Replacement of ALK inhibitors as an effective strategy for reducing drug toxicity in non-small cell lung cancer patients with ALK gene rearrangement

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Abstract
This case report examines the effects of replacement of anaplastic lymphoma kinase inhibitor (ALKi) as a strategy to reduce drug toxicity in patients with non-small cell lung cancer (NSCLC) with ALK gene rearrangements. A 61-year-old female patient with lung adenocarcinoma encountered difficulties in ALK abnormalities diagnosis: the expression of abnormal ALK protein was not detected by the immunohistochemistry (IHC) assay, but ALK gene rearrangement was present in next generation sequencing (NGS) and fluorescence in situ hybridization (FISH) assays. The patient was initially treated with second-generation ALKi (alectinib). However, the patient experienced severe hepatotoxicity. She was successfully switched to brigatinib (another second-generation ALK inhibitor). During brigatinib therapy, a transient increase in creatinine kinase concentration was observed, which required brigatinib dose reduction. Effectiveness of both anti-ALK agents was observed (partial response to treatment, followed by disease stabilization). This case report illustrates the difficulties in diagnosing ALK gene rearrangements and the possibility of replacing ALK inhibitors without compromising treatment efficacy.

Key words: ALK rearrangement, alectinib, brigatinib, hepatotoxicity, lung adenocarcinoma

Introduction
The anaplastic lymphoma kinase (ALK) gene rearrangement occurs in approximately 4.5% of non-small-cell lung cancer (NSCLC) patients. It is found mainly in young and non-smoking patients with adenocarcinoma. It is the third most common driver alteration in lung adenocarcinoma after mutations in the kirsten rat sarcoma virus (KRAS) and epidermal growth factor receptor (EGFR) genes. There are different fusion partners for the ALK gene, and some variants are very rare. The most common is the fusion of exon 13 of the echinoderm microtubule-associated protein-like 4 (EML4) gene and exon 20 of the ALK gene (variant 1). Slightly more seldom, exon 20 of the EML4 gene is fused with exon 20 of the ALK gene (variant 2) or exon 6 of the EML4 gene with exon 20 of the ALK gene (variant 3a or 3b). The genetic fusion partner for the ALK gene and the fusion variant may determine the usefulness of different methods of ALK gene diagnosis and the effectiveness of treatment with ALK inhibitors (ALKi) [1].
Three generations of ALKi are available for locally advanced or advanced NSCLC patients with ALK gene rearrangement. The first-generation crizotinib, which is characterized by relatively low efficacy and poor penetration to the central nervous system (CNS) is rarely used. Alectinib, brigatinib, ceritinib, and ensartib belong to the second generation of ALKi. These drugs are more effective than crizotinib, especially in the treatment of CNS metastases, and can be used both in the first line of treatment and after crizotinib treatment failure. The third generation of ALKi is lorlatinib — it has the highest CNS penetration and high intracranial and extracranial efficacy. Lorlatinib can be used in the first-line treatment and patients with failure of first- and second-generation ALKi therapy. All these drugs differ in their toxicity profile [2].

The patient presented in this report experienced ALKi treatment toxicity, which was managed by switching the inhibitor. The patient also had difficulties in the diagnosis of ALK gene rearrangement probably due to the presence of a rare EML4-ALK fusion variant. The patient gave her written consent to participate in research following approval of the local bioethics committee at the Medical University of Lublin (No. KE-0254/160/2021).

**Case report**

A 61-year-old female patient, a former cigarette smoker, was unsuccessfully treated in July 2021 for bronchitis with a persistent dry cough. Comorbidities included multinodular thyroid goiter, hypertension, anemia, neutropenia, and type 2 diabetes. The patient had a good performance status. A chest X-ray revealed the presence of pleural effusion on the left side. The presence of pleural effusion was confirmed on computed tomography (CT) imaging, and a tumor in the left hilum, the prevertebral soft-tissue lesion measuring 34 × 26 mm at the carina level and fluid in the pericardium were revealed. In August 2021, a left-sided diagnostic thoracotomy was performed with partial resection of rib VI and decortication of the left lung. In the material from the pleura, infiltration of lung adenocarcinoma with the expression of cytokeratin 7 and 19 (CK7 and CK19) and epithelial membrane antigen (EMA) was found. This material did not show mutations in EGFR gene which was examined by real-time PCR method with Entrogen reagent kit and COBAS Z480 real-time equipment. ALK abnormal protein expression was not detected by immunohistochemistry (IHC) method using the Ventana D5F3 antibody clone and BenchMark GX autostainer. ROS1 gene rearrangement was excluded by fluorescent in situ hybridization (FISH) method using ZytoVision ROS1 Dual Color Break Apart Probe.

