Identification of a Novel HIP1-ALK Fusion Variant in Non–Small-Cell Lung Cancer (NSCLC) and Discovery of ALK I1171 (I1171N/S) Mutations in Two ALK-Rearranged NSCLC Patients with Resistance to Alectinib

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Abstract: Huntington-interacting protein 1 (HIP1) has recently been identified as a new fusion partner fused to anaplastic lymphoma kinase (ALK) in non–small-cell lung cancer (NSCLC). To date, two variants of HIP1-ALK (H21; A20) and (H28; A20) have been identified in NSCLC. However, the response of patients with NSCLC harboring HIP1-ALK to ALK inhibitors and potential resistance mechanisms to such remain unknown. Here, we report a patient with NSCLC harboring a novel HIP1-ALK fusion variant (H30; A20). This patient and another patient with EML4-ALK variant 3a/b initially responded sequentially to crizotinib and then alectinib, a next-generation ALK inhibitor, but developed acquired resistance to alectinib with the presence of a mutation in amino acid residue 1171 (I1171N and I1171S respectively) located in the hydrophobic regulatory spine (R-spine) of the ALK kinase in both the cases as identified by a comprehensive next-generation sequencing-based assay performed on biopsies of new liver metastases that developed during alectinib treatment.

Key Words: HIP1-ALK, ALK I1171N, ALK I1171S, Alectinib resistance, ALK-rearranged NSCLC, Next generation sequencing, Hydrophobic regulatory spine.

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as compared with cytotoxic chemotherapy as either first-line or second-line treatment of patients with ALK-rearranged non–small-cell lung cancer (NSCLC) diagnosed based on an U.S. Food and Drug Administration–approved fluorescence in situ hybridization test.1,2 However, resistance to crizotinib invariably develops and many next-generation ALK inhibitors are in development. ALK-rearranged NSCLC is a heterogeneous molecular subgroup of lung cancer with at least four known fusion partners identified (EML4-, KIF5B-, TFG-, KLC1-).3 Recently Huntington-interacting protein-1 (HIP1) was the latest and fifth fusion partner identified to be fused to ALK in NSCLC with two different HIP1-ALK variants reported (H28; A20) and (H21; A20) to date.4 Analogous to the known multiple variants of EML4-ALK, it is likely that other HIP1-ALK fusion variants exist in NSCLC also. Here, we describe a patient with NSCLC harboring a novel HIP1-ALK fusion variant together with another patient with EML4-ALK variant 3a/b both of whom have been treated with crizotinib and then alectinib in which secondary mutations at amino acid residue 1171 in the hydrophobic regulatory-spine (R-spine) of the ALK kinase domain were identified.

CASE DESCRIPTIONS

Case one is that of a Dutch-Indonesian female never smoker who was diagnosed with de novo stage IV NSCLC adenocarcinoma at age 58. Her tumor was found to be positive for ALK-rearrangement by fluorescence in situ hybridization and she was enrolled onto PROFILE 1014 (NCT01154140) (crizotinib versus platinum/pemetrexed in treatment-naive advanced ALK-rearranged NSCLC) and was randomized to receive crizotinib. She had a partial response (PR) although on crizotinib but developed progression after 5 months. She was then enrolled onto the phase 1 dose escalation portion of the alectinib trial (AF-002JG, NCT01588028) at 900 mg orally twice daily. No rebiopsy was performed after crizotinib progression. Preacteinib baseline positron emission tomography–computed tomography scan showed the progressive disease in the mediastinum but no evidence of liver metastases (Fig. 1A). The washout period from the last dose of crizotinib to the first dose of alectinib was 20 days. She tolerated the alectinib dose well without any adverse events, dose interruption or dose...
reduction, and achieved a confirmed complete response (CR) after 2 months on alectinib that lasted for 12 months after starting alectinib when several new liver lesions were noted (Fig. 1B). Computed tomography–guided needle biopsy of a liver metastasis confirmed adenocarcinoma and was subjected to genomic profiling through clinical grade next-generation sequencing assay (FoundationOne, Foundation Medicine, Inc., Cambridge, MA) as previously described. These results revealed that this tumor specimen harbored a new HIP1-ALK fusion variant involving rearrangement and fusion of exons 1 to 30 of HIP1 to exons 20 to 29 of ALK (H30; A20) (Fig. 2). In addition, a single amino acid substitution at position 1171 changing the hydrophobic isoleucine residue to a hydrophilic asparagine residue was comprising approximately 15% of the sequencing runs was identified where tumor purity in the biopsy sample was estimated to be 30% (Fig. 3A).

Case 2 is that of a Caucasian male never-smoker who was diagnosed with stage IV ALK-rearranged NSCLC at age 38.
He was enrolled onto single-arm crizotinib trial PROFILE1005 (NCT00932451) and received crizotinib 250 mg twice daily. He achieved a confirmed PR after 5 months but developed both intrathoracic progression and asymptomatic brain metastasis after 21 months of crizotinib treatment. He was then enrolled onto the same trial as the patient in case 1 and received alectinib 900 mg twice daily. The period between the dose of crizotinib and the first dose of alectinib was 15 days. The alectinib dose was eventually reduced to 750 mg twice daily after 4 months of treatment due to headache. He achieved a PR with alectinib treatment after 6 weeks but after 12 months of alectinib treatment a new 18-fluorodeoxyglucose-avid liver lesion was identified (Fig. 1C), a biopsy of which was done and assayed with FoundationOne. The tumor purity in the sample was estimated to be 70%. The analysis revealed the EML4-ALK 3a/b variant and a base substitution occurring at 36% frequency in the codon encoding amino acid residue 1171 that resulted in the substitution of isoleucine to serine (I1171S) (Fig. 3B).

