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ALK Kinase Mutagenesis in Non-small Cell Lung Cancer

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Abstract

Lung cancer is a leading cause of mortality worldwide, accounting for approximately 18% of global cancer deaths and representing a significant public health burden, particularly in countries with high smoking rates and pollution. This review examines the critical role of anaplastic lymphoma kinase (ALK) in non-small cell lung cancer (NSCLC) and explores how ALK mutagenesis analysis is a therapeutic strategy for this malignancy. ALK gene rearrangements, occurring in 3–7% of NSCLC cases, most commonly result in the EML4-ALK fusion protein, which drives uncontrolled cancer cell proliferation through constitutive activation of downstream signaling pathways including PI3K/AKT, RAS/MAPK, JAK/STAT, and PLCγ. The discovery of these molecular alterations has revolutionized lung cancer treatment, enabling the development of targeted ALK inhibitors ranging from first-generation drugs like crizotinib to advanced third-generation inhibitors such as lorlatinib. However, acquired resistance through secondary ALK mutations and bypass signalling pathways remains a significant clinical challenge. This study focuses on ALK protein mutagenesis analysis as a cornerstone of precision oncology. By systematically characterizing resistance mutations such as L1196M, G1269A, F1174L, and G1202R, we can predict treatment failure, guide therapeutic selection, and design next-generation inhibitors with improved efficacy. Through techniques such as site-directed mutagenesis, CRISPR-Cas9 gene editing, and structure-based drug design supported by crystallographic studies, researchers are developing more potent compounds tailored to mutant ALK protein conformations. Our research aims to advance personalized medicine by integrating molecular diagnostics, real-time mutation monitoring, and adaptive treatment strategies. Understanding the complex interplay between ALK mutagenesis and therapeutic response will enable clinicians to overcome resistance mechanisms, optimize combination therapies, and ultimately improve survival outcomes for ALK-positive lung cancer patient. This approach represents a critical step toward transforming lung cancer from a uniformly fatal disease to a manageable chronic condition through precision-targeted interventions.

Keywords: Anaplastic Lymphoma Kinase (ALK), Non-Small Cell Lung Cancer (NSCLC), ALK Mutagenesis Analysis, Targeted Therapy and Drug Resistance, Precision Oncology

1. Introduction

Lung cancer is one of the most common and deadliest forms of cancer globally. It ranks second when it comes to death due to cancer. It occurs when abnormal cells in the lung grow uncontrollably, often forming tumours that interfere with lung function. The two main types are **non-small cell lung cancer (NSCLC)**, which accounts for approximately 85% of cases, and **small cell lung cancer (SCLC)**, which is more aggressive and rapidly spreading. The primary causes include long-term smoking, exposure to environmental pollutants, and genetic mutations. Lung cancer often remains asymptomatic in its early stages, leading to late diagnosis and poor prognosis in many cases. NSCLC predominantly occurs in individuals who are passive smokers, but SCLC is seen in active smokers mainly. The former rises mainly due to adenocarcinoma, that is, cancer or hyperproliferation of glandular cells. Men mostly die due to lung cancer, and this incidence is mounting day-by-day.

1.1 World Disease Percentage

Lung cancer is a leading cause of cancer-related deaths worldwide. According to the World Health Organization (WHO), lung cancer accounts for about **11.4% of all diagnosed cancers globally** and nearly **18% of all cancer deaths**. It is more prevalent in countries with high rates of smoking, industrial pollution, and aging populations. Despite advances in cancer treatment, the five-year survival rate for lung cancer remains relatively low, emphasizing the need for early diagnosis and innovative therapeutic strategies.

1.2 Percentage in India

In India, lung cancer represents a significant public health concern. It is the second most common cancer in men and the fifth in women. The incidence rate is estimated to be around 6.9 per 100,000 individuals, and it contributes to about **8.1% of all cancer-related deaths** in the country. The increasing prevalence of smoking, especially among males, along with rising air pollution in urban centres, has led to a notable rise in lung cancer cases. Moreover, awareness, early screening, and access to modern treatments remain limited in many parts of the country, contributing to higher mortality rates.

1.3 Mode of Action of Lung Cancer

The development of lung cancer begins at the cellular level, where genetic and environmental factors cause mutations in DNA. These mutations disrupt the normal cell cycle, leading to uncontrolled growth and the formation of tumours. Key mechanisms include:

A. **Oncogene activation**: Gain-of - function mutation of genes like EGFR, KRAS, and ALK are frequently mutated or overexpressed, leading to excessive cell proliferation.

B. Tumour suppressor gene inactivation: Loss or mutation of genes like TP53 removes the natural checks on cell division.

C. Angiogenesis: Tumours stimulate the growth of new blood vessels to supply nutrients, allowing them to grow and spread.

D. Metastasis: Cancer cells can break away from the original tumour, travel through the blood or lymph, and form new tumours in other organs.

These molecular changes are complex and often involve multiple pathways, making treatment a challenge, especially in advanced stages.

