



ALK Inhibitors in Nonsmall Cell Lung Cancer

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The management of advanced nonsmall cell lung cancer (NSCLC) has gone a definitive change over time with the discovery of small-molecule inhibitors. In 2004, the first initial description of actionable mutation of epidermal growth factor receptor (EGFR) was provided, and since then, almost 10 actionable mutations have been identified.¹ These include EGFR, anaplastic lymphoma kinase (ALK), ROS, MET, rearranged during transfection (RET), Kirsten rat sarcoma viral oncogene (KRAS), raf murine sarcoma virus oncogene homolog B (BRAF), and many others. After the development of the drugs against these receptors, these were approved by US Food and Drug Administration (FDA) for used against NSCLC with the receptor mutations.

The most common receptor tyrosine kinase mutations in adenocarcinoma lung are as follows:²

1. KRAS mutation in 25% of cases of NSCLC
2. EGFR mutation—10 to 30%
3. MET amplification or MET exon 14 mutation—5%
4. ALK mutation—3 to 7% cases
5. BRAF mutations—2 to 4%
6. Rest others: RET rearrangements, MEK1 mutation, FGFR1 amplification, Her2 mutation, ROS1 rearrangements, and NRAS mutation occur in 1% each
7. The majority are still unknown—40%.

The drugs are very effective for patients with actionable mutant NSCLC with the response rates of 60 to 85% in the first-line setting. The main barrier is the development of resistance after a few months of treatment, which limits the duration of response to ~1 to 2 years for most of the drugs.³

Anaplastic Lymphoma Kinase Rearrangements

ALK being the first reported oncogene in NSCLC in 2007 is usually found fused to echinoderm microtubule-associated protein-like 4 (EML4) gene, leading to its permanent

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activation, and hence, leading to cellular proliferation. The other fusion loci are TFG, KIF5B, and KLC1.⁴⁻⁸

It is detected in 3 to 7% cases of NSCLC and seen in younger patients who are light or never smokers, and their presentation is usually at the advanced stage.⁸ Since 2007, small-molecule ALK tyrosine kinase inhibitors (TKIs) are being developed and are found to be highly sensitive in ALK-rearranged NSCLC.

Diagnostic tools for targeted mutation in carcinoma lung:⁹

1. Fluorescence in situ hybridization (FISH) analysis: It is the gold standard technique for ALK mutation testing. Vysis ALK break apart FISH probe kit manufactured by Abbot was approved by FDA for the use in 2011. The probe has two fluorophores—red binding to 3' end of ALK gene and green binding to 5' end. When the translocation is seen, the probe shows separated red and green fluorophores, or has loss of the green signal, but we have to examine 15% or more cells for the mutation to be labeled as positive. The demerits of FISH are as follows: (1) costly, (2) it requires expert and experienced pathologist for interpretation, (3) provides information only about the translocation of ALK gene and not about the fusion partners, and (4) is time-consuming. However, it is very accurate and detects all the ALK rearrangements.
2. Immunohistochemistry (IHC): ALK can be readily identified by IHC, but the protein levels in NSCLC are lower compared with anaplastic large-cell lymphoma that makes it difficult to attempt. The antibodies commonly used are D5F3, ALK clone ZAL4, and 5A4, and in India, we are commonly using D5F3 antibodies. It is still not a preferred method because of the problems faced during tissue preparation, antibody choice, signal enhancement systems, and the optimal scoring system, which are not standardized as yet. It is helpful because of low cost, easy to be performed and interpreted by pathologist, quick results and with the background histological information.

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Other problem with IHC is the discordant results went compared with FISH, which are in the range of 1 to 10%. This problem may be due to intrachromosomal translocations that are not detected on FISH and the patients are labeled as IHC+/FISH-ve. These patients when treated with crizotinib show similar objective response rate (ORR) and disease control rate as compared with FISH positive patient in PROFILE

1001 trial. Hence, IHC+/FISH-ALK-positive patients can still be treated with ALK TKIs.¹⁰

3. Polymerase chain reaction (PCR) by either reverse transcriptase or 5' rapid amplification of cDNA ends-PCR helps finding out the fusion protein when specific primers are added. It helps in identifying DNA from a diluted sample with good sensitivity and specificity. However, PCR fails

