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Guidelines

Part III - Post-analytical phase

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Summary
Precision oncology requires standardized and clinically meaningful reporting of molecular test results to support therapeutic decision-making. Next-generation sequencing (NGS), increasingly used in routine diagnostics, must be accompanied by clear, structured, and up-to-date interpretative reports. This document provides updated guidance for the annotation, interpretation, and reporting of variants detected through NGS, encompassing both small targeted panels and large-scale comprehensive genomic profiling (CGP) assays. Emphasis is placed on structured reporting, clinical applicability, and harmonization across institutions. The recommendations also address critical aspects of quality assurance, standardization for both tissue and liquid biopsy samples, with the aim to streamline molecular report generation, improve multidisciplinary communication, and facilitate the integration of NGS into everyday oncology practice in Italy.

Key words: Pathology, recommendations, molecular reporting, next-generation sequencing (NGS), comprehensive genomic profiling.

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Introduction

Next-generation sequencing (NGS) has become a fundamental tool in precision oncology, enabling simultaneous analysis of multiple genomic alterations across cancer types to support diagnosis, prognosis, and therapeutic decision-making [1,2]. Recognizing its clinical relevance, both national and international guidelines now endorse the integration of NGS into routine oncology practice [3,4]. Despite its growing implementation, significant challenges remain – particularly in the post-analytical phase, where the complexity of genomic data must be effectively translated into clear and clinically meaningful reports. The lack of standardized, structured reporting formats can compromise the interpretability and utility of NGS results. A well-designed report is critical for guiding ther-

apeutic decisions, improving communication among healthcare professionals, and ensuring consistency across institutions (5-8). Standardized reporting not only enhances clarity for clinicians but also supports inter-laboratory comparability, quality assurance, and the reproducibility of genomic findings [9,10]. As precision medicine continues to evolve, harmonized NGS reporting becomes essential to maximize the clinical value of molecular diagnostics and to promote equitable access to biomarker-driven therapies. In this context, specific attention must also be given to the reporting of results related to BRCA genes and homologous recombination deficiency (HRD). Pathogenetic and likely pathogenetic variants (PV/LPV) in BRCA1/2 and other homologous recombination repair (HRR) genes carry significant implications – not only for selecting targeted therapies such as PARP inhibitors but also for assessing hereditary cancer risk and informing patient management strategies [11,12].

Structure and Content of a Molecular Report: Diagnostic Gene Panel (DGP)

According to ESMO recommendations on clinical reporting of genomic tests, a molecular report should be concise but thorough, complete, synoptic and easy-to-interpret. The report may include both text and tables, and the use of graphical solutions may be required to facilitate comprehension of complex alterations [13]. Different studies suggest formatting options such as column formats, and shorter sentences contribute to better understanding and faster information retrieval, preventing overwhelming the users [14]. Ideally, to facilitate readability by all healthcare users the report should be divided in two sections, an upfront part including all the key genomic findings for clinical decision making, followed by technical details and an appendix containing all supplementary information.
The report should include the following information:

1. Testing facility identification
2. Patient identification, sample characteristics and pathological evaluation
3. Indication for testing: test requested and clinical context
4. Methodology: platform and assay used
5. Diagnosis: summary of most relevant findings and their clinical interpretation
6. Results: about inquired test
7. Other results
8. Test details: molecular assay and data analysis characteristics
9. Appendix
10. Date and Signature

DGP Section 1. Testing facility identification

Details about the laboratory or institution conducting the test to ensure traceability and accountability.

- Name of the hospital or healthcare institution;
- Department or unit (e.g., Pathology, Molecular Diagnostics Unit);
- Location (city and address);
- Contact information (e.g., phone number, email);
- Laboratory certifications or accreditations (e.g., ISO, CE-IVD compliance).

DGP Section 2. Patient identification, sample characteristics and pathological evaluation

A section outlining the key demographic, clinical and sample characteristics should be included at the beginning of molecular reports. The goals are to help contextualize genomic data and to allow for accurate biospecimen identification and monitoring.

Patient identification

- Anonymous identifier (ID) assigned to the patient (patient ID, progressive number);
- Patient name;
- Date of birth;
- Age;
- Gender;
- Personal tax identification number (if applicable).

Sample characteristics

- Sample ID: histological/cytological case ID and paraffin blocks/sections identification;
- Primary tumor type: histopathological/cytopathological diagnosis (tumor histotype) and clinical stage;
- Sample type (e.g., cytological specimen, tissue biopsy, surgical resection, plasma, other) and site of biopsy (organ)
- Sample origin (the sample comes from a primary or metastatic tumor) and preservation mode (formalin-fixed paraffin-embedded [FFPE], frozen tissue, fresh tissue)

Pathological evaluation

- Tumor content: pathology-based estimation of tumor content by percentage of neoplastic cells and absolute number of viable tumor cells; indication of enrichment by micro- or macrodissection, if performed;

- It should be stated here if the material was unsuitable for molecular analysis due to either quantitative or qualitative reasons.

Other information

- Sample collection date;
- Sample receipt date;
- Sample origin: for internal samples, the department and/or requesting clinician; for external samples, the referring institution and requesting clinician.

DGP Section 3. Indication for testing: test requested and clinical context

In this section the 'reason for testing' should be indicated, in particular the biomarkers to be identified. Moreover, the working group recommends reporting any additional clinical context details provided to the laboratory (e.g. sample obtained at the time of diagnosis or at time of disease progression). We are aware that most laboratories performing molecular tests have limited access to detailed medical histories of individual patients, but these details may be relevant for optimal interpretation of genomic biomarkers and for their potential clinical relevance. Therefore, the results of other relevant auxiliary tests (e.g., immunohistochemistry (IHC) for PD-L1 or Mismatch Repair (MMR) status, Fluorescent in Situ Hybridization (FISH)) should be included in this section of the report, if available.

- Genes tested as requested (e.g. for advanced lung adenocarcinoma - test requested: analysis of the mutational status of the following genes: *ALK, BRAF, EGFR, ERBB2, KRAS, MET, NTRK1/2/3, RET, ROS1*). It is recommended to write gene names in italics throughout the entire report to ensure clarity and adherence to scientific conventions;
- Relevant clinical details (e.g., cancer stage, sampling performed at diagnosis or post-treatment, previous or auxiliary test performed, consent statement obtained if applicable).

DGP Section 4. Methodology: platform and assay used

A brief overview of the platform and assay used should be included in this section, while a detailed description of them will be provided in a subsequent section (Section 8. Molecular assay and data analysis characteristics).

- Type of molecular test (NGS);
- Platform and assay names, with their degree of validation (e.g., CE-IVD or RUO-LDT);
- Nucleic acid analyzed (e.g., DNA, RNA, circulating free DNA (cfDNA), circulating free RNA (cfRNA), circulating free total nucleic acids (cfNA).

Molecular profiling of certain tumor types may require other analytical methods in addition to NGS (e.g. promoter methylation analysis performed on the same paraffin block also analyzed for NGS). Ideally, if more than one test is requested on a given sample, all tests performed should be reported in different sections with the same accession number, although for administrative accountability different tests performed on the same sample may also be reported with individual accession numbers.

DGP Section 5. Diagnosis: summary of most relevant findings and their clinical interpretation

To facilitate the interpretation process, a concise but comprehensive summary of the most important results of the NGS test should appear on the first page of a genomic report. This narrative summary should directly answer the clinical question of the physician who ordered the test and provide him/her with a brief but complete and integrated interpretation of all results and potential limitations, including the recommendation of follow-up or confirmatory testing, if appropriate. In this section, alternative scientific nomenclature with a one letter code (International Union of Pure and Applied Chemistry [IUPAC]), for example *EGFR* p.(E746_A750del), or alternative description as *EGFR* exon19 deletion, may be accepted because they can better assist physicians of different training and experience. In case the sample cannot be analyzed the following statement should be reported: sample not adequate for molecular testing (if the sample is unsuitable the most likely reason must be specified: e.g., over-fixation, low neoplastic cell percentage, technical errors in the processing phase, decalcification procedures, other reasons). Likewise, inconclusive results or gene regions under the minimal required sequencing coverage criteria should be reported.

Although annotating clinical actionability is a difficult undertaking, this information is crucial to the report since it can help with the clinical review of the NGS results. To reduce the possibility of misinterpreting data that may be important for treatment decisions, it is imperative that this process be standardized and carried out in accordance with the highest standards.

Regarding actionable mutations, each alteration should be linked with a corresponding level of clinical relevance according to classification systems developed to value the clinical significance (actionability) of genomic alterations.

Among these systems there is the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT). This system classifies the clinical relevance of genomic alterations in six tiers of actionability [15]. Another useful knowledge-based database of actionable genomic variations is the public Precision Oncology Knowledge Base (OncoKB), which is powered by the Memorial Sloan Kettering Cancer Center (https://www.oncokb.org/) [16]. Concerning the inclusion of available therapies targeting the genomic alteration identified during NGS-based molecular profiling, the working group, in accordance with Italian guidelines, consensually decided to exclude this type of information from NGS reports [17].

