ESMO Open
Cancer Horizons

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

Anastasia Kougioumtzi,[1] Panagiotis Ntellas,[2,3] Eirini Papadopoulou,[4] George Nasioulas,[4] Eleftherios Kampletsas,[2,3] George Pentheroudakis [2,3]

To cite: Kougioumtzi A, Ntellas P, Papadopoulou E, *et al*. Molecular findings reveal possible resistance mechanisms in a patient with ALK-rearranged lung cancer: a case report and literature review. *ESMO Open* 2019;**4**:e000561. doi:10.1136/esmoopen-2019-000561

Received 26 June 2019
Revised 29 July 2019
Accepted 30 July 2019

[1]Laboratory of Clinical Chemistry, University of Ioannina School of Medicine, Ioannina, Greece
[2]Medical Oncology, University of Ioannina, Ioannina, Greece
[3]Society for Study of Clonal Heterogeneity of Neoplasia (EMEKEN), Ioannina, Greece
[4]Diagnostics Laboratory, GeneKor Medical SA, Athens, Greece

Correspondence to
Dr George Pentheroudakis; gpenther@otenet.gr

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 oncogenes 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.

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?

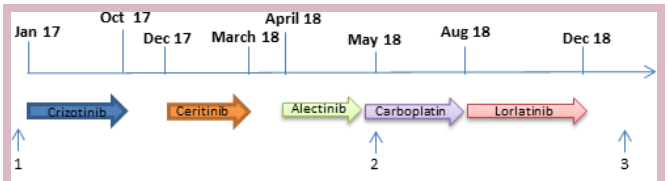
- Serial tumour profiling is not routinely performed in clinical practice, which adds to the value of the reported case.
- Our data offer information about the mechanisms of acquired resistance of an *ALK*-rearranged NSCLC treated with ALK tyrosine kinase inhibitors.

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.

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 a 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).



Molecular profiling	Biomarker	MAF (%)	Methodology	Sample
1	EML4-ALK fusion	-	FISH (Zytovision)	Tissue
2	ALK : c3604G>A (p.G1202R)	9.1%	NGS:(Ion AmpliSeq™ Panel, ThermoFisher)	Tissue
3	ALK: c3604G>A (p.G1202R) c.3806G>C (p.G1269A) EML4(6)-ALK(20)	14.1% 3.5% 6 copies	NGS: (Oncomine Lung cfDNA and Ion S5™ System , ThermoFisher)	Plasma

Figure 1 Tumour molecular profiling and treatment strategy. Sequential therapeutic strategy of ALK tyrosine kinase inhibitors (TKI) and chemotherapy over the course of time together with detected molecular findings in patient tissue and plasma. FISH, fluorescent in situ hybridisation; MAF, mutant allele frequency; NGS, next generation sequencing.

Despite a greater efficacy of targeted ALK inhibitorscompared with standard chemotherapy, development of acquired resistance is often a matter of time and disease progression is imminent.[4] 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 outpatients 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,[5] 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,[6] 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.[7] 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.[8 9] The *EML4-ALK* fusion gene results from the paracentric inversion of chromosome 2 and was first reported in NSCLC in 2007[10]; to date at least 15 variants of *EML4-ALK* have been identified.[11] 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.[12] 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.[13 14] To date, evidence concerning the response to crizotinib among patients with different tumour fusion variants is conflicting[15 16]; a recent study by Lin *et al*[17] 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.[8 18 19] 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.[20 21] De Carlo *et al* have reported that the plasma levels of the G1202R mutation are liable to change during treatment with ALK TKIs.[22] 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.[23] 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.[24] Recently, a study[25] 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.[25] 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 myofibroblastic tumours incriminating G1269A mutation as a resistance mechanism to crizotinib,[26 27] 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.

Contributors Study design and drafting of the manuscript were equally performed by AK and PN. Study conception was performed by GP. Review of the clinical data was performed by EK. EP and GN reviewed the integrity of the mutational analyses. Critical revision was performed by GP and EK. GP conceived and finally reviewed the manuscript. All the authors contributed to the writing of the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests GN and EP are employees of GeneKor Medical SA, Athens, Greece.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iD

