ALK Translocation in ALK-Positive Mesenchymal Tumors

Diagnostic and Therapeutic Insights

Minsun Jung, MD, PhD; Kyung Chul Moon, MD, PhD; Jeongmo Bae, MD, PhD; Tae Min Kim, MD, PhD; Miso Kim, MD, PhD; Yoon Kyung Jeon, MD, PhD; Cheol Lee, MD, PhD

• ***Context.***—**A wide spectrum of mesenchymal tumors harboring *ALK* gene rearrangements has been identified outside the archetypal example of ALK-positive inflammatory myofibroblastic tumors.**

Objective.—**To evaluate the molecular pathology of unusual ALK-positive mesenchymal tumors and their response to ALK-targeted treatments.**

Design.—**Seven patients with ALK-positive mesenchymal tumors, including inflammatory epithelioid cell sarcoma, undifferentiated sarcoma, histiocytic neoplasm, smooth muscle tumor of uncertain malignant potential (STUMP), and atypical fibrohistiocytic tumor, were included on the basis of aberrant ALK immunoexpression. Patients with inflammatory myofibroblastic tumors were excluded from the study. *ALK* gene rearrangement was investigated either by fluorescence in situ hybridization or next-generation sequencing.**

Results.—**ALK was immunolabeled in all patients, diffusely (≥50%) in 6 patients and partially (10%–50%) in 1 patient. *ALK* gene rearrangement was discovered in 5 of the 6 available patients. The 3′-partners of *ALK* fusion were identified in 3 of 4 investigated patients as follows: *PRKAR1A-ALK* (ALK-positive histiocytic neoplasm), *TNS1-ALK* (STUMP), and *KIF5B-ALK* (ALK-positive atypical fibrohistiocytic tumor). We failed to discover *ALK* translocation in 1 patient with ALK-positive inflammatory epithelioid cell sarcoma. However, transcriptomic investigation showed that this tumor was significantly enriched with *ALK*-related pathways, which suggested activation of *ALK* through a nontranslocation pathway, as a constitutive oncogenic mark in this tumor. ALK-targeted inhibitors, which were administered to 3 patients with metastatic diseases, achieved partial remission in 1 patient with ALK-positive inflammatory epithelioid cell sarcoma and stable disease in patients with ALK-positive undifferentiated sarcoma and STUMP.**

Conclusions.—**Molecular investigation of ALK-positive mesenchymal neoplasms could allow for an accurate diagnosis and personalized treatment.**

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The *ALK* gene, located on chromosome band 2p23.2–2p23.1, encodes the receptor tyrosine kinase family protein ALK. Since this gene was first described in anaplastic large cell lymphoma in 1994,[1] translocation and activation of *ALK* have been demonstrated in diverse neoplasms of epithelial, melanocytic, hematolymphoid, and mesenchymal origin.[2–11] In addition to the archetypal example of ALK-positive inflammatory myofibroblastic tumor (IMT) and the rarer ALK-positive epithelioid inflammatory myofibroblastic sarcoma (EIMS), it is intriguing that a wide spectrum of mesenchymal tumors, including undifferentiated pleomorphic sarcoma, smooth muscle tumor of uncertain malignant potential (STUMP), leiomyosarcoma, histiocytosis, and fibrohistiocytic tumor, can harbor *ALK* gene rearrangements.[10,12–16] *ALK*-translocated neoplasms have predilections for certain fusion partners. For example, *TPM3/4-ALK* and *CLTC-ALK* are frequent in IMT, whereas *RANBP2-ALK* is frequent in EIMS.[17,18] Next-generation sequencing (NGS) is revealing an increasing complexity of *ALK* alterations as the list of *ALK* translocations grow.[5,11,12,18] Moreover, the recent advent of ALK-targeted tyrosine kinase inhibitors in ALK-positive mesenchymal tumors creates an urgent need to better understand the fundamental molecular pathology associated with *ALK* gene alterations so that these new regimens can be applied appropriately.[10,12,19–22] However, the molecular characteris-

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From the Departments of Pathology (Jung, Moon, Bae, Jeon, Lee) and Internal Medicine (TM Kim, M Kim), Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea; the Department of Pathology, Severance Hospital, Yonsei University College of Medicine (Jung), Seoul, Republic of Korea; Kidney Research Institute, Medical Research Center, Seoul National University College of Medicine (Moon), Seoul, Republic of Korea; and Seoul National University Cancer Research Institute (TM Kim, M Kim, Jeon), Seoul, Republic of Korea.

Jeon and Lee contributed equally to this work.

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Corresponding author: Cheol Lee, MD, PhD, Department of Pathology, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea (email: fe98134@snu.ac.kr).

Table 1. Clinical Details and Treatment Outcomes of Patients With ALK-Positive Mesenchymal Tumors

Pts	Age, y/Sex	Disease Extent	Diagnosis	*ALK* Rearrangement		Treatment	Best ALKi Response (PFS)	Outcome (Post Diagnosis)
				State	Method			
1	32/M	Systemic (mesentery, liver, peritoneum)	ALK-positive IES	Present	FISH	Excision→CT→crizotinib[a] (third-line)→ ceritinib[a] (fourth-line)→CT, TACE	PR (crizotinib: 10 mo; ceritinib: 7 mo)	DOD (3 y)
2	41/F	Localized (lung)	ALK-positive IES	NA	NA	Lobectomy, MLND	NA	NED (3 y)
3	28/F	Systemic (buttock, lung)	ALK-positive IES	Absent	FISH, TGS, RNA-seq	Excision, PORT→lung excision (after 34 mo)	NA	NED (3 y)
4	63/M	Systemic (leg, lung, brain)	ALK-positive US	Present	FISH	CT→ceritinib[a] (second-line)→GKS	SD (6 mo)	DOD (9 mo)
5	13/M	Systemic (both knees, LN)	ALK-positive histiocytic neoplasm	Present	FISH, TGS	Wide excision→CT→ICI→second excision	NA	NED (4 y)
6	53/F	Systemic (pelvis, bone, peritoneum)	ALK-positive STUMP	Present	FISH, TGS	Excision→HT, bone RT→peritoneum debulking→alectinib[a] (first-line)→ HT, bone RT	SD (5 mo)	AWD (7 y)
7	24/F	Localized (nipple)	ALK-positive AFHT	Present	FISH, TGS	Excision	NA	NED (1 y 8 mo)

Abbreviations: AFHT, atypical fibrohistiocytic tumor; ALKi, ALK-targeted inhibitor; AWD, alive with disease; CT, cytotoxic chemotherapy; DOD, died of disease; FISH, fluorescence in situ hybridization; GKS, gamma knife surgery; HT, hormone treatment; ICI, immune-checkpoint inhibitor; IES, inflammatory epithelioid cell sarcoma; LN, lymph node; MLND, mediastinal lymph node dissection; NA, not available; NED, no evidence of disease; PFS, progression-free survival; PORT, postoperative radiation therapy; PR, partial response; Pts, patients; RNA-seq, mRNA sequencing; RT, radiation therapy; SD, stable disease; STUMP, smooth muscle tumor of uncertain malignant potential; TACE, transarterial chemoembolization; TGS, targeted gene sequencing; US, undifferentiated sarcoma.

