65 91.2% BRCA2 G1376Afs*11 89.4%	FANCA A746V 10.8% BARD1 R150G 5.8% MRE11 E600Q 41.4%	
4	SEROUS		ATM T2333I 63.2%	
5	CLEAR CELL	PIK3CA H1047R 34% ARID1A Y417Tfs*16 76.3%	CDK12 S987F 37.1%	
6	SEROUS	BRIP1 R798* 84.7% TP53 H179Y 65.9%	FANCD2 M782T 49.3% ATM R2580S 35.9%	
7	ENDOMETROID	KRAS G12V 43.4%	MRE11 V646I 47.9% BARD1 L239Q 48.5%	
8	SEROUS	TP53 Y220C 59.8%	BRCA2 E2599del1 55%	BRCA2 Y42C 77.9%
9	SEROUS	TP53 E271* 68.9%	PALB2 L332H 59.8%	
10	SEROUS	TP53 N239Pfs*16 72.2%	FANCA R756C 14.7% ATR Y291D 16.4%	
11	CLEAR CELL	ARID1A Q878* 38.8% PTEN T167Lfs*16 13.4% PTEN P248Tfs*5 14.2%	RAD50 R327H 53%	
12	SEROUS	TP53 c.920-1G>T 91.4%	ATM T2333I 8.1%	
13	SEROUS	TP53 C242F 4.2% TP53 C124* 39.9%		
14	MIXED	PIK3CA E542K 27.6% KRAS G12A 61.8%		
15	SEROUS	TP53 H193R 86.1%	FANCD2 P732L 45%	
16	SEROUS	CDK12 c.2964-1G>A 13.9% TP53 R280G 33.3%	FANCD2 R1452I 48.4%	
17	SEROUS	TP53 C242S 82.8%	BRCA1 E1494K 18.1% BRCA1 M1783T 81.9%	
18	SEROUS	BRCA2 N404Mfs*26 20.8% TP53 Y107* 37.3%	BRCA1 A1708V 60.7%	
19	SEROUS	TP53 R175H 31.2%		
20	ENDOMETROID	CTNNB1 S37C 26.7%	ATR R668W 48.3% FANCE R69Q 31.4%	FANCE G246del 46.5%
21	SEROUS	TP53 P75Lfs*48 74.7% BRCA1 E23Vfs*17 90.2%	NBN P199S 94.2% ATR K764E 69.3%	
22	MUCINOUS	TP53 G245S 21.5% KRAS G12V 15%		
23	SEROUS	TP53 W146* 40.8%		
24	SEROUS	TP53 L111R 20.5%	ARID1A A45V 18.3% ATR Y2132D 52.4%	
25	CLEAR CELL	TP53 C141Afs*29 63.9%	BRCA2 S1115P 14.7%	
26	MUCINOUS	KRAS G12V 40.5% TP53 L330Ffs*15 49.2%	RAD50 R726H 48.3% FANCD2 R779H 29.9%	FANCD2 F386V 30.3%
27	SEROUS			TP53 P72R 99.9%
28	CLEAR CELL			BRCA1 P871L 47.3%
29	MUCINOUS	TP53 Q38KFS*6 62.7 BRAF V600E 38.3		
30	SEROUS	TP53 R273H 45.8		
31	SEROUS			BRCA1 P871L 84.9%

Table 1: DNA mutations detected by NGS in the study cases

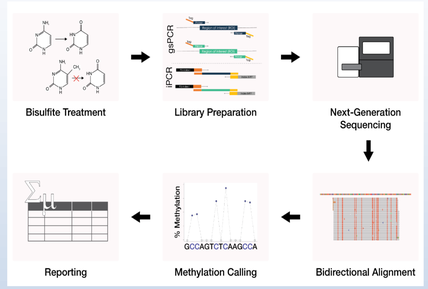

Fig 2: Flowchart for the SLIMamp NGS methylation panel

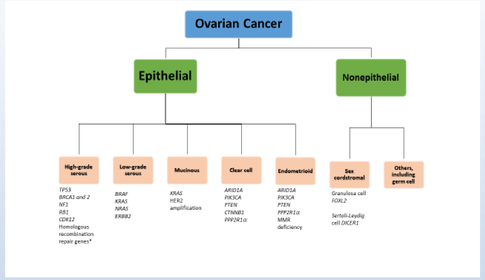

Fig 3: Histological and molecular subtypes of ovarian cancers; some can benefit by investigation of HRD status

Conclusions

This study provides encouraging preliminary evidence for the possibility of implementing in house based HRD testing in routine clinical practice. The oncoReveal methylation assay also provides a unique approach to investigate HRD in ovarian carcinoma.

# Methylation Sites =>		Gene-Level Raw Average Methylation				Amplicon-Level Average Methylation									
		21	26	10	25	BRCA2 5	BRCA2 4	BRCA2 12	XRCC3 4	XRCC3 3	XRCC3 9	XRCC3 6	XRCC3 4	BRCA1 7	BRCA1 3	RAD51C 15	RAD51C 10
Sample_ID	Sequencing_ID	BRCA2	XRCC3	BRCA1	RAD51C	N-Bis04.trg40.BRCA2.tgt2	N-Bis05-Deg.trg40.BRCA2.tgt2	N-Bis06.trg40.BRCA2.tgt2	XRCC1.Intron6B-news	XRCC3.5UTR-new	XRCC3.Intron6A-new	N-Bis02.trg86.XRCC3.tgt	N-Bis01.trg86.XRCC3.tgt	BisSeq04.BRCA1.Prom1	BisSeq05.BRCA1.Prom2	BisSeq02.RAD51C.Prom2	BisSeq03.RAD51C.Prom3
Sample_1	RDvSMpMethyld230614iN4-07A_Sample-1	0.00	44.28	0.25	0.42	1.80	0.32	0.18	56.84	50.32	85.60	0.26	0.24	0.29	0.16	0.44	0.40
Sample_2	RDvSMpMethyld230614iN4-07B_Sample-2	1.12	36.79	0.49	0.64	3.27	0.40	0.46	51.42	41.86	69.07	0.40	0.32	0.52	0.42	0.74	0.49
Sample_3	RDvSMpMethyld230614iN4-07C_Sample-3	1.32	44.85	0.54	0.69	3.84	0.56	0.52	80.65	49.78	76.46	0.65	0.49	0.51	0.60	0.72	0.65
Sample_4	RDvSMpMethyld230614iN4-07D_Sample-4	0.86	46.84	0.26	0.32	2.68	0.31	0.29	72.13	70.79	79.35	0.31	0.27	0.27	0.22	0.32	0.31
Sample_5	RDvSMpMethyld230614iN4-07E_Sample-5	0.67	50.44	0.28	0.41	1.72	0.36	0.33	74.37	67.19	89.90	0.32	0.34	0.29	0.25	0.47	0.34
Sample_6	RDvSMpMethyld230614iN4-07F_Sample-6	0.50	46.44	0.26	0.49	1.19	0.32	0.28	70.83	61.69	81.77	0.34	0.28	0.28	0.21	0.56	0.39
Sample_7	RDvSMpMethyld230614iN4-07G_Sample-7	0.77	40.22	0.29	0.61	2.38	0.27	0.27	70.25	65.90	62.62	0.36	0.28	0.29	0.28	0.77	0.37
Sample_8	RDvSMpMethyld230614iN4-07H_Sample-8	0.68	35.68	0.45	0.61	1.24	0.64	0.47	74.00	49.78	53.08	0.49	0.42	0.50	0.34	0.63	0.58
Sample_9	RDvSMpMethyld230614iN4-08A_Sample-9	0.76	49.76	29.44	1.37	1.37	0.59	0.56	80.14	75.60	82.28	0.61	0.54	32.28	22.82	1.77	0.76
Sample_10	RDvSMpMethyld230614iN4-08B_Sample-10	2.65	45.39	51.50	1.39	9.41	0.69	0.49	77.92	68.50	72.99	0.66	0.53	47.76	60.22	1.72	0.90
Sample_11	RDvSMpMethyld230614iN4-08C_Sample-11	1.67	30.82	0.49	0.53	5.36	0.56	0.51	29.27	22.42	67.96	0.57	0.49	0.46	0.58	0.52	0.55
Sample_12	RDvSMpMethyld230614iN4-08D_Sample-12	0.50	46.06	0.46	2.12	0.79	0.52	0.38	81.07	67.39	74.01	0.53	0.43	0.48	0.40	3.13	0.61
Sample_13	RDvSMpMethyld230614iN4-08E_Sample-13	0.93	44.23	0.58	0.99	1.88	0.58	0.65	80.69	70.58	67.69	0.66	0.59	0.65	0.42	1.18	0.70
Sample_14	RDvSMpMethyld230614iN4-08F_Sample-14	1.87	41.69	0.44	0.57	5.97	0.56	0.59	51.07	45.53	81.91	0.59	0.56	0.46	0.39	0.49	0.68
Sample_15	RDvSMpMethyld230614iN4-08G_Sample-15	0.74	38.24	0.61	0.62	0.93	0.84	0.62	70.56	54.49	60.19	0.70	0.64	0.61	0.62	0.68	0.53
Sample_16	RDvSMpMethyld230614iN4-08H_Sample-16	0.78	45.41	0.53	0.66	1.87	0.40	0.45	60.66	57.72	84.46	0.51	0.45	0.57	0.46	0.75	0.53
Sample_17	RDvSMpMethyld230614iN4-09A_Sample-17	1.34	52.23	0.22	0.34	4.77	0.27	0.26	85.95	78.81	86.13	0.31	0.16	0.22	0.20	0.32	0.36
Sample_18	RDvSMpMethyld230614iN4-09B_Sample-18	1.02	48.56	0.28	0.50	3.45	0.26	0.26	73.91	66.36	85.08	0.26	0.15	0.31	0.23	0.59	0.35
Sample_19	RDvSMpMethyld230614iN4-09C_Sample-19	1.41	37.41	0.39	0.72	4.81	0.32	0.36	74.00	56.15	56.12	0.33	0.28	0.43	0.30	0.92	0.43
Sample_20	RDvSMpMethyld230614iN4-09D_Sample-20	1.58	40.00	0.34	0.46	5.56	0.39	0.32	50.99	42.25	78.40	0.36	0.36	0.36	0.30	0.52	0.36
Sample_21	RDvSMpMethyld230614iN4-09E_Sample-21	0.96	50.44	0.57	0.87	2.46	0.45	0.51	80.15	78.20	83.60	0.44	0.32	0.59	0.54	1.07	0.57
Sample_22	RDvSMpMethyld230614iN4-09F_Sample-22	0.63	33.53	0.33	0.54	1.38	0.37	0.41	42.82	32.03	66.69	0.40	0.42	0.34	0.33	0.65	0.39
Sample_23	RDvSMpMethyld230614iN4-09G_Sample-23	0.97	39.86	34.90	0.41	3.13	0.29	0.29	67.90	60.15	64.66	0.25	0.22	31.81	42.11	0.49	0.29
Sample_24	RDvSMpMethyld230614iN4-09H_Sample-24	1.00	35.26	0.44	0.79	2.91	0.33	0.42	61.65	47.70	58.15	0.41	0.31	0.52	0.25	0.94	0.55
Sample_25	RDvSMpMethyld230614iN4-10A_Sample-25	0.55	39.97	0.28	0.39	1.38	0.32	0.28	56.98	38.64	76.97	0.25	0.30	0.28	0.28	0.44	0.31
Sample_26	RDvSMpMethyld230614iN4-10B_Sample-26	0.50	39.91	0.25	0.28	1.38	0.25	0.21	51.86	56.28	73.23	0.27	0.18	0.29	0.16	0.30	0.25
Sample_27	RDvSMpMethyld230614iN4-10C_Sample-27	0.55	33.86	46.91	0.69	1.40	0.31	0.28	82.66	68.03	38.00	0.43	0.29	44.95	51.49	0.85	0.45
Sample_28	TEMPLE-METH-28-1	0.60	48.20	0.53	0.73	0.93	0.66	0.47	68.86	63.84	86.69	0.55	0.51	0.50	0.59	0.79	0.63
Sample_29	TEMPLE-METH-29-2	0.69	42.61	0.59	0.77	0.84	0.77	0.62	59.32	58.48	76.52	0.63	0.67	0.60	0.57	0.85	0.61
Sample_30	TEMPLE-METH-30-3	0.60	37.48	0.65	0.98	0.77	0.47	0.59	53.09	43.60	69.43	0.64	0.64	0.64	0.67	1.15	0.68
Sample_31	TEMPLE-METH-31-4	0.55	51.24	24.05	0.74	0.50	0.75	0.50	87.24	81.10	81.51	0.66	0.59	24.22	23.65	0.73	0.76

Table 2: Promoter methylation detected by NGS in the study cases

SAMPLE NO	TUMOR TYPE	PATHOGENIC MUTATIONS	METHYLATION
2	SEROUS	TP53 L289Pfs*56 4.7% BRCA1 Q1756Pfs*74 50.4%	Neg
3	SEROUS	TP53 P58Qfs*65 91.2% BRCA2 G1376Afs*11 89.4%	Neg
6	SEROUS	BRIP1 R798* 84.7% TP53 H179Y 65.9%	Neg
18	SEROUS	BRCA2 N404Mfs*26 20.8% TP53 Y107* 37.3%	Neg
21	SEROUS	TP53 P75Lfs*48 74.7% BRCA1 E23Vfs*17 90.2%	Neg
9	SEROUS	TP53 E271* 68.9%	29.44
10	SEROUS	TP53 N239Pfs*16 72.2%	51.5
23	SEROUS	TP53 W146* 40.8%	34.9
27	SEROUS		46.91
31	SEROUS		24.05

Table 3: Specimens detected by NGS that exhibit HRD and benefit from PARP inhibitors

Results

The NGS results were analyzed by the Pillar PIVAT pipeline.

- Table 1 shows pathogenic HRD mutations were detected in 28/31 (90%) specimens.
- The most common mutations detected were in *TP53*, 68% and *BRCA1/2* 13% followed by mutations in genes such as *ARAD1A, KRAS, PTEN, CTNNB1, BRIP1, PIK3CA* and *CDK12.* (Table 1).
- Table 2 shows the average amplicon and gene level methylation for *BRCA 1, BRCA 2, XRCC3* and *RAD51C*. Promoter methylation was observed in five high grade serous carcinoma specimens in *BRCA1*.
- Promoter methylation in XRCC3 was also identified in clear cell, mucinous as well as high grade serous carcinoma patients.
- Table 3 is a compilation of patient specimens that will show HRD using the Pillar HRD assay. These specimens either harbor a HRD related mutation or show promoter methylation in HRD genes.