DGP Section 6. Results: about inquired test

The "Results" section ought to be emphasized and positioned in the middle of the first page. A descriptive list of findings for each type of modification might be used to tabulate the summary of genomic changes found in the sample.

Only those alterations with an expected impact on the gene function, that is oncogenic or likely oncogenic variants, should be included and evaluated for clinical actionability [18]. Polymorphisms, benign and likely benign variants should not be reported (Section 8: test limitations). Variants of Uncertain Significance (VUS) will be included in the following section (Section 7: Other results).

Genomic variants should be reported using the standard gene nomenclature recommended by the Human Genome Organisation (HUGO) Gene Nomenclature Committee (HGNC) (http://www.genenames.org) and the Human Genome Variation Society (HGVS) (http://www.hgvs.org) [19,20].

For all types of genomic alterations, it is recommended to specify the gene altered, the effect, the class and the level of clinical-therapeutic actionability of the detected variant. Wild type molecular results should be reported specifying that no variants were detected in the target regions of the genes under investigation, in order to increase the readability of the report. Inconclusive results are also deemed necessary components to incorporate in this part.

- Gene variants (SNV, indel): it is recommended to specify the altered gene and the type of variant identified. This section should also contain the gene name and the Matched Annotation from NCBI and EMBL-EBI (MANE) select transcript used for genetic mapping (e.g., NM_004985.5). The sequence alteration should be annotated and reported using "c." and "p." prefixes, respectively, for nucleotide and amino acid variation, with protein changes without experimental evidence (no RNA or protein sequence analyzed) given in parentheses. Moreover, for protein description the HGVS recommended using the three letter amino acid code, e.g., *KRAS* c.34G > T and p.(Gly12Cys). A separate variant/mutant allele frequency (VAF/MAF) field should be dedicated in the results table because of its significant clinical implications. Infact VAF quantifies the proportion of DNA sequences carrying a specific genetic variant, providing insights into the clonal composition of a tumor and the patient's overall disease burden. This information is crucial for assessing the variant's significance, understanding its potential impact on disease, and guiding therapeutic decisions.
- Gene fusions: HGNC recommends the use of a double colon (::) between approved gene symbols to separate the genes involved in gene fusions (e.g., *KIF5B::ROS1* or *TP53::NTRK1*). Both fusion partners, including exons, should be reported. The 5′ partner gene should always be listed first, before the double colon, irrespective of chromosomal location or gene orientation. Furthermore, transcript reference sequence and relative read counts mapping gene fusion should be reported. Regarding the *EML4::ALK* fusions, it is recommended to specify the particular variant name (e.g., *EML4* [20]*::ALK* [20]*. - Variant 2/V2*);
- CNV: should be described including gene name, cytogenetic location (chromosome number and cytogenetic band designation) and Matched Annotation from NCBI and EMBL-EBI (MANE) select transcript. CNV can be reported in a continuous or categorical manner, according to NGS panel and analysis software. In the first case, reporting of numerical copy number changes is recommended. Otherwise, a canonical format as "copy number gain" or "loss" can be reported [21];
- Genomic signatures detected, e.g., HRD, Microsatellite Instability (MSI), if applicable;
- Any confirmatory tests should also be mentioned, e.g., IHC, digital PCR;
- Limited information on the detection of possible germline alterations in certain genes related to hereditary cancer susceptibility may be included in this section. Specify that the information reported must be confirmed by germline testing following oncogenetic counseling, if appropriate, as reported variants are not germline annotated.

DGP Section 7. Other results

At the bottom of the previous section, it is further rec-

ommended to report:

- Any other oncogenic/likely oncogenic variant detected in the other genes of the panel, reported as described in Section 6;
- Optional: any therapeutic, prognostic and diagnostic implications of the variants detected can be reported;
- Any VUS (clearly labeled) detected, both in genes under investigation or in other genes;
- Wild type molecular results should also be reported (e.g., no oncogenic variants were identified in the tested regions of the other genes included in the panel), since other not inquired genes may still have clinical-therapeutic relevance.

DGP Section 8. Test details: Molecular assay and data analysis characteristics

This section should provide a concise overview of the assay's capabilities, and it would be appropriate to report the nucleic acid extraction method and technical specifications of the NGS platforms adopted.

- Nucleic acid extraction platform and kit;
- Platform and assay name with the total number of genes tested for each genomic variant type (SNV and indels, CNV, rearrangements/imbalance, splicing isoforms);
- The limit of detection (LOD) of the panel;
- Clinical cut-off values selected for the annotation of molecular alteration;
- Bio-informatics software applied for variant analysis, including software version;
- Reference genome [e.g., Genome Reference Consortium Human Build 37 patch release 13 (GRCh37.p13/Hg19)];
- Test limitations: (e.g. the only reported variants are those found in the interrogated regions of the genes - normal population variations, single nucleotide polymorphisms [SNP], and benign variants are not included in this report).

It should be stated that the test was designed for detection of somatic tumor variants and is not intended to be a germline test and that somatic results do not necessarily reflect the patient's germline status.

In addition to the general assay characteristics (unchangeable across different reports using the same assay), a subsequent paragraph should explain how the assay performed on the specific sample under study (quality metrics).

- Assay quality evaluation: median sequencing coverage: on target coverage, mean depth, uniformity.

DGP Section 9. Appendix

In this section the following should be reported:

- Information regarding internal/external assurance programs (European Quality Assurance (EQA)), including the type of evaluation and the date of the most recent successful evaluation;
- Knowledge bases consulted for the biological and functional annotation of each detected variant;
- Knowledge bases consulted for the annotation of clinical actionability of the pathogenic and likely pathogenic detected variants;
- ESCAT score system and/or OncoKB Therapeutic Level of Evidence;
- Any relevant literature references;
- Any other supplementary information required.

DGP Section 10. Date and Signature

At the bottom of the molecular report must be reported:

- Date of signature, in order to indicate the laboratory's Turn-Around Time (TAT).
- **Signature:** The test must be signed by the qualified laboratory biologist who overseed performance of the assay (primarily responsible for technical aspects and data interpretation) and co-signed by the pathologist (primarily responsible for sample selection and histological assessment). If the molecular test is performed in a unit other than the Pathology Department or if a pathologist's co-signature is not possible, the report must indicate the pathologist who selected the sample and assessed the percentage of neoplastic cells.

Supplementary Material: a molecular diagnosis of non-small cell lung cancer (NSCLC) was presented as a pilot example for NGS reporting on tissue samples using small/medium gene panels (Supplementary Figure 1).

Structure and Content of a Molecular Report: Comprehensive Genomic Profiling

Broad molecular profiling or CGP is an efficient and cost-effective NGS approach that uses a single assay to assess hundreds of genes and to simultaneously detect different types of molecular biomarkers. CGP allows it to detect not only SNVs, indels, CNVs and rearrangements/fusions, but also complex biomarkers and genomic signatures, like MSI and HRD [22-25].

Interpretation and report of complex sequencing

readouts can be challenging, according to the large amounts of information and genomic data generated by CGP. Only few genomic alterations are known oncogenic events with most detected alterations being of unknown significance [26]. In this context, CGP can identify both actionable, potentially actionable variants and VUS, so standardization of variant interpretation and clinical reports and subsequent result discussion within molecular tumor board (MTB) are crucial to correctly translate genomic information in clinical practice [14,25].

CGP Section 1. Testing facility identification

Unchanged compared to the general scheme proposed.

CGP Section 2. Patient identification, sample characteristics and pathological evaluation

Unchanged compared to the general scheme proposed.

CGP Section 3. Indication for testing: test requested and clinical context

In comparison to the general scheme, biomarkers to be identified are not usually listed. Instead, if the CGP test is routinely used for molecular diagnostics, it is recommended to follow the indications provided in "DGP Section 3" specifying the biomarkers to be tested. In any case, the reason for CGP analysis should be clearly indicated (i.e. GCP test for routine patient testing or oncology research, evaluation of one or more biomarkers that require a GCP test; tumors of unknown primary; rare tumors; metastatic cancers with no more available lines of standard therapy, CGP required in order to facilitate drug administration in expanded access programs, compassionate use or enrollment in clinical trials) [13]. Relevant clinical context details and data should be reported (i.e. smoking history, tumor site, clinical stage of disease, previously performed molecular tests and treatment). In this section it is also advisable to specify whether the CGP analysis was requested by the MTB, as established by many Italian health authorities, or by clinical oncologists [27].

CGP Section 4. Methodology: platform and assay used

Unchanged compared to the general scheme proposed.

