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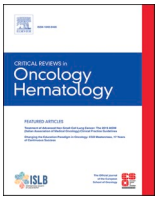

Practical tricks and recommendations on BRCA1/2 testing in prostate cancer: From therapy to cancer prevention

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ABSTRACT

Prostate cancer (PC) is the most frequently diagnosed cancer among men in the Western World. When it is diagnosed and treated at localized stages, it has a 97 % 5-yr cancer-specific survival compared to 30 % in the metastatic setting. Recently, inhibitors of the Poly(ADP-ribose) polymerase (PARPi) were shown to be effective treatments for metastatic PC harbouring defects in the homologous recombination mechanism of DNA repair (HRR), mainly in the *BRCA1/2* genes. The *BRCA1/2* pathogenetic variants are currently used, in clinical setting, as predictive markers for PARP-i therapy eligibility in patients with metastatic castration-resistant PC (mCRPC). *BRCA1/2* pathogenetic variants are also responsible for inherited cancer predisposition syndromes when they are detected in the germinal setting. For this reason, it is of paramount importance that germline genetic testing be triggered following the identification of *BRCA* somatic variants in order to implement appropriate prevention pathways for subjects with high cancer risk. To date, there are no standardised protocols for this dual path of personalized therapy and prevention through the identification of subjects at high risk of cancer. AIFET and SIURO in this paper analyse the critical points of this dual path and propose a working model.

1. Introduction

Prostate cancer is the most common cancer in adult men and the third lead cause of cancer-related death in the Western World.

When it is diagnosed and treated at localized stages it has a 97 % 5-yr cancer-specific survival compared to 30 % in the metastatic setting (Siegel et al., 2022).

The mainstay treatment for metastatic (m)PC remains targeting of the androgen receptor pathway, although patients will invariably develop resistance to such treatments, known as castration resistant (CR) status.

Recently, inhibitors of the Poly(ADP-ribose) polymerase (PARPi)

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[1] **AIFET** (Associazione Italiana Familiarita' Ereditarieta' Tumori) was founded in 2021 but can be traced back to the Italian association for Gastrointestinal Familial Hereditary Tumours (AIFEG) established in 2002. AIFET has been conceived as a multidisciplinary resource for studying all hereditary tumours, to optimize personalized prevention, diagnosis and treatment. It aims to be a reference point for geneticists, oncologists, surgeons and physicians of different disciplines to understand better how to manage patients and family at risk. This Association is active also in multidisciplinary research and training and participates to the drawing up and editing of the guidelines of the Italian Association of Medical Oncology (AIOM). **SIURO** (Societa' Italiana di Uro-Oncologia) has been founded in 1990. It gathers all the different Specialists (Urologists, Oncologists, Pathologists, Radiotherapists, etc) involved in the prevention, diagnosis and treatment of urinary tract tumours. The Association is active also in multidisciplinary research and training and participates to the drawing up and editing of the guidelines of the Italian Association of Medical Oncology (AIOM).

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were shown to be effective treatments for mCRPCs harbouring defects in the homologous recombination mechanism of DNA repair (HRR), mainly in the *BRCA1/2* genes (Messina et al., 2023), while still uncertain is the relevance of other HRR alteration in terms of PARPi sensitivity (Rescigno et al., 2020)

HRR alterations are present in up to 27 % of mCRPCs (Teyssonneau et al., 2021), with approximately 1/3rd affecting the *BRCA1/2* genes. Differently from ovarian and breast cancers, *BRCA1/2* defects in PC are primarily somatic (Mateo et al., 2019; Gallagher et al., 2022), and approximately half of *BRCA* alterations are of germline origin. Germline *BRCA1/2* mutant PCs appear to be more aggressive, usually present as metastatic from diagnosis and have shorter responses to standard treatment for mCRPC (Castro et al., 2019). Therefore, the prognostic and predictive role of these alterations has triggered genomic and genetic testing in prostate cancers.

Nevertheless, there is not unanimous consensus on the right timing of testing (diagnosis of PC, diagnosis of mPC, diagnosis of mCRPC) as well as on the type of test that should be performed (sequencing of tissue biopsies, circulating tumour DNA, whole blood).

