Crizotinib in *ALK*-Rearranged Inflammatory Myofibroblastic Tumor

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SUMMARY

Inflammatory myofibroblastic tumor (IMT) is a distinctive mesenchymal neoplasm characterized by a spindle-cell proliferation with an inflammatory infiltrate. Approximately half of IMTs carry rearrangements of the anaplastic lymphoma kinase (*ALK*) locus on chromosome 2p23, causing aberrant ALK expression. We report a sustained partial response to the ALK inhibitor crizotinib (PF-02341066, Pfizer) in a patient with *ALK*-translocated IMT, as compared with no observed activity in another patient without the *ALK* translocation. These results support the dependence of *ALK*-rearranged tumors on ALK-mediated signaling and suggest a therapeutic strategy for genomically identified patients with the aggressive form of this soft-tissue tumor.

Inflammatory myofibroblastic tumors (IMTS) occur primarily during the first two decades of life and typically arise in the lung, retroperitoneum, or abdominopelvic region.[1,2] Abdominal tumors may be multifocal. Lesional cells are predominantly myofibroblasts in a myxoid to collagenous stroma admixed with inflammatory cells.[2,3] Local recurrence may occur after initial surgery, with a low risk of distant metastases,[1,2] so that IMTs are considered to be soft-tissue tumors of intermediate biologic potential, with a small fraction behaving aggressively.[4]

Rearrangements involving the *ALK* locus on chromosome 2p23 have been documented in approximately 50% of IMTs.[5,6] ALK aneuploidy has also been described, with a gain in copy number without rearrangement.[5] Among cancers with rearrangements, several fusion partners have been identified that serve to constitutively activate ALK.[7–10] ALK expression reliably correlates with *ALK* rearrangement.[11] Distant metastases occur primarily in ALK-negative IMTs, but local recurrence occurs regardless of ALK expression.[5,12]

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Several ALK fusion proteins, including TPM3-ALK found in IMT, induce transformation in cell lines and animal models,[13] a finding that suggests that *ALK* rearrangement may define a subgroup of IMTs that is sensitive to targeted kinase inhibition. We therefore enrolled two patients with IMT in a dose-escalation phase 1 trial of crizotinib, an orally bioavailable ATP-competitive inhibitor of the ALK and MET tyrosine kinases.[14,15]

CASE REPORTS

Patient 1 was a 44-year-old man who had been well until May 2007, when he reported having early satiety and abdominal pain. Computed tomography (CT) of the abdomen and pelvis revealed ascites, a mass in the right upper quadrant, and omental caking. The results of esophagogastroduodenoscopy and colonoscopy were unremarkable. The patient then underwent paracentesis. Combined ^{18}F-fluorodeoxyglucose positron-emission tomography and CT (FDG-PET–CT) revealed hypermetabolic masses in the abdomen and pelvis. In June 2007, he underwent exploratory laparotomy, which showed massive omental caking with discrete, round, gelatinous, grape-size tumor nodules and extensive peritoneal disease. Maximal tumor debulking was performed along with catheter placement to facilitate administration of a hyperthermic peritoneal perfusion of cisplatin, doxorubicin, and mitomycin C. The tumor was composed of predominantly epithelioid cells with large vesicular nuclei, prominent nucleoli, and amphophilic cytoplasm, embedded in a myxoid stroma with prominent neutrophils (Fig. 1A). On immunohistochemical analysis, tumor cells were positive for desmin, a finding that is consistent with a myofibroblastic origin, and ALK (Fig. 1B) and were negative for SMA, cytokeratin, and myogenin. Fluorescence in situ hybridization (FISH) with the use of break-apart probes showed *ALK* rearrangement (Fig. 1C). The nuclear membrane pattern of ALK staining suggested the *RANBP2* fusion partner, which encodes a nuclear pore protein.[16] This rearrangement was confirmed by reverse-transcriptase–polymerase-chain-reaction (RT-PCR) and sequencing assays (Fig. 1D and 1E).

