MYC amplification identified in an EML4-ALK-positive lung adenocarcinoma with primary resistance to targeted therapy

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Guang Yang, MD, PhD; Michelle Dolan, MD
Haixia Qin, MD, PhD; Manish R. Patel, DO
Sophia Yohe, MD; Andrew C. Nelson, MD, PhD

The advent of genomically targeted therapy and immunotherapy has greatly altered the clinical management of advanced non-small cell lung cancer (NSCLC). Molecular testing is recommended for sensitizing EGFR mutations, ALK fusions, BRAF V600E, NTRK fusions, RET fusions, and MET exon 14 skipping alterations. Anaplastic lymphoma kinase (ALK) gene rearrangements are identified in about three to seven percent of NSCLC cases, with the echinoderm microtubule-associated protein-like 4 (EML4) gene being the most common fusion partner.

Multiple small molecule inhibitors targeting ALK fusion proteins have been developed, and the use of these new therapies has significantly improved the overall survival of patients with ALK-rearranged NSCLC. However, as with many targeted therapies, eventual resistance to ALK inhibitors and disease progression are often observed in NSCLC patients. Primary resistance refers to a lack of tumor response from the initiation of the targeted therapy, whereas secondary resistance is defined as disease progression after an initial partial or complete response to treatment. Although most studies focus on various secondary-resistance mechanisms along with therapeutic strategies, the molecular mechanisms of primary resistance to ALK inhibitors in ALK-rearranged NSCLC are not well documented.

We report a case with primary resistance to ALK inhibitors in EML4-ALK-positive lung adenocarcinoma with concomitant MYC amplification. We also review the related literature and discuss possible therapeutic strategies to overcome this primary resistance to ALK inhibitors.

Case presentation. A 68-year-old Caucasian female nonsmoker presented with a two-month history of dry cough, shortness of breath, worsening mid-thoracic back pain, and new onset mid-sternal chest pain. Chest computed tomography pulmonary angiogram with contrast showed two 10–12-mm solid pulmonary nodules in the right upper lobe. A positron emission tomography scan of the neck, chest, abdomen, and pelvis demonstrated widespread metastases in bones, liver, adrenal, and retroperitoneal lymph nodes. Magnetic resonance imaging of the brain detected numerous punctate brain metastases.

Endobronchial ultrasound-guided fine-needle aspiration of station 10L lymph node showed non-small cell carcinoma. Tumor cells demonstrated weak, patchy nuclear positivity for TTF-1 and were negative for p40. Based on these findings, a diagnosis of adenocarcinoma of pulmonary origin was made (Fig. 1, next page). PD-L1 immunohistochemistry (Ventana clone SP263) showed low expression (tumor proportion score between one and two percent).

DNA-based next-generation sequencing was performed using a custom-designed targeted 61-gene hotspot panel, which covers single nucleotide variants (SNV) and small insertions/deletions (indel). Library preparation was carried out by tagmentation following Nextera protocols (Nextera XT library preparation, Illumina). The enriched libraries were sequenced on an Illumina MiSeq instrument to a target of 1.5 million reads per sample. No actionable SNV or small indel were detected in the genes sequenced (ALK, BRAF, EGFR, ERBB2, IDH1, IDH2, KRAS, MET, NRAS, PIK3CA, RET, and TP53).

A parallel RNA-based targeted NGS assay was performed for the simultaneous assessment of fusions, exon skipping, and other expression targets frequently observed in NSCLC. Targeted RNA-Seq libraries were pre-
pared using the Quantidex NGS RNA Lung Cancer Kit (Asuragen) according to the manufacturer’s instructions. EML4-ALK gene rearrangement (EML4 exon 20 fused to ALK exon 20) was detected. Based on this finding, the patient was treated with steroids and later with palliative whole brain radiotherapy. Several days later she was admitted with sepsis due to suspected aspiration pneumonitis, postobstructive pneumonia, and right-sided pleural effusion. After admission the patient was started on antibiotics, but her overall condition rapidly deteriorated and she died of acute respiratory failure three months after starting targeted therapy.

Discussion. Over half of newly diagnosed patients with lung cancer have metastatic disease at the time of diagnosis, making surgical intervention ineffective. Therapy targeted at the underlying molecular abnormality has significantly improved the clinical outcomes of subgroups of NSCLC patients with actionable mutations in EGFR, ALK, ROS1, RET, BRAF V600E, MET exon 14, and NTRK. Multiple ALK tyrosine kinase inhibitors, including crizotinib, ceritinib, alectinib, brigatinib, and lorlatinib, have been approved by the Food and Drug Administration for the treatment of patients with advanced ALK-positive NSCLC.

