

## Rapid Acquisition of Alectinib Resistance in *ALK*-Positive Lung Cancer With High Tumor Mutation Burden

### Abstract

**Introduction:** The highly selective *ALK* inhibitor alectinib is standard therapy for *ALK*-positive lung cancers; however, some tumors quickly develop resistance. Here, we investigated the mechanism associated with rapid acquisition of resistance using clinical samples. **Methods:** Autopsied samples were obtained from lung, liver, and renal tumors from a 51-year-old male patient with advanced *ALK*-positive lung cancer and who had acquired resistance to alectinib in only 3 months. We established an alectinib-resistant cell line (ABC-14) from pleural effusion and an alectinib/crizotinib-resistant cell line (ABC-17) and patient-derived xenograft (PDX) model from liver tumors. Additionally, we performed next-generation sequencing (NGS), direct DNA sequencing, and quantitative real-time reverse-transcription polymerase chain reaction.

**Results:** ABC-14 cells harbored no *ALK* mutations and were sensitive to crizotinib while also exhibiting *MET* gene amplification and *amphiregulin* overexpression. Additionally, combined treatment with crizotinib/erlotinib inhibited cell growth. ABC-17 and PDX tumors harbored *ALK G1202R*, and PDX tumors metastasized to multiple organs *in vivo*, whereas the third generation *ALK*-inhibitor, lorlatinib, diminished tumor growth *in vitro* and *in vivo*. NGS indicated high tumor mutation burden (TMB) and heterogeneous tumor evolution. The autopsied lung tumors harbored *ALK G1202R* (c. 3604 G>A) and the right renal metastasis harbored *ALK G1202R* (c. 3604 G>C); the mutation thus comprised different codon changes. **Conclusions:** High TMB and heterogeneous tumor evolution might be responsible for rapid acquisition of alectinib resistance. Timely lorlatinib administration or combined therapy with an *ALK* inhibitor and other receptor tyrosine-kinase inhibitors might constitute a potent strategy.

**Keywords:** NSCLC, alectinib, *ALK G1202R*, *MET*, *amphiregulin*