Clinical response to a lapatinib-based therapy of a Li-Fraumeni Syndrome patient with a novel HER2-V659E mutation

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Abstract

Genomic characterization of recurrent breast and lung tumors developed over the course of 10 years in a 29-year-old patient with a germline p53 mutation (Li-Fraumeni Syndrome) identified oncogenic alterations in the HER2 and EGFR genes across all tumors, including HER2 amplifications, an EGFR-exon 20 insertion, and the first-in-human HER2-V659E mutation showing a phenotypic convergent evolution towards HER2 and EGFR alterations. Following the identification of HER2-activating events in the most recent lung carcinoma and in circulating tumor cells, we treated the reminiscent metastatic lesions with a lapatinib-based therapy. A clinical response both symptomatic and radiologic was achieved. HER2-V659E sensitivity to lapatinib was confirmed in the laboratory.

Statement of Significance

The precise knowledge of the genomic alterations present in tumors is critical to select the optimal treatment for each patient. Here, we report the molecular characterization and clinical response to a lapatinib-based therapy of the tumors of a Li-Fraumeni patient showing prevalence of HER2 and EGFR genomic alterations.
Introduction

We describe the case of a 29-years-old non-smoker female who, in 2003, underwent a bilateral mastectomy due to a malignant phyllodes tumor (MPT), and to a bilateral ductal carcinoma in situ (DCIS) (Fig. 1A). The patient was treated with adjuvant tamoxifen from 2003 until 2008 (Fig. 1B). In November 2007, a nodule in the lower lobe of the left lung was detected in a control chest x-ray and confirmed by computed tomography (CT) scan. A CT-guided needle aspiration of the lesion showed positivity for CK7+ and TTF1+, suggesting a primary lung adenocarcinoma. A left lower lobectomy and lymphadenectomy was subsequently performed and the pathology analysis showed a moderately differentiated invasive lung adenocarcinoma with a bronchioloalveolar pattern (NSCLC left) with visceral pleural invasion and involvement of three N1 lymph nodes, without N2 involvement (pT2N1). The patient received 4 cycles of post-operative adjuvant cisplatin/docetaxel chemotherapy. In December 2011, a follow-up CT-scan detected a 1 cm nodule in the right lower lobe of the lung. In parallel, the left lung images could not rule out the presence of pulmonary lymphangitic carcinomatosis (PLC). A segmentectomy of the right lower lung lobe and lymph node sampling was performed showing a 1 cm TTF1+ invasive adenocarcinoma (suggesting lung cancer origin, NSCLC right) and extensive invasion of N2 lymph nodes in 2R, 4R and 7 regions (N2(2R), N2(4R), N2(7)). Given the pattern of tumors developed by the patient, genetic counseling was provided and revealed that the patient carried a TP53-R248W germline mutation leading to the diagnosis of a Li-Fraumeni Syndrome (LFS). A post-operative CT-scan confirmed the presence of pulmonary lymphangitic carcinomatosis in the left lung and the patient started cisplatin / pemetrexed.
chemotherapy. After two chemotherapy cycles, the patient referred the appearance of pain in the lower left thorax and a CT-scan showed the presence of a new pleural effusion (PE) in the left lung, a 2 cm nodule in the left hilus and the previously detected PLC. As a result of whole exome sequencing of her most recent tumor, the NSCLC right, an activating mutation HER2-V659E was identified (Fig. 2). This result led us to consider that the tyrosine kinase inhibitor lapatinib could be a beneficial treatment option. While this clinical decision was hypothesis-driven, we confirmed that HER2-V659E expressing cells were sensitive to lapatinib (Fig. 3). Lapatinib was given daily at a dose of 1000 mg in combination with paclitaxel 80 mg/m². Shortly after starting therapy, the patient experienced pain relief at the lower left thorax and a pronounced improvement in breathing. Two months after the beginning of the treatment, a CT-scan revealed a reduction in the pleural effusion in addition to shrinkage of the nodule in the left hilus (Fig. 4). Three months after the beginning of the lapatinib / paclitaxel treatment the administration of paclitaxel was discontinued due to toxicity. Treatment with lapatinib continued and trastuzumab, and anti-HER2 antibody, was added (initial dose of 8 mg/kg intravenously followed by 6 mg/kg every 3 weeks). The observation that HER2 was amplified in circulating tumor cells (CTCs) at this point in time, in addition to reports describing clinical benefit of trastuzumab in the context of HER2-mutant NSCLC supported this therapeutic approach (1, 2). The disease was further stabilized for additional 6 months. In total, the clinical benefit lasted over 9 months.
Results