[a] ALK-targeted inhibitors.

tics of ALK-positive mesenchymal tumors outside of IMT are not fully understood because of their rarity. Immunohistochemical and genetic analyses of ALK-driven mesenchymal neoplasms may allow for a molecularly driven diagnosis and personalized treatment. In this study, we investigated the clinical, histopathologic, and molecular characteristics of ALK-positive mesenchymal neoplasms. Aberrant expression and translocation of *ALK* were identified in a small cohort of rare and in some cases, difficult-to-classify mesenchymal tumors in which ALK inhibitors demonstrated promising results.

MATERIALS AND METHODS

Discovery of ALK-Positive Mesenchymal Tumors

First, we retrieved tumors that were immunostained for ALK from the pathologic depository of Seoul National University Hospital (SNUH; Seoul, Republic of Korea) between 2012 and 2018. After eliminating nonmesenchymal tumors, including carcinoma, lymphoma, melanoma, glioma, germ cell tumor, and neuroblastoma, 2 pathologists (M.J. and C.L.) reviewed every diagnosis, ALK expression, and *ALK* gene translocation, using glass slides, fluorescence in situ hybridization (FISH) images, and NGS data. To exclusively examine unusual mesenchymal tumors with aberrant ALK immunoexpression, or "ALK-positive mesenchymal tumors," we screened out the classical examples of IMT as based on the latest 2020 World Health Organization classification.[23] Tumors showing patchy (<10%) expression of ALK were also excluded. Clinical information was obtained from the medical records. Oncologic response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria.[24] The institutional review board of SNUH approved this study (institutional review board No. H-2102-036-1195).

Immunohistochemical Investigation

A series of immunohistochemical staining, including ALK (1:50; clone 5A4, Leica, Wetzlar, Germany), was carried out with 4-μm-thick formalin-fixed, paraffin-embedded (FFPE) tissue (Supplemental Table 1, see supplemental digital content at https://meridian.allenpress.com/aplm in the December 2022 table of contents). ALK immunohistochemical staining was conducted with a BenchMark autostainer (Ventana, Basel, Switzerland). The expression of ALK was measured and determined to be partial (10%–50%) or diffuse (≥50%). The expression of other proteins was semiquantified as negative (0%), low expression (1%–30%), or high expression (>30%). The Ki-67 proliferative index was digitally analyzed within the highest area by using a QuPath viewer.[25]

Fluorescence In Situ Hybridization

Interphase FISH was performed, and the results were assessed under conditions that have been previously described.[26] Briefly, a Vysis *ALK* dual-color break-apart probe kit (Abbott Molecular, Abbott Park, Illinois) was hybridized on available 3-μm-thick FFPE slides. The slides were examined with an Allegro Plus with a Solo Touch Workstation (BioView Ltd, Rehovot, Israel) and were reviewed manually under an Olympus BX51TRF microscope (Olympus, Tokyo, Japan) equipped with the appropriate filters. Translocation of *ALK* was interpreted as recommended: break-apart green and red signals or isolated red signals were considered positive for *ALK* translocation.[27]

Next-Generation Sequencing

We further investigated *ALK* gene fusion and potential druggable targets in 4 of the 5 available patients (80%) through targeted gene sequencing (TGS) using in-house hybrid capture–based panels of SNUH (FIRST-Cancer Panel and/or FIRST-Lung Cancer Panel) that cover both the exonic and intronic regions of *ALK* (Table 1). Briefly, nucleotides were extracted from FFPE tumor tissue by using a QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, California) and

then fragmented by using adaptive focused acoustics (Covaris, Woburn, Massachusetts) following the manufacturer's protocols. The libraries were prepared by using the SureSelect XT Reagent Kit (Agilent, Santa Clara, California).

In addition, mRNA sequencing (RNA-seq) was used to confirm the *ALK* fusion status and the functional significance of ALK overexpression in patient 3 by comparing the transcriptional profiles of tumor versus normal tissue (Macrogen, Seoul, Republic of Korea). To this end, total RNA was extracted from FFPE specimens, followed by selective enrichment of mRNA with a poly-A tail and preparation of a cDNA library using the TruSeq RNA Access Library Prep Kit (Illumina, San Diego, California). Paired-end sequencing was performed on a HiSeq2500 platform (Illumina). Differentially expressed genes were identified with an absolute fold change of 2 or greater as a cutoff. Enrichment of gene sets was investigated from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database[28] and the MsigDB gene sets.[29] The detailed methods have been previously published.[30]

RESULTS

Patient Characteristics

Patient data from a total of 7 individuals with ALK-positive mesenchymal tumors were analyzed (Table 1). The ages at diagnosis varied and ranged from 13 to 63 years. Three patients with malignancies (patients 1, 3, and 5) initially had distant metastasis. Patient 4 developed lung metastasis 19 months after diagnosis. Patient 2 had an ALK-positive inflammatory sarcoma confined to the lung. In contrast, patient 6 was diagnosed with STUMP, which recurred with multiple metastases. Patient 7, who was diagnosed with an ALK-positive atypical fibrohistiocytic tumor, had localized disease in the nipple, which was suspected to have borderline malignant potential.

Clinical Course

Table 1 summarizes the clinical course and treatment response of the patients. Among the 5 patients with systemic diseases (patients 1, 3, 4, 5, and 6), 3 (60%; patients 1, 4, and 5) received cytotoxic chemotherapy and 3 (60%; patients 1, 4, and 6) received ALK-targeted tyrosine kinase inhibitors. Patient 5 initially underwent wide excision, followed by systemic treatment, after which he received a second excision for the chemotherapy-resistant lesion. Patient 3 received localized treatments for both primary and metastatic lesions. The other patients (2 and 7) with localized disease underwent complete surgical excision. After treatments, patients 1 and 4 died of the disease, patient 6 was alive with the disease for 7 years, and the other patients were free of disease.

ALK Aberration and Diagnostic Considerations

The histopathologic characteristics, including immunostaining for ALK and *ALK* gene rearrangement, are described in Tables 1 and 2. All patients (7 of 7, 100%) had aberrant ALK immunoreaction, which was diffuse (≥50%) in 6 patients (86%) and partial (<50%) in 1 patient (14%) in the cytoplasm and other subcellular locations, including the cytoplasmic membranes (patients 3 and 4), the nuclear membrane (patient 4), and the nucleus (patient 7) (Table 2). Rearrangement of *ALK* was identified in 5 of the 6 investigated patients (83%) with either FISH or NGS (TGS or RNA-seq) (Table 1). Patient 2, a consultation case from an outside hospital, had no material for the molecular assay.

