Guidance document for the Tumor-First testing approach for patients recently diagnosed with ovarian cancer

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Introduction

All patients with ovarian carcinoma have an indication for screening for hereditary predisposition. The Tumor-First approach detects both somatic (acquired) and germline (inherited) mutations, but it does not distinguish between the two types. Genetic testing of tumor tissue in patients with ovarian carcinoma provides information for therapy selection, as well as about a possible genetic predisposition. It also serves as a selection criterion for germline diagnostics. Because it allows patients to use genetic counseling more selectively, this care pathway is more efficient and less stressful for the patient.
Given that the tumor test is negative in most women, the test on tumor tissue replaces a germline blood test for a large proportion of women. For this reason, the test falls under the scope of the Special Medical Procedures Act ('WBMV'), in which the clinical genetics disciplines are tasked with monitoring the quality of diagnostics.

Several conditions have been defined for the implementation of this workflow:

1. The quality of the Tumor-First test is guaranteed and is comparable to a germline blood test, in which all genes relevant for family research are analyzed.
2. The Tumor-First test is performed only within centers in which a pathologist, clinical scientist in molecular pathology, and clinical laboratory geneticist work in close collaboration.
3. National standards have been set for reporting Tumor-First analyses, and these standards have been met.
4. Communication with the patient about the test and its results is guaranteed.
5. Funding for the Tumor-First test should be arranged.

This document focuses on Items 1 to 3, and it was prepared as part of a project of the KWF Dutch Cancer Society entitled "Tumor-First workflow: Nationwide implementation of ovarian cancer heredity prescreening, to stratify both genetic testing and treatment options" (project number 12732). Within this project, work package 2 focuses on the preconditions and quality of the test, as well as on its reporting. For a practical translation into daily practice, consensus was sought with the professionals involved. In February 2021, a survey was distributed to clinical scientists in molecular pathology, clinical laboratory geneticists, and pathologists from all the university medical centers

(UMCs) and the Antoni van Leeuwenhoek Netherlands Cancer Institute (AVL) regarding ovarian carcinoma testing for the presence of known cancer predisposition genes and related reporting and organization. This inventory was used as a basis for developing a set of recommendations, which were submitted to clinical scientists in molecular pathology, clinical laboratory geneticists, pathologists, and clinical geneticists in May 2021. These recommendations and the percentage of respondents who agreed with them have been included as an appendix to this document (only available in Dutch). These recommendations and the resulting discussions form the basis of this guidance document.

Definition of the Tumor-First test

An analysis on ovarian tumor tissue of genes which, according to the Dutch guideline for hereditary ovarian cancer, are included in a germline analysis. The sensitivity of the Tumor-First test for detection of germline variants is comparable to that of a germline blood test, and it also allows the detection of somatic changes in these genes. For optimal alignment and quality, the Tumor-First test should be performed at a center in which professionals from the Pathology Department and the Genetics Department work in close collaboration. In addition, the test should be approved by both disciplines for this application.

Request and inclusion of tumor material

1. With the Tumor-First test, the gynecopathologist of the center conducting the test plays a central role in requesting the test, inspecting the quality of the tissue, integrating the molecular results into the PA report, and the discussion in the multidisciplinary team meetings (MDTs) . Parts of this process (e.g., tumor delineation and tumor percentage assessment) can also be assessed by other pathologists.
2. In addition to the gynecopathologist at the center performing the test, gynecopathologists at other hospitals also have the responsibility to request Tumor-First testing, guided by agreements made with the multidisciplinary treatment team in the region, the integration of the findings into the local pathology report and the discussion of the test results in the MDTs.
3. In principle, the Tumor-First test is requested at the moment ovarian carcinoma is diagnosed by the pathologist, provided the patient has not opted out and a Tumor-First analysis has not been previously registered in the Palga database. The local internal and external application procedures are followed in this process.
4. If a Tumor-First analysis is requested, the laboratory performing the test may assume that the patient has not used the opt-out option for the Tumor-First test.
5. In accordance with the revision of the guideline for hereditary ovarian carcinoma (2021/2022), all ovarian carcinomas, tuba carcinomas, or carcinomas at the site of the peritoneum are eligible for the test, regardless of histological type. With the advent of the most recent classification system (WHO2014 and/or WHO2020 Classification of Ovarian Cancer), however, this may be curtailed in time.
6. The starting material on which the Tumor-First test is performed can vary (e.g., ascites, biopsies, resections), and also if some form of therapy has been given previously. There is no evidence that this has a negative impact on the test results.
7. Within the pathology laboratory, consideration may be given to periodically checking whether or not a Tumor-First test has been performed (or considered).

