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Effect of ALK-inhibitors in the treatment of non-small cell lung cancer: a systematic review and meta-analysis

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Abstract. – OBJECTIVE: Lung cancer is the leading cause of cancer-related mortality. Over 80% of all lung cancer cases are non-small-cell lung cancer (NSCLC) and approximately 5% of NSCLC patients are positive for anaplastic lymphoma kinase (ALK) gene rearrangement or fusion with echinoderm microtubule-associated protein-like 4 (EML4). NSCLC patients with positive ALK-EML4 gene fusion are highly sensitive to ALK-inhibitors. While the efficacy of the ALK-inhibitors in the treatment of NSCLC has been consistently reported, a limited number of randomized, large-scale clinical trials have been reported. The current study was, therefore, designed to systematically review and appraise current knowledge and conduct a meta-analysis on phase I, II, and III clinical trials in which ALK-inhibitors were used to treat NSCLC.

MATERIALS AND METHODS: The PubMed online database was thoroughly searched. A total of 26 articles were included in a qualitative systematic review, and four of them were used to conduct the quantitative meta-analysis.

RESULTS: We found that ALK inhibitors significantly improved the overall survival (OS) and progress free survival (PFS) of NSCLC patients, especially of ALK or ROS1 gene fusion-positive cases. ALK inhibitors contributed to better therapeutic outcomes regarding increased one-year and two-year OS, PFS, and ORR (Odds ratio: 4.393, 95% CI: 3.302-5.845, $p < 0.001$). Visual disturbance was the most common side effect observed in the patients treated with crizotinib, whereas mild gastrointestinal reactions, such as diarrhea and nausea, were most frequent in the patients treated with the 2nd generation of ALK inhibitors.

CONCLUSIONS: ALK inhibitors are safe and effective in the treatment of NSCLC patients, especially those with positive ALK-EML4 gene fusion or rearrangement.

Key Words:
NSCLC, Anaplastic lymphoma kinase, Echinoderm microtubule-associated protein-like 4, Meta-analysis.

Introduction

Lung cancer remains the leading cause of cancer-associated mortality, with non-small-cell lung cancer (NSCLC) accounting for 85-90% of the cases[1]. Genetic alterations of the anaplastic lymphoma kinase (ALK) gene have been reported in 2-7% of the patients with NSCLC[2,3], and the most common alteration of ALK is the fusion of the *ALK* gene with the Echinoderm microtubule-associated protein-like 4 (*EML4*) gene[4,5]. NSCLC with positive *ALK-EML4* gene fusion is highly sensitive to ALK inhibition by molecules designed to target tyrosine kinases.

The *ALK* gene rearrangements or fusion of *ALK-EML4* genes in NSCLC can be detected in clinical samples using several techniques, primarily fluorescence in situ hybridization (FISH), reverse transcriptase polymerase chase reaction (RT-PCR), and immunohistochemistry (IHC). The methods for detection of *ALK* gene fusion or rearrangement have been rapidly developed, and ALK-inhibitors have been recently approved to use in clinic and are increasingly applied for the treatment of ALK-positive NSCLC patients.

Crizotinib, the first generation of ALK-targeted molecule, is an oral tyrosine kinase inhibitor (TKI) that has been shown to improve overall survival (OS), progression-free survival (PFS), and objective response rate (ORR) in NSCLC patients compared with standard second-line

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chemotherapy[6]. Crizotinib was approved for use in clinic for the treatment of ALK-positive NSCLC patients in the U.S. within four years of its discovery. It has subsequently been approved in many European countries, and effective outcomes from phase I and II clinical trials are consistently reported[4,7-9]. However, acquired resistance to crizotinib could be developed within one year of application of this molecule in most NSCLC patients. To overcome the acquired resistance to crizotinib, a second generation of ALK inhibitors, such as ceritinib and alectinib, have recently been developed and approved for use in clinical settings. These second generation of ALK inhibitors are not only highly selective, but also more potent than crizotinib in their activity against ALK [10].

While consistent efficacy findings from phase I and II studies have been reported, a limited number of randomized clinical trials on the use of ALK-inhibitors to treat NSCLC have been reported. The current review is, therefore, designed to systematically review the scientific literature and summarize the outcomes of phase I and II clinical studies as well as to conduct a meta-analysis on limited reports of phase III clinical studies.

