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ALK mutation and inhibition in lung cancer

Tri Le[1] and **David E. Gerber**[1,2]

[1]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas Texas, USA

[2]Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas Texas, USA

Abstract

The advent of precision medicine in non-small cell lung cancer has remarkably altered the direction of research and improved clinical outcomes. The identification of molecular subsets with differential response to targeted therapies began with the identification of epidermal growth factor receptor mutated tumors in subsets of non-small cell lung cancer (NSCLC). Emboldened by unprecedented response rates to kinase inhibitors seen in that subset, the oncologic community searched for other molecular subsets featuring oncogene addiction. An early result of this search was the discovery of NSCLC driven by activating rearrangements of the anaplastic lymphoma kinase (ALK) gene. In an astoundingly brief period following the recognition of ALK-positive NSCLC, details of the biology, clinicopathologic features, development of targeted inhibitors, mechanisms of therapeutic resistance, and new generations of treatment were elucidated. This review summarizes the current understanding of the pathologic features, diagnostic approach, treatment options, resistance mechanisms, and future research areas for ALK-positive NSCLC.

Keywords

adenocarcinoma; crizotinib; oncogene addiction; personalized medicine; precision medicine; targeted therapy

Introduction

Lung cancer accounts for more than a quarter of all cancer-related morality [1]. The identification of molecular subsets of lung cancer with targetable driver mutations has altered the landscape of treatment. Advanced lung cancers that harbor druggable oncogenic alterations are highly responsive to molecularly targeted therapies. Thus, discerning tumor-specific, oncogenic driver mutations from passenger mutations has become a concerted

Corresponding author: David E. Gerber, MD, Division of Hematology-Oncology, Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Mail Code 8852, Dallas, Texas 75390-8852, Phone: 214.648.4180, Fax: 214.648.1955, david.gerber@utsouthwestern.edu.

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effort in the clinical and research oncology communities, ushering in the era of precision medicine. In 2007, one such molecular alteration, activating fusions of the anaplastic lymphoma kinase (ALK) gene, was discovered in a subset of patients with non-small cell lung cancer (NSCLC) [2]. Only three years later, the first-generation ALK inhibitor crizotinib was found to have a response rate of 57% and 6-month progression free survival of 72% in patients with previously treated advanced NSCLC harboring *ALK* rearrangement confirmed by fluorescence in situ hybridization (FISH) [3]. In the ensuing years, translational research has yielded further insight into the biology, epidemiology, clinical features, alternative therapies, and mechanisms of resistance in *ALK*-rearranged NSCLC. Here, we review the current understanding and future directions of biology, epidemiology, clinicopathology, therapy, mechanisms of resistance, and strategies to counter resistance in *ALK*-rearranged NSCLC.

Biology of *ALK*-rearranged NSCLC

The ALK gene is located on chromosome 2 and encodes a transmembrane tyrosine kinase that is normally restricted at low levels to the small intestine, nervous system, and testes in adult humans [4, 5]. It is developmentally regulated and appears to play a role in neurodevelopment based on studies in mice and Drosophila [6, 7]. Normal activation of ALK is mediated by extracellular, ligand-induced dimerization (Figure 1) [8]. Molecular alterations leading to heightened ALK activation have been implicated in several cancers including non-Hodgkin's lymphoma, rhabdomyosarcomas, renal cell carcinoma, thyroid cancer, neuroblastoma, and NSCLC [6].

Activating alterations of *ALK* obviate ligand-dependence, render ALK constitutively active via hyperphosphorylation and/or overexpression, lead to downstream activation of proliferative and anti-apoptotic signals via intracellular pathways (including STAT3, PI3K, mTOR, and MEK), and culminate in oncogenesis [6, 9, 10]. In ALK-activated NSCLC, the predominant molecular event leading to *ALK* activation is juxtaposition of the N-terminal portion of the protein encoded by the echinoderm microtubule-associated protein like 4 (EML4) gene with the intracellular domain of the ALK tyrosine kinase [2]. Several variants of *EML4-ALK* rearrangements have been identified. These resulting fusion proteins promote oncogenesis via constitutive activation of downstream pathways (Figure 1). Less commonly, other fusion partners, including *KIF5B*, and intrinsic activating mutations of *ALK* have been described [11]. In other malignancies such as diffuse large B cell lymphoma and inflammatory myofiboblastic tumors, other ALK 5′ fusion partners, including Ran-binding protein 2 (RANBP2) and Clathrin, have been described [12, 13]. To date, the *EML4-ALK* fusion appears unique to NSCLC [6, 14].