Table 1: Country-wise ALK Mutagenesis Type and Prevalence in Lung Cancer (2020-2025)

Country/Region	ALK Prevalence (%)	Predominant ALK Mutation/Fusion Type	Most Common Cancer Subtype	Patient Demographics	Effects on Treatment Outcomes	Reference
United States	4.3-5.0%	EML4-ALK fusion (most common); L1196M, G1202R resistance mutations	Adenocarcinoma (predominant)	Younger patients (18-44 yrs: 16.2%); Asian Americans: 6.3%; Non-smokers	High response to crizotinib (60%); Median survival 6.8 years with targeted therapy	Allen et al., 2020; Desai et al., 2021
China	5.1-5.8%	EML4-ALK fusion (81.5%); KIF5B-ALK (1.5%); Resistance: L1196M, G1269A, G1202R	Invasive mucinous adenocarcinoma and acinar subtype	Predominantly non-smokers; Younger age (median 52 years); Female predominance	Excellent response to 1st gen TKIs; Resistance develops within 11-14 months; 2nd/3rd gen inhibitors extend PFS	Tarigopula et al., 2020; Zaric et al., 2016
Taiwan	5.73%	EML4-ALK fusion	Adenocarcinoma (71% EGFR+)	Non-smokers; Asian ethnicity; Younger patients	High response rate; Better PFS with alectinib vs crizotinib; Overall favorable prognosis	Lin et al., 2025
Japan	4-5%	EML4-ALK fusion variants (V1, V3a/b predominant)	Adenocarcinoma	Non-smokers; Younger median age	Superior outcomes with 2nd gen ALK-TKIs (alectinib); Lower CNS metastasis with early treatment	Multiple studies 2020-2023
South Korea	5-6%	EML4-ALK fusion; Secondary mutations after treatment	Adenocarcinoma	Never-smokers; Female predominance	High initial response; Sequential therapy with multiple generations of ALK-TKIs improves OS	Tarigopula et al., 2020
India	2.7-3.0%	EML4-ALK fusion; Limited data on specific resistance mutations	Adenocarcinoma (81.9%)	Peak age 36-50 years; Both smokers and non-smokers	Response similar to global data but limited access to newer TKIs affects outcomes	Tarigopula et al., 2020
Europe (Overall)	3.7-4.9%	EML4-ALK fusion; G1202R and L1196M resistance mutations	Adenocarcinoma	Younger patients; Non-smokers; No significant gender difference	Good response to ALK-TKIs; Access to 3rd gen inhibitors (lorlatinib) improves resistant cases	Multiple European studies 2020-2024
Eastern Europe (Serbia)	5.1%	EML4-ALK fusion (acinar subtype correlation)	Acinar subtype of adenocarcinoma	Caucasian population; Similar demographics to Western Europe	Comparable outcomes to Western populations with appropriate therapy	Zaric et al., 2016
Middle East & North Africa (MENA)	8.7% (range: 2.2-19.6%)	EML4-ALK fusion; Limited mutation profiling data	Adenocarcinoma	Younger age; Non-smokers; No EGFR co-mutation	Variable outcomes due to testing availability; Egypt highest (19.6%), Lebanon lowest (2.2%)	AlJassim et al., 2025
Saudi Arabia	3-5%	EML4-ALK fusion	Adenocarcinoma	Mixed smoking history	Limited data on outcomes; Testing increasingly available	AlJassim et al., 2025
Egypt	19.6%	EML4-ALK fusion	Adenocarcinoma	Predominantly younger patients	Higher prevalence may indicate population-specific factors; Good response to available TKIs	Al-Shamsi et al., 2021
Lebanon	2.2%	EML4-ALK fusion	Adenocarcinoma	Mixed demographics	Lower prevalence similar to Western populations	Al-Shamsi et al., 2021
Kuwait, Bahrain, UAE	3-8%	EML4-ALK fusion; BRAF testing recent	Adenocarcinoma	Younger age; Non-smokers	Routine testing now standard; Good access to targeted therapies	AlJassim et al., 2025
Levant Region (Lebanon, Jordan, Iraq)	3-5%	EML4-ALK translocation by FISH	Adenocarcinoma	Mean age 63.4 years; 66% male	Limited access to newer ALK-TKIs; Primarily crizotinib available	Multiple studies 2017-2025
Latin America (Overall)	3.7-9.5%	EML4-ALK fusion	Adenocarcinoma	Mixed ethnicity; Genetic ancestry influences frequency	Chile: 3.7%; Costa Rica: 9.5%; Variable access to testing and treatment	Laguna et al., 2024
Brazil	5-7%	EML4-ALK fusion; KRAS mutations (24.2%)	Adenocarcinoma	Admixed population; Variable smoking history	Outcomes depend on access to molecular testing and targeted therapies	Laguna et al., 2024
Argentina	6-8%	EML4-ALK fusion; KRAS mutations (23%)	Adenocarcinoma	Similar to Brazil	Limited molecular testing in practice; Most patients managed without mutation data	Laguna et al., 2024

1.4 Role of Alk on Lung Cancer

The anaplastic lymphoma kinase (ALK) gene plays a crucial role in a subset of non-small cell lung cancer (NSCLC). The kinase is encoded by the ALK gene located on chromosome 2, particularly on the 2p23 region. In about 3–7% of NSCLC patients, a chromosomal rearrangement causes the gene to fuse with another gene, most commonly EML4. This fusion leads to the production of a constantly active ALK fusion protein, which drives cancer cell growth, division, and survival. The discovery of rearrangements has had a major impact on the treatment of lung cancer. ALK-positive patients often respond well to targeted therapies, particularly ALK inhibitors such as crizotinib, ceritinib, alectinib, and newer third-generation drugs like lorlatinib. These treatments specifically block the activity of the ALK protein, significantly slowing disease progression and improving survival rates.

1.5 Our Aim to Cure Lung Cancer by Alk Protein Mutagenesis Analysis

Our research is focused on understanding and combating lung cancer through ALK protein mutagenesis analysis. This involves studying how specific mutations in the ALK gene affect its structure and function, as well as its interaction with targeted drugs. By identifying mutations that lead to drug resistance or hyperactivity of the protein, we can design more effective, next-generation inhibitors.