CGP Section 5. Diagnosis: summary of most relevant findings and their clinical interpretation

The summary, along with relevant interpretations, should be clear and easily accessible to reduce the risk of missed opportunities for matching patients with appropriate therapies or clinical trials. In addition to actionable driver mutations, the report may also highlight potentially relevant co-mutations for targeted therapies or immunotherapy. This section can facilitate multidisciplinary discussions to clarify the clinical significance of the co-mutational profile and inform treatment decisions. Moreover, any prognostic and diagnostic implications of the detected variants can be reported.

CGP Section 6. Results

The CGP report should provide a clear, comprehensive, and detailed overview of all actionable or potentially actionable alterations and genomic signatures in a single results section. To facilitate readability for all healthcare professionals, it is recommended to present the detected alterations in a table with separate sections, annotated according to ESCAT levels, as illustrated below (Tab. I).

1) Mutations (SNV, INDELS)
2) CNV
3) Fusions
4) Genomic signatures (MSI, HRD)

Moreover, negative results for common actionable genes including any caveats regarding the potential for false-negatives results should be included in this section.

While details of sequence variants (SNV, INDEL), fusion variants and CNV have already been reported in previous sections, the genomic signature biomarker must be described as follows:

- MSI status should be clearly reported as MSI-high (MSI-H) or stable (MSS) indicating, if possible, the related score [28,29].
- For HRD reporting see the specific paragraph below.
- If the CGP test is routinely used for molecular diagnostics, it is recommended to follow the procedure described for DGP. Additional genomic data

Table I. Proposed format for reporting genomic alterations detected through a comprehensive genomic panel. The results are categorized into SNV/INDEL, CNV, gene fusions, and genomic signatures, following a structured approach. Each alteration is reported with relevant details, including the affected gene, variant effect, variant classification and ESCAT level. Genomic signatures such as MSI and HRD/GIS are included with their corresponding values, reference thresholds, and ESCAT classification where applicable.

SNV, INDEL variants									
Example	**Gene**	**Effect**	**Exon**	**Transcript ID**	**Nucleotid Variant**	**Protein Variant**	**Variant class**	**VAF**	**ESCAT**
1	***EGFR***	Missense	21	NM_005228.5	c.2573T > G	p.(Leu858Arg)	Oncogenic	73%	IA
2	***EGFR***	Insertion in frame	20	NM_005228.5	c.2303_2311dup	p.(Ser768_Asp770dup)	Oncogenic	22%	IA
3	***BRCA1***	Splice acceptor variant	N/A	NM_007294.4	c.5075-2A > C	p.?	Oncogenic	52%	IA
4	***TERT***	Promoter variant	N/A	NM_198253.3	c.-124C > T	N/A	Oncogenic	14%	IV

Copy Number Variations								
Example	**Gene**	**Effect**	**Transcript ID**	**Region**	**Event**	**Variant class**	**Gene Copy Number**	**ESCAT**
1	***MET***	Amplification	NM_001127500.3	7q31.2	gain	Oncogenic	20	IIB
2	***PTEN***	Deletion	NM_000314.8	10q23.31	loss	Oncogenic	0	IIIA

Fusions								
Example	**Gene**	**Effect**	**Transcript ID**	**Specific fusion**	**Phasity**	**Variant class**	**Read counts**	**ESCAT**
1	***ALK***	Fusion	*EML4*: NM_019063; *ALK*: NM_004304.3	***EML4::ALK*** (*EML4* exon 13::*ALK* exon 20)	in frame	Oncogenic	800/188.000	IA
2	***NRG1***	Fusion	*CD74:* NM_004355.4*; NRG1:* NM_013956.5	***CD74::NRG1*** (*CD74* exon 6::*NRG1* exon 6)	in frame	Oncogenic	1890/166.000	IIB

Genomic Signature				
Biomarker (example)	**Score**	**Results**	**Threshold**	**ESCAT**
MSI	4	MSS	18	N/A
HRD/GIS	45/23	Positive/High	42/16	IA

can be included in a separate table, or the center may choose to report only the results for the requested genes, specifying that complete molecular data can be provided upon request.

- Clinical Trial Information Note: below the results table, a note can be added indicating that the center, upon request from the oncologist or the MTB, is able to provide information on active clinical trials related to the identified biomarkers, including detailed study descriptions, eligibility criteria, and contact information for trial enrollment. Alternatively, clinical trials matched for variant (or genomic signatures) and disease, can be presented in a separate table, as outlined in the ESMO reporting recommendations [30].
- Incidental findings: alterations that may be a sign of hereditary tumor syndrome should be clearly marked in the report, both in the summary of most relevant findings and in results section (additional notes of table results). We recommend adhering to the ESMO precision medicine working group (PMWG) 2023 to identify individuals who may benefit from referral to cancer genetics specialists [31,32].
- Inconclusive results or gene regions under the minimal required sequencing coverage criteria should be reported.
- The Tumor Mutational Burden (TMB) was not considered in these recommendations for molecular reporting, as it is not yet recognized as a validated predictive biomarker for the efficacy of immune checkpoint inhibitors across various cancers in clinical practice, according to the current AIOM (Associazione Italiana di Oncologia Medica) guidelines. While studies suggest that TMB levels can predict responses to immunotherapy, the optimal thresholds and methodologies for its assessment have yet to be standardized [33].

CGP Section 7. Other results: Variants of unknown clinical significance (VUS)

At the bottom of the previous section, it is further recommended to report any VUS detected, because, although VUS may lack immediate clinical relevance, their documentation allows for future reassessment as new data emerge, potentially informing clinical management updates.

CGP Section 8. Test details: molecular assay and data analysis characteristics

In addition to what is reported in the general proposed scheme, some considerations are required for MSI testing. Different NGS assays perform MSI testing with a great heterogeneity in calculation, interpretation, and reporting approaches. In this context, besides technical information, like the type of assay and sequencing platforms, it is recommended to specify size, type of genomic regions analyzed and the defined threshold for clinical actionability [29,34].

CGP Section 9. Appendix

Interpretation of CGP variants usually makes it necessary to consult more databases than standard routine tests, to correctly annotate biological/functional effect and clinical actionability of identified variants. Considering that content and data aggregation of each database can be different, in this section, all employed databases should be clearly mentioned, as well as bioinformatic tools used to eventually match identified variants with ongoing clinical trials [15,35,36].

CGP Section 10. Date and Signature

Unchanged compared to the general scheme proposed.

Supplementary Material: a molecular diagnosis of NSCLC was presented as a pilot example for NGS reporting on tissue samples using a comprehensive genomic panel (Supplementary Figure 2).

Structure and Content of a Molecular Report for *BRCA1*, *BRCA2* and HRD testing (BRCA/HRD)

It is important to dedicate a separate section to the development of recommendations for the reporting of somatic BRCA and HRD testing due to their significant clinical implications and the potential need for genetic counseling. These tests are crucial for patients with advanced cancers, such as ovarian, breast, pancreatic, and prostate cancer, as the results can directly influence treatment decisions, including the use of poly (ADP-ribose) polymerase (PARP) inhibitors.

Pathogenic *BRCA* variants play a critical role in disrupting the HRR pathway, which is essential for repairing DNA double-stranded breaks. As a result, cells are forced to rely on the alternative base excision repair (BER) pathway, where PARP inhibitors can effectively target key components [11,12,37]. In addition to *BRCA* status, another important biomarker for sensitivity to PARP inhibitors is HRD [38,39]. This condition arises from the inefficiency of HRR genes and is associated with distinct markers of genomic instability, including loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale transitions (LST) [40-42].

According to the latest recommendations (Italian Association of Medical Oncology (AIOM)-ESMO), it is preferable to first investigate pathogenic *BRCA* variants in tumor tissue, as the likelihood of detecting mutations is higher than with germline analysis. If somatic pathogenic *BRCA* mutations are found, patients should be referred for genetic counseling and germline testing to identify potential hereditary variants. Additionally, somatic testing should be considered for patients who initially underwent germline testing that did not identify a pathogenic variant but are candidates for PARP inhibitor treatment [13,43-45]. In cases of advanced prostate cancer where tissue analysis is insufficient, ctDNA (liquid biopsy) offers an alternative method for detecting pathogenic variants (see technical characteristics described in the specific section liquid biopsy analysis) [46,47].

A well-structured reporting framework ensures that clinicians have clear, actionable information to guide therapeutic choices and improve patient outcomes. Somatic *BRCA* and HRD tests can uncover hereditary cancer risks, making genetic counseling a vital part of the process. In this section, some recommendations are provided for both the interpretation of results and the integration of genetic counseling, which is essential to inform patients about potential hereditary risks and implications for family members.

BRCA/HRD Section 1. Testing facility identification

Unchanged compared to the general scheme proposed.

BRCA/HRD Section 2. Patient identification and sample characteristics

Unchanged compared to the general scheme proposed.

