Elevated levels of pre-treatment lactate dehydrogenase are an unfavorable predictor factor in patients with EML4-ALK rearrangement non-small cell lung cancer treated with crizotinib

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Background: Targeted therapy is an important treatment for advanced non-small cell lung cancer (NSCLC) patients with specific genetic mutations, crizotinib can prolong survival in advanced NSCLC patients with echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) rearrangement. We performed a retrospective analysis to investigate the association between the lactate dehydrogenase (LDH) levels and progression-free survival (PFS) in patients with EML4-ALK rearrangement NSCLC receiving treatment with crizotinib.

Methods: Advanced (stage IIIb-IV) NSCLC patients with EML4-ALK rearrangement receiving treatment with crizotinib were enrolled between January 2007 and January 2016 at Peking Union Medical College and Cancer Hospital Chinese Academy of Medical Sciences.

Results: Overall, 212 patients were enrolled. Kaplan–Meier univariate analysis showed that elevated pre-treatment LDH level (7.9 vs 14.1 months, HR =1.251, CI: 1.008–1.553, P=0.004) was significantly associated with shorter PFS, while the post-treatment mean-LDH level (13.3 vs 14.3 months, HR=1.439, 95% CI: 0.994–2.082, P=0.970) was not significantly associated with PFS. Cox proportional hazards model also identified that pre-treatment LDH level (HR=2.085, 95% CI: 1.150–3.781, P=0.016) was associated with the PFS. Logistic regression analysis showed that post-treatment LDH level was associated with creatine kinase (OR=6.712, 95% CI 3.395–13.273, P<0.01), creatine kinase isoenzyme (OR=6.297, 95% CI 2.953–13.427, P<0.01), and hemoglobin (OR=4.163, 1.741–9.56, P<0.001).

Conclusion: An elevated pre-treatment serum LDH level (>250 U/L) was significantly associated with shorter PFS in patients with EML4-ALK rearrangement NSCLC. Post-treatment elevated serum LDH level was not significantly associated with PFS, which related to adverse events including muscle damage and anemia.

Keywords: non-small cell lung cancer, lactate dehydrogenase, crizotinib, echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase, progression-free survival

Introduction
Lung cancer is a leading cause of cancer-related mortality worldwide and non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancer. Targeted therapy is an important treatment for advanced NSCLC patients with specific genetic mutations, which can significantly improve their outcomes.
protein-like 4-anaplastic lymphoma kinase (EML4-ALK) rearrangement was one of the known therapeutic targets. The incidence rate of EML4-ALK rearrangement in NSCLC patients is 3.3–6.1%. A small molecule tyrosine kinase inhibitor, crizotinib, can prolong survival in EML4-ALK rearrangement advanced NSCLC patients. Lactate dehydrogenase (LDH) is a glycolytic enzyme that can convert pyruvate into lactic acid in an anaerobic environment, contribute to anaerobic glycolysis, and produce adenosine triphosphate for cells. A previous study reported that there was a significant association between the pre-treatment serum LDH level and poor survival in NSCLC patients receiving treatment with EGFR-tyrosine kinase inhibitor (EGFR-TKI), PD-1/PD-L1 inhibitors, or standard chemotherapy, which may be related to that LDH can provide energy for tumor cells, enhance tumor invasion, and angiogenesis. However, little is known about the association between LDH level and progression-free survival (PFS) in NSCLC patients with EML4-ALK rearrangement receiving treatment with crizotinib. We also found that many patients with EML4-ALK rearrangement developed elevated LDH levels after treatment with crizotinib. The reason for the elevated LDH level and its influence on prognosis in patients remains unclear. Recently, major therapeutic advances have occurred in the management of advanced EML4-ALK rearrangement NSCLC patients. Several second- and third-generation ALK inhibitors have shown clinical benefits in these patients, and have been shown to dramatically prolong overall survival (OS) in this group. Thus, it has become even more important to identify prognostic factors and create appropriate follow-up schedules for advanced ALK+ NSCLC patients. We thus performed a retrospective analysis to investigate the association between the pre-treatment and post-treatment serum LDH levels and PFS in patients with EML4-ALK rearrangement receiving treatment with crizotinib.

