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ORIGINAL ARTICLE

Clinical features and outcomes of *ALK* rearranged non-small cell lung cancer with primary resistance to crizotinib

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Abstract

Background: Crizotinib is associated with a favorable survival benefit in patients with *ALK*-positive non-small cell lung cancer (NSCLC); however, a subset of patients harboring *ALK* rearrangement shows a poor response.

Methods: We collected the clinical features and survival outcomes of 28 primary-resistant responders (PRR) with progression-free survival (PFS) of < 3 months on crizotinib and compared these with 78 long-term responders (LTR) that achieved > 24 months PFS (control).

Results: Primary resistance was observed in 6.5% of the patients. The median PFS of the PRR and LTR groups was 1.2 months (95% confidence interval [CI] 0.70–1.73) and 47.0 months (95% CI 34.39–59.64), respectively. A better Eastern Cooperative Oncology Group performance status score was significantly associated with longer PFS (odds ratio 0.06, 95% CI 0.01–0.33; $P = 0.001$). The median overall survival (OS) of the PRR group was 8.4 months (95% CI 3.47–13.42) and crizotinib as first-line treatment was an independent predictive factor for survival outcome ($P = 0.005$). Patients administered ALK-tyrosine kinase inhibitors after crizotinib progression had significantly longer survival than the PRR group treated with best supportive care ($P = 0.007$), but no significant difference was found between ALK-tyrosine kinase inhibitor treatment and single chemotherapy ($P = 0.944$).

Conclusion: Patients with primary resistance to crizotinib displayed unfavorable survival outcomes and the underlying mechanism cannot be identified in clinical features. Nevertheless, next-generation ALK inhibitors and chemotherapy after crizotinib progression could confer a therapeutic and survival benefit in this population.

Introduction

Lung cancer is the leading cause of cancer-related death worldwide.[1] Approximately 40% of patients with non-small cell lung cancer (NSCLC) present with metastatic or locally advanced disease.[2] Over the past decade, therapies have been developed to inhibit irregular oncogenic pathways in lung cancer and these pathways represent promising potential targets for antitumor therapy. Indeed, the development of targeted therapy, such as EGFR and ALK tyrosine kinase inhibitors (TKIs), has led to different molecular pathology classifications in terms of targeted therapies for lung cancer.

ALK gene rearrangements are found in approximately 3–7% of NSCLC patients.[3] Treatment with crizotinib, the

first-generation small molecule inhibitor of ALK kinase activity, has yielded high objective response rates (ORRs) of > 60% and median progression-free survival (PFS) of seven months to one year in advanced *ALK*-positive NSCLC patients, based on a series of PROFILE clinical trials.[4–8] Although most patients with *ALK*-positive NSCLC experience a substantial clinical benefit from crizotinib, disease relapse is inevitable as a result of acquired resistance. The mechanisms of acquired resistance have previously been reported, including secondary mutations in the ALK tyrosine kinase domain, copy number alterations of the *ALK* fusion gene, upregulation of bypass signaling pathways, and limited penetration of the central nervous system.[9–11] However, despite the presence of *ALK* rearrangement, approximately 30% of patients do not respond to ALK-TKIs and the underlying mechanisms of such poor responses have not been fully elucidated.[12,13] First-line TKI treatment failure is rarely reported, contributing to the lack of comprehensive information about the clinical features and treatment outcome of this subset of *ALK*-positive NSCLC patients.

We evaluated the clinical features and survival outcomes of primary-resistant responders (PRR, PFS < 3 months) to crizotinib compared to long-term responders (LTR, PFS > 24 months). We also analyzed the effects of subsequent treatments on survival following crizotinib progression and explored the possible mechanisms for primary resistance.

Methods

Patient eligibility and treatment

We retrospectively reviewed the medical records of 428 patients who were histologically or cytologically diagnosed with locally advanced, recurrent, or metastatic NSCLC at five cancer centers in China between January 2013 and November 2017 (each center enrolled at least 10 eligible patients). All enrolled patients tested *ALK*-positive and received at least 21 days of crizotinib treatment. *ALK* translocation was determined by one of following methods: fluorescence in situ hybridization (FISH) assay, Ventana immunohistochemistry, anti-ALK (D5F3), or real-time reverse transcription PCR. In this study, crizotinib was administered at a dose of 250 mg twice daily, and proper dose adjustments were made. Clinical responses were evaluated one month after the first administration of crizotinib and then approximately every two months during crizotinib treatment until drug withdrawal. Routine hematology tests, biochemistry analyses, and electrocardiograms were also performed. The ethics committee of the Cancer Hospital, Chinese Academy of Medical Sciences, approved the study.