This approach allows us to:

- Predict resistance mutations that may emerge during treatment.
- Develop personalized therapies tailored to individual mutation profiles.
- Enhance drug design by understanding the molecular structure of mutant ALK proteins.
- Improve long-term outcomes for ALK-positive lung cancer patients.

Through this focused analysis, we aim to advance precision medicine strategies and move closer to curing ALK-driven lung cancer.

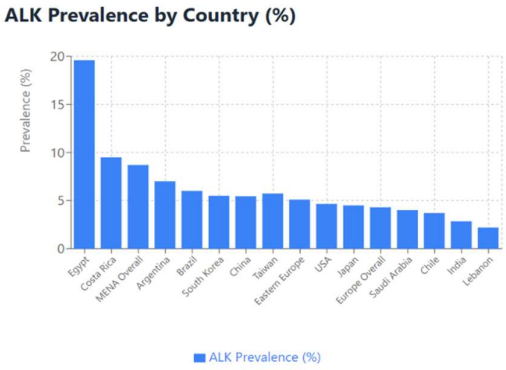

Figure. 1 ALK mutation rate country wise

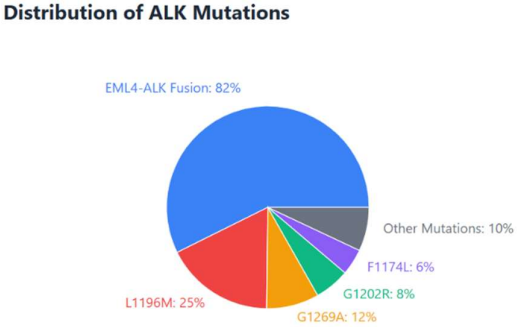

Figure 2. Distribution of ALK mutation

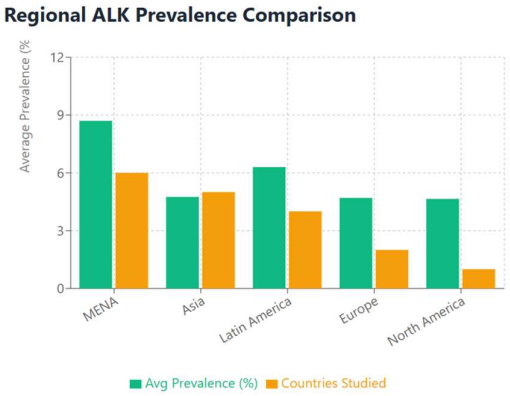

Figure.3. Comparison of ALK mutation

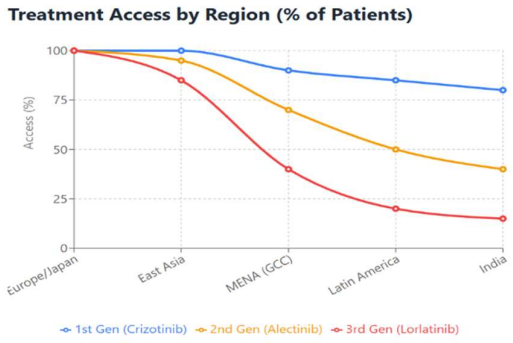

Figure 4. Treatment access by region

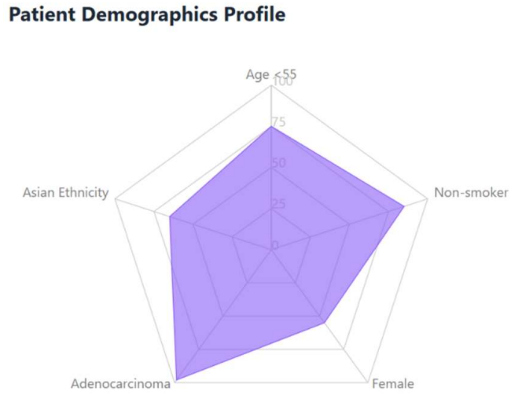

Figure 5. Patient demographic profile

2. Lung Cancer Effects on Physiology

Lung cancer significantly impacts the human body, not only due to the presence of malignant cells in the lungs but also through its systemic effects on various physiological systems. As the disease progresses, it disrupts normal lung function, impairs oxygen delivery, alters metabolism, and weakens the immune system. These physiological disruptions can lead to serious complications and ultimately affect the entire body. The most immediate and profound effect of lung cancer is on the respiratory system. Tumours can grow within lung tissue or bronchial passages, leading to obstruction of airways, causing shortness of breath (Dyspnea), wheezing, and persistent coughing. There is diminished gas exchange, where oxygen intake and carbon dioxide removal become less efficient due to the destruction of alveoli and capillaries. Pleural effusion is the buildup of fluid between the lungs and chest wall, which compresses the lungs and makes breathing more difficult. Haemoptysis (coughing up blood), often a result of tumour invasion into blood vessels. These issues cause a chronic lack of oxygen (hypoxia), which affects nearly all other body systems.

Lung cancer weakens the immune system, both directly and through treatments like chemotherapy and radiation. The tumour itself can suppress immune responses by releasing immunosuppressive signals, allowing it to grow unchecked. Cancer also spreads via the lymphatic system, affecting lymph nodes and reducing the body's ability to fight infections. As lung cancer progresses, it can lead to muscle wasting (cachexia), characterized by severe weight loss, fatigue, and loss of muscle mass. Metastasis to bones is common, causing bone pain, fractures, and elevated calcium levels in the blood (hypercalcemia), which can lead to confusion, kidney issues, and muscle weakness.

