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Transformation to neuroendocrine carcinoma as a resistance mechanism to lorlatinib

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ABSTRACT

Objectives: Small cell transformation is a well-recognized mechanism of resistance to EGFR-TKI therapy in EGFR-mutant NSCLC, yet it remains a poorly-described phenomenon in ALK-rearranged NSCLC.
Material and methods: Chart and literature review. Results: We report a case of a patient with ALK-rearranged lung cancer progressing on three-lines of ALK-targeted therapies, with development of acquired resistance to lorlatinib, with both transformation to a neuroendocrine carcinoma, and acquisition of ALK 1196 M.
Conclusions: Given the inevitable development of resistance in ALK + NSCLC, if feasible, re-biopsy on progression should be standard over liquid biopsy. Neuroendocrine carcinoma transformation remains an important mechanism of acquired resistance to lorlatinib.

1. Introduction

In non–small-cell lung cancer (NSCLC), anaplastic lymphoma kinase (*ALK*) rearrangements define a distinct subset, occurring in approximately 5% of cases [1]. Indeed, the therapeutic landscape for advanced ALK-positive NSCLC has been transformed in recent years, by the development of increasingly potent and selective ALK inhibitors. Despite these advances, however, following initial responses, patients inevitably progress due to acquired resistance. While small cell transformation is a well-recognized mechanism of resistance to EGFR-tyrosine kinase inhibitor (TKI) therapy in EGFR-mutant advanced NSCLC [2], it remains a poorly-described phenomenon in ALK-rearranged lung adenocarcinomas.

Multiple mechanisms of acquired resistance to ALK inhibitors have been reported, with acquired mutation within the *ALK* kinase domain, including the recalcitrant solvent-front mutation ALK G1202R and gatekeeper mutation L1196M [3], identified in approximately one third of patients with acquired resistance to a next generation ALK TKI. Further mechanisms of resistance include amplification of the *ALK* fusion gene [4], epithelial-mesenchymal transition [5], and rarely, small cell lung cancer (SCLC) transformation [6,7]. Furthermore, SCLC transformation and the ALK solvent-front mutation G1202R have also been noted in the same patient [8].

The third generation ALK inhibitor loralatinib (PF-6463922) is highly active against ALK and ROS1 kinases [9]. It is specifically designed to have CNS penetration, and preclinical activity is reported across all reported acquired ALK kinase resistance genotypes. Durable clinical responses have been demonstrated in patients with progression on crizotinib, ceritinib, alectinib and brigatinib, with evidence of activity against the G1202R mutation [9]. Despite this clinical activity, little has been reported on acquired resistance mechanisms with data on ALK inhibitor-multitreated patients demonstrating additional compound ALK kinase mutations [10](eg G1202R/L1196 M, G1202R/L1198 F), as well as loss of lorlatinib-sensitive ALK mutations. SCLC transformation as a lorlatinib resistance mechanism has been rarely reported [8].

Here, we report a case of a patient with ALK-rearranged lung cancer progressing on three-lines of ALK-targeted therapies, with development of acquired resistance to lorlatinib, and both transformation to a neuroendocrine carcinoma, and acquisition of ALK 1196 M, highlighting the need to re-biopsy patients on development of acquired resistance to lorlatinib, highlighting the pitfalls of relying on liquid biopsies.

2. Case description

A 38-year-old male never-smoker was diagnosed with T4N2M1a EGFR wild-type TTF-1 positive adenocarcinoma, with bilateral lung metastases in an external centre in 2011. Initially declining systemic treatment, he developed haemoptysis and dyspnoea, and was treated with palliative radiotherapy to the right upper lobe (20 Gy in 5 fractions

