



# Emergence of a *HER2*-amplified clone during disease progression in an *ALK*-rearranged NSCLC patient treated with *ALK*-inhibitors: a case report

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**Abstract:** Anaplastic lymphoma kinase tyrosine kinase inhibitors (*ALK*-TKIs) are the standard treatment for advanced *ALK*-positive non-small cell lung cancer (NSCLC) allowing survivals up to 5 years. However, duration of responses is limited by the almost certain occurrence of drug resistance. Here, we report a case of a never smoker, 59-year-old female with metastatic *ALK*-positive adenocarcinoma, solid and signet ring patterns, who developed resistance to alectinib, a second-generation *ALK*-TKI, mediated by *HER2* gene amplification. The patient received 22 months of crizotinib as first-line and subsequently 1-year of alectinib therapy. A study of resistance mechanism was performed with next generation sequencing (NGS) on tissue re-biopsy. A *HER2*-amplified emerging clone was identified by NGS in a liver metastasis and confirmed by fluorescent *in situ* hybridization (FISH) analysis. The resistant clone was detectable 2 months before disease progression in plasma cell-free DNA (cfDNA) using digital droplet PCR (ddPCR) copy number variation (CNV) assay and it was retrospectively traced in rare cells of the lung primary by FISH. To our best knowledge, this is first evidence of *HER2* gene amplification as a resistance mechanism to *ALK*-TKI in a NSCLC. Future strategies against oncogene-addicted NSCLC might benefit of combined drug treatments, such as *ALK* and *HER2* inhibition.

**Keywords:** Anaplastic lymphoma kinase-rearranged (*ALK*-rearranged); non-small cell lung cancer (NSCLC); *HER2*-amplification; resistance mechanism; cell-free DNA (cfDNA); case report

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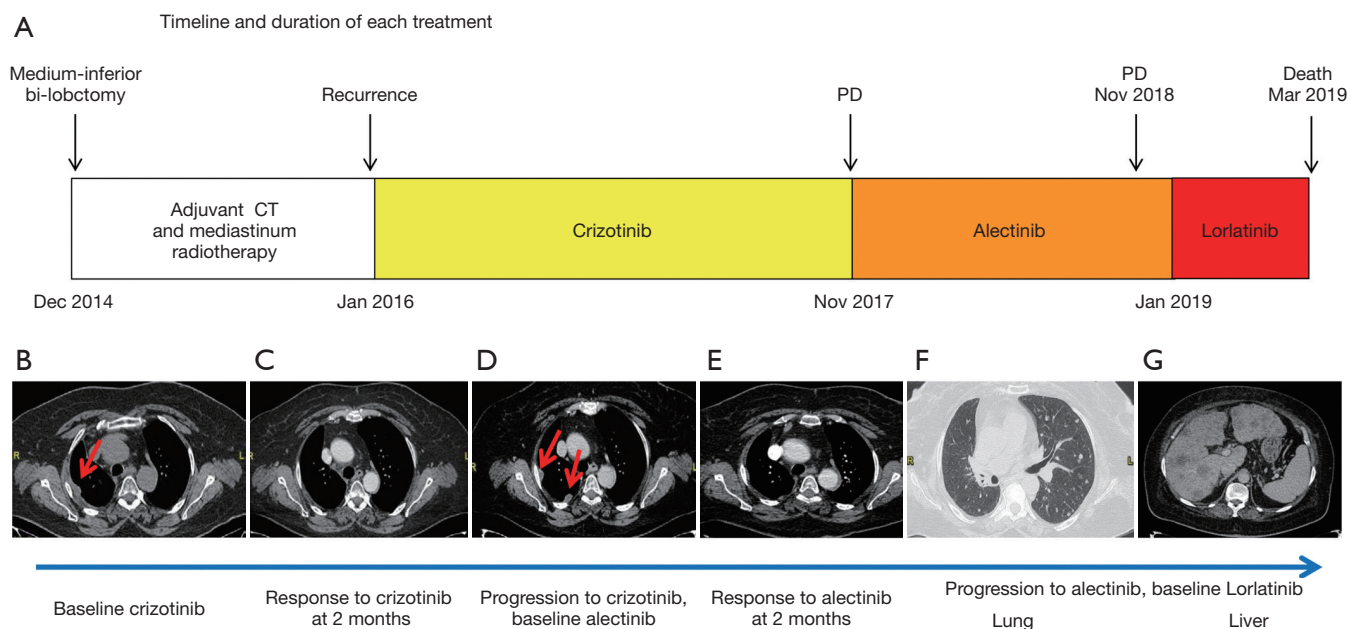
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## Introduction