**Methods**
The treatment regimen was crizotinib 250 mg/time, twice daily orally until disease progression or intolerable adverse events occurred. All patients were evaluated regularly, and were carried out every 2–3 months by imaging examinations, including chest and abdominal computed tomography, enhanced head magnetic resonance imaging and scintigraphy. PFS was calculated from the beginning of crizotinib administration until the date of disease progression (according to RECIST 1.1 criteria) or the date of mortality done. All patients were followed up regularly in outpatient department.

The normal value of LDH level was according to the analyzer standard of Peking Union Medical College Hospital and Cancer Hospital. LDH levels were evaluated using the Siemens ADVIA2400 unit and its corollary reagent, and LDH >250 U/L is defined as elevated LDH level. Pre-treatment LDH level was divided into the LDH-elevated group and the LDH-normal group. Post-treatment LDH levels were evaluated after the initiation of treatment with crizotinib until the disease progression or mortality, and multiple values were thus obtained. The post-treatment mean-LDH level was defined as the mean value of multiple LDH detecting levels in one patient who have received crizotinib, which was calculated and divided into the ≤250U/L group (normal post-treatment mean-LDH group) and >250 U/L group (elevated post-treatment mean-LDH group).

**Patients and methods**

**Study design and patients**
The present study retrospectively enrolled 212 patients with advanced EML4-ALK rearranged NSCLC who received treatment with crizotinib from January 2007 to January 2018 at Peking Union Medical College Hospital and Chinese Academy of Medical Sciences Cancer Hospital. The patients’ clinical data were based on CAPTRA-Lung (NCT03334864) database. The study was approved by the Institutional Review Board (IRB) (Approval Number: JS-1410), and it was conducted in accordance with the Declaration of Helsinki. All the patients provided written informed consent for the collection of their clinical data. The inclusion criteria were as follows: 1) age ≥18 years, 2) histologically or cytologically confirmed NSCLC, 3) stage IIIb (do not meet surgery or radical chemoradiotherapy criteria) or stage IV, 4) histology positive for EML4-ALK rearrangement, and the evaluation method for ALK fusion should be fluorescence in situ hybridization (FISH), next-generation sequence (NGS), or Ventana immunohistochemistry (IHC with D5F3 antibody), 5) patients receiving treatment with crizotinib, 6) LDH level was detected before or after treatment. Exclusion criteria were as follows: 1) only sputum pathology specimens available, 2) genetic results from sputum or blood samples, and 3) ALK evaluation methods that did not fulfill the inclusion criteria.
Data collection
In this retrospective study, the following patient data were collected: gender, age, smoking history, pathological types, disease stage, sites of metastasis, EGFR mutation status, number of treatment regimens received previously and LDH levels.

Statistical analyses
Statistical analyses were performed by IBM SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± SD. PFS curves were drawn according to the Kaplan–Meier method. The univariate analysis of PFS was performed by the Kaplan–Meier method and the log-rank test. The multivariate analysis was performed by the Cox proportional hazard model and calculation of HRs using the 95% CI. The correlation between LDH serum levels and clinicopathological parameters was performed using Fisher’s exact test and logistic regression analysis. All tests were two-sided, and P≤0.05 was considered statistically significant.

Results
Patients’ characteristics
Between January 2007 and January 2018, a total of 212 patients fulfilled the criteria and were included in the analysis at Peking Union Medical College Hospital and Chinese Academy of Medical Sciences Cancer Hospital. Among these patients, the most common pathological type was adenocarcinoma (203 cases), followed by unclassified carcinomas (3 cases), squamous cell carcinoma (2 cases), adenosquamous carcinoma (2 cases), mucoepidermoid carcinoma (1 case), and carcinosarcoma (1 case). There were 12 patients with EGFR-active mutations, 190 with wild-type EGFR, and 10 patients with unknown EGFR mutations due to insufficient pathological specimens. The ALK fusion detection methods were FISH method in 124 cases, IHC targeting D5F3 method in 103 cases, and NGS method in 10 cases. Table 1 summarizes the other clinical pathological characteristics of 212 NSCLC patients.