Diffuse immunoexpression and genetic aberrations of ALK, together with histopathologic findings, indicated ALK-positive inflammatory epithelioid cell sarcoma with features of EIMS as a diagnosis for patients 1 through 3. Among these patients, patients 1 and 2 had initially been diagnosed otherwise, with malignant perivascular epithelioid cell tumor and intimal sarcoma, respectively, at outside hospitals. In patients 1 (Figure 1, A and B) and 2 (Figure 1, C and D), the tumor cells were spindled to epithelioid, arranged in a fascicle or in a solid growth pattern, and infiltrated by numerous inflammatory cells, including lymphocytes, plasma cells, and/or eosinophils. Along with diffuse ALK immunolabeling, *ALK* translocation was confirmed in patient 1 (Figure 1, B, inset). Similarly, patient 3 demonstrated round to polygonal tumor cells with diffuse ALK immunoexpression that were admixed with lymphocytes and plasma cells (Figure 1, E and F). Although structural variation in the *ALK* gene was undetected by FISH (Figure 1, F, inset), TGS, and RNA-seq (Table 1), gene expression profiling identified *ALK*-driven molecular pathways, as described below, which suggested ALK-positive inflammatory epithelioid cell sarcoma in this patient.

Given that patient 4 had highly pleomorphic tumor cells and no intermixing inflammatory cells, ALK-positive undifferentiated sarcoma was diagnosed despite diffuse ALK expression and *ALK* translocation (Figure 1, G and H). Patient 5 had a 7.0-cm hypermetabolic mass in the right infrapatellar fat pad with involvement of the adjacent bone (Figure 2, A and B). A positron emission tomography scan also revealed other lesions in the left knee and right inguinal/external iliac lymph nodes, which raised the possibility of bilateral malignancy with lymph node metastasis (Figure 2, B). Excisional biopsy of the primary mass revealed discohesive, round to polygonal cells containing round nuclei, prominent nucleoli, and vacuolated cytoplasm, which frequently showed hemophagocytosis (Figure 2, C). Immunohistochemical staining for ALK was diffuse-positive, and FISH analysis revealed *ALK* translocation (Figure 2, D). On the basis of positivity for histiocytic markers, including CD68, CD163, and lysozyme (Table 2), this patient was diagnosed with an ALK-positive histiocytic neoplasm. Patient 6, who had been treated for STUMP, had locoregional recurrence and bony metastases. Recurred tumors also construed as STUMP showed brisk mitoses without coagulative necrosis and myxoid changes (Figure 2, E; Table 2). Although ALK was weakly and partially (<50%) expressed in tumor cells, rearrangement of *ALK* was demonstrated by FISH and TGS (Figure 2, F). Finally, patient 7 had a tumor in the nipple involving the epidermis to deep dermis that showed solid or fascicular proliferation of spindle cells with mild cytologic atypia and lymphoplasma cell-rich stroma (Figure 2, G and H). Assessment of ALK revealed diffuse immunoreaction and *ALK* translocation (Figure 2, I). Further studies showed histiocytic differentiation, with positivity for CD68 and CD163 but negativity for smooth muscle–related antigens, including smooth muscle actin (SMA), desmin, calponin, and caldesmon (Table 2). The dermal-centered location and absence of smooth muscle differentiation suggested an ALK-positive atypical fibrohistiocytic tumor.

Characterization of Genetic Alterations of ALK

NGS studies were conducted to further characterize *ALK* gene alterations in 4 patients (3, 5 through 7), including patient 3, in whom we failed to identify *ALK* gene translocations by FISH. The results indicated the following fusion of the 3′ domains of *ALK* to 5′ partners (Figure 3, A): *PRKAR1A-ALK* (patient 5; Figure 3, B), *TNS1-ALK* (patient

Table 2. Pathologic Characteristics of Patients With ALK-Positive Mesenchymal Tumors

Pts	Primary Site	Size, cm	ALK Expression/ Pattern[a]	*ALK* Fusion Partner	Immunohistochemistry[b]	Histopathology	Dx Consideration
1	Mesocolon	17.0	Diffuse/C	Unknown	(+): SMA, vimentin, TFE3 (low), EMA (low), Ki-67 (45.8%) (−): CD34, CK, S100, HMB-45, melan-A, c-Kit, desmin	Epithelioid tumor cells admixed with lymphocyte and plasma cell infiltration	Originally malignant PEComa
2	Lung	1.5	Diffuse/C	NA	(+): SMA, desmin (low), Ki-67 (33.4%) (−): CK, TTF-1, S100, HMB-45, melan-A, CD34, p63, CD30, MDM2	Spindled to epithelioid tumor cells with lymphocyte, plasma cell, and eosinophil infiltration	Originally intimal sarcoma
3	Buttock	12.8	Diffuse/C, CM	No fusion	(+): vimentin (low), Ki-67 (14.8%) (−): SMA, desmin, myogenin, S100, ERG, FLI-1, CD99, PAX-8, NUT, c-Kit, inhibin-α, INI1 (no loss), CK, CK5/6, CK7, EMA, CD68, CD163, lysozyme, HMB-45, WT-1, HBME-1, calretinin, D2-40, CD3, CD20, CD30, CD43, CD45, CD56, CD79a, MUM-1, PAX-5, EBER	Sheets of round to polygonal tumor cells admixed with lymphocytes and plasma cells	Negative smooth muscle–related markers
4	Thigh	3.6	Diffuse/C, CM, NM	Unknown	(+): SMA (low), p53, CD68, CD163 (low), Ki-67 (56.8%) (−): CD1a, CD3, CD20, CD21, CD30, CD31, CD34, CD43, CD45, CK, S100, desmin, MDM2, TTF-1, EBER	Bizarre pleomorphic cells, no inflammatory cell infiltration	DDx: EIMS
5	Knee	7.0	Diffuse/C	*PRKAR1A-ALK*	(+): CD68, CD163, lysozyme, CD43, CD45 (low), PD-L1 (10%) (−): S100, langerin, CD1a, CD3, CD4, CD20, CD38, CD138, CD34, CD30, MPO, EBER, HHV-8, CK, EMA, SMA, desmin, myogenin, INI-1 (no loss)	Discohesive round to polygonal cells with abundant, vacuolated cytoplasm, frequent hemophagocytosis, invasion to bone	
6	Pelvis	6.6	Partial/C	*TNS1-ALK*	(+): SMA, desmin, ER, PR, WT-1, p16 (low), p53 (low), Ki-67 (7.9%) (−): S100, CK	Atypical spindle cells in fascicular pattern, focal ischemic necrosis, mitosis 18/2 mm^2	
7	Nipple	2.0	Diffuse/C, N	*KIF5B-ALK*	(+): Vimentin, CD68, CD163, Ki-67 (7.3%) (−): SMA, desmin, calponin, caldesmon, CD30, CK, EMA, lysozyme, S100, melan-A	Spindle cell proliferation in dermis and nipple stroma, lymphocyte infiltration	DDx: IMT

Abbreviations: C, cytoplasm; CK, cytokeratin (AE1/AE3); CM, cytoplasmic membrane; DDx, differential diagnosis; Dx, diagnosis; EIMS, epithelioid inflammatory myofibroblastic sarcoma; EMA, epithelial membrane antigen; ER, estrogen receptor; HHV-8, human herpesvirus-8; IMT, inflammatory myofibroblastic tumor; N, nucleus; NA, not available; NM, nuclear membrane; PEComa, perivascular epithelioid cell tumor; PR, progesterone receptor; Pts, patients; SMA, smooth muscle actin; STUMP, smooth muscle tumor of uncertain malignant potential.