Anaplastic lymphoma kinase (*ALK*) rearranged disease account for 2–5% of non-small cell lung cancers (NSCLC) and it is an indication to administer *ALK* tyrosine kinase inhibitors (TKIs) in first and following lines of treatment of advanced stage patients (1). The therapeutic landscape of *ALK*-positive NSCLC is rapidly evolving thanks to the discovery of different molecules. Crizotinib (first-generation), ceritinib, alectinib, brigatinib (second-generation) and lorlatinib (third-generation) are currently available in clinical practice, while additional inhibitors are in development (e.g.,

ensartinib, entrectinib, repotrectinib) (2). The use of *ALK*-TKIs allows long survivals up to 5–7 years (3).

Whether *ALK*-inhibitors should be used in sequence and which is the best first are open issues in the scientific community (4). The main clinical issue, however, is the expected development of resistance mainly caused by the acquisition of *ALK* secondary mutations (5,6). *ALK* gene amplification can also cause resistance (7) as well as different by-pass *ALK*-independent mechanisms, such as epidermal growth factor receptor (EGFR), KIT, and insulin-like growth factor 1 receptor (IGF-1R) activation



**Figure 1** Timeline, duration and computed tomography scan evaluation of each treatment. (A) Patient's clinical timeline; (B,C,D,E,F,G) computed tomography scans of the patient during ALK-TKI treatment. CT, chemotherapy; PD, progression of disease; ALK-TKI, anaplastic lymphoma kinase tyrosine kinase inhibitors.

(5-8). Here, we present a case of an ALK-positive NSCLC patient treated with ALK-TKIs and progressed to alectinib treatment (Figure 1) via the emergence of a *HER2*-amplified clone (Figure 2).