LDH results
Among all patients, pre-treatment LDH level was evaluated in 129 patients, with normal LDH levels in 100 cases and elevated LDH levels in 29 cases. Pre-treatment LDH levels were not associated with any patients’ clinical pathological feathers (Table 2). Post-treatment LDH level was evaluated in 193 patients, with normal post-treatment mean-LDH levels in 64 cases and elevated post-treatment mean-LDH levels in 129 patients. With normal post-treatment LDH levels in 28 cases and elevated post-treatment LDH levels in 165 patients. Post-treatment LDH levels were not associated with any patients’ clinical pathological feathers (Table 2). Among patients with elevated post-treatment LDH level, 41.2% (68/165) patients had LDH elevated for the first time in the first month after crizotinib treatment (Table 3) (Figure S1). The mean value of LDH levels evaluated in each month among all patients who have received crizotinib fluctuated between 248 and 281 U/L (Table 3).

To further analyze the reason of elevated post-treatment LDH levels, we performed analysis of association between LDH levels and concurrent liver function indicators (alanine aminotransferase/aspartate aminotransferase), renal function indicators (creatinine), muscle enzymes (creatine kinase/creatine kinase isoenzyme), and hemoglobin levels (hemoglobin). Logistic regression analysis showed that elevated post-treatment LDH levels were significantly consistent with creatine kinase (OR=6.712, 95% CI 3.395–13.273, P<0.01), creatine kinase isoenzyme (OR=6.297, 95% CI 2.953–13.427, P<0.01), and hemoglobin (OR=4.163, 1.741–9.956, P<0.001) (Table 4).

PFS
The efficacy was evaluated in 212 patients: 128 (60.4%) with partial response, 51 (24.1%) with stable disease, 5 (2.4%) with progression disease, 28 (13.2%) with uncertain, and with an objective response rate of 60.4% and a disease control rate of 84.5%. At the end of follow-up, the median follow-up time was 15.9 months, and the median PFS was 13.4 months (95% CI 10.6–16.3 months). Of all patients, only 21.1% (45/212) were followed up to death, and it was immature for analysis of OS.

In the Kaplan-Meier univariate analysis, PFS in patients with elevated pre-treatment LDH level was shorter than that in patients with normal pre-treatment LDH level (7.9 vs 14.1 months, HR=1.251, 95% CI: 1.008–1.553, P=0.004) (Figure 1). PFS was not significantly related to post-treatment mean-LDH level (13.3 vs 14.3 months, HR=1.439, 95% CI: 0.994–2.082, P=0.970) (Figure 2). In addition, Kaplan-Meier univariate suggested liver metastasis (10.9 vs 15.0 months, P=0.032) and adrenal metastasis (5.0 vs 14.3 months, P=0.001) were also associated with shorter PFS (Table 1). All clinical pathological factors were included in the Cox regression model.
Table 1 Log-rank test analysis of clinical pathological characteristics and PFS in 212 ALK-positive NSCLC patients