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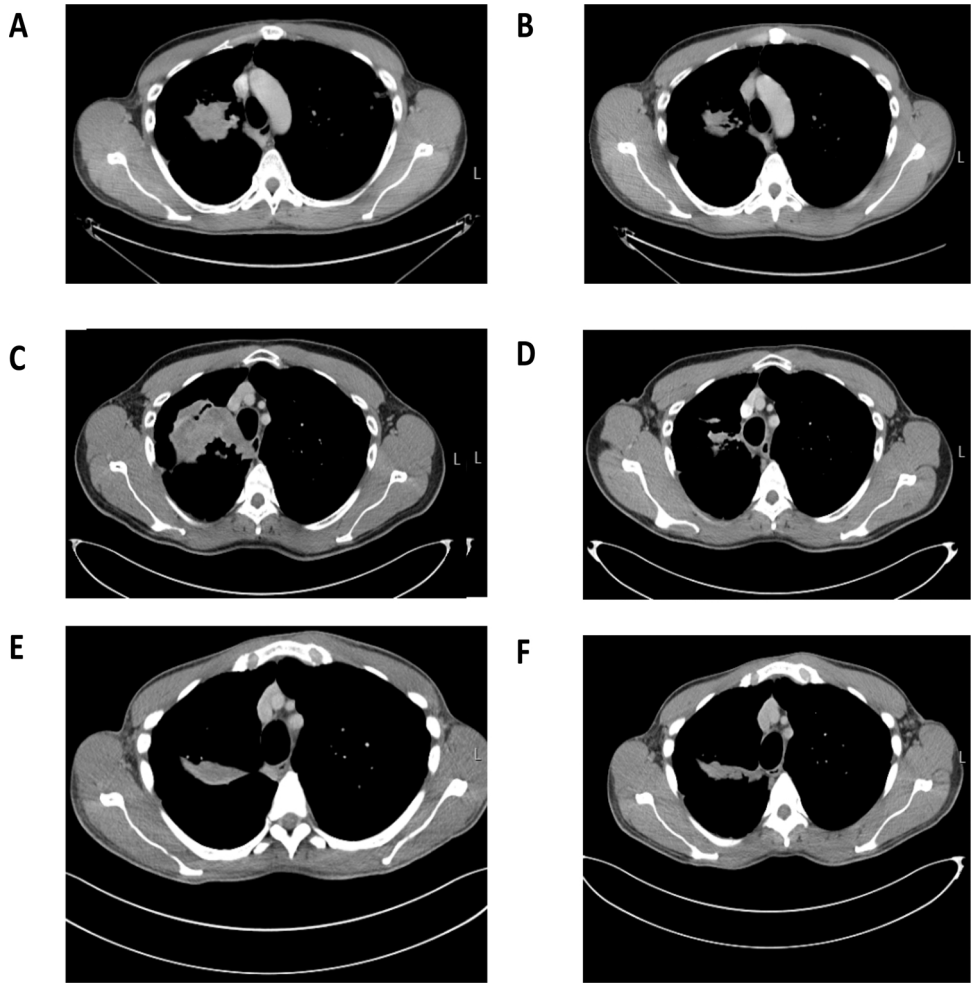

Fig. 1. CT images showing right hilar disease and responses to ALK-targeted therapy. (a) Before and (b) after two months of crizotinib therapy; partial response before (c) and following (d) two months ceritinib; slight reduction is seen before (e) and after two months lorlatinib therapy (f).

to right lung mass). Initial response on CT imaging was followed by disease progression with bilateral lung nodules. The patient was referred to our service, where he received first-line cisplatin and pemetrexed chemotherapy. Following implementation of routine ALK testing, the tumour was confirmed ALK positive by immunohistochemistry (D5F3 clone) and validated thereafter by FISH (Vysis break apart). CT imaging demonstrated a good partial response to 6 cycles of cisplatin-pemetexed.

Six months later, progressive disease was identified by CT imaging, and the patient was commenced on crizotinib 250 mg twice daily, which was well tolerated requiring no dose reduction. A partial response was identified after two months on treatment (Fig. 1, images (a) and (b)). However, after 14 months on treatment, MRI brain surveillance identified oligometastatic CNS progression with an asymptomatic solitary fronto-parietal lobe lesion, but no extra-cranial progressive disease. After multidisciplinary discussion of therapeutic options, the patient received definitive surgical debulking of right parietal metastasis, followed by stereotactic radiotherapy to the tumour bed postoperatively. Histology confirmed metastatic TTF-1 positive adenocarcinoma, CD56-negative, appearances and immunoprofile in keeping with origin from known lung primary. The patient continued crizotinib 250 mg twice daily throughout this period and remained well with performance status (PS) 0. Crizotinib was continued until further multifocal progression was identified on CT imaging five months later. He then switched to ceritinib 750 mg once daily, with a rapid partial response, MRI brain confirming no CNS progression (Fig. 1(c) and (d)); this response was maintained for a further seven months, until disease progression was identified by CT imaging.