Programmed death ligand 1 (PD-L1) expression was visualised on < 1% tumor cells and was examined by IHC method using Ventana SP263 antibody clone and BenchMark GX autostainer.

A decision about performing in-depth diagnostics was made. In September 2021, a positron emission tomography–computed tomography (PET-CT) examination was performed. Pleural effusion accumulated FDG [(18)F-FDG, (18)F-2-fluoro-2-deoxy-D-glucose] in the left costophrenic angle, where maximum standardized uptake value (SUVmax) was 3.3. Numerous, metabolically active nodules were present in the left pleura. The primary lesion measuring 65 × 45 mm was present at the level of the lower part of the left hilum (SUVmax = 15.7 with a central cold area, indicating tumor disintegration). A prevertebral soft-tissue lesion modulating the lumen of the esophagus shown on CT did not accumulate FDG, suggesting a reservoir of encapsulated, thick fluid. A 13 × 10 mm nodule was present at the proximal part of the descending aorta (SUVmax = 13.0). Numerous enlarged and metabolically active lymph nodes were visualized in the lower part of the left lung hilum (22 × 13 mm, SUVmax = 15.1), aortopulmonary window (17 × 12 mm, SUVmax = 13.5), pulmonary trunk (15 × 13 mm, SUVmax = 9.0), at the apex of the heart (20 × 12 mm, SUVmax = 12.6), suprarenal and paraspinal on the left side (20 × 14 mm, SUVmax = 10.7). Increased FDG uptake was also observed postoperatively in the stump of rib VI (SUVmax = 5.8). On this basis, stage IVA of lung adenocarcinoma (pT4N2M1A according to the 8th Edition of TNM in Lung Cancer) was diagnosed (Fig. 1A–C).

In addition, RNA-based next-generation sequencing (NGS) was performed on the material obtained during thoracotomy to qualify the patient for a clinical trial. The assay used RNA isolated from the formalin-fixed paraffin-embedded (FFPE) material. The first fusion variant of the EML4-ALK gene was detected (fusion of exons 13 and 20). The FISH method (using Vysis ALK Break Apart FISH Probe Kit) was performed due to discrepancies between the IHC and NGS results in the assessment of the presence of ALK gene rearrangements. Single red signals were found in 20% of tumor cell nuclei, which allowed for recognition of ALK gene rearrangement.

In November 2021, alectinib therapy at the standard dose of 600 mg twice a day was started. During the first month, the treatment was interrupted for a week due to the onset of herpes zoster, which required acyclovir therapy. However, on the first follow-up CT scan, partial remission was observed. The primary tumor was reduced to 25 mm in the longest diameter (baseline — 65 mm). Pleural effusion was encapsulated. Mediastinum and subaortic lymph nodes regressed and were not enlarged on the short axis (Fig. 2A, B). The amount of fluid in the pericardium decreased.
After two months of treatment with alectinib, an increase in the activity of liver enzymes was noted — the increase in alanine transferase (ALT) values was 89 U/L, and asparagine transferase (AST) of 89 U/L [grade 1 toxicity according to Common Terminology Criteria for Adverse Events (CTCAE)] was detected. Alectinib therapy was continued with close observation. However, at the beginning of February 2022, grade 4 hepatotoxicity was found. The AST level increased to 905 U/L, the ALT level — to 732 U/L, and the bilirubin level — to 2 mg/dL. Alectinib therapy was interrupted until liver enzymes were normal. After 6 weeks, the patient received a reduced dose (300 mg twice a day) of alectinib, unfortunately, the activity of liver enzymes increased again. Alectinib therapy was permanently discontinued. After a significant decrease in liver enzymes in April (AST — 69 U/L, ALT — 58 U/L), brigatinib (another second-generation ALK inhibitor) was administered.