BRCA/HRD Section 3. Indication for testing: test requested and clinical context

This section should not only specify the biomarkers for which the test is requested, but also include the Consent Statement, confirming that the Clinical Consultant has followed the appropriate counseling procedures to obtain the patient's informed consent for the sample analysis [32,46]. Additionally, the reason for the referral must be clearly stated, along with a detailed description of the clinical information necessary for accurate result interpretation. This includes information about the tumor stage, any previous tests performed (whether germline or tumor tests), treatments received prior to testing, and the patient's family history of cancer.

BRCA/HRD Section 4. Methodology: platform and assay used

Unchanged compared to the general scheme proposed.

BRCA/HRD Section 5. Diagnosis: summary of the most relevant clinical findings

This section should provide a concise overview of the detected genomic alterations and recommend genetic counseling if pathogenic or likely pathogenic variants in HRR genes with high or medium penetrance are identified. Furthermore, it should suggest complementary tests, such as germline multiplex ligation-dependent probe amplification (MLPA), to address the limitations of the test in detecting large rearrangements.

BRCA/HRD Section 6. Results: about test inquired

As previously reported, this section should present the main genomic alterations in a tabular format. For the *BRCA1* and *BRCA2* genes, as well as other HRR genes both the absence of variants (when no variants are detected) and the presence of pathogenic or likely pathogenic variants must be described. For each variant detected, details should be provided, including the reference transcript of the gene, the exon or intron involved, the nucleotide and amino acid variations, the molecular consequence, the variant class (pathogenic or likely pathogenic), the allele frequency, and the level of clinical actionability. Additionally, the variant's classification must be included according to the 5-category pathogenicity scheme established by International Agency for Research on Cancer (IARC), American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) [48,49].

BRCA/HRD Section 7. Other results

At the bottom of the previous section, it is further recommended to report any other oncogenic/likely oncogenic variant detected in the other genes of the panel. Moreover, this section should list VUS class 3, with a recommendation for periodic reassessment of their significance. For comprehensive gene panels, any alterations in non-HRR genes with uncertain clinical relevance should also be reported.

BRCA/HRD Section 8. Test details: molecular assay and data characteristics

In addition to the general characteristics of the assay, as described above, the criteria for evaluability of the sequencing should be specified. The minimum requirements include an analytical sensitivity of the method greater than 99%, with allele frequencies of variants ≥ 5%, and a reading depth for each amplicon greater than 500x. It is also important to emphasize that the gene panel covers the entire coding sequence and intron-exon boundaries, ensuring the detection of all possible variants across both coding and splicing regions. Furthermore, this section should address, if necessary, the limitations of the test in detecting large rearrangements in FFPE samples, as well as the potential for false negatives and false positives, depending on the method used.

BRCA/HRD Section 9. Appendix

In addition to what has already been indicated, this section should include all the databases consulted (e.g. BRCA Exchange, https://brcaexchange.org/)

as well as the guidelines and criteria used for the pathogenicity classification of variants (e.g. Criteria: ENIGMA Rules 2017-06-29-v2.5.1: https://enigmaconsortium.org/; ClinGen ENIGMA BRCA1 and BRCA2 Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines https://www.clinicalgenome.org/). Public databases like BRCA Exchange and ClinVar are essential for accurate *BRCA* variant interpretation, offering standardized, up-to-date classifications. Citing these sources in reports ensures consistency with global standards, enhances reliability, and supports informed clinical decisions and genetic counseling.

BRCA/HRD Section 10. Date and Signature

Unchanged compared to the general scheme proposed.

Supplementary Material: a molecular report on *BRCA1*, *BRCA2* and HRD evaluation for serous ovarian cancer is presented as a pilot example (Supplementary Figure 3).

Structure and Content of a Molecular Report for Liquid Biopsy Analysis

In the era of personalized medicine, the rapidly evolving scenario of predictive biomarkers in the clinical management of patients with a solid tumor has revolutionized the clinical decision paradigm [50]. At the sight of the clinical implementation of an increasing number of predictive biomarkers, NGS platforms are recommended to comprehensively evaluate druggable molecular biomarkers in diagnostic routine settings [50,51]. Despite the widespread diffusion of ultra-sensitive and technically informative NGS assays in detecting actionable alterations in predictive biomarkers, tissue specimens are affected by low amount and/or high fragmented nucleic acids drastically impacting on successful rate of molecular testing [30,36]. In this scenario, liquid biopsy became a tissue integrating biological source of nucleic acids for molecular analysis [52]. Among several analytes detectable in torrent blood (cfNA, circulating tumor cells (CTCs), extracellular vesicles (EVs), microRNAs (miRNA(miRNAs), ctDNA from peripheral blood is the only clinically approved target to profile molecular biomarkers in clinical practice solid tumor patients [53]. Of relevance, liquid biopsy (LB) has been approved to evaluate clinically actionable mutations in predictive biomarkers when matched tissue specimen is not enough to successfully carry out genomic profiling or to target acquired resistance *EGFR* exon 20 p.(T790M) mutation in NSCLC patients relapsing after first line of tyrosine kinase inhibitors (TKIs) [53,54]. Moreover, liquid biopsy based emerging applications may be found in diagnostic routine settings for advanced stage tumor patients. In particular, *ESR1* emerged as a molecular target electing hormone therapy resistant estrogen receptor-positive (ER+), HER2- metastatic breast cancer (ER+/HER2- BC) patients [55,56].

Moreover, liquid biopsy has been also suggested to detect *KRAS*/*NRAS* clonal resistance mutations in colorectal cancer (CRC) patients receiving monoclonal antibodies (mABs) selecting patients to “re-challenge therapy” on the basis of molecular assessment [57,58]. In this scenario, plasma represents an easy- to-collect, dynamic and less invasive sampling approach able to integrate tissue-based analysis for detecting molecular alterations in actionable biomarkers [59,60]. Several key factors (like as pre-analytical managing procedures, clonal hematopoiesis, low abundance of ctDNA in torrent blood) drastically impact on the accuracy rate of liquid biopsy testing in clinical practice; harmonized technical reports play a crucial role in sharing molecular records with clinical oncologists to reduce the gap between intention to treat and clinical decision making for tumor patients [60-63].

LB Section 1. Testing facility identification

Unchanged compared to the general scheme proposed.

LB Section 2. Patient identification and sample characteristics

This section should include pre-analytical quality checks (blood collection time and receipting time from molecular test institution shipping biological sample) and sample type (plasma/serum) should also be clearly and concisely stated. In addition, starting volume of peripheral blood (total amount of entire blood before processing samples) is also crucial to be defined [30,61,64]. Additionally, total volume of plasma/serum separated by centrifugation procedures should be reported in this section [30,61,64]. If available, cfDNA concentration should be added and considered relevant data to technically validate molecular results. Moreover, tumor fraction (TF) represents an emerging parameter able to measure tumor derived from nucleic acids on total cfDNA fragments [65-67]. It has been ascertained that TF significantly decreases

the clonal impact of hematopoiesis on molecular data. For this reason, if available, TF should be integrated in the analytical part of the report. Furthermore, since cfRNA has a very short half-life, storage conditions play a crucial role to preserve and maintain cfNAs and should be added and, if available, the hemolysis fraction should be annotated [68].

LB Section 3. Indication for testing: test requested and clinical context

When available, additional clinically relevant data (smoking history, tumor site, clinical stage of disease and eventually molecular analysis previously performed on matched tissue sample) should be integrated in the report [50,61,62]. Moreover, first line therapy on tissue samples or other therapeutical approaches on previously managed liquid biopsies should be also cited [50].

LB Section 4. Methodology: platform and assay used

In this section the following need to be defined:

- cfDNA/RNA purification protocol, manual and/or platform integrated automatized assays;
- Technical strategies (assay/platform) to perform molecular analysis;
- Technical parameters (LOD, referenced genes covered, medium coverage depth) inspected for data interpretation.

LB Section 5. Diagnosis: summary of most relevant findings and their clinical interpretation

Unchanged compared to the general scheme proposed.

LB Section 6-7. Results: about inquired test and Other results

Unchanged compared to the general scheme proposed, but when clinically relevant molecular alterations are not identified, “not detected alterations” should be used instead of “wild type” accepting false negative results depending on the shedding of ctDNA in torrent blood [64].

Variants with a VAF below the limit of detection (LOD) (e.g., < 0.3%) should be reported as ‘equivocal’. In such cases, orthogonal confirmation testing (e.g., digital PCR), analysis on corresponding tissue if available, or a repeat liquid biopsy should be recommended. Negative results should be clearly stated as ‘the requested mutation was not detected [68]’. Variants suspected to originate from clonal hematopoiesis rather than the tumor should be reported as ‘potential variant related to clonal hematopoiesis’. Furthermore, variants in cancer susceptibility genes with a VAF suggestive of germline origin should be specifically highlighted, and if a putative germline variant is reported, genetic counselling and/or germline testing should be recommended. Each report should also include a disclaimer noting that the presence of mutations below the LOD cannot be excluded.