In addition, given the role of *BRCA1/2* genes also in cancer predisposition, it is cost/effective to integrate the therapeutic path with the prevention path.

Here we discuss key relevant issues related to *BRCA 1/2* testing in PC, including:

- Selection of the appropriate sample
- Quality of cancer samples
- BRCA testing and sensitivity
- Classification of BRCA variants
- Assessment of constitutional or somatic origin
- Impact of positive results on prevention
- Informed consent

2. General implications of *BRCA* gene status in prostate cancer:

It is widely recognised that genes involved in the HRR pathway are also involved in genetic predisposition to cancer. Among these, *BRCA1* and *BRCA2* are the most relevant and best studied ones.

Carriers of germline *BRCA 1/2* pathogenic and likely pathogenic variants that are collectively named PVs in this paper are at high risk to develop breast and ovarian carcinomas and other cancers, including prostate and pancreatic carcinomas when compared with the general population (Carter, 2018; Zayas-Villanueva et al., 2019; Daly et al., 2023).

Carriers of a *BRCA1* PV have an estimated 7–26 % cumulative lifetime risk of prostate cancer, while the cumulative lifetime risk is 19–61 % for carriers of a *BRCA2* PV (Lecarpentier et al., 2017; Li et al., 2022; Nyberg et al., 2020). There is evidence that advanced or metastatic prostate cancer is associated with *BRCA2* PVs, while it is not yet known if an aggressive phenotype is also associated with *BRCA1* PVs (Abida et al., 2017; Pritchard et al., 2016; Darst et al., 2021).

An international study including 5545 patients with PC of European ancestry showed that the frequency of *BRCA2* PVs was significantly higher in patients with aggressive disease (ie, dead from PC, metastatic disease, T4 disease, or T3 with Gleason score $\geq$ 8) than in patients with non-aggressive disease (OR, 3.19; 95 % CI, 1.94–5.25) (Darst et al., 2021).

When a cancer patient is identified as a carrier of *BRCA1/2* PV, cascade genetic testing is useful to identify their high-risk relatives. The identification of a healthy female carrier of *BRCA1/2* PV allows their enrolment in clinical prevention programs including risk-reducing surgery and intensive clinical screening for both ovarian and breast cancers to reduce mortality and morbidity (Owens et al., 2019). Even if there are not clear evidences that intensive clinical screening for PCs can reduce mortality in male carrier of *BRCA1/2* PVs, the National Comprehensive Cancer Network (NCCN) guidelines and the Italian Association of Medical Oncology (AIOM) recommendations advise an intensive screening program for PC prevention and early diagnosis in male carriers of *BRCA1* and *BRCA2* PVs with a PSA test and consultation with an urologist from the age of 40 years onwards.

Currently, *BRCA1/2* testing for the identification of hereditary cancer predisposition is usually performed on peripheral blood (germline or constitutional) mainly by next-generation sequencing (NGS).

The sequencing analysis of the coding portion and exon/intron junctions of the *BRCA1* and *BRCA2* genes allows the detection of point variations (single nucleotide substitutions and insertions/deletions of one or a few bases) in the DNA sequence and encompasses about 90 % of *BRCA* pathogenic variants. 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 %. Analyses using NGS methods allow to predict with a certain degree of reliability large rearrangements in *BRCA1/2*, which are usually confirmed by techniques such as *Multiplex Ligation Probe dependent Amplification* (MLPA) or *Multiplex Amplicon Quantification* (MAQ). Generally, MLPA and MAQ should be complementarily used, to circumvent technical failures (Darst et al., 2021; Owens et al., 2019). According to available data, it is expected that half of somatic pathogenic *BRCA* variants identified in prostate cancer are constitutional, inherited from one parent or arisen *de novo* (less than 1 % of cases) and, therefore, transmissible to the offspring (50 % probability for each child). On the other hand, the other half of the PVs are exclusively somatic and thus confined to tumour tissue.

The *BRCA* test conducted on tumour tissue ("somatic test") identifies the variants acquired as somatic mutations in addition to constitutional variants. Hence, in the event of a positive result, the alteration must be verified in normal tissues, usually peripheral blood leukocytes, in order to ascertain if the origin of the PV is constitutional or somatic.