Molecular characteristics of tumors and CTCs

In order to identify potential actionable mutations, molecular pathological analysis and exome sequencing of the MPT, DCISs, NSCLCs and N2(4R) were performed (Fig. 1A, 1B).

Tumors were analyzed for the expression level of HER2, EGFR, estrogen receptor (ER), progesterone receptor (PR) and PTEN by immunohistochemistry (IHC) and HER2 and EGFR gene amplification by fluorescence in situ hybridization (FISH) (Fig. 2A). High expression of EGFR was found in the MPT and in the N2(4R). HER2 overexpression was observed in both DCIS by IHC and HER2 gene amplification was confirmed by FISH. All three lung lesions showed Chr17 polysomy (the chromosome that bears HER2). HER2 gene amplification was observed in NSCLC right in 65% of tumor cells whereas no amplification was found in NSCLC left. Interestingly, 23% of tumor cells presented HER2 amplification in N2(4R) (Supplementary Fig. 1). Similarly, CTCs exhibited HER2 gene amplification. PTEN (and PIK3CA later on) were assessed because of their potential negative predictive value towards HER2/EGFR therapy (3-6). While PIK3CA was wild type in all samples, PTEN expression displayed inter- and intratumoral heterogeneity (Fig. 2A).

Whole exome sequencing was performed on DNA extracted from FFPE tumor samples and from peripheral blood. We first identified the TP53-R248W mutation in the blood sample.
confirming the diagnosis of LFS. However, the analysis of somatic variants presented two main difficulties. The percentage of tumoral cells within the samples was diverse, and the tumor samples were fixed and conserved in paraffin for up to 10 years, generating DNA of variable quality. The analysis of MPT was not possible due to the low quality of the DNA, and for the other samples a high number of otherwise low frequency C>T/G>A transitions was detected. This likely represents cytosine deamination events caused by the fixation / storage of the samples and not tumor-specific somatic mutations, as suggested by the linear correlation between number of C>T/G>A substitutions and the length of storage of the sample (data not shown). Both factors prompted us to use stringent criteria for the identification of genomic alterations (Fig. 2B). Among the somatic mutations, we identified a transmembrane domain mutation of HER2, HER2-p.V659E (69% mutation allele frequency, MAF), in the NSCLC right. This mutation was a dinucleotide change (659 V(GTT)-> E(GAA)) that has not been previously identified in human tumors and is orthologous to the well described oncogenic mutation found in rat HER2 (664 V(GTG)->E(GAG) (7, 8)) (Supplementary Fig. 2A). The high MAF of the HER2-p.V659E mutation was in agreement to the HER2 amplification identified by FISH. In addition, we identified an indel in exon 20 of EGFR (p.A767_S768insSVD, (9)), in the 2008 tumor, NSCLC left, (25% MAF), that was also present in the N2(4R) lymph metastases (11% MAF). EGFR exon 20 insertions are infrequent, accounting for up to 4% of all EGFR mutations (9) and although no functional studies of our patient's specific EGFR indel have been reported, neighboring insertions positioned at the loop following the C-helix of EGFR (for example p.M766_A767insASV) have been shown to be activating mutations indicating that the insertion harbored by our patient is likely to result in constitutive receptor activation. HER2 and EGFR somatic mutations were confirmed by capillary sequencing (Supplementary Fig.
Interestingly, all tumors presented alterations in either HER2 or EGFR, both members of the HER family of tyrosine kinase receptors (Fig. 2C). This indicates that HER-mediated signaling confers a selective advantage to the tumors and exemplifies a phenotypic convergent evolution towards a hyperactive HER pathway in all tumors of the Li-Fraumeni patient.

