

COMMENTARY

Recommendations for the implementation of *BRCA* testing in the care and treatment pathways of ovarian cancer patients

Carmine Pinto^{*1}, Maria Angela Bella², Ettore Capoluongo³, Paola Carrera⁴, Claudio Clemente⁵, Nicoletta Colombo⁶, Laura Cortesi⁷, Gaetano De Rosa⁸, Francesca Fenizia⁹, Maurizio Genuardi¹⁰, Stefania Gori¹¹, Valentina Guarneri¹², Antonio Marchetti¹³, Paolo Marchetti¹⁴, Nicola Normanno¹⁵, Barbara Pasini¹⁶, Sandro Pignata¹⁷, Paolo Radice¹⁸, Enrico Ricevuto¹⁹, Antonio Russo²⁰, Piersandro Tagliaferri²¹, Pierfrancesco Tassone^{21,22}, Mauro Truini²³ & Liliana Varesco²⁴

First submitted: 13 April 2016; Accepted for publication: 10 May 2016; Published online: 31 May 2016

In the last 20 years, following the identification of the *BRCA1* and *BRCA2* genes (hereinafter referred to as the *BRCA* genes), preventive pathways have been developed for the identification and clinical management of individuals at high risk

KEYWORDS

• *BRCA1* • *BRCA2* • genetic testing • germline mutations • ovarian cancer • PARP inhibitors • somatic mutations

¹Medical Oncology S.Maria Nuova Hospital – IRCCS Reggio Emilia, Italy

²Medical Oncology Unit, University Hospital of Parma, Italy

³Institute of Biochemistry & Clinical Biochemistry, Catholic University of the Sacred Heart, Rome, Italy

⁴Unit of Genomics for Diagnosis of Human Pathologies, Division of Genetics & Cell Biology, & Laboratory of Clinical Molecular Biology, IRCCS Ospedale San Raffaele, Milan, Italy

⁵Pathology & Cytopathology Unit, Casa di Cura San Pio X & IRCCS Policlinico San Donato, Milano, Italy

⁶European Institute of Oncology 'IEO', Milan, Italy

⁷Department of Oncology, Hematology & Respiratory Diseases, University Hospital of Modena, Italy

⁸Department of Advanced Biomedical Sciences, Pathology Section, University of Naples Federico II, Naples, Italy

⁹Pharmacogenomic Laboratory, Centro di Ricerche Oncologiche di Mercogliano (CROM), Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione Giovanni Pascale" – IRCCS Naples, Italy

¹⁰Institute of Genomic Medicine, 'A Gemelli' School of Medicine, Catholic University of the Sacred Heart, Rome, Italy

¹¹Department of Oncology, Ospedale Sacro Cuore Don Calabria, Negrar-Verona, Italy

¹²Department of Surgery, Oncology & Gastroenterology, University of Padova; Medical Oncology 2, Istituto Oncologico Veneto IRCCS, Padova, Italy

¹³Center of Predictive Molecular Medicine, University-Foundation, CeSI Biotech Chieti, Italy

¹⁴Clinical & Molecular Medicine Department, Sapienza University, Rome, Italy

¹⁵Cell Biology & Biotherapy Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori 'Fondazione Giovanni Pascale' – IRCCS Naples, Italy

¹⁶Department of Medical Science, University of Turin, Turin, Italy

¹⁷Uro-Gynecological Oncology, Istituto Nazionale per lo Studio e la Cura dei Tumori 'Fondazione Giovanni Pascale' – IRCCS Naples, Italy

¹⁸Unit of Molecular Bases of Genetic Risk & Genetic Testing, Department of Preventive & Predictive Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

¹⁹Oncology Network ASL1 Abruzzo, Oncology Territorial Care Unit, Division of Medical Oncology, Department of Biotechnological & Applied Clinical Sciences, University of L'Aquila, Italy

²⁰Department of Surgical, Oncological & Oral Sciences, Section of Medical Oncology, University of Palermo, Italy

²¹Department of Experimental & Clinical Medicine, Magna Graecia University, Salvatore Venuta University Campus, Catanzaro, Italy

²²Sbarro Institute for Cancer Research & Molecular Medicine, Center for Biotechnology, College of Science & Technology, Temple University, Philadelphia, PA, USA

²³Pathological Anatomy Histology & Cytogenetics, Niguarda Cancer Center, Niguarda Ca' Granda Hospital, Milan, Italy

²⁴Unit of Hereditary Cancers, IRCCS AOU San Martino – IST, Genoa, Italy

*Author for correspondence: Tel.: +39 0522 296614; Fax: +39 0522 296854; pinto.carmine@asmn.re.it

of developing breast and ovarian cancer due to the presence of a pathogenic variant in either of these genes. These pathways are aimed at educating high-risk subjects on programs targeted toward early diagnosis and cancer risk reduction.