| Variables                      | No. (%)      | PFS                      |        |        | P-value (log-rank) |
|--------------------------------|--------------|--------------------------|--------|--------|-------------------|
|                                |              | Median (months)          | CI (months) |       |                   |
| Gender                         |              |                          |        |        |                   |
| Male                           | 101 (47.6%)  | 12.8                     | 10.2–15.4 | 0.440  |
| Female                         | 111 (52.4%)  | 14.6                     | 10.4–18.7 |        |
| Age                            |              |                          |        |        |                   |
| Median (range)                 | 49.5 (22–82) |                          |        |        |                   |
| ≤50 years old                  | 115 (54.2%)  | 15.0                     | 11.7–18.4 | 0.407  |
| >50 years old                  | 97 (45.8%)   | 12.6                     | 9.7–15.5  |        |
| Smoking history                |              |                          |        |        |                   |
| Never                          | 153 (72.2%)  | 13.3                     | 9.9–16.7  | 0.940  |
| Ever                           | 59 (27.8%)   | 14.6                     | 10.5–18.7 |        |
| Pathology                      |              |                          |        |        |                   |
| Non-squamous                   | 210 (99.18%) | 13.4                     | 10.6–16.3 | 0.552  |
| Squamous                       | 2 (0.9%)     | 9.9                      |        |        |                   |
| Stage                          |              |                          |        |        |                   |
| IIIb                           | 28 (13.2%)   | 16.8                     | 11.8–21.7 | 0.300  |
| IV                             | 184 (86.8%)  | 13.0                     | 10.6–15.4 |        |
| EGFR status                    |              |                          |        |        |                   |
| Wild type                      | 190 (89.6%)  | 13.4                     | 10.2–16.7 | 0.640  |
| Mutant type                    | 12 (5.7%)    | 12.2                     | 0.0–30.7  |        |
| Unknown                        | 10 (4.7%)    | –                        | –       |        |                   |
| ALK evaluation method          |              |                          |        |        |                   |
| FISH                           | 124 (58.5%)  | 15.3                     | 11.1–19.6 | 0.636  |
| IHC (DSF3)                     | 103 (48.6%)  | 14.1                     | 9.9–18.3  |        |
| NGS                            | 10 (4.7%)    | 20.7                     | 8.5–33.0  |        |
| Previous treatment             |              |                          |        |        |                   |
| Never                          | 138 (65.1%)  | 16.5                     | 14.1–19.0 | 0.148  |
| Ever                           | 74 (34.9%)   | 11.5                     | 9.5–13.6  |        |
| Lung metastases                |              |                          |        |        |                   |
| Yes                            | 80 (37.7%)   | 14.6                     | 9.2–20.0  | 0.694  |
| No                             | 132 (62.3%)  | 13.3                     | 9.6–17.0  |        |
| Brain metastases               |              |                          |        |        |                   |
| Yes                            | 43 (20.3%)   | 16.5                     | 10.3–22.7 | 0.961  |
| No                             | 169 (79.7%)  | 13.0                     | 10.5–15.5 |        |
| Liver metastases               |              |                          |        |        |                   |
| Yes                            | 29 (13.7%)   | 10.9                     | 7.3–14.6  | 0.032  |
| No                             | 183 (86.3%)  | 15.0                     | 12.1–18.0 |        |
| Bone metastases                |              |                          |        |        |                   |
| Yes                            | 71 (33.5%)   | 10.9                     | 8.8–13.1  | 0.052  |
| No                             | 141 (66.5%)  | 16.5                     | 13.7–19.3 |        |
| Pleural metastases             |              |                          |        |        |                   |
| Yes                            | 75 (35.4%)   | 13.4                     | 9.0–17.9  | 0.698  |
| No                             | 137 (64.6%)  | 13.0                     | 9.2–16.8  |        |

(Continued)
Table 1 (Continued).

| Variables                          | No. (%)     | PFS Median (months) | 95% CI (months) | P-value (log-rank) |
|-----------------------------------|-------------|---------------------|-----------------|-------------------|
| Adrenal metastases                |             |                     |                 |                   |
| Yes                               | 11 (5.2%)   | 5.0                 | 2.0–8.0         | 0.001             |
| No                                | 201 (94.8%) | 14.3                | 11.5–17.1       |                   |
| Pre-treatment LDH level           |             |                     |                 |                   |
| ≤250 U/L                          | 100 (47.2%) | 14.1                | 10.3–18.0       | 0.004             |
| >250 U/L                          | 29 (13.7%)  | 7.9                 | 5.7–10.0        |                   |
| Post-treatment mean-LDH level     |             |                     |                 |                   |
| ≤250 U/L                          | 64 (30.2%)  | 14.3                | 10.9–17.7       | 0.970             |
| >250 U/L                          | 129 (60.8%) | 13.3                | 9.2–17.4        |                   |

Abbreviations: LDH, lactate dehydrogenase; PFS, progression-free survival; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequence; ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer.