George Pentheroudakis http://orcid.org/0000-0002-6632-2462

REFERENCES

1 Novello S, Barlesi F, Califano R, *et al*. Metastatic non-small-cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2016;27:v1–27.
2 Pakkala S, Ramalingam SS. Personalized therapy for lung cancer: striking a moving target. *JCI Insight* 2018;3. doi:10.1172/jci.insight.120858
3 Pikor LA, Ramnarine VR, Lam S, *et al*. Genetic alterations defining NSCLC subtypes and their therapeutic implications. *Lung Cancer* 2013;82:179–89.
4 Ziogas DC, Tsiara A, Tsironis G, *et al*. Treating ALK-positive non-small cell lung cancer. *Ann Transl Med* 2018;6.
5 Kazandjian D, Blumenthal GM, Chen H-Y, *et al*. Fda approval summary: crizotinib for the treatment of metastatic non-small cell lung cancer with anaplastic lymphoma kinase rearrangements. *Oncologist* 2014;19:e5
6 Shaw AT, Felip E, Bauer TM, *et al*. Lorlatinib in non-small-cell lung cancer with ALK or ROS1 rearrangement: an international, multicentre, open-label, single-arm first-in-man phase 1 trial. *Lancet Oncol* 2017;18:1590–9.
7 Iwama E, Okamoto I, Harada T, *et al*. Development of anaplastic lymphoma kinase (ALK) inhibitors and molecular diagnosis in ALK rearrangement-positive lung cancer. *Onco Targets Ther* 2014;7
8 Lin JJ, Riely GJ, Shaw AT. Targeting ALK: precision medicine takes on drug resistance. *Cancer Discov* 2017;7:137–55.
9 Rothenstein JM, Chooback N. Alk inhibitors, resistance development, clinical trials. *Curr Oncol* 2018;25:59–67.
10 Soda M, Choi YL, Enomoto M, *et al*. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007;448:561–6.
11 Sabir SR, Yeoh S, Jackson G, *et al*. Eml4-Alk variants: biological and molecular properties, and the implications for patients. *Cancers* 2017;9. doi:10.3390/cancers9090118
12 Yang HH, Hu Y, Lee MP, *et al*. Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer. *Cancer Res* 2008;68:3075.
13 Heuckmann JM, Balke-Want H, Malchers F, *et al*. Differential protein stability and ALK inhibitor sensitivity of EML4-ALK fusion variants. *Clin Cancer Res* 2012;18:4682–90.
14 Bayliss R, Choi J, Fennell DA, *et al*. Molecular mechanisms that underpin EML4-ALK driven cancers and their response to targeted drugs. *Cell Mol Life Sci* 2016;73:1209–24.
15 Yoshida T, Oya Y, Tanaka K, *et al*. Differential crizotinib response duration among ALK fusion variants in ALK-positive non-small-cell lung cancer. *J Clin Oncol* 2016;34:3383–9.
16 Cha YJ, Kim HR, Shim HS. Clinical outcomes in ALK-rearranged lung adenocarcinomas according to ALK fusion variants. *J Transl Med* 2016;14.
17 Lin JJ, Zhu VW, Yoda S, *et al*. Impact of EML4-ALK variant on resistance mechanisms and clinical outcomes in ALK-positive lung cancer. *J Clin Oncol* 2018;36:1199–206.
18 Katayama R. Therapeutic strategies and mechanisms of drug resistance in anaplastic lymphoma kinase (ALK)-rearranged lung cancer. *Pharmacol Ther* 2017;177:1–8.
19 Sharma G, Mota I, Mologni L, *et al*. Tumor resistance against ALK targeted therapy-Where it comes from and where it goes. *Cancers* 2018;10:62.
20 Toyokawa G, Seto T. Updated evidence on the mechanisms of resistance to ALK inhibitors and strategies to overcome such resistance: clinical and preclinical data. *Oncol Res Treat* 2015;38:291–8.
21 Akamine T, Toyokawa G, Tagawa T, *et al*. Spotlight on lorlatinib and its potential in the treatment of NSCLC: the evidence to date. *Onco Targets Ther* 2018;11:5093–101.
22 De Carlo E, Schiappacassi M, Urbani M, *et al*. Therapeutic decision based on molecular detection of resistance mechanism in an ALK-rearranged lung cancer patient: a case report. *Onco Targets Ther* 2018;11:8945–50.
23 Doebele RC, Pilling AB, Aisner DL, *et al*. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. *Clin Cancer Res* 2012;18:1472–82.
24 Yoda S, Lin JJ, Lawrence MS, *et al*. Sequential *ALK* Inhibitors Can Select for Lorlatinib-Resistant Compound *ALK* Mutations in *ALK*-Positive Lung Cancer. *Cancer Discov* 2018;8:714–29.
25 Okada K, Araki M, Sakashita T, *et al*. Prediction of ALK mutations mediating ALK-TKIs resistance and drug re-purposing to overcome the resistance. *EBioMedicine* 2019;41:105–19.
26 Michels SYF, Scheel AH, Wündisch T, *et al*. Alk G1269A mutation as a potential mechanism of acquired resistance to crizotinib in an ALK-rearranged inflammatory myofibroblastic tumor. *npj Precision Onc* 2017;1.
27 Xu X, Li H, Peng K, *et al*. ALK-G1269A mutation in epithelioid inflammatory myofibroblastic sarcoma after progression on crizotinib: a case report. *Oncol Lett* 2019;17:2370–6.