Materials and Methods

Data Sources

We searched the relevant literature published up to July 2016 on PubMed site of using the following phrases: "anaplastic lymphoma kinase", OR "ALK inhibitor", AND "lung cancer", AND "clinical trial" OR "clinical study". The search was limited to English-language publications.

Inclusion Criteria

To be included in the current systematic review, each study had to meet the following criteria: (1) It was a phase I, II, or III clinical study on the treatment of lung cancer with an ALK inhibitor regardless of whether it was first- or second-generation ALK inhibitor; (2) The study was a full-text article.

Data Extraction

Information was carefully extracted from all included literature sources. The following data was collected: study title, name of the first author, year of publication, study design, total number of cases treated with ALK inhibitors (crizotinib or ceritinib), median survival months, one-year survival rate, 2-year survival rate, and adverse effects of ALK inhibitors.

Statistical Analysis

For the data entry, event number treated with an ALK inhibitor, total number of the cases treated with an ALK inhibitor, event number treated without ALK inhibitor, and the total number of the cases treated without an ALK inhibitor was used. The effect of the treatment with an ALK inhibitor on NSCLC outcome was determined by the odds ratio and 95% confidence interval (95% CI). A fixed-effects model was adopted for statistical analysis when no heterogeneity was observed among the studies; otherwise, a random-effect model was applied. The heterogeneity between studies was assessed by the Q-test and I^2 statistic, and values of $p < 0.10$ and $I^2 > 50\%$ were considered to indicate heterogeneity between the studies[11]. The meta-analysis was performed using the Comprehensive Meta-analysis software (Version 3, NJ, USA).

Results

Features of the Clinical Studies

The processes of literature screening and final selection of the articles are outlined in Figure 1. After careful reading of the Abstract sections of the initially selected publications, a total of 60 full-text articles were retrieved. The retrieved full-text articles were then independently asses-

Table I. Studies on the methods of ALK detection.

Authors	Year	Country	Detection methods	No. of samples	References
Cutz, JC	2014	Canada	FISH and IHC comparison	28	30
Fu, S	2015	China	FISH, qRT-PCR, sequencing	173	26
Zwaenepoel, K	2015	Italy	Automation of FISH assay	40	28
Ma, D	2016	China	FISH and IHC comparison	6	27

FISH: Fluorescence in-situ hybridization; qRT-PCR: quantitative reverse transcriptase polymerase chain reaction; IHC: immunohistochemistry.

Table II. Study designs included in qualitative synthesis.

Authors	Year	Country	Study design	ALK-inhibitor	No. of patients	References
Kwak, EL	2010	USA	Phase I	crizotinib	82	4
Mosse, YP	2013	USA	Phase I	crizotinib	79	7
Seto, T	2013	Japan	Phase I and II	CH5424802	70	23
Sargis, RM	2015	USA	?	crizotinib	7	15
Shaw, AT	2014	USA	Phase I	crizotinib	50	14
Costa, DB	2015	USA	Retrospective	crizotinib	888	9
Mazieres, J	2015	France	Retrospective	crizotinib	31	31
Gadgeel, SM	2014	USA	Phase I and II	alectinib	47	16
Nishio, M	2015	Japan	Phase I	ceritinib	20	24
Kim, DW	2016	USA	Phase I	ceritinib	255	17
Li, T	2016	USA	Phase I	ASP3026	33	18
Shaw, AT	2016	USA	Phase I	alectinib	87	25

Table III. Studies on resistance of ALK-inhibitors.

Authors	Year	Country	Mechanism of resistance	Overcome resistance	References
Katayama, R	2011	USA	EML4-ALK gene mutation	2nd-generation ALK-TKIs or Hsp90 inhibitors	20
Ceccon, M	2014	Italy	Point mutation		29
An, R	2016	USA		CRKL silence or inhibition	21
Dong, XY*	2016	USA	Overexpression of p-ALK p-EGFR, p-HER3, p-IGFR-1R Upregulation of NRG1	Combination of ALK inhibitor and afatinib	22
Katayama, R*	2016	Japan	p-glycoprotein (P-gp/ABCB1) overexpression	Combination of P-gp and ALK inhibitor	25
Lin, YT	2016	Taiwan	L1196M and 2nd domain mutation		32