The cardiovascular system is indirectly affected by lung cancer. Hypoxia forces the heart to work harder to deliver oxygen to

tissues, potentially leading to tachycardia and high blood pressure as compensatory mechanisms. There is failure of right-side of the heart due to high pressure in the lungs from blocked blood vessels or a tumour mass. Anaemia, either from chronic disease or cancer-related bleeding, can further reduce the oxygen-carrying capacity of the blood. In advanced stages, cancer cells may enter the bloodstream and metastasize to distant organs, further complicating physiological functions. Lung cancer can spread to the liver, impairing its function and leading to symptoms like jaundice, fluid accumulation (ascites), and liver failure. Additionally, nausea, vomiting, and loss of appetite are common—partly from the cancer itself and partly from side effects of treatment—further reducing nutritional status and overall health. Hence, it disrupts vital physiological systems through direct tumour growth, metastasis, and the body's response to chronic illness. Understanding these effects is critical for managing symptoms, improving quality of life, and guiding treatment strategies.

3. Alk Expression and Signalling Pathway

The Anaplastic Lymphoma Kinase (ALK) is a receptor tyrosine kinase (RTK) that plays a crucial role in the development of the nervous system. While ALK expression is typically low or absent in healthy adult tissues, its abnormal activation through mutations, gene rearrangements, or amplification has been strongly associated with several cancers, including non-small cell lung cancer (NSCLC), anaplastic large cell lymphoma (ALCL), and neuroblastoma. In normal physiology, ALK is expressed predominantly in embryonic neural tissues, where it helps regulate cell growth, differentiation, and survival. In adults, its expression is minimal, but in cancers, ALK can become abnormally activated through mechanisms such as Gene fusion, point mutations and duplications. The most common mechanism in NSCLC, where ALK fuses with another gene, most frequently is EML4 (Echinoderm Microtubule-associated protein-Like 4). This results in the EML4-ALK fusion protein, which is constitutively active and no longer regulated by normal cellular mechanisms. Point mutations are found in neuroblastoma and other cancers, which cause abnormal activation of the ALK protein. An increase in the number of copies of the ALK gene can lead to overexpression of the protein. These alterations result in continuous signalling through downstream pathways that promote cancer cell proliferation and survival.

When ALK is abnormally activated, either by mutation or gene fusion, it triggers a cascade of downstream signalling pathways that are vital for cell survival, proliferation, and resistance to apoptosis. The major downstream pathways activated by ALK include:

A. PI3K/AKT Pathway:

The phosphoinositide 3-kinase (PI3K)/AKT signalling cascade plays a major role in promoting cell growth, metabolism, and survival. When ALK is activated, it recruits PI3K, which in turn activates AKT. This pathway inhibits apoptosis and promotes resistance to chemotherapy, making the cancer more aggressive.

B. RAS/MAPK Pathway:

This pathway is involved in cell proliferation and differentiation. Activated ALK stimulates RAS, which then activates the RAF-MEK-ERK kinase cascade. Continuous stimulation of this pathway leads to uncontrolled cell division, a hallmark of cancer.

C. JAK/STAT Pathway:

ALK also activates the Janus kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway, particularly STAT3. This results in the transcription of genes that promote cell survival, proliferation, and immune evasion.

D. PLCγ Pathway:

Activation of phospholipase C gamma (PLCγ) leads to the production of diacylglycerol (DAG) and inositol triphosphate (IP3), which increase intracellular calcium levels and activate protein kinase C (PKC). This contributes to changes in cell adhesion and motility, enhancing the potential for metastasis.

Understanding ALK signalling is crucial in modern oncology as it would help us find which pathway or molecules to target for controlling cancer progression.

4. Therapeutic Use of Alk Mutagenesis

The discovery of ALK rearrangements in NSCLC has revolutionized treatment, allowing for targeted therapy using ALK inhibitors like crizotinib, ceritinib, alectinib, brigatinib, and lorlatinib. These drugs specifically block ALK's kinase activity, shutting down the aberrant signaling pathways and slowing tumour growth. The first breakthrough in ALK-targeted therapy came with the development of **Crizotinib**, a first-generation ALK inhibitor. It specifically targets ALK fusion proteins in ALK-positive NSCLC and blocks their activity. However, over time, many patients develop resistance due to additional mutations in the ALK gene, such as **L1196M, G1269A, and F1174L**. These mutations change the ALK protein's shape, reducing drug binding and allowing the cancer to progress. However, resistance often develops through secondary mutations in ALK or activation of bypass pathways. **Gatekeeper mutations** (e.g., L1196M) introduce steric hindrance at the ATP-binding site, as a consequence, the first-generation inhibitors lose potency. **Solvent-front mutations** (e.g., G1202R) alter the entrance to the ATP pocket rendering most second-gen inhibitors are ineffective; third-gen inhibitors can accommodate these changes. **Macrocyclic design** (third-gen) locks inhibitors into conformations that avoid clashes and stabilize inactive kinase, leading to a broad resistance coverage.

Ceritinib has shown efficacy in preclinical studies and phase 1 trials, inhibiting ALK secondary mutations during crizotinib therapy. In one phase 1 study involving 114 NSCLC patients, the overall response rate was 58%, with notable responses in patients with ALK gene amplification or mutations. Another phase 1 study yielded a response rate of 55% among 20 patients. The ASCEND-1 trial, which included 246 ALK-rearranged NSCLC patients, reported an overall response rate of 72% in ALK inhibitor-naïve patients and 56% in those pretreated. Additionally, the ongoing ASCEND-2 study reported a response rate of 38.6% in 140 ALK-rearranged NSCLC patients who had failed prior treatments (Holla et al.,2017).