The somatic analysis enables physicians to identify a fraction of PC patients with a pathogenic *BRCA* variant that would remain unknown if the test were performed only on peripheral blood (Ellison et al., 2018).

Nonetheless, despite several existing commercial systems that are CE-IVD (In Vitro Diagnostics) certified for *BRCA1/2* analysis on tumour tissue, many of them are currently not equipped with bioinformatic software applications dedicated to the implementation and interpretation of *BRCA* analysis. Such methods may not be accurate for the assessment of large rearrangements, while they are as reliable as blood tests for the detection of point variants (Ellison et al., 2018; Capoluongo et al., 2017).

3. Selection of the appropriate sample

Since PARPi are approved for metastatic prostate disease, *BRCA1/2* analysis should be performed on metastatic tissue samples, such as surgically removed sample, bone biopsy, lymph node biopsy or other biopsy material, if possible. Sequencing of circulating tumour DNA (ctDNA) can be considered when tissue testing fails.

The original study that established PARPi olaparib as an effective treatment for mPC (de Bono et al., 2020) has also clearly shown that up to 50–60 % of the times sequencing of the tissue samples failed the quality control. Failure rate is dependent on tissue age, tumour content, DNA yield and DNA quality. Some of these pre-analytic issues can be overcome by the acquisition of fresh tumour biopsies, usually lymph-node or prostate biopsies, that are the easiest to reach and provide the most abundant material for sequencing purposes. The TOPARP-B study (Mateo et al., 2020) clearly showed that DNA extracted from biopsies of the metastatic sites acquired during the trial screening period gave the highest success rate (87 %) in terms of likelihood of deriving sequencing results.

It is however known that the majority of mPC patients present also (90 %) or exclusively with (40 %) bone disease (Lorente et al., 2016),

with most having disease involving the pelvic bones. Selection of the bone lesion is crucial to derive a positive DNA yield. Cimadamore et al. have recently produced recommendations for optimal procedures for BRCA testing of tissue derived from bone biopsies (Cimadamore et al., 2023).

4. Quality of the cancer sample

To avoid BRCA analysis failure it is mandatory to perform genomic testing on well preserved samples. Overall, the tissue more used for NGS analysis is an archival sample fixed in formalin and included in paraffin blocks (FFPE). Sample age has been evidenced as a critical feature: 68.1 % of samples that were less than one year old generated an interpretable NGS result, compared to 47.3 % of samples that were 10 years old.

The pre-analytical procedures influence the DNA preservation, and the more adequate fixation is buffered formalin for no more than 72 h in order to avoid DNA degradation and fragmentation (Capoluongo et al., 2017; Zhong et al., 2018).

Considering that the most relevant site of metastatic disease is bone, in case of a bone biopsy, the pre-analytic process is extremely critical due to the use of intensive decalcification process that would not preserve DNA. In these cases, the use of mild decalcification with EDTA or preliminary separation of the soft tissue fraction to avoid its exposure to the decalcification procedure is mandatory.

Another critical point is the percentage of tumoral cells: a PC is adequate for molecular analysis when the pathologist has observed the presence of $\geq$ 20 % of neoplastic cells. The pathologist must also evaluate the characteristics of the tissue block under examination and consider, if necessary, manual macrodissection to select areas with higher tumour cellularity within the specimen (Cimadamore et al., 2023). When prostate samples have insufficient tumour content or purity or insufficient tissue size (the minimum tissue volume required is 0.2 mm^3) it is recommended to enrich the tumour amount using more samples even from different blocks. Importantly, an irradiated prostatic tissue cannot be used for genetic testing.

If the tumour tissue is not adequate for NGS analysis, plasma circulating tumour DNA (ctDNA) could be used. However, this is not a standardised technique and it is also burdened by false negative results in about 20 % of the cases. False positive results can be derived from clonal haematopoiesis of indeterminate potential (CHIP). Genes mutated in CHIP partially overlap with solid tumour drivers and could lead to false-positive calls in CHIP-related genes (e.g., *TP53, ATM*). CHIP was reported in about 14 % of plasma DNA from metastatic prostate cancer patients (Pascual et al., 2022; Mayrhofer et al., 2018). Routine collection of buffy coat (enriched for WBC) from patients undergoing plasma ctDNA testing is recommended, to have available material to rule out CHIP if necessary (Cimadamore et al., 2023).