The approval of a novel class of drugs, the PARP enzyme inhibitors, for the treatment of ovarian cancer patients carrying high-risk *BRCA* pathogenic variants has changed this scenario. *BRCA* testing, in addition to providing information on the risk of disease, has become also a predictive marker of drug response in ovarian carcinoma patients. These recommendations prepared by Associazione Italiana di Oncologia Medica (AIOM), Società Italiana Genetica Umana (SIGU), Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica (SIBIOC) and Società Italiana di Anatomia Patologica e Citologia Diagnostica – Italian Division of the International Academy of Pathology (SIAPEC-IAP) are focused on the implementation of *BRCA* testing in the care and treatment pathways of ovarian cancer patients.

“The timing of *BRCA* testing should be chosen with the patient in order to respect her needs for the decision-making process.”

***BRCA* testing for the prediction of treatment efficacy**

Recent population studies have highlighted that the prevalence of constitutional *BRCA* pathogenic variants in ovarian cancer patients is >10%, independently of age of diagnosis and of family history of breast/ovarian cancer [1,2]. The prevalence of pathogenic variants progressively increases in patients with serous ovarian carcinoma (17–20%), high-grade serous carcinoma (23–25%) and in platinum-sensitive patients (30–40%) [3–6]. Furthermore, approximately 25% of women carrying pathogenic *BRCA* variants have a diagnosis of ovarian carcinoma over 60 years of age [1–3].

Retrospective studies suggest that heterozygosity for a *BRCA* hereditary pathogenic variant in ovarian cancer patients is associated with a significantly more favorable prognosis and is predictive of sensitivity to combination therapies containing platinum derivatives [3,7].

Somatic mutations of the *BRCA* genes have been identified in approximately 6% of serous ovarian carcinomas [4,6], thus suggesting that inactivation of these genes can also occur during cancer progression.

Importantly, it has been shown that *BRCA* gene mutations, whether constitutional or somatic, are a biomarker of sensitivity to

treatment with PARP inhibitors, a class of pharmacological agents involved in the repair of single-strand DNA breaks, in patients with advanced ovarian cancer [8,9]. The therapeutic effect of PARP inhibitors in ovarian cancer is due to ‘synthetic lethality’, which occurs in cells with an inactive double-strand DNA repair mechanism mediated by homologous recombination (HR). The *BRCA1/2* proteins play an essential role in HR [8–13], and their loss of function due to constitutional or somatic *BRCA* gene mutations can be one of the major causes of HR dysfunction [6,14–15]. Following clinical trials, the PARP inhibitor olaparib has been registered in October 2014 by EMA as a maintenance therapy in patients with relapsed high-grade serous platinum-sensitive ovarian cancer, Fallopian tube cancer and primary peritoneal cancer [5,9,16].

Hence, *BRCA* testing is now a prerequisite for the indication of PARP inhibitor therapy. However, it is advisable to consider offering the test at the time of initial diagnosis to all patients with nonmucinous and nonborderline ovarian epithelial carcinoma, fallopian tube carcinoma and primary peritoneal carcinoma. This approach would allow us to obtain early the information on the potential sensitivity to PARP inhibitors and at the same time it would also pave the way for cancer genetic counseling and prevention. The timing of *BRCA* testing should be chosen with the patient in order to respect her needs for the decision-making process. In addition, the patient must be provided with appropriate and thorough information regarding all issues associated with a positive test outcome.

Types of *BRCA* testing

The *BRCA* test on peripheral blood detects constitutional/hereditary variants – that is, those which can be transmitted to the offspring (50% probability for each child). On the other hand, the test conducted on tumor tissue can also identify the variants acquired by somatic mutations during tumor development and progression, in addition to constitutional defects. This implies that when a variant is identified in tumor DNA, its origin (constitutional or somatic) must be established by analyzing normal tissue (blood or other tissue).

