Molecular findings reveal possible resistance mechanisms in a patient with ALK-rearranged lung cancer: a case report and literature review

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ABSTRACT

Background: Non-small-cell lung cancer (NSCLC) is recognised as a particularly heterogeneous disease, encompassing a wide spectrum of distinct molecular subtypes. With increased understanding of disease biology and mechanisms of progression, treatment of NSCLC has made remarkable progress in the past two decades. Molecular testing is considered the hallmark for the diagnosis and treatment of NSCLC, with liquid biopsies being more and more often applied in the clinical setting during the recent years. Rearrangement of the ALK gene which results in the generation of fusion oncoproteins is a common molecular event in NSCLCs. Among ALK fusion transcripts, EML4-ALK fusion is frequently observed and can be targeted with ALK tyrosine kinase inhibitors (TKI). However, acquired resistance and disease progression in many cases are inevitable.

Method: Here, we present the case of a patient with NSCLC treated with TKIs, in which molecular profiling of the tumour was performed with different methods of tissue and plasma testing at each disease progression. A review of the literature was further conducted to offer insights into the resistance mechanisms of ALK-rearranged NSCLC.

Conclusions: Based on the results, the EML4-ALK fusion initially detected in tumour tissue was preserved throughout the course of the disease. Two additional ALK mutations were later detected in the tissue and plasma and are likely to have caused resistance to the administered TKIs. Continued research into the mechanisms of acquired resistance is required in order to increase the benefit of the patients treated with targeted ALK TKIs.

INTRODUCTION

Non-small-cell lung cancer (NSCLC) is a prominent example of personalised therapy application, as over the last 10 years targeted molecular therapies are available for the treatment of different molecular subtypes of this malignancy. In recent years, along with histomorphological examination, molecular testing is considered the hallmark for the diagnosis and treatment of NSCLC, as it provides the ability to detect driver oncogenic mutations or immunogenic markers.1 A plethora of NSCLC distinct phenotypes and/or genotypes have been recognised; among them, adenocarcinomas with EGFR, BRAF, ALK and ROS1 alterations can nowadays be treated with targeted therapies.1,2 Moreover, many selective inhibitors for other actionable molecular targets such as RET, MET, HER2 and NTRK are undergoing development. ALK rearrangement results to the EML4-ALK fusion oncogene which is found in approximately 5% of NSCLCs with distinct clinicopathological characteristics.3 This particular translocation leads to oncogenic transformation of the cell through a constitutively active ALK kinase and can be effectively targeted through the available tyrosine kinase inhibitors (TKI).

Key questions

What is already known about this subject?

- Non-small-cell lung cancer (NSCLC) encompasses a wide spectrum of molecular subtypes.
- EML4-ALK fusion is detected in 5% of patients with NSCLC and is crucial for the design of an effective treatment strategy with ALK inhibitors.
- Tissue or liquid-based genetic tumour molecular profiling in different stages during treatment could provide information about the mechanisms of resistance.

What does this study add?

- Serial tumour profiling is not routinely performed in clinical practice, which adds to the value of the reported case.
- Our data offer information about the mechanisms of acquired resistance of an ALK-rearranged NSCLC treated with ALK tyrosine kinase inhibitors.

How might this impact on clinical practice?

- As the optimal use of targeting agents remains challenging, the only way forward would be to collect and analyse the molecular data that are generated.
- This case report also highlights the importance of liquid biopsy markers in disease monitoring and therapy decision.
Despite a greater efficacy of targeted ALK inhibitors compared with standard chemotherapy, development of acquired resistance is often a matter of time and disease progression is imminent. Therefore, identification of resistance mechanisms after targeted inhibition of the EML4-ALK fusion oncogene is crucial for designing an effective sequential treatment strategy. Here, we present a case of a patient with metastatic adenocarcinoma of the lung carrying an EML4-ALK rearrangement and treated sequentially with different generations of ALK TKIs in the course of the disease.