- *Sequencing from tissue remains the gold standard for determination of BRCA1/2 in mCRPC.*
- *This is challenged by the 'age' of the tissue and the tumour content for small core biopsies.*
- *Freshly collected tissue gives the highest chance of extracting good quality DNA for sequencing.*

5. *BRCA1/2* testing and sensitivity

NGS methods allow to detect somatic and germline variants in tumour tissue (Ellison et al., 2018), but they often fail to identify medium- or large-sized rearrangements as well as low frequency allelic variants in tumour specimens. The development of specific algorithms for the identification of large DNA rearrangements could help to make NGS analysis more sensitive.

At present, only few labs conduct the test on tumour tissue with the previously discussed limitations however tumour testing is a gold standard method to ascertain somatic and germline *BRCA1/2* variants. An ever-increasing number of labs perform the test on DNA from peripheral blood thus excluding the identification of somatic variants.

To implement *BRCA1/2* testing properly, the laboratory must have a proven experience of test validation, meet specific laboratory standards for performing tests on human samples and take part in approved external quality control programs. There are specific methodological recommendations for the development of a NGS data analysis workflow on prostate tumour tissue for the detection of *BRCA1/2* variants (de Bono et al., 2020; Cimadamore et al., 2023; Mosele et al., 2020).

For PARP inhibitor treatment eligibility, the *BRCA1/2* tests can be performed both on tumour and on plasma circulating tumour DNA (ctDNA), although tissue testing is currently considered the gold standard. Plasma ctDNA obtained by liquid biopsy is essentially small fragments of DNA that originate from cancer cells released into the bloodstream.

Even if prostate tissue testing is considered a gold standard technique, the majority of existing commercial systems certified for *BRCA1/2* analysis on tumour tissue are currently not equipped with bioinformatic software applications for the assessment of *BRCA1/2* large rearrangements, that usually account for 7–10 % of all *BRCA1/2* PVs. This limitation of NGS analysis should be considered especially when a patient affected by metastatic prostate cancer has a positive family history for breast, prostate, pancreas or ovarian cancers.

The sensitivity of *BRCA* somatic testing could also be affected by contamination of non-neoplastic cells, tumour heterogeneity and low allelic frequency in relationship to clonal evolution timing. Usually using NGS analysis, PVs with an allelic frequency (VAF) $>$ 5–10 % should be taken into consideration for treatment decision making. However, a low VAF could not reflect the real variant abundance in the prostate tumour if it is observed in a sample with a low percentage of neoplastic cells.

Only few ctDNA *BRCA1/2* tests are currently approved by FDA. The Blood Profiling Atlas in Cancer Consortium (BloodPAC) has released a series of analytical protocols for validating NGS-based ctDNA assays, which the consortium believes will help accelerate the development and regulatory reviews of new tests (Godsey et al., 2020).

Of note when the ctDNA fraction is $<$ 1 %, the sensitivity of the genetic test drops to 68 % (Chi et al., 2023; Carreira et al., 2021). Assays that are able to measure ctDNA fraction/purity in plasma DNA should report the ctDNA fraction to assist clinicians in estimating the likelihood that failure to detect a somatic variant is due to the variant not being present in the tumour rather than to insufficient ctDNA amount in the specimen. Moreover, the sensitivity of ctDNA testing varies according to the type of variant, ranging from high for frameshift/indels (86 %), nonsense (93 %) and splice (87 %) alterations to lower values for large structural alterations, that is, homozygous loss (27 %) and large unbalanced rearrangements (63 %) (Chi et al., 2023). ctDNA testing can detect genetic alterations that may include somatic (not inherited) or germline (inherited) alterations, but does not distinguish between them. In case of identification of a genetic variant by liquid biopsy, a germline test should be performed to evaluate the germline or somatic nature of the PV.