[a] Expression of ALK is presented in terms of the extent, diffuse (≥50%) or partial (10%–50%), and the reaction sites (C, CM, N, NM).
[b] Immunohistochemistry other than ALK is measured as negative, low expression (1%–30%), or high expression (>30%).

6; Figure 3, C), and *KIF5B-ALK* (patient 7; Figure 3, D). Such chimeric proteins were expected to encompass the tyrosine kinase motifs of ALK. The same *TNS1-ALK* fusion was also discovered in the FoundationOne Heme panel (Foundation Medicine, Cambridge, Massachusetts) in patient 6.

Although *ALK* translocation was absent in patient 3 by FISH, TGS, and RNA-seq, significant overexpression of the *ALK* gene was verified in the tumor compared to adjacent normal tissue (Figure 4, A). Transcriptomic data suggested that tumor cells were enriched with downstream cascades of *ALK* activation, including the phosphatidylinositol 3-kinase (PI3K)–Akt and mitogen-activated protein kinase (MAPK) pathways, which were identified among the top 5 significantly enriched KEGG pathways (Figure 4, B). In addition, gene sets associated with E2F targets (Figure 4, C) and the G2/M checkpoint (Figure 4, D) were enriched in the tumor, but the Akt (Figure 4, E) and ALK (Figure 4, F) pathways were downregulated in normal tissue. These results collectively suggested that *ALK* gene activation might be a driver oncogene in patient 3 through a nontranslocation pathway.

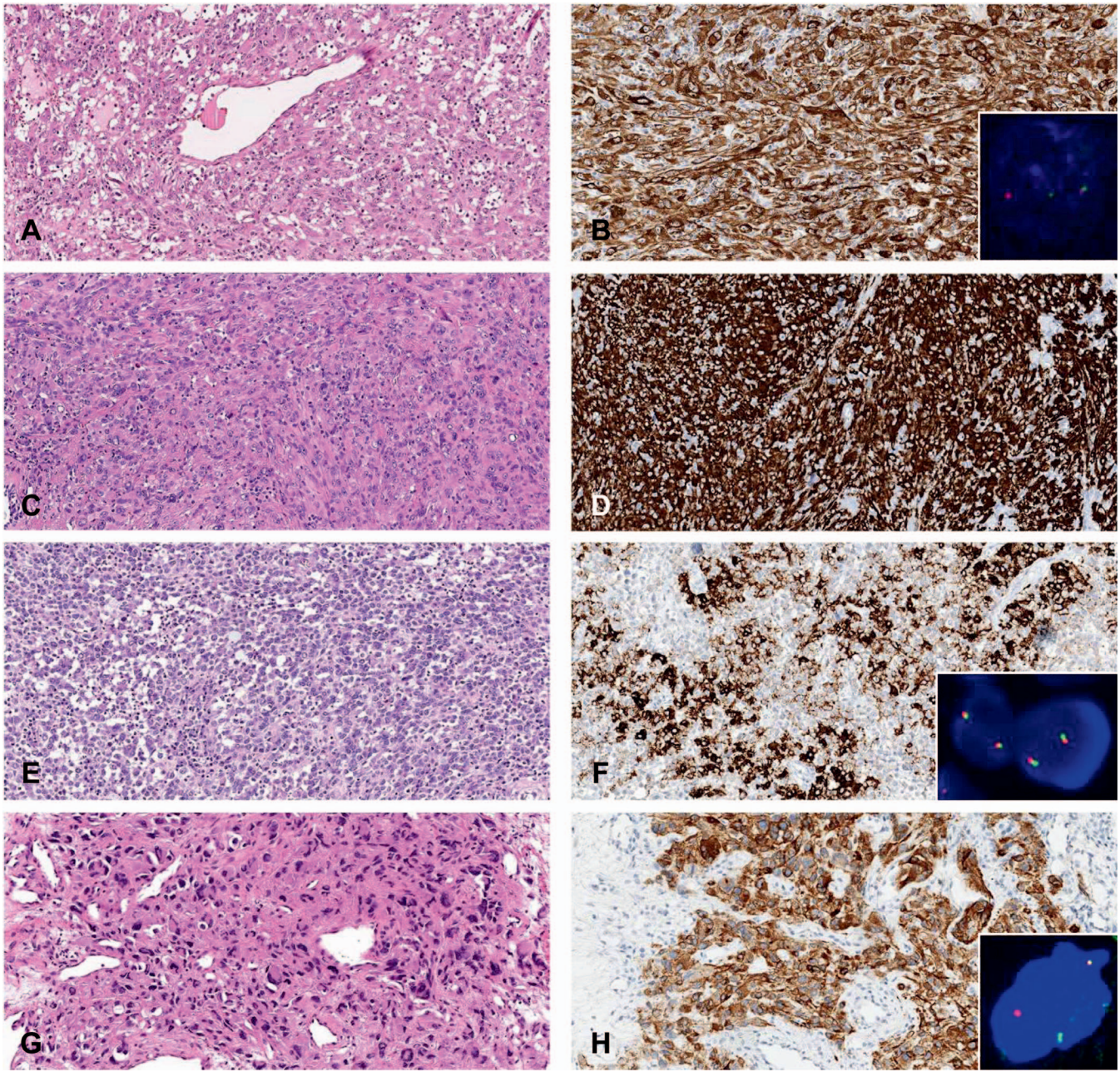

Figure 1. *A through F, Inflammatory epithelioid cell sarcoma. A, C, and E, Epithelioid tumor cells admixed with lymphocytes and/or plasma cells (patients 1 through 3). B, D, and F, Diffuse ALK expression and* ALK *translocation (patients 1 through 3). G and H, Undifferentiated sarcoma (patient 4). G, Pleomorphic tumor cells without inflammatory cells. H, Diffuse ALK expression and* ALK *translocation (hematoxylin-eosin, original magnification, ×200 [A, C, E, and G]; ALK, original magnification ×200 [B, D, F, and H]; fluorescence in situ hybridization, original magnification ×1000 [insets B, F, and H]).*

