MEDGENOME | Prima Cancer Genetics

AstraZeneca

White Paper

Germline vs Tumor HRR gene panel (including BRCA1/2 genes) NGS testing:

The preferred approach for clinical decision making

Background

Inhibition of poly (ADP-ribose) polymerase (PARP) activity induces synthetic lethality in HRR (homologous recombination repair) gene mutated cancers by selectively targeting tumor cells that fail to repair DNA double strand breaks (DSBs). Gene mutations in HRR genes can be divided into two distinct categories – germline and somatic gene mutations.

1. Germline alterations include highly penetrant susceptible mutations and common genetic variants that are heritable from generation to generation. These types of variants are present in all cells of an individual, and can be useful as predictive biomarkers for drug response.

2. On a contrary, somatic mutations are acquired randomly following exposure to agents that have the potential to damage DNA in cells. In the context of cancer, these somatic mutations accumulate in the cancer cells and are commonly used as drug targets[1].

Tumour tissue-based genetic testing can detect both – germline and somatic variants. This is cost effective and saves time since only one test needs to be performed to identify all patients with deleterious variants that may benefit from PARPi (PARP inhibitor) treatment[2]. The somatic analysis enables physicians to identify a fraction of around 7% of ovarian cancer patients with a pathogenic BRCA variant that would remain unknown if only a test in peripheral blood is performed[3], yet germline testing is routinely pursued at diagnosis.

This white paper evaluates the ability to detect somatic and germline variants from different solid tumor samples in Indian context using a next generation sequencing (NGS) system.

PARP inhibitor and HRR gene mutations:

- PARP plays a vital role in the repair of single-strand DNA breaks through the base excision repair pathway.
- PARPi are thought to become trapped at the sites of single-strand DNA breaks leading to double-strand DNA breaks when DNA replication is attempted[4].
- The double-strand DNA breaks would normally be repaired by the process of homologous recombination repair (HRR), which is a complex process including many proteins, notably BRCA1/2[5].
- Tumours with an HRR deficiency, such as those found in BRCA-mutated cancers, cannot accurately repair DNA damage when PARP protein function is also disrupted and both the base excision and HRR DNA repair pathways are rendered inoperative.
- In these tumours, DNA repair by low fidelity repair mechanisms such as non-homologous end joining can cause the accumulation of genomic instability, ultimately resulting in cell death; a concept referred to as synthetic lethality (Fig 1)[6].
- Additionally, preclinical data suggest that PARPi may also benefit patients whose tumours are sensitive to platinum-based chemotherapy and who have an HRR deficiency caused by mutations other than those in the BRCA1/2 genes[7].
- The clinical and molecular profiles of high-grade serous ovarian cancer (SOC) and metastatic castration resistant prostate cancer (mCRPC) appeared well matched to PARPi biology. These tumors are noted for genome instability thought to be driven by HRR deficiency, repeated and prolonged platinum sensitivity, and a high frequency of deficiency in BRCA and other candidate HRR proteins[8].

