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Original Article

Spectrum of Resistance Mechanisms to ALK TKIs in NSCLC: Largest Single-Center Experience from India

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Abstract

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Introduction Anaplastic lymphoma kinase (ALK) rearranged non-small cell lung carcinoma (NSCLC) has emerged as a distinct entity with growing number of potent ALK tyrosine kinase inhibitors (TKIs). Despite showing durable responses and promising survival rates, resistance to these ensue. This is the largest series of repeat biopsies from patients of ALK-positive NSCLC progressing on ALK-directed therapy from this part of the world. Using a combinatorial approach of genomics and histology, we describe the spectrum of various resistance mechanisms encountered.

Methods This is a cross-sectional study recruiting ALK-positive NSCLC cases who have progressed on any line ALK TKI and have undergone repeated biopsies followed by genomic sequencing by next-generation sequencing (NGS).

Results Thirty-two ALK-positive NSCLC patients progressed on TKI were enrolled. Median age was 53 years (range: 36–75 years) with a male predilection (male:female 1.3:1). Twenty-seven (84.4%) cases harbored an additional resistance mechanism. Eighteen of these harbored an on-target ALK alteration, with L1196M gatekeeper mutation being the most common, in 11 cases, and G1202 alteration in 3 cases. In 9 cases an off-target alteration was detected, the most frequent being TP53 mutation in 8 cases, KRAS mutation in 4 cases and MET amplification in 3 cases. Four patients underwent sequential NGS testing and allele frequency changes in ALK fusion and resistance mechanisms were demonstrated. Sixteen patients have been offered lorlatinib therapy, the median progression-free survival of which has not yet been reached.

Conclusion This is the largest series depicting ALK resistance mechanisms from a single center to date. The SPACEWALK study which demonstrated ALK TKI resistance

Keywords
- ALK
- resistance
- liquid biopsy
- NGS

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mechanisms using plasma-based genotyping was a multicentric study. The spectrum encountered in this study is distinct from the rest of the world, thus highlighting heterogeneity within ALK-rearranged tumors. Comprehensive clinical evaluation at disease progression coupled with NGS-based genotyping will pave the way for lucid understanding of disease biology, thus aiding in the institution of optimal therapy.

Introduction

Anaplastic lymphoma kinase (ALK) rearranged non-small cell lung carcinoma (NSCLC) has emerged as a distinct entity with a growing number of potent ALK tyrosine kinase inhibitors (TKIs). Crizotinib, a first-generation TKI, emerged as the forerunner among these, and has shown remarkable responses as evidenced in the PROFILE study.[1] However, resistance mechanisms ensue and newer generation alectinib and ceritinib were developed to overcome these.[2,3] Despite showing durable responses and promising survival rates, resistance to these have also been reported. The newest third-generation lorlatinib has now gained approval for use in the first-line setting (Food and Drug Administration data sheet 2021),[4] and has been described to overcome resistance to the second-generation TKIs.

Resistance to TKIs can broadly be categorized as on-target alterations which include ALK kinase domain alterations (the most common being G1202R, L1196M, G1269A) and ALK amplification; and the second category includes off-target mechanisms involving upregulation of other bypass pathways (*EGFR*, *SRC*, *MEK/ERK*, *KIT*, and others).[5–10] Description of resistance mechanisms to crizotinib have been extensively reported in literature with almost one-third cases showing on-target resistance mechanisms.[11] However, the same for second-generation drugs is limited to in vitro studies, few case series and reports, and anecdotal cohorts. Resistance to lorlatinib has also emerged and still needs real-world elucidation.[12,13]

In this study, we present the largest series of repeat biopsies (tissue and/or liquid) from patients of ALK-positive NSCLC who have progressed on ALK-directed therapy from this part of the world. Using a combinatorial approach of genomics and histology, we describe the spectrum of various resistance mechanisms encountered as patients relapse on ALK TKIs.