Among these TKIs, alectinib is currently considered the preferred first-line treatment because of its clinical activity and favorable toxicity profile. According to a recent study, alectinib is more effective than standard chemotherapy in patients with advanced or metastatic ALK-positive NSCLC, with a median progression-free survival significantly longer with alectinib than with chemotherapy (9.6 months versus 1.4 months). Studies have also demonstrated that in patients with advanced ALK-rear-
ranged NSCLC, alectinib has a higher brain penetration\textsuperscript{15} and overall central nervous system response rate\textsuperscript{15} than crizotinib (85.7 percent versus 71.4 percent, respectively).

Although the use of ALK TKIs has led to marked improvements in response and survival, patients with ALK-rearranged NSCLC inevitably develop secondary resistance.\textsuperscript{16} Current research on resistance to ALK inhibitors in NSCLC largely focuses on identifying and characterizing the molecular mechanisms causing secondary resistance, such as mutations or amplification of ALK and “bypass track” activation via mutations in EGFR and PIK3CA, amplification of MET and KIT, and IGF1R activation.\textsuperscript{7} Secondary mutations in the ALK kinase domain were observed in about 50 percent of the ALK-rearranged NSCLC cases with resistance to second-generation ALK TKIs.\textsuperscript{17} In a study of 51 ALK-positive NSCLC patients who had progressive disease upon treatment with crizotinib, the most common ALK resistance mutations were L1196M and G1269A, detected in seven percent and four percent of these patients, respectively. Other mutations identified were C1156Y (two percent), G1202R (two percent), I1171T (two percent), S1206Y (two percent), and E1210K (two percent).\textsuperscript{18} However, mechanisms of primary resistance to ALK inhibitors are less well characterized.

Our patient was treated with a second-generation ALK inhibitor, alectinib, shortly after her diagnosis of metastatic \textit{EML4-ALK}-positive lung adenocarcinoma. Her tumor showed no objective response to this targeted therapy, with radiologic evidence of progression within one month of therapy initiation. Because the median overall survival for stage III/IV ALK-positive NSCLC patients treated with alectinib is 48.2 months,\textsuperscript{19} this patient’s rapid deterioration and death after ALK targeted therapy was unexpected. In light of the rapid disease progression while on alectinib, we hypothesized that this patient’s aggressive clinical course was likely due to a primary resistance mechanism not characterized in the guideline-focused genomic assays performed at diagnosis.

The cfDNA assay performed at the time of progression identified high-level MYC amplification, which was retrospectively confirmed to be present at diagnosis by IHC and FISH studies performed on pretreatment specimens.

Resistance to ALK inhibitors due to MYC amplification has been reported. Rihawi, et al.,\textsuperscript{20} reported the presence of \textit{ALK} rearrangement together with MYC amplification in a patient with stage IV NSCLC who failed to respond to crizotinib and ceritinib and had rapid disease progression. Based on in vitro studies using a human \textit{EML4-ALK}-rearranged NSCLC cell line with MYC overexpression, these cells showed reduced sensitivity to crizotinib and alectinib that was reversed by MYC inhibitors and CDK4/6 inhibitors.\textsuperscript{20}

Another study by Alidousty, et al.,\textsuperscript{21} investigated a possible mechanism for MYC-dependent resistance to ALK inhibitors in \textit{TP53}-mutated

![c-Myc 20X](image-url)

\textit{Fig. 2.} Endobronchial ultrasound-guided fine-needle aspiration of station 10L lymph node. c-Myc 20×: tumor cells show strong nuclear positive for c-Myc (clone Y69 from Abcam) (upper image). FISH using a dual-color breakapart probe to the \textit{MYC} locus (Abbott Laboratories) shows amplification of the 5’ (orange, centromeric) portion of the probe; two signals for the green (3’ telomeric) probe (lower image).
ALK-positive NSCLC patients. They propose that MYC amplification leads to increased expression of the EML4-ALK fusion gene by binding its promoter, causing resistance to ALK inhibitors.

Our case further supports the notion that MYC amplification may be pathogenetically linked to primary resistance in ALK-rearranged NSCLC and is associated with a poorer outcome. MYC inhibition (either directly or through CDK4/6 inhibitors) may overcome this resistance mechanism.

However, it is worth mentioning that MYC amplification-related potential primary resistance to EGFR TKIs was also reported in lung adenocarcinoma patients with EGFR-activating mutations.

**Conclusion.** We report a case of primary resistance to ALK inhibitors in EML4-ALK-positive lung adenocarcinoma with concomitant MYC amplification. The few similar cases reported in the literature have had a more aggressive clinical course compared with other ALK-rearranged NSCLC cases. We highlight this case because it demonstrates the important role of MYC amplification in the molecular mechanism of primary resistance to ALK inhibitors; assessment of MYC amplification for such patients may help guide prognostication and therapy. Patients with ALK-rearranged NSCLC and MYC amplification may need other agents against downstream targets, such as CDK4/6 inhibitors, to overcome MYC amplification-associated primary resistance to ALK inhibitors. However, given the limited number of cases reported, further study is needed to make definitive conclusions regarding precision therapy in this clinical setting.