- *Sequencing of ctDNA is complemental and or alternative to tissue sequencing considering the accessibility of blood sample collection.*
- *There is a good concordance between tissue and ctDNA sequencing.*
- *Low tumour fraction remain the main challenge for determination of copy number estimation with ctDNA sequencing.*

6. Classification of BRCA1/2 variants

The spectrum of allelic variants of the *BRCA1* and *BRCA2* genes is very broad. Indeed, variant classification is an important aspect of the testing process, particularly when considering that quite often clinical testing detects genomic alterations not reported in the scientific

literature. Therefore, although several standards exist for the classification of constitutional *BRCA1/2* variants (Richards et al., 2015), it is important that laboratories use the updated criteria developed by the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA), available on the consortium website (https://enigmaconsortium.org/), that are gene specific and based on a broad consultation of international experts. ENIGMA classifies variants in five groups, according to IARC recommendations (Plon et al., 2008; Gori et al., 2019): benign, likely benign, uncertain, likely pathogenic and pathogenic.

Likely pathogenic and pathogenic variants, that are collectively named PVs in this paper, should be considered for PARP-i treatment and for preventive purposes.

It is important to highlight that the before mentioned criteria were developed in order to define the meaning of the variants of *BRCA1/2* genes as predictive of hereditary risk. At this time, information on the effect of the different *BRCA1/2* variants in terms of response to treatment is much more limited and specific classification criteria for this purpose have not yet been developed.

It is therefore necessary that laboratory use and make available the protocol used, including the interpretation process and indicate in the test report the clinical significance of the *BRCA1/2* gene variants identified along with a list of the essential information used for the classification (Claustres et al., 2014). Laboratories should take part in external quality control programs and contribute to a systematic and centralized, national and international, collection of all *BRCA1/2* variants observed, with the aim to improve their classification (Gori et al., 2019; Yvonne Wallis et al., 2019), regarding the implications for both the definition of hereditary risk and the prediction of response to anticancer treatments.

Furthermore, it is also recommended that the classification of a variant be periodically verified, when appropriate. Each reclassification must be notified to the referring physician, who usually is the person reporting the results of those tests. Of particular importance is the revision of variants of unknown significance (VUS, class 3 variants). These can over time be re-classified as pathogenetic or benign variants leading to modifications of the therapeutic decisions and of preventive advice. As described in the Fig. 1 (FLOW-CHART) discussion of the significance of VUS in relation to family history and molecular data should be encouraged during meetings of the molecular tumour board.

Each PV identified on tumoral tissue should be confirmed with a second technique to limit false positive, such as Sanger sequencing. In addition, large deletions and duplications should be confirmed using MLPA or MAQ approaches.

- *Determination of pathogenic variant might change over time.*
- *Many of these variants are defined as pathogenic based on study on hereditary risks and their impact on sensitivity to PARPi might be understudied.*

7. Assessment of constitutional or somatic origin

The *BRCA1/2* test conducted on tumour tissue or on ctDNA ("somatic test") identifies the variants acquired as somatic mutations in addition to constitutional variants. Hence, in case of a positive result, the alteration must be verified on DNA from peripheral blood to ascertain if the origin of the PV is constitutional or somatic.

It is well known that VAF greater than 50 %, is suggestive for a constitutional variant, however, because of the *BRCA1/2* somatic testing is strongly influenced by the number of neoplastic cells, it is mandatory to check all PVs identified. It is not so uncommon to identify constitutional PVs in presence of *BRCA1/2* variants showing less than 20 % of VAF.

In relation to *BRCA1/2* variants, it is mandatory to check on peripheral blood all PVs (class 4 and 5 variants). It is noteworthy that VUS (Class 3 variant), even if it is associated to unknown clinical significance, could be associated to a positive cancer family history, and also could be reclassified in the future.

The identification of constitutional/germinal origin of *BRCA1/2* PVs is crucial for cancer prevention not only for the affected patient but also for their relatives.

For these reasons and to facilitate the engagement of consanguineous relatives with cascade testing, the identification of *BRCA1/2* PV origin through peripheral blood analysis should be organised through a Cancer Genetic Clinic (Fig. 1).