LB Section 8. Test details: molecular assay and data characteristics

Compared to the general scheme proposed, the type of blood collection tube (e.g., EDTA, Streck) should be specified too.

LB Sections 9-10.

Unchanged compared to the general scheme proposed.

Supplementary Material: a molecular report on *ESR1* detection in liquid biopsy for breast cancer is presented as a pilot example (Supplementary Figure 4).

RECOMMENDATION STATEMENTS

1. Diagnostic Gene Panel (DGP)

a. A molecular report should strike a balance between being concise and comprehensive. It must present essential clinical findings upfront, followed by technical and supplementary details to enhance interpretability for healthcare professionals. A well-structured format incorporating textual and tabular elements ensures efficient analysis and understanding of complex genomic data.

b. The report should include key information such as testing facility details, patient and sample identification, clinical context, and molecular findings to support clinical decision-making. Standardizing gene variant annotation, highlighting clinically actionable findings, and adhering to international nomenclature standards are crucial for improving clarity and usability.

c. The report must clearly distinguish and present actionable variants, providing clinical interpretations to guide treatment decisions (ESCAT actionability level). The summary section should directly

address the clinical question and offer integrated insights on the most significant results, ensuring relevance to patient care.

2. Comprehensive Genomic Profiling (CGP)

a. CGP enables the simultaneous assessment of multiple molecular biomarkers, in terms of SNV, insertions and deletions, CNV, fusions, and complex biomarkers such as MSI and HRD. Due to the vast amount of genomic data generated, it is essential to provide a clear and well-structured molecular report that includes actionable, potentially actionable variants, and VUS. Standardizing variant interpretation and reporting through CGP is vital for accurate clinical decision-making and should involve multidisciplinary discussions within a MTB.

b. CGP reports must present actionable and potentially actionable genomic alterations, alongside co-mutations that are relevant for targeted therapies or immunotherapy. Results should be displayed in a table format, organized by mutation type and clinical relevance, as ESCAT levels. Clinical trial information should be available upon request, and incidental findings linked to hereditary cancer syndromes should be highlighted for further evaluation.

3. BRCA/HRD

a. The structured reporting of BRCA and HRD tests is crucial for guiding therapeutic decisions and identifying hereditary cancer risks. Including pathogenic or likely pathogenic variant classifications, actionable findings, and genetic counseling recommendations supports evidence-based clinical management. Citing public databases further ensures standardized and accurate variant interpretations. Moreover, the inclusion of technical assay details, test limitations, and relevant references strengthens the report's utility in precision medicine.

4. Liquid Biopsy

a. Clinical interpretation of molecular records from LB samples requires a concise and comprehensive description of pre-analytical and analytical parameters of the diagnostic sample. In particular,, pre-analytical key points (including collecting time, total volume of entire blood, shipment conditions) should be annotated to be easily interpretable by the clinicians.

b. TF enables to directly measure abundance of ctDNA on total cfDNA. The implementation of this parameter in the clinical report increases technical interpretability of molecular alterations.

Conclusions

These updated recommendations for NGS molecular reporting respond to the increasing complexity of precision oncology by promoting standardized, clinically meaningful, and user-friendly reporting practices. Developed through the collaboration of molecular biologists, pathologists, geneticists, and oncologists, the new templates are designed to be adaptable to various gene panel sizes and testing contexts, including liquid biopsy and BRCA/HRD analysis. Reports are structured to prioritize clarity, beginning with a concise summary of key genomic findings to guide therapeutic decisions, followed by detailed technical and interpretative information. This format improves readability and facilitates integration into clinical workflows, while the use of synoptic tables enhances the accessibility of complex data. Practical examples in non-small cell lung, breast, and ovarian cancers illustrate the versatility of the templates across tumor types. Although the implementation of standardized reporting may require adjustments to existing laboratory software and processes, the proposed structure lays the groundwork for harmonized molecular diagnostics aligned with international recommendations. Continued evolution of the model is anticipated to reflect advances in science, technology, and clinical practice.

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Authors' contributions

Starting from an initial draft prepared by Davide Seminati, all authors contributed substantially to the development of the manuscript and reviewed its final version. Simonetta Buglioni coordinated the writing team and provided extensive input in both drafting and revising the text. Nicola Fusco served as the overall coordinator of the guidelines project, ensuring harmonization across all documents and conducting critical revision and editing of the manuscript. Umberto Malapelle and Fabio Pagni provided critical feedback and input throughout the writing process. Giancarlo Pruneri acted as the final reviewer.

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MOLECULAR DIAGNOSTIC REPORT

Name of the healthcare institution: XXX
Department or Unit: XXX
Location: City and address:XXX
Contact information: XXX
Laboratory certifications or accrediations:XXX

Section 1

Section 2

Patient ID: XXX	**Sample ID:** XXX	**Collection date: XXX**
Patient name: XXX	**Primary Tumor type and clinical stage:** advanced lung adenocarcinoma – stage IV	**Received date:** XXX
Date of Birth: XXX	**Sample type and site of biopsy:** Core needle biopsy left upper lobe	**Ordering Physician**: XXX
Sex: XXX	**Sample origin and preservation mode**: primary tumor FFPE	
	Tumor content: 60% neoplastic cells; no micro dissection; viable tumor cells >100	

Section 3

Test requested: mutational (molecular) analysis of the following genes *ALK, BRAF, EGFR, ERBB2, KRAS, MET, NTRK1/2/3, RET, ROS1*
Clinical context: molecular diagnostics at the time of diagnosis to evaluate treatment with target therapy; non-smoking patient; PD-L1 negative

Section 4

Methodology: molecular analysis was carried out using NGS technology; Sequencer: XXX;
CE-IVD gene panel: XXX(DNA and RNA)

Section 5

SUMMARY OF THE MOST RELEVANT FINDING

One clinically relevant variant was detected in this case: The *EGFR* p.E746_A750del (Exon 19 deletion) variant is known to be oncogenic. Since this is a sensitizing mutation, the patient may be eligible for treatment with anti-EGFR drugs.

No other clinically relevant molecular alterations detectable by this assay were identified. Pertinent negatives include the absence of *ALK, ROS1, RET, NTRK1/2/3* rearrangements, *BRAF, ERBB2* and *KRAS* mutations and *MET* exon 14 skipping mutations.

Section 6

RESULTS

Gene	Effect	Transcript ID	Exon	Nucleotide variant	Protein variant	Variant class	VAF	*ESCAT
EGFR	Inframe deletion	NM_005228.5	19	c.2235_2249del	p.(Glu746_Ala750del)	Oncogenic	35%	IA
No other alterations were detected in the target regions of the panel genes								

Abbreviations: FFPE: Formalin-fixed, paraffin-embedded; NGS: Next Generation Sequencing; VAF: Variant Allele frequency; ESCAT: ESMO Scale for Clinical Actionability for Molecular Targets; ESMO: European Society for Medical Oncology

OTHER RESULTS: Section 7

Gene	Effect	Transcript ID	Exon	Nucleotide variant	Protein variant	Variant class	VAF	*ESCAT
TP53	Missense	NM_000546.5	5	c.524G>A	p.(Arg175His)	Oncogenic	20%	IV
No other alterations were detected in the target regions of the panel genes								

TEST DETAILS: MOLECULAR ASSAY AND DATA ANALYSIS CHARACTERISTICS Section 8

Nucleic acid extraction platfom and used kit: XXX; **Molecular analysis** was carried out using NGS technology, platform: XXX; gene panel name: XXX; The gene panel can detect the following alterations: SNV, INDEL,CNV and fusions.
Panel gene list (number of genes: XXX): *ALK, BRAF, EGFR, ERBB2* etc….
Analytical characteristics: this test has an analytical sensitivity for detecting 5% SNV and INDEL mutated sequences in a background of non-mutated DNA sequences. Lower limit of detection VAF ≥5%; sensitivity XX%; specificity XXX%; lower limit of sequencing coverage
-List of genes for CNVs detection: XXX; accuracy and molecular evaluation parameters: XXX
-List of genes (RNA) for detection of target rearrangements: XXX; accuracy and molecular evaluation parameters: XXX
Variant analysis: Software XXX (version XXX)
Reference genome: GRCh37.p13 (hg19)
Test limitations: the only reported variants are those found in the interrogated regions of the genes. The test is unable to detect variants present outside of the regions under investigation. Normal population variations, SNPs, and benign variants are not included in this report.
The test was designed for detection of somatic tumor variants and is not intended to be a germline test and that somatic results do not necessarily reflect the patient's germline status.
Assay quality evaluation: Median sequencing coverage:XXX; on target coverage: XXX; mean depth: XXX; uniformity: XXX.