Composition of the gene panel

1. The Tumor-First test is used to analyze predisposition genes for ovarian carcinoma. In accordance with the revision of the guideline for hereditary ovarian carcinoma (2021/2022), this concerns the following: *BRCA1*, *BRCA2*, *BRIP1*, *PALB2, RAD51C,* and *RAD51D* (the core genes for hereditary ovarian carcinoma).
2. To demonstrate all clinically relevant variants, analysis should be performed for single-nucleotide variants (SNVs) and multi-nucleotide variants (MNVs), as well as for copy number variants (CNVs) that lead to the deletion (and, in rare cases, duplication) of one or more exons.
3. The detection of SNVs and MNVs is performed using a next-generation sequencing (NGS) analysis with complete coverage of the coding sequences, including the flanking intron regions. These regions of interest (ROIs) contain at least 5 nt on either side of the exons. Sequencing of 20 nt flanking intron sequence is, however, recommended, particularly on the 5' side of the exon (the acceptor site side).
4. Current NGS techniques are not always capable of detecting all CNVs. The Tumor-First test will therefore consist primarily of a combination of NGS technique and Multiplex Ligation-dependent Probe Amplification (MLPA). Given the frequency of the occurrence and clinical relevance of CNVs, it is acceptable to limit an MLPA analysis to the analysis of intragenic deletions in *BRCA1* and to focus the analysis on germline variants, so that only the loss of more than 50% of copies has to be detected.
5. The analysis of a gene panel broader than *BRCA1*, *BRCA2*, *BRIP1*, *PALB2, RAD51C,* and *RAD51D* may be considered, provided that it does not reduce the reliability of the analysis of the Tumor-First core genes and measures are taken to prevent this expanded analysis from leading to incorrect treatment choices or unwarranted referral to a clinical geneticist.

Validation of the test

The Tumor-First test must have been validated for its intended application. Considerations to be included in this regard are as follows:

1. Validation and optimization of the detection of pathogenic variants in mononucleotide repeat stretches in *BRCA1* and *BRCA2*.
2. The depth of sequencing should be sufficient over the entire ROI of the core genes, in order to provide a reliable demonstration of both germline variants and somatic variants. The minimum depth of sequencing therefore depends on the percentage of neoplastic cells.
3. Cutoff values for the depth of sequencing should be defined for the reliable detection of (1) germline variants and (2) somatic variants. These values can be substantiated by validation samples and/or through the use of unique molecular identifiers (UMIs), which can be used to determine the number of template molecules analyzed. These values should be used: (1) to reject individual cases if the required depth of sequencing is not achieved at 100% (depth for germline variants), or at 90% (depth for somatic variants) over the entire ROI of the core genes; and (2) to avoid analyzing individual cases because the variant allele frequency (VAF) to be detected for the detection of somatic variants cannot be achieved with the workflow used.
4. Validation and optimization of detection of exon deletions and, where possible, duplications in formalin-fixed paraffin-embedded (FFPE) material, focusing on the 510 bp deletion of exon 22

(61bp) and the 3835 bp deletion of exon 13 (172 bp) of *BRCA1*, which frequently occur in the Netherlands.