Clinicopathologic features

ALK-rearranged NSCLC comprises 2% to 5% of all NSCLC cases [2]. Compared to patients with pan-wild-type NSCLC (i.e. patients who have NSCLC without a known targetable, driver oncogene such as *EGFR* or *ALK*), patients who harbor *ALK*-activated NSCLC tend to be light or never smokers (100% vs 42%), younger (median age 52 years vs 64 years, P=0.005), male (58% vs 32%, P=0.039) [15–17], and at more advanced stage

(89% vs 58% with stage IV disease (P=0.051). Histologically, *ALK*-rearranged NSCLC cases are more likely to be adenocarcinoma histology, in particular signet-ring cell type with abundant intracellular mucin. Molecularly, *ALK* alterations appear to be mutually exclusive of *EGFR* and *KRAS* mutations [2, 15, 16, 18, 19].

Screening

Given the relative infrequency of *ALK*-rearranged NSCLC, the optimal approach to screening for these cases has received considerable attention [6]. If all advanced NSCLC cases are screened, only 1.6% would be ALK-positive. Employing the screening recommendations of the College of American Pathologists and the Association for Molecular Pathology, if only adenocarcinoma cases are screened (approximately 40% of advanced NSCLC), the rate of ALK-positivity increases to 4%. However, an estimated 13% of ALK-positive cases would be missed. The National Comprehensive Cancer Network recommends screening the slightly broader population of non-squamous histology (which includes large cell, NSCLC not otherwise specified, and other rare histologic subtypes in addition to adenocarcinoma). Restricting screening to adenocarcinoma cases in never smokers (an estimated 6% of advanced NSCLC), the rate of ALK-positivity increases to 14%, but up to 50% of cases are missed. If one were to further enrich advanced NSCLC for ALK-positivity by limiting testing only to those adenocarcinoma cases in never smokers that harbor neither *EGFR* nor *KRAS* mutations (representing 2% of advanced NSCLC), an estimated 36% of cases will be ALK-positive, but approximately 55% of ALK-positive cases may be missed.

Methods of testing

Accurate, reproducible, and widely-accessible testing for *ALK* alterations is essential for clinically meaningful identification and targeting of molecular subtypes. Available assays include fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR), and DNA sequencing [20–23]. FISH is the most commonly performed and is approved by the United States Food and Drug Administration (FDA). The assay employs uniquely labeled split-signal probes on the 5′ and 3′ termini of *ALK*. Wild-type *ALK* appears as a fused signal whereas *ALK* rearrangements appear as separately colored signals (e.g. fused yellow signal in wild-type and separate red and green signal in *EML4-ALK* rearrangement) [24]. Sensitivity and specificity with this technique approach 100% using thresholds of >15% of cells and examination of 4+ fields. However, there are challenges to large-scale FISH screening, including cost, equipment requirements, labor training, and time intensiveness.

Immunohistochemistry, due to its low cost, wide availability, and ease of use relative to FISH, has been proposed as an alternative initial screening test and is now FDA-approved [25–27]. IHC was initially fraught with use-limiting insensitive antibodies but newer antibodies (i.e. 5A4 by Novocastra and D5F3 by Cell Signaling Technology with ADVANCE) have demonstrated sensitivities of 96–100% [25, 28]. IHC has been employed as the enrollment biomarker in some clinical trials and has been shown in some case reports to identify ALK-inhibitor responsive tumors that were FISH-negative for *ALK*

rearrangements [22]. Nevertheless, various IHC platforms have differing sensitivity and specificity (Table 1) [29]. It has been proposed IHC and FISH complement one another and that combined testing may enhance the detection of ALK-positive cases [30, 31].

T-PCR-based techniques are available for both the detection of *ALK* rearrangements [29, 32] and for the quantification of the *ALK* kinase domain, capitalizing on low expression in normal lung tissue relative to *ALK*-altered tumors. The former relies on specific messenger RNA transcripts and are limited as such. Next generation sequencing has also demonstrated the ability to detect a subset of ALK-rearranged NSCLC not detected by FISH [33, 34] and may have a role in future diagnostics.