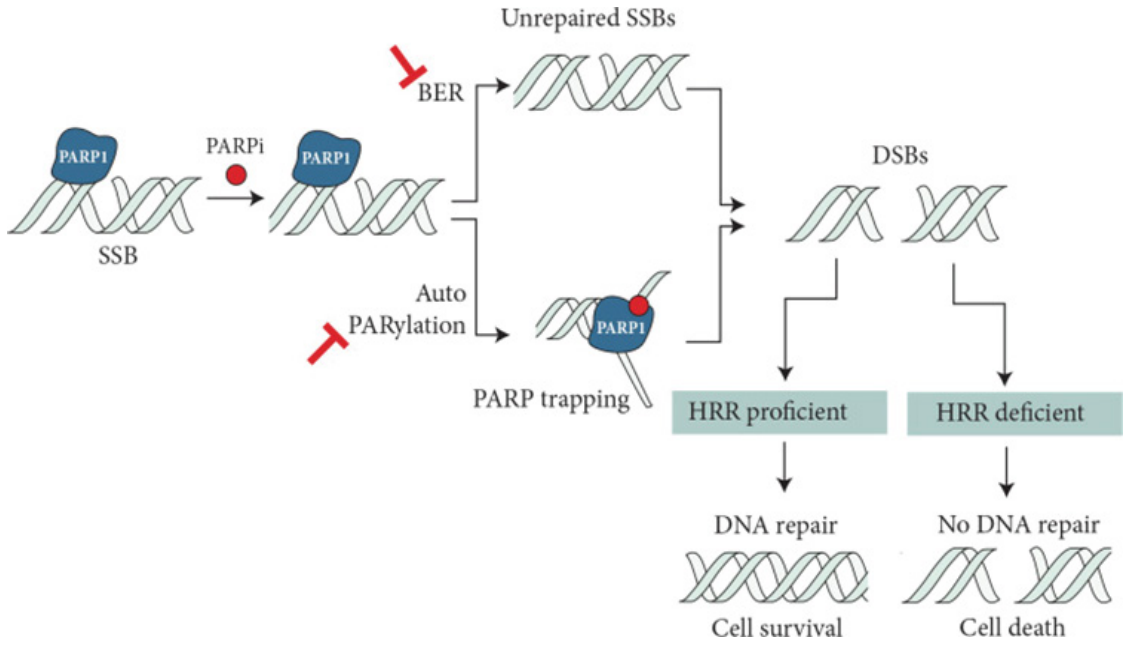

Figure 1: Mechanisms for PARPi activity in HRR-deficient cells. PARP inhibition impairs repair of single strain breaks (SSBs) by disrupting the base excision repair (BER) pathway and causing PARP1 trapping by inhibiting auto-PARylation and/or PARP release from DNA. These result in unresolved DNA double-strand breaks (DSBs) that in homologous recombination repair (HRR)-deficient cells lead to cell death. (Adopted from: Mateo J, Lord CJ, Serra V, Tutt A, Balmaña J, Castroviejo-Bermejo M, Cruz C, Oaknin A, Kaye SB, de Bono JS. A decade of clinical development of PARP inhibitors in perspective. Ann Oncol. 2019 Sep 1;30(9):1437-1447.)

- Several PARPi in clinical development have different potencies as PARP1 catalytic inhibitors and as PARP-'trappers'.
- It has been suggested that PARPi that are weak PARP1 trappers (e.g. veliparib), fail to elicit the same scale of synthetic lethality in pre-clinical models, compared with effective trappers (e.g. rucaparib, olaparib, talazoparib, niraparib)[9].
- Clinical utility has been demonstrated with PARPi in four tumour types (ovarian, prostate, breast, and pancreatic) where patients were selected for tumours displaying homologous recombination deficiency.
- The US FDA approved PARPi - olaparib for the maintenance treatment of adult patients with deleterious or suspected deleterious germline or somatic BRCA-mutated (gBRCAm or sBRCAm) advanced epithelial ovarian, fallopian tube or primary peritoneal cancer which are incomplete or partial response to first-line platinum-based chemotherapy[10] and for adult patients with deleterious or suspected deleterious germline or somatic HRR gene-mutated metastatic castration-resistant prostate cancer (mCRPC), which have progressed following prior treatment with enzalutamide or abiraterone[11].

Thus, HRR gene testing has become important to identify eligible patients who may benefit from treatment with olaparib.

HRR, involved genes and diagnostic tests:

- Homologous recombination repair is a form of DNA recombination often used to repair DNA double strand breaks (DSBs).
- HRR predominantly acts in S and G2 phases of the cell cycle and is a conservative process, restoring the original DNA sequence at the site of damage.
- During HRR, part of the DNA sequence around the DSB is removed (resection), revealing regions of single stranded DNA (ssDNA).
- The DNA recombinase RAD51 binds ssDNA and invades the DNA sequence on a homologous sister chromatid, using this as a template for the synthesis of new DNA at the DSB site.
- Crucial proteins involved in mediating HRR include those encoded by BRCA1, BRCA2, RAD51, RAD51C, RAD51D, ATM, PALB2 etc[12].
- A defect in DNA repair by hampered HRR is homologous recombination deficiency (HRD). In cancers, this is often caused by loss of function mutations in BRCA1, BRCA2, RAD51C, RAD51D or PALB2, promoter hypermethylation of the BRCA1 gene (leading to reduced expression of BRCA1) or a series of yet to be defined causes[12].

There are two principal approaches to detect tumours with defects in HRR capability and the associated HRD phenotype (Fig 2).

HRR and HRD are important features in DNA damage response that relate to prognostic and treatment outcomes for patients

HRR: Homologous Recombination Repair	**HRD:** Homologous Recombination Deficiency
HRR is essential for error-free DNA damage repair and maintenance of genome integrity	HRD is the consequence of losing HRR capability and characterised by genomic instability
• HRR fixes DNA double-stranded breaks (DSBs), which is the most common genomic form of DNA damage	• Mutation and/or epigenetic changes in one or more of the HRR genes can compromise some tumor cells ability to perform HRR
• HRR when compromised leads to genetic alterations and genomic instability	• Cells with malfunctioning HRR rely on error-prone pathways such as Non-Homologous End Joining to repair DSBs, leading to the accumulation of genetic aberrations and genomic instability
• The BRCA genes are considered the archetypal and most well understood components of the HRR pathway	• **This phenotype of loss of HRR capability and the associated genomic instability is called Homologous Recombination Deficiency, or HRD**
• HRR can be detected by identifying the mutations in genes involved through a NGS gene panel test	• HRD phenomenon is identified by HRD assay or HRD Scar test

Figure 2: What are HRR and HRD?

How to detect HRR gene variants and HRD phenotype?

A. One method is to sequence HRR genes to look for pathogenic, or deleterious, mutations that disrupt function. This is called an HRR gene panel test, which can be thought of as testing for mutations that cause HRD. HRR genes include *ATM*, *BRCA1*, *BRCA2*, *BRIP1*, *BARD1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D*, *and RAD54L*.

B. Another approach is to detect and quantify the genomic aberrations that result from loss of HRR capability and are characteristic of the HRD phenotype. This is called an HRD genomic instability assay/HRD Score, also known as a scar test.

Germline and somatic gene mutations:

Cancer occurs from mutations, or harmful changes from alterations in a gene's DNA sequence. Most mutations involve changes in the sequence of the purine or pyrimidine bases, including substitutions, deletions, additions, or frameshifts. Mutations can be divided into two broad categories, germline or somatic. (Fig 3).

