WHOLE GENIX

COMPREHENSIVE CANCER
GENOMIC ANALYSIS

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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

Patient is a 63-year-old man with a stage IV non-small cell lung cancer (NSCLC). Sample under analysis is a formalin-fixed, paraffin embedded (FFPE) biopsy of the primary tumor with a diagnosis of adenocarcinoma and a 70% tumor infiltration.

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
HER2 (ERBB2, EGFR2, Neu)	Mutaded	Mutation p.E770_A771insAYVM	The mutation correlates with response to anti-HER2 therapies (refs 1-4). There are clinical trials open for NSCLC patients with HER2 mutations

Results for other relevant genes in CPNM

GEN	STATUS	COMMENTS
ALK	wt (no fusions)	--
BRAF	wt	--
CDKN2A	wt	--
EGFR	wt	--
KRAS	wt	--
FBXW7	Mutation p.W365*(inactivating)	Good response to temsirolimus (mTOR inhibitor) has been reported in a NSCLC patient with a FBXW7 mutation (ref 5). Inactivating mutations in FBXW7 have also been related with resistance to anti-tubulin agents (taxanes) in NSCLC (ref 6, 7).
FGFR1-4	wt (not amplified)	--
MET	wt (not amplified)	--
MTOR	Wt	--
PIK3CA	Wt	--
PTEN	Wt	--
RET	wt (no fusions)	--
ROS	wt (no fusions)	--
STK11	wt	--
TP53	wt	The wt genotype for the TP53 gene has been associated with a better overall survival in stage IIIB-IV NSCLC (ref 8)

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

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Discussion

Relevant findings: Activating mutations in the HER2 gene

Whole Exome Sequencing (WES) revealed a somatic mutation in the *HER2* oncogene, also known as *ErbB2* o *Neu*, which codes for a receptor tyrosin kinase (RTK). The mutation is an "in frame" insertion of 12 base pairs (4 amino acids) in the exon 20 that leads to a constitutive activation of the HER2 receptor.

The membrane receptors of the ErbB family activate several intracellular signal transduction pathways (such as MAPK, PIK/Akt, PKC or STAT3 pathways) that, in turn, stimulate cell proliferation and inhibit apoptosis. In normal cells, the activity of the ErbB family receptors is tightly regulated and their deregulation (due to mutation, amplification or other mechanisms) is associated to the development and progression of many tumor types. In the case of *HER2*, the gene is amplified in 30% of human breast tumors and mutated at lower frequencies (usually less than 5%) in other neoplasms, such as NSCLC. In all these cases, the HER2 receptor represents a therapeutic target of drugs approved for clinical use or in advanced clinical trials. The mutations in the *HER2* gene are mutually exclusive with mutations in other genes of the ErbB family (such as *EGFR*) o in the *KRAS* gene. Finally, the patient does not present translocations in any gene of clinical relevance in NSCLC (*ALK*, *RET*, *ROS*).

In the most extensive study published so far, *HER2* mutations were detected in 65 of 3800 NSCLC patients analyzed (1.7%). The average age of the mutated patients was 60 years, with a majority of women (69%) and non-smokers (52%). All tumors were adenocarcinomas, no *HER2* mutations were found in squamous cell carcinomas.

A total of 22 patients were treated second-line (after chemotherapy) with anti-HER2 therapies. Disease control rate (complete and partial responses plus stable disease) was 93% for trastuzumab (n=15) and 100% for afatinib (n=3). Median survival for stage IV patients was 23 months, significantly longer than the median survival in unselected stage IV NSCLC.

Therapeutic implications

Given the presence of an activating mutation in the *HER2* gene, the administration of an anti-HER2 drug would likely be of therapeutic benefit, either as a first line treatment or in subsequent lines of therapy. Although anti-HER2 drugs have not been approved by the health authorities for the treatment of *HER2* mutated tumors, the patient can be eligible for compassionate use or recruited in a clinical trial for *HER2* mutated NSCLC. One of those trials, the PUMA-NER-5201, is currently open worldwide. It is an open-label, phase II study of neratinib in patients with solid tumors with somatic human *EGFR -EGFR*, *HER2*, *HER3*- mutations or *EGFR* gene amplification. Neratinib is an ErbB2 pan-inhibitor that has demonstrated activity against *HER2*-mutated NSCLC in combination with temsirolimus in a Phase I clinical trial (ref 4).

The absence of translocations of therapeutic significance or *EGFR* mutations indicates that the first-line administration of drugs targeted to those alterations (such as crizotinib or erlotinib) would not report any therapeutic benefit. Finally, regarding the *FBXW7* inactivating mutation detected in the patient, two reports (Refs 6, 7) suggest that such mutations might be related to resistance to anti-tubulin drugs, such as taxanes. In consequence, if chemotherapy was to be administered to the patient, a platin-based regime will probably be most adequate to the genetic prolife of the tumor.

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ANNEX 1: OTHER Somatic mutations of UNCLEAR therapeutic significance

GENE	FUNCTION	MUTATION
ADAMTS17	Extracellular matrix metalloproteinase	p.R710Q
AICDA	Citidine desaminase	p.R171C
AIFM3	Factor inducing programmed cell death (apoptosis)	p.R378*
CDH10	Cell adhesion protein	p.R472H
EPHA2	Receptor tirosine kinase In squamous carcinoma of the lung, some EPHA2 mutations have been correlated with poor prognosis. Its significance in adenocarcinoma is unclear	p.147L
GK2	Glicerol kinase. Glucid metabolism is often altered in tumor cells	p.R330C
KPNA2	Carioferine involved in nucleus-citoplasm transport	p.R30C
LAMA3	Laminine related to invasion and metastasis. Mutations in this gen have been describe in some solid tumor types, but its significance is unclear	p.A433V
PLEC	Plectine. Involved in migration and plastic properties of the cells.	p.E343fs*88
PTPN5	Protein kinase phosphatase	p.D345E
STK24	Serine Threonine Kinase 24	p.A134T
TEP1	Telomere associated protein	p.G1171E
WINT7A	Protein of the Wnt/beta-catenin pathway with antitumor properties	p.G285S

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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 100bp 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 (HER2 and FBXW7 mutations) were validated by standard PCR plus sequencing (Sanger method) of the tumor sample.

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Referencias

1. Mazières J et al. Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. J Clin Oncol. 2013 Jun 1;31(16):1997-2003.

2. Landi L, Capuzzo F. HER2 and lung cancer Expert Rev Anticancer Ther. 2013 Oct;13(10):1219-28.

3. Rita RG et al. NSCLC & HER2, Between lights and shadows. J Thorac Oncol. 2014 Sep 22. [Epub ahead of print]

4. Gandhi L et al. Phase I study of neratinib in combination with temsirolimus in patients with human epidermal growth factor receptor 2-dependent and other solid tumors. J Clin Oncol. 2014 Jan 10;32(2):68-75

5. Villaruz LC, Socinski MA.Temsirolimus therapy in a patient with lung adenocarcinoma harboring an FBXW7 mutation. Lung Cancer 2014 Feb;83(2):300-1.

6. Wertz IE et al. Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. Nature 2011 Mar 3;471(7336):110-4.

7. Yokobori T et al. FBXW7 mediates chemotherapeutic sensitivity and prognosis in NSCLCs. Mol Cancer Res. 2014 Jan;12(1):32-7.

8. Molina-Vila MA, Bertran-Alamillo J et al. Nondisruptive p53 mutations are associated with shorter survival in advanced non-small-cell lung cancer patients. Clin Cancer Res. 2014 Sep 1;20(17):4647-59

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