APPENDIX Section 9

Databases consulted: COSMIC (Catalogue of Somatic Mutations in Cancer https://cancer.sanger.ac.uk/cosmic); ClinVar (National Center for Biotechnology information https://www.ncbi.nlm.nih.gov/clinvar/variation/); Varsome (https://varsome.com/); OncoKB (https://www.oncokb.org/); Franklin by genoox https://franklin.genoox.com/clinical-db/home)

***ESCAT[1]: clinical-therapeutic significance levels of molecular variants**
I: **ready for routine use**; **IA:** prospective randomized clinical trials show the alteration-drug match in a specific tumour type results in a clinically meaningful improvement of a survival; **IB**: prospective non-randomized clinical trials show that the alteration-drug match in a specific tumour type, results in clinically meaningful benefit; **IC**: clinical trials across tumour types or basket clinical trials show clinical benefit associated with the alteration-drug match, with similar benefit observed across tumour types
II: **investigational molecular variant**; **IIA:** retrospective studies show that alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown; **IIB**: prospective studies show that alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown.
III: hypothetical molecular target; **IIIA**: clinical benefit demonstrated in patients with the specific alteration but in different tumor type; **IIIB**: clinical benefit demonstrated in patients with an alteration in the same gene or in similar pathway
IV: hypothetical molecular target pre-clinical evidence of actionability
V: the molecular alteration-drug match is associated with objective response, but without clinically meaningful benefit;
X: There is no evidence, clinical or pre-clinical, that the genomic alteration is a potential therapeutic target

EQA: The laboratory participates in the European Molecular Genetics Quality Network (EMQN) program and successfully passed the evaluation for the year 2023.

References

1) J. Mateo, D. Chakravarty, R. Dienstmann, et al. *A* framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol 2018, 29(9): 1895-1902

Date and Signature Section 10

Abbreviations: NGS: Next Generation Sequencing; VAF: Variant Allele frequency; ESCAT: ESMO Scale for Clinical Actionability for Molecular Targets; ESMO: European Society for Medical Oncology; ; SNV: Single Nucleotide Variant; INDEL: small Insertions and Deletions; CNV: Copy Number Variation; SNP: single nucleotide polymorphisms; GRCh37.p13(hg19): Genome Reference Consortium Human Build 37 patch release 13 (synonimus: human genome 19); EQA: External quality assessment

MOLECULAR DIAGNOSTIC REPORT

Section 1

Name of the healthcare institution: XXX
Department or Unit: XXX
Location: City and address:XXX
Contact information: XXX
Laboratory certifications or accreditations:XXX

Section 2

Patient ID: XXX	**Sample ID:** XXX	**Collection date: XXX**
Patient name: XXX	**Primary Tumor type and clinical stage:** advanced lung adenocarcinoma - stage IV	**Received date:** XXX
Date of Birth: XXX	**Sample type and site of biopsy:** Liver core needle biopsy	**Ordering Physician**: XXX
Sex: XXX	**Sample origin and preservation mode**: metastatic lesion FFPE	
	Tumor content: 80% neoplastic cells; microdissection; viable tumor cells >100	

Section 3

Test requested: CGP testing for treatment decision making
Clinical context: Patient with metastatic lung adenocarcinoma positive for *EGFR* p.(L858R) variant. Patient received first line treatment with a third-generation EGFR-TKI and is now in progression. Smoking patient. PD-L1 80%.

Section 4

Methodology: molecular analysis was carried out using NGS technology; Sequencer: XXX; gene panel: XXX

Section 5

SUMMARY OF THE MOST RELEVANT FINDING

In this case, the following clinically relevant alterations were detected: the *EGFR* p.(L858R) driver mutation present in the primary tumor, the *EGFR* p.(C797S) mutation and MET amplification, both described as mechanisms of resistance to third-generation EGFR-TKIs in patients with advanced EGFR-mutated NSCLC. In addition, the oncogenic loss of function variant *TP53* p.(Y220C) was detected. An evaluation of MTB, taking into account clinical and molecular aspects, is necessary to assess the best therapeutic approach. No other clinically relevant molecular alterations detectable by this test were identified.

Section 6

RESULTS

SNV, INDEL VARIANTS								
Gene	**Effect**	**Transcript ID**	**Exon**	**Nucleotide Variant**	**Protein Variant**	**Variant Class**	**VAF**	**ESCAT ***
EGFR	Missense	NM_005228.5	21	c.2573T>G	p.(Leu858Arg)	Oncogenic	73%	IA
EGFR	Missense	NM_005228.5	20	c.2390G>C	p.(Cys797Ser)	Oncogenic	19%	IA
TP53	Missense	NM_000546.6	6	c.659A>G	p.(Tyr220Cys)	Oncogenic	14%	IIB

CNV							
Gene	**Effect**	**Transcript ID**	**Region**	**Event**	**Variant Class**	**Gene copy number**	**ESCAT**
MET	Amplification	NM_001127500.3	7q31.2	gain	Oncogenic	13	IIB

FUSIONS							
Gene	**Effect**	**Transcript ID**	**Specific fusion**	**Phasity**	**Variant class**	**Read Counts**	**ESCAT**
NO FUSION TRANSCRIPT DETECTED							

CANCER GENOMIC SIGNATURE				
Biomarker	**Score**	**Results**	**Threshold**	**ESCAT**
TMB	2 Muts/Mb	Low	10 Muts/Mb	**N/A**
MS	5.4	MSS	18	**N/A**
HRD/GIS	Negative/Low	10	16	**N/A**

Section 7

		OTHER RESULTS (VUS)						
Gene	**Effect**	**Transcript ID**	**Exon**	**Nucleotide Variant**	**Protein Variant**	**Variant Classification**	**VAF**	**ESCAT**
MYC	Missense	NM_002467.6	1	c.62G>A	p.(Ser21Asn)	VUS	14%	N/A

Abbreviations: FFPE: Formalin-fixed, paraffin-embedded; CGP: Comprehensive Genomic Profiling; TKI: Tyrosine Kinase Inhibitor; NGS: Next Generation Sequencing; NSCLC: Non-Small Cell Lung Cancer; MTB: Molecular Tumor Board; SNV: Single Nucleotide Variant; INDEL: small Insertions and Deletions; VAF: Variant Allele frequency; CNV: Copy Number Variation; ESCAT: ESMO Scale for Clinical Actionability for Molecular Targets; ESMO: European Society for Medical Oncology; TMB: Tumor Mutational Burden; Muts/MB: number of mutations per megabase; MS: Microsatellite Status; MSS**:** microsatellite stability; HRD: Homologous recombination deficiency; GIS: Genomic Instability Status; N/A: Not Applicable;

Section 8

TEST DESCRIPTION: MOLECULAR ASSAY AND DATA ANALYSIS CHARACTERISTICS

Nucleic acid extraction platform and used kit: XXX; Molecular analysis was carried out using NGS technology, platform: XXX; gene panel: XXX; The gene panel can detect the following alterations: SNVs, INDELs, CNVs), traslocations (fusions), and cancer genomic signature (TMB, MSI, HRD).
Panel gene list (number of genes: XXX): *ALK, BRAF, EGFR, ERBB2* etc….
This test has an analytical sensitivity for detecting 5% SNV and INDEL mutated sequences in a background of non-mutated DNA sequences. Lower limit of detection VAF ≥5%; sensitivity XX%; specificity XXX%; lower limit of sequencing coverage: XXX
-CNV: the gene test include the following genes: XXX; molecular evaluation cut-off: XXX
-Fusions: the gene test for translocations include the following genes : XXX; Molecular evaluation cut-off: XXX
-TMB: was calculated as an index of the number of mutations per megabase (muts/Mb) harbored by tumor cells from this neoplasm . TMB is considered high if it exceeds a threshold of XXXmuts/Mb
-Microsatellite status: MS is categorized as MSI-H, MSS, or MS-Equivocal according to the fraction of microsatellite loci determined to be altered or unstable. MSI is evaluated based on a genome-wide analysis across >XXX genes microsatellite loci. The final fraction unstable loci score is calculated as the number of unstable microsatellite loci divided by the number of evaluable microsatellite loci.
- HRD status: HRD positive is defined as either a tBRCA mutation and/or an HRD score* ≥XXX; HRD negative is defined as either non–tBRCA-mutated and/or HRD score <XXX; *HRD score: is intended as the Genomic instability status
Variant analysis: Software XXX (version XXX)**.**
Reference genome: GRCh37.p13 (hg19)
Test limitations: the only reported variants are those found in the interrogated regions of the genes. The test is unable to detect variants present outside of the regions under investigation. Normal population variations, SNPs and benign variants are not included in this report.
The test was designed for detection of somatic tumor variants and is not intended to be a germline test and somatic results do not necessarily reflect the patient's germline status.
Assay quality evaluation: Median sequencing coverage: XXX; on target coverage: XXX; mean depth: XXX; uniformity: XXX.