Ongoing research into the structure and mutagenesis of ALK is helping to develop next-generation inhibitors and combination therapies to overcome resistance. To overcome this, second- and third-generation ALK inhibitors were developed such as Ceritinib and Alectinib. They are second-generation inhibitors that target both wild-type and mutated forms of ALK. Brigatinib is effective against a wider range of resistance mutations. Lorlatinib is a third-generation inhibitor designed to overcome nearly all known resistance mutations, including those that cause

resistance to second-generation drugs. The development of these drugs is directly linked to the study of ALK mutagenesis, demonstrating how therapeutic research is driven by mutation profiling. First-generation inhibitors, such as Crizotinib, primarily target the ATP-binding site of wild-type ALK, forming hydrogen bonds with the hinge region of the kinase domain. However, their binding affinity is often compromised by gatekeeper mutations (e.g., L1196M) that sterically hinder inhibitor interaction. Second-generation inhibitors, including Ceritinib and Alectinib, exhibit enhanced potency and selectivity by adopting a more flexible binding mode, allowing them to accommodate several resistance mutations. Third-generation inhibitors, exemplified by Lorlatinib, incorporate structural modifications that enable binding to both the wild-type and highly resistant ALK mutants, such as G1202R, by stabilizing the kinase in a specific inactive conformation and avoiding steric clashes. These mechanistic insights provide a structural rationale for the stepwise development of ALK inhibitors and their ability to overcome acquired resistance in non-small cell lung cancer.

Despite the success of ALK inhibitors, acquired resistance remains a major challenge. Resistance can develop through secondary mutations in ALK that reduce drug efficacy and activation of bypass signalling pathways, such as EGFR, MET, or KRAS. Phenotypic transformation (e.g., transformation from NSCLC to small-cell lung cancer) is another way of developing resistance.

Through **mutagenesis analysis**, researchers can identify these resistance mechanisms at the molecular level. By sequencing tumour DNA from patients who relapse after therapy, clinicians can detect new ALK mutations and adjust treatment accordingly. For instance, if a patient develops the **G1202R mutation**, they may respond better to Lorlatinib than to earlier inhibitors.

This kind of real-time monitoring and adaptive treatment strategy — also called precision oncology — relies heavily on understanding and tracking ALK mutagenesis over the course of the disease.

In the lab, ALK mutagenesis is used to model drug resistance and discover new inhibitors. Techniques like site-directed mutagenesis and CRISPR-Cas9 gene editing allow scientists to artificially introduce specific mutations into cancer cell lines or animal models. These models help to understand how each mutation affects drug sensitivity, predict how resistance may develop in patients and design inhibitors that fit the altered structure of mutant ALK proteins. This has led to a structure-based drug design approach, where inhibitors are tailored to the 3D conformation of ALK with specific mutations. Structural studies, often supported by cryo-electron microscopy or X-ray crystallography, guide medicinal chemists in developing next-generation compounds.

Studies of ALK mutagenesis in non-small cell lung cancer employ a combination of genetic, biochemical, cellular, and computational approaches to comprehensively analyze the functional and therapeutic consequences of specific mutations. Site-directed mutagenesis is frequently used to introduce precise point mutations or small insertions/deletions into ALK cDNA, typically via PCR-based methods or commercial mutagenesis kits. The resulting constructs are transfected into cell lines, and successful mutation is confirmed by sequencing, while expression and activity are validated through

immunoblotting and kinase assays. CRISPR-Cas9 gene editing enables the introduction of mutations directly into the endogenous ALK locus in cells or animal models, preserving native regulatory contexts. Following CRISPR modification, genomic sequencing, protein expression analysis, and functional assays—including proliferation, apoptosis, and drug response studies—are performed to evaluate the effects of the mutations in physiologically relevant settings. Complementing these approaches, overexpression systems allow controlled comparison between wild-type and mutant ALK, while in vitro kinase assays with purified recombinant proteins directly measure catalytic activity and inhibitor sensitivity, isolating kinase-intrinsic effects from cellular context. Structural modeling and molecular dynamics simulations provide mechanistic insights, revealing how specific mutations alter the ATP-binding pocket, affect inhibitor binding, and confer resistance. High-throughput mutagenesis and screening approaches, such as saturation mutagenesis libraries, further enable systematic identification of resistance hotspots and unexpected mutations that impact inhibitor efficacy. By integrating these methodologies, researchers can dissect the molecular mechanisms underlying ALK activation, mutation-driven drug resistance, and therapeutic response, providing a detailed framework for the rational design and optimization of next-generation ALK inhibitors.

The therapeutic use of ALK mutagenesis has already improved survival and quality of life in many cancer patients. It plays a central role in

A. **Molecular diagnostics**: Testing for ALK rearrangements and mutations guides treatment decisions.

B. **Treatment selection**: Patients receive therapies best suited to their mutation profile.

Table 2: ALK Mutations and Their Effects on Lung Cancer Treatment

Generation	Inhibitor	Target (WT / Mutants)	Molecular Mechanism / Resistance Overcoming
First	Crizotinib	WT ALK; some activity vs L1196M (limited)	Binds the ATP-binding site of ALK kinase, forming hydrogen bonds with hinge region residues. Gatekeeper mutation L1196M introduces steric hindrance, reducing binding affinity and leading to resistance.
	Ceritinib	WT ALK	Similar ATP-competitive binding; more potent than crizotinib, but less effective against solvent-front mutations like G1202R.
Second	Alectinib	WT ALK, L1196M, C1156Y, F1174C, others	Flexible binding to ATP pocket allows accommodation of bulky gatekeeper and adjacent mutations. Hydrophobic interactions with αC-helix and hinge region stabilize binding despite mutations.
	Brigatinib	WT ALK, L1196M, G1269A, F1174C	Binds ATP pocket with extended substituents to avoid steric clashes from gatekeeper mutations; hydrogen bonding network allows activity against solvent-front and αC-helix mutations.
	Ceritinib (later considered second-gen)	WT ALK, L1196M, G1269A	Similar to alectinib; improved interactions with hydrophobic pocket reduce sensitivity to gatekeeper mutations.
Third	Lorlatinib	WT ALK; resistant mutants including G1202R, L1196M, F1174C, I1171N	Stabilizes ALK in inactive DFG-out conformation, avoiding steric hindrance from gatekeeper and solvent-front mutations. Overcomes almost all clinically relevant resistance mutations.
	Ensartinib	WT ALK; many resistant mutants (L1196M, G1269A, F1174C)	Designed to maintain hydrogen bonding and hydrophobic contacts even in the presence of bulky substitutions in the ATP-binding pocket; flexible binding mode allows activity against multiple mutation classes.

