WHOLE GENIX

COMPREHENSIVE CANCER
GENOMIC ANALYSIS

COMPREHENSIVE CANCER GENOMIC ANALYSIS

Patient ID	xxx xxx xxx
Patient Name	xxx xxx xxx
Date of birth	xxx xxx xxx

Biopsy ID	xxx xxx xxx
Physician	xxx xxx xxx
Physician Institution	xxx xxx xxx

Date of sample reception: ___/___/___
Date of report: ___/___/___

Clinical History

Fifty-two year-old female with stage IV breast cancer. The analyzed samples is a biopsy from the primary tumor, paraffin embedded, with previous diagnosis of triple negative carcinoma of the breast (TNBC) with an infiltration rate of 85%.
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Testing performed

1. Whole Exome Sequencing (WES) (“average depth > 200x”)
2. Somatic copy number alteration (SCNA) analysis
3. Whole transcriptome sequencing

Note: For a more detailed description of methods, see Annex II of this report.

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Relevant Finding

GEN	STATUS	ALTERATION	CLINICAL RELEVANCE
BRCA1	Mutated	p.E23fs*8 (inactivating mutation)	Inactivating mutations of BRCA1/2 are associated with response to PARP inhibitors (refs 1-5) and platinum agents (refs 5, 6). There are clinical trials for patients with this type of mutations. It is also recommended to perform a study in leukocytes in order to determine if it's a germinal mutation.

Results for other relevant genes in TNBC

GEN	STATUS	COMMENTS
BRAF	wt	--
CDKN2A	wt	--
FGFR1	wt. (not amplified)	--
FGFR2	wt. (not amplified)	--
HER2	(not amplified)	Expected result in TNBC
KRAS	wt	--
NRAS	wt	--
PTEN	wt	--
RB1	wt	--
TP53	Mutation c.993+1G>T	Mutation of uncertain significance. It could affect TP53 splicing, thus producing a truncated protein.
BRAF	wt	--
CDKN2A	wt	--
FGFR1	wt. (not amplified)	--
FGFR2	wt. (not amplified)	--
HER2	(not amplified)	Expected result in TNBC

Other somatic alterations of unclear therapeutic significance are listed in Annex I

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Discussion

Relevant findings: Activating mutations in the BRAC1

Whole Exome Sequencing (WES) revealed a somatic mutation in BRCA1, which codifies for a DNA rpair protein. The mutation detected is an insertion in codon 23, which provokes a reading frame shift and the production of a truncated, totally inactive, protein.

The BRCA1 protein has a key role in two DNA repair mechanisms. The first one is by homologous recombination repair, which eliminates double-strand brakes in DNA originated by several environmental agents (such as radiation). The second one is called nucleotide-excision repair that corrects the damage in a single DNA strand and is especially important in the elimination of thymine dimmers provoked by UV radiation. Inactivating mutations in BRCA1 significantly perturb the repair by homologous recombination of DNA.

BRCA1 mutations can either be somatic or germinal. In the first case, they are only detected in the tumor; in the second one, they may appear in any cell of the body (i.e. peripheral blood leukocytes). Most mutations of BRCA1 in TNBC are germinal; although there is a small percentage of somatic mutations.

Germinal BRCA1 mutations are linked to hereditary predisposition to breast cancer. As a matter of fact, BRCA1 is the most frequently mutated gene (24%) in the families affected by the Hereditary Breast and Ovarian Cancer Syndrome (HBOC) (ref 7). In the most extensive study published so far (ref 8), 1824 patients with TNBC were studied, without selection by neither family history nor by an early age at diagnosis. BRCA1 mutations were found in 8.5% of the cases, and mutations in other genes of the homologous recombination pathway (such as BRCA2, PALB2, RAD51) were found in an additional 6.1%. Finally, the frequency of somatic mutations in TNBC might be around 1 and 2% of the patients.

Therapeutic implications

The presence of an inactivating mutation in BRCA1 indicates that the patient may benefit from the administration of a poly-ADP-ribose polymerase (PARP) inhibitor; as well as from platinum agents (cisplatin, carboplatin).

Poly-ADP-ribose polymerase (PARP) is another one of the enzymes implicated in DNA repair, in concrete single-strand breaks of DNA. Several studies and clinical trials have demonstrated the correlation between mutations of the genes of the homologous recombination pathway (BRCA1 and 2) and the response to PARP inhibitors such as olaparib. The FDA approved by the end of 2014 the use of olaparib in advanced carcinoma of the ovary harboring BRCA1 and BRCA2 mutations based on the results obtained in a phase II clinical trial where the patients treated with olaparib reached benefit of 7 months in overall survival. The approval will become definitive when the results from phase III studies comparing olaparib against standard treatment.