CASE REPORT
A 39-year-old never smoker, Caucasian woman presented at the outpatient’s office with gradual onset of dyspnoea, cough and left-sided pleuritic pain over the last 3 months. Imaging by means of a thorax CT showed the presence of a 52×61×62-mm mass in the left upper lobe of the lung. Subsequent workup revealed a stage IV adenocarcinoma of the lung with bone, liver and left adrenal distant metastases. Molecular analysis of the lung biopsy was performed, detecting an EML4-ALK translocation via fluorescent in situ hybridisation (ZytoVision) (figure 1). Following the current guidelines, the patient was given crizotinib, a TKI first to be approved for the management of patients with metastatic NSCLC who carry an ALK rearrangement, resulting in a partial response in the lung primary tumour and stable disease at the metastatic sites after 5 months of therapy. However, 9 months from therapy initiation, the patient progressed with an increase in the number and size of the liver metastases. Crizotinib was discontinued and ceritinib followed by alectinib were given successively (figure 1), however, with very short duration of responses.

Subsequently, treatment with targeting agents was switched to chemotherapy with six cycles of carboplatin and pemetrexed (figure 1), resulting in short-term disease stabilisation. At the time of chemotherapy administration, a repeat biopsy of the liver metastasis was performed for ALK mutations in exons 22, 23 and 25 using Ion AmpliSeq Targeted Sequencing Technology (ThermoFisher); testing revealed the presence of a G to A point mutation in exon 23 of the ALK gene that results in G1202R substitution of the ALK tyrosine kinase receptor (figure 1). The patient was then given lorlatinib, a third-generation TKI for patients with NSCLC who carry ALK rearrangement mutations who have progressed after receiving ALK-targeted drugs (first or second-generation TKIs). Treatment with lorlatinib delayed disease progression for 5 months. On further disease progression, the patient’s plasma was sent for circulating-free tumour DNA (cfDNA) analysis. The isolated cfDNA was tested for mutation hotspot regions in 12 genes (ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1, RET, TP53) as well as for ALK, ROS1 and RET rearrangements using Oncomine Lung cell free total nucleic assay (ThermoFisher) and Ion S5 Next Generation Sequencing platform (ThermoFisher). Liquid biopsy revealed a new ALK point mutation, G1269A resulting from a G to C nucleotide change in exon 25, which was observed in 3.5% of reads (figure 1). Additionally, cfDNA testing verified the ongoing presence of the G1202R mutation at a frequency of 14.5%, as well as the subsistence of the variant 3a/b EML4(6)-ALK(20) translocation. A repeat liquid biopsy a month later showed no differences. Soon after, the patient succumbed to her disease.

DISCUSSION
The ALK gene has been found to be involved in multiple chromosomal translocations in NSCLC that result in oncogenic fusions generating a transforming, activated tyrosine kinase. The development of ALK TKIs including the first to be approved, crizotinib, as well as second and third-generation inhibitors (ie, ceritinib/alectinib/brigatinib and lorlatinib, respectively) has changed the landscape for the treatment of patients who carry an ALK rearrangement in their tumours. The EML4-ALK fusion gene results from the paracentric inversion of chromosome 2 and was first reported in NSCLC in 2007, to date at least 15 variants of EML4-ALK have been identified. In the present report, our patient carried the variant 3a/b (exon 6a/b of EML4 fused to exon 20 of ALK (E6a/b;A20)), which along with variant 1 (exon 13 of EML4 fused to exon 20 of ALK (E13;A20)) constitutes two of the most frequently detected variants in NSCLC. Although, overall, EML4-ALK variants result to constitutive activation of the ALK tyrosine kinase receptor and lead to downstream oncogenic signalling activation (ie, PI3K/Akt, JAK/STAT and RAS/RAF/MEK/ERK),
different variants have been recognised to demonstrate unique molecular properties, which may affect disease progression, sensitivity to ALK-TKIs and mechanisms of acquired resistance. To date, evidence concerning the response to crizotinib among patients with different tumour fusion variants is conflicting; a recent study by Lin et al. demonstrated no difference in progression-free survival (PFS) between patients who carried either variant 1 or 3 and treated with ALK-TKIs, despite variant 3 being mostly associated with the development of G1202R resistant mutation, as also seen in our case.