Data extraction

The demographics and clinical characteristics of the enrolled patients were collected, including age, gender, tumor stage, histological type, Eastern Cooperative Oncology Group performance status (ECOG PS) score, smoking history, and previous treatment regimens. The authors independently reviewed imaging data to evaluate the best treatment response and disease progression according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Survival information was obtained from clinical records or telephone follow-up by investigators at each center.

Definitions and study endpoints

The study endpoints were PFS (from crizotinib initiation to the first RECIST-defined progression or death from any cause) and OS (from crizotinib initiation to death or the last follow-up). Patients that had not progressed at the time of analysis were censored at the date of their last contact with our institution. The groups were determined based on their tumor response to crizotinib treatment. The PRR group included patients who experienced disease progression within three months without any evidence of an objective response while receiving crizotinib treatment, while patients with PFS > 24 months were categorized as the LTR group. The subsequent drugs administered following crizotinib failure were also monitored.

Statistical analysis

All analyses were carried out at the final follow-up date (30 November 2017) using SPSS version 19.0. For categorical variables, differences between the PRR and LTR groups were compared using two-sided Fisher's exact or chi-square tests. The Kaplan–Meier method was applied to estimate survival curves for OS, and the log-rank test was performed to compare the survival outcomes between different subgroups. Cox proportional hazard models were used to evaluate independent prognostic factors and the differences in PFS were assessed by multivariate logistic regression. The results were presented as odds ratios (ORs) for logistic regression or hazard ratios (HRs) for Cox regression with their corresponding 95% confidence intervals (CIs). A two-sided P value of < 0.05 was considered to indicate statistical significance.

Results

Patient characteristics and treatments

Primary resistance was observed in 6.5% of the patients (28/428). The cohort included 11 (39.3%) women and 17 (60.7%) never smokers. The median age of the PRR group was 52 years (range: 24–69) and all patients were at

Table 1 Baseline characteristics of the population at the time of crizotinib initiation (n = 106)

Characteristics, n (%)	All patients	PRR group	LTR group	*P*
Age, years	50 ± 10.7	52 ± 9.7	49 ± 10.9	
< 60	83 (78.3)	20 (71.4)	63 (80.8)	0.304
≥ 60	23 (21.7)	8 (28.6)	15 (19.2)	
Gender				
Male	56 (52.8)	17 (60.7)	39 (50.0)	0.330
Female	50 (47.2)	11 (39.3)	39 (50.0)	
Smoking status				
Never smoker	72 (67.9)	17 (60.7)	55 (70.5)	0.341
Former smoker	34 (32.1)	11 (39.3)	23 (29.5)	
Histology				
Adenocarcinoma	100 (94.3)	25 (89.3)	75 (96.2)	0.383
Other†	6 (5.7)	3 (10.7)	3 (3.8)	
ECOG PS				
0–1	96 (90.6)	20 (71.4)	76 (97.4)	< 0.001
2–3	10 (9.4)	8 (28.6)	2 (2.6)	
Line of therapy before crizotinib				
0	64 (60.4)	13 (46.4)	51 (65.4)	0.079
≥ 1	42 (39.6)	15 (53.6)	27 (34.6)	
Metastasis site				
Brain	25 (23.6)	8 (28.6)	17 (21.8)	0.469
Lung	47 (44.3)	11 (39.3)	36 (46.2)	0.530
Pleural	42 (39.6)	10 (35.7)	32 (41.0)	0.622
Liver	11 (10.4)	6 (21.4)	5 (6.4)	0.061
Bone	28 (26.4)	10 (35.7)	18 (23.1)	0.193
Lymph node	64 (60.4)	19 (67.9)	45 (57.7)	0.346
Others‡	8 (7.5)	1 (3.6)	7 (9.0)	0.609