In the case of TNBC with BRCA1/2 mutations, a first phase I clinical trials published in 2010 (ref 1) demonstrated that the treatment with olaparib is well tolerated and the patients had a benefit in the objective response rate. In a more recent clinical trial using single-agent treatment with olaparib in several tumors harboring BRCA1/2 muations, TNBC patients had a combined response rate and stable disease of 60% (ref 4). Finally, a phase I/Ib clinical trial demonstrated that the combination of olaparib with carboplatin is well tolerated. Of the 8 patients with breast cancer included, one had a complete response (maintained over 23 months), and 7 a partial response (with a median progression free survival of 10 months) (ref 2).

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It should also be pointed out that recent comprehensive genomics analyses have unveiled numerous molecular similarities among TNBC and high-grade serous carcinoma of the ovary; even suggesting that a common treatment algorithm should be used for these conditions (ref 10).

Although the use of olaparib has not yet been approved in breast cancer patients, the medication may be accessed through extended access programs or clinical trials for TNBC with BRCA1/2 mutations. Among them, there are studies comparing monotherapy with olaparib against chemotherapy (ensayo OlympiaAD, NCT02000622, opened worldwide, including Perú and México) or radiotherapy in combination with olaparib in non-operable breast carcinoma (NCT02227082).

Recommendation of germinal mutation analysis and genetic counseling

There is an elevated possibility that the detected BRCA1 mutation is of germinal origin, thus DNA analysis from peripheral blood leukocytes is encouraged. A positive result would indicate that the patient has HBOC syndrome. In such case, genetic counseling for the female members of the patient's family is recommended.

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ANNEX 1: OTHER Somatic mutations of UNCLEAR therapeutic significance

GENE	FUNCTION	MUTATION
ABHD2	Negative regulator of cellular migration.	p.P283L
ALS2CR8 (CARF)	Regulator of senescence and apoptosis	p.H237R
ARAP3	Cytoskeletal rearrangement. Mutations in this gene have been related to metastasis of breast cancer to the lymph nodes (ref 13).	p.P129A
BID	Key regulator in apoptosis	p.K203_A206 delKKVA
CAD	Promotion and regulation of the synthesis of pyrimidine. Promotes cellular division.	p.D791H
DDP4	Invasiveness and angiogenesis Aberrant expression of this protein has been described in several tumors, including breast.	p.G741E
EPHB3	Tyrosine-kinase receptor. Mutations in the EPH family receptors appear in several tumors.	p.R649P
MDN1 (MINA)	Histone demethylase It has been proposed that mutations in this gene are related to resistance to hormone therapy in Luminal B breast cancer (ref 14).	p.V211L
NSMCE4A	Component of SMC5-SMC6 complex that intervenes in DNA repair by homologous recombination.	p.L218V
TLE2	Wnt-induced transcription repressor.	p.E52K
ABHD2	Negative regulator of cellular migration.	p.P283L
ALS2CR8 (CARF)	Regulator of senescence and apoptosis	p.H237R
ARAP3	Cytoskeletal rearrangement. Mutations in this gene have been related to metastasis of breast cancer to the lymph nodes (ref 13).	p.P129A

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ANNEX 2: ASSAY METHOD AND INFORMATION

Genomic DNA was purified from normal and tumor tissue using the FFPE Qiagen kit and assessed for quality and quantity by spectrophotometric analysis, gel agarose electrophoresis and Q-PCR. Samples were prepared for whole exome sequencing using the Agilent SureSelectXT Human All Exon (50 Mb) V5 kit.

Libraries were generated and sequenced on the Illumina HiSeq2500 platform in order to generate at least 125 million, 2 x 150bp paired-end reads for the tumor and 75 million reads for the normal samples. Tumor and normal exome reads were aligned to the reference human genome (hg19) and BAM files were generated. Somatic variants were identified computationally using a variety of bioinformatics tools (GATK Unified Genotype, samtools mpileup, SHORE, Annovar, Indelocator). Three independent predictions were obtained, combined and filter with two additional tools ("GATK VariantFiltration and intersected with GATK CombineVariants") to generate the final list of somatic alterations in the tumor sample. Somatic copy number alterations (SCNAs) were identified by comparing normalized read counts within each gene in the tumor to a panel of normal tissues.

Finally, the two most relevant alterations (BRCA1 and TP53 mutations) were validated by standard PCR plus sequencing (Sanger method) of the tumor sample.

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