*Resistance to 2nd-generation ALK inhibitors; CRKL: CRK-like protein, downstream of ALK signaling; HER3: human epidermal growth factor receptor3; NRG1: neuregulin1, is a ligand for HER3

sed by two investigators (GL and WD). Of the 60 full-text articles, 34 articles, consisting mostly of case reports and review articles, were excluded, while 26 articles were included for systematic review (qualitative synthesis), and four articles were included in the meta-analysis (quantitative synthesis)[6,12-14]. Among the 26 articles included for systematic review and meta-analysis, 13 articles were from the USA[4,7,9,12,14-22], three of the articles reported on studies performed in multiple countries[6,13,14], three articles were from Japan[23-25], two articles were from China[26,27], two articles were from Europe[28,29], one article was from Canada[30], one article was from France[31], and one article was from Taiwan[32]. Of them, four articles reported on *ALK* gene fusion detection methods (Table I), 12 articles were in phase I and/or II studies or retrospective analyses (Table II), six articles concerned studies on resistance to ALK inhibitors (Table III), and four articles reported in phase III clinical studies (Table IV).

Efficacy of ALK Inhibitors

Of the 26 articles selected for our systematic review, four were phase III clinical trials or compared the efficacy of treatment with an ALK inhi-

Table IV. Randomized clinical studies.

First author	Year	Country	No. of cases	ALK inhibitor	References
Shaw, AT	2011	USA	438	crizotinib	12
Shaw, AT	2013	USA	347	crizotinib	6
Solomon, BJ	2014	Multi-country	343	crizotinib	13
Shaw, AT	2014	USA	130	ceritinib	14

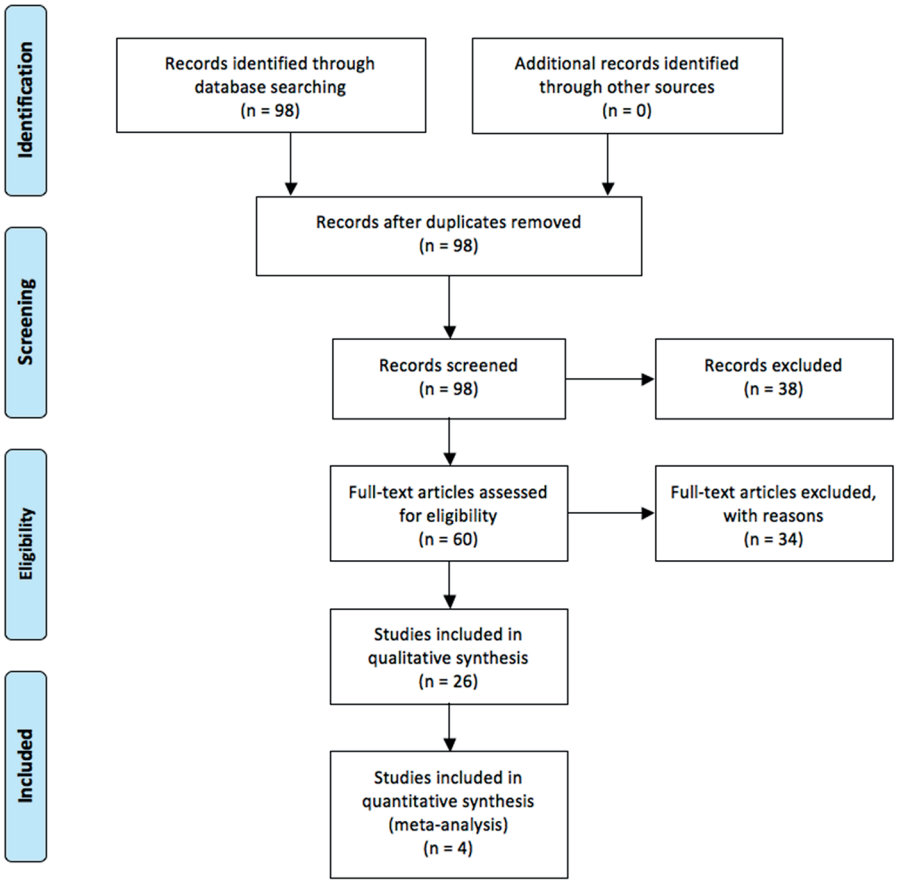

Figure 1. Flow diagram of literature search and eligible publication selection.

bitor *vs.* systemic chemotherapy, 12 studies were phase I and/or II clinical trials with one-arm, open-labeled for ALK-inhibitors without comparison with a control or chemotherapy treatment. All results of the one-arm, open-labeled phase I or II studies indicated that the first and second generation of ALK inhibitors (crizotinib, ceritinib, and alectinib) significantly improved the overall survival (OS) and progress free survival (PFS), of patients with non-small cell lung cancer especially in the patients with positive *ALK* or *ROS1* gene fusion. However, the data of phase I or II studies were not included in the meta-analysis due to the absence of a proper control group.