According to available data, 2/3 of the pathogenic *BRCA* variants identified in patients affected by ovarian cancer are constitutional (present in every cell of the individual) and

1/3 somatic (confined to tumor tissue) [4,6]. Constitutional *BRCA* variants are nearly always inherited from one parent; less than 1% of cases are due to *de novo* mutations, occurring in the parental germ cells or *in utero*.

In the clinical setting, mutation screening of *BRCA1* and *BRCA2* is usually carried out by sequencing of all coding exons ($n = 22$ and $n = 26$, respectively) and corresponding exon/intron junctions. This allows detection of point variations (single nucleotide substitutions and insertions/deletions of one or a few bases) in the DNA sequence. These alterations encompass more than 90% of *BRCA* pathogenic variants occurring in the coding regions of both genes. In order to maximize sensitivity, the test must include the search for large genomic rearrangements (i.e., deletions or duplications of one or more exons, or of the whole gene), which account for a variable proportion of constitutional *BRCA* variants across populations, usually not exceeding 10%. These types of changes can be investigated by multiplex ligation probe dependent amplification or multiplex amplicon quantification [17,18].

Currently, sequencing analyses for the detection of constitutional pathogenic *BRCA* gene variants on peripheral blood is performed in most laboratories using different methods. These include well-established techniques, namely Sanger sequencing, as well as novel technologies – that is, next-generation sequencing, which are under validation [19–24].

There are as yet no standardized tests for *BRCA* analysis in tumor samples. *BRCA* testing in tumor tissue is more complex than other molecular somatic tests already included in routine cancer diagnosis. This is due to the high degree of heterogeneity of potentially pathogenic variants, which can be observed along the entire sequence of *BRCA1* and *BRCA2* genes. Furthermore, in addition to the already mentioned different types of DNA variations (point variations, large deletions/duplications), epigenetic alterations (methylation of the regulatory regions) can also occur [4,15]. This makes it difficult to assess the predictive value, in terms of response to treatment, of the different *BRCA* variants, in particular those identified at somatic level. Indeed, the available classification algorithms have been devised to identify constitutional variants associated with a high risk of developing breast and ovarian cancer (see below). While *BRCA* mutation test in

blood DNA is routinely applied in the search of constitutional pathogenic variants, the actual feasibility of the somatic test by the diagnostic laboratory should be verified in advance, in consideration of the inherent methodological difficulties. Laboratories should carry out technical assessment procedures and take part in external quality control programs of the tests they offer (constitutional and/or somatic) [25].

Interpretation of *BRCA* genetic variants

The mutation spectrum of the *BRCA* genes is very broad, and the interpretation of the clinical significance of identified variants is not always straightforward. Indeed, variant classification is an important aspect of the *BRCA* testing process, particularly when considering that quite often clinical testing detects genetic alterations not reported in the scientific literature [26]. Therefore, although no consolidated standards exist for the classification of constitutional *BRCA* variants, it is important that laboratories use updated classification criteria, and that reports be written up in accordance with good laboratory practice recommendations.

The Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) has recently developed specific criteria for interpreting the clinical significance, in relation to cancer risk, of constitutional variants of *BRCA* genes. These are available on the consortium website [27]. However, it must be considered that studies in mouse models have highlighted that not all *BRCA* gene variants associated with high risk of developing cancer are predictors of response to anti-PARP therapy [8].

It is therefore necessary that laboratory reporting protocols, including the interpretation process, be made publicly available. In addition, the clinical significance of the *BRCA* gene variants identified should be clearly stated, along with a list of the essential information used for the classification [26,28]. In this regard, laboratories, in addition to taking part in external quality control programs, should contribute to a systematic and centralized collection of all *BRCA* variants observed with the aim to improve their classification [25].

BRCA testing in the care & treatment pathway

The use of *BRCA* testing as a treatment decision tool implies that it should be readily accessible

“The use of *BRCA* testing as a treatment decision tool implies that it should be readily accessible to all those patients who may benefit from it and that the test results be made available within a time compatible with the clinical needs.”

to all those patients who may benefit from it and that the test results be made available within a time compatible with the clinical needs.