The patient received doxorubicin and ifosfamide from August through November 2007, followed by maintenance therapy with imatinib until February 2008, when follow-up CT revealed asymptomatic, multifocal, recurrent peritoneal nodules. After meeting eligibility criteria and providing written informed consent, the patient began receiving crizotinib on March 25, 2008, at a dose of 200 mg twice daily. On May 21, 2008, and June 19, 2008, CT scanning showed reductions of 40% and 53%, respectively, in the sum of unidimensional measurements of target lesions, which was classified as a partial response, according to the Response Evaluation Criteria in Solid Tumors (RECIST)[17] (Fig. 2). The maximal response was achieved in October 2008. At that time, despite the continued partial response of multiple mesenteric and peritoneal lesions, growth of three lesions (hepatic, peripancreatic, and perirectal masses) was noted.

In December 2008, further growth of these masses occurred, and the patient subsequently underwent exploratory laparotomy to resect the growing lesions with maximal debulking (Fig. 1F and G). After he had recovered from the operation and with approval from the sponsor and the institutional review board, treatment with crizotinib was restarted at a dose of 250 mg twice daily, which had been defined as the maximum tolerated dose.[15] As of September 2010, he remained in complete radiographic remission. While receiving crizotinib, the patient has had edema in the legs and feet, intermittent joint aches, hypocalcemia, hypophosphatemia, leukopenia, and anemia, all of grade 1 severity, according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). He has not reported fatigue (the known dose-limiting toxic effect) and has not had

aminotransferase elevations, a finding that has complicated the treatment course in a small percentage of patients.[15]

Patient 2 was a 21-year-old man who presented with vomiting and new-onset jaundice in November 2007. CT scanning revealed a calcified mass near the greater curvature of the stomach in the region of the pancreatic head. Endoscopic retrograde cholangiopancreatography was performed, and samples that were obtained on biopsy were not diagnostic. In December 2007, increasing abdominal pain led to exploratory laparotomy for debulking, partial gastrectomy, partial right colectomy, cholecystectomy, and splenectomy and placement of internal and external biliary draining devices.

The tumor showed a multinodular growth pattern involving the gastric and colonic wall. Morphologically, the tumor had a heterogeneous appearance, ranging from hypercellular areas arranged in intersecting fascicles and associated with an abundant lymphoplasmacytic inflammatory infiltrate (Fig. 3A and 3B) to hypocellular and sclerotic regions with dystrophic calcification. The tumor cells ranged from slender, fusiform cells with fibrillary cytoplasm and open chromatin, reminiscent of myofibroblasts, to epithelioid cells with abundant eosinophilic cytoplasm and macronucleoli, resembling ganglion cells. A diagnosis of IMT was made on the basis of the histologic analysis. The tumor cells were focally positive for muscle-specific actin (HHF35), with a characteristic tram-track pattern of staining, a finding that is consistent with myofibroblastic differentiation. All other markers were negative, including CD117, CD34, desmin, SMA, AE1:AE3, S-100, CD21, CD35, clusterin, and CD68. The cellular morphologic features, prominent inflammatory infiltrate, and negativity for these markers ruled out entities that mimic IMT, such as spindle-cell melanoma, sarcomatoid carcinoma, gastrointestinal stromal tumor, follicular or interdigitating dendritic-cell sarcoma, inflammatory leiomyosarcoma, and desmoid fibromatosis. ALK immunohistochemical analysis was also negative, and FISH studies did not reveal a rearranged ALK gene (Fig. 3C and 3D).

After the patient had recovered from surgery, in February 2008, treatment with prednisone and ibuprofen was started.[18] He had increasing abdominal pain, and progressive disease was documented on imaging. In July 2008, after meeting eligibility criteria and providing written informed consent, the patient began to receive crizotinib at a dose of 250 mg twice daily. In August 2008, crizotinib therapy was interrupted because of an increased level of total bilirubin. Repeat imaging showed worsening of abdominal disease, and the patient was removed from the trial.

DISCUSSION

Patient 1 had advanced IMT with *ALK* rearrangement and had only a short disease-free interval after initial surgery with aggressive treatment, consisting of hyperthermic peritoneal chemotherapy perfusion, followed by adjuvant systemic doxorubicin–ifosfamide and empirical imatinib. This patient's disease showed a rapid and substantial partial response to crizotinib that lasted at least 6 months, despite an extensive tumor burden. This response suggests a primary role of aberrantly expressed and constitutively activated ALK in the growth and maintenance of the patient's tumor. Consistent with this biologic hypothesis is the finding that the tumor in Patient 2, which lacked *ALK* rearrangement, had no response to crizotinib. Together, these cases support the dependence on ALK-mediated signal transduction in a subgroup of IMTs.