Response to ALK-Targeted Treatments

ALK inhibitors were administered to patients 1, 4, and 6, who achieved partial remission (patient 1) or stable disease (patients 4 and 6) (Table 1). Patient 1, who was diagnosed with *ALK*-translocated inflammatory epithelioid cell sarcoma, was enrolled in clinical trials for the ALK inhibitors crizotinib (NCT01121588) and ceritinib (NCT01283516) after worsening of liver metastases despite cytotoxic chemotherapy. Both crizotinib, as a third-line treatment, and subsequently ceritinib, as a fourth-line treatment, successfully alleviated the tumor burden for 10 and 7 months, respectively (Figure 5, A). Patient 4, who was diagnosed with ALK-positive undifferentiated sarcoma, developed pulmonary metastases shortly after doxorubicin-based chemotherapy, had stable disease after 4 months of ceritinib treatment, and showed a slight decrease in metastatic nodules (Figure 5, B). Later, this patient developed a brain metastasis, and he died of the disease 9 months after diagnosis. Patient 6, who was diagnosed with STUMP, experienced a leiomyosarcoma-like clinical course with aggravating metastases in the spine and ileum. These bony

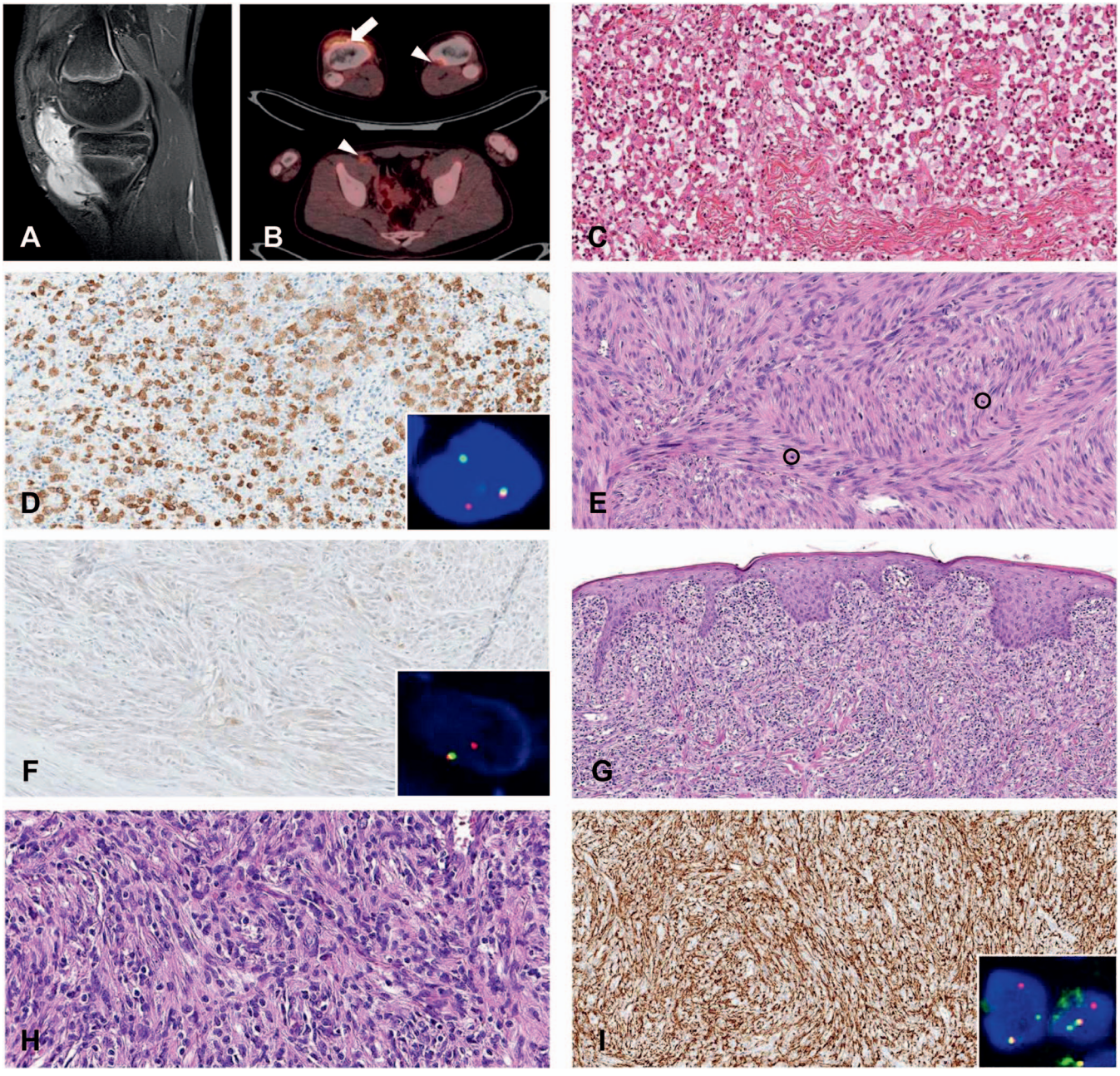

Figure 2. *A through D, ALK-positive histiocytic neoplasm (patient 5). A, Right knee mass. B, Main mass (arrow) and other lesions/lymph nodes (arrowheads). C, Hemophagocytosis in the primary tumor. D, Diffuse ALK expression and* ALK *translocation. E and F, Smooth muscle tumor of uncertain malignant potential (patient 6). E, Mitoses (circle). F, Partial ALK expression and* ALK *rearrangement. G through I, ALK-positive atypical fibrohistiocytic tumor (patient 7). G and H, Spindle cells with lymphocytes in the epidermis-dermis. I, Diffuse ALK expression and* ALK *translocation (hematoxylin-eosin, original magnifications ×200 [C and E], ×150 [G], and ×400 [H]; ALK, original magnifications ×200 [D and I] and ×400 [F]; fluorescence in situ hybridization, original magnification ×1000 [insets D, F, and I]).*

lesions exhibited metabolic responses to alectinib for 6 months and showed a 30% or greater reduction in standardized uptake value (Figure 5, C). She had been living with the disease for 7 years.