Table 2 Chi-square test analysis of LDH level and clinical pathological characteristics

| Variables                          | Pre-treatment LDH level | Post-treatment mean-LDH level |
|-----------------------------------|-------------------------|------------------------------|
|                                   | ≤250 U/L | >250 U/L | P-value (Fisher) | ≤250 U/L | >250 U/L | P-value (Fisher) |
| Gender                            |          |          |                  |          |          |                  |
| Male                              | 53       | 15       | 1.000            | 36       | 54       | 0.067            |
| Female                            | 47       | 14       |                  | 28       | 75       |                  |
| Age                               |          |          |                  |          |          |                  |
| Median (range)                    |          |          |                  |          |          |                  |
| ≤50 years old                     | 55       | 14       | 0.534            | 41       | 66       | 0.094            |
| >50 years old                     | 45       | 15       |                  | 23       | 63       |                  |
| Smoking history                   |          |          |                  |          |          |                  |
| Never                             | 67       | 20       | 0.819            | 44       | 95       | 0.499            |
| Ever                              | 33       | 8        |                  | 20       | 34       |                  |
| Pathology                         |          |          |                  |          |          |                  |
| Non-squamous                      | 99       | 29       | 1.000            | 62       | 124      | 1.000            |
| Squamous                          | 1        | 0        |                  | 2        | 5        |                  |
| Stage                             |          |          |                  |          |          |                  |
| IIIb                              | 10       | 3        | 1.000            | 8        | 17       | 1.000            |
| IV                                | 90       | 26       |                  | 56       | 112      |                  |
| EGFR status                       |          |          |                  |          |          |                  |
| Wild type                         | 92       | 24       | 1.000            | 60       | 115      | 0.312            |
| Mutant type                       | 7        | 2        |                  | 1        | 9        |                  |
| Unknown                           | 1        | 3        |                  | 3        | 5        |                  |
| Previous treatment                |          |          |                  |          |          |                  |
| Never                             | 65       | 14       | 0.131            | 47       | 78       | 0.081            |
| Ever                              | 35       | 15       |                  | 17       | 51       |                  |
| Lung metastases                   |          |          |                  |          |          |                  |
| Yes                               | 39       | 7        | 0.187            | 23       | 52       | 0.639            |
| No                                | 61       | 22       |                  | 41       | 77       |                  |

(Continued)
### Table 2 (Continued)

| Variables          | Pre-treatment LDH level | Post-treatment mean-LDH level |  |
|--------------------|-------------------------|-------------------------------|---|
|                    | ≤250 U/L | >250 U/L | P-value (Fisher) | ≤250 U/L | >250 U/L | P-value (Fisher) |
| Brain metastases   |           |           |                 |           |           |                 |
| Yes                | 20        | 8         | 0.444           | 13        | 26        | 1.000           |
| No                 | 80        | 21        |                 | 51        | 103       |                 |
| Liver metastases   |           |           |                 |           |           |                 |
| Yes                | 12        | 8         | 0.076           | 7         | 19        | 0.512           |
| No                 | 88        | 21        |                 | 57        | 110       |                 |
| Bone metastases    |           |           |                 |           |           |                 |
| Yes                | 31        | 15        | 0.051           | 22        | 44        | 1.000           |
| No                 | 69        | 14        |                 | 42        | 85        |                 |
| Pleural metastases |           |           |                 |           |           |                 |
| Yes                | 40        | 8         | 0.277           | 24        | 44        | 0.749           |
| No                 | 77        | 21        |                 | 40        | 85        |                 |
| Adrenal metastases |           |           |                 |           |           |                 |
| Yes                | 4         | 4         | 0.075           | 0         | 8         | 0.054           |
| No                 | 96        | 25        |                 | 64        | 121       |                 |

Abbreviation: LDH, lactate dehydrogenase.

### Table 3 Evaluation of post-treatment LDH levels in every month

| Months | No. of patients with LDH evaluated | No. of patients with LDH elevated | No. of patients with LDH elevated for the first-time | Mean value of LDH level |
|--------|-----------------------------------|----------------------------------|-----------------------------------------------------|-------------------------|
| 1      | 119                               | 68                               | 68                                                  | 289                     |
| 2      | 86                                | 39                               | 25                                                  | 248                     |
| 3      | 115                               | 64                               | 21                                                  | 266                     |
| 4      | 75                                | 37                               | 11                                                  | 256                     |
| 5      | 107                               | 66                               | 9                                                   | 281                     |
| 6      | 79                                | 44                               | 6                                                   | 262                     |
| 7      | 93                                | 59                               | 3                                                   | 280                     |
| 8      | 77                                | 39                               | 3                                                   | 267                     |
| 9      | 74                                | 44                               | 3                                                   | 277                     |
| 10     | 69                                | 37                               | 0                                                   | 262                     |
| >10    | 310                               | 195                              | 16                                                  | 276                     |

Abbreviation: LDH, lactate dehydrogenase.