A different type of toxicity was observed after one month of brigatinib therapy at the standard dose (90 mg once a day for the first 7 days, then 180 mg once a day) in the form of an increased concentration of creatine kinase at 863 U/L. Brigatinib therapy was interrupted,
and after normalization of the enzyme concentration, the treatment was resumed at the same dose. After restarting treatment, the concentration of creatine kinase increased again to 1218 U/L. The enzyme concentration was rapidly normalized after withholding treatment. Currently, the patient continues brigatinib therapy at the reduced dose of 120 mg once a day. On subsequent follow-up CT, the disease remains stable (the size of the target lesion in the left lung is currently 21 × 11 mm).

Discussion

ALK gene rearrangement can be detected by IHC, FISH, and NGS. However, each of them has limitations. The gold standard was the FISH method, which was used in early clinical trials with crizotinib. It was validated during clinical trials of ALK inhibitors, but due to the costs and difficulties in interpretation of the results, FISH is increasingly replaced by other methods. The IHC method is not expensive and quite simple, which makes it useful in screening for the presence of abnormal ALK proteins. However, uncertain IHC results should be confirmed by FISH or NGS methods. Mattsson et al. [4] studied 712 patients using both IHC (clone D5F3) and FISH methods. The FISH method detected ALK rearrangements in 13 patients and the IHC method in 14 patients. In 9 patients, the results from both methods coincided, however, in 5 patients the results were not confirmed. The study showed that sensitivity and specificity of the IHC method, compared to the FISH method, were 61.5% and 99.6%, respectively [4].

Currently, the NGS method is beginning to gain recognition because it allows detection of all fusion partners of the ALK gene. The most common fusion partner for the ALK gene is the EML4 gene, however, there are several different variants of rearrangements. The other partners are the KIF5B, KLC1, TFG, and PTPN3 genes. Not all diagnostic methods detect them all [3]. Siraj et al. examined patients using the NGS method. Of over a thousand patients, 47 were diagnosed with ALK gene rearrangements — in most patients (41 cases), the EML4-ALK fusion gene was detected while the remaining (6 cases) were KIFB-ALK, CLTC-ALK, TFG-ALK, EIF2AK-ALK, PPM1B-ALK, and PRKAR1A-ALK. Of these patients, 31 were also eligible for FISH and 11 of them had negative FISH test results. FISH failed to detect EIF2AK3-ALK, PRKAR1A-ALK, and one of the EML4-ALK variants [5]. In 2022, Zhao et al. [6] tested nearly 15000 patients using the NGS and IHC methods, including 12533 cases examined by DNA-based NGS and 2361 cases examined by RNA-based NGS tests. Based on DNA examination, they showed the presence of ALK gene rearrangements in 439 (3.5%) patients. RNA analysis identified fusion variants in 52 (2.2%) patients. At the same time, expression of ALK abnormal protein in the IHC test was detected in 455 patients from the DNA-tested group (3.6%) and 62 patients in the RNA-tested group (2.6%). Overall percentage agreement (OPA), positive percentage agreement (PPA), and negative percentage agreement (NPA) of NGS vs IHC test results were calculated. In the DNA-tested group, OPA, PPA, and NPA were 99.60%, 92.75%, and 99.86%, respectively. In the group of patients with adenocarcinoma, the PPA was 95.69%. Regarding the RNA-tested group, these values were as follows: 99.49%, 82.26%, and 99.96%, and in the group of patients with adenocarcinoma, the PPA was 82.26%.

The percentage distribution of specific fusion partners was similar to the results of other studies. It is noteworthy that in one case where the FAM114A1-ALK variant was detected, it was not confirmed by any of the other methods (FISH, IHC, or RT-PCR) [6]. The results showed that only the NGS method could detect all partners of the ALK gene and all their variants, and perhaps in the future, it should become the gold standard in diagnosis of ALK gene rearrangement.

In the presented case report, ALK gene rearrangement was detected by the NGS and FISH methods. The IHC method failed to detect the abnormal ALK protein although the presence of the most common, first EML4-ALK fusion variant was confirmed. The fusion of exons 13 and 20 of these genes is not always the same. Exon 20 of the ALK gene contains 187 nucleotides. The most common exon 20 breakage points are known. However, DNA breakage could occur in different parts of exon 20. This influences the differences in the structure of the ALK protein, which may affect the effectiveness of IHC tests. Moreover, the ALK protein may be damaged during FFPE material formation. ALK protein expression may then not be visible in IHC assays. On the other hand, improper fixation of tissue material may damage DNA and RNA, which results in non-diagnostic FISH and NGS test results. In our patient, the results of genetic tests were diagnostic, which translated into the effectiveness of ALKi treatment.