Table 1 Anaplastic lymphoma kinase inhibitors with their properties and evidence

Drug	Gen	Dose	RR	PFS (months)	AEs	Evidence
Crizotinib	1st	250 mg twice a day (with/without food)	74% (1st line) 65% (2nd line)	10.9 (1st line) 7.7 (2nd line)	Visual disturbances Transaminitis Nausea/vomiting Diarrhea/abdominal pain Cytopenia Fatigue Edema Hypophosphatemia QTc prolongation Renal cysts	PROFILE1014 ¹⁷ (1st line) PROFILE1007 ¹⁸ (2nd line)
Ceritinib	2nd	750 mg fasting or 450 mg with food ¹⁹	72.5% (1st line)	26.3 (no brain mets:1st line) 16.6 (brain mets:1st line) 5.4 (2nd line)	Transaminitis Nausea/vomiting Diarrhea/abdominal pain Cytopenia Hyperglycemia Hypophosphatemia Fatigue Increased creatinine QTc prolongation	ASCEND-1 ²⁰ ASCEND-5 ²¹ (both 2nd line) ASCEND-4 ²² (1st Line)
Alectinib	2nd	600 mg twice a day after food	92% (1st line)	25.7 (1st line) 7.5–11 (2nd line, postcrizotinib) ^{23,24}	LFT derangement Cytopenia Elevated CPK Hyperglycemia Hyponatremia Hypophosphatemia Raised creatinine Bradycardia Fatigue Edema Constipation	J-ALEX Global ALEX ²⁵ (1st line)
Brigatinib	2nd	90 mg for 7 days than 180 mg once a day (with/without food)	54% in crizotinib refractory	12.9 (1st line)	Early ILD Transaminitis Hyperglycemia Hypertension Bradycardia Fatigue Nausea Diarrhea Headache Increased CPK	ALTA phase II trial ²⁶
Lorlatinib	3rd	100 mg once a day (with/without food)	46% in heavily pretreated patients	11.4 and has high CNS penetration unlike other P-gp drugs ¹³	Hypercholesterolemia Hypertriglyceridemia Edema Neurocognitive dysfunction Mood lability Peripheral neuropathy Second-degree AV block or Complete heart blocks Interstitial lung disease Contraindicated when strong CYP P450 inducers are used	

Abbreviations: AE, adverse effect; AV, atrioventricular; CNS, central nervous system; CPK, creatine phosphokinase; CYP, cytochrome P450; Gen, generation; ILD, interstitial lung disease; LFT, liver function test; PFS, progression-free survival; p-gp, glycoprotein-P; RR, response rate.

- to identify unknown fusions or novel fusions affected by sample contamination and quality of sample.
- Next-generation sequencing: The technique of genomic profiling of the sample DNA to identify the ALK mutation or fusions missed by FISH. It can identify complex rearrangements in the genome and helps in identifying the mutations in patients who are IHC positive and FISH negative. It helps in the diagnosis of driver mutations not identified by other methods, but is time-consuming and costly.
 - Exon-array profiling can be used for the detection of alk-*eml4* fusion by the expression of transcripts at 3' and 5' end of ALK gene. The details of ALK inhibitors are given with evidence in ►Table 1.

Resistance Mechanism

First Generation

Usually, patients experience progression after 1 to 2 years of treatment. About 20 to 30% cases of resistance to first-line ALK inhibitors are due to ALK tyrosine kinase domain facing steric hindrance at the adenosine 5'-triphosphate binding site (L1196M-analog of T790M of EGFR). At progression, as only 20 to 30% ALK-positive NSCLC will have ALK TDK mutations after the use of crizotinib, compared with EGFR where it is in range of 60%. Hence, the reassessment with repeat biopsy or circulating free DNA is not required in ALK positive cases progressed on first-line ALK inhibitors.

The other mechanisms are mutation in solvent-exposed kinase region binding site (G1202R) and I1151Tins, ALK amplification leading to single-crystal fibers amplifications, and bypass pathways such as EGFR, MET, and KRAS. Even in few cases, histological transformation may be a cause known as epithelial to mesenchymal transition. Among these ALK mutations, 10% cases are where ALK amplification leads to the resistance to crizotinib.^{11,12}

Second Generation

In the second-generation, ALK TKI's most important mechanism is a change in the ALK kinase domain or the solvent-exposed kinase region.

However, still certain studies have found patients acquiring compound mutations over time.

Other off-target pathways are the same as the first-generation drugs. The therapy and sequencing of ALK inhibitors for ALK positive NSCLC are shown in ►Fig. 1.