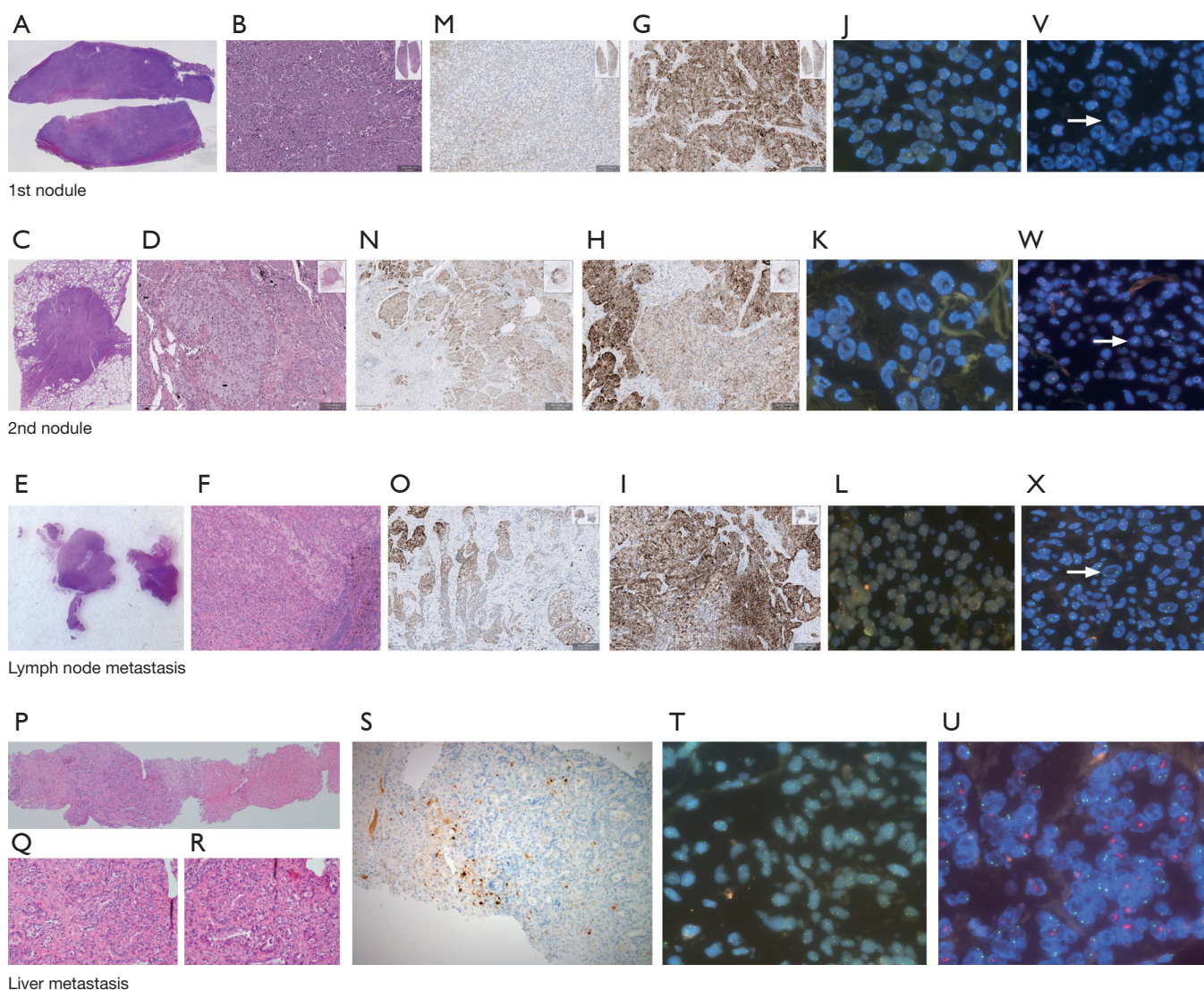
### Case presentation

A 52-year-old never smoker woman, without a significant medical history, underwent in 2014 to medium-inferior bi-lobectomy for lung adenocarcinoma (Figure 1A). The pathological staging was T3N2, considering two neoplastic nodules in right inferior lobe (Figure 2A,B,C,D) and multiple hilio-subcarinal lymph nodal involvement (Figure 2E,F). In both nodules and in the metastasis, two distinct cell components were observed characterized by a solid and signet ring pattern. Molecular study of the nodules and the metastasis revealed *KRAS* and *EGFR* wild-type status. ALK (D5F3, Ventana BenchMark XT, OptiView AMP Kit) immunostaining was positive (Figure 2G,H,I) but fluorescent *in situ* hybridization (FISH, ALK Break Apart FISH, ABBOTT VYSIS) was negative (Figure 2J,K,L). Spatial variation in staining intensity (Figure 2G,H,I) was observed in all samples that was not caused by a fixation defect as it did not show zonal differences between peripheral (Figure 2M,N,O) and central areas of the tumor (Figure 2G,H,I). Conversely,

the ALK staining pattern tended to segregate with the different cytotypes, the signet ring cells being mostly ALK-negative (Figure 2M,N,O).

Despite adjuvant chemotherapy and mediastinum radiotherapy, in January 2016, right pleura, mediastinal lymph node and bone recurrences occurred (Figure 1A,B). Based on the immunohistochemistry (IHC) ALK positivity, the patient received crizotinib treatment and radiotherapy on a L4 bone metastasis (Figure 1A). After 2 months of therapy, an improvement of the pleural involvement with stability of other lesions was observed (Figure 1C). Crizotinib was continued without relevant toxicity until November 2017 when progression in right pleura was observed (Figure 1D). A second-line treatment with alectinib was started with improvement of the pleura and stability at other sites (Figure 1E). In November 2018, after 1-year of alectinib therapy without adverse effects, lung and liver progression occurred (Figure 1F,G). A liver biopsy was obtained to study alectinib resistance. Written informed consent for molecular analysis was collected. The biopsy showed an adenocarcinoma (Figure 2P,Q,R) with homogeneous solid pattern, similar to that observed in the lung primary, but ALK-negative both by IHC and FISH (Figure 2S,T).