At this time, progressive hilar disease was biopsied, confirming TTF-1 positive adenocarcinoma, ALK positive by IHC (ALK1); next generation sequencing (NGS) further confirmed an *EML4-ALK* fusion with no additional kinase mutations, but sample was insufficient to identify other somatic variants. He then commenced brigatinib with initial transient symptomatic improvement but subsequent further bone disease progression confirmed on MRI spinal imaging following three cycles of brigatinib. Due to clinical deterioration, unsuitable tissue to rebiopsy and unavailability of ctDNA NGS, he next switched to lorlatinib with a marked symptomatic improvement during the first cycle of treatment. Lorlatinib was well tolerated with no required dose-reductions. Response evaluation CT-imaging following two months of lorlatinib demonstrated a partial response (Fig. 1(e) and (f)). Response continued for an additional five months with CT surveillance then demonstrating progression due to new liver metastases. Image-guided liver-biopsy identified TTF-1 negative NSCLC, expressing CD56, synaptophysin, and chromogranin, interpreted as NSCLC with neuroendocrine differentiation (Fig. 2). PDL1 testing was negative (22C3) and ALK immunohistochemistry (D5F3) was positive, validated by FISH (Vysis break apart). NGS confirmed an *EML4-ALK* fusion, but also an L1196 M gatekeeper mutation, and CDKN2A homozygous deletion, also confirmed by FISH, with no *RB1* loss. The patient commenced carboplatin-vinorelbine chemotherapy for two cycles but clinically deteriorated through this and rapidly thereafter, consistent with clinical progression. The patient's care was transferred to the community palliative care services, and he subsequently passed away. The sequence of anti-

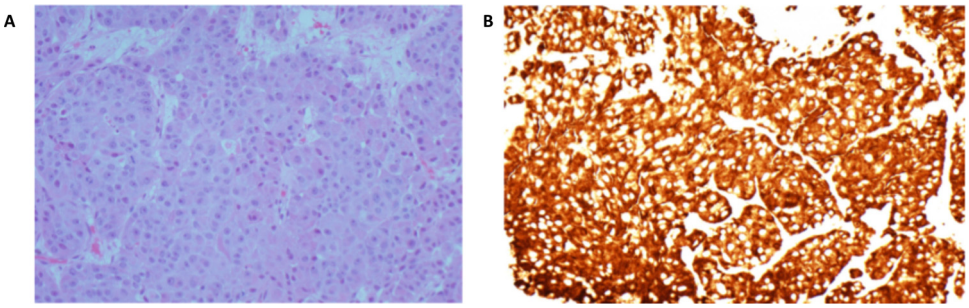

Fig. 2. (a) Liver core biopsy demonstrates NSCLC with neuroendocrine differentiation, (H&E, x20). (b) Staining for ALK (D5F3) is positive.

Table 1
Sequence of anti-cancer treatments.

Date	Treatment
Jul-11	Presentation with bilateral lung masses following admission to A&E for acute abdominal pain. Patient declined initial treatment.
Oct-11	Patient developed haemoptysis, received palliative radiotherapy to RUL lesion (20 Gy in 5 fractions).
May 2012 – September 2012	Received cisplatin and pemetrexed chemotherapy (6 cycles).
February 2013 – November 2014	Crizotinib.
Apr-14	Surgical debulking of right parietal brain metastasis, followed by SRS to tumour bed; patient continued crizotinib.
December 2014 – July 2014	Ceritinib; PR as best response.
August 2016 - November 2016	Brigatinib with PD as best response.
December 2016 – May 2017	Lorlatinib; PR as best response.
June 2017 – August 2017	Carboplatin-vinorelbine chemotherapy, with PD as best response.

Abbreviations: SRS, stereotactic radiosurgery; PD, progressive disease, PR, partial response.

cancer treatments is summarised in Table 1.