A comparison of the somatic mutations and indels found in the five tumors analyzed showed that both DCIS were independent primary tumors since they did not share any genomic alteration between them or with the rest of the tumors (Fig. 2B and Supplementary Table 1). However, the analysis of the NSCLCs and the N2(4R) metastasis showed that these three samples were related. In support of this assessment, the NSCLC left and the N2(4R) shared five indels (including EGFR p.A767_S768insSVD) and two non-synonymous mutations, indicating that N2(4R) is a metastasis derived from the NSCLC left. On the other hand, N2(4R) and NSCLC right contained cells with HER2 amplification and, moreover, both NSCLCs shared one indel as well as polysomy of chromosomes 14, 16 and 17 (Supplementary Fig. 3A,B). These results suggested a phylogenetic relationship between them.

**Functional analysis of HER2-V659E**

The identification of the HER2-V659E in the most recent tumor prompted us to consider a therapeutic approach based on lapatinib. Studies using the MMTV-c-neu mouse model (a mouse model for breast cancer generated by the overexpression of the rat orthologue of HER2-V659E) have shown evidence of the antitumor activity of lapatinib (10, 11).
We decided to functionally validate the sensitivity of HER2-V659E to lapatinib in a preclinical in vitro model. HER2-V659E was overexpressed in MCF10A cells and, as expected, high levels of HER2-V659E led to hyperphosphorylation of the receptor and activation of the downstream signaling (Fig. 3A). Lapatinib inhibited HER2-V659E and EGFR signaling in a dose-dependent manner. At low concentration of lapatinib the HER2-V659E mutant cells were less sensitive to the treatment than control cells suggesting that the mutation could confer resistance to the treatment under these conditions, an observation that was extended to other EGFR/HER2-targeting agents (Supplementary Fig. 4). Importantly, when the inhibitor was used at a clinically achievable concentration of 2μM (12), MCF10A expressing HER2-V659E exhibited the same sensitivity to lapatinib than control cells (Fig. 3B). This reinforced our hypothesis that lapatinib at clinically achievable concentrations could be beneficial for the patient.
Discussion

The identification of tumor driver genetic alterations, like the ones observed in the HER-family of tyrosine kinase receptors, has led to novel therapeutic approaches that have dramatically improved patient outcomes. The paradigmatic example is the discovery of HER2 gene amplification in breast cancer and the clinical response of HER2-amplified tumors to the anti-HER2 monoclonal antibody trastuzumab (13, 14). In addition to trastuzumab, the tyrosine kinase inhibitor lapatinib has also been approved for the therapy of HER2 amplified tumors, thanks to its ability to reversibly inhibit the intracellular tyrosine kinase activity of HER2 and downstream pathways, such as the PI3K/Akt and ERK (15, 16).

In addition to HER2 gene amplification, recent studies have shown that the HER2 gene can also be altered by somatic gene point mutations and insertions and deletions (indels) (17-23). Functional analysis in preclinical models have shown that some of these HER2 gene mutations can induce oncogenesis and sensitize tumors to anti-HER2 treatments (23). However, little is known about the clinical response of HER2 mutant tumors to anti-HER2 therapies (1, 2) and novel irreversible EGFR/HER2 inhibitors are currently under clinical evaluation for the treatment of HER2-mutant metastatic breast cancer (NCT01111825) and NSCLC (NCT01827267). Here, we report the molecular characterization and clinical response to a lapatinib-based therapy of the tumors of a Li-Fraumeni patient showing prevalence of HER2 and EGFR oncogenic driver alterations.
In this study, we have performed whole exome sequencing of the breast and lung tumors of a LFS patient and identified driver genomic alterations that are actionable. The observation of a phenotypic convergent evolution of the tumors of the patient towards a hyperactive HER2 or EGFR pathways and the discovery of the HER2-V659E mutation (to our knowledge yet unreported in human samples) in her most recent tumors, as well as the identification of HER2 gene amplification in the patient CTCs, has allowed us to select a beneficial treatment for our patient. This work is an example of how personalized characterization of tumors can lead to therapeutic success. Despite the difficulty of using FFPE archived material, we have observed that the patient generated different independent primary tumors and that the three most recent tumors were interrelated. Our data suggests that the two lung tumors and the lymph node metastasis located at N2(4R) have common origins and share tumor clones most likely due to cross-seeding of metastasis. Despite the complexity and diversity of genomic alterations as well as the intricate relationship between the tumors, we observed alterations in HER2 and EGFR across all tumors. These results suggest a predisposition towards genomic alterations in HER-family receptor tyrosine kinases in the context of the p53 germline mutation of the LFS. Whether this constitutes a general mechanism in LFS patients and the exact mechanism involved in this phenomenon is something that needs to be evaluated.

