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Early View

Research letter

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A new KIF5B-ERBB4 gene fusion in a lung adenocarcinoma patient.

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Research Letter

Dear Editor,

In cancer patients, few *ERBB4* fusions have been reported in the literature with different gene partners (*IKZF, B4GALT5, EZR* [1,2,3]) allowing dimerization and constitutive activation of ERBB4 tyrosine kinase domain. Here we report a new fusion of *ERBB4* with the coiled-coil domain encoded by *KIF5B* gene, a universal partner in lung cancer gene fusions, in a patient with a stage IV lung adenocarcinoma.

A 69-year-old female former smoker at 40 pack years who had ceased smoking 9 years ago, was diagnosed with an upper right lobe non-mucinous lung adenocarcinoma with symptomatic cystic brain metastases (cT2aN0M1c, 8th edition of the TNM classification for Lung cancer, figure 1a). Immunohistochemistry analyses on CT-guided lung biopsy sample revealed TTF1 positive immunostaining (figure 1b), but negative PD-L1, ROS1, and ALK staining. A first next-generation sequencing (NGS) analysis was performed on tumor sample DNA [*Oncomine Tumor solid DNA kit (OST panel) and complementary panel (OST+V2)* - Thermo-Fisher, scientific, San Francisco, CA, US] and RNA extract [*Oncomine Focus Assay (OFA*), Thermo-Fisher, scientific, San Francisco, CA, US] failed to detect any addictive gene alteration. Since initial general condition was poor with ECOG PS= 2, she first received whole brain radiation therapy and was then treated with weekly paclitaxel plus carboplatin (4 cycles) in the first line setting, with stable disease as best tumor response according to RECIST 1.1. Because of early relapse at 3 months, she then received single-agent pemetrexed as a second-line treatment with disease progression after two cycles. A third line consisted of nivolumab with rapid metastatic progression (lung, liver), and finally single agent gemcitabine was proposed without any clinical benefit while general condition worsened after one cycle. Taking into account for the long period elapsed since smoking cessation, and since tumor disease failed to show any response to four lines of treatment, even showing fast progression upon checkpoint inhibitor and a pulmonary miliary pattern of progression on CT-scan, we suspected that patient's tumor could

contain a yet unknown addictive mutation. We thus decided during our monthly institutional Molecular multidisciplinary Tumor Board (North-Paris TMB, Assistance Publique-Hôpitaux de Paris, at Bichat University Hospital), to investigate the patient' sample by a new enlarged NGS panel for further addictive mutations and potential therapeutic targets. Two different analysis panels were used to maximize the likelihood of significant results. First, we used Oncomine™ Comprehensive Assay v3 (Life Technologies-Thermo Fisher Scientific), for DNA and RNA analyses (including 86 hotspot gene mutations, 48 full length genes, 47 copy number variations and gene fusions across 45 genes with 760 possible fusions), and second, FusionPlex® Lung Tumor, for Ion Torrent (Archer DX, Boulder, Colorado), which allows the identification of additional gene fusions involving 14 genes.

The Oncomine™ Comprehensive Assay V3 kit only identified a new *KIF5B* (15)-*ERBB4* (18) fusion transcript (figure 1c). To check that such *fusion* was not an artifact, we performed an additional RNA sequencing analysis with the FusionPlex® Pan-Solid Tumor panel for Ion Torrent (Archer DX, Boulder, Colorado), which allows analysis of fusions across 103 genes including ERBB4. This expanded third analysis did confirm the existence of the *KIF5B* (Chr10:g.323117356)-*ERBB4* (Chr2:g.212488769) fusion.