Methods

Patient Accrual

All patients of ALK-positive NSCLC treated with any ALK TKI in any line and progressed on the same during their disease course were considered for enrolment in this study. Only those patients with a rebiopsy (tissue) or frozen plasma sample at the time of progression on TKI were recruited. Those with insufficient tissue in the formalin-fixed paraffin-embedded (FFPE) block/suboptimal nucleic acid quantity or quality were excluded from the study.

Patient Details

The basic demographic and clinical details of the patients were retrieved from the electronic medical record archives of the hospital. Histologic evaluation was done independently performed by two experts and the details were collated. The patients were followed up telephonically. This study conforms to the Declaration of Helsinki and no animal experiments were performed for the same. The study has been approved by the Institutional Review Board.

Genomic Sequencing

Comprehensive Genomic Profiling (Tissue)

The tumor block was examined for adequacy of cellularity by an experienced pathologist. Blocks with $>20\%$ cellularity were considered optimal for further genomic sequencing. Comprehensive genomic profiling was done using Oncomine Focus Assay (Thermofisher Scientific, Lifetechnologies, United States) encompassing 52 genes, including both deoxyribonucleic acid (DNA)- and ribonucleic acid (RNA)-based alterations. The libraries were prepared, and the templates were enriched on Ion Chef using Ion One Touch 2. The prepared libraries were quality checked on TapeStation (Agilent). The final libraries were optimized and equalized and then sequenced on Ion Torrent S5 platform. The run was quality checked on TorrentSuite v5.10 (Thermofisher Scientific, Lifetechnologies) and the variants were called using Torrent Variant Caller and Oncomine Knowledge Reporter (Thermofisher Scientific, Lifetechnologies). The called variants were visualized on the Integrative Genomics Viewer to ascertain the validity of the call. The variants were determined against the tumor cellularity of the tumor block, and variants with allele frequency of less than 5% were not considered.

Comprehensive Genomic Profiling (Liquid)

Liquid biopsy next-generation sequencing (NGS) was done for those patients with insufficient material in FFPE blocks and for those who did not consent for a tissue rebiopsy. Comprehensive genomic profiling of cell-free DNA extracted from plasma was done using Lung Cell-Free Total Nucleic Acid panel (Thermofisher Scientific, Lifetechnologies) encompassing 11 genes, including both DNA- and RNA-based alterations. Twelve milliliters of ethylenediaminetetraacetic acid anticoagulated peripheral blood was centrifuged at 3,000 revolutions per minute for 10 minutes. The plasma was carefully separated, and cell-free total nucleic acid was extracted using QiaAMP cell-free extraction kit

from Qiagen. The library was prepared, and the template was enriched on Ion Chef using Ion One Touch 2. The prepared libraries were quality checked on TapeStation (Agilent). The final libraries were optimized and equalized and then sequenced on Ion Torrent S5 platform. The run was quality checked on TorrentSuite v5.10 (Thermofisher Scientific, Lifetechnologies) and the variants were called using Torrent Variant Caller and Oncomine Knowledge Reporter (Thermofisher Scientific, Lifetechnologies). The called variants were visualized on the Integrative Genomics Viewer to ascertain the validity of the call.

Statistical Analysis

All of the statistical analyses were performed on SPSS version 23 for windows (SPSS Inc, Chicago, Illinois, United States). The categorical variables were presented in frequencies along with respective percentages. Graphs were generated using Microsoft Excel.

Results

Between 2015 and 2021, a total of 32 patients with ALK-positive NSCLC, who progressed on any ALK TKI, were enrolled in this study based on the availability of a rebiopsy specimen at disease progression. The median age was 53 years (range: 36–75 years) with a male predilection (male:female 1.3:1). ALK immunohistochemistry was positive in all the 32 cases at diagnosis, whereas on NGS an ALK fusion was detected in 30 out of the 32 cases. The two cases which did not reveal a fusion on NGS, were confirmed on fluorescence in situ hybridization and showed break-apart signals. ►**Table 1** depicts the basic demographic details, preenrolment, and postenrolment treatment details.

