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A Case of Metastatic Atypical Neuroendocrine Tumor with ALK Translocation and Diffuse Brain Metastases

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Disclosures of potential conflicts of interest may be found at the end of this article.

ABSTRACT

A challenge in precision medicine requires identification of actionable driver mutations. Critical to such effort is the deployment of sensitive and well-validated assays for mutation detection. Although identification of such alterations within the tumor tissue remains the gold standard, many advanced non-small cell lung cancer cases have only limited tissue samples, derived from small biopsies or fine-needle aspirates, available for testing. More recently, noninvasive methods using either circulating tumor cells or tumor DNA (ctDNA) have become an alternative method for identifying molecular biomarkers and screening patients eligible for targeted therapies. In this article, we present a case of a 52-year-old never-smoking male who presented with widely metastatic atypical neuroendocrine tumor to the bones and the brain. Molecular genotyping using DNA harvested from a bone metastasis was unsuccessful due to limited material. Subsequent ctDNA analysis revealed an ALK translocation. The clinical significance of the mutation in this particular cancer type and therapeutic strategies are discussed.

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KEY POINTS

- To our knowledge, this index case represents the first reported ALK translocation identified in an atypical carcinoid tumor.
- Liquid biopsy such as circulating tumor DNA is a feasible alternative platform for identifying sensitizing genomic alterations.
- Second-generation ALK inhibitors represent a new paradigm for treating ALK-positive patients with brain metastases.

PATIENT STORY

A 52-year-old never-smoking male initially presented with acute-on-chronic back pain, which gradually worsened to involve the hips and shoulders. After several months of conservative management without relief, lumbar spine magnetic resonance imaging (MRI) showed multifocal abnormal marrow enhancement suggesting an infiltrative process. Bone scan showed abnormal uptake throughout the axial skeleton, including the skull, sternum, bilateral ribs, and the sacroiliac joints. Computed tomography (CT) of the chest, abdomen, and pelvis identified a large spiculated mass in the right middle lobe of the lung spanning 1.2 × 1.0 cm and a lytic lesion in the manubrium. Brain MRI revealed a 4.3-cm mass to the right of the falk cerebri with extensive calcifications and vasogenic edema, a rim-enhancing lesion in the posterior aspect of the left superior frontal gyrus, and numerous punctuate foci of enhancement infra- and supra-tentorially and within the calvarium (Fig. 1A). The patient complained of floaters, and fundoscopic examination showed bilateral choroidal metastases (Fig. 1B).

CT-guided, fine-needle aspiration of the manubrium lesion and the right middle lobe lesion both revealed monomorphic tumor cells with moderate amount of eosinophilic cytoplasm, forming organoid nests with small foci of coagulative necrosis, that stained diffusely positive for TTF-1, CK7, synaptophysin, and neural-specific enolase and focally positive for chromogranin. High-grade features were not identified in this specimen. Ki67 was approximately 30%. These cells were negative for CK5/6 and p40. The morphological appearance, positive neuroendocrine markers, and intermediate Ki67 all suggest a neuroendocrine tumor of the atypical carcinoid subtype assuming lung origin (Fig. 2). Molecular genotyping could not be completed due to insufficient material.

Planned gamma knife radiotherapy was aborted due to innumerable brain metastases. Because the patient was...
Figure 1. Regression of brain and choroidal metastases after alectinib. (A): T1 gadolinium enhanced MRI images pre- and post-treatment showing shrinkage of the dominant brain lesion. (B): Right fundus at presentation with a small metastasis in the temporal macula. (C): Left fundus with tumors inferior to the optic disc and in the temporal macula. (D): Right fundus 2 months later with tumor regression. (E): Left fundus 2 months later with regression of the juxtapapillary tumor and disappearance of the temporal metastasis. Ultrasound scans showing left juxtapapillary tumor at presentation, when the thickness was 1.3 mm (F), and after 2 months, when the thickness had diminished to 0.5 mm (G). The metastases are indicated by red arrows.