Efficacy of first- and second- line crizotinib compared to chemotherapy on ALK-positive NSCLC

Pre-clinical evaluation of ALK inhibitors and subsequent clinical translation developed rapidly after the initial identification of the *EML4-ALK* rearrangement [2, 35, 36]. This expeditious advancement was aided by previous experience with other tyrosine kinase inhibitors (TKI) and the fact that crizotinib, initially developed as a MET inhibitor but subsequently found to inhibit ALK as well, was already under clinical development [37]. Crizotinib, the first approved targeted therapy for ALK-positive NSCLC, is an oral small-molecule TKI that inhibits ALK, MET, and ROS1 [17, 38, 39]. Crizotinib yielded promising outcomes comparable to EGFR inhibitors in *EGFR*-mutant NSCLC (Tables 2 and 3). In the first-line setting, crizotinib had an overall response rate (RR) of 74% and median progression-free survival (PFS) of 11 months, which was superior to standard first-line platinum-pemetrexed chemotherapy (RR of 45% and median PFS of 7 months) [40]. In the second-line setting, crizotinib demonstrated similarly impressive superiority over conventional chemotherapy with a RR of 65% versus 20% and median PFS of 8 months versus 3 months [41].

Interestingly, in the study that demonstrated crizotinib's superiority over conventional chemotherapy in ALK-positive NSCLC, pemetrexed demonstrated an advantage over docetaxel in the second line (RR of 29% versus 6% and median PFS of 4.2 months versus 2.6 months). ALK-positivity was previously shown to be a marker for pemetrexed sensitivity when compared to EGFR-mutated or wild-type NSCLC (RR of 46.7 % versus 16.2% versus 4.7%, respectively, P=0.001; PFS 9.2 versus 2.9 versus 1.4 months, P=0.001) [42]. This enhanced sensitivity to pemetrexed is postulated to be a result of decreased thymidylate synthase mRNA levels in ALK-positive cells.

Mechanisms of crizotinib resistance

Unfortunately, resistance to crizotinib invariably occurs. Mechanisms of resistance can be divided into two broad categories: ALK-dominant (i.e. mechanisms dependent on ALK signaling) and ALK-non dominant (i.e. mechanisms that are only partially or independent of ALK signaling) [43, 44]. Among ALK-dominant mechanisms, three means of resistance occur: mutations in the *ALK* kinase domain [3, 45], copy number gain (CNG) of the *ALK* fusion gene [44, 46], and central nervous system (CNS) progression. The CNS represents a

sanctuary site in which approximately 40% of ALK-positive cases treated with crizotinib develop progression [47], which has been attributed to poor drug penetration of the blood brain barrier [48].

In contrast to EGFR secondary resistance mutations—which are singularly dominated by T790 mutations—the range of mutations within the *ALK* kinase domain that confer crizotinib resistance appear to be quite wide and broadly distributed [43, 44, 46, 49]. Fundamentally, these mutations impact crizotinib binding and adenosine triphosphate (ATP) affinity. Camidge and Doebele have posited that this difference in range of kinase domain resistance mutations between ALK-positive and *EGFR* mutant tumors lies in the difference in selective pressure of such mutations [43]. In ALK-positive NSCLC cells with kinase domain mutations, there appears to be no growth disadvantage but, rather, increased proliferation when compared to wild-type [44]. In contrast, EGFR T790M conveys a selective disadvantage to EGFR-positive NSCLC cells compared to wild-type [50, 51]. The lower tolerance of mutations within the EGFR kinase domain relative to the ALK domain suggests higher constraints on the structure of EGFR rendering it less tolerable to amino acid substitutions than is ALK.

ALK non-dominant mechanisms of resistance involve the emergence of a second mutated, overexpressed, or amplified oncogene relative to the pre-treated sample. These include *EGFR*, *KRAS*, *BRAF*, *MET*, *HER2*, and *KIT* [43, 44, 46, 52]. In one case series, lung adenocarcinomas harboring both mutant EGFR and an ALK rearrangement responded to erlotinib [53]. Histologic transformation to small cell lung cancer has also been reported after treatment with the ALK inhibitor alectinib possibly as a mechanism of resistance [54].