The models of cancer genetic counseling developed in the preventive setting are currently insufficient to meet the increasing number of *BRCA* test requests, particularly when the test for genetic predisposition also has a predictive value for treatment which needs to be determined in a short time. The optimal model of genetic counseling within the prevention pathway involves detailed information and discussion of the genetic aspects right from the pretest stage. On the other hand, the need to obtain test results in a timely manner in order to implement treatment planning implies that also oncologists and gynecologists experienced in oncology can directly request the *BRCA* test to the laboratory. Even when the test is performed in the cancer treatment setting, the arrangement of full care pathways is mandatory, to ensure the correct interpretation of the results for clinical purposes, the correct way to manage family members at risk if a hereditary pathogenic variant is identified and the correct genetic assessment of cases with a noninformative *BRCA* test result [25–26,28].

Physicians who request the *BRCA* test must receive appropriate updated training in genetic oncology. Since prescribers bear final responsibility for the use of the test result, they must verify that the laboratory uses appropriate protocols for correct clinical interpretation. Furthermore, protocols must be defined for the referral of patients with inherited pathogenic variant to a clinical cancer genetics team so that family members can receive appropriate care, as well as for particular cases warranting further genetic investigation [28].

Each center must provide clear indications of the management pathways to the patients and their relatives, outlining the duties and responsibilities of the oncology team, of the laboratory and of the clinical cancer genetics team across the different phases of the defined care pathway. Should recognized standards not be available, one should consider submitting these pathways to verification via planned audits, with the aim to improve service quality.

Essential items of the informed consent

The *BRCA* test for prediction of response to PARP inhibitors may be prescribed by clinical geneticists, oncologists and gynecologists with oncologic expertise. The prescriber has the

responsibility to provide appropriate information to the patients on the genetic aspects associated with the results in order to enable them to make informed choices. The information provided to the patient should cover the potential therapeutic benefits of treatment with a PARP inhibitor, together with the possibility of detecting a high cancer risk condition for the patient and her relatives. The timing at which informed consent to genetic testing is obtained, as well as the modalities, must respect the will of the patient, who should be given the possibility to discuss all the different implications of genetic testing, such as whether or not to tell other family members about the test results, before taking the decision.

Physicians who prescribe a *BRCA* test should abide to an appropriate communication and written informed consent collection protocol, using specific information material and consent forms. Oncologists and gynecologists with oncologic expertise who do not have experience in cancer genetics must follow a training program which includes ethical aspects of *BRCA* testing. Finally, the care pathway must clearly identify the cancer genetics team to be contacted, should the patient require a closer examination of the genetic aspects before deciding whether or not to undergo the test, as well as for those cases which present particular issues.

Conclusion

In conclusion, *BRCA* mutations represent a biomarker predictive of sensitivity to treatment with PARP inhibitors, in addition to cancer risk assessment. As highlighted in this recommendation, several issues are associated with diagnostic *BRCA* testing: the screening methods vary across laboratories, and yet no standardized tests exist; *BRCA* analyses can reveal changes of unclear clinical significance, in addition to variants with a pathogenic or neutral role; finally, there is a need to devise the most appropriate report models, to avoid misinterpretations among clinicians and confusion among the patients. In this respect, the combined goal of this recommendation by the Italian scientific societies is to improve the outcome of therapy, to promote prevention and finally to reduce mortality of ovarian cancer.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes

“...*BRCA* mutations represent a biomarker predictive of sensitivity to treatment with PARP inhibitors, in addition to cancer risk assessment.”

employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Open access

This work is licensed under the Creative Commons Attribution-NonCommercial 4.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>