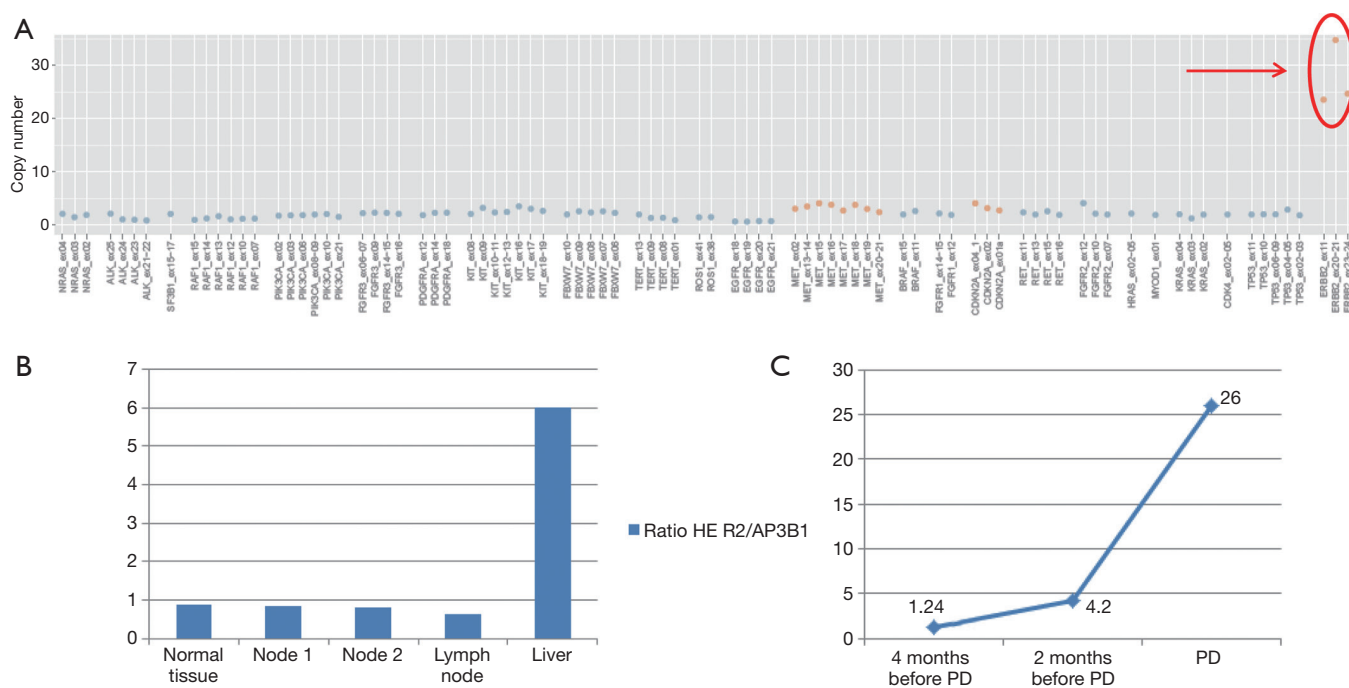
DNA was extracted from the liver biopsy and studied by



**Figure 2** Tissue staining and molecular characterization. H/E staining: (A,C,E,P) the panoramic photos exhibit H/E staining of the two lung nodules and lymph node metastasis at the onset of the disease and liver metastasis, respectively; (B,D,F,Q,R) H/E high power resolution obtained with VENTANA DP 200 slide scanner; IHC ALK analysis: (G,H,I,M,N,O,S) ALK-IHC (clone D5F3) high power resolution obtained with VENTANA DP 200 slide scanner; FISH analysis of *ALK*: (J,K,L,T) images are negative for *ALK* rearrangement demonstrated by FISH with fused green and red dots (ALK Break Apart FISH, ABBOTT VYSIS); blue fluorescence corresponds to DAPI staining of nuclei; original magnification 1,000 $\times$ ; FISH analysis of *HER2/Neu*: (V,W,X) images are negative for *HER2/Neu* (Pathvision HER probe, ABBOTT VYSIS) displaying GCN =2; green dots represent chromosome 17 centromeres; blue fluorescence corresponds to DAPI staining of nuclei; the arrow shoes a single nucleus amplified with GCN =7 (red dots) and centromeres number =2; (U) *HER2* amplified in liver metastasis; the red dots are uncountable and forming clusters. Original magnification 1,000 $\times$ . H/E, hematoxylin and eosin; IHC, immunohistochemistry; GCN, gene copy number; ALK, anaplastic lymphoma kinase; FISH, fluorescent *in situ* hybridization.

next generation sequencing (NGS) (Solid Tumor Solution, Sophia Genetics, on MiSeq Platform, Illumina, USA). A *HER2* gene amplification was observed (Figure 3A), confirmed with FISH analysis (Figure 2U) and digital

droplet PCR (ddPCR, Bio-Rad, USA) (Figure 3B); the tumor was *HER2*-negative by immunostains. Plasma cell-free DNA (cfDNA) collected during ALK-TKI treatment was retrospectively analyzed with ddPCR and *HER2*-