Second-generation ALK-inhibitors: certinib, alectinib, and beyond

With the inevitable development of crizotinib resistance came the search for new ALK inhibitors that could overcome the aforementioned mechanisms of resistance. Two second-generation ALK inhibitors, ceritinib [55] and alectinib [56], are currently FDA approved (Tables 2 and 3) Ceritinib is an oral, small-molecule, ATP competitive TKI of ALK [55, 57] that has demonstrated impressive response rates in patients with *ALK*-rearranged NSCLC in both crizotinib-naïve and crizotinib-resistant patients. Among patients previously treated with crizotinib, the overall RR was 56% (95% CI, 45% to 67%), and median PFS was 6.9 months (95% CI, 5.3 to 8.8). Among patients naïve to crizotinib, the RR was 62% (95% CI, 44% to 78%) [55], and median PFS was 10.4 months (95% CI, 4.6 to could not be estimated). PFS appeared to be similar between patients with and without CNS disease at baseline (6.9 and 7.0 months, respectively; P = 0.37). The impressive response of ceritinib in both crizotinib-resistant and crizotinib-naïve patients may be attributed to several possible mechanisms: increased potency (20 times) against ALK, activity against ALK with secondary mutations in the tyrosine kinase domain, improved CNS activity, and/or inhibition of other tyrosine kinases not targeted by crizotinib, including IGF-1 [55].

Alectinib is a highly-selective ALK inhibitor with activity against both wild-type ALK and ALK harboring secondary mutations conferring crizotinib resistance [56, 58, 59]. In patients naïve to ALK inhibitors and who were resistant to or intolerant of crizotinib, RRs were

noted to be 94 % (95 % CI 82% to 98%) and 55%, respectively [56, 60]. Among crizotinib-resistant patients with CNS disease, alectinib demonstrated promising activity with a RR of 52% [56].

What remains to be answered is the optimal sequencing of TKIs in ALK-positive NSCLC to maximize benefit while limiting toxicity, ultimately leading to greatest prolongation of overall survival. As previously mentioned, both ceritinib and alectinib have activity in both crizotinib-naïve and crizotinib-exposed patients. Moreover, PFS seems to be improved when ceritinib is used in the first line as compared to crizotinib. Further head-to-head studies looking at different TKI sequencing will be needed to better clarify this question.

Additionally, the search for and validation of new, more efficacious ALK-inhibitors are underway. These include brigatinib, entrectanib, loratinib, and belizatinib (table 4), for which outcomes data in ongoing clinical trials are pending.

Alternative treatment strategies: HSP90 inhibition and immunotherapy

In addition to the development of newer generation ALK inhibitors, efforts have been made to overcome crizotinib resistance by targeting ALK function via ALK-independent pathways [61]. One such alternative strategy is inhibition of heat shock protein 90 (Hsp90). Hsp90 is a molecular chaperone protein that guides the normal folding and turnover of intracellular growth factors and is implicated in the stabilization of several oncoproteins, including ALK [62–65]. Inhibition of Hsp90 activity results in aggregation and degradation of its client proteins and disrupts associated signaling pathways involved in cellular proliferation. Early preclinical studies with Hsp90 inhibitors in *EML4-ALK* rearranged NSCLC cells demonstrated encouraging results [61]. Ganetespib, a trazolone inhibitor of Hsp90, when used in used as a single agent *in vitro* against ALK-positive NSCLC resulted in loss of ALK expression and depletion of several oncogenic signaling proteins. In tumor xenografts, ganetespib resulted in improved survival and antitumor activity comparable to crizotinib. These effects were amplified when used in combination with crizotinib. In cells with induced crizotinib-resistance, whether mediated by secondary mutations in the tyrosine kinase domain of ALK or by ALK amplification, ganetespib overcame these barriers and demonstrated anti-tumor benefit. Early clinical trials have suggested that Hsp90 inhibition may have some activity in patients with *ALK* rearranged NSCLC (Table 2) [66, 67]. Several ongoing trials are exploring the sequencing of Hsp90 inhibitors alone or in combination with ALK inhibitors.

