BRCA1 and BRCA2 Mutations in Ovarian Cancer

Oncology is in the throes of a great revolution with molecular biology at the hub of it. New pathways of carcinogenesis and tumor progression are being unveiled. Many among these are being examined for drug development and companion biomarker testing.

DNA damage response pathways are the new kids on the block and are being harnessed to convert carcinogenic, sublethal genetic alterations to lethal, and cancer cell-destroying change by the phenomenon of synthetic lethality. The presence of *MGMT* methylation and consequent gene silencing has been well described as a predictor of response to temozolomide and RT in gliomas.[1,2] Contrarily, overexpression of excision repair cross-complementing1 leading to rapid repair of interstrand cross-linking is associated with drug resistance to cisplatin in human gliomas.[3]

The latest clamor is on "Homologous Recombination (HR) Defects" as a marker for identifying "Hereditary Breast and Ovarian Cancer Families" and treating the advanced ovarian cancers with poly (ADP-ribose) polymerase (PARP) inhibitors.

HR repair pathway corrects the double-stranded DNA breaks using the homologous sequence in sister chromatid or on the second chromosome as a template. The process is efficient and restores the DNA to its pristine state. Failure of HR leads to the use of alternative nonhomologous end joining pathway of DNA repair which yields a repaired but altered DNA sequence. Accumulation of such mutagenic event is carcinogenic.

The HR repair pathway employs a horde of proteins of which BRCA1 and BRCA2 are most significant; other being MRN complex, BRIP1, PALB2, RAD51C, RAD51D, and BARD1. The germline loss of function mutations in BRCA1 and BRCA2 is responsible for an important group of inherited cancer syndromes such as hereditary breast-ovarian cancer syndrome and hereditary site-specific ovarian cancer syndrome [Figure 1].

It is understood that 5%–10% of breast cancer and 15% of all ovarian cancer are caused by germline mutations in BRCA1 and BRCA2 genes.[4,5] Defects in other HR proteins such as BRIP1, PALB2, RAD51C, RAD51D, and BARD1 also contribute to hereditary breast and ovarian cancer but far less commonly [Figure 2].[6]

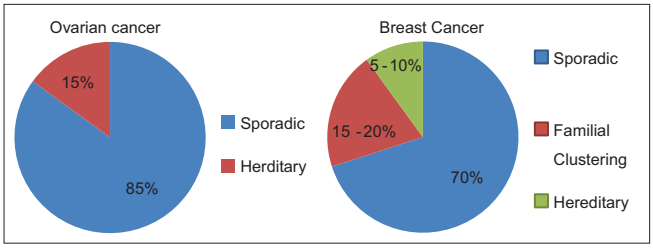

Figure 1: The risk of ovarian and breast cancer as part of HBOC syndrome

Looking at it the other way, the BRCA gene mutation carriers have a high lifetime risk of developing breast and ovarian cancer. Table 1 exhibits the penetrance of BRCA gene mutations by 70 years of age.[7]

An important fact that emerges from Table 1 above is the high concentration of BRCA1 and BRCA2 mutations in patients with ovarian and male breast cancer. How this is important in schemes of things becomes obvious when I discuss the preventive strategy for ovarian cancer.

Why Test for BRCA Mutations in All Ovarian Cancer?

Universal BRCA testing has been recommended for patients with ovarian cancer.[8] The reasons for such recommendations are two-fold [Figure 3].

Table 1: Incidence of breast and ovarian cancer in individuals with pathogenic BRCA mutations

Cancer type	Risk in general population (%)	BRCA1 (%)	BRCA2 (%)	Number of times risk raised
Female breast cancer	12.5	50-70	40-50	4-fold
Ovarian cancer	1-1.5	40-50	20-30	30-fold
Male breast cancer	0.12	01.2	06.8	50-fold

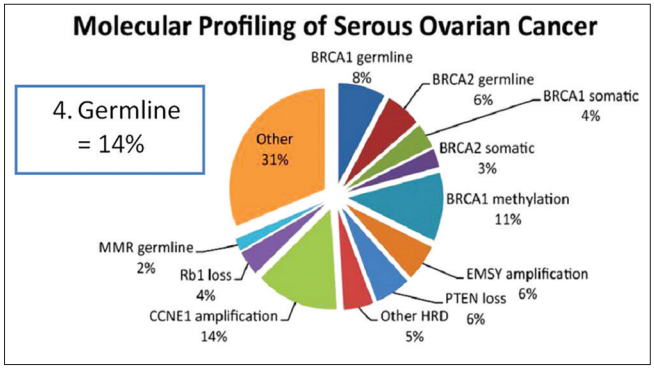

Figure 2: Germline mutations in BRCA genes account for 14% of ovarian cancers. In addition biallelic somatic loss of function mutations also cause ovarian cancer but at half the rate of germline mutations

From the Perspective of Patients

From a patient perspective, the major benefits are as follows: (I) forecasting prognosis, (II) use of BRCA mutation as a predictive marker for the use of PARP inhibitors, and (III) encouraging the application of risk reduction strategies for breast cancer in long-term survivors.