The traditional pre-test models of cancer genetic counselling developed in the risk-assessment setting are currently insufficient to face the

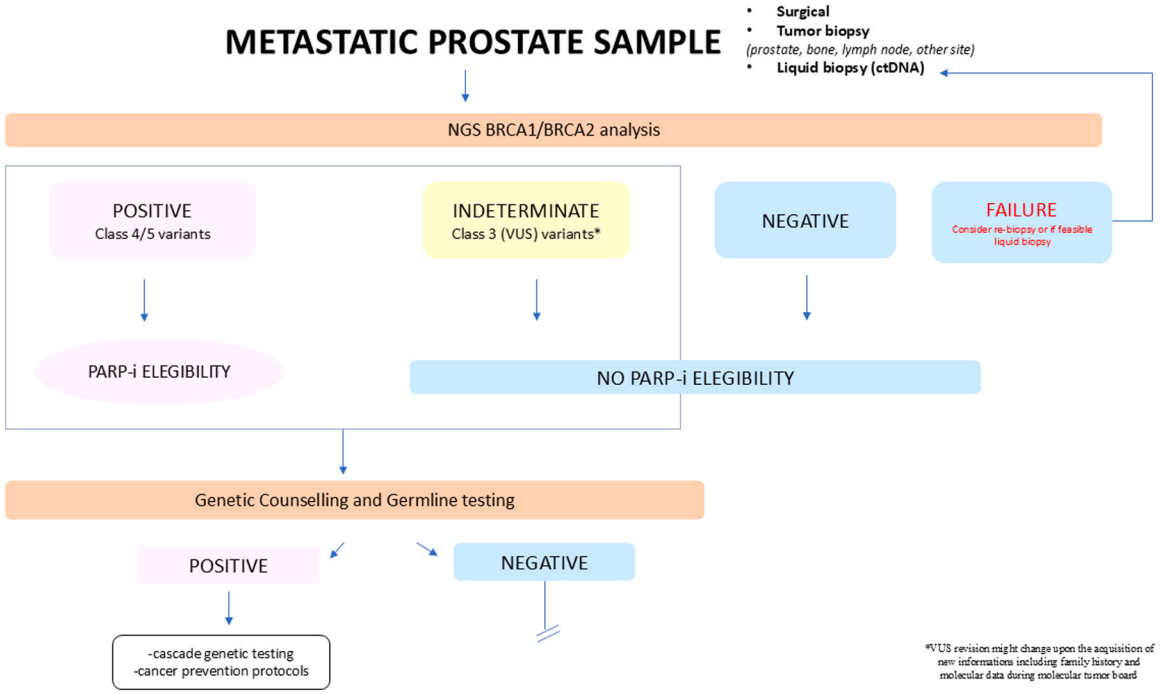

Fig. 1. Flow-chart for *BRCA1/2* testing in prostate cancer.

increasing number of *BRCA1/2* test requests, particularly when the test for genetic predisposition has also a predictive value for treatment, which a timely turnaround.

The optimal model of genetic counselling for cancer risk assessment involves detailed information and discussion of the genetic aspects beginning from the pre-test stage. However, the need to obtain test results in a timely manner to implement specific treatments implies that also oncologists experienced in genetics can directly request the *BRCA1/2* test to the laboratory using a *mainstreaming* model. This model includes identifying patients for whom a genetic test is indicated, obtaining informed consent, completing the administrative work required for prescribing the genetic test and providing cancer genetic counselling when the genetic test result is a pathogenic or likely pathogenic *BRCA1/2* variant or when, in absence of BRCA1/2 variant, a cancer family history suggests an inherited condition for other cancer predisposing genes (Sigu recommendations 2021, https://sigu.net/).