The results of the four phase III studies showed that the average of the median survival length was significantly greater in the patients treated with ALK-inhibitors than in the patients without therapy with ALK inhibitors[6,12-14]. However, quantitative comparison of median survival length was not conducted by this meta-analysis since it was "not reached" in the ALK-inhibitor-treated groups in three out of the four studies. Nevertheless, a meta-analysis was conducted to compare the OS, PFS, or objective response rate (ORR) between the two groups of patients treated with or without ALK inhibitors. It is noteworthy that ALK inhibitors were found to have exerted positive effects on the improved therapeutic outcome regarding one-year OS, two-year OS, PFS, and ORR (Odds ratio: 4.393, 95% CI: 3.302-5.845, $p < 0.001$, Figure 2).

Side Effects of ALK-Inhibitors

Of the 26 articles included in the current systematic review and meta-analysis, seven studies reported adverse effects of crizotinib[4,6-8,13,14,23], and four studies reported adverse effects of 2nd-generation ALK inhibitors[16,17,19,24]. As shown in Table V, visual disturbance was the most common side

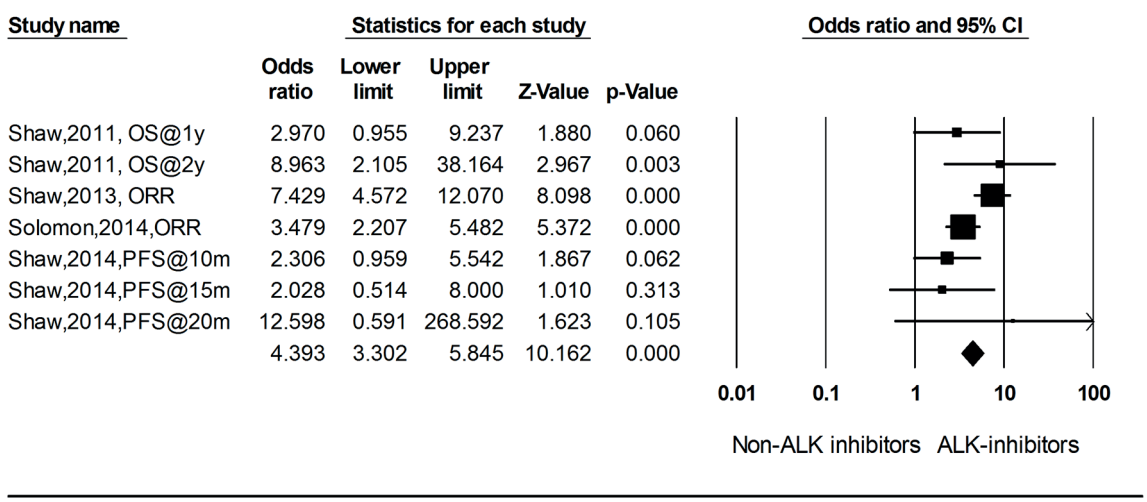

Figure 2. Forest plot for therapeutic effect of ALK-inhibitors. The following parameters were used to perform the meta-analysis: one-year and two-year overall survival (OS) extracted from the study conducted by Shaw et al (2011) on crizotinib used as 2nd- or 3rd-line therapy *vs.* any 2nd-line chemotherapy treatment; objective response rate (ORR) obtained by Shaw et al (2013) in the study of oral crizotinib *vs.* pemetrexed or docetaxel; ORR obtained by Solomon et al (2014) in the study of oral crizotinib administration *vs.* chemotherapy; progress-free survival (PFS) at 10, 15, and 20 months established by Shaw et al (2014) in the study of ceritinib application in the non-prior crizotinib treatment *vs.* prior treatment of crizotinib. Overall odds ratio (OR) was 4.393 with 95% CI: 3.302-5.845, $p < 0.001$. A fixed model was used.