†Includes squamous, adenosquamous, and large cell carcinomas. ‡Includes adrenal and subcutaneous metastases. BM, brain metastasis; ECOG PS, Eastern Cooperative Oncology Group performance status; LTR, long-term responder; NSCLC, non-small cell lung cancer; PRR, primary-resistant responder.

stage IV disease at crizotinib initiation. Most of the patients with primary resistance had good ECOG PS of 0–1 (20/28, 71.4%); 89.3% (25/28) of patients had adenocarcinoma; and 8 (28.6%) patients had brain metastasis at baseline. Thirteen (46.4%) patients received crizotinib as a first-line regimen. A total of 78 patients with PFS > 24 months were included in the LTR group. The baseline clinical characteristics of the groups are summarized in Table 1.

The demographic and clinical features were compared between the groups, and multivariate analyses of logistic regression revealed that better ECOG PS was significantly associated with longer PFS (OR 0.06, 95% CI 0.01–0.33; P = 0.001). Other features, including age (P = 0.814), gender (P = 0.722), brain metastases (P = 0.805), and lines of crizotinib treatment (P = 0.308), were not significantly different between the groups (Table 2).

Table 2 Logistic regression analysis of factors associated with primary resistance and long-term PFS underlying crizotinib treatment

Risk factor	OR	95% CI	*P*
Age			
< 60 (Reference)	0.87	(0.26–2.85)	0.814
≥ 60			
Gender			
Male (Reference)	1.27	(0.34–4.67)	0.722
Female			
Smoking status			
Never smoker (Reference)	0.53	(0.15–1.91)	0.334
Former smoker			
ECOG PS			
0–1 (Reference)	0.06	(0.01–0.33)	0.001
2–3			
Brain metastasis			
No (Reference)	0.87	(0.29–2.62)	0.805
Yes			
Line of therapy before crizotinib			
0 (Reference)	0.60	(0.22–1.61)	0.308
≥ 1			

CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; OR, odds ratio; PFS, progression-free survival.

Survival analysis

The median PFS of the PRR and LTR groups was 1.2 months (95% CI 0.70–1.73, range: 0.9–3.0) and 47.0 months (95% CI 34.39–59.64, range: 24.2–53.3),