Of these 32 cases, 27 (84.4%) cases were found to harbor an additional resistance mechanism. Among these 27 cases, 12 (44.4%) received crizotinib as the first line, 4 (%) received alectinib, and 11 received ceritinib. Eighteen of these 27 cases harbored an on-target ALK alteration, with L1196M gatekeeper mutation being the most common, seen in 11 cases, and G1202R alteration seen in 3 cases. One case harbored the I1171T mutation after first-line alectinib, and three cases showed ALK amplification. In 9 cases a potential off-target alteration was detected, the most frequent being TP53 mutation in 8 cases, followed by KRAS mutation in 4 cases and MET amplification in 3 cases. ►**Fig. 1** depicts the spectrum of resistance alterations detected along with their frequencies and related drugs.

A total of four patients underwent serial rebiopsies after subsequent lines of treatment. ►**Fig. 2** Three out of these four patients were treated with crizotinib in the first-line setting, of which two developed L1196M solvent front mutation, one patient developed a G1202del, and one patient treated with alectinib developed KRAS mutation and MET dysregulation. The two patients who developed L1196M were offered ceritinib, and post-ceritinib progression NGS revealed additional D1203N mutation in one patient along with increased allele frequency of the existing L1196M mutation, and the other developed an ALK amplification with a copy number of 8.4. The patient with G1202del was offered alectinib, and post-alectinib progression revealed stable allele frequency of the G1202del mutation, with an additional TP53 alteration (allele frequency: 2.3%, possibly indicative of clonal hematopoiesis of indeterminate potential) on liquid biopsy-based NGS profiling. The patient with KRAS (p.G12V), MET exon 14 skipping mutation, showed an increase in variant allele frequency of the KRAS alteration (from 3.2 to 4.6%).

Table 1 Basic demographic details, preenrolment, and postenrolment treatment details of the patients in the study population

Characteristics	Number	%
Age: Median	53	–
Range	36–75 y	–
Gender		
Male	18	56.3
Female	14	43.7
Preenrolment TKI		
Crizotinib	12	37.5
Ceritinib	12	37.5
Alectinib	8	25
Postresistance treatment given		
Ceritinib	9	28.1
Alectinib	7	21.9
Lorlatinib	16	50

Abbreviation: TKI, tyrosine kinase inhibitor.

Discussion

This is a real-world experience of ALK TKI resistance from this part of the world. A total of 32 patients who progressed on any ALK TKI and who underwent NGS-based testing for detection of potential resistance mechanisms were enrolled. Using targeted NGS, resistance mutations were detected in 27 (84.4%) cases, with 18 (56.3%) being on-target ALK mutations and 9 (28.1%) being secondary off-target mechanisms.

Our positive rate of resistance mutations in rebiopsied samples (84.4%) is substantially higher when compared to contemporary real-world studies, or controlled clinical trials. Lin et al reported a positive rate of 39%, as well as Gainor et al reported a positive rate of 38%.[5,6,14] This may be attributed to the enriched nature of the population in our study, as only those who underwent NGS-based testing were included. This proposition has also been exemplified by the high 52% positive rate from Guardant database where mutations were encountered in 16/31 patients enrolled.[15] Additionally, ethnic and geographic differences, analogous to *EGFR*-mutated NSCLC, may also contribute to the same.[16,17]

As reported by other real-world studies and trials, L1196M, G1202R, and D1203N are known mechanisms of resistance to crizotinib.[18,19] Also, crizotinib was the most common drug, after which resistance mechanisms developed. While G1202 mutations have been reported to be