**Figure 3** CNV analysis performed with NGS and ddPCR. (A) Plot of normalized coverage levels is shown. Blue dots correspond to target regions without gene amplification (normal regions), orange dots to potential amplified genes; in particular the arrow indicate *HER2* gene amplification; (B) ddPCR confirmation on DNA used for NGS analysis, extracted from tissue biopsy, was performed with CNV Assay for *HER2* (dHsaCP1000116) and AP3B1 as recommend reference assay (dHsaCP2500348) with ddPCR QX200 Droplet Reader (Bio-Rad, USA) according to the manufacturer's protocol; (C) CNV studied in cfDNA retrospectively from disease progression (PD) sample backward. CNV, copy number variation; NGS, next generation sequencing; ddPCR, digital droplet PCR; cfDNA, cell-free DNA.

amplified circulating DNA was identified 2 months before clinical disease progression (Figure 3C). *HER2* FISH analysis was then retrospectively performed on the primary tumor samples revealing a limited clone with *HER2* amplification (Figure 2V,W,X). It was not possible to define the ALK status of these *HER2*-amplified cells given their small number and negative *HER2* immunostains.

After liver biopsy and discontinuation of alectinib in January 2019, the clinical conditions of the patient rapidly worsened. A treatment with carboplatin-pemetrexed was not feasibly due to the poor performance status. Lorlatinib was started in February 2019 with a temporary clinical improvement (Figure 1A). The patient died in March 2019.

## Discussion

We described a case of an ALK-positive NSCLC patient who developed clinical resistance to ALK-TKI therapy due to the emergence of a *HER2*-amplified clone. The primary tumor had heterogeneous histology, non-homogeneous

ALK immunoreactivity and it was negative for *ALK* gene rearrangements by FISH. It harbored a minor *HER2*-amplified clone selected by ALK-TKI therapy to become the dominant component upon disease progression.

Most studies consistently report that ALK status, as assessed by IHC and FISH, is stable in primary NSCLC and its metastasis (9-11). However, at the cellular level, there is evidence of a significant intra-tumor heterogeneity of ALK expression that may underlie biological and clinical diversity (12). In the present case, the primary tumor was characterized by zonal variation in ALK immunostaining that in part segregated with different cell types, and it showed discordance between ALK IHC and FISH status in all samples. IHC and FISH discordance can occur, nonetheless, IHC positive/FISH negative NSCLCs have a high probability of response to ALK-inhibitors (13), as confirmed in present case. The patient, in fact, underwent first- and second-line ALK-TKI therapy with clinical benefit but, ultimately, she progressed. Biopsy of a liver metastasis showed an ALK-negative, histologically monomorphic

tumor with diffuse *HER2* gene amplification, as assessed by NGS sequencing and FISH. The emergence of this *HER2*-amplified clone could be traced 2 months earlier than the development of clinical resistance by the analysis of circulating tumor DNA with ddPCR. When retrospectively studied by FISH, both primary lung nodules and lymph node metastasis showed rare *HER2*-amplified cells (Figure 2V,W,X). Based on all these evidences, we conclude that the ALK-negative/*HER2*-amplified disease observed at progression derived from the selective pressure exerted by ALK-TKI therapy on a heterogeneous tumor harboring a primary resistant minor clone. The persistence and emergence of the resistant clone under sequential crizotinib and alectinib treatments suggests that *HER2* amplification might cause resistance to both drugs.

To the best of our knowledge, this is the first report of *HER2* gene amplification as a resistance mechanism to ALK-TKIs, both first- and second-generation. *HER2* is known to cause resistance in *EGFR*-mutated patients treated with different generation of *EGFR*-TKIs (14,15). There is also limited evidence that it might be involved in resistance of ALK-positive NSCLC, evidence deriving from a preclinical study (16) and the analysis of a single case with several concomitant oncogenic drivers (17). Future treatment strategies of ALK-addicted NSCLC might benefit from a combined inhibition of both ALK and *HER2* to minimize the development of resistance.

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## Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tlcr.2020.04.03>). MT serves as an unpaid editorial board member of *Translational Lung Cancer Research* from Dec 2019 to Nov 2021. MT reports grants and personal fees from Astra-Zeneca, personal fees from Pfizer, personal fees from Eli-Lilly, personal fees from BMS, personal fees from Novartis, personal fees from Roche, personal fees from MSD, grants and personal fees from Boehringer Ingelheim, personal fees from Otsuka, personal fees from Takeda, personal fees from Pierre Fabre, outside the submitted work. The other authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

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