Sample Reports:

Example 1: *BRCA1/2* clinically significant variant was detected.

Name: Last, First

DOB: DD/MM/YYYY

Patient Reference Number:

Tissue Reference Number:

Tissue Type: {Cytology, Biopsy, Resection – Omentum, Fallopian Tube, Ovary, etc.}

Provisional diagnosis: high grade serous carcinoma

Tumour Cellularity:

Test: Tumour *BRCA1* and *BRCA2* sequence and copy number variants analysis

RESULTS

A clinically actionable sequence variant c.5851_5854del, p.(Ser1951TrpfsTer11) was detected in the *BRCA2* gene. The variant was present in approximately 60% of sequenced fragments.

NGS quality parameters:

Average depth of coverage: 1,120x

Regions with suboptimal depth of coverage where presence of variants cannot be conclusively assessed: none

INTERPRETATION

Presence of clinically actionable *BRCA1/2* variants can be associated with a favourable response to PARP inhibitors treatment in patients responsive to platinum-chemotherapy [1; 2]. The tumour specimen provided contains a clinically actionable variant c.5851_5854del, p.(Ser1951TrpfsTer11) in the *BRCA2 gene* Presence of this variant is likely to be associated with increased sensitivity to PARP inhibitors treatment.

The current analysis was limited to tumour tissue and we are therefore unable to determine whether this is a somatic (acquired) or germline (inherited) variant.

A high proportion of *BRCA1/2* variants identified in ovarian tumour samples are also present in the germline. Germline pathogenic variants in the *BRCA1/2* genes are associated with an inherited cancer predisposition syndrome that may confer an increased cancer risk for this individual and family members.
Germline variants in genes other than *BRCA1/2* that are known to be associated with high grade serous carcinoma were not examined.

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Referral to hereditary cancer clinic for genetic counselling and hereditary cancer risk assessment is recommended.

Variant(s) summary:

This *BRCA2* c .5851_5854del, p.(Ser1951TrpfsTer11) variant is a four nucleotides deletion and is predicted to result in a frameshift/ of open reading frame and premature stop codon. This variant is expected to result in loss-of-function due to a truncated BRCA2 protein and/or nonsense-mediated decay. This variant {has /has not} been previously reported in the ClinVar patient database (Variation ID: _____) and {has /has not} been catalogued in the COSMIC database of somatic variants.

TEST SUMMARY & DISCLAIMERS:

Background: Loss of function *BRCA1/BRCA2* mutations in high grade serous ovarian, fallopian tube or primary peritoneal tumors can be associated with favorable response to treatment with the PARP inhibitors and improved overall survival [1;2]. A high proportion of *BRCA1/BRCA2* variants identified in ovarian tumour samples are also present in the germline [2]. Germline pathogenic variants in the *BRCA1/BRCA2* genes are associated with an inherited cancer predisposition syndrome that may confer an increased cancer risk for this individual and family members [3].

Genes Tested: *BRCA1* (NM_007294.3 *, exons 2-24), *BRCA2* (NM_000059.3, exons 2-27)**

Methodology: DNA was extracted from the paraffin-embedded tissue and tested using a custom designed next-generation sequencing (NGS) protocol. Coding exons and 10 bp of flanking intronic regions were enriched using a [targeted capture/amplicon-based]protocol ([Add vendor]). Sequencing was performed using either a […] instrument. Sequence alignment and variant calling was performed using […] software, version X ([add vendor]).

Sequence variants are annotated using the […] software , version [] ([add vendor]). This test was validated to detect sequence variants with a variant allele frequency of 10% or higher. Test sensitivity is estimated to be [>98%] for detection of single nucleotide variants and insertions/deletions smaller than [ ] nucleotides.

Exon level copy number variants (CNVs) were analyzed using the […] algorithm in [..] software, version [] ([add vendor]). This method was validated to detect exon level deletions and duplications in the tumour tissue similar to those in germline samples (ie. at an allele frequency of approximately 50% or greater). Sensitivity of CNV detection might be lower in tumours compared to peripheral blood samples due to tumour biology and quality of DNA from FFPE samples.

Variants are interpreted and classified using ACMG guidelines [4]. Variants that are classified as Pathogenic (ACMG 1), Likely Pathogenic (ACMG 2) are reported as clinically actionable, variants of Uncertain Significance (ACMG 3) are not considered to be clinically actionable and are reported separately to inform future germline testing. Variants classified as Likely Benign (ACMG 4) or Benign (ACMG 5) are not reported but are available upon request.

