Response to Crizotinib in ROS1 Fusion–Positive Intrahepatic Cholangiocarcinoma

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INTRODUCTION

ROS1 (*ROS1* proto-oncogene receptor tyrosine kinase) encodes a tyrosine kinase of the insulin receptor family that plays a role in regulating cellular growth and differentiation. *ROS1* is activated by chromosomal rearrangement involving its kinase domain (exons 36 to 42) in a variety of human cancers and is most commonly associated with non–small-cell lung cancer (NSCLC).[1] Several techniques are used to detect these *ROS1* fusions, including DNA sequencing, RNA sequencing, reverse transcription polymerase chain reaction (RT-PCR), and fluorescence in situ hybridization (FISH). Immunohistochemistry (IHC) of the ROS1 protein can also be considered as a prescreening test because high-expression staining is suggestive of possible activating fusions present in the sample.

ROS1 aberrations are present in 1% to 2% of patients with NSCLC, and ROS1 inhibitor therapy (crizotinib,[2] ceritinib,[3] lorlatinib[4]) is active in these tumors. In the expansion cohort of the phase I study of crizotinib in this indication, the objective response rate was 72%, and the median progression-free survival was 19.2 months. ROS1 inhibitors have also shown evidence of activity in other tumor types, including pediatric inflammatory myofibroblastic tumors (crizotinib),[5-7] melanoma (entrectinib),[8] pediatric high-grade glioma (entrectinib),[6] acral lentiginous melanoma (entrectinib),[9] and pancreatic cancer (entrectinib).[10] Here we report the successful treatment of an intrahepatic cholangiocarcinoma (ICC) harboring a ROS1 fusion with crizotinib.

CASE REPORT

A 56-year-old non-Hispanic white woman with a past medical history of nontoxic goiter and hypothyroidism presented to her primary care provider with right-sided abdominal pain and lower extremity swelling of 2 months duration. Abdominal ultrasound demonstrated a large liver mass that was later visualized on a computed tomography (CT) scan. The mass was approximately 7 cm in its largest dimension in the right lobe of the liver. Several prominent periportal/portocaval lymph nodes were also noted. Biopsy of the mass showed moderately differentiated adenocarcinoma consistent with intrahepatic biliary tract cancer (BTC) or cholangiocarcinoma (immunostaining was positive for CK7, CK19, and MUC-1 and negative for CK20, CDX-2, GATA-3, and thyroid transcription factor); S100 showed weak nonspecific staining). Laboratory evaluation showed mildly elevated alkaline phosphatase and carbohydrate antigen 19-9 (CA19-9) of 278 U/mL. Her case was presented at a multidisciplinary tumor board and she was determined to have unresectable disease because of the presence of involved nonregional lymph nodes.

Systemic therapy with gemcitabine and cisplatin was initiated. She tolerated the chemotherapy well, but repeat CT imaging approximately 10 weeks after the start of chemotherapy showed progression with new lesions in segments 2 and 8 of the liver in addition to the continued presence of lesions in segments 6 and 4 and the caudate. Disease progression was also noted in the periportal/portocaval lymph nodes. CA19-9 had increased to 482 U/mL.

Molecular profiling of the tumor (FoundationOne CDx, Foundation Medicine) revealed an *RDX-ROS1* fusion as well as a *TERT* promotor 124 C>T mutation. Variants of undetermined significance were noted in *BCORL1*, *BRCA2*, *LTK*, and *PDK1* genes. Second-line treatment was initiated with the ROS1 inhibitor crizotinib at the standard oral dose of 250 mg twice per day. She tolerated crizotinib well; on review, her only complaints were intermittent constipation along with mild abdominal pain and indigestion. A few weeks after initiating systemic therapy with crizotinib, her CA19-9 declined from 482 to 178 U/mL, and approximately 4 months after the start of crizotinib it declined to 33 U/mL (Fig 1). CT evaluation at month 3 showed a significant tumor response. Her dominant lesion decreased from 7.3 cm to 4.1 cm (–43.8%). The segment 2 lesion and enlarged periportal/portocaval lymph nodes comparably decreased in size, and the lesions in segments 4, 8, and the caudate lobe were no longer identified (Fig 1). The patient's response to crizotinib is ongoing at the time of this report approximately 6 months after initiating therapy. The patient provided consent to publish this case report.

DISCUSSION

Cholangiocarcinomas arise from epithelial cells lining the biliary tree. Standard treatment options are limited, and the median survival for patients with advanced

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