Clinical response to ALKi might vary in ALK fusion subtypes, it can also change among different variants. Due to the limited number of rare fusion cases, it is difficult to compare the reasons for the differential responses of different rare fusions to ALKi and their resistance mechanisms [7].

The presented report describes the case of an NSCLC patient with ALK gene rearrangements treated with two different ALKi (alectinib followed by brigatinib) and various adverse events in the course of administering both medications. As first-line therapy, a highly selective, central nervous system-active drug — alectinib — was used. Alectinib is the second-generation ALKi, and it is characterized by good penetration of...
the blood-brain barrier (BBB). Alectinib could achieve higher concentrations in cerebrospinal fluid (CSF) than a first-generation ALK inhibitor (crizotinib) [8]. A calculated CSF/plasma ratio in stable state is about 0.75 [9]. Indeed, good penetration to CSF seems to be a result of alectinib lipophilic properties [10]. Alectinib and its main active metabolite CH5468924000 (M4) showed high (more than 99%) binding to human plasma protein, however, protein-binding capacity did not depend on the concentration in vitro [11]. Moreover, in human studies, unchanged alectinib and M4 were found as major circulating moieties in plasma, where about 61% accounted for the parent compound. Similarly, both molecules were excreted primarily via the fecal route and unchanged alectinib contributed to 84% of administered dose [8]. The metabolism of alectinib is mostly mediated by hepatic cytochrome CYP3A, and gut metabolism seems to be negligible. M4 is developed as a result of demethylation at the morpholine ring via some intermediate metabolites, but predominantly M4 shows similar pharmacodynamic activity against ALK as a parent compound [10].

The mechanism of alectinib-induced hepatotoxicity remains unclear, however, studies on ALKi (including first-generation ALKi — crizotinib) in human hepatocyte cell lines suggest that mitochondrial failure and inhibition of glycolysis as well as reactive oxygen species (ROS) — dependent DNA damage may play an important role in liver failure caused by alectinib [12, 13].

Brigatinib is a second-generation, highly selective ALKi with a unique molecular structure and physicochemical features among the group of anti-ALK agents. In particular, such properties include a dimethylphosphine oxide (DMPO) group, attached to the C4 aniline substituent and a specific solubilization region connected to the phenyl ring at C2. DMPO group increases brigatinib activity against ALK, whereas the solubilization region is attributed to several pharmacokinetic properties such as low lipophilicity, low binding to human plasma protein (approx. 66%), and robust metabolic stability [14, 15]. When compared to alectinib, brigatinib shows apparent differences in excretion, for only 65% of the orally administered dose is found in feces, whereas 25% is eliminated via renal pathways. Metabolism of brigatinib is primarily mediated via CYP3A4 and CYP2C8, while N-demethylation and cysteine conjugation are found to be the main metabolic pathways. It is noteworthy that over 92% of the administered dose in plasma accounted for unchanged brigatinib and only 3.5% for primary active metabolite — AP26123. What is more, AP26123 showed about three-fold weaker activity against ALK than the parent compound [10].

Although structural and pharmacokinetic features of brigatinib are well described [14, 15], it remains unclear whether those differences may have any impact on the lower risk of brigatinib-induced hepatotoxicity in comparison with other ALKi [16]. Alterations in creatine kinase (CK) are recognized as common adverse events in patients treated with ALKi for solid tumors [17]. Based on a 2022 meta-analysis, the prevalence of brigatinib-induced CK elevation in NSCLC patients is approximately 30% [18]. CK is an essential enzyme for maintaining energy homeostasis, especially in tissues with high and floating energy requirements like cardiac and skeletal muscles. An elevation of CK may be assigned to concurrent inhibition of both ABL (ABL proto-oncogene) and AMP (Adenosine Monophosphate)-activated protein kinase (AMPK). In patients receiving ALKi therapy, skeletal muscle cells may share mutual tyrosine kinase metabolic pathways with NSCLC, and those pathways probably could be inhibited simultaneously [13]. Notably, there is some evidence that significant elevation of CK as a response to administered ALKi is connected with improved clinical efficacy and prolongation of survival [17, 19].