The use of targeted inhibitors to disrupt mutant signaling pathways that cancers require for continued growth has resulted in substantial progress in cancer treatment. Other examples include imatinib inhibition of BCR-ABL in chronic myeloid leukemia[19] and of KIT in

gastrointestinal stromal tumors[20] and the inhibition of epidermal growth factor receptor (EGFR) by tyrosine kinase inhibitors (gefitinib or erlotinib) in non–small-cell lung cancers with activating EGFR mutations.[21] Crizotinib has also shown striking clinical activity in non–small-cell lung cancers with *EML4-ALK* rearrangements.[22,23]

Several histologic patterns of IMT have been described, including those with compact spindle-cell, myxoid–vascular, and hypocellular fibrous morphologic features.[2] However, it is not possible to predict which tumors will have *ALK* rearrangement or *ALK* aneuploidy. Rearrangement correlates well with ALK expression and kinase domain activation, both of which probably predict ALK dependence and crizotinib sensitivity. ALK immunoreactivity has not been reliably shown in cases of aneuploidy,[5] and the dependence on ALK signaling or propensity to respond to targeted treatment in this subgroup of tumors is unknown.

Although there have been no clear associations among ALK expression, histologic analysis, and disease recurrence, tumors that have *RANBP2-ALK* fusion, including samples obtained from Patient 1, have common morphologic features and biologic behavior.[16,24] Histologically, such tumors are dominated by epithelioid or round cells and have prominent myxoid stroma and a neutrophilic inflammatory infiltrate. In addition, nearly all patients with such tumors have presented with intraabdominal disease, many with multifocal tumors. These patients have had a locally aggressive clinical course, with early recurrence after initial surgery. Although not all cases of IMT with round-cell transformation express RANBP2-ALK, it is possible that specific fusion partners influence both histologic features and prognosis. Crizotinib therapy along with surgery may be useful in cases that are complicated by local recurrences. Similarly, unresectable IMTs may respond to crizotinib, facilitating their complete surgical removal.

Despite the impressive rates of tumor regression and control achieved with the use of targeted tyrosine kinase inhibitors in diseases such as EGFR-mutant non–small-cell lung cancer or KIT-mutant gastrointestinal stromal tumors, resistance develops in most patients within 1 to 2 years. Resistance results from the acquisition or selection of secondary mutations in the targeted kinase, which serve to inhibit drug binding through steric effects or increase affinity for ATP, or involves the activation of alternative tyrosine kinases that the cancer requires.[25] Although Patient 1 did not have objective disease progression according to RECIST after 8 months of receiving crizotinib, three masses were growing at that time and were resected. These tumors are under study to determine the mechanism of resistance to crizotinib. Various sites of solid- tumor metastases may behave heterogeneously, and several other IMT lesions were continuing to respond at the time of surgery. We therefore elected to continue crizotinib therapy after surgical tumor resection, and an additional 19 months have passed without evidence of recurrence.