Section 9

APPENDICES

Databases consulted: COSMIC (Catalogue of Somatic Mutations in Cancer https://cancer.sanger.ac.uk/cosmic); ClinVar (National Center for Biotechnology information https://www.ncbi.nlm.nih.gov/clinvar/variation/); Varsome (https://varsome.com/); OncoKB (https://www.oncokb.org/); Franklin by genoox https://franklin.genoox.com/clinical-db/home); Knowledge bases consulted for the biological and functional annotation of each detected variant:XXX; Knowledge bases consulted for the **EQA:** The laboratory participates in the European Molecular Genetics Quality Network (EMQN) program and successfully passed the evaluation for the year 2023.

***ESCAT[1]: clinical-therapeutic significance levels of molecular variants**
I: **ready for routine use**; **IA:** prospective randomized clinical trials show the alteration-drug match in a specific tumour type results in a clinically meaningful improvement of a survival; **IB**: prospective non-randomized clinical trials show that the alteration-drug match in a specific tumour type, results in clinically meaningful benefit; **IC**: clinical trials across tumour types or

basket clinical trials show clinical benefit associated with the alteration-drug match, with similar benefit observed across tumour types
II: **investigational molecular variant**; **IIA:** retrospective studies show that alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown; **IIB**: prospective studies show that alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown.
III: hypothetical molecular target; **IIIA**: clinical benefit demonstrated in patients with the specific alteration but in different tumor type; **IIIB**: clinical benefit demonstrated in patients with an alteration in the same gene or in similar pathway
IV: hypothetical molecular target pre-clinical evidence of actionability
V: the molecular alteration-drug match is associated with objective response, but without clinically meaningful benefit;
X: There is no evidence, clinical or pre-clinical, that the genomic alteration is a potential therapeutic target

Abbreviations: FFPE: Formalin-fixed, paraffin-embedded ;CGP: Comprehensive Genomic Profiling; TKI: Tyrosine Kinase Inhibitor; NGS: Next Generation Sequencing; NSCLC: Non-Small Cell Lung Cancer; MTB: Molecular Tumor Board; SNV: Single Nucleotide Variant; INDEL: small Insertions and Deletions; VAF: Variant Allele frequency; CNV: Copy Number Variation; ESCAT: ESMO Scale for Clinical Actionability for Molecular Targets; ESMO: European Society for Medical Oncology; TMB: Tumor Mutational Burden; Muts/MB: number of mutations per megabase; MS: Microsatellite Status; MSS**:** microsatellite stability; HRD: Homologous recombination deficiency; GIS: Genomic Instability Status; N/A: Not Applicable; VUS: Variant of Uncertain Significance; SNP: single nucleotide polymorphisms; GRCh37.p13(hg19): Genome Reference Consortium Human Build 37 patch release 13 (synonimus: human genome 19); EQA: External quality assessment

References

1) J. Mateo, D. Chakravarty, R. Dienstmann, et al. *A* framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol 2018, 29(9): 1895-1902

Section 10

Date and signature

MOLECULAR DIAGNOSTIC REPORT

Section 1

Name of the healthcare institution: XXX
Department or Unit: XXX
Location: City and address:XXX
Contact information: XXX
Laboratory certifications or accrediations:XXX

Section 2

Patient ID: XXX	**Sample ID:** XXX	**Collection date: XXX**
Patient name: XXX	**Primary tumor type and clinical stage:** serous ovarian cancer – stage IV	**Received date:** XXX
Date of Birth: XXX	**Sample type and site of biopsy:** surgical resection of ovarian cancer	**Ordering Physician**: XXX
Sex: XXX	**Sample origin and preservation mode**: primary tumor FFPE	
	Tumor content: 70% neoplastic cells; no micro dissection; viable tumor cells >100	

Section 3

Test requested: HRD and tumor BRCA1 (NM_007294.4) and BRCA2 (NM_000059.4) testing.
The Clinical Consultant followed appropriate counseling procedures to obtain the patient's informed consent for the sample NGS analysis.

Section 4

Clinical context: The patient has been diagnosed with serous ovarian cancer and has undergone platinum-based chemotherapy, to which her tumor responded. She is now being evaluated for maintenance treatment with PARP inhibitors. No previous germline testing was performed.

Methodology: molecular analysis was carried out using NGS technology; Sequencer: XXX; gene panel: XXX

Section 5

SUMMARY OF THE MOST RELEVANT FINDING

In this case was detected a pathogenic variant in *BRCA1* gene p.(Gln1756ProfsTer74) and a High Genomic Instability (GIS-H) since the tumor is HRD positive. Therefore the patient may be elegible for PARP inhibitor therapy. No other clinically relevant molecular alterations detectable by this assay were identified. Pertinent negatives include the absence of alterations in *BRCA2* gene. Genetic counseling is strongly recommended

Section 6

RESULTS

Gene	Effect	Transcript ID	Exon	Nucleotide variant	Protein variant	Variant Class *IARC/ACMG/ AMP	VAF	**ESCAT
BRCA1	Frameshift	NM_007294.4	20	c.5266dup	p.(Gln1756ProfsTer74)	Pathogenic (5 class)	89%	IA
BRCA2	NO PATHOGENIC /LIKELY PATHOGENIC VARIANT DETECTED							

Genomic instability status	Genomic instability metric	BRCA status	HRD status	**ESCAT
High	42	Positive	**Positive**	IA

Abbreviations - FFPE: Formalin-fixed, paraffin-embedded; HRD: Homologous recombination deficiency; PARP: poli-ADP ribosio polimerasi; NGS: Next Generation Sequencing; GIS-H: Genomic Instability Status-High; VAF: Variant Allele frequency; IARC: International Agency for Research on Cancer; ACMG-AMP: American College of Medical Genetics and Genomics in collaboration with the Association for Molecular Pathology; ESCAT: ESMO Scale for Clinical Actionability for Molecular Targets; ESMO: European Society for Medical Oncology;

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OTHER RESULTS: Section 7

Gene	Effect	Transcript ID	Exon	Nucleotide variant	Protein variant	variant class	VAF	*ESCAT
TP53	Splice variant	NM_000546.5	N/A	c.920-1G>A	p.?	Pathogenic	42%	N/A
BRCA2	missense	NM_000059.4	25	c.9302T>C	p.(Leu3101Pro)	VUS[§]	15%	N/A
No other alterations were detected in the target regions of the panel genes								

[§]Note: a six month periodic reassessment of the variant significance will be performed.

Section 8

TEST DETAILS: MOLECULAR ASSAY AND DATA ANALYSIS CHARACTERISTICS

Nucleic acid extraction platfom and used kit: XXX; **Molecular analysis** was carried out using NGS technology, platform: XXX; gene panel name: XXX; The gene panel can detect the following alterations: SNV, INDEL, CNV in a select group of genes;
Panel gene list (number of genes: XXX): *BRAC1, BRCA2,* and other HRR genes
This test has an analytical sensitivity for detecting 5% SNV and INDEL mutated sequences in a background of non-mutated DNA sequences. Lower limit of detection VAF ≥5%; sensitivity XX%; specificity XXX%; lower limit of sequencing coverage (coverage depth): 500x
The gene panel covers the entire coding sequence and intron-exon boundaries of *BRCA1,2* genes ensuring the detection of all possible variants across both the coding and splicing regions.
-CNV: the gene test include the following genes: XXX; molecular evaluation cut-off: XXX
BRCA Status positive or negative: presence of pathogenic /likely pathogenic variant or absence of pathogenic /likely pathogenic variant
Genomic instability status High: genomic instability metric>16
Variant analysis: Software XXX (version XXX)
Reference genome: GRCh37.p13 (hg19)
Test limits: the only reported variants are those found in the interrogated regions of the genes. The test is unable to detect variants present outside of the regions under investigation. Benign, likely benign and low clinical relevance variants are not included in this report.
The method currently used does not rule out the presence of genomic rearrangements in the genes analyzed. The test was designed for detection of somatic tumor variants and is not intended to be a germline test and that somatic results do not necessarily reflect the patient's germline status. The incidence of false negative/positive due to sample processing errors is estimated to be <1%. Test sensitivity: Failure to identify a pathogenic variant within the regions analyzed using these methodologies is estimated to be <2%.
Assay quality evaluation: Median sequencing coverage:XXX; on target coverage: XXX; mean depth: XXX; uniformity: XXX.