Several pooled analysis, single-center studies, and meta-analysis have revealed favorable outcome for patients with BRCA mutated ovarian cancer.[9-12]

In addition, BRCA mutation status is used as a predictive marker for the use of PARP inhibitors (PARPi) in ovarian cancer. PARP is a versatile enzyme with several key physiological functions, one among which is single-strand DNA break repair by "Base excision repair pathway." With PARP blockade by PARPi, the single strand breaks are not repaired and are converted into double-stranded breaks with cell replication. The absence of functional BRCA does not allow the repair of double-stranded breaks with consequent accumulation of fragmented DNA incompatible with cellular viability. This concept of coupling one dysfunctional DNA damage pathway with externally induced dysfunction in another is called "Synthetic Lethality." Synthetic lethality is the basis of the use of PARPi in ovarian cancer. PARPi is used in two clinical settings:

- Single-agent therapeutics
- Maintenance therapy – postplatinum chemotherapy.

Current Food and Drug Administrations approvals as single agent therapy are for following two agents

- Olaparib as a single-agent treatment for patients with ovarian cancer with a documented "germline BRCA mutation" who have received a minimum of three prior lines of cytotoxic chemotherapy[13]
- Rucaparib as third-line single-agent treatment for women with ovarian cancer germline BRCA and somatic BRCA mutations.[13]

In addition, and more significantly, PARPi are also approved as maintenance therapy following a partial or complete response to a platinum-based therapy. The current approval is for Olaparib in germline BRCA mutated ovarian cancer post ≥2 lines of therapy (SOLO2) and Niraparib following the attainment of a second-line (or later) response (complete or partial) to platinum-based chemotherapy. The latter drug has shown median progression-free survival (PFS) of 21 months against 5.5 months for placebo in germline BRCA mutated ovarian cancer. A somewhat mooted response has also been seen in BRCA wild-type patients with a median PFS of 9.3 months against 3.9 months for placebo. However, even in this latter group, patients harboring HR deficiencies were the ones with median PFS of 12.9 months[14,15] (NOVA Trial).

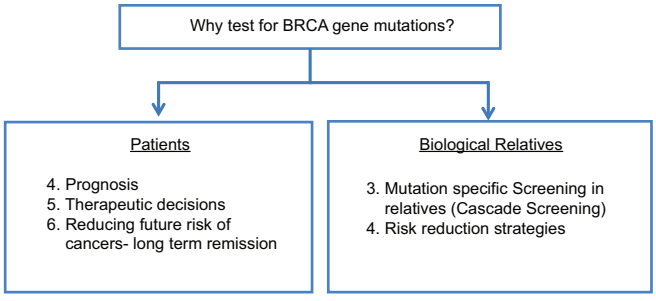

Figure 3: Benefits of BRCA testing

From the Perspective of Biological Relatives

The patient with germline BRCA mutation acts as a proband for further testing of first degree relatives (FDR) from three generations because these FDR are at 50% risk of carrying the harmful mutation. The patient undergoes comprehensive BRCA testing where the entire BRCA1 and BRCA2 genes are sequenced, and pathogenic mutation is identified. Take as an example, the presence of c. 800C>G (p.Ser267Ter) BRCA1 in the patient (proband). Hereafter, the relatives at risk are only tested for c. 800C>G (p.Ser267Ter) in BRCA1. This restricted testing is called "Single site – Predictive testing." Such testing is a very powerful preventive strategy. Why so? Take a look at Table 2. Given the incidence of 1 mutation carrier in 800 patients, testing 800 patients will yield one mutation carrier while testing 100 cases of ovarian cancer will identify 15 mutation carriers. Assuming that approximately 3 FDR undergo further testing, 50 single site tests will identify 25 new mutation carriers from the community. To achieve similar results 20,000 full BRCA tests were otherwise needed.

Enormous resource saving in identifying a population at risk is the main benefit of universal BRCA testing in ovarian cancer patients. Encouraging these newly identified individuals to practice risk reduction strategies can provide full longevity to >90% of them.