Although the role of crizotinib in this prolonged disease-free interval cannot be conclusively established, our observations suggest a long-term response of certain tumor-cell populations. In addition, Patient 1 continues to have an excellent performance status and only mild side effects, supporting the tolerability of the long-term administration of crizotinib.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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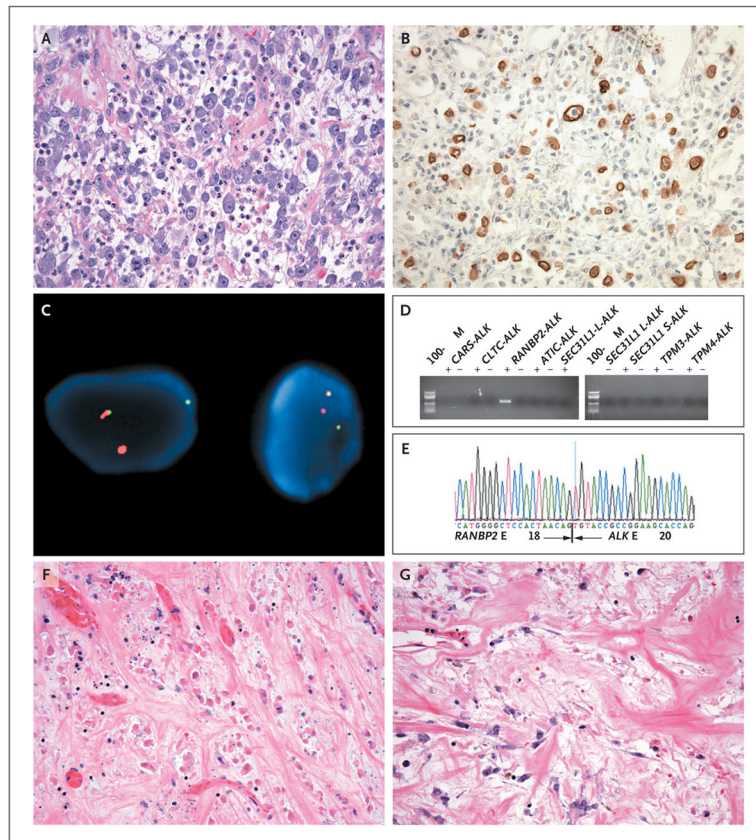
Figure 1. Histologic, Immunohistochemical, and Molecular Analyses of IMT Samples from Patient 1