Another strategy that has gained significant attention in NSCLC therapy is immunotherapy. NSCLC tumor cells have been shown to express programmed cell death ligand 1 (PD-L1), which when bound to programed cell death protein 1 on T cells, initiates apoptosis of T cells. This attenuation of T cell activity provides a mechanism for immune escape in NSCLC [68–70]. Nivolumab is a humanized monoclonal antibody directed against the PD1 and PD-L1 interaction and is currently approved for use in both squamous and non-squamous metastatic NSCLC that has progressed after platinum-based therapy. Checkpoint blockade in ALK-positive NSCLC has yet to be fully elucidated, but *in vitro* studies have shown that ALK-rearranged NSCLC upregulates PD-L1 expression via activation of PI3K-

ALT, MEK-ERK, HIF-1α, and STAT3 signaling [68, 71, 72]. The implications for treatment are currently under investigation in early clinical trials.

Conclusion

ALK-rearranged NSCLC represents an exciting opportunity in the realm of personalized medicine in which identification of a molecular subset of patients with cancer can yield tailored therapy with superior outcomes. There are up to 10,000 cases per year of ALK-rearranged NSCLC in the United States [37, 73] from which further research into improved molecular screening, therapeutic options, and counter-therapies to developed resistance can produce better treatment.

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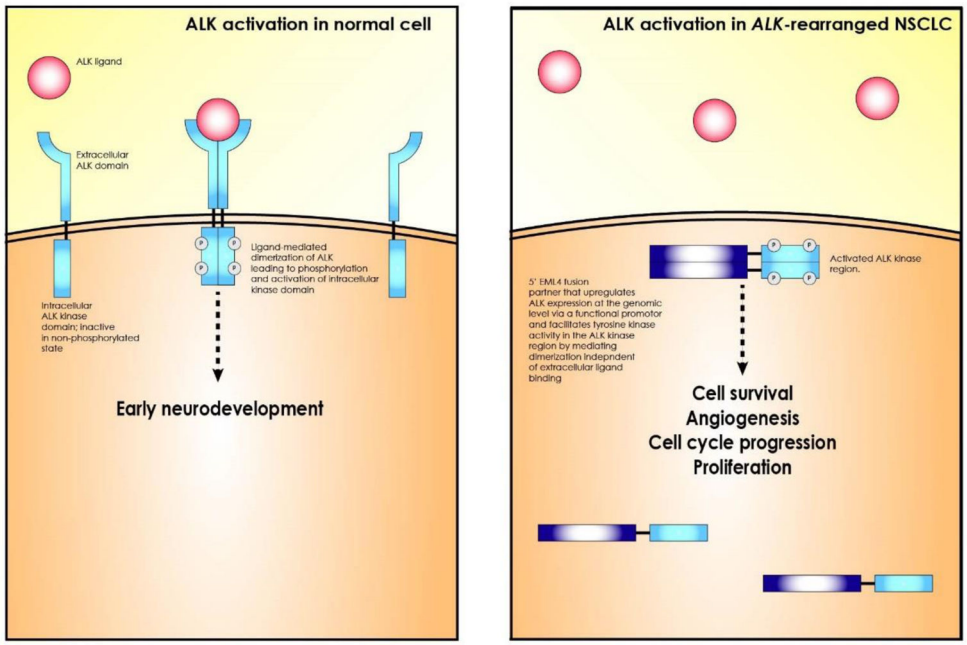

Figure 1.

ALK biology and oncogenesis. Activation of ALK is developmentally regulated and sparse in adult tissue. It appears to be involved in neurodevelopment and is largely restricted to the gut, CNS, and testes in adulthood. Native ALK signaling occurs via extracellular ligand-mediated dimerization of ALK and subsequent autophosphorylation and activation of the intracellular tyrosine kinase domain. The native ligands that bind to and activate ALK have remained unknown, although recent evidence suggests that heparin may be one [74, 75]. In ALK-rearranged NSCLC, ALK activation occurs independently of ligand-mediation. The 5′ fusion partner of *ALK* provides a functional promotor that escapes normal *ALK* regulation and expresses a domain in the functional protein that facilitates dimerization. In this way, the kinase domains of separate ALK proteins, which are entirely intracellular in ALK-rearranged cells, are brought into proximity for autophosphorylation. This ultimately results in downstream activation of signaling pathways that enhance cell survival, angiogenesis, cell survival and cell cycle progression [10]. Implicated pathways include STAT3, mTOR, PI3K, Ras, and MEK.