3. Discussion

We report a case of neuroendocrine carcinoma transformation as a mechanism of resistance to the next generation ALK-inhibitor lorlatinib, following three previous three lines of ALK-targeted therapy. To the best of our knowledge, this is the first report of neuroendocrine carcinoma transformation as a resistance mechanism to lorlatinib following three generations of ALK inhibition. SCLC transformation has been reported following progression on crizotinib alone [6,11] post alectinib after crizotinib [12–14], post ceritinib after crizotinib [7], and following lorlatinib after crizotinib [8]. Consistent with all these cases where re-biopsied SCLC tissue was examined [6,11–14], ALK rearrangement was retained in the transformed SCLC – indicating true transformation, and not outgrowth of an existing SCLC clone. Our case therefore further confirms neuroendocrine carcinoma transformation as a resistance mechanism that is not exclusive to crizotinib use.

Multiple molecular mechanisms can trigger acquired resistance to ALK inhibitors, including secondary mutations encoding variants in the ALK kinase domain in approximately 20% and 50% of patients following treatment with first-generation (crizotinib) and second-generation

(ceritinib and alectinib) ALK inhibitors [3]. Further mechanisms of resistance include *ALK* gene amplification [4,15], activation of different kinases such as EGFR [15], KIT [15], nonreceptor tyrosine kinase (SRC) [16], and insulin like growth factor 1 receptor [17]. These resistance mechanisms are thought to occur due to maintained activation of downstream ERK and/or PI3K–AKT signaling despite ALK inhibition. Ou et al [8] recently reported a complimentary similar case of the ALK G1202R solvent-front mutation and SCLC transformation as resistance mechanisms to second generation ALK inhibitors, without prior exposure to crizotinib. We report the first case of dual occurrence of the ALK L1196 M ALK gatekeeper mutation and neuroendocrine transformation as a resistance mechanism to lorlatinib. Our case, therefore, again highlights the risk of relying on liquid biopsies in the clinic for oncogene-addicted NSCLC, as this would have simply detected the L1196 M ALK variant. Whilst L1196 M has been identified as an acquired variant associated with resistance to crizotinib, ceritinib, and alectinib, but not brigatinib, the identification of this variant after brief exposure to brigatinib and longer exposure to lorlatinib suggests the L1196 M variant was associated with resistance to lorlatinib. This is, however, unusual, since in preclinical models this mutation results in lorlatinib sensitivity [10]. Nevertheless, we cannot exclude a competing role in acquired resistance of the CDKN2A loss identified, or that L1196 M developed on brigatinib.

The exact mechanisms for how SCLC transformation ALK + SCLC occurs, and leads to unresponsiveness to ALK inhibitors remains unknown, though inactivation of tumour suppressor genes retinoblastoma (Rb1) and p53 [2], defining lesions of SCLC, have been postulated. In the parallel model of SCLC transformed EGFR mutant NSCLC, baseline RB loss was predictive of those cases likely to transform to this neuroendocrine carcinoma [18]. Intriguingly, in our patient, NGS of the neuroendocrine carcinoma revealed inactivation of *CDKN2A,* but not *RB1*, with TP53 status unknown (not analysed on the panel), thus suggesting perhaps inactivation of *CDKN2A* led to neuroendocrine transformation in this particular patient. Moreover, the lack of a dominant SCLC morphology in the re-biopsy specimen is a novel finding for ALK-driven NSCLC, and that neuroendocrine morphology is associated with acquired kinase inhibitor resistance is consistent with observations in EGFR- mutant lung tumours.

Our patient was treated with carboplatin and vinorelbine prior to confirmation of neuroendocrine transformation, and while combination of carboplatin and vinorelbine is active against SCLC, it tends to be poorly tolerated. Although our patients' condition deteriorated before further strategies with increased SCLC activity could be trialled, we have previously reported lack of benefit from immune checkpoint inhibitors from such transformed tumours [19], and the lack of PDL1 expression is consistent with other reports in transformed EGFR mutant neuroendocrine carcinomas.

Given the inevitable development of resistance in ALK-positive NSCLC patients, our case highlights that, if feasible, re-biopsy on progression should be standard over liquid biopsy, and reaffirms the role of neuroendocrine carcinoma transformation as an acquired resistance

mechanism to lorlatinib, observing for the first time, an additional acquired resistance mutation, L1196 M.

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