C. **Monitoring disease progression**: Repeating genetic tests during and after treatment helps detect emerging resistance.

D. **Personalized medicine**: ALK mutagenesis allows for highly individualized treatment planning, reducing trial-and-error approaches. Looking ahead, combining ALK inhibitors with agents targeting parallel pathways, immunotherapies, or chemotherapy may improve outcomes further. Ongoing studies continue to investigate novel mutations, combination strategies, and biomarkers of response to optimize ALK-targeted therapy.

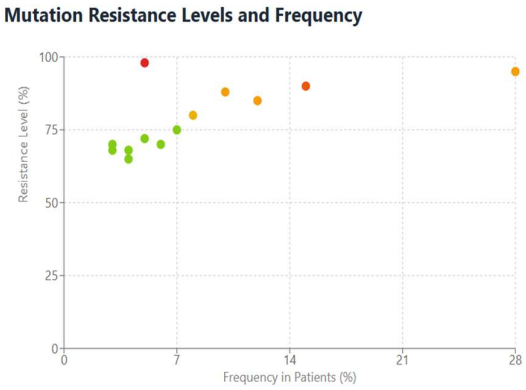

Figure. 6 Mutation resistance level and frequency

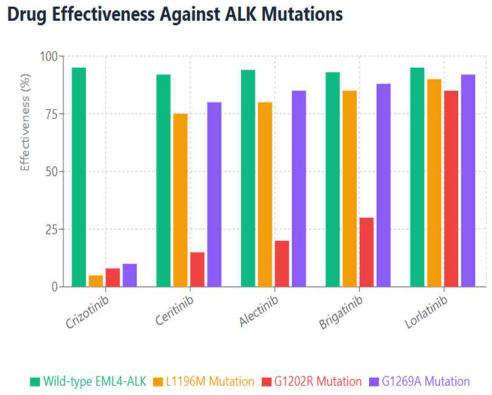

Figure 7. Drug effectiveness against ALK mutation

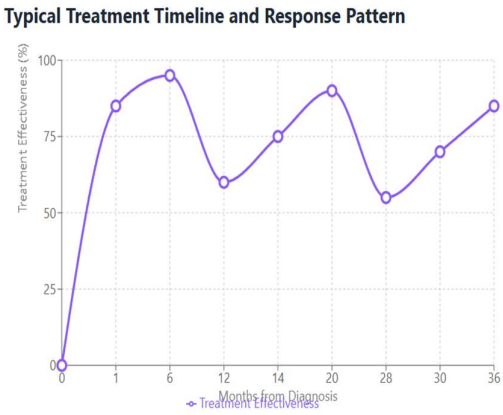

Figure 8. Treatment response pattern

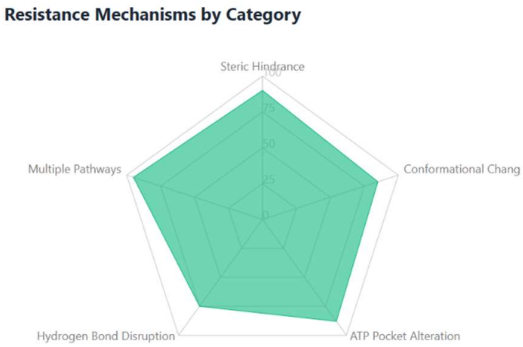

Figure 9. Resistance mechanism by category

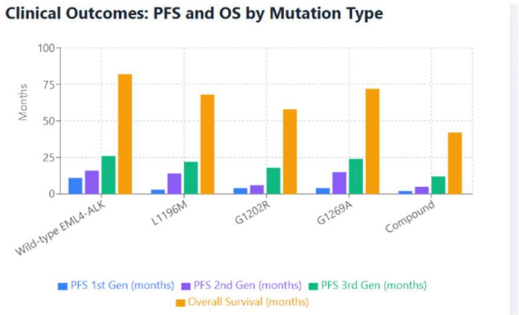

Figure 10. Clinical outcome

Table 3. Overcoming resistance in tabular form

Mutation	Location	Generation of Inhibitor Affected	Mechanism of Resistance	Clinical Effect	Effective Treatment	Reference
L1196M	Kinase domain (gatekeeper position)	First-generation (Crizotinib)	Steric hindrance blocks drug binding to ATP pocket	High resistance to crizotinib; disease progression	Ceritinib, Alectinib, Brigatinib	Choi et al., 2010; Katayama et al., 2012
G1269A	Kinase domain	First-generation (Crizotinib)	Conformational change reduces drug affinity	Moderate to high resistance; reduced progression-free survival	Ceritinib, Alectinib, Lorlatinib	Doebele et al., 2012
F1174L	Kinase domain	First-generation (Crizotinib)	Altered ATP-binding pocket structure	High resistance; associated with aggressive disease	Ceritinib, Brigatinib, Lorlatinib	Choi et al., 2010
G1202R	Kinase domain (solvent front)	First and Second-generation	Steric clash and altered hydrogen bonding	Resistance to both crizotinib and second-gen inhibitors	Lorlatinib (third-generation)	Katayama et al., 2012; Shaw et al., 2020
L1152R	Kinase domain	First-generation	Disrupts drug binding interface	Moderate resistance	Alectinib, Brigatinib	Doebele et al., 2012
C1156Y	Kinase domain	First-generation	Alters ATP pocket configuration	Moderate to high resistance	Ceritinib, Lorlatinib	Katayama et al., 2012
I1171T/N/S	Kinase domain	First and some second-generation	Changes hydrophobic interactions	Variable resistance depending on substitution	Brigatinib, Lorlatinib	Lin et al., 2017
V1180L	Kinase domain	First-generation	Structural alteration of binding pocket	Moderate resistance	Alectinib, Brigatinib	Doebele et al., 2012
F1245C	Kinase domain	First-generation	Reduces drug binding affinity	Moderate resistance	Second and third-generation inhibitors	Katayama et al., 2012
S1206Y	Kinase domain	First-generation	Conformational change in ATP pocket	Moderate resistance	Ceritinib, Alectinib	Lin et al., 2017
D1203N	Kinase domain	First-generation	Disrupts critical hydrogen bonds	Moderate resistance	Second-generation inhibitors	Doebele et al., 2012
E1210K	Kinase domain	First-generation	Electrostatic repulsion affects drug binding	Moderate resistance	Alectinib, Brigatinib	Lin et al., 2017
Compound Mutations (e.g., G1202R + L1196M)	Multiple positions	Second and third-generation	Multiple resistance mechanisms acting synergistically	Extreme resistance; very poor prognosis	Limited options; experimental compounds	Shaw et al., 2020
EML4-ALK (wild-type fusion)	Chromosomal rearrangement (2p23)	Drug-sensitive	Constitutive kinase activation without secondary mutations	Excellent initial response to ALK inhibitors	Crizotinib, Alectinib (first-line)	Soda et al., 2007; Solomon et al., 2014

Benefits of ALK Mutagenesis Analysis in Lung Cancer

