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Response to crizotinib in advanced *ALK*-rearranged non-small cell lung cancers with different *ALK*-fusion variants

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ABSTRACT

Introduction: Anaplastic lymphoma kinase (*ALK*) rearrangements are present in approximately 5% of non-small-cell lung cancers (NSCLCs). NSCLCs with *ALK*-rearrangement can be effectively treated with crizotinib. However, magnitude and duration of responses are found to be heterogeneous. This study explored the clinical efficacy of crizotinib in different *ALK* variants.

Methods: Among 96 *ALK*-rearrangement patients treated with crizotinib, 60 patients were identified with tumor specimens that could be evaluated by next-generation sequencing (NGS). We retrospectively evaluated the efficacy of crizotinib in different *ALK* variants.

Results: The median Progression-free survival (PFS) of the 96 *ALK*-rearrangement patients was 14.17 months. Among the 60 patients with NGS results, the most frequent variants were variant 3a/b (33.33%), variant 1 (23.33%) and variant 2 (15.00%). The percentage of rare *EML4-ALK* variants and non *EML4-ALK* variants were 10.00% and 18.33%. Survival analysis showed that patients with variant 2 appeared to have longer PFS than others (P = .021); also, patients with *TP53* mutation seemed to have an unfavorable PFS than those with *TP53* wild-type with a borderline *p* value (P = .068). After adjusting for other baseline characteristics, *EML4-ALK* variant 2 was identified as an important factor for a better PFS of crizotinib. We also found that patients with variant 3a/b had shorter duration of response to crizotinib; however, no significant difference of PFS was observed between the PFS of variant3a/b and non-v3 *EML4-ALK* variants.

Conclusions: Our results indicate prolonged PFS in patients with *EML4-ALK* variant 2.

1. Introduction

Targeted therapy is at the forefront of malignant tumor treatment. Over the past decade, the treatment of non-small cell lung cancer (NSCLC) has been revolutionized by the usage of tyrosine kinase inhibitors (TKIs) with the approach to the driven genetic events of the tumor. Anaplastic lymphoma kinase (*ALK*) gene rearrangements are found in approximately 5%-7% of lung adenocarcinomas [1].

In lung adenocarcinomas, the echinoderm microtubule-associated protein-like 4 (*EML4*) was the most common fusion partner of *ALK* rearrangements [1]. To date, at least 21 *EML4-ALK* variants have been reported in NSCLC [2]. Moreover, other predominant 5′-partners in *ALK* fusion-positive NSCLC have also been reported, including kinesin family member 5B (*KIF5B*), TRK-fused gene (*TFG*), kinesin light chain 1 (*KLC1*) and so on. The exact frequency and clinical significance of these fusion genes are still being investigated [3–5].

As the first *ALK* tyrosine kinase inhibitor approved by FDA in 2011 for *ALK*-rearranged NSCLCs, crizotinib has shown remarkable response in a series of clinical trials [6,7]. However, the response magnitude and duration are heterogeneous; also, most patients benefiting from crizotinib will develop acquired resistance. Several studies have reported different response to crizotinib among different *ALK* variants. However, some of the conclusions are contradictory. Tatsuya Yoshida reported that patients with *EML4-ALK* variant 1 had a longer progression-free survival (PFS) than those with non-v1 *EML4-ALK* variants [8]. Heuckmann et al. also tried to analyze different response to *ALK* inhibitors

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and reported a different result [9]. In their *in vitro* study using the Ba/F3 and NIT3T3 cell lines, Heuckmann showed that variant 2 was significantly more sensitive to crizotinib [9]. Tatsuya Yoshida's study was limited by the small sample size; also, the difference of PFS among non-v1 variants was not reported. Heuckmann's study was *in vitro* study, and need to be confirmed in clinical settings with large cohorts. Therefore, study on the association between clinical responses with *ALK* variant is still needed.

Recently, a Phase III study demonstrated that alectinib, the second-generation *ALK* inhibitor, might have a better response to *ALK*-rearrangement patients than crizotinib [10]. Should alectinib replace crizotinib as the frontline treatment for *ALK*-rearranged patients? Or should those patients be treated with a sequential approach frontline crizotinib followed by alectinib when the crizotinib resistance occurs? To answer these questions, more study should be done to investigate the heterogeneous response to crizotinib.

In our study, we explored the PFS to crizotinib of *ALK*-rearranged lung cancer. By using next-generation sequencing technology (NGS), we also aimed to investigate the responsiveness of various *ALK*-rearrangement variants to crizotinib.