The development of secondary mutations that render ALK-TKIs ineffective is a typical resistance mechanism against these inhibitors. Multiple molecular alterations may promote resistance to crizotinib and other ALK-TKIs such as secondary mutations of the ALK gene, amplification of the ALK fusion protein and activation of alternative bypass signalling pathways, such as mutations of EGFR, KRAS, KIT or IGF1R and finally, histological transformation. The most typical mutations that confer resistance to ALK-TKIs occur at residues 1151, 1152, 1156, 1174, 1202, 1203 and 1206 of the ALK kinase domain. The G1202R solvent front mutation, one of the most frequently detected resistance mutations, has been spotted in the tissue and plasma of patients treated with first-generation ALK-TKIs; it occurs in the crizotinib-binding side of the tyrosine kinase, diminishing the binding activity of crizotinib as well as other second-generation inhibitors such as ceritinib and alectinib, but sensitivity seems to remain for brigatinib and lorlatinib. De Carlo et al have reported that the plasma levels of the G1202R mutation are liable to change during treatment with ALK TKIs. In our case, we observed that the levels of G1202R mutation (mutant allele frequency 14.4%) in plasma were not affected by lorlatinib treatment, however, we cannot confirm whether the levels remained constant between first to second and second to third-generation TKIs.

The second mutation that our patient’s tumour developed, G1269A, resides in exon 25 of ALK gene and has been identified in the plasma of our patient after lorlatinib treatment; it occurs in the ATP-binding pocket and also has the capacity to inhibit crizotinib binding, but unlike G1202R it remains sensitive to later generation TKIs, such as ceritinib and alectinib. As Yoda et al have demonstrated for patients treated with a multiple sequence of ALK-TKIs, the G1269A mutation matters because when combined with the G1202R mutation, both of them together confer resistance to lorlatinib, a third-generation TKI. Recently, a study on patient tumour xenografts demonstrated that the combination of G1202R plus G1269A confers resistance to lorlatinib, with brigatinib remaining still active, however, our experience on the use of brigatinib for double-mutant tumours in the clinic remains limited and further investigation is needed. It is postulated that patients progressing after multiple lines of therapy will not benefit significantly from single TKIs due to the resulting high genetic complexity of their tumours. In the present case, except for the two secondary resistance mutations mentioned, no other mechanisms of resistance were identified; neither activation of bypass pathways (ie, mutations or fusions of other genes) nor amplification of the EML4-ALK fusion gene (ie, only six copies were detected). Therefore, in the presented case of NSCLC bearing EML4-ALK rearrangement, the coexistence of G1202R and G1269A mutations after lorlatinib treatment is presented as resistance mechanisms, however the clinical significance of G1269A mutation cannot be evaluated as no other ALK-TKIs have been followed by lorlatinib. There is anecdotal evidence from myofibroplastic tumours incriminating G1269A mutation as a resistance mechanism to crizotinib, potentially relevant for lung cancer as well. Further evaluation of the role of G1269A mutation as resistance mechanism after sequential treatment with ALK inhibitors is of paramount significance.

In the era of precision medicine, treatment decisions are based on clinical and pathological characteristics and depend on an extended molecular profile of the tumour, since it enables the identification of actionable molecular targets, giving access to available targeted therapies. Liquid biopsies, being a less invasive method along with tissue biopsies, allow us to determine the genetic make-up of the tumour at the time of diagnosis as well as during treatment. Repeat tissue biopsies are associated with morbidity and the inherent limitations of tumour heterogeneity, since only a small amount of material can be obtained. In contrast, liquid biopsies are easily repeated and detect genetic material released into the bloodstream by all tumour sites, overcoming the problem of sampling bias. In fact, researchers hypothesise that malignant genetic aberrations detected via liquid biopsies are the biologically relevant ones originating from higher volume or more aggressive malignant clones that will define patient outcome. Despite the usefulness of tissue or liquid-based genetic tumour profiling, a significant number of genetic aberrations cannot presently be exploited in the clinical setting, since they are not actionable or are of unknown clinical significance. Even for targetable aberrations, the optimal use of targeting agents in sequence or combination remains challenging and resistance becomes soon a problem. To overcome this conundrum, the only way forward would be to meticulously collect and analyse the abundance of molecular data that are generated nowadays. In the emerging era of big data and artificial intelligence, genetic data collected in prospective registries, annotated with clinicopathological and outcome information, could help us understand the mechanisms of resistance of malignancies under the therapeutic pressure of selective oncogene inhibition, and allow us to design an optimal and successful therapeutic strategy for each of our patients.

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