The introduction of ALKi into treatment significantly affected the quality and length of life of NSCLC patients with ALK gene rearrangement. In the ALEX study, superior effectiveness of alectinib over crizotinib was confirmed. Several side effects have been shown in patients treated with alectinib. Of the 79 patients who experienced serious adverse reactions (≥ 3 grade), 8 (5.3%) had an increase in AST and 7 (4.6%) in ALT activities, which in turn led to discontinuation of the drug [20]. For brigatinib, the ALTA clinical trial was the pivotal study. This study compared the effectiveness of brigatinib and crizotinib. Several adverse reactions have been observed in patients in association with the administration of brigatinib, the most commonly reported being an increase in CK concentration. There were 36 cases of this side effect, representing 20% of all patients [21]. Therefore, the toxicities that occurred in our patient were consistent with those observed in clinical trials conducted in patients treated with alectinib and brigatinib. Our approach to dose reduction or discontinuation of ALKi was also consistent with clinical trial results. However, managing toxicity by replacing one ALKi with another is unusual, and the decision must be made on an individual basis.

The ALEX and ALTA study demonstrated the efficacy of brigatinib and alectinib over crizotinib [20, 21]. However, there are several studies in which patients received brigatinib after chemotherapy or other ALK inhibitors, including alectinib, which demonstrated the efficacy of brigatinib over other second-generation inhibitors. Lin et al. [22] described 22 patients who were treated with brigatinib immediately after progression or toxicity during alectinib therapy. Of the 18 patients who had measurable disease, 3 had a partial response (PR).
and had stable disease (SD). The mean PFS duration was 4.4 months, and the mean duration of treatment was 5.7 months. In addition, patients were re-biopsied after alectinib treatment and before brigatinib administration. Among 9 patients with detected resistance mutations such as G1202R, I1171N, I1171T, and V1180L, some achieved PR or SD during brigatinib therapy, which may indicate the effectiveness of brigatinib against tumor cells with some resistance mutations [22].

Nishio et al. [23] also studied ALK-rearranged NSCLC patients treated with alectinib. The studied group included 47 Japanese patients treated with first-line alectinib or alectinib after failure of crizotinib therapy. These patients, after progression on alectinib therapy, received brigatinib. The overall response rate to brigatinib therapy was 34%, and PR was achieved in 34% of patients, whereas SD was observed in 45% of patients. The duration of the response was 11.8 months. Resistance mutations after alectinib treatment were also detected among the subjects. The most common are G1202R, L1196M, I1171N, I1171S [23].

Popat et al. [24] observed brigatinib-treated patients who had previously been treated with ALK inhibitors, including alectinib. Of the 104 patients enrolled in the study, 93 benefited from brigatinib therapy. A complete response (CR) or PR was achieved in 37 patients (39.8%), and disease stabilization was obtained in 52 patients (55.9%). The mean PFS rate was 11.3 months, and mean overall survival (OS) was 23.3 months. More lines of treatment used before brigatinib therapy shortened both PFS and OS.

**Conclusion**

Our case report presents three difficult issues related to the diagnosis and treatment of NSCLC in patients with ALK gene rearrangement. First, the methods for ALK gene rearrangement diagnosis are not equally effective in some patients. It appears that NGS will become the preferred technique used for this purpose in the near future. Second, toxicities of different ALK inhibitors vary. Management of toxicity in patients with ALK gene rearrangement may include reduction of the ALKi dose or discontinuation of treatment, but also the replacement of inhibitors may be of value. Third, not only lorlatinib shows efficacy after second-generation of ALKi. It may be possible to continue successfully therapy if the second-generation ALKi is switched to a different one of the same generation due to toxicity.

**Ethics statement**

Not applicable.

**Author contributions**

M.G., P.K., M.S.: conceptualization; M.G., I.C., P.N., M.S.: resources; M.G., I.C., P.N., P.K., M.S.: writing — original draft preparation; P.K., M.S.: writing — review and editing; P.K., J.M.: supervision.

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