2. Materials and methods

2.1. Patients

Ninety-Six Chinese consecutive patients who were diagnosed with advanced NSCLC and treated with crizotinib between May 2011 to September 2016 at the Cancer Hospital, Chinese Academy of Medical Sciences (CAMS, Beijing, CHINA) were enrolled in this study. Among these 96 patients, we identified 60 cases with tumor specimens that could be evaluated for *ALK* fusion status by NGS. In addition, the tumor specimens used were Formalin-Fixed Paraffin-Embedded tissues from the tissue bank of the Department of Pathology, Cancer Hospital, CAMS.

2.2. Study design

This retrospective study included 96 *ALK*-positive advanced NSCLC patients who have received crizotinib treatment (Fig. 1). Patients' *ALK*-rearrangement status was assessed by FISH or IHC. Clinicopathological parameters were collected through electronic medical records. The tumor histology was classified using the WHO criteria [11]. All patients have been followed up for at least 6 months since their first dose of crizotinib, and 30th April 2017 was the cutoff date for the statistical analysis. *ALK* variants analysis was performed in 60 patients with evaluable tumor tissues using targeted NGS. These 60 patients started crizotinib treatment between May 2011 to September 2016.

2.3. Next-generation sequencing analysis

Genomic DNA of the 60 cases with evaluable tumor tissue specimens were isolated. Extracted DNA were quantitated on the Qubit dsDNA Assay (Thermo Fisher Scientific, Waltham, MA). Targeted NGS was performed as previously reported [12].

2.4. Clinical response evaluation

The initial dose of crizotinib was 250 mg twice per day, and the drug was orally administered until progressive disease (PD) or unacceptable toxicity. When experienced toxicity, patients underwent dose reduction or interruption. Patients underwent image testing such as computed tomography (CT) or magnetic resonance imaging (MRI) at the screening and every 6–8 weeks thereafter to assess the response. The response evaluation was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST), VERSION 1.1 [13] by independent medical oncologist. PFS was calculated from the date of initiation of crizotinib to the date of disease progression. Cases were censored at the latest date if patients were still not progressed, or voluntarily withdrew. The cutoff date was April 30th 2017, when all patients have completed at least six months of follow-up. The objective response rate (ORR) was calculated as the total percentage of patients with optimal efficacy of complete response (CR) or partial response (PR). The disease control rate (DCR) was calculated as the total percentage of patients with CR, PR or stable disease (SD).

2.5. Statistics

All statistical analyses were performed by Statistic Analysis System software (SAS v9.1, SAS Institute, Cary, NC). Differences between groups were compared using the Pearson's χ^2 test for categorical data, and *t*-tests for continuous data. The survival curves were estimated

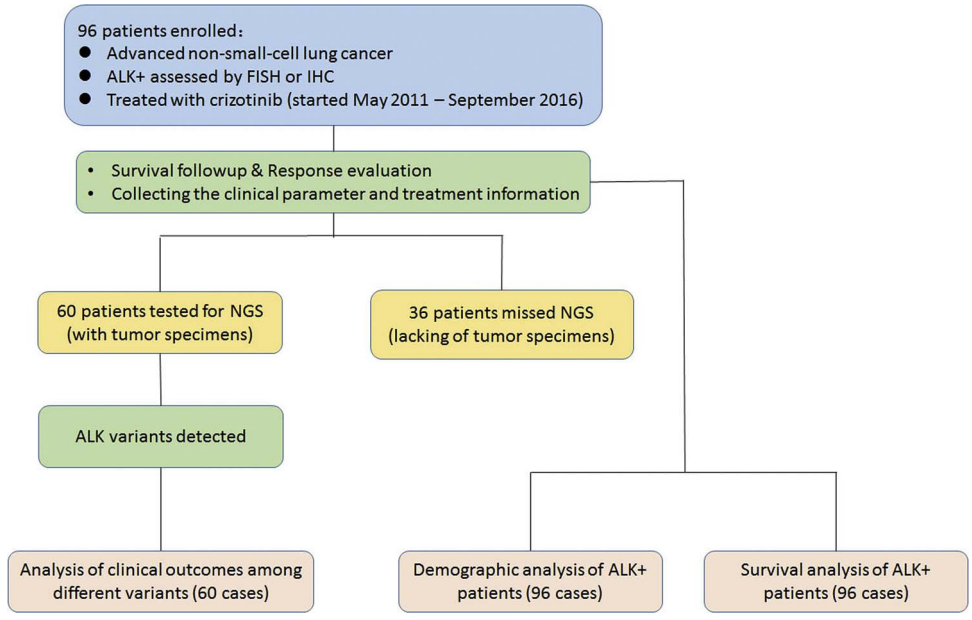

Fig 1. Study Flow Chart. Abbreviations: *ALK* = anaplastic lymphoma kinase; FISH = fluorescence in situ hybridization; IHC = immunohistochemistry; NGS = next-generation sequencing.