Germline mutation

Originate in germinal cells (eggs or sperm) and transmitted to conception to offspring, where they are replicated in every cell of the body

Somatic mutations

Acquired during an individual's life, present only in cells descended from the cel in which the mutation originated and not heritable.

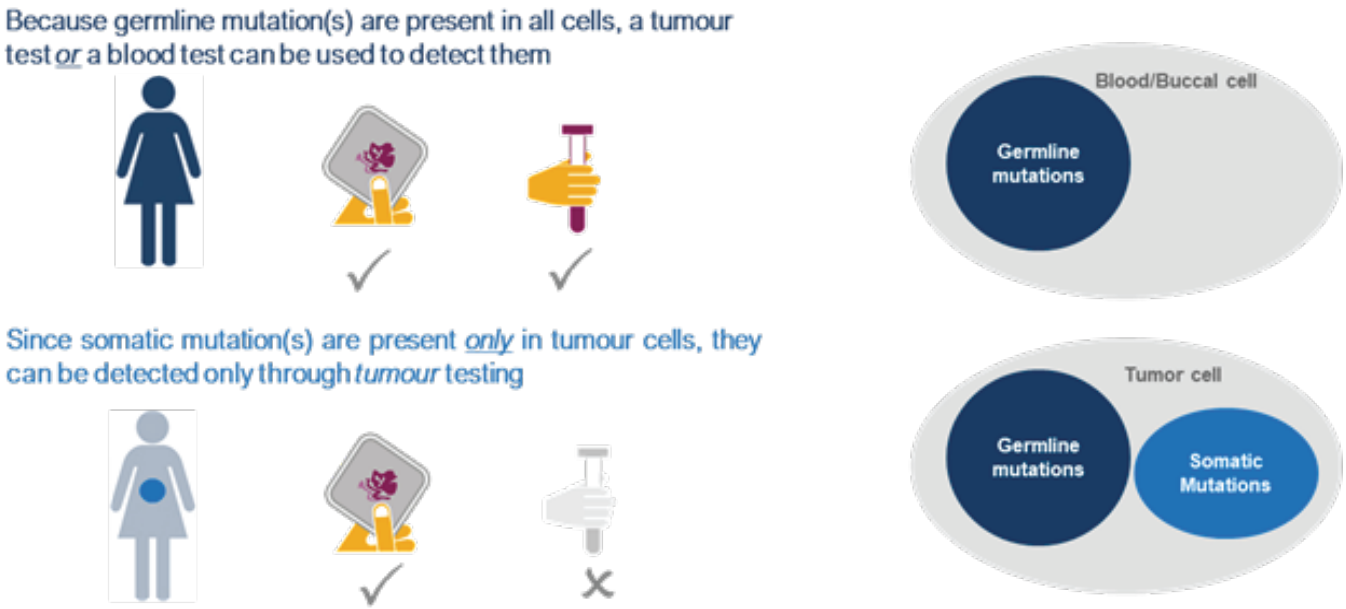

Figure 3: Only tumour HRR gene testing enables the detection of both germline and somatic mutations

Germline mutation

- A germline mutation occurs in a sperm cell or an egg cell and is passed directly from a parent to a child at the time of conception.
- As the embryo develops, the mutation from the initial sperm or egg cell is copied into every cell in the body.
- Because the mutation affects reproductive cells, it can pass from generation to generation.
- Germline mutations are present in all the cells of an individual thus, buccal cells in saliva or peripheral blood cells are convenient to utilize as samples in a genetic testing to identify these mutations.
- Presence of cancer predisposition germline mutations increases the risk of developing malignancies in an individual. An individual may carry a germline mutation and not get malignancy in his/her lifetime however, the presence of these mutations increases the relative risk of developing cancer.

Somatic or Acquired mutations

- Somatic or acquired mutations are the most common cause of cancer.
- These mutations occur from damage to genes in an individual cell during a person's life.
- Somatic mutations are not found in every cell in the body and they are not passed from parent to child.
- Some common carcinogens that cause these mutations include tobacco use, ultraviolet radiation, viruses, chemical exposures, and aging.
- Somatic mutations are present only in the cancerous cells thus, tumor samples used for detection of these mutations.

DNA sequencing techniques can identify both germline and somatic mutations by comparing the sequence of DNA with that in normal cells. Germline mutations can be identified by utilizing a saliva sample that contains buccal cells or a peripheral blood sample. Genetic testing in the tumor can be utilized to identify genetic changes in cancer cells that may be driving the growth of an individual's cancer. This information may help determine which therapies might be most effective for treating a particular malignancy. Testing the tumor samples can in principle detect both somatic and germline variants. The limitations of tumor testing must, however, be considered. It is difficult to distinguish between germline and somatic mutations by analyzing tumor tissue in isolation and it is therefore inappropriate to draw any conclusions concerning familial risk based on this analysis alone. Any patient found to have a deleterious BRCA1/2 mutation in a tumor specimen should be offered genetic counseling to undergo germline analysis to assess the existence of the mutation in germline DNA, a finding affecting familial risk[2].