DISCUSSION

We described the clinicopathologic and molecular properties of patients with ALK-positive mesenchymal tumors and their response to ALK-targeted therapies. Genetic rearrangement of *ALK* was identified in 5 of 6 available cases and was not observed in patient 3, in whom *ALK*-driven oncogenic pathways were suggested by transcriptomic analysis. The oncogenic action of *ALK* depends on the ligand-independent activation of the intracytoplasmic tyrosine kinase moiety at the 3′ end of *ALK*. In *ALK*-translocated neoplasms, such tyrosine kinase regions are dissociated and fuse with new partners at the 5′ end, as identified in patients 5 (*TNS1-ALK*), 6 (*PRKAR1A-ALK*), and 7 (*KIF5B-ALK*) of the present study.[5,11] Expression of ALK and/or its translocation were key to changing the diagnoses of patients 1 through 3. In addition, we suggest that altered expression of ALK could be found outside of conventional IMT/EIMS. For instance, we

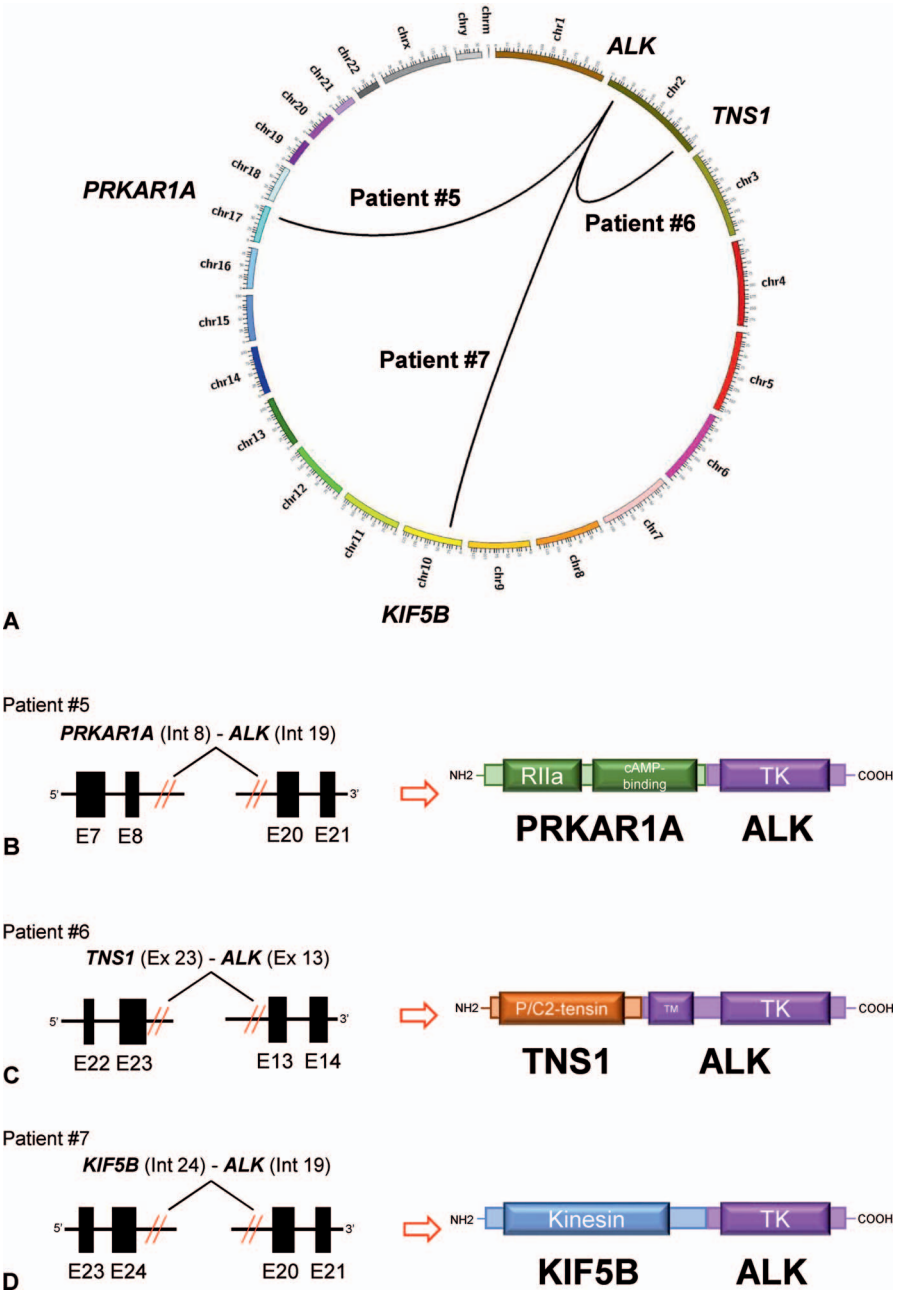

Figure 3. *A,* ALK *gene fusions identified in patients 5 through 7. B through D, Illustrations showing each fusion transcript and protein. These data are based on Genome Browser (https://genome.ucsc.edu) and UniProt (https://www.uniprot.org) without reflecting the actual scale.*

discovered positivity for ALK in a handful of poorly defined mesenchymal and histiocytic neoplasms, including 3 inflammatory epithelioid cell sarcomas exhibiting features of EIMS, undifferentiated sarcoma, and a histiocytic neoplasm of soft tissue. Positive ALK immunolabeling could lead to further molecular studies and ALK-targeted treatments, which demonstrated promising results in patients with metastatic sarcomas included in this study. ALK expression in tumors is closely related to the underlying genetic alterations of *ALK*.[11,21] Therefore, it is plausible to argue that immunohistochemical investigation of ALK is an efficient method that can aid in the correct diagnosis of unusual mesenchymal tumors and in the selection of personalized treatment for aggressive sarcomas.

Constitutively activated ALK enhances the proliferation, survival, and migration of tumor cells through multiple pathways, including Ras-MAPK, PI3K-Akt, phospholipase Cγ, and Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signals, or less commonly, through epigenetic deregulation.[5,11] This pathogenicity centered around *ALK* has become a basis for targeted therapies in *ALK*-driven diseases, including mesenchymal neoplasms. For example, crizotinib, a small-molecule inhibitor of ALK and other tyrosine kinases, is a treatment of choice for *ALK*-rearranged non–small cell lung cancer and has also become the standard treatment for locally advanced or metastatic ALK-positive IMT/EIMS.[19,21,31–33] Consistent with this, the *ALK*-translocated inflammatory epithelioid cell sarcoma in patient 1 showed robust shrinkage after crizotinib treatment, which was followed by similar responsiveness to ceritinib.[33] In addition, as seen in patients 4 and 6, who had response to ALK inhibitors, off-label treatment with ALK inhibitors has resulted in treatment effects in *ALK*-driven mesenchymal tumors, such as STUMP, leiomyosarcoma,