effect of crizotinib (55 ± 11%, reported in five of the seven studies), followed by diarrhea (44 ± 20%, reported in six of the seven studies), nausea (41 ± 9%, reported in all of these seven studies), vomiting (39 ± 7%, reported in six of the seven studies), peripheral edema (34 ± 7%, reported in four of the seven studies), and constipation (31 ± 5%, reported in six of the seven studies). Other side effects of crizotinib included elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine, as well as presence of dysgeusia, , abdominal pain, decreased appetite, fatigue, dizziness, rash, neutropenia, dyspnea, anemia, hypophosphatemia, and lymphopenia. As can be seen in Table VI, the most common adverse effect caused by 2nd-generation ALK inhibitors (alectinib and ceritinib) was diarrhea (61 ± 20%, reported in three of the four studies), followed by nausea (54 ± 21%, reported in the four studies), decreased appetite (44 ± 6%, reported in two of the four studies), creatinine elevation (39 ± 22%, reported in two of the four studies) and fatigue (37 ± 3%, reported in the four studies). Other adverse effects include vomiting, constipation, ALT elevation, cough, abdominal pain, myalgia, peripheral edema, AST elevation, dyspnea, headache, dizziness, rash, and neutropenia.

Discussion

The fusion of *ALK* with *EML4* is a common genetic alteration in NSCLC. ALK-positive tumors are highly sensitive to ALK inhibition, and thus,

Table V. Adverse events of crizotinib.

Adverse Event	% (Mean ± SEM)	References
Visual disturbance	55 ± 11	4,6,7,13,14
Diarrhea	44 ± 20	4,6-8,14
Nausea	41 ± 9	4,6,7,13,14,23
Vomiting	39 ± 7	4,6-8,13,14
Peripheral edema	34 ± 7	4,6,8,13
Constipation	31 ± 5	4,6,7,13,14,23
ALT elevation	25 ± 10	4,7,8,14,23
Dysgeusia	25 ± 3	6,13,14,23
AST elevation	24 ± 9	4,7,8,14,23
Creatinine elevation	24 ± 2	7,23
Abdominal pain	23 ± 3	7,13
Decreased appetite	22 ± 9	4,13
Fatigue	21 ± 4	4,6,7,13,14
Dizziness	18 ± 2	4,6,8,14
Rash	18 ± 9	6,23
Neutropenia	16 ± 5	4,7,8,13,23
Dyspnea	16 ± 3	6,13
Anemia	15 ± 6	7,13
Hypophosphatemia	8 ± 7	4,14
Lymphopenia	7 ± 5	4,7

it is an important target for anticancer drug therapies in NSCLC. The current study systematically reviewed phase I, II, and III clinical studies and found that ALK-inhibitors significantly improved the overall survival (OS) and progress free survival (PFS) of non-small cell lung cancer patients as evidenced by the results of the meta-analysis of the phase III studies. Treatment with ALK-inhibitors was specifically favored in the patients that were positive for fusion of *ALK* or *ROS1* genes. However, more than 50% of the patients treated with crizotinib had visual disturbance and over 50% of the participants treated with 2nd-generation ALK inhibitors (ceritinib or alectinib) had gastrointestinal adverse effects, such as diarrhea and nausea.

Previous researches[2,3] reported that 3-7% of NSCLC patients had ALK and/or MET fusion or mutation. With the discovery of the abnormal fusion of *ALK-EML4* genes, crizotinib, an oral tyrosine kinase inhibitor (TKI), has been developed and approved for use in clinic. In this regard, in a multicenter phase I study, Shaw et al[8] reported that crizotinib was associated with an objective response rate (ORR) of 72%, a median duration of response of 17.6 months, and a median PFS of 19.2 months among 50 patients with advanced *ROS1* rearrangement NSCLC. Also, several phases I and II clinical investigations[4,7-9,15,23] have also reported a reduction in key lung cancer-related symptoms including cough, pain, and dyspnea. Consistent with the outcomes of phase I and II clinical studies, a phase III trial (N = 347) conducted by Shaw et al[6] demonstrated that median PFS after treatment with crizotinib was 7.7 months *vs.* 3.0 months of chemotherapy with pemetrexed or docetaxel (hazard ratio, 0.49; $p < 0.0001$); the ORR with crizotinib was more than three times that observed with chemotherapy (65% *vs.* 20%, $p < 0.0001$). These findings suggest that crizotinib is an effective antitumor agent for NSCLC treatment, especially in cases positive for *ALK-EML4* gene fusion.