ERBB4 (also known as HER4) belongs to the ErbB/HER family of protein tyrosine kinases, which also includes EGFR (ErbB1), HER2 (ErbB2), HER3 (ErbB3). These cell membrane receptor proteins are able to dimerize upon ligand stimulation, and then activate multiple signaling pathways including PI3K/AKT and Ras/Raf/MAPK cascades, transmitting signals that could trigger cell mitosis and proliferation or cell differentiation. ERBB4 dysfunction has been reported to be involved in multiple human cancers, such as colorectal adenocarcinoma, breast carcinoma, gastric adenocarcinoma, or melanoma[4]. *ERBB4* was also reported to carry missense mutations in 4.8% of lung carcinomas[5]. Only rare ERBB4 fusions have been described so far in human cancers, notably with EZR [3] (in a mucinous lung adenocarcinoma), IKZF2 [1] (in T cell lymphoma and ovarian tumors), or B4GALT5 [2] (in a HPV-positive oro-pharyngeal squamous cell carcinoma). All these protein fusions retained the full ERBB4

kinase domain and, either the polypeptide corresponding to the coiled-coil domain of EZR or *IKZF2*, leading to dimerization of two ERBB4-containing fusion proteins, and most probably trans-phosphorylation of their kinase domains, or the protein fragment encoded by the first exon of *B4GALT5*, of which function remains unclear, but in each case probably causing aberrant activation of the ERBB4 kinase domain. Nakaoku T *et al* reported that EZR-ERBB4 fusions constitutively activated the ERBB4 kinase function by showing downstream signaling pathways activation. Moreover, fusion-induced anchorage-independent growth and tumorogenicity of NIH3T3 cells stably expressing such plasmid-encoded fusions, were shown to be efficiently suppressed by already approved, but repositioned, tyrosine kinase inhibitors [3].

KIF5B (Kinesin Family Member 5B) is involved in the normal distribution of mitochondria and lysosomes in cells, and regulates centrosome and nuclear positioning during mitotic entry. It is also a well-known fusion partner in NSCLC gene fusions especially with *RET*, *MET* and *ALK*[6]. Fusions with these genes, include the KIF5B coiled-coil domain, involved in protein dimerization leading to trans-phosphorylation and constitutive activation of RET, ALK or MET kinase domains respectively. In this new *KIF5B* (15)-*ERBB4* (18) fusion transcript, the KIF5B component encodes for the KIF5B coiled-coil domain that should lead to dimerization and thus abnormal ERBB4 kinase activation (Figure 1d).

With respect to this finding, the MTB, based on scarce literature data and taking into account the lack of other validated efficient drugs in a fifth-line setting for NSCLC, suggested to offer the patient Afatinib treatment, a second generation pan-ErbB tyrosine kinase inhibitor, at 40mg/day, as already described in NSCLC with EGFR mutation [7]. This treatment had actually been reported to display some efficacy in a lung squamous cell carcinoma patient with a tumor missense mutation in the *ERBB4* gene (p.Arg847His)[8], and in an urothelial carcinoma patient with *ERBB3* missense mutations[9]. Indeed, Afatinib inhibits *in vitro* all members of the ErbB family. Unfortunately, due to worsening neurological condition , the patient never received Afatinib and passed away.

Other alternative treatments could also be considered for ERBB4 alterations such as lapatinib or tarloxotinib. However, *ERBB4* gene mutations seem to confer a resistance mechanism to lapatinib in breast cancers with HER2 amplifications[10]. Tarloxotinib (EGFR/HER inhibitor) is currently tested for NSCLC in cases of ERBB fusions, including ERBB4 fusion (https://clinicaltrials.gov/show/NCT03805841).

In conclusion, we report a new *ERBB4* fusion transcript, in a non-mucinous lung adenocarcinoma with *KIF5B* as partner, never previously described to our best knowledge. Other *ERBB4* fusion transcripts were previously shown to be oncogenic and targetable, but they are probably under-diagnosed, since the current genetic tests do not explore this gene. In fact, routine genetic tests lack several unusual or rare genetic alterations that could be targeted, especially in never or former smoker patients. We think such enlarged second-line panel strategy should be discussed more systematically in MTB, when no common genetic alteration is found in the first-line NGS (especially, with no *EGFR*, *ROS*, *ALK*, *KRAS*, *MET* or *RET* alterations), possibly earlier than in the current case. Second-line NGS approach could lead to treating these patients with registered oral TKI initially developed to target other related genes or with drug in clinical trials, with the potential to prolong patient survival.