- *Once a BRCA1/2 somatic pathogenic variant is identified, this should trigger germline testing and eventually cascade testing if the variant is confirmed of germline origin.*

8. Impact of positive results on prevention

As previously reported, 2 % of mPCs has germinal *BRCA1/2* PVs, 5.3 % of which in *BRCA2* and 0.9 % in *BRCA1* (Fasulo et al., 2023). For this reason, somatic genomic analysis of BRCA1/2 genes for prediction of therapeutic response is also a valuable approach for the identification of the HBOC syndrome in patients affected by PC. Male carriers of *BRCA1/2* PVs have 0.2–7 % risk at 70 years to develop also breast cancer as reported by NCCN guidelines and Li et al. (Daly et al., 2023; Li et al., 2022). In addition, male carriers of *BRCA1/2* PVs have a 50 % chance of transmitting the PV to each of their offspring. A daughter who has inherited the PVs has $>$ 60 % of lifetime risk to develop breast cancer, and a 13 %-58 % of lifetime risk to develop ovarian cancer. It is well documented that cancer prevention protocols including personalised surveillance and risk reducing surgery can reduce morbidity and mortality of breast and ovarian cancers in *BRCA* carriers.

Although surveillance protocols for female carriers of *BRCA1/2* genes are well established, effective follow-up programs for male carriers still lack. NCCN guidelines recommend PSA testing 10 years earlier that the age of insurgence of prostate cancer in the affected relative or starting at age 40 yrs, whichever is younger. However, in sexually active men, there is a higher chance of detecting of high PSA values that are commonly associated with infective/inflammatory disease.

For these reasons, even when the test is performed in the cancer treatment setting, the arrangement of comprehensive care pathways is mandatory, to ensure the correct interpretation of the results for clinical purposes, the correct management of family members at risk if an hereditary PV was identified and the correct genetic assessment of cases with a non-informative test results (Richards et al., 2015; Plon et al., 2008; Gori et al., 2019).

Each centre must provide clear information on the management pathways to the patients and their relatives, outlining the duties and responsibilities of the oncology team, of the laboratory and of the cancer genetics team across the different phases of the defined care pathway. In the absence of recognized standards, one should consider submitting these pathways to verification via planned audits, with the aim to improve service quality. It is desirable that local health authorities render the *BRCA1/2* test free of charge for healthy relatives of patients carrying an identified *BRCA1/2* pathogenic variant. Currently the genetic test for healthy relatives in Italy is free only in some regions.

- *While prevention protocols are well established for female carriers of BCRA1/2 alterations, there are not standardised protocol for male carriers.*

9. Informed consent

The *BRCA* test for prognosis and prediction of response may be prescribed by clinical geneticists or oncologists who have the responsibility to provide appropriate information to the patients on the genetic aspects associated with the results. The information provided to the patient should cover the potential benefits in terms of prognostic and therapeutic significance, together with the possibility of detecting a high secondary cancer risk and the presence of a cancer predisposition in 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 to tell other family members about the test results, before taking a decision.

Physicians who prescribe a *BRCA* test should abide to an appropriate communication and protocols for collection of specific written informed consent, possibly using *ad hoc* information material. Oncologists without 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 which the patient should be referred, when requiring a deeper evaluation of the genetic aspects, before deciding whether or not to undergo the test, as well as in particular circumstances, such as families with nonspecific hereditary predisposition to cancer.

- *Informed consent is a key part of the diagnostic process of a BRCA1/2 alterations and should be conducted by trained personnel.*

10. Conclusions

The availability of PARP inhibitor therapies for the treatment of metastatic prostate cancer has opened new possibilities for the treatment of this disease, and today the definition of *BRCA1/2* gene mutations to determine eligibility for PARP inhibitors has entered in clinical practice.

The purpose of this work is to analyse and to highlight all critical aspects of the BRCA testing process, including selection of the appropriate tumour sample and choosing an adequate NGS approach to obtain a good sensitivity of the *BRCA* analysis.

Of more interest, the involvement of *BRCA* genes in this therapeutic pathway has opened the possibility of identifying patients carrying germline *BRCA1/2* PVs with hereditary cancer predisposition, allowing an important opportunity to identify healthy people at high risk of cancer for whom specific cancer prevention strategies are advised.

For these reasons it is crucial that the therapeutic process with PARP inhibitors be closely related to the cancer genetic counselling process to maximize personalized cancer prevention in prostate cancer patients and in their families. This model involving the *BRCA1/2* genes that includes therapy and prevention could be useful in the future also for other cancer predisposition genes.

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Declaration of Competing Interest

All the authors of this manuscript declare no conflicts of interest.

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