5. The validation should focus on avoiding a standard disclaimer indicating a deficiency in the test as compared to a regular germline test.
6. Within a given center, the clinical scientist in molecular pathology and the clinical laboratory geneticist, in consultation with the gynecopathologist and clinical geneticist, are jointly responsible for the validation of the method and the associated criteria for introducing the test within the framework of Tumor-First (i.e., for prescreening or for germline diagnostics and therapy stratification).

Interpretation and reporting of the test results

1. The classification of variants in the core genes is done according to the agreements of the National Consultation on Breast Cancer Diagnostics ('LOB'). It is important for the variants in the core genes to be interpreted by specialized clinical laboratory geneticists who are affiliated with the LOB, so that the interpretation of individual variants is the same throughout the Netherlands, and thus within individual families. The classification of variants is increasingly being performed according to the systematics of the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP), based on gene-specific modifications defined by expert panels.
2. To increase the quality and efficiency of variant classification, it is recommended that the departments of Genetics and Pathology use a joint database and agree on the role of clinical laboratory geneticists in the interpretation of previously classified variants.
3. Variants classified as "probably pathogenic" (Class 4) and "pathogenic" (Class 5) are always reported, and those that are classified as "benign" (Class 1) and "probably benign" (Class 2) are never reported. It is recommended that variants with unknown pathogenicity (Class 3) tending toward Class 4 be reported as well. These include exceptional variants, which may introduce a defect in splicing, which can be investigated by RNA analysis, or variants identified as such by the LOB. It is acceptable not to report the remaining Class 3 variants, as there is only a small chance that they will become clinically relevant. Reporting these variants could lead to confusion in the clinic, in addition to causing distress for the patient. These Class 3 variants must nevertheless be registered in the joint database.
4. Homologous repair deficiency is expected only upon inactivation of both alleles of a core gene. The *a priori* risk of loss for the 2nd allele for a clinically relevant variant in *BRCA1* and *BRCA2* is so high that this information is of no added value for these genes in ovarian carcinomas. Estimating the inactivation of the 2nd allele, which is often associated with loss of heterozygosity, is particularly important when considering experimental or other therapy in clinically relevant variants in *BRIP1*, *RAD51C*, *RAD51D,* and *PALB2*. Patients are usually not eligible for such therapy, except in the case of bi-allelic inactivation of these genes. Given the potential difficulty of determining inactivation of the 2nd allele (e.g., in certain percentages of neoplastic cells and in the absence of informative SNPs), it is not essential to include this information in the results. If the status of the 2nd allele eventually does become relevant, this information can be generated from the available data and/or additional analyses.

Reporting

1. Reporting of Tumor-First analysis takes place within the pathology system, so that the reports can also be viewed by other pathology laboratories through Palga.
2. For purposes of monitoring the use of Tumor-First analysis, it is requested that the term “Tumor-First analysis ovarian carcinoma” be included in the report.
3. Reporting follows the regular structure and includes the standard items required by ISO 15189.

Conclusion text (conclusion based on the molecular results in microscopy section and/or final conclusion of the pathology report)

1. The conclusion text should include a statement that a Tumor-First test was performed, along with the clinically relevant test results.
2. The preferred approach is to use scenario-specific standard formulations, which can be adapted to specific cases as needed. For sample texts, see the text box.
3. When reporting a variant that is (or is likely to be) pathogenic, or one that tends toward probably pathogenic in one of the core genes, attention should be drawn to the possible germline origin of the variant, and a recommendation/referral for genetic counseling and a germline test should be included.
4. If desired, the implications of the result for the likelihood of response to PARP inhibitors can be included as well. For pathogenic variants in *BRCA1* and *BRCA2*, the response rate is generally relatively good, while the response rate for the other core genes in the panel currently remains unclear. GenQA and EMQN assessments will assign deductions if no such information is provided.
5. If no clinically relevant variant is reported, it is desirable to alert the requester that a referral to the clinical geneticist may nevertheless be appropriate in the event of a positive family history.
6. If the submitted material is unsuitable for Tumor-First analysis because the percentage of neoplastic cells is too low for reliable somatic analysis or because the quality or quantity of DNA is insufficient for analysis, reference should be made to the possibility of submitting material to a follow-up procedure or the possibility of referral for clinical genetic counseling and germline testing.
7. If a specific test is technically suboptimal, thereby increasing the likelihood that a germline variant was missed, this should be stated in the conclusion.
8. If other genes besides the core genes are included in the panel, finding a clinically relevant variant is not necessarily a reason for referral to a clinical geneticist, unless an exception to this end has been made by the project group for tumor and heredity diagnostics (see Recommendations of the project group for tumor and heredity diagnostics (vkgn.org))