During the clinical laboratory study of somatic cancer causing mutations, it is important to distinguish acquired somatic variants from inherited germline variants. Germline variants are inherited variants that are passed down from their biological parents and can be inherited through generations. The germline variants typically is present in 100% of cells, leading to allelic fractions of nearing 50% in heterozygous mutations and 100% in homozygous mutations. Somatic variants are acquired after birth and typically result from errors in DNA replication or repair or from environmental insults. Allelic fractions for somatic variants are usually <50% because of the presence of contaminating normal tissue, even in apparently pure tumor samples[13]. Although in some samples with high tumor content may show high allele fraction.

	Tumor testing	Blood testing
Advantages	Can detect both somatic and germline mutations Identifies a greater number of patients who may benefit from PARPi therapy Potentially requires less extensive genetic counselling at the outset, and less involvement for the wider family Reverted BRCA1/2 mutations can identify patients resistant to treatment	Validated methods are available and professionals are experienced in testing and interpreting variants Patient protocols, pathways and procedures are well established Evidence is strong for the association between BRCA germline mutations and response to PARPi therapy Sample is easily obtained and contains high–quality DNA Analysis feasible in 100% of cases
Disadvantages	Validated methods not yet widely available Types of mutations not well defined Only preliminary data are available on the response to PARPi associated with somatic mutations Sample with sufficiently high percentage of tumor cells/quality of DNA may be hard to obtain, leading to the need for repeat testing and/or biopsy Analysis not always possible for technical reasons. Investment in new NGS technology may be required as most traditional methods are unsuitable due to limited DNA Requires additional expertise in pathology to determine sample adequacy	Does not identify patients with somatic mutations who could benefit from PARPi therapy Genetic profile of the tumor may change with disease progression and chemotherapy

Table 1: Advantages and disadvantages of tumor testing versus blood germline testing.[2]

Recommendations for tumor based HRR testing:

Cancers are characterized by a complex and changing genetic profile; consequently, tumor based HRR gene panel testing results can potentially vary depending on disease stage, sample and testing methodology. Tumor testing be performed on primary tumors, as this is the most likely sample type available. However, it should be noted that the analysis of metastatic tissue at the time of progression may provide a more accurate indication of tumors likely to respond to PARPi treatment, due to the evidence supporting the association of revertant mutations and treatment resistance.

Surgeons responsible for patients need to be made aware of the potential need for tissue testing further down the line and thus the need for adequate collection of tumor samples prior to surgery (ie, multiple biopsy specimens with a high tumor content) (Table 2)[2]. Moreover, a validated molecular testing should be performed in an accredited facility using the most recent available tumor tissue[21].

Sample recommendations for tumor based HRR testing:

Parameter considerations	Recommendations
Fixation method	10% buffered formalin
Fixation time	8–48 hours, depending on size of specimen
Prevention of cross-contamination	Replace knife blades before each new FFPE tissue block is cut Use disposable plasticware to transfer sections to glass slides
Use of DNAzap wipes or bleach on microtome blades	Should be avoided, as are likely to degrade DNA and inhibit PCR
Percentage of neoplastic cells	3X the limit of detection of the method
Selection of tumor area	Avoid areas of inflammation or immune infiltrate, areas of necrosis, and selecting many different small regions
Thickness of section	5–10 µm sections are normally suitable. The amount is dependent on the surface of the dissected area
Macro-dissection	Careful dissection under a magnifier glass is recommended. Pre-wet the scalpel or pipette tip to avoid flakes of tissue coming off the slides
DNA extraction	Any method including a purification step suitable for small DNA fragments can be used
Use of decalcification	Not advised – reduces DNA yield and quality. If decalcification is needed (ie, bone biopsies), EDTA must be used instead of acidic decalcification

Abbreviations: DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; FFPE, formalin fixed paraffin embedded; PCR, polymerase chain reaction

- Next generation sequencing (NGS) is highly recommended as the sequencing method of choice for tumor testing, due to the less quantity of DNA available from tumor samples and the size of the coding regions and ultrasenstivity of NGS.
- Sanger sequencing should not be used in tumor testing, as it is not sensitive enough for tumor tissue sample analysis, especially in samples with <50% tumor cells, and it also requires a large amount of DNA for the screening of this large gene panel (2).

The accuracy of tumor testing is influenced by multiple variables, including

Specimen's percentage of neoplastic cells and the sensitivity	Specificity	Lower limit of detection [2]

Bioinformatic approaches using NGS data analysis of copy number variation from capture–enrichment strategies are recommended for identification of large deletions and duplications, if previously validated (2).

With this background, diagnostic yield of germline and somatic variant testing in paired tumour and blood samples from the same patient has been compared.