Although the initial response to crizotinib treatment is significant in most of phase I and II clinical studies, development of resistance has been observed in several studies[20-22,25,29,32]. The mechanisms of the development of resistance to crizotinib can be classified into two general categories. The first category involves a genetic alteration of the target, i.e., *ALK* or *ELM4*, by mutation or gene amplification[20,29,32]. The secondary category of resistance mechanisms involves the activation of alternative signaling pathways that can bypass ALK. For example, upregulation of EGFR signaling has been observed in ALK-positive cell lines made resistant to crizotinib *in vitro*[21,22,25]. Due to the increasing number of cases of acquired resistance to crizotinib, 2nd-generation ALK-inhibitors have been developed and are being examined in the most of the clinical trials[16,17,19,24]. In this regard, preclinical trial[10] of ceritinib, a second-generation multi-targeted oral tyrosine kinase inhibitor, has been demonstrated to be approximately 20 times more potent against ALK than crizotinib. Moreover, previous clinical trials on ceritinib have shown the antitumor activity in both crizotinib-resistant and crizotinib-sensitive tumors[5], and thus, in 2014, the FDA granted accelerated approval of ceritinib for the treatment of ALK-positive metastatic NSCLC in patients who are intolerant to or have progressed despite therapy with crizotinib[33]. Based on the aforementioned mechanisms of acquired resistance development, targeting ALK signaling may also be an alternative strategy in addition to developing a new generation of ALK-inhibitors. In this context Katayama et al[25] reported that a P-glycoprotein inhibitor with ceritinib could overcome the ceritinib or crizotinib resistance mediated by P-glycan protein overexpression. Meanwhile, An et al[21] reported that the treatment with CRKL, a novel downstream effector of ALK signaling, and the combined inhibition of ALK and CRKL may be an effective strategy for treating ALK-rearranged NSCLC patients.

Detection of *ALK* gene fusion or rearrangements in NSCLC is achieved by the following three techniques: fluorescence *in situ* hybridization (FISH), quantitative reverse transcriptase PCR (qRT-PCR), and immunohistochemistry (IHC), each of them having its advantages and limitations. FISH is the gold standard because it has been clinically validated in trials with crizotinib and approved by the U.S. FDA for this indication. The FISH assay, using ALK break-apart probes, involves labeling of the 5' and 3' ends of the *ALK* gene with differently colored fluorescent probes (typically, red and green). In at least 15% of the cells counted, rearrangements result in a split appearance of the signal (in approximately 70% of cases) or loss of the 5' signal in approximately 30%)[34]. FISH can be performed on formalin-fixed paraffin-embedded tissue as well as on snap-frozen samples. Nevertheless, the FISH technique is expensive and technically challenging. Quantitative reverse transcriptase PCR (qRT-PCR) is highly sensitive and detects mRNA for ALK with

both gene fusion and mutation. A disadvantage of qRT-PCR is the requirement for RNA extraction from frozen or fresh tissues or cells. IHC is a readily available, inexpensive, and rapid technique, and thus, it may be suitable for ALK screening[30]. However, the validation of this method in larger-scale studies might be required for quality control before its routine use in clinic[35].

We found that gastrointestinal adverse events, such as diarrhea, nausea, and vomiting, were commonly experienced by the patients treated with crizotinib, although these symptoms were mild to moderate. By systematic review, the current study found that visual disturbance was the most common side effect for crizotinib, while gastrointestinal reactions, such as diarrhea and nausea, were the most common adverse effect in patients treated with 2nd-generation ALK inhibitors. Furthermore, visual disturbance, lymphopenia, hypophosphatemia, anemia, and dysgeusia were not reported in the patients treated with 2nd-generation ALK inhibitors; conversely, other side effects, such as myalgia, cough, and headache, were reported. These findings indicated that first- and second-generation ALK inhibitors have slightly different targets of tissue or organ.

Conclusions

This systematic review and meta-analysis show that the first-generation ALK inhibitor crizotinib is effective in the treatment of NSCLC, especially in cases positive for *ALK-ELM4* gene fusion. Side effects of ALK-inhibitors are mild or moderate. However, the development of acquired resistance to crizotinib is a problem facing the long-term clinical application of ALK inhibitors. Therefore, combined strategies to overcome the acquired resistance to ALK inhibitors are urgently required.

Conflict of interest

The authors declare no conflicts of interest.

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