Table 1

Sensitivity and specificity of FISH by and IHC for the detection of ALK rearrangement in NSCLC cancer by technique and probe

Screening Test		Sensitivity (%)	Specificity (%)
FISH	*Number of tumor areas examined under high-power microscopy fields*		
	2	98.6	96.6
	3	99.3	100
	4	100	100
IHC	*Probe and Detection System*		
	5A4 by Novocastra with ADVANCE [25]	100	87.5
	5A4 by Nichirei with Histofine [25]	100	62.5
	D5F3 by Cell Signaling with ADVANCE [25]	100	75
	ALK1 by DAKO with FLEX [25]	66	100
	ALK1 by DAKO with ADVANCE [25]	66	87.5
	5A4 by Novocastra with i-view [27]	100	95.8
	5A4 by Novocastra with BenchMark XT [28]	96	100
	D5F3 by Cell Signaling BenchMark XT [28]	96	100
	5A4 by Novocastra with Bond-MAX [28]	96	100
	5A4 by Abcam with Bond-MAX and UltraView Universal DAB [31]	69	99.3
	5A4 by Novocastra with Envision Plus [76]	93	100
	5A4 by Abcam with Benchmark Ventana [77]	95	100
	D5F3 by Ventana with Optiview [78]	98	100
	5A4 by Novocastra with Optiview [78]	98	100

Abbreviations: FISH, fluorescence in situ hybridization; IHC, immunohistochemistry

Table 2

Published clinical trial outcomes in ALK-rearranged NSCLC

Clinicaltrials.gov Identifier	Trial	Study drug	Trial Phase	Description	RR (%)	Median PFS (months)
NCT00585195	PROFILE 1001 [79]	crizotinib	1/2	Safety of crizotinib in ALK-rearranged NSCLC, mostly in the second line and beyond	57	9.7
NCT00932451	PROFILE 1005 [80]	crizotinib	2	Activity of crizotinib in ALK-rearranged NSCLC	53	8.5
		crizotinib	3	Outcomes of ALK-rearranged NSCLC treatment in the second line	65	7.7
NCT00932893	PROFILE 1007 [41]	docetaxel	3	Outcomes of ALK-rearranged NSCLC treatment in the second line	6	2.6
		pemetrexed	3	Outcomes of ALK-rearranged NSCLC treatment in the second line	29	4.2
NCT01154140	PROFILE 1014 [40]	crizotinib	3	Outcomes of ALK-rearranged NSCLC treatment in the first line	74	10.9
		Platinum-based chemotherapy	3	Outcomes of ALK-rearranged NSCLC treatment in the first line	45	7.0
NCT01283516	ASCEND-1 [55, 81]	ceritinib	1	Efficacy and safety of ceritinib in ALK-rearranged NSCLC in patients who are ALK inhibitor-naïve	72% in ALK inhibitor-naïve and 56% in ALK inhibitor-pretreated	18.4 months in ALK inhibitor-naïve and 6.9 months in ALK inhibitor-pretreated
JapicCTI *-101264	AF-001JP [60]	alectinib	1/2	Activity and safety of ceritinib in ALK-rearranged NSCLC in ALK inhibitor-naïve patients	94	n/a
NCT01801111	NP28673 trial [82]	alectinib	2	Activity of alectinib in crizotinib-refractory ALK-rearranged NSCLC	50	8.9
NCT01871805	Alectinib in ALK - positive, crizotinib-resistant, non-small-cell lung cancer: a single-group, multicenter, phase 2 trial [83]	alectinib	2	Activity of alectinib in crizotinib-refractory ALK-rearranged NSCLC	48	8.1
JapicCTI *-132316	J-ALEX [84]	alectinib	3	Alectinib versus crizotinib in treatment-naive ALK rearranged advanced NSCLC	Not reported	Not reached; PFS HR of alectinib arm to crizotinib arm was 0.35 99.6826% CI:

Clinicaltrials.gov Identifier	Trial	Study drug	Trial Phase	Description	RR (%)	Median PFS (months)
						0.17–0.70, stratified log-rank p<0.0001)
		crizotinib	3	Alectinib versus crizotinib in treatment-naive ALK rearranged advanced NSCLC	Not reported	10.2
NCT01449461	Safety and efficacy of brigatinib (AP26113) in advanced malignancies, including ALK+ non–small cell lung cancer (NSCLC) [85].	brigatinib	1/2	Safety and efficacy of brigatinib in ALK rearranged NSCLC	74 (69 in crizotinib-treated subset and 100 in crizotinib-naïve subset)	14 (11.8 in crizotinib-pre-treated subset)
NCT00431015	Activity of IPI-504, a Novel Heat-Shock Protein 90 Inhibitor, in Patients With Molecularly Defined Non–Small-Cell Lung Cancer [66, 86]	IPI-504	2	Activity of IPI-504 in molecularly defined NSCLC	only 3 patients (partial response in 2 and stable disease in 1)	n/a
NCT01031225	A Multicenter Phase II Study of Ganetespib Monotherapy in Patients with Genotypically Defined Advanced Non–Small Cell Lung Cancer [67]	ganetespib	2	Activity and tolerability of ganetespib in the second line in NSCLC	50	8.1

* Registered with Japan Pharmaceutical Information Center

Abbreviations: ALK, anaplastic lymphoma kinase; NSCLC, non-small cell lung cancer

Table 3

ALK inhibitor characteristics: dosing, toxicity, IC50, and additional molecular targets

Drug	Standard Dose	Toxicities	ALK IC_{50}	Additional Targets
Crizotinib [39–41, 87]	250 mg twice daily (with or without food)	Visual effects (60%), nausea (45%), diarrhea (40%), edema (25%), constipation (25%), decreased appetite (18%), fatigue (15%)	24 nM	MET, ROS1, VEGFR2, PDGFRβ, IRK, Lck, Tie2, TrkA, TrkB, RON, Axl
Ceritinib [55, 57, 81]	750 mg (five 150-mg capsules) daily (without food)	Nausea (82%; 5% Gr 3–4), diarrhea (75%; 7% Gr 3–4), vomiting (65%; 5% Gr 3–4), fatigue (47%; 5% Gr 3–4), ↑ ALT (35%; 21% Gr 3–4), constipation (32%), abdominal pain (30%), decreased appetite (29%), ↑ AST (25%; 11% Gr 3–4)	200 pM	IGF1-R, INSR, STK22D
Alectinib [56, 59, 88]	600 mg twice daily	Fatigue (30%), myalgia (17%), edema (15%), ↑ CPK (15%), nausea (15%), ↑ ALT (13%), photosensitivity (13%), constipation (11%)	3 nM	LTK, GAK
Brigatinib (AP26113) [85, 89]	Standard dose not yet established (30–300 mg daily)	nausea (45%), diarrhea (36%), fatigue (36%), cough (26%), headache (26%), Early-onset pulmonary events, observed ≤7 d after starting treatment, included dyspnea, hypoxia, or new pulmonary opacities on chest computed tomography suggestive of pneumonia or pneumonitis (9%)	0.37 nM	FLT3, ROS1, IGF1E, INSR
Entrectinib (RXDX-101) [86, 90, 91]	Standard dose not yet established (800 mg/m^2 used in ALK rearranged NSCLC)	paraesthesia (42%) nausea (37%), myalgia (34%), asthenia (27%), dysgeusia (27%), vomiting (21%), arthralgia (19%) and diarrhoea (19%)	12 nM	ROS1, Pan-TRK
Loratinib (PF-06463922) [92–94]	Standard dose not yet established (10–200 mg daily)	hypercholesterolemia (48%), peripheral oedema (23%) and peripheral neuropathy (21%)	1.3 nM	ROS1, pan-TRK, LTK, FER, FRK, PTK1, PTK2B, FES
Belizatinib (TSR-011) [95]	No standard dose (30 to 480 mg total per daily, administered 1,2, or 3 times daily)	fatigue (26.1%), diarrhea (21.7%), QTc prolongation (21.7%), headache (17.4%), decreased appetite (15.2%), urinary tract infection (15.2%), vomiting (15.2%), anemia (13%), asthenia (13%), constipation 13%), dysgeusia (10.9%)	0.7 nM	Pan-TRK