Assessment methodology:

1. The HRR gene (BRCA1, BRCA2, ATM, BRIP1, BARD1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, and RAD54L) variants were studied in 9 tumor tissue samples and matched peripheral blood samples collected from a random set of five prostate and four ovarian cancer samples to understand the tumor specific somatic and germline variants.
2. In the current study, paired blood and tumor tissue samples from the same patient were subjected to HRR genes sequencing. Germline testing of variants in HRR genes was performed with peripheral blood. DNA was extracted from the peripheral blood using standard column-based extraction methods using commercial kits. Tumor specific variants of HRR genes were identified from formalin fixed paraffin embedded (FFPE) tissue blocks. Tissue sections (8 µm) were made from the FFPE block and subjected to tumor content estimation. Blocks with more than 20% tumor content were selected for DNA extraction using commercial kits. The extracted DNA was subjected to QC check for quantity and quality. Highly fragment DNA samples were rejected from this analysis.
3. The captured DNA was utilized for Illumina sequencing compatible library preparation. The libraries were sequenced to mean >250X coverage for tumor DNA and 80-100X coverage for blood DNA on Illumina sequencing platform. For germline data, the raw reads were adapter trimmed and mapped to the human reference genome (GRCh37.p13/hg19) using the BWA algorithm following the GATK protocol using the Sentieon (v201808.01) analysis package(22). Gene, disease, prediction of impact and population allele frequency annotations of the variants were performed using in-house variant annotation pipeline – VariMAT 2.4.4. Gene annotation of the variants was performed using VEP program against the Ensemble release 91 human gene model. Somatic mutations were identified using LoFreq (version 2) variant caller. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation.
4. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases ClinVar, OMIM, GWAS, HGMD, SwissVar, cBioPortal, OncoMD (MedGenome's lab curated somatic database, that includes TCGA and COSMIC). Common variants were filtered based on minor allele frequency (MAF) in 1000 Genome Phase 3, ExAC, gnomAD, dbSNP141, 1000 Japanese Genome and our internal Indian population database. The biological effect of a non-synonymous variant is calculated using multiple prediction algorithms such as PolyPhen, SIFT, Mutation Taster2, and LRT. Reportable mutations were prioritized and reported based ACMG/AMP-ASCO-CAP guidelines.

Outcome of HRR gene panel variant analysis from tumor and matched blood samples:

Tumor cellularity was assessed by an in-house pathologist prior to sequencing. Samples with a tumor content >20% included in this analysis. HRR gene panel NGS analyses were successful in all nine blood samples and 7 matched FFPE tumor specimens. Two tumor samples failed at NGS library QC analysis due to poor quality of DNA materials however, both the failed samples passed the DNA QC (Table 3). Among the seven NGS successful samples six genetic variants were identified in four genes (BRCA1, BRCA2, RAD54L and BARD1) in five samples – three ovarian cancer samples (No. 5, 6 and 7) and two prostate cancer samples (No. 3 and 4) (Table 3).

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9
Sample type	Prostate	Prostate	Prostate	Prostate	Ovary	Ovary	Ovary	Prostate	Ovary
gHRR									
tHRR									

Not Detected | Detected | Failed

gHRR Blood based Germline HRR Gene Panel result

tHRR FFPE block based Tumor-specifc HRR Gene Panel results

Table 3: Sample types and mutational variant characteristics of HRR gene panel testing in matched tumor and blood samples

Out of nine NGS successful blood samples, one sample (No. 7) detected two germline variants in two different genes (BRCA2 and BARD1) (No. 7) (Table 4). The variant detected in BRCA2 has been classified as pathogenic variant and BARD1 variant classified as uncertain significance (VUS).

Somatic

	Gene	Amino acid variant/Exon no	Amino Acid Variant/ Exon No/Transcript ID	Variant Classification
Sample 3	BRCA2	p.Glu908Ter/Exon 11 ENST00000544455.1	30.70%	Tier1: variant of strong clinical significance
Sample 4	BRCA2	p.Tyr2997CysfsTer2/Exon23 ENST00000544455.1	34.80%	Tier1: variant of strong clinical significance
Sample 5	BRCA1	p.Glu237Ter/Exon10 ENST00000471181.2	74.90%	Tier1: variant of strong clinical significance
Sample 6	RAD54L	P,Cys391Ter/Exon10 ENST00000371975.4	15.40%	Tier1: variant of strong clinical significance
Sample 7	BRCA2	p.Asn718Ile/Exon11 ENST00000544455.1	42.80%	Tier1: variant of strong clinical significance
Sample 7	BARD1	p.Asn718Ile/Exon11 ENST00000260947.4	52.50%	VUS*

Germline

	Gene	Amino acid variant/Exon no	Amino Acid Variant/ Exon No/Transcript ID	Variant Classification
Sample 3	BRCA2	Not Detected		
Sample 4	BRCA2	Not Detected		
Sample 5	BRCA1	Not Detected		
Sample 6	RAD54L	Not Detected		
Sample 7	BRCA2	P.Lys1777SerfsTer4/Exon11 ENST00000544455.1	44.40%	Pathogenic
Sample 7	BARD1	p.Asn718Ile/Exon11 ENST00000260947.4	46.20%	VUS

Table 4: Cases with germline and tumor specific HRR mutations

The same variants are also identified in the corresponding tumor sample. The allelic fraction of the variants in the tumor sample were 42.8% (BRCA2) and 52.5% (BARD1), also suggesting germline origin. Tumor testing provided additional information on clinically actionable variants when germline testing was unable to identify any variants. Four tumor samples detected with four variants of strong clinical significance (Tier 1 variants) in three different genes (BRCA1, BRCA2 and RAD54L) (Table 4) with allelic fraction <50%, except BRCA1 variant (c.709G>T; p.Glu237Ter/Exon 10) was having allelic fraction of 74.9%. The sample 5 had a high allele fraction of 74.9% that may be attributable to the high tumor content is tissue (>60%).