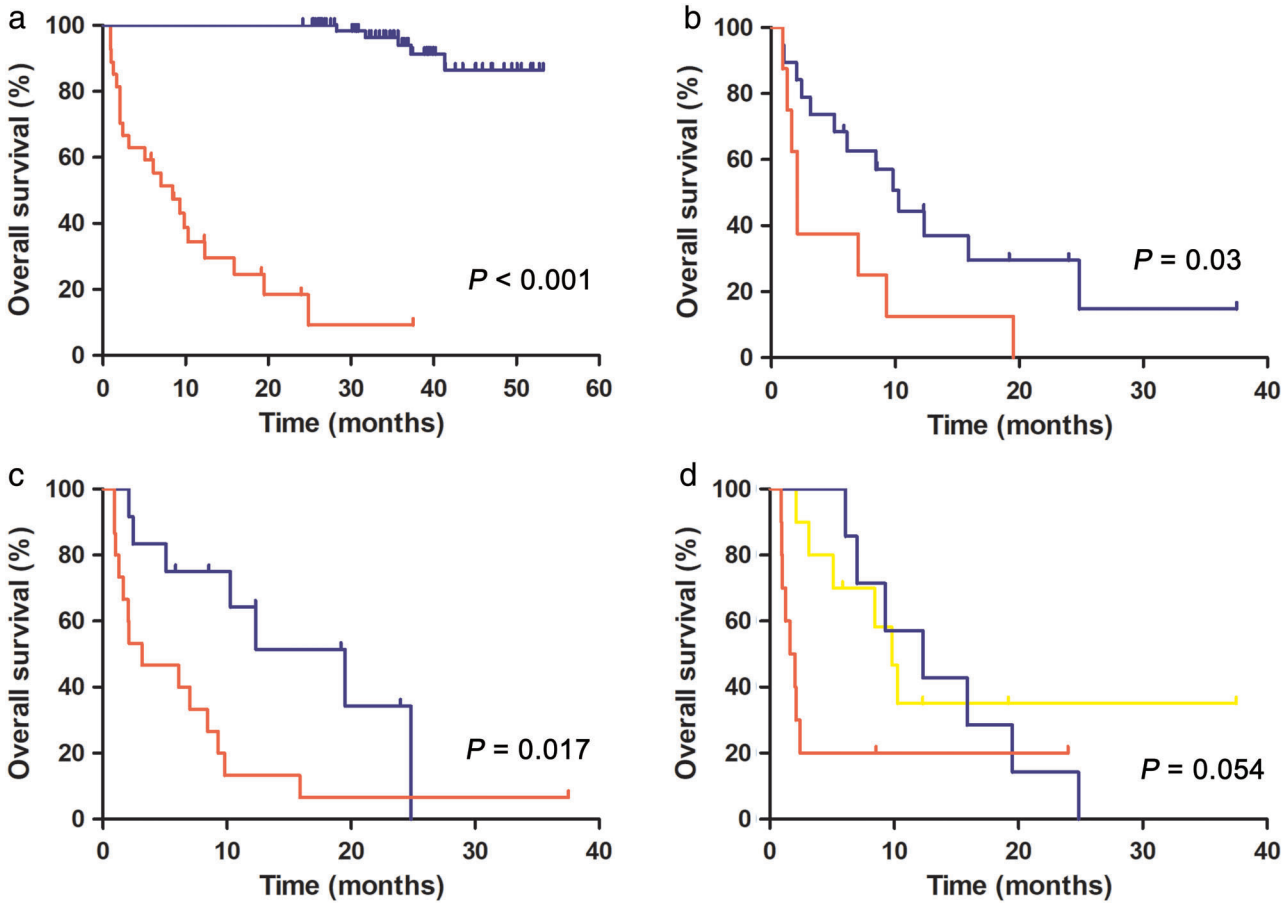

Figure 1 Overall survival (OS) of patients with *ALK*-positive non-small cell lung cancer (NSCLC) treated with crizotinib. Kaplan–Meier curves of OS (**a**) in the primary-resistant responder (PRR) versus long-term responder (LTR) group, and (**b–d**) in the PRR group according to Eastern Cooperative Oncology Group performance status (ECOG PS) score, line of crizotinib treatment, and subsequent therapy after crizotinib progression, respectively, including *ALK* inhibitors (ALKis), chemotherapy, and best supportive care (BSC). —— PRR group, —— LTR group, —— ECOG 0-1, —— ECOG 2-3, —— First line, —— Non-first line, —— ALKis, —— Chemotherapy, —— BSC.

respectively. The median OS of the PRR group was 8.4 months (95% CI 3.47–13.42) and 6 patients were still alive at the last follow-up. Notably, OS was significantly shorter in the PRR than in the LTR group (8.4 months vs. not reached; $P < 0.001$) (Fig 1a).

We further analyzed the effects of clinical factors on OS in the PRR group. A log-rank test demonstrated that better ECOG PS ($P = 0.030$) (Fig 1b) and first-line crizotinib treatment ($P = 0.017$) (Fig 1c) were significantly associated with favorable survival outcomes. Multivariate Cox analyses revealed that crizotinib as a first-line regimen was an independent predictive factor of OS in *ALK*-positive patients with primary resistance (HR 5.24, 95% CI 1.64–16.74; $P = 0.005$) (Table 3).

Six patients (21.4%) in the PRR group continued crizotinib after disease progression, four of which experienced disease re-progression. Four patients were administered next-generation ALK-TKIs and nine patients were administered chemotherapy as the first subsequent regimen after crizotinib withdrawal. The ORRs of next-generation ALK-TKIs and chemotherapy were 25.0% (1 partial response [PR], 2 stable disease [SD], and 1 progressive disease [PD]) and 11.1% (1 PR, 5 SD, 2 PD, and 1 unassessable), and the median PFS rates were 2.9 months (95% CI 0.61–5.11) and 4.0 months (95% CI 1.56–6.39), respectively. Statistical analysis showed that patients administered ALK-TKIs (including crizotinib and next-generation ALK inhibitors [ALKis], $n = 10$, median OS 9.8 months, 95% CI 7.26–12.39) had significantly better survival outcomes than those treated with best supportive care ($n = 11$, median OS 1.6 months, 95% CI 0.47–2.81), but there was no significant difference to patients who received single chemotherapy ($n = 7$, median OS 12.3 months, 95% CI 4.56–20.08) (Fig 1d, Table 3).