References

- 1 Soegaard M, Kjaer SK, Cox M *et al.* *BRCA1* and *BRCA2* mutation prevalence and clinical characteristics of a population-based series of ovarian cancer cases from Denmark. *Clin. Cancer Res.* 14(12), 3761–3767 (2008).
- 2 Song H, Cicek MS, Dicks E *et al.* The contribution of deleterious germline mutations in *BRCA1*, *BRCA2* and the mismatch repair genes to ovarian cancer in the population. *Hum. Mol. Genet.* 23(17), 4703–4709 (2014).
- 3 Alsop K, Fereday S, Meldrum C *et al.* *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.* 30(21), 2654–2663 (2012).
- 4 George J, Alsop K, Etemadmoghadam D *et al.* Nonequivalent gene expression and copy number alterations in high-grade serous ovarian cancers with *BRCA1* and *BRCA2* mutations. *Clin. Cancer Res.* 19(13), 3474–3484 (2013).
- 5 Ledermann J, Harter P, Gourley C *et al.* Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by *BRCA* status in a randomised Phase 2 trial. *Lancet Oncol.* 15(8), 852–861 (2014).
- 6 Tcga: Integrated genomic analyses of ovarian carcinoma. *Nature* 474(7353), 609–615 (2011).
- 7 Bolton KL, Chenevix-Trench G, Goh C *et al.* Association between *BRCA1* and *BRCA2* mutations and survival in women with invasive epithelial ovarian cancer. *JAMA* 307(4), 382–390 (2012).
- 8 Drost R, Jonkers J. Opportunities and hurdles in the treatment of *BRCA1*-related breast cancer. *Oncogene* 33(29), 3753–3763 (2014).
- 9 Wiggins AJ, Cass GK, Bryant A, Lawrie TA, Morrison J. Poly(ADP-ribose) polymerase (PARP) inhibitors for the treatment of ovarian cancer. *Cochrane Database Syst. Rev.* (5), CD007929 (2015).
- 10 Bouwman P, Jonkers J. Molecular pathways: how can *BRCA*-mutated tumors become resistant to PARP inhibitors? *Clin. Cancer Res.* 20(3), 540–547 (2014).
- 11 Kaelin WG Jr. The concept of synthetic lethality in the context of anticancer therapy. *Nat. Rev. Cancer* 5(9), 689–698 (2005).
- 12 Lord CJ, Tutt AN, Ashworth A. Synthetic lethality and cancer therapy: lessons learned from the development of PARP inhibitors. *Annu. Rev. Med.* 66, 455–470 (2015).
- 13 Michels J, Vitale I, Saparbaev M, Castedo M, Kroemer G. Predictive biomarkers for cancer therapy with PARP inhibitors. *Oncogene* 33(30), 3894–3907 (2014).
- 14 Curtin NJ. DNA repair dysregulation from cancer driver to therapeutic target. *Nat. Rev. Cancer* 12(12), 801–817 (2012).
- 15 Hilton JL, Geisler JP, Rathe JA, Hattermann-Zogg MA, Deyoung B, Buller RE. Inactivation of *BRCA1* and *BRCA2* in ovarian cancer. *J. Natl Cancer Inst.* 94(18), 1396–1406 (2002).
- 16 EMA: CHMP assessment report. www.ema.europa.eu
- 17 Hogervorst FB, Nederlof PM, Gille JJ *et al.* Large genomic deletions and duplications in the *BRCA1* gene identified by a novel quantitative method. *Cancer Res.* 63(7), 1449–1453 (2003)
- 18 Kumps C, Van Roy N, Heyrman L *et al.* Multiplex amplicon quantification (MAQ), a fast and efficient method for the simultaneous detection of copy number alterations in neuroblastoma. *BMC Genomics* 11, 298 (2010).
- 19 Ellard S, Charlton R, Yau M Clinical Molecular Genetics Society *et al.* Practice guidelines for Sanger sequencing analysis and interpretation. Birmingham, UK. www.acgs.uk.com
- 20 Grada A, Weinbrecht K. Next-generation sequencing: methodology and application. *J. Invest. Dermatol.* 133(8), e11 (2013)
- 21 Mardis ER. Next-generation DNA sequencing methods. *Annu. Rev. Genomics Hum. Genet.* 9, 387–402 (2008).
- 22 Meldrum C, Doyle MA, Tothill RW. Next-generation sequencing for cancer diagnostics: a practical perspective. *Clin. Biochem. Rev.* 32(4), 177–195 (2011).
- 23 Rizzo JM, Buck MJ. Key principles and clinical applications of “next-generation” DNA sequencing. *Cancer Prev. Res. (Phila.)* 5(7), 887–900 (2012).
- 24 Walsh T, Lee MK, Casadei S *et al.* Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. *Proc. Natl Acad. Sci. USA* 107(28), 12629–12633 (2010).
- 25 Wallis Y, Payne S, McAnulty C *et al.* Practice guidelines for the evaluation of pathogenicity and the reporting of sequence variants in clinical molecular genetics ACGS /VGKL. www.acgs.uk.com
- 26 Plon SE, Eccles DM, Easton D *et al.* Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum. Mutat.* 29(11), 1282–1291 (2008).
- 27 ENIGMA. <https://enigmaconsortium.org/>
- 28 Claustres M, Kozich V, Dequeker E *et al.* Recommendations for reporting results of diagnostic genetic testing (biochemical, cytogenetic and molecular genetic). *Eur. J. Hum. Genet.* 22(2), 160–170 (2014).