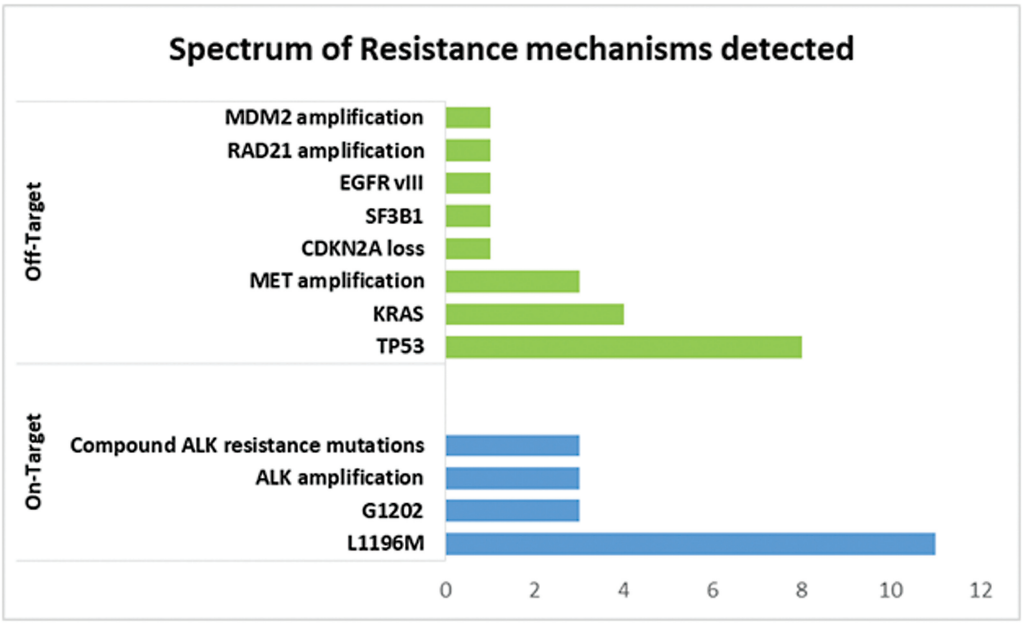

Fig. 1 Spectrum of on-target and off-target resistance mechanisms encountered in the study cohort posttreatment with anaplastic lymphoma kinase (ALK) tyrosine kinase inhibitor therapy.

common after second-generation ALK TKIs, in our study, this was not encountered as most cases were post-crizotinib. Secondary ALK mutations like L1196M and I1171T[20,21] have been shown to be sensitive to ceritinib and not alectinib. One patient subsequent to treatment with first-line alectinib, developed I1171T mutation, which has been reported to be sensitive to ceritinib. The response to ceritinib in this patient was partial response and the patient is currently on second-line ceritinib therapy with an ongoing response.

The role of rebiopsy versus a liquid biopsy-based assessment of circulating tumor DNA and newly emerged mutations have been widely discussed in literature.[22–25] Whereas tissue is the gold-standard material to ascertain any emergent and evolved clones/mutations, liquid biopsy has