Abbreviations: MRI, magnetic resonance imaging.
The Expanding Role of Liquid Biopsies

Liquid biopsies utilizing ctDNA offer an alternate means of detecting genomic alterations to inform treatment. Compared with traditional tissue biopsies, liquid biopsies are faster, more economical, and less invasive, thereby reducing the health risk to patients. Liquid biopsies are most useful when a patient's clinical condition precludes a tissue diagnosis or the lesion in question is anatomically inaccessible. Because tissue biopsies usually sample only a small portion of the tumor, mutations may be missed due to tumor heterogeneity. Liquid biopsies have the potential to sample all metastatic sites, to monitor therapeutic response, and to predict recurrences through serial sampling. Finally, because ctDNA samples do not undergo formalin fixation, background is reduced.

The greatest challenge of liquid biopsies is the false negative rates. Although several series report mutation detection rates between 60%–80% for ctDNA, some cancers may simply shed little DNA into circulation because of small size, limited metastatic spread, or other factors [1]. Liquid biopsies may miss co-occurring mutations and tumors with mixed histology.

**Genotyping Results and Interpretation of the Molecular Results**

Molecular profiling was performed in a Clinical Laboratory Improvement Amendments-certified laboratory using a hybrid capture ctDNA platform. Briefly, 10–20 mL peripheral, whole blood was collected from the patient and spun down to collect 5–10 mL of plasma. Greater than 50 ng of ctDNA was extracted from the sample and quantitated using smear analysis. An adaptor-ligation library was constructed and hybrid capture was performed using a panel of 2,695 5' biotinylated single-stranded DNA oligonucleotides to isolate exonic regions of 62 genes and introns of six genes often involved in genomic rearrangement in cancer. A sequencing library was prepared with short oligonucleotide sequences called fragment-barcodes inserted between the sequencing adaptor and the sample barcode that allow downstream analysis to uniquely identify the original double-stranded DNA fragment. Multiplexed sequencing was performed using 2 × 175 paired-end on the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, https://www.illumina.com). Analyses included error correction across duplicate ctDNA molecules, as identified by the fragment barcodes, to <0.02% and target depth post-correction of >5,000× unique coverage. Variant calling included indels, substitutions, rearrangements, and amplifications. Reporting of ctDNA was done without a matched normal sample from the patient and compared with known driver alterations in the Catalogue of Somatic Mutations in Cancer (COSMIC) database.

tDNA allows for sampling across all metastatic sites. ctDNA from this patient detected only two mutations: the ALK translocation and a variant in the PDCD1LG2 gene of unknown significance. The ALK rearrangement in this patient occurred at the canonical intron 19 breakpoint and contained the kinase domain of ALK. This event was a translocation between chromosome 2 and chromosome 9 leading to an out-of-strand rearrangement with SMC5 intron 18 where the SMC5 gene was in the opposite transcriptional orientation as ALK (Fig. 3). The median exon coverage for this sample was 5,986. Even though, to our knowledge, ALK translocations have not been reported previously in atypical carcinoid cancer, and SMC5 has never been identified as a fusion partner for ALK, we hypothesized that this fusion was likely the driver mutation because it contained an intact kinase domain capable of downstream signaling. Although we could not rule out the presence of a subclonal population harboring other driver mutations, given the paucity of mutations identified, we believe the patient's tumor was relatively homogeneous and the ALK rearrangement represented a truncal mutation that disseminated early during evolution. Because ctDNA is a new platform, the sensitivity and specificity of the test remains uncertain. Extrapolating from a prior report using circulating tumor cells, investigators were able to identify ALK translocation with 100% sensitivity and specificity [2].
Furthermore, not all tumors shed DNA into the bloodstream. Therefore, we would surmise that the actual false negative rate for detecting ALK translocation would be higher than the false positive rate. Being able to detect the translocation at all in the blood sample suggests it was likely an actionable driver mutation in this patient.