*VUS:- Variant of uncertain significance

The observations from the outcome of HRR gene panel test:

- In India, PARPi Olaparib is now approved for clinical use in different indications of ovarian and prostate cancers with impaired HRR function. Hence, the introduction of a diagnostic test able to identify both germline and somatic HRR variants including BRCA1/2 alterations in tumor specimens has become critical to guide treatment choice.
- Traditionally, the genetic testing (e.g. BRCA1/2 gene testing) has been performed in peripheral blood/saliva to detect pathogenic variants. Recent reports demonstrated, blood based germline testing may miss the tumor tissue specific somatic variants (23).
- The HRR gene panel testing in tumor and matched blood sample demonstrated the feasibility of detecting somatic variants as well as germline variants from tumor samples.
- Somatic variants are specific to the tumour samples and hence will not be detected by blood based germline testing. Thus, tumor testing maximizes the identification of eligible patients for treatment with PARPi.
- Presence of germline variants in a patient may have familial implications. Tumor testing can identify both somatic and germline variants however, cannot differentiate. Thus, a targeted blood based germline testing by Sanger sequencing is recommended post tumor testing to confirm the origin of the identified variant.
- The allelic fraction of a variant may provide a preliminary indication about the origin of the variant. Allelic fractions for germline variants are usually >50% because of the presence in all the cells. However, this needs to be confirmed through a blood sample testing.
- Large gene panel testing increases the diagnostic yield and simultaneously increases the prevalence of a variant of uncertain significance (variant of no known clinical significance). Clinical decision should not be taken based on VUS. The patient should be referred to a genetic counsellor to be aware of the impact.
- FFPE tissues may undergo extensive degradation and chemical modification of DNA resulting from formalin fixation and paraffin embedding[24]. The failure of NGS analysis is mainly related to the quality of DNA which is derived from archival samples. In the context of an organised healthcare network, it is important to verify the suitability of FFPE specimens from each pathology unit referring samples to the reference laboratory[25].

Tumor HRR gene panel test workflow in clinical diagnostic setting:

Tumor based HRR gene panel testing is feasible, effective, to detect both germline and somatic mutations in a single test. Tumor specific somatic variants cannot be detected in DNA from blood, testing tumor DNA as the first step can maximize the identification rate of patients who stand to benefit most from PARP inhibitors. Pujol et al. proposed a model (Figure 4) of a genetic testing pathway starting from tumor analysis based on oncologist information given to the patient before the test[26]. The model portrays the schemes for managing patients with mutations in tumor analyses and a multidisciplinary approach involving an oncologist, molecular biologist/pathologist, and geneticist in case of germline findings[26].

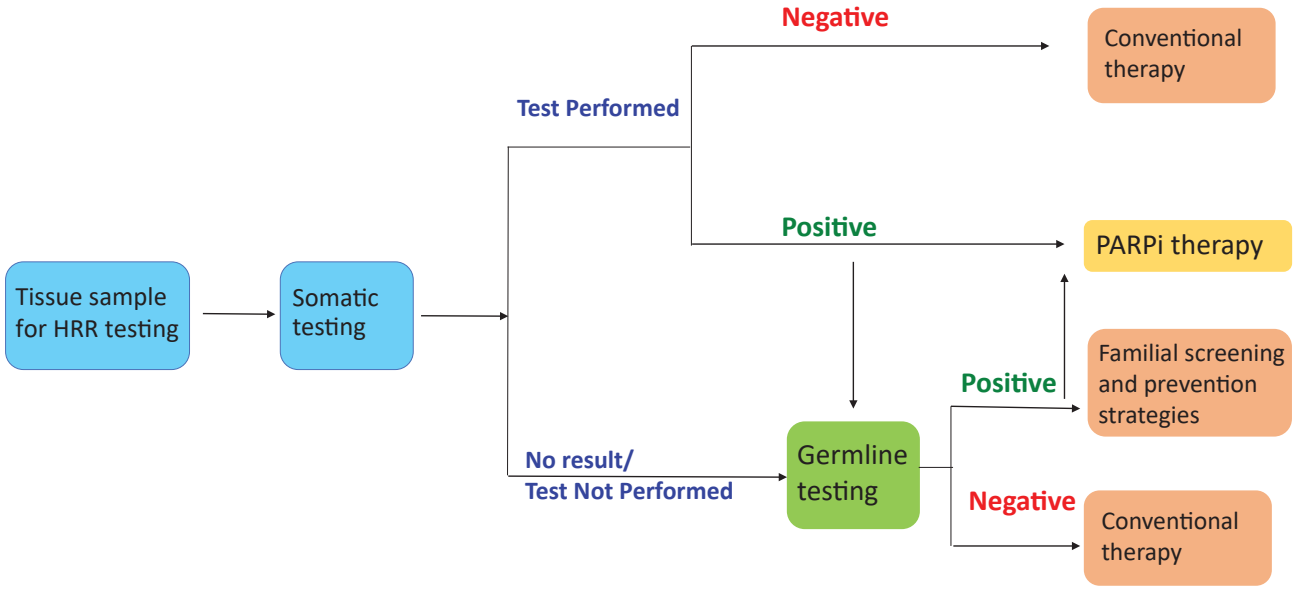

Figure 4: HRR gene panel testing for targeted therapies. PARPi: poly(ADP)ribose polymerase inhibitors. Not contributive: insufficient quantity of tumor cells or uninterpretable results. [Modified from Pujol P, et al. Diagnostics. 2019 Jul 26;9(3):83].