Newer Anaplastic Lymphoma Kinase Small Molecules

- Lorlatinib—Dual ALK and ROS1 inhibitor and active against most of the ALK resistance mutations. It is even used in the combination when patients are found to be having compound mutations. This third-generation ALK TKI is a low efflux substrate of P-gp and hence has high central nervous system (CNS) penetration and activity, even in highly pre-treated patients with >1 ALK TKIs. Lorlatinib showed ORR of 46% and PFS of 11.4 months in phase I of phase I/II clinical trial and ORR of 42% and PFS of 9.2 months in patient treated with >2 lines of ALK inhibitors.¹³ The recommended dose is 100 mg once a day and major Grade 3 adverse effects (AEs) are hypercholesterolemia, hypertriglyceridemia, and edema that are most commonly seen. Reversible CNS side effects are neurocognitive disorders, mood lability, and peripheral neuropathy. Combined mutations have shown to cause resistance to lorlatinib than single-gene mutations, for example, C1156Y+L1198F. Mutation to third-generation ALK inhibitors occur due to complex mechanism and not due to acquisition of secondary mutations in ALK-EM4. These mutations occur due to activation of by-pass mechanisms like loss of NF2 or EMT, this mechanism is called loss of ALK dependency, and compound mutations.^{14,15}

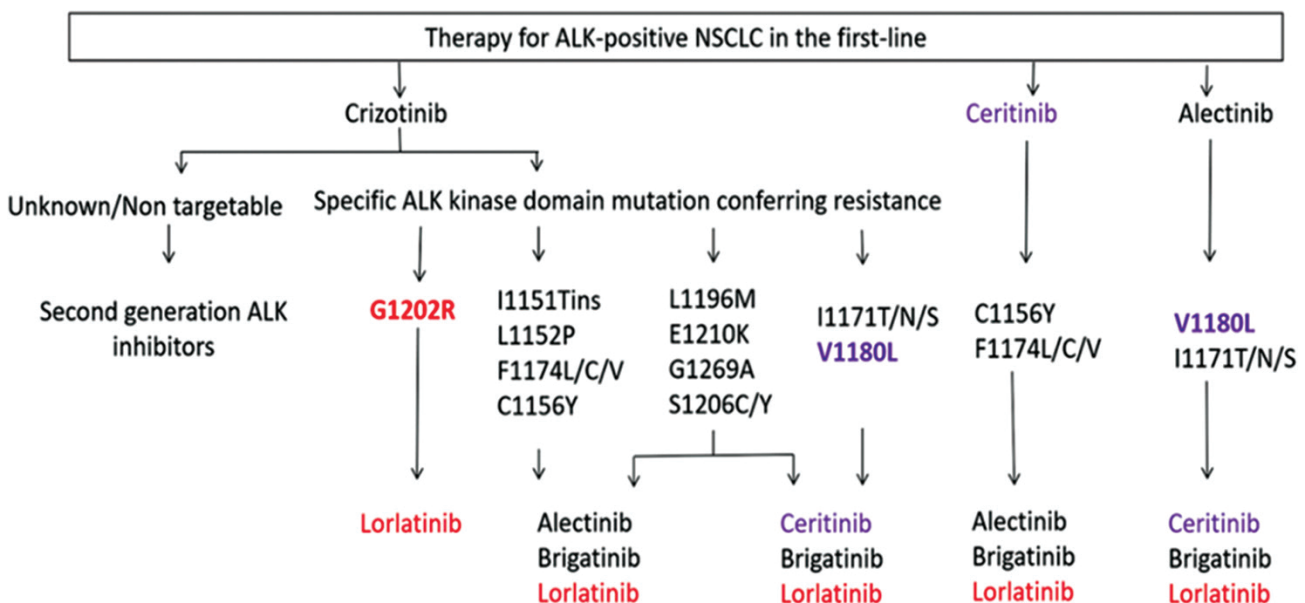


Fig. 1 Therapy for anaplastic lymphoma kinase positive nonsmall cell lung cancer at progression guided by the next-generation sequencing.²⁷

2. Ensartinib—Another third-generation molecule with activity against—ALK, ROS1, MET, AXL. It has good CNS penetration. In phase II trials, responses shown are 77% in crizotinib naïve and 73% in crizotinib-treated patients.¹⁶ The dose recommended is 225 mg once a day till progression or unacceptable toxicities. Most common AEs are mild rash, nausea, vomiting, fatigue, and pruritus.
3. Repotrectinib (known as TPX-0005)—It has activity against ALK, ROS, and TRK kinases. It was specially designed to overcome G1202R mutation but is affective against SRC amplification and FAC mutations. It is undergoing phase I study at present.

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Conflict of Interest

There are no conflict of interest.

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