The HER2-V659E mutation identified in our patient is orthologous to the HER2/neuT (HER2-V664E) allele that was originally identified in rat tumors induced by chemical mutagenesis (7, 8). Preclinical models that allowed the successful development of anti-HER2 therapies were based on this mutant form of HER2 (24, 25). Nevertheless, mutations affecting the V659 residue within the transmembrane domain of HER2 have not been
identified in human tumors until now. Of note, the human HER2-V659E mutation necessarily implies a tandem dinucleotide change (TT>AA), a type of mutation that may arise from induced pyrimidine adducts in response to radiotherapy (26). The patient did not receive radiotherapy; however the dinucleotide genomic alteration might have been caused by other type of irradiation including the one from the frequent x-ray / CT scans performed on the patient.

In summary, the thorough molecular characterization of tumors from a patient with LFS has led to the identification of alterations in HER2 and EGFR, among those the first-in-human HER2-V659E mutation. The genomic characterization of the patient, together with the preclinical analysis showing that cells bearing the HER2-V659E mutation remain sensitive to clinically achievable doses of lapatinib supported a lapatinib-based treatment for our patient. Notably, this therapeutic regime led to clinical benefits with objective decrease in tumor size and pleural effusion, relief of thoracic pain and breathing improvement. The potential role of HER tyrosine kinase inhibitors in the therapy of HER2-mutant tumors deserves a systematic evaluation; therefore, a clinical trial with the irreversible HER2 inhibitor neratinib in metastatic breast cancer is currently underway (NCT01111825) and a “basket” clinical trial in patients with HER2 mutations from any other histological origin is also in an advanced planning stage.
Methods

Please see Supplementary Data for additional Methods.

MCF10A cells were cultured in complete growth media as recommended by the vendor (ATCC) supplemented with selection antibiotic (hygromycin). MCF10A cells were not authenticated since they had undergone few passages after their purchase.

Exome sequencing

The patient provided written informed consent for somatic and germline DNA analysis. Formalin-fixed paraffin embedded (FFPE) tumor samples from six primary tumors including a malignant phyllodes tumor (MPT), two DCIS (right and one left), two non-small cell lung cancers (NSCLC left and right) and one metastatic lymph node N2(4R) along with a blood sample from the patient were subjected to whole exome sequencing. Samples were initially assessed for tumor content based on a hematoxylin and eosin staining (Fig. 2A). Genomic DNA was extracted from FFPE tumor samples using DNA RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Ambion) according to manufacturer’s instructions. Germline DNA was extracted from whole blood using the DNA QIAampDNA blood midi kit (Qiagen). Library preparation was performed following the standard Illumina protocol (Genomic Sample Prep) with slight modifications for FFPE-derived DNA samples. One µg of DNA was decross linked by heat incubation, fragmented, ends repaired and an adenine was ligated to each of the 3’ends, where sample-specific adaptors were linked. Libraries were amplified using 8-12-cycles of polymerase chain reaction (PCR), and exome enrichment was performed using specific biotinylated probes.
(SureSelect XT Human All Exon 50Mb, Agilent). After enrichment, the exome libraries were PCR-amplified, quantified and loaded in a HiSeq2000 sequencer (Illumina). Paired-end 100 base sequences were obtained and aligned to the reference genome using BWA. This yielded a median depth of coverage in targeted regions between 13x and 80x, and with more than 83% of the exome having at least 10 reads. Tumor samples were compared to normal DNA as previously described (27). To remove artifacts likely caused by cytosine deamination due to fixation / long-term storage of FFPE samples, and due to the presence of a low tumor cell content of some samples, the following filtering criteria were applied: somatic variants were called when supported by at least three reads, representing at least 10% of the total reads and with a minimum coverage of 35x. Putative somatic mutations were manually inspected to remove false positives in homopolymer stretches and close to indels.
Acknowledgments