A sample of the inflammatory myofibroblastic tumor (IMT) obtained on biopsy shows epithelioid cells containing vesicular nuclei, prominent nucleoli, and amphophilic cytoplasm embedded in a myxoid stroma containing prominent neutrophils (Panel A, hematoxylin and eosin). Immunohistochemical analysis for ALK shows positive staining in tumor cells, with a nuclear membrane pattern (Panel B). Dual-color fluorescence in situ hybridization (FISH) shows rearrangement of centromeric (green) and telomeric (orange) probes flanking the *ALK* locus at 2p23 (Panel C). Gel electrophoresis of polymerase-chain-reaction (PCR) products after reverse-transcriptase PCR (RT-PCR) is shown for primers directed at known *ALK* translocation partners in IMT, including *CARS, CLTC, RANBP2, ATIC, SEC31L1* (which generates both long-form [L] and short-form [S] fusion transcripts), *TPM3,* and *TPM4*[4,9] (Panel D). Only *RANBP2-ALK* primers produce an amplification product in the presence of (but not in the absence of) reverse transcriptase. Sequencing of the PCR product confirmed that *RANBP2* exon 18 is fused in frame with *ALK* exon 20 (Panel E). Sections from progressing tumor masses in the liver (Panel F) and perirectal region (Panel G), which were resected after approximately 8 months of crizotinib administration, show histologic heterogeneity, with cellular areas similar in appearance to the initial biopsy sample but also revealing extensive areas suggestive of a treatment effect, with foci of tumor-cell necrosis in the liver sample and marked stromal hyalinization in the perirectal sample (hematoxylin and eosin in Panels F and G). Methods for immunostaining, FISH, and RT-PCR are described in the Supplementary Appendix, available with the full text of this article at NEJM.org.
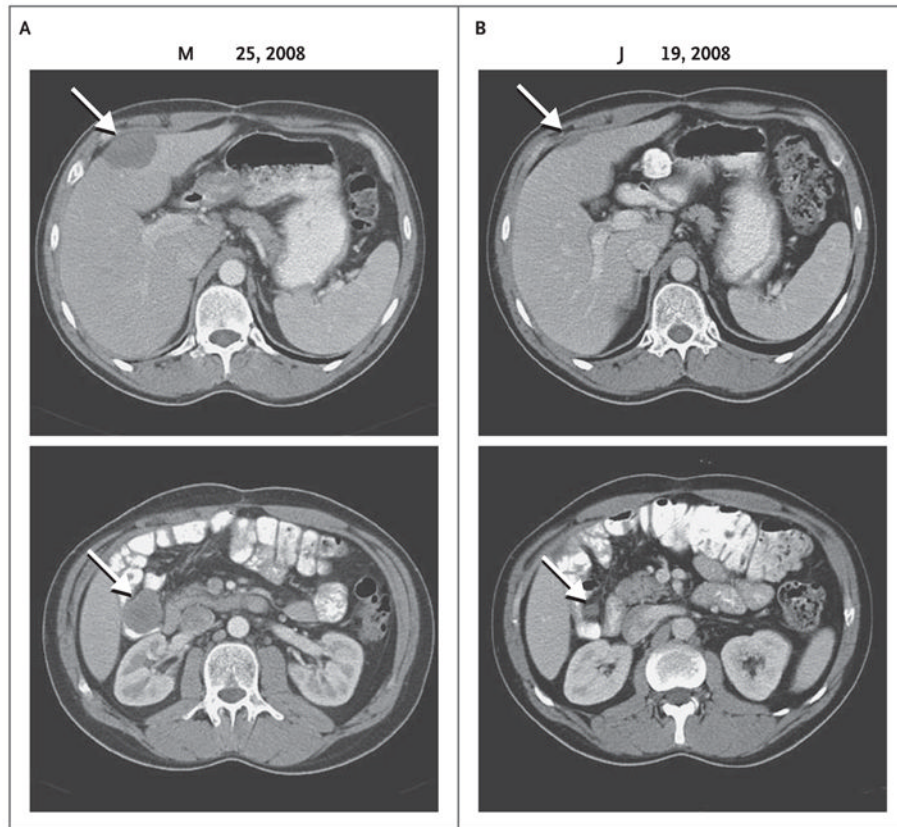
Figure 2. CT Scans Showing the Response to Crizotinib in Patient 1
The baseline abdominal CT scan shows a hepatic mass measuring 4.8 by 3.3 cm (top) and
one of several mesenteric masses measuring 3.8 by 3.3 cm (bottom) (Panel A, arrows). After
13 weeks of treatment with crizotinib, the hepatic and mesenteric masses measured 2.3 by
0.8 cm and 1.3 by 1.2 cm, respectively (Panel B, arrows). In October 2008, these masses
measured 3.6 by 2.2 cm and 0.5 by 0.5 cm, respectively (not shown), indicating that the
hepatic mass had regrown, despite a continued response, according to the Response
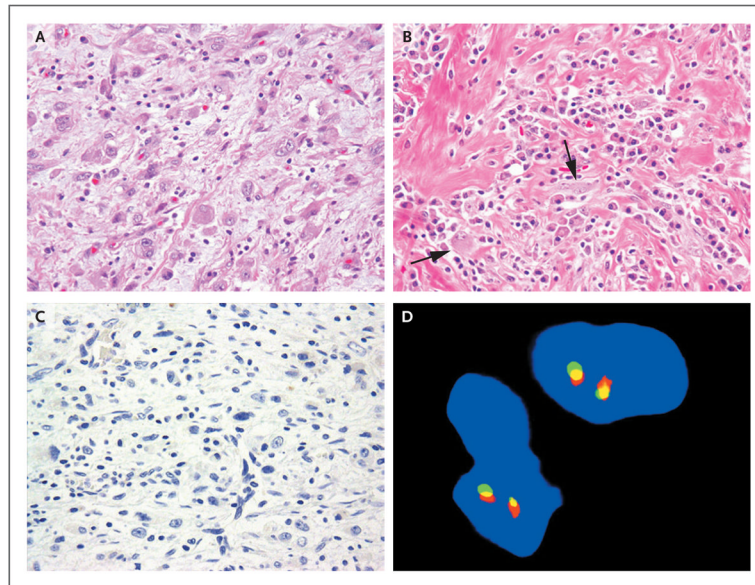Evaluation Criteria in Solid Tumors.

Figure 3. Histologic and Immunohistochemical Analyses of IMT Samples from Patient 2
A sample of the inflammatory myofibroblastic tumor (IMT) obtained on biopsy shows
ganglion-like, plump epithelioid cells with abundant eosinophilic cytoplasm and nuclei with
open chromatic and prominent nucleoli; scattered small lymphocytes are in the background
(Panel A, hematoxylin and eosin). A different area of the tumor shows a prominent
inflammatory component that is composed primarily of plasma cells, dense fibrous stroma,
and scattered plump spindle and ganglion-like cells (Panel B, arrows; hematoxylin and
eosin). Results of immunostaining for ALK are negative (Panel C), and FISH analysis shows
no *ALK* rearrangement (Panel D).