Table 3 Cox multivariate analysis of survival from the first crizotinib dose in patients with primary resistance (n = 28)

			Univariate analysis			Multivariate analysis		
Variables	Median OS (95% CI)	Log-rank test	HR	95% CI	*P*	HR	95% CI	*P*
Age								
< 60 (Reference)	9.3 (4.19–14.40)	0.146	2.02	(0.77–5.32)	0.155			
≥ 60	1.6 (0.72–2.57)							
Gender								
Male (Reference)	7.0 (2.43–11.57)	0.911	0.95	(0.4–2.29)	0.911			
Female	9.8 (0–21.53)							
Smoking status								
Never smoker (Reference)	7.0 (0–14.73)	0.532	0.75	(0.30–1.86)	0.535			
Former smoker	8.4 (1.22–15.66)							
ECOG PS								
0–1 (Reference)	10.3 (6.88–13.69)	0.03	2.63	(1.06–6.53)	0.037	2.77	(0.90–8.50)	0.075
2–3	2.1 (1.49–2.72)							
Brain metastases								
No (Reference)	8.4 (4.40–12.49)	0.402	0.65	(0.24–1.80)	0.406			
Yes	3.2 (0.00–19.51)							
Line of therapy before crizotinib								
0 (Reference)	19.5 (8.81–30.23)	0.017	2.93	(1.16, 7.39)	0.023	5.24	(1.64, 16.74)	0.005
≥ 1	3.2 (0–8.30)							
Subsequent therapy after crizotinib PD								
ALKis (Reference)	9.8 (7.26–12.39)	0.054			0.069			0.007
Chemotherapy	12.3 (4.56–20.08)		1.26	(0.42–3.77)	0.682	0.96	(0.27–3.42)	0.944
BSC	1.6 (0.47–2.81)		3.31	(1.11–9.86)	0.032	6.98	(1.71–28.58)	0.007

ALKis, ALK inhibitors; BSC, best supportive care; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; OS, overall survival; PD, progressive disease.

Next-generation sequencing

Next-generation sequencing was performed in two patients in tumor or blood plasma specimens. New additional intracranial involvement was discovered in a 58-year old man treated with crizotinib as first-line treatment after 2.6 months. The baseline lymph node formalin-fixed and paraffin-embedded specimen was detected as FISH-positive and a 509-panel NGS platform was used to analyze genomic profiling in the baseline tumor and plasma. The tumor specimen harbored *EML4_E13*:*ALK_E19* fusion and the matched blood plasma was detected with a novel *LTBP1_E2*:*ALK_E11* fusion variant with *EML4_E13*:*ALK_E19* and concomitant *L1196M* mutation at disease progression. Another patient, a 24-year old woman administered crizotinib as a second-line regimen, developed intracranial progression within one month. The baseline lymph node was detected as Ventana immunohistochemistry-positive and the NGS results of baseline blood plasma showed a *TP53* mutation concurrent with *EML4_E20*:*ALK_E19* fusion.

Discussion

Currently, little data is available concerning primary resistance to ALK-TKIs. To our knowledge, our study is the first to include a relatively large sample to investigate the clinical features and survival outcomes of *ALK*-positive NSCLC patients with a poor response to crizotinib. Primary resistance was observed in 6.5% of the patients, which is consistent with 5–7% reported in previous crizotinib trials.[4,6,7] The clinical characteristics of the *ALK*-positive patients with primary resistance in our study were similar to the general *ALK* population (i.e. adenocarcinoma histology, never smokers, and young age).[14]

Because long-term PFS can be translated into favorable survival prognoses, it is critically important to identify factors that can effectively discriminate PRRs from LTRs prior to crizotinib treatment. However, the results of our study showed no specific baseline clinicopathologic factors between the groups, with the exception of ECOG PS. Thus, our findings emphasize the need for further studies investigating reliable biomarkers that can predict the therapeutic efficacy of ALK-TKIs and explore the mechanisms underlying primary resistance.