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Limitations: This test is limited to *BRCA1* and *BRCA2* [coding exons and 10 bp of flanking intronic regions]**.

References: [1] Ledermann et al, Lancet Oncol. 2016 Nov;17(11):1579-1589 (PMID: 27617661); [2] Konstantinopoulos et al, J Clin Oncol. 2020 Apr 10;38(11):1222-1245., Br J Cancer. 2016 Nov 8;115(10):1157-1173, (PMID: 31986064); [3] GeneReviews, 2016 (PMID 20301425); [4] Richards et al Genet Med 2015; 17:405-424 (PMID: 25741868).

Disclaimers: This test is unable to distinguish between a somatic and a germline variants. Any interpretation is provided without knowledge if the variants detected are somatic or germline and assuming and that the pathology diagnosis and tumor % is correct.

Report was reviewed and approved by:

Date:

*Alternatively Locus Reference Genomic numbers LRG_293t1 (BRCA1) and LRG_293t1 (BRCA2) could be used

** At minimum coding regions of *BRCA1* & *BRCA2* and 10 bp of flanking regions should be tested, additional non-coding region could be added as knowledge about *BRCA1/2* disease causing variants evolve.

[] – indicate the parameters that are laboratory specific

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Example 2: No Variants Detected in the *BRCA1/2*.

Name: Last, First

DOB: DD/MM/YYYY

Patient Reference Number:

Tissue Reference Number:

Tissue Type: {Cytology, Biopsy, Resection – Omentum, Fallopian Tube, Ovary, etc}

Provisional diagnosis: high grade serous carcinoma

Tumour Cellularity:

Test: Tumour *BRCA1* and *BRCA2* sequence and copy number variants analysis

RESULTS

Clinically actionable sequence or copy number variants were NOT detected in either the *BRCA1 or BRCA2* genes.

NGS Quality Parameters: Average depth of coverage: 1,080 x

Regions with suboptimal depth of coverage where presence of variants cannot be conclusively assessed: none

INTERPRETATION

No clinically actionable variants in the *BRCA1* and *BRCA2* genes were detected in the tumour specimen provided. Absence of clinically significant *BRCA1/BRCA2* variants can be associated with a less favourable response to PARP inhibitors treatment.

Germline variants in genes other than *BRCA1/2* that are known to be associated with high grade serous ovarian carcinomas were not examined and a negative test for *BRCA1/2* does not rule out an inherited etiology.

Referral to hereditary cancer clinic for genetic counselling and hereditary cancer risk assessment is recommended.

Test Summary and Disclaimer as above

Report was reviewed and approved by:

Date:

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Example 3: *BRCA1/2* variant of uncertain clinical significance was detected.

Name: Last, First

DOB: DD/MM/YYYY

Patient Reference Number:

Tissue Reference Number:

Tissue Type: {Cytology, Biopsy, Resection – Omentum, Fallopian Tube, Ovary, etc.}

Provisional diagnosis: high grade serous carcinoma

Tumour Cellularity:

Test: Tumour *BRCA1* and *BRCA2* sequence and copy number variants analysis

RESULTS

Clinically actionable sequence or copy number variants were NOT detected in either the *BRCA1* or *BRCA2* genes.

NGS quality parameters: Average depth of coverage: 1,119 x

Regions with suboptimal depth of coverage where presence of variants cannot be conclusively assessed: none.

INTERPRETATION

No clinically actionable variants in the *BRCA1* and *BRCA2* genes were detected in the tumour specimen provided. Absence of clinically significant *BRCA1/BRCA2* variants can be associated with a less favourable response to PARP inhibitor treatment.

However, a variant of uncertain significance was detected in the *BRCA1* gene (see variant summary below).

Germline variants in genes other than *BRCA1/2* that are known to be associated with high grade serous ovarian carcinomas were not examined and a negative test for *BRCA1/2* does not rule out an inherited etiology.

Referral to hereditary cancer clinic for genetic counselling and hereditary cancer risk assessment is recommended.

Variant(s) summary:

This sample is positive for *BRCA1* c.1333G>C, p.(Glu445Gln) missense sequence variant. The variant was detected in 25% of sequenced fragments. The clinical significance of this variant is uncertain, and no recommendation can be made regarding PARP inhibitors sensitivity. The

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information about this variant is provided to inform future germline testing in this individual or her family.

Test Summary and Disclaimer as above

Report was reviewed and approved by:

Date:

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