1. Precision Medicine and Personalized Treatment

ALK mutagenesis analysis enables clinicians to tailor treatment strategies based on individual patient mutation profiles (Lin et al., 2017). By identifying specific ALK gene rearrangements and mutations, oncologists can select the most effective targeted inhibitor, moving away from the traditional trial-and-error approach and significantly improving treatment outcomes.

2. Early Detection of Drug Resistance

Through continuous molecular monitoring, resistance mutations can be detected before clinical relapse becomes apparent (Katayama et al., 2012). This early detection allows for timely adjustment of therapeutic strategies, such as switching to more potent third-generation inhibitors like lorlatinib when secondary mutations emerge, thereby extending progression-free survival (Shaw et al., 2020).

3. Development of Next-Generation Inhibitors

Understanding the structural and functional consequences of specific ALK mutations guides the design of novel inhibitors (Choi et al., 2010). Structure-based drug design, informed by crystallographic studies of mutant ALK proteins, has already led to the development of increasingly effective drugs that can overcome resistance mechanisms (Lin et al., 2017).

3.1 Fourth-Generation ALK Inhibitors

TPX-0131 (Zotizalkib)

TPX-0131 is a compact macrocyclic molecule designed to fit within the ATP-binding boundary to inhibit ALK fusion proteins, with superior potency against the G1202. TPX-0131 is potent against the G1202R/L1196M compound mutation in cell proliferation assays with an IC50 of 0.7 nmol/L, while other ALK inhibitors had modest to no measurable (Murray et al., 2021). Against the L1196M gatekeeper mutation, TPX-0131 is 76-fold more potent than lorlatinib and 11- to 550-fold more potent than first- and second-generation inhibitors (Murray et al., 2021). Following repeat oral administration to rats, brain levels of TPX-0131 were approximately 66% of those observed in plasma (Murray et al., 2021).TPX-0131 is currently being studied in the phase I/II FORGE-1 trial, with the phase I portion determining safety, tolerability, pharmacokinetics, and recommended phase 2 dose, while the phase II portion will include patients with advanced ALK-positive NSCLC who have received fewer than three prior lines of ALK TKI therapy (Desai & Lovly, 2023).

3.2.NVL-655 (Neladalkib)

NVL-655 is a novel ALK inhibitor designed to have activity against single and compound ALK mutations while sparing TRKB, with in vitro data showing single digit nanomolar efficacy against G1202R, G1202R/L1196M, G1202R/G1269A, and G1202R/L1198F (Desai & Lovly, 2023).

3.3. Proteolysis-Targeting Chimeras (PROTACs)

Novel strategies including development of fourth-generation macrocyclic TKIs and proteolysis targeting chimeras are currently under development to overcome compound mutations that represent an area of major unmet need for patients whose disease progresses on lorlatinib (Desai & Lovly, 2023).

3.4.Antibody-Drug Conjugates (ADCs)

For patients who develop tumor progression due to off-target ALK-

independent resistance, options may include combination therapies targeting ALK and other downstream or parallel pathways, novel antibody drug conjugates, or combinations of ALK inhibitors with chemotherapy and immunotherapy (Desai & Lovly, 2023). ADCs are a hybrid molecule that combines biologics, made up of an antibody scaffold covalently connected by a chemical linker with small molecular payloads, combining the principles of both chemotherapy and immunotherapy (Khan et al., 2023). While ALK-targeted ADCs remain largely in preclinical development, this approach offers a mechanism distinct from kinase inhibition.