**FUNCTIONAL AND CLINICAL SIGNIFICANCE OF THE ALK MUTATION**

ALK encodes the anaplastic lymphoma kinase, a receptor tyrosine kinase that belongs to the insulin receptor superfamily and induces downstream activation of pathways associated with cell survival, angiogenesis, and proliferation [3]. It was first identified as a fusion with the echinoderm microtubule-associated protein-like 4 (EML4) gene in lung cancer in 2007, and this fusion is sufficient to transform 3T3 cells in vitro and induce tumor formation in nude mice in vivo [4]. Since then, other fusion partners have been identified with ALK, including TFG and KIF5B [5, 6]. The rearrangement in this tumor has a breakpoint within ALK intron 19, resulting in separation of the ALK kinase domain (exons 20–29) from the N-terminal regions (exons 1–19). When expressed in cultured cells, the kinase domain of ALK exhibits oncogenic activity [7, 8]. We hypothesize the fusion in this patient’s tumor to be oncogenic because it contains the intact ALK kinase domain. Clinically the EML4-ALK gene fusion has been observed in ~3%–7% of non-small cell lung cancer (NSCLC) cases, and these patients tend to be younger, non-smoking males of Asian heritage [9]. Notably, ALK mutation has not been observed in 12 atypical lung carcinoid-endocrine samples analyzed in the COSMIC database to date.

**POTENTIAL STRATEGIES TO TARGET THE PATHWAY AND IMPLICATIONS FOR CLINICAL PRACTICE**

The ALK inhibitors crizotinib, ceritinib, and alectinib have been approved for the treatment of patients with metastatic NSCLC whose tumors test positive for ALK rearrangement [10–14]. Several others, such as brigatinib and lorlatinib, are currently in preclinical studies [15, 16]. The first-generation ALK inhibitor crizotinib is the preferred initial therapy for patients whose tumor harbors the ALK fusion because results of a phase III trial comparing crizotinib with chemotherapy demonstrated improved progression-free survival (PFS), response rates (RR), and quality of life [17]. The incidence of brain metastases in newly diagnosed stage IV ALK-positive NSCLC is approximately 20%–30%, comparable to those without the ALK fusion [17, 18].

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**Table 1. Treatment options**

| ALK inhibitor | Generation | Responses in patients with CNS disease |
|---------------|------------|---------------------------------------|
| Crizotinib    | First; FDA approved | PROFILE 1014 [18]: ORR 30/39 (77%) with crizotinib; 11/40 (28%) with chemotherapy |
| Alectinib     | Second; FDA approved | J-ALEX [26]: 1 event/14 with alectinib; 16 events/29 with crizotinib; PFS HR 0.08 favoring alectinib (CI95 0.01–0.61) |
| Ceritinib     | Second; FDA approved | ASCEND-4 [31]: IC-ORR 25/54 (46.3%) with ceritinib; 11/52 (21.2%) with chemotherapy |
| Brigatinib    | Phase II   | ALTA [30]: IC-ORR 36% 90 mg daily and 67% 180 mg |
| Lorlatinib    | Phase II   | 10/32 (31%) [29] |