Examples of conclusion texts are provided in Box 1.

Box 1: Sample conclusion texts
Short template text without therapy choice
1. *With a pathogenic/probably pathogenic variant* A pathogenic [probably pathogenic] variant in *BRCA1/BRCA2/BRIP1/PALB2/RAD51C/RAD51D* was found in this tumor. Because the analysis was performed on tumor DNA, it can be either a somatic or a germline variant. Referral to a clinical geneticist for further investigation of an inherited predisposition is advised. 2. *With a variant of uncertain significance (VUS) tending toward Class 4 (due to a possible splicing defect or based on the judgement of the LOD)*

A suspected variant of unknown pathogenicity in *BRCA1/BRCA2/BRIP1/PALB2/RAD51C/RAD51D* was found in this tumor. [This variant could possibly affect gene splicing.] No clinical implications can be associated with this variant at this time. Because the analysis was performed on tumor DNA, it can be either a somatic or a germline variant. Referral to a clinical geneticist for further investigation of an inherited predisposition is advised.

3. *Without a pathogenic variant*
   No pathogenic variant in *BRCA1, BRCA2, BRIP1, PALB2, RAD51C* or *RAD51D* was detected in this tumor. A referral to a clinical geneticist may nevertheless be considered if warranted by the family history.
4. *Without pathogenic variant, but with a deficiency in the analysis*
   No pathogenic variant in *BRCA1, BRCA2, BRIP1, PALB2, RAD51C* or *RAD51D* was detected in this tumor. Because the MLPA of BRCA1 could not be analyzed reliably, there is a small chance that a clinically relevant germline variant was missed. A referral to a clinical geneticist may be considered, particularly if warranted by the family history.
5. *Tissue inadequate for reliable analysis*
   Reliable analysis of the *BRCA1*, *BRCA2*, *BRIP1*, *PALB2*, *RAD51C,* and *RAD51D* genes is not possible on the available material. A request for this analysis on material from a possible follow-up procedure may be considered. If no timely tumor test can be performed, there is cause for referral to a clinical geneticist for further investigation of an inherited predisposition to breast and ovarian carcinoma.

Long template text with therapy choice

1. *With a pathogenic/probably pathogenic variant*
   a. A pathogenic [probably pathogenic] variant in *BRCA1/BRCA2* has been detected in the tumor DNA of this ovarian carcinoma. Ovarian carcinomas with such variants respond relatively well to PARP inhibitors. This variant may indicate an inherited predisposition to breast and ovarian carcinoma, with associated cancer risks for the patient and her family members. This result provides cause for further investigation of an inherited predisposition. Referral to a clinical geneticist is advised.
   b. A pathogenic [*probably pathogenic*] variant in *BRIP1/PALB2/RAD51C/RAD51D* has been detected in the tumor DNA of this ovarian carcinoma. The implications of this finding for response to PARP inhibitors is as yet unclear. This variant may indicate an inherited predisposition to breast and ovarian carcinoma, with associated cancer risks for the patient and her family members. This result provides cause for further investigation of an inherited predisposition. Referral to a clinical geneticist is advised.
2. *With a variant of uncertain significance (VUS) tending toward Class 4 (due to a possible splicing defect or based on the judgement of the LOD)*
   A suspected variant with unknown pathogenicity *in BRCA1/BRCA2/BRIP1/PALB2/RAD51C/RAD51D* has been detected in the tumor DNA of this ovarian carcinoma. [This variant could possibly affect the splicing of the gene.] The implications of this finding for response to PARP inhibitors is as yet unclear. This variant may possibly indicate an inherited predisposition to breast and ovarian carcinoma, with associated cancer risks for the patient and her family members. This result provides cause for further investigation of an inherited predisposition. Referral to a clinical geneticist is advised.
3. *Without a pathogenic variant*
   No pathogenic variant in *BRCA1*, *BRCA2*, *BRIP1*, *PALB2*, *RAD51C,* or *RAD51D* was detected in the tumor DNA of this ovarian carcinoma. Ovarian carcinomas without a pathogenic variant in *BRCA1* or *BRCA2* are generally less sensitive to PARP inhibitors. This outcome also provides no evidence of a hereditary predisposition to breast and ovarian carcinoma. Nevertheless, there can be cause to consider referral to a clinical geneticist, if warranted by the family history.
4. *Without a pathogenic variant, but with a deficiency in the analysis*