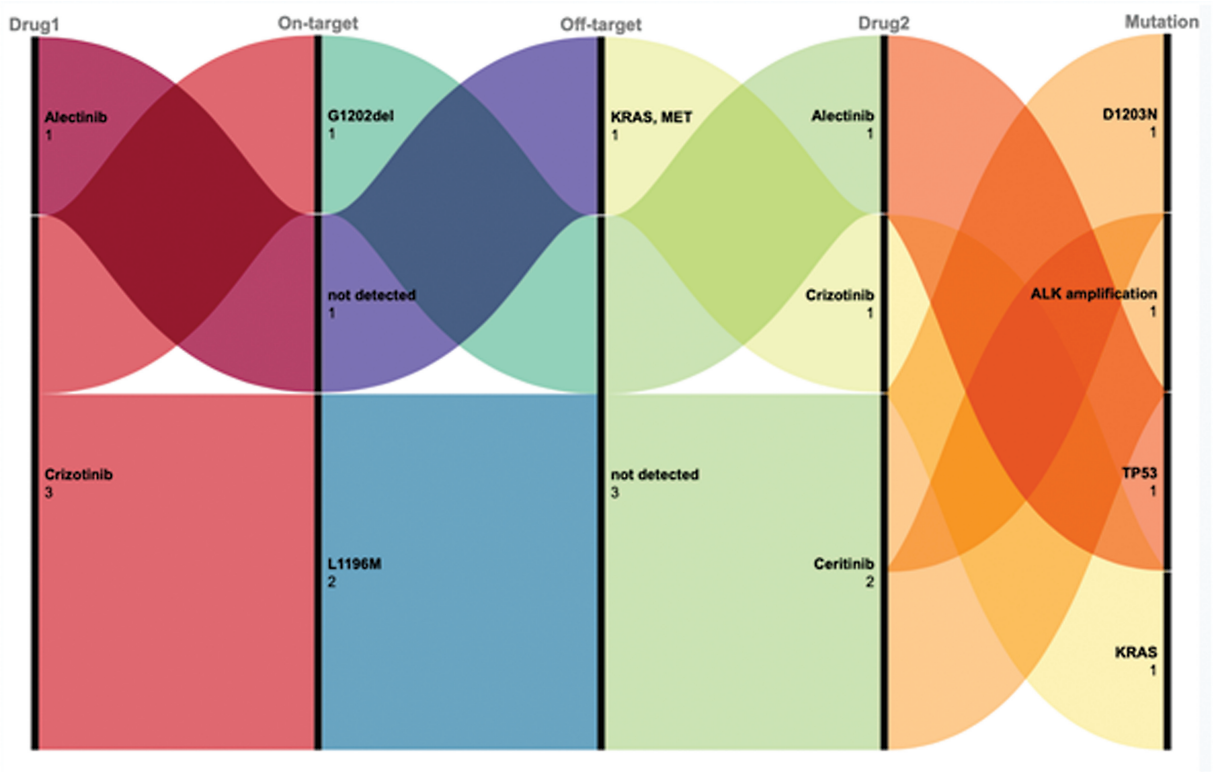

Fig. 2 Alluvial diagram depicting the sequence of alterations and sequential tyrosine kinase inhibitor (TKI) therapy in the four patients who underwent serial biopsies after every line of treatment.

emerged as an effective surrogate for the same, especially in the metastatic setting where the disease burden is high. Issues related to tumor heterogeneity with dynamic clones present in different areas of a tumor are also taken care of when using liquid biopsy-based testing.[26,27] In a study by Shaw et al,[28] on sequencing of ALK TKIs, they reported that in cases who progress on alectinib, the underlying cause in almost 50% cases is a secondary ALK mutation, and hence rebiopsy in such cases revealed both pan-TKI resistant mutations as well as secondary mutations which were proven sensitive to ceritinib as well as crizotinib, both in vitro and in vivo.[29]

This is a single-center real-world experience, the largest from the Indian peninsula, depicting resistance profiles to ALK TKIs. Owing to ethnic and geographic heterogeneity in terms of prevalence of biomarker-driven process in both EGFR and ALK altered NSCLC in the Asian population, this study becomes relevant depicting the clinical behavior and responses, including the need for rebiopsy (tissue/liquid) to ascertain the underlying molecular causes for resistance or disease progression. Future artificial intelligence-based algorithms may be developed to predict these mechanisms of resistance for instituting optimal therapy.[30]

Publication/Presentation Statement

This study has been presented at the European Lung Cancer Congress 2022 as a poster.

Competing Interests

The authors have no relevant financial or nonfinancial interests to disclose.

Authors' Contributions

U.B. contributed to the study conception and design. Material preparation, data collection and analysis were performed by U.B., S.N., M.S., A.B.P., S.D., D.K., A.M., and S.V. The first draft of the manuscript was written by S.N. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The supervision for the entire study was by U.B.

Data Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Rajiv Gandhi Cancer Institute and Research Center (RGCIRC/IRB-BHR/165/2021).

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None.

Conflict of Interest

None declared.

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