3.5. Combination Strategies

To enhance ADC efficacy, they are increasingly being combined with other therapeutic strategies, including immune checkpoint inhibitors, chemotherapy, small-molecule inhibitors, anti-angiogenic agents, and CAR-T cell therapies, with these combination therapies aiming to overcome resistance mechanisms, improve tumor targeting, and boost immune responses (Mao et al., 2025). The single-arm pilot study ALKTERNATE investigated fixed alternating cycles of lorlatinib intercalated with crizotinib in individuals resistant to second-generation ALK inhibitors, revealing safety, feasibility, and effectiveness with a median time-to-treatment failure of 10 months (Itchins et al., 2024).

4. Improved Survival Rates and Quality of Life

ALK-targeted therapies have dramatically improved outcomes for ALK-positive NSCLC patients compared to traditional chemotherapy (Solomon et al., 2014; Peters et al., 2017). The five-year survival rate for these patients has increased substantially, and targeted inhibitors generally cause fewer side effects than conventional treatments, preserving patient quality of life (Costa et al., 2015).

5. Advancement of Cancer Biology Knowledge

Studying ALK mutagenesis deepens our understanding of cancer signaling pathways, including PI3K/AKT, RAS/MAPK, JAK/STAT, and PLCγ cascades (Hallberg & Palmer, 2013). This knowledge extends beyond lung cancer and contributes to therapeutic strategies for other ALK-driven malignancies such as anaplastic large cell lymphoma and neuroblastoma (Morris et al., 1994).

6. Cost-Effectiveness in the Long Term

Although molecular testing and targeted therapies are initially expensive, personalized treatment reduces unnecessary exposure to ineffective drugs, minimizes hospitalizations due to treatment-related complications, and ultimately proves more cost-effective than traditional approaches with poorer outcomes.

7. Foundation for Combination Therapies

Mutagenesis analysis reveals bypass resistance mechanisms involving alternative pathways like EGFR, MET, or KRAS (Doebele et al., 2012; Yasuda et al., 2012). This insight enables the rational design of combination therapies that simultaneously target multiple pathways, potentially preventing or delaying resistance development.

Challenges of ALK Mutagenesis Analysis in Lung Cancer

1. Emergence of Multiple Resistance Mechanisms

Cancer cells can develop resistance through various mechanisms including secondary ALK mutations, activation of bypass signaling pathways, and phenotypic transformation (such as conversion to small cell lung cancer) (Katayama et al., 2012; Doebele et al., 2012). Managing these diverse resistance patterns requires continuous monitoring and increasingly complex treatment strategies.

2. Limited Accessibility and High Costs

Advanced molecular testing, including next-generation sequencing and mutation profiling, remains expensive and inaccessible in many regions, particularly in developing countries like India where awareness and access to modern diagnostics are limited (Indian Council of Medical Research, 2023). This creates disparities in treatment outcomes between different socioeconomic groups and geographic regions.

3. Tumour Heterogeneity

Lung tumours often contain multiple clonal populations with different mutations. A single biopsy may not capture the full mutational landscape, leading to incomplete treatment planning. Spatial and temporal heterogeneity means that mutations present at diagnosis may differ from those at relapse (Lin et al., 2017).

4. Technical Complexity and Expertise Requirements

Conducting mutagenesis analysis requires sophisticated laboratory techniques such as CRISPR-Cas9 gene editing, site-directed mutagenesis, cryo-electron microscopy, and X-ray crystallography. These methods demand specialized equipment, highly trained personnel, and substantial infrastructure investment.

5. Time Lag in Mutation Detection

Current diagnostic approaches often identify resistance mutations only after clinical progression becomes evident (Katayama et al., 2012). Real-time monitoring technologies are still evolving, and the delay between mutation emergence and detection can allow resistant clones to expand, limiting treatment options.

6. Incomplete Understanding of All Mutations

While major resistance mutations like L1196M, G1269A, and G1202R are well-characterized (Choi et al., 2010), rare or novel mutations continue to emerge. The clinical significance and optimal treatment approaches for these uncommon variants remain uncertain, complicating treatment decisions.

7. Drug Development Lag

Despite rapid progress, the development of new inhibitors to overcome emerging resistance mutations takes years (Camidge et al., 2018). Regulatory approval processes, clinical trials, and manufacturing scale-up mean that patients may develop resistance faster than new drugs become available.

8. Sample Acquisition Challenges

Obtaining adequate tumour tissue for comprehensive mutation analysis can be difficult, especially for repeated biopsies during disease monitoring. Liquid biopsies using circulating tumour DNA show promise but are not yet standardized or universally available, and their sensitivity varies.

9. Limited Applicability

ALK rearrangements occur in only 3–7% of NSCLC patients (Soda et al., 2007), meaning this approach benefits a relatively small subset of lung cancer cases. The majority of patients with other driver

mutations or no identified targetable alterations cannot benefit from ALK-specific therapies.

10. Psychosocial and Ethical Considerations

Continuous genetic monitoring and the possibility of developing untreatable resistance mutations can cause significant psychological distress for patients. Additionally, questions arise regarding informed consent, data privacy, and equitable access to precision medicine technologies.

Conclusion

While ALK mutagenesis analysis represents a significant advancement in lung cancer treatment with clear benefits in survival and quality of life (Solomon et al., 2014; Peters et al., 2017), addressing the challenges of accessibility, cost, tumour complexity, and evolving resistance mechanisms remains critical. Future research must focus on developing more affordable diagnostic tools, improving real-time monitoring capabilities, accelerating drug development pipelines, and ensuring equitable access to precision oncology worldwide (Lin et al., 2017; Shaw et al., 2020).

Conflict of interest

There is no conflict of interest among the authors of the manuscript

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