APPENDIX Section 9

Database and tools: BRCA Exchange (https://brcaexchange.org/); COSMIC (Catalogue of Somatic Mutations in Cancer https://cancer.sanger.ac.uk/cosmic); ClinVar (National Center for Biotechnology information https://www.ncbi.nlm.nih.gov/clinvar/variation/); Varsome (https://varsome.com/); OncoKB (https://www.oncokb.org/); Franklin by genoox https://franklin.genoox.com/clinical-db/home) Knowledge bases consulted for the biological and functional annotation of each detected variant:XXX

Criteria: ClinGen ENIGMA BRCA1 and BRCA2 Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines, IARC/ACMG/AMP Criteria for the Interpretation of Sequence Variants; ClinGen-CGC-VICC Guidelines

*IARC/ACMG-AMP 5-category scheme (classes)[3,4]:
- Pathogenic (causative; class 5)
- Likely pathogenic (class 4)
- Uncertain clinical significance ('VUS'; class 3)
- Likely benign (class 2)
- Benign (class 1)

***ESCAT[1]: clinical-therapeutic significance levels of molecular variants**
I: **ready for routine use**; **IA:** prospective randomized clinical trials show the alteration-drug match in a specific tumour type results in a clinically meaningful improvement of a survival; **IB**: prospective non-randomized clinical trials show that the alteration-drug match in a specific tumour type, results in clinically meaningful benefit; **IC**: clinical trials across tumour types or basket clinical trials show clinical benefit associated with the alteration-drug match, with similar benefit observed across tumour types
II: **investigational molecular varian**t; **IIA:** retrospective studies show that alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown; **IIB**: prospective studies show that alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown.
III: hypothetical molecular target; **IIIA**: clinical benefit demonstrated in patients with the specific alteration but in different tumor type; **IIIB**: clinical benefit demonstrated in patients with an alteration in the same gene or in similar pathway
IV: hypothetical molecular target pre-clinical evidence of actionability
V: the molecular alteration-drug match is associated with objective response, but without clinically meaningful benefit;
X: There is no evidence, clinical or pre-clinical, that the genomic alteration is a potential therapeutic target

EQA: The laboratory participates in the European Molecular Genetics Quality Network (EMQN) program and successfully passed the evaluation for the year 2023.

References

1)J. Mateo, D. Chakravarty, R. Dienstmann, et al. *A* framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol 2018, 29(9): 1895-1902

2) Mosele MF, Westphalen CB, Stenzinger A, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. Ann Oncol 2024;35(7):588-606.

3) IARC:Sharon E. Plon, Diana M. Eccles, Douglas Easton et al. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat 2008 Nov;29(11):1282-91

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5) Michael T Parsons, Miguel de la Hoya , Marcy E Richardson et al. Evidence-based recommendations for gene-specific ACMG/AMP variant classification from the ClinGen ENIGMA BRCA1 and BRCA2 Variant Curation Expert Panel. Am J Hum Genet 2024 Sep 5;111(9):2044-2058.

Section 10

Date and Signature

Abbreviations - FFPE: Formalin-fixed, paraffin-embedded; HRD: Homologous recombination deficiency; PARP: poli-ADP ribosio polimerasi; NGS: Next Generation Sequencing; GIS-H: Genomic Instability Status-High; VAF: Variant Allele frequency; ESCAT: ESMO Scale for Clinical Actionability for Molecular Targets; ESMO: European Society for Medical Oncology; SNV: Single Nucleotide Variant; INDEL: small Insertions and Deletions; CNV: Copy Number Variation; HRR: Homologous recombination repair; GRCh37.p13(hg19): Genome Reference Consortium Human Build 37 patch release 13 (synonimus: human genome 19); EQA: External quality assessment. IARC: International Agency for Research on Cancer; ACMG-AMP: American College of Medical Genetics and Genomics in collaboration with the Association for Molecular Pathology

MOLECULAR DIAGNOSTIC REPORT FOR LIQUID BIOPSY (LB)

Section 1

Name of the healthcare institution: XXX
Department or Unit: XXX
Location: City and address:XXX
Contact information: XXX
Laboratory certifications or accrediations:XXX

Section 2

Patient ID: XXX	**Sample ID:** 025-001	**Collection date:** 20/01/2025
Patient name: XXX	**Primary tumor type and clinical stage:** Breast cancer ER+/HER2- ; stage IV	**blood collection time**: 10:30 AM
Date of Birth: XXX	**Sample type:** peripheral blood. **Starting volume of peripheral blood:** total amount of entire blood before processing samples: 10 ml; **Type of blood collection tube**: EDTA	**receipting time**: 10:42 AM
Sex: XXX	**Total volume of plasma**: 4 ml**; storage conditions: XXX;**	**Ordering Physician**: XXX
	cfDNA concentration: 5 ng/µl; **Tumor Fraction** (TF): XXX	**Hemolysis fraction: XX**

Section 3

Test requested: mutational analysis of the ***ESR1*** gene

Clinical context and Clinically relevant data: The patient was first diagnosed with ER+/HER2- breast cancer. She received adjuvant endocrine therapy and, after disease progression, was treated with aromatase and CDK4/6 inhibitors. After further disease progression (metastatic lung nodules), plasma analysis for ESR1 variants was requested to aid clinical decision-making.

Section 4

Method of molecular analysis: cfDNA/RNA purification protocol; manual and/or platform integrated automatized assays; molecular analysis was carried out using NGS technology; Sequencer: XXX; gene panel: XXX

Section 5

SUMMARY OF THE MOST RELEVANT FINDING

Exon 5 of *ESR1* gene: p.(E380Q). This alteration is able to elect the patient to aromatase inhibitors and to SERDs treatment.

Section 6

RESULTS

Gene	Effect	Transcript ID	Exon	Nucleotide variant	Protein variant	Variant class	VAF	*ESCAT
ESR1	Missense	NM_001122740.1	5	c.1138G>C	p.(Glu380Gln)	Likely oncogenic	3.5 %	IA

Abbreviations - cfDNA: cell free DNA; TF: Tumor Fraction; NGS: Next Generation Sequencing; SERDs: Selective Estrogen receptor degraders; SNV: Single Nucleotide Variant; VAF: Variant Allele frequency; ESCAT: ESMO Scale for Clinical Actionability for Molecular Targets; ESMO: European Society for Medical Oncology

Section 7

OTHER RESULTS: No other alterations were detected in the target regions of the panel genes

Section 8

TEST DESCRIPTION: MOLECULAR ASSAY AND DATA ANALYSIS CHARACTERISTICS

cfDNA purification protocol: XXX; platform integrated automatized assays: XXX;
ctDNA TF was quantified by the following method: XXX
Molecular analysis was carried out using NGS technology, platform: XXX; gene panel name: XXX; The gene panel can detect the following alterations: SNV and INDEL in a select group of genes; **Panel gene list** (number of genes: XXX): *ESR1, ….*
This test has an analytical sensitivity for detecting 0.5% SNV and INDEL mutated sequences in a background of non-mutated DNA sequences. Lower limit of detection VAF ≥0.1%; sensitivity >99%; specificity XXX%; lower limit of sequencing coverage 10000x
Variant analysis: Software XXX (version XXX)
Reference genome: GRCh37.p13 (*hg19*)
Test limitions: the only reported variants are those found in the interrogated regions of the genes. The test is unable to detect variants present outside of the regions under investigation. Normal population variations, SNPs, and benign variants are not included in this report.
The test was designed for detection of somatic tumor variants and is not intended to be a germline test and that somatic results do not necessarily reflect the patient's germline status.
Assay quality evaluation: Median sequencing coverage:XXX; on target coverage: XXX; mean depth: XXX; uniformity: XXX.

APPENDIX Section 9

Databases consulted: COSMIC (Catalogue of Somatic Mutations in Cancer https://cancer.sanger.ac.uk/cosmic); ClinVar (National Center for Biotechnology information https://www.ncbi.nlm.nih.gov/clinvar/variation/); Varsome (https://varsome.com/); OncoKB (https://www.oncokb.org/); Franklin by genoox https://franklin.genoox.com/clinical-db/home)

***ESCAT[1]: clinical-therapeutic significance levels of molecular variants**
I: **ready for routine use**; **IA:** prospective randomized clinical trials show the alteration-drug match in a specific tumour type results in a clinically meaningful improvement of a survival; **IB**: prospective non-randomized clinical trials show that the alteration-drug match in a specific tumour type, results in clinically meaningful benefit; **IC**: clinical trials across tumour types or basket clinical trials show clinical benefit associated with the alteration-drug match, with similar benefit observed across tumour types
II: **investigational molecular variant**; **IIA:** retrospective studies show that alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown; **IIB**: prospective studies show that alteration-drug match is associated with antitumor activity, but magnitude of benefit is unknown.
III: hypothetical molecular target; **IIIA**: clinical benefit demonstrated in patients with the specific alteration but in different tumor type; **IIIB**: clinical benefit demonstrated in patients with an alteration in the same gene or in similar pathway
IV: hypothetical molecular target pre-clinical evidence of actionability
V: the molecular alteration-drug match is associated with objective response, but without clinically meaningful benefit;
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Section 10

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Abbreviations - cfDNA: cell free DNA; TF: Tumor Fraction; NGS: Next Generation Sequencing; INDEL: small Insertions and Deletions; VAF: Variant Allele Sequencing; SNP: single nucleotide polymorphisms; GRCh37.p13(hg19): Genome Reference Consortium Human Build 37 patch release 13 (synonimus: human genome 19); EQA: External quality assessment