No pathogenic variant in *BRCA1*, *BRCA2*, *BRIP1*, *PALB2*, *RAD51C,* or *RAD51D* was detected in the tumor DNA of this ovarian carcinoma. Because the MLPA of BRCA1 could not be analyzed reliably, there is a small chance that a clinically relevant germline variant was missed. Ovarian carcinomas without a pathogenic variant in *BRCA1* or *BRCA2* are generally less sensitive to PARP inhibitors. This outcome also provides no evidence of a hereditary predisposition to breast and ovarian carcinoma. Referral to a clinical geneticist may be considered, particularly if warranted by the family history.

5. *Tissue inadequate for reliable analysis*
   A reliable analysis of the *BRCA1*, *BRCA2*, *BRIP1*, *PALB2, RAD51C,* and *RAD51D* genes is not possible with this material. A request for this analysis on material from another T-number may be considered. If this is not available, there is cause for referral to a clinical geneticist for further investigation of an inherited predisposition to breast and ovarian carcinoma and the associated sensitivity of the tumor to PARP inhibitors.

[...] depending on the context, the text in square brackets may be omitted.

Results text (microscopy section)

1. The variant allele frequency (VAF) of a clinically relevant variant can be included in the results text, but not in the conclusion text.
2. A low VAF of a clinically relevant variant is not a reason to waive referral for genetic counseling and germline testing.
3. The sensitivity/reliability of the test should be included in the results text.
4. If the test (or any part thereof) does not meet the specified quality criteria, this should be indicated.
5. The classifications of “VUS,” “probably pathogen,” and “pathogen” should be included in the result, especially for missense and possible splice-site variants. It is recommended to provide the background information that formed the basis on which the classification was determined.

Quality

1. In accordance with ISO 15189 standards, regular participation in external quality rounds is mandatory. For Tumor-First analysis on ovarian carcinomas, participation in the EMQN/GenQA assessments is recommended. For the purpose of implementing the Tumor-First working method, participation in this assessment in 2022 is desirable.
2. Because the Tumor-First analyses are performed in close cooperation between pathologists, the clinical scientists in molecular pathology and the clinical laboratory geneticist, it is desirable to establish working agreements within the relevant quality systems. We recommend that this guidance document be included in such agreements.

Option of a germline test after a Tumor-First analysis

If a germline test is requested after the detection of a clinically relevant variant in the Tumor-First analysis, a targeted analysis of the variant detected in the tumor is an option, provided it is ensured that the variant detected in the tumor can be shown. If not, a complete gene panel is recommended.

Funding

To promote the implementation of the Tumor-First working method, it is not desirable to bill gynecologists through a mutual services system. Because the Tumor-First analysis prevents many referrals to clinical genetics and germline testing, the heads of the clinical genetics departments have given the space to bill for these analyses through a clinical genetics fee. As a result, the bill goes directly to the patient's health insurance. Representatives of several health insurers support this working method. It is recommended that procedures to this end be set up within the respective centers.