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CGT Laboratory

OncoHRD

NGS test for identification of HRD status through the use of the OncoPan® panel.

Introduction

OncoHRD is Cogentech's proposed molecular test based on an advanced technological process called Next Generation Sequencing (NGS) to derive the biomarker of genomic instability '**GI index**' (Genome Instability) to be added to the assessment of mutational analysis of *BRCA1* and *BRCA2* genes to evaluate the HRD (Homologous Recombination Deficiency) status of a sample.

The test is offered at the somatic level in order to evaluate the possibility of offering Parp inhibitor therapy to patients with ovarian cancer who have a mutation in the *BRCA1* or *BRCA2* genes, or a homologous recombination (HR) deficiency, due to different alterations, and evidenced by the presence of genomic instability. The identification of HRD status turns out, therefore, to be fundamental in clinical practice in order to direct therapeutic decisions and improve patient survival. In fact, the Italian Drug Agency (AIFA) has approved and made reimbursable Olaparib in combination with bevacizumab in ovarian cancers with genomic instability even in the absence of a *BRCA1/2* mutation.

The test includes:

- the use of the OnconPan® panel developed by Cogentech's CGT Lab, which is required for assessment of the mutational status of *BRCA1* and *BRCA2* genes, including both substitutions/small deletions/insertions (SNVs) and large rearrangements (CNVs);
- lpWGS (low pass Whole Genome Sequencing) analysis to detect the GI index, obtained through the bioinformatics tool GIInger (Sophia Genetics).

The test is for BRCA genes only, although the use of the OncoPan® panel allows for possible additional analyses to investigate the mutational status of other genes in the panel.

How the test is carried outt

The test is performed on genomic DNA extracted from material taken from the tumor, previously fixed in kerosene (**somatic sample**), with a tumor content (cellularity) greater than 30 percent. The isolated material is subjected to an enzymatic fragmentation process and then divided into two aliquots for :

- the sample enrichment step related to the genes of interest by means of the technique termed 'capture,' in which probes drawn through a criterion are used to specifically select only well-defined regions. In detail, the probes were created by exploiting Agilent Sure Select technology, including the nucleotide regions of the exons of the selected genes and at least 20 bases of the adjacent intronic regions;
- The creation of the material for lpWGS analysis.

The samples thus generated are then sequenced through a process employing NGS techniques and the use of instruments provided by the Illumina company.

Variants of uncertain significance (VUS) and pathogenic variants found are confirmed by direct Sanger sequencing or Multiplex Ligation-dependent Probe Amplification (MLPA). The ABI3500 Dx Genetic Analyzer platform (Applied Biosystems) is used for both Sanger method sequence identification and MLPA fragments. Computer processing of these data is done with Mutation Surveyor and Gene Marker software, respectively, both sold by the company SoftGenetics.

Bioinformatics analysis

At the end of the NGS sequencing phase, the data undergo two different bioinformatics analysis (*pipeline*):

1. an automated procedure developed by Cogentech's CGT Lab, in collaboration with bioinformatics expert firm enGenome, which allows the pairing of the data obtained with the corresponding regions of the genome chosen as a reference (GRCh37_hg19), to define the presence of any SNV or CNV variants;
2. the use of the online software GIInger, developed by Sophia Genetics, which highlights GI status.

Combining the data from these two analyses allows the HRD status of the sample to be defined.

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Verification of the test

1. The OncoPan® panel has been validated as per the specific data sheet (accessible upon request).
2. Verification of the reliability of the workflow leading to the production of data for lpWGS analysis was carried out:

- By the company Sophia Genetics (as per Attachment1);
- by CGT Lab, which prepared at its site the material for lpWGS analysis, which was done by using the GIInger software, to compare the results with the expected ones.

Table 1 shows the concordance of the GI parameter between two different workflows, considering 8 in-house controls + 2 controls provided by the company Sophia Genetics, for a total of 10 samples previously analyzed with the HRD Sophia DDM Solution kit.

		GI status (GIInger)	
		Positive	Negative
GI status (HRD Sophia DDM Solution)	Positive	6	-
	Negative	-	4

Table 1: concordance of GI Status

Panel limitations

This analysis is unable to detect deep intronic variants, genomic rearrangements, triplet expansions, somatic mutations below 10 percent frequency of the least represented allele.

Conclusioni

The **OncoHRD** test proved reliable within the limits of the reported values, combining the advantages of the OncoPan panel with the robustness of the bioinformatic analysis provided by Sophia Genetics' GIInger software. The **concordance** between the results obtained with the new workflow and that previously adopted by CGT Lab is **100%**. Somatic CNV analysis also makes use of verification by MLPA, consistent with the success of the 'experiment, the MLPA technique not being currently validated by the vendor for its use on DNA extracted from paraffin-embedded tissue.