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OncoPan®

NGS genetic test developed by Cogentech's CGT Laboratory

Introduction

OncoPan® is the innovative molecular test proposed by Cogentech, based on an advanced technological process called Next Generation Sequencing (**NGS**), which enables the detection of variations at the level of the patient's genome. These variations include point mutations (substitutions, small deletions/insertions, or SNVs) and large rearrangements (CNVs) due to deletion/duplication of one or more exons of the genes under investigation. The test is offered at both **germline** and **somatic levels**.

- The **germinal** analysis concerns genes involved in **predisposition to** cancers of the mammmella and ovary, colon, endometrium, stomach, prostate, pancreas, melanomas, and known cancer syndromes. The panel also includes 313 SNPs for the calculation of Polygenic Risk Score (PRS), which are useful in improving the definition of individual breast and ovarian cancer risk of the analyzed patients. The "z score" value obtained, can be used by clinicians in the calculation developed by the CanRisk software (www.canrisk.org), according to the BOADICEA integrated risk prediction model, which considers personal risk factors, family oncology history, genetic testing for the presence of genes conferring moderate or high risk, polygenic risk scores (PRS), and mammographic density.
- **Somatic** analysis looks for alterations present in tumor tissue that may have **therapeutic value**, indicating sensitivity or resistance to particular drugs, or **prognostic value**, giving indications regarding expectations in the prognosis of the disease.

BRCA1 and *BRCA2* gene analysis is used to evaluate the possibility of offering Parp inhibitor therapy (Olaparib, Rucaparib,Talazoparib) to ovarian cancer patients.

POLE gene analysis is used for al evaluation for prognostic purposes in endometrial carcinomas.

KRAS and *BRAF* gene analysis is used to assess resistance to anti-EGFR monoclonal antibodies (Cetuximab, Panitumumab) in colon cancer.

Analysis of *EGFR* and *NRAS* genes assesses sensitivity or resistance to therapies with Gefitinib or Erlotinib in metastatic non-small cell lung cancer (NSCLC).

Mutations in the *BRAF* gene confer sensitivity to Vemurofenib in metastatic melanoma.

Patients with breast cancer may benefit from treatment with Trastuzumab, Lapatinib or Pertuzumab in the presence of *HER2* mutations or Alpelisib in combination with Fulvestrant in the presence of *PIK3CA* mutations.

Mutations in the *c-KIT* and *PDGFR-α* genes are used to confirm the diagnosis of gastrointestinal stromal tumor (GIST) and to assess the risk of progression and resistance to therapies (Imatinib).

The genes included in the current version (OncoPan Vs_4.0) of the panel are listed below:

ACD, APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1,CDKN2A (α and *β), CDK4 (exon 2), CHEK2, CTNNA1, EPCAM, FANCM, GREM1, HOXB13, MC1R, MITF (exon 9), MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NTHL1, PALB2, PMS2, POLD1, POLE, POT1, PTEN, RAD51C, RAD51D, RNF43, SMAD4, STK11, TERF2IP, TERT, TP53, KRAS, NRAS, BRAF, EGFR, HER2(ERBB2), PIK3CA, c-KIT,* and *PDGFR-α.*

How the test is carried out

The test is performed on genomic DNA extracted from a patient's blood sample (**germ sample**) or from tumor material, previously fixed in kerosene (**somatic sample**). The isolated material is enriched for genes of interest by a technique termed 'capture,' in which probes designed by 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 set of genomic fragments 'captured' by the probes represents the enriched regions of the genes of interest (*library*), which are subsequently sequenced through a process employing NGS techniques and the use of Illumina's **MiSeq Dx** or **NextSeq 550 Dx** instruments.

Variants found are confirmed by direct Sanger sequencing or by Multiplex Ligation-dependent Probe Amplification (MLPA), on second aliquots of blood, if they are VUS (variants of uncertain significance) or pathogenic variants. In the case of SNV variants in *PMS2* or *CHEK2*, Long Range PCR is expected to be used to select the gene against pseudogenes. 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 software is Mutation Surveyor and Gene Marker, respectively, both sold by the company SoftGenetics.

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Bioinformatics analysis

At the end of the NGS sequencing phase, the data undergo a series of advanced **bioinformatic analysis** that include:

- The *primary analysis*, that is, the generation of sequences (reads) and the evaluation of their quality;
- the *secondary analysis*, which consists of pairing the data obtained with the corresponding regions of the genome chosen as reference (GRCh37-hg19): the presence of any SNV or CNV variants is defined;
- *tertiary analysis*, which allows the interpretation of variants, which is eventually followed by the issuance of a molecular diagnostic report.

While the primary analysis is performed by the NGS sequencer, the others are performed by an automated procedure (*pipeline*) developed in collaboration with an expert firm in the field (enGenome). The *pipeline* was created so as to::

- highlight any regions of interest with low sequencing coverage, i.e., that have a read depth (number of sequences) less than 50X (for somatic samples) and 30X (for germline samples). These areas will be reanalyzed by sequencing with the Sanger method to always ensure 100 percent coverage of the region of interest in the analysis;
- Generate results related only to the required genes.

Panel verification

The OncoPan® panel has been updated over the years as the number of regions analyzed, but the choice of methodology for creating the *library* and the type of instrumentation used have remained constant.

To check the robustness of the panel, data obtained with the current panel were compared with those obtained from the same sample but with the previous version of the panel, which was originally validated by comparing data generated through the Sanger method, MLPA (Multiplex Ligation-dependent Probe Amplificatio) or NGS (whose libraries were created with a different approach). *Specificity* and *sensitivity* values were assigned, investigating some genes in the panel, relative to coding exons and adjacent intronic sequences (-21 or +7 base pairs from splicing junctions, for SNVs).

	SNV		CNV	
	GERMINAL	SOMATIC	GERMINAL	SOMATIC
Sensitivity	99.9%	99.9%	99.9%	99.9%
Specificity	100%	100%	99.9%	80%

Lists the outcomes of this analysis in OncoPan®_Vs.4.0

Panel limitations

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

Conclusions

The OncoPan® panel proved reliable within the limits of the reported values. To increasingly improve the accuracy of the CNV analysis, additional data will be added to harden the '*baseline*,' i.e., the set of controls necessary for the process to assess the variation in the read depth of the regions being investigated, with particular attention to those genes that have corresponding pseudogenes in the human genome (*PMS2* and *CHEK2*). At present, somatic CNV analysis also makes use of verification by MLPA, where the kit is available for the requested gene and consistent with the success of the 'experiment, the MLPA technique not being currently validated by the vendor company for its use on DNA extracted from paraffin-embedded tissue. The optimal average **read depth** (calculated with respect to all regions of each sample) was estimated to be around 200X for germinal samples, and 300X for somatic samples, with a minimum of 30X and 50X respectively.

Finally, each region sequenced by NGS technique is visualized using the Broad Institute's Integrative Genomics Viewer (IGV) program to further reduce the possibility of false negatives.