References

1. Boddicker RL, Razidlo GL, Dasari S, et al. Integrated mate-pair and RNA sequencing identifies novel, targetable gene fusions in peripheral T-cell lymphoma. ***Blood***. 2016;128(9):1234-1245.

2. Guo T, Gaykalova DA, Considine M, et al. Characterization of functionally active gene fusions in human papillomavirus related oropharyngeal squamous cell carcinoma. ***Int J Cancer***. 2016;139(2):373-382.

3. Nakaoku T, Tsuta K, Ichikawa H, et al. Druggable oncogene fusions in invasive mucinous lung adenocarcinoma. ***Clin Cancer Res***. 2014;20(12):3087-3093.

4. Rudloff U, Samuels Y. A growing family: adding mutated Erbb4 as a novel cancer target. ***Cell Cycle***. 2010;9(8):1487-1503.

5. Kurppa KJ, Denessiouk K, Johnson MS, Elenius K. Activating ERBB4 mutations in non-small cell lung cancer. ***Oncogen****e*. 2016;35(10):1283-1291.

6. Takeuchi K, Soda M, Togashi Y, et al. RET, ROS1 and ALK fusions in lung cancer. ***Nat Med***. 2012;18(3):378-381.

7. Wang S, Li J. Second-generation EGFR and ErbB tyrosine kinase inhibitors as first-line treatments for non-small cell lung cancer. ***Onco Targets Ther*****.** 2019; 12:6535-6548.

8. Jian H, Han Y, Yu Y, Lu S. Long-term efficacy of afatinib in a patient with squamous cell carcinoma of the lung and multiple ERBB family aberrations: afatinib in ERBB+ lung squamous cell carcinoma. ***Anticancer Drugs***. 2019;30(8) :873-878.

9. Choudhury NJ, Campanile A, Antic T, et al. Afatinib Activity in Platinum-Refractory Metastatic Urothelial Carcinoma in Patients With ERBB Alterations [published correction appears in J Clin Oncol. 2017 Feb;35(4):478]. ***J Clin Oncol*****.** 2016;34(18):2165-2171.

10. Canfield K, Li J, Wilkins OM, et al. Receptor tyrosine kinase ERBB4 mediates acquired resistance to ERBB2 inhibitors in breast cancer cells [published correction appears in Cell Cycle. 2015;14(8):1339-41]. **Cell Cycle.** 2015;14(4):648-655.

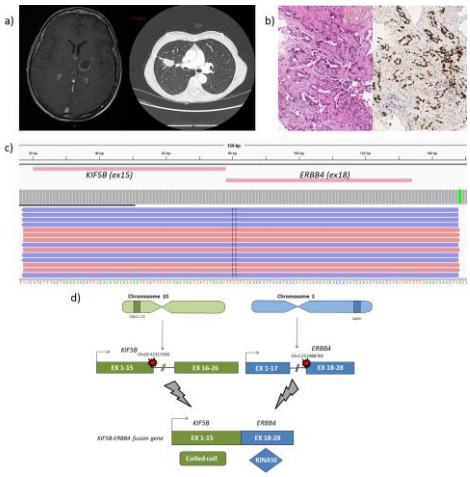

FIGURE 1. a) Baseline imaging characteristics with upper right lobe mass on CT scan and multiple nodular and cystic brain metastasis (MRI T1 sequence with gadolinium). b) Bronchial biopsy showing an acinar adenocarcinoma (HES staining, x100 magnification). The tumoral cells display a diffuse nuclear immunostaining of TTF1 (x100 magnification) c) Alignment of sequence reads mapping to KIF5B and ERBB4 from RNA-seq data issue of OCAV3 panel. The vertical line indicates the fusion point. d) A schematic of fusion genes with exons from KIF5B and ERBB4 and the genomic position of breakpoint.