Studies on the mechanism of primary resistance are relatively rare. Several studies have revealed that concurrent gene alterations in *ALK*-rearranged tumors may negatively impact the PFS of crizotinib in patients with *ALK*-rearranged NSCLC. Yu *et al.* demonstrated that concurrent *ALK* activation mutations were more common in patients

administered multiple lines of TKI treatment compared to single agent crizotinib, and a co-existing *TP53* mutation was correlated to unfavorable survival in *ALK*-positive NSCLC patients treated with crizotinib.[15] We also found a *TP53* mutation in the baseline blood plasma of one patient with primary resistance, which might be related to her poor response to crizotinib. Furthermore, a small number of case reports revealed that the intrinsic factors in *ALK*-rearranged lung cancer cells, such as *KRAS* mutations,[16,17] *MYC* amplification[18] and the *Bim* deletion polymorphism,[19] might be responsible for primary resistance to crizotinib. Different *EML4-ALK* translocation variants might generate a distinct response to ALKis[3,20] and the significance of diverse *ALK* fusion partners has not yet been fully elucidated.[21–24] In our study, a novel *LTBP1-ALK* fusion was detected by NGS technology at the baseline specimen of a patient with primary resistance, and his "gold standard" FISH assay result was *ALK*-positive. This fusion gene has not been reported previously, but an EML4-ALK fusion with atypical LTBP1 insertion could responded well to crizotinib.[25] In future research, we will explore the underlying mechanism for this result. Because NGS technology has displayed impressive capability for identifying the underlying molecular profile of cancers, its clinical application as a molecular screening test might favorably alter the clinical outcomes of *ALK*-positive NSCLC patients.[26]

To further explore the therapeutic options that can overcome primary resistance to crizotinib, we investigated the clinical efficacy of subsequent therapies after crizotinib failure and analyzed their impacts on survival outcomes. The results demonstrated that *ALK*-positive patients with primary resistance to crizotinib can obtain therapeutic and survival benefits from second-generation ALKis or chemotherapy, and no significant differences were found between these two regimens. Previous research has indicated that next-generation ALKis, such as alectinib, ceritinib, and lorlatinib, show a favorable response to crizotinib resistance.[27–30] These ALK-TKIs can overcome the acquired resistance caused by "ALK-dependent" alterations, such as *ALK* tyrosine domain mutations or amplification of the *ALK* gene. However, the efficacy of novel ALKis in patients with primary resistance to crizotinib has not been fully documented. Facchinetti *et al.* first reported a case of an *ALK*-rearranged NSCLC patient with primary resistance to crizotinib who experienced a partial and durable response to ceritinib.[31] Preclinical evidence has also confirmed the potential benefit of ceritinib in overcoming crizotinib-resistant mutations.[27] It may therefore be presumed that the presence of resistance mutations in the *ALK* kinase domain at baseline may give rise to the lack of crizotinib efficacy, which can be successfully interrupted by the more potent compound next-generation ALKis. However, other resistance mechanisms, including pharmacokinetic issues and interpatient variability in drug bioavailability, may also have an impact on crizotinib efficacy. The current availability of a wide spectrum of ALK-TKIs makes it imperative to obtain multiplex molecular genetic profiling for lung cancer before making final therapeutic decisions.

There were some limitations to the current study that cannot be ignored. First, this was a retrospective study. The patient sample was small and the characteristics of the groups were partially imbalanced because of selection bias. Second, limited patients received sequencing treatment after crizotinib progression, and we were not able to compare the efficacy of different ALKis and chemotherapy regimens. Given the crossover of ALKis and chemotherapy in subsequent treatment, the results on survival outcomes need to be interpreted with caution. Third, only two patients had adequate specimens to conduct NGS testing, thus the association between gene alterations and primary resistance to crizotinib remains unknown.

In conclusion, this study demonstrated that clinical variables cannot successfully predict the survival outcomes of patients with primary resistance to crizotinib treatment. This subset of patients can obtain therapeutic and survival benefits from next-generation ALKis and chemotherapy. The mechanism of primary resistance to crizotinib requires further investigation, and NGS technology might be a good complementary method for screening genetic alterations and making final treatment decisions.

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Disclosure

No authors report any conflict of interest.

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