Abbreviations: CNS, central nervous system; FDA, Food and Drug Administration; IC-ORR, intracranial overall response rate; PFS HR, progression free survival hazard ratio; ORR, overall response rate.
18. With improved control of systemic disease using crizotinib, however, approximately 60% of patients will ultimately develop brain metastases [19–21]. This observation is in part due to the poor central nervous system (CNS) penetration of crizotinib [22]. Until recently, surgery and/or radiation have been the mainstay of treatment for patients with brain metastases, including those with ALK fusions, although next-generation ALK inhibitors may alter this treatment paradigm because of their impressive intracranial activity (Table 1). A small phase II trial of alectinib consisting of 21 crizotinib-refractory patients showed a complete response in 29% (6), partial response in 24% (5), stable disease in 38% (8), and progression only in 10% (2) [23]. In a subsequent phase II study, objective responses were observed in 67% of the patients (10 complete responses and two partial responses) with baseline brain metastases who had not received prior CNS directed treatment, 28% with stable disease, and only 6% (1 patient) with CNS progression [13]. Similarly, other second-generation ALK inhibitors, ceritinib and brigatinib, have demonstrated intracranial overall RR between 36% to 63% in early phase studies, depending on whether the patient has had prior exposure to an ALK inhibitor [24, 25]. A recent interim analysis of the phase III J-ALEX trial, where 207 treatment-naïve, Japanese, ALK-positive patients were randomized either to receive alectinib or crizotinib in the first-line setting, showed improved PFS and tolerability with alectinib. The median PFS for crizotinib was 10.2 months (HR 0.34, 95% CI 0.17–0.7, p < .0001) but the PFS has not been reached in the alectinib arm [26]. A global phase III study comparing crizotinib with alectinib in the upfront setting (ALEX) has finished accrual with results expected next year. In this case of NSCLC with diffuse CNS disease and a suspected oncogenic ALK translocation, the molecular tumor board recommended initiating therapy with the second-generation ALK inhibitor alectinib.

Patient Update

In the case of this 52-year-old man with newly diagnosed metastatic ALK-positive atypical carcinoid tumor, the patient was started on alectinib with rapid shrinkage of his disease after 1 month, notably the brain and choroidal metastases (Fig. 2). He achieved a partial response with approximately 60% shrinkage of his dominant brain metastatic lesion and resolution of many smaller lesions. All the choroidal lesions resolved. The impressive clinical response supports that the novel ALK translocation identified using ctDNA is the driver mutation in this patient’s tumor. His disease remains stable after 5 months and the patient continues on alectinib.

Conclusion

Given the dramatic response to alectinib, the question was raised whether to pursue stereotactic radiation to the remaining brain lesions. Evidence on this topic is limited, although case reports have shown an increased risk of radio necrosis with alectinib [27]. Therefore, we recommended expectant management with close surveillance. At progression, efforts will be made to identify resistance mechanisms via direct biopsy of accessible sites of progression or by utilization of ctDNA. Treatment options directed at CNS progression include alectinib dose escalation (from 600 mg p.o. b.i.d to 900 mg p.o. b.i.d) to re-induce CNS response versus the third-generation ALK inhibitor lorlatinib, which has shown high CNS penetration and response in a heavily pre-treated patient cohort [28, 29]. Therapies for systemic progression include chemotherapy such as platinum and pemetrexed combination versus lorlatinib.

Glossary of Genomic Terms and Nomenclature

Circulating tumor cells: tumor cells identified circulating in the patient’s blood thought to be derived from tumor shedding

Circulating tumor DNA: tumor DNA identified in the patient’s blood thought to be released upon tumor cell death

Hybrid capture-based comprehensive genomic profiling: method of sequencing DNA using probes to pull down exons and fusions of interest

InDel: insertions or deletions of bases in the DNA

Median exon coverage: average number of times an exon is read during sequencing

Pair-end sequencing: sequencing from both ends of a DNA as opposed to just from one end (single-end)

Substitution: a DNA mutation that exchanges one base for another

Target depth correction: an error-correction process where fragment barcodes are employed to identify polymerase chain reaction duplicates of unique ctdNA molecules and merge them into a single consensus read

Translocation or rearrangement: a chromosome abnormality whereby parts of non-homologous chromosomes join together

Author Contributions

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Disclosures

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Editor’s Note: See the related commentary, “ALK Fusion Detection in Circulating Free DNA: Finding an Important Needle in the Haystack,” by Meghan J. Mooradian and Justin F. Gainor on pages 759–761 of this issue.