### Table 4 Logistic regression analysis of elevated post-treatment LDH levels

| Variables | B     | SE   | Wals | Sig   | Exp (B) | 95% CI  |
|-----------|-------|------|------|-------|---------|---------|
| ALT       | -0.329| 0.398| 0.684| 0.408 | 0.720   | 0.330–1.569 |
| AST       | 0.300 | 0.502| 0.358| 0.550 | 1.350   | 0.505–3.613 |
| CK        | 1.904 | 0.348| 29.960| <0.001| 6.712   | 3.395–13.273 |
| CKMB      | 1.840 | 0.386| 22.679| <0.001| 6.297   | 2.953–13.427 |
| Cr        | -0.271| 0.314| 0.742| 0.389 | 0.763   | 0.412–1.412 |
| HGB       | 1.426 | 0.445| 10.277| <0.001| 4.163   | 1.741–9.956 |

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; Cr, creatinine; CK, creatine kinase; CKMB, creatine kinase isoenzyme; HGB, hemoglobin; LDH, lactate dehydrogenase.
multivariate analysis. The results showed that only pre-treatment LDH levels (HR=2.085, 95% CI 1.150–3.781, $P=0.016$) and adrenal metastasis (HR=3.827, 95% CI 1.307–11.205, $P=0.014$) were associated with shorter PFS (Table S1).

**Discussion**

There are several previous studies on the relationship between LDH levels and chemotherapy, PD-1/PD-L1 inhibitors or EGFR-TKI treatment. To our knowledge, this is the first study of the relationship between LDH levels and crizotinib treatment. The present study revealed the existence of a significant association between the pre-treatment serum LDH level and PFS in patients with EML4-ALK rearranged NSCLC receiving treatment with crizotinib, while post-treatment mean-LDH levels were not associated with PFS. Furthermore, elevated post-treatment LDH level was associated with multiple factors including muscle damage, and anemia.

Previous studies showed that elevated pre-treatment serum LDH level had a shorter PFS in inoperable NSCLC patients. Zhu et al analyzed the prognostic factors in 105 patients with advanced NSCLC receiving first-line chemotherapy. The results showed that patients with high LDH levels before treatment had shorter PFS (3.6 vs 6.6 months, $P=0.005$) and shorter OS (10.8 vs 17.0 months, $P=0.014$) than those with lower LDH levels. Multivariate analysis also suggested that patients with elevated LDH levels had shorter PFS ($P=0.019$) and OS ($P=0.006$). Minehiko Inomata et al analyzed the prognostic factors affecting patients with EGFR mutation-positive NSCLC treated with gefitinib or erlotinib. A total of 65
patients were included in the study. The results showed that patients with high LDH levels before treatment had shorter PFS (6.2 vs 13.2 months, \( P < 0.01 \)) and OS (10.5 vs 36.1 months, \( P < 0.01 \)) than those with lower LDH levels. Cox regression multivariate also suggested that patients with higher LDH levels had shorter PFS (\( P = 0.05 \)) and shorter OS (\( P < 0.01 \)).\(^9,12\) Mezquita et al analyzed the prognostic impact of pre-treatment LDH levels on advanced NSCLC receiving immunotherapy (a PD-1/PD-L1 inhibitor). A total of 466 NSCLC patients were classified as high LDH levels group (n=179) and normal LDH level group (n=287). The results showed that patients with higher LDH level had a shorter OS time (\( P < 0.01 \)) than the normal LDH level group.\(^7\)