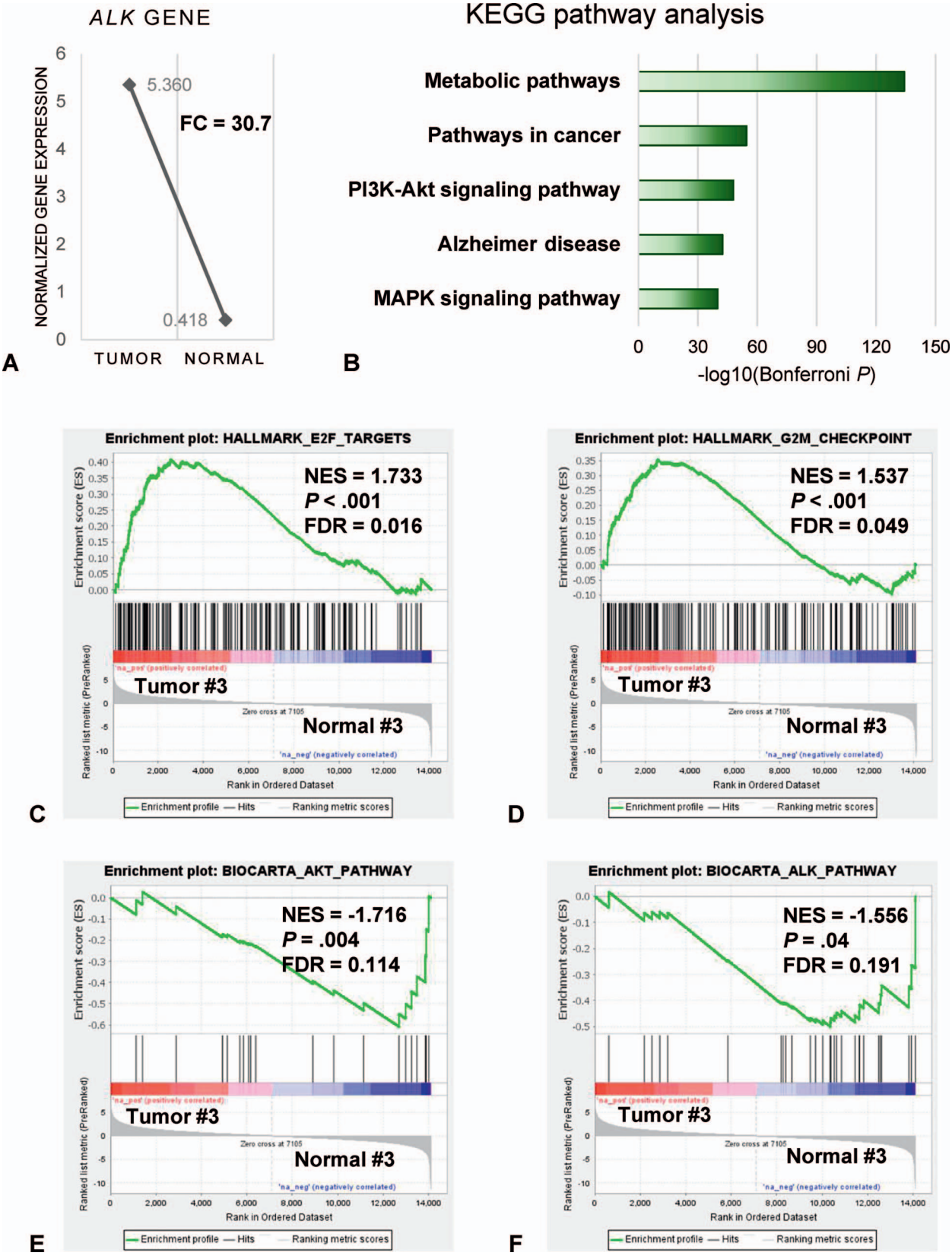

Figure 4. *Transcriptomic analysis in patient 3. A,* ALK *gene overexpression in the tumor compared to adjacent normal tissue. B, Top 5 significant KEGG pathways represented by DEGs between the tumor and adjacent normal tissue. DEGs were compared against the KEGG molecular pathways. C through F, Gene set enrichment analysis calculates enrichment scores on the basis of distribution of the predetermined gene sets either at the top (upregulated in tumor, positive score) or at the bottom (downregulated in tumor, negative score) of the DEGs. Abbreviations: DEGs, differentially expressed genes; FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; NES, normalized enrichment score.*

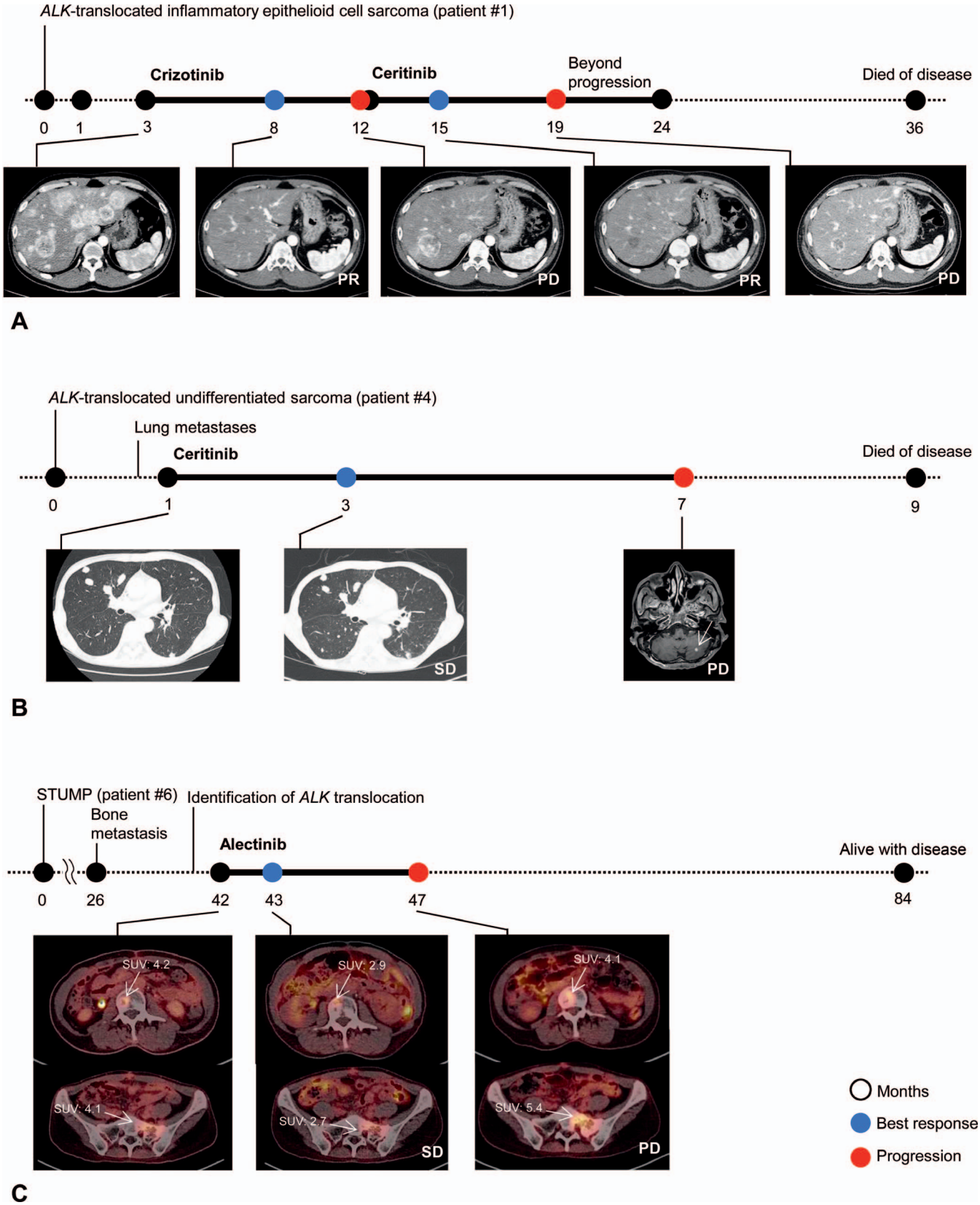

Figure 5. *Responses to ALK inhibitors. A, Initial partial remission, followed by disease progression after crizotinib and ceritinib treatment in inflammatory epithelioid cell sarcoma (patient 1). B, Response to ceritinib in metastatic undifferentiated sarcoma (patient 4). C, A metabolic response to alectinib in metastatic STUMP (patient 6) in both the spine (upper) and iliac bone (lower). Bold lines denote the duration of ALK-targeted treatment. Abbreviations: PD, progressive disease; PR, partial remission; SD, stable disease; STUMP, smooth muscle tumor of uncertain malignant potential; SUV, standardized uptake value.*