**DISCUSSION**

Here we report a novel HIP1-ALK (H30; A20) fusion transcript, the third HIP1-ALK fusion variant described in NSCLC to date. The fusion breakpoint of HIP1 at intron 29 is the same breakpoint as the Huntingtin-interacting protein 1-platelet-derived growth factor receptor beta (HIP1-PDGFRβ) gene fusion in chronic myelomonocytic leukemia, the only other malignancy where HIP1 has been reported to be rearranged to kinases leading to aberrant signaling and malignant transformation.7 The full-length HIP1 protein contains 1037 amino acids encoded by 31 exons and contains an AP180 N-terminal domain between the amino acids 32 to 160, a coiled-coil domain between amino acids 368 to 644, and a talin-homology domain between 771 to 1012 amino acids. Within the coiled-coil domain there is also a pseudo dia-autoregulatory domain (pDAD) domain that may mediate apoptosis.8 HIP1-ALK (H30; A20) is generated by in-frame rearrangement and fusion of the exons 1 to 30 of HIP1 to exon 20 to 29 of anaplastic lymphoma kinase (ALK) resulting in a fusion lacking only 18 amino acids of the c-terminal domain of HIP1 and retaining all the three functional domains of HIP1 (AP180 N-terminal, coiled-coil, talin-homology) (Fig. 2). Fang et al.4 has demonstrated that HIP1-ALK (H28; A20) is responsive to crizotinib in vitro whereas Hong et al.5 treated their HIP1-ALK (H21; A20) patient with adjuvant crizotinib after complete surgical resection with no recurrence at the time of...
their report but cannot confirm that the lack of recurrence is due to crizotinib. The current report is the first to describe that a patient with NSCLC harboring the HIP1-ALK fusion variant is responsive to multiple ALK inhibitors.

Isoleucine to asparagine change at amino acid residue 1171 in the ALK kinase domain (I1171N) was first identified as a rare activating mutation in familial neuroblastoma. Mutations at amino acid residue 1171 (I1171T/N/S/I) was

FIGURE 3. A, Diagrammatic representation of the I1171N mutation identified from case 1 indicating approximately 15% of the next generation sequencing run showed the presence of resistant I1171N substitution. B, Diagrammatic representation of the I1171S mutation identified from case 2 indicating approximately 36% of the next-generation sequencing run showed the presence of resistant I1171S substitution.
also identified as one of the most common acquired resistance mechanisms from an in vitro acceleration mutagenesis screen in EML4-ALK cell lines in the presence of increasing concentration of crizotinib. Furthermore, I1171N was also identified from a resistance selection assay in a nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) cell line (SPU-M2) that confer high level of acquired resistance to several ALK inhibitors (crizotinib, AP26113, TAE684). When engineered into Ba/F3 NMP-ALK cell lines, I1171N results in 6 to 127 fold increase in the concentration that inhibits 50% (IC50) against the same three ALK inhibitors. When Zdzalik et al. separately selected for Karpas299 (driven by NPM-ALK rearrangement) cell line that is resistant to crizotinib (Karpas299 CR), an I1171T mutation emerged that increased the IC50 to both crizotinib and alectinib by at least 10 fold. Consistent with this, our cases indicated that I1171X can also confer resistance to alectinib as I1171N/S were both identified from new liver metastases. These liver metastases were neither present during the initial crizotinib treatment nor present during the first 12 months of alectinib treatment in both the patients. Amino acid residue 1171 is situated on the hydrophobic regulatory spine (R-spine) that connects the two major lobes of the ALK kinase and I1171N results in ALK kinase preferentially in the active conformation. The inhibitory activity of alectinib against many of the known acquired ALK mutations was recently published but I1171N was not among the ALK mutations described. Despite the in vitro data and clinical presentation, notably we could not definitively conclude that mutation at I1171 confers resistance to alectinib as tumor specimens after crizotinib and before alectinib treatment was not available. However, the characterization of Karpas299CR (I1171T) cells strongly suggests that if the presence of a I1171X mutation appeared during and confer resistance to crizotinib treatment before the use of alectinib, the I1171 mutation would have conferred resistance to alectinib and not the prolonged CR and PR observed with alectinib in our two patient cases. 

Recently another second generation ALK inhibitor, ceritinib may be able to overcome acquired resistance conferred by an I1171T mutation. An implication of this finding is that doing a rebiopsy of the NSCLC cases with acquired resistance to targeted therapy can provide additional treatment options. Furthermore, from the results of the sequencing runs only a portion of the tumor samples harbored the resistance I1171 mutation from our two patients cases respectively indicating the tumors in both of our patients likely continue to depend on ALK signaling and switching to another ALK inhibitor that can potentially overcome I1171X resistance such as ceritinib may be the optimal approach for our two patients. In summary, comprehensive and fully informative genomic profiling allows the identification of both known and novel ALK fusion partners and variants, and alterations in the ALK gene that underlie acquired resistance and which can be targeted to likely further benefit patients who have progressed on first-line or even second-line targeted therapy.

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REFERENCES


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