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References


Figure legends

Figure 1. Tumor sites and treatment timelines. Panel A) shows the different tumor localization for the MPT, malignant phyllodes tumor of 2cm, the DCIS (ductal carcinoma in situ) being the right one of 1cm (pT1mic, grade 3) and the left ones of 0.8 and 1cm (pTis, grade 3), the NSCLC (non small cell lung cancer) of the left lung, 1.6cm (pT2N1); and the NSCLC of the right lung, 1cm (T1N2M1). The PLC (pulmonary lymphangitis carcinomatosis) and the PE (pleural effusion) were not measurable. The hillar lymph nodes N1(10) (n=2, of 0.5cm), peribronchial N1(12) (n=1, of 1cm), subcarinal N2(7) (n=2, of 9 and 3mm) and paratracheal N2(2R) (n=2, of 1.6 and 1cm) and N2(4R) (n=1, of 3cm), N1(10) (n=1, of 2cm by CT scan) are also shown. Panel B) indicates the treatment timelines received by the patient. From 2003 until 2008, the patient received adjuvant tamoxifen (TAM). In 2008, following the lobectomy she received six cycles of cisplatin / docetaxel (CDDP/DOC). After the segmentectomy in 2012 she received 2 cycles of CDDP/PM, cisplatin / pemetrexed. Based on the identification of HER2-V659E, she received Lap/P, (oral daily lapatinib 1000mg/intravenous weekly paclitaxel 80 mg/m^2).

Figure 2. Molecular characterization of the tumors. In panel A) the Estrogen receptor (ER), progesteron receptor (PR), HER2, EGFR and PTEN levels were quantified by IHC or FIS. ND, not determined. Panel B) shows exome sequencing analysis in five tumor samples. Blue represents mutations and yellow insertion-deletion alterations. In bold, Cancer Gene Census annotated genes. The table in panel C) summarizes the HER-family molecular alterations in the five tumors analyzed. Amp, amplification.
**Figure 3. HER2-V659E response to lapatinib in MCF10A cells.** In panel A) MCF10A overexpressing empty vector control, HER2 wild type or HER2-V659E were treated with increasing doses of lapatinib (0, 0.125, 0.5 and 2μM). Immunoblotting of the total and phosphorylated proteins were performed. pERK, pAKT and p4EBP1 are downstream biomarkers of HER2 activation. Panel B) shows MCF10A cells overexpressing empty vector control, HER2 wild type or HER2-V659E treated with increasing doses of lapatinib. Cell proliferation was superior in HER2-V659E overexpressing cells. Error bars indicate standard deviation of six independent experiments. Patient plasma concentration of 2.43 μg/ml = 2.58 μM based on a 1250mg/daily dose.

**Figure 4. Radiologic response to lapatinib / paclitaxel**

The figure shows images from a computer tomographic scan of the pleural effusion (PE and N2(10) lymph node adenopathy, green arrows) and the pulmonary lymphangitic carcinomatosis (PLC, diffused image) previous to and their reduction under lapatinib / paclitaxel treatment.
Figure 1. Serra et al
### A Molecular alterations

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<th>Year</th>
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### B Exome sequencing

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### C HER2 and EGFR alterations

Figure 2. Serra et al
### A Biochemical activity of lapatinib

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### B Antiproliferative activity of lapatinib

![Graph showing the antiproliferative activity of lapatinib](image)

**Figure 3. Serra et al**
Radiologic response to lapatinib/paclitaxel

March 19, 2012

June 11, 2012

PE

PLC

Figure 4. Serra et al
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