Who among the FDR shall be tested is the next question to answer? The FDR from 3 generations from that side of the family where proband lies become the test subjects [Figure 4].

On finding a positive carrier, the testing is expanded to the three generations of this newly discovered mutation carrier. This concept of widening the mutation testing with a small site-specific test is called cascade testing and is recommended by ACOG.[16]

Table 2: Benefit of limited BRCA testing in first degree relatives of patients of ovarian cancer

Subjects	*n*	Positive mutation carriers
General population	800 individuals	1+
Ovarian cancer	100 cases	15+
FDR	50 individuals	25+

50 single site predictive tests in FDR identify 25 mutation carriers. To achieve same 800×25=20,000 full BRCA tests were needed. FDR: First degree relatives

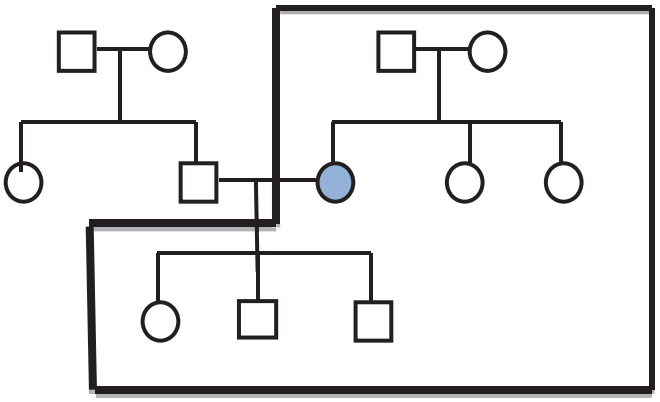

Figure 4: - - Patient of ovarian cancer (proband). The FDR in the black box are the test subjects

What test to employ is the other question that is frequently asked? While companion diagnostics from foundation medicine and Myriad are approved for BRCA testing in the USA, such tests are expensive and not accessible to all in India. Next-generation sequencing-based test covering all coding regions and splice sites are available. One problem with this test is its inability to identify 10% of pathogenic alterations in the form of long genetic rearrangements (big Indels). Subjects with negative results, therefore, need to undergo further testing by Multiplex Ligation Probe Amplification assay capable of identifying such big Indels.

For FDR site-specific testing is adequate.

Patients with ovarian cancer negative for germline BRCA mutation and being considered for Rucaparib as monotherapy can undergo somatic BRCA testing using formalin fixed paraffin embedded tumor tissue [Figure 5].

The test results should be reported as per ENIGMA classes.[17] Most results clearly fall as pathogenic or benign. However, a small percentage ~10% fall in "no man's land" and are given the appellation of "Variants of Undetermined significance." Remember, these are not negative results. Good laboratory and an astute physician will keep track of these mutations overtime to see which side of the fence these will finally fall and what are the implications for the subject.

Conclusion

- BRCA1 and BRCA2 – biallelic loss of function mutations: Cause cancer(s)
- These could be germline or somatic
- Other members of HR can also be responsible-but penetrance is low
- BRCA testing help determine therapeutic options for the patient
- BRCA testing of an ovarian cancer patient and cascade screening thereof is a powerful preventive strategy that can help reduce the incidence of this dreadful malignancy.

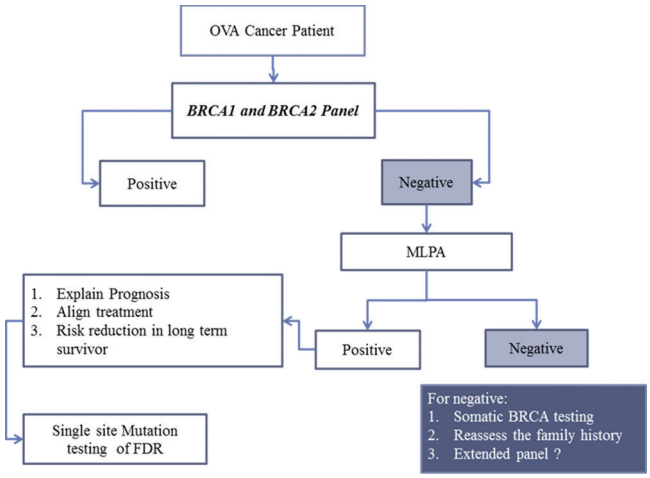

Figure 5: The workflow of breast and ovarian cancer susceptibility gene testing in a patient of ovarian carcinoma and her biological relatives

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