Unlike previous studies, this present study showed that elevated serum LDH levels before treatment with crizotinib were significantly associated with shorter PFS in patients with NSCLC harboring ALK-rearrangement. The reasons may be as follows: 1) NSCLC relies on the anaerobic metabolism of glucose and has a phenotype closely related to the clinical invasion behavior of tumor cells.\(^13,29\) LDH is a glycolytic enzyme that catalyzes the conversion of pyruvate to lactic acid in anoxic environment, producing adenine triphosphate and nicotinamide adenine dinucleotide, which is beneficial to the growth of tumor cells and increases tumor burden in vivo;\(^14,30\) 2) LDH can increase the invasive ability and angiogenic ability of tumor cells.\(^10,16,17\)

Our study also suggested that post-treatment LDH levels were not associated with PFS. The elevated post-treatment LDH level was significantly associated with creatine kinase, creatine kinase isoenzyme, and hemoglobin levels. Therefore, elevated post-treatment LDH levels may be mainly caused by muscle damage, and anemia, and therefore do not reflect the real ability of growth, infiltration, and invasion of tumor cells. In addition, activation of the receptor tyrosine kinase, c-Met, by hepatocyte growth factor (HGF) leads to increased cell motility, proliferation, invasion, and metastasis. Crizotinib is also a c-mesenchymal-epithelial transition (c-MET) inhibitor. It is reported that an LDH inhibitor can reduce c-Met activation and HGF-induced cell motility, indicating a potential connection between LDH activity and the c-Met signaling axis.\(^31\) Thus, we assume that crizotinib can inhibit the c-MET/HGF axis, which can stimulate an increase in LDH levels to re-active c-MET signaling. Similarly, NSCLC patients have 13 EML4-ALK fusion variants, which contain exons 20–29 of ALK and eight different EML4 exons.\(^32\) This variants induce the phosphorylation of one or more of the juxtamembrane tyrosine residues and then active the downstream signaling, including the Ras/Raf/MEK/ERK1/2 pathway, the Janus activated kinase (JAK)/STAT pathway, and the phosphatidylinositol 3-kinase/Akt (PKB) pathway.\(^33\) Activation of these pathways can mediate tumor cell proliferation and survival. While LDH can contribute to anaerobic metabolism of glucose in tumor cells and increase their invasive ability and angiogenic ability. Thus, we assume that the survival environment of tumor cells are poor after crizotinib inhibits the EML4-ALK procedure, which, in turn, stimulates the production of LDH and contributes to the survival of tumor cells.

Brain metastasis (BM) is a common complication of NSCLC. Because of its poor ability to penetrate the blood-brain barrier (0.0026), crizotinib has a lower efficacy against BMs.\(^34,35\) The incidence of BM increases with increasing duration of the disease, with up to 60% of patients receiving crizotinib developing BMs for the first time during treatment.\(^36\) In recent studies, second- or third-generation ALK inhibitors have shown favorable intracranial activities in patients with ALK-rearranged NSCLC and BMs.\(^37–41\) However, in our study, BMs were not found to be associated with PFS in ALK+ NSCLC patients treated with crizotinib. We suspect the following reasons: First, local therapies have been the primary method for treatment of patients with BMs, including surgery, whole-brain radiation therapy, and stereotactic radiosurgery. Most patients with baseline BMs received local therapies, which effectively controlled intracranial symptoms and inhibited intracranial progression to some extent. Second, only 43 patients had baseline BMs, and the sample size needs to be increased to further study the association between baseline BM and PFS.

Our research has several limitations. First, this was a retrospective research, and the result should be confirmed in further studies. Second, we only tested serum total LDH levels, but did not detect LDH subtypes. Third, there are many factors affecting the increase of LDH levels. We only analyzed the four most common influencing factors: liver dysfunction, renal dysfunction, muscle damage, and anemia.

**Conclusion**

In conclusion, the present study indicates the existence of an association between pretreatment serum LDH levels and PFS in NSCLC patients with EML4-ALK rearrangement receiving treatment with crizotinib, while post-treatment...
LDH levels were found to be mainly associated with adverse effects and not with PFS.

Data sharing statement
No additional unpublished data are available.

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Disclosure
The authors report no conflicts of interest in this work.

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