rhabdomyosarcoma, and myofibroblastic sarcoma, in vitro and in vivo.[16,20,34,35] To our knowledge, this is the first report to share the real-world experience of responses of patients with ALK-positive undifferentiated sarcoma to ALK inhibitors.

Case 3 is an unusual inflammatory sarcoma with features of EIMS that did not reveal any signs of differentiation. Although there was no structural variation in *ALK*, the *ALK* gene was significantly overexpressed, consistent with the immunohistochemical staining for ALK. Moreover, we demonstrated enrichment of the PI3K-Akt and MAPK pathways, upregulation of molecular hallmarks related to proliferation, including E2F targets and G2/M checkpoint proteins, and downregulation of Akt and ALK pathways in this tumor, suggesting that *ALK*, which was overexpressed, functioned as a driver oncogene in this patient. Similar immunohistochemical and genetic discrepancies were previously reported in ALK-positive IMT.[18] In addition to translocation, activating events in the *ALK* gene, including mutation, amplification, or epigenetic dysregulation, have been reported in different sarcomas.[36–38] The exact genetic alteration of *ALK* in this patient has yet to be confirmed by further investigation.

Like in EIMS, patients 1 through 3 had epithelioid or polygonal tumor cells and patients 1 and 3 also had infiltrating inflammatory cells. However, there were some discrepancies between inflammatory epithelioid cell sarcomas diagnosed in patients 1 through 3 and EIMS. For example, EIMS has been typified by recurrent *RANBP2-ALK* or *RRBP1-ALK* translocation, ALK immunoreactivity in the nuclear membrane or perinuclear cytoplasm, and frequent expression of desmin and CD30.[17,39] In patients 1 through 3, however, *ALK* translocation, which was studied in 1 patient, and such immunohistochemical characteristics were not observed, as ALK was expressed predominantly in the cytoplasm and/or cytoplasmic membrane in all patients, desmin was expressed in 1 patient (33%), and CD30 was nonreactive in 2 included patients (0%). In addition, patients 2 and 3 had tumors in the lung and buttock, respectively, which are unusual sites for EIMS.[17] Therefore, we classified these tumors as ALK-positive inflammatory epithelioid cell sarcomas with features of EIMS, although the data on ALK-positive epithelioid cell sarcoma are very limited.[40] In addition, ALK immunostaining associated with *RANBP2*/*RRBP1-ALK*–rearranged EIMS was shown in ALK-positive undifferentiated sarcoma (nuclear membrane; patient 4) and ALK-positive atypical fibrohistiocytic tumor (nucleus; patient 7) in the present study. This result suggests that other fusion partners may also produce immunohistochemical patterns similar to those related to the molecular topology of *RANBP2*/*RRBP1-ALK* fusions.[11,17,39]

The ALK-positive histiocytic neoplasm of patient 5 is a rare, ill-defined entity. ALK-positive histiocytosis, which has been reported in fewer than 20 patients, is possibly an appropriate classification for this neoplasm.[12,15] In agreement with the findings for patient 5, ALK-positive histiocytosis may also diffusely express ALK, show hemophagocytosis, and involve distant organs.[10,12,15,41] *KIF5B*, and less frequently, *COL1A2*, *EML4*, or *TPM3*, have been identified as partners of *ALK* fusion in this rare entity.[10,12] We found a novel transcript, *PRKAR1A-ALK*, in ALK-positive histiocytic neoplasm, which was reported in ALK-positive IMT.[18] ALK inhibitors, as previously reported in ALK-positive histiocytosis, might be a promising treatment option for this patient in the future.[12] It is worth mentioning that unlike what has been described in ALK-positive histiocytosis, patient 5 had a large and infiltrative soft tissue mass that exhibited cytologic atypia and presented with lymph node metastasis; this patient also had to undergo a second excision for persistent disease, even after systemic treatment following wide excision, and did not show any hematologic abnormality. On the contrary, ALK-positive histiocytosis is usually reported to have an indolent clinical course, even in disseminated conditions.[12] Therefore, we reasoned that the ALK-positive histiocytic neoplasm in patient 5 might represent a distinct entity that may benefit from more intensive treatments than what has been described for ALK-positive histiocytosis.[12] In addition, histiocytic sarcoma could be another differential diagnosis for case 5, which may be distinguished by the absence of "monster" cells and the presence of ALK expression in our patient.[42]

Patient 7, who was diagnosed with ALK-positive atypical fibrohistiocytic tumor, had a *KIF5B-ALK* fusion, and intriguingly, this is the same fusion that is frequently observed in ALK-positive histiocytosis.[12,43] Histiocytic differentiation by immunohistochemical staining in patient 7 might signify a connection of this fusion to histiocytic differentiation. The main differential diagnosis for this patient was ALK-positive IMT, although myofibroblastic differentiation was absent.[23] For example, the same *KIF5B-ALK* fusion was discovered in an ALK-positive spindle cell tumor of the brain that was negative for SMA and desmin and that was classified as IMT by the authors.[44] Epithelioid fibrous histiocytoma, which is characterized by consistent expression of ALK and *ALK* gene rearrangements, also requires diagnostic consideration.[13] In addition to its classical exophytic, well-circumscribed, and epithelioid morphologies, nontypical forms containing spindle cells admixed with inflammatory cells have been recognized.[45] ALK-positive skin tumors encompass a wide spectrum of diseases, from benign to malignant tumors, which may share pathologic features.[13] The results of the present study would aid in overcoming this diagnostic challenge in the future.

We investigated ALK expression by using a standardized method across all patients. However, the small number of patients included in this study limited the detailed determination of the immunohistochemical-genetic associations of ALK.[5] In addition, comprehensive assessment of the types of *ALK* fusions implicated in the response to ALK-targeted therapy in ALK-positive mesenchymal tumors is urgently needed. Finally, the unambiguous classification of ALK-positive undifferentiated (patient 4), histiocytic (patient 5), and fibrohistiocytic (patient 7) neoplasms has not yet been determined owing to their rarity, which warrants further study.

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