Under current practice of germline testing first, up to 85-90% of patients would receive two genetic tests because they are negative for a hereditary germline variant and would need a subsequent tumor test to identify tumor specific somatic variants. On the other hand 22-29% of ovarian and prostate cancer patients harbour a tumor specific (includes both germline and somatic) mutation. Thus, only they will undergo a germline testing to confirm the presence of germline mutations to address familial implications. Thus, tumor testing first approach reduces the number of patients receiving an unneeded and expensive double-test procedure [23, 27].

In conclusion, this whitepaper demonstrates tumor HRR testing detects additional tumor specific somatic variants along with germline alterations thus, maximizing the identification of patients who may benefit from PARPi treatment. A workflow in which tumor testing is requested by the treating physician and is integrated in routine care for the patients is outlined. This allows more efficient patient selection for precision medicine, genetic counselling, and preventive options. Awareness of family history remains important, and referral to genetic services should be based on both the detection of variants in the tumor test and the presence of affected cases in family histories.

References

1. Chan, H.T., Chin, Y.M. & Low, SK. The Roles of Common Variation and Somatic Mutation in Cancer Pharmacogenomics. Oncol Ther 7, 1–32 (2019).
2. Capoluongo E, Ellison G, López-Guerrero JA, Penault-Llorca F, Ligtenberg MJL, Banerjee S, Singer C, Friedman E, Markiefka B, Schirmacher P, Büttner R, van Asperen CJ, Ray-Coquard I, Endris V, Kamel-Reid S, Percival N, Bryce J, Röthlisberger B, Soong R, de Castro DG. Guidance Statement On BRCA1/2 Tumor Testing in Ovarian Cancer Patients. Semin Oncol. 2017 Jun;44(3):187-197.
3. Ellison G, Ahdesmäki M, Luke S, Waring PM, Wallace A, Wright R, Röthlisberger B, Ludin K, Merkelbach-Bruse S, Heydt C, Ligtenberg MJL, Mensenkamp AR, de Castro DG, Jones T, Vivancos A, Kondrashova O, Pauwels P, Weyn C, Hahnen E, Hauke J, Soong R, Lai Z, Dougherty B, Carr TH, Johnson J, Mills J, Barrett JC. An evaluation of the challenges to developing tumor BRCA1 and BRCA2 testing methodologies for clinical practice. Hum Mutat. 2018 Mar;39(3):394-405.
4. Pommier, Y., O'Connor, M. J. & de Bono, J. Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. Sci. Transl. Med. 8, 362ps17 (2016).
5. Roy, R., Chun, J. & Powell, S. N. BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nat. Rev. Cancer 12, 68–78 (2011).
6. Farmer, H. et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434, 917–921 (2005).
7. O'Connor, M. J. et al. Generating preclinical models to assess bone marrow toxicity induced by the PARP inhibitor olaparib in combination with chemotherapy. Cancer Res. 73, 4420 (2013).
8. Patch, A. M. et al. Whole-genome characterization of chemoresistant ovarian cancer. Nature 521, 489–494 (2015).
9. Shen Y, Rehman FL, Feng Y, Boshuizen J, Bajrami I, Elliott R, Wang B, Lord CJ, Post LE, Ashworth A. BMN 673, a novel and highly potent PARP1/2 inhibitor for the treatment of human cancers with DNA repair deficiency. Clin Cancer Res. 2013 Sep 15;19(18):5003-15.
10. https://www.fda.gov/drugs/fda-approved-olaparib-lynparza-astrazeneca-pharmaceuticals-lp-maintenance-treatment-adult-patients (Accessed on 24.05.2021)
11. https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-olaparib-hrr-gene-mutated-metastatic-castration-resistant-prostate-cancer (Accessed on 24.05.2021)
12. Miller RE, Leary A, Scott CL, Serra V, Lord CJ, Bowtell D, Chang DK, Garsed DW, Jonkers J, Ledermann JA, Nik-Zainal S, Ray-Coquard I, Shah SP, Matias-Guiu X, Swisher EM, Yates LR. ESMO recommendations on predictive biomarker testing for homologous recombination deficiency and PARP inhibitor benefit in ovarian cancer. Ann Oncol. 2020 Dec;31(12):1606-1622.
13. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, Tsimberidou AM, Vnencak-Jones CL, Wolff DJ, Younes A, Nikiforova MN. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan;19(1):4-23.

14. Hennessy BT, Timms KM, Carey MS, Gutin A, Meyer LA, Flake DD 2nd, Abkevich V, Potter J, Pruss D, Glenn P, Li Y, Li J, Gonzalez-Angulo AM, McCune KS, Markman M, Broaddus RR, Lanchbury JS, Lu KH, Mills GB. Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer. J Clin Oncol. 2010 Aug 1;28(22):3570-6.

15. Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, Dobrovic A, Birrer MJ, Webb PM, Stewart C, Friedlander M, Fox S, Bowtell D, Mitchell G. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. J Clin Oncol. 2012 Jul 20;30(21):2654-63.

16. Teyssonneau D, Margot H, Cabart M, Anonnay M, Sargos P, Vuong NS, Soubeyran I, Sevenet N, Roubaud G. Prostate cancer and PARP inhibitors: progress and challenges. J Hematol Oncol. 2021 Mar 29;14(1):51.

17. McCuaig JM, Stockley TL, Shaw P, Fung-Kee-Fung M, Altman AD, Bentley J, Bernardini MQ, Cormier B, Hirte H, Kieser K, MacMillan A, Meschino WS, Panabaker K, Perrier R, Provencher D, Schrader KA, Serfas K, Tomiak E, Wong N, Young SS, Gotlieb WH, Hoskins P, Kim RH; BRCA TtoT Community of Practice. Evolution of genetic assessment for BRCA-associated gynaecologic malignancies: a Canadian multisociety roadmap. J Med Genet. 2018 Sep;55(9):571-577.

18. Dougherty BA, Lai Z, Hodgson DR, Orr MCM, Hawryluk M, Sun J, Yelensky R, Spencer SK, Robertson JD, Ho TW, Fielding A, Ledermann JA, Barrett JC. Biological and clinical evidence for somatic mutations in BRCA1 and BRCA2 as predictive markers for olaparib response in high-grade serous ovarian cancers in the maintenance setting. Oncotarget. 2017 Jul 4;8(27):43653-43661.

19. Swisher EM, Lin KK, Oza AM, Scott CL, Giordano H, Sun J, Konecny GE, Coleman RL, Tinker AV, O'Malley DM, Kristeleit RS, Ma L, Bell-McGuinn KM, Brenton JD, Cragun JM, Oaknin A, Ray-Coquard I, Harrell MI, Mann E, Kaufmann SH, Floquet A, Leary A, Harding TC, Goble S, Maloney L, Isaacson J, Allen AR, Rolfe L, Yelensky R, Raponi M, McNeish IA. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. Lancet Oncol. 2017 Jan;18(1):75-87.

20. Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, Colombo N, Weberpals JI, Clamp A, Scambia G, Leary A, Holloway RW, Gancedo MA, Fong PC, Goh JC, O'Malley DM, Armstrong DK, Garcia-Donas J, Swisher EM, Floquet A, Konecny GE, McNeish IA, Scott CL, Cameron T, Maloney L, Isaacson J, Goble S, Grace C, Harding TC, Raponi M, Sun J, Lin KK, Giordano H, Ledermann JA; ARIEL3 investigators. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017 Oct 28;390(10106):1949-1961.

21. NCCN Guidelines; https://www.nccn.org/guidelines/category_1 (Accessed on 24.05.2021)

22. Freed, D., et al., The Sentieon Genomics Tools—a fast and accurate solution to variant calling from next-generation sequence data. bioRxiv 2017.

23. Marchetti C, Minucci A, D'Indinosante M, Ergasti R, Arcieri M, Capoluongo ED, Pietragalla A, Caricato C, Scambia G, Fagotti A. Feasibility of tumor testing for BRCA status in high-grade serous ovarian cancer using fresh-frozen tissue based approach. Gynecol Oncol. 2020 Sep;158(3):740-746.

24. Morganti S, Tarantino P, Ferraro E, D'Amico P, Duso BA, Curigliano G. Next Generation Sequencing (NGS): A Revolutionary Technology in Pharmacogenomics and Personalized Medicine in Cancer. Adv Exp Med Biol. 2019;1168:9-30.

25. Rivera D, Paudice M, Gismondi V, Anselmi G, Vellone VG, Varesco L; Ligurian BRCA Working Group. Implementing NGS-based BRCA tumour tissue testing in FFPE ovarian carcinoma specimens: hints from a real-life experience within the framework of expert recommendations. J Clin Pathol. 2020 Sep 7:jclinpath-2020-206840.

26. Pujol P, De La Motte Rouge T, Penault-Llorca F. From Targeting Somatic Mutations to Finding Inherited Cancer Predispositions: The Other Side of the Coin. Diagnostics (Basel). 2019 Jul 26;9(3):83.

27. Vos JR, Fakkert IE, de Hullu JA, van Altena AM, Sie AS, Ouchene H, Willems RW, Nagtegaal ID, Jongmans MCJ, Mensenkamp AR, Woldringh GH, Bulten J, Leter EM, Kets CM, Simons M, Ligtenberg MJL, Hoogerbrugge N; OPA Working Group. Universal Tumor DNA BRCA1/2 Testing of Ovarian Cancer: Prescreening PARPi Treatment and Genetic Predisposition. J Natl Cancer Inst. 2020 Feb 1;112(2):161-169.

MEDGENOME

For more information

Call (Toll Free): 1800-103 3691
www.medgenome.com
diagnostics@medgenome.com

MedGenome Labs Pvt. Ltd.
3rd Floor, Narayana Netralaya Building,
Narayana Health City,
258/A, Bommasandra, Hosur Road,
Bangalore - 560 099, India.

Bangalore | Cochin | California | Singapore

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

Document Number: IN-7797, Approved for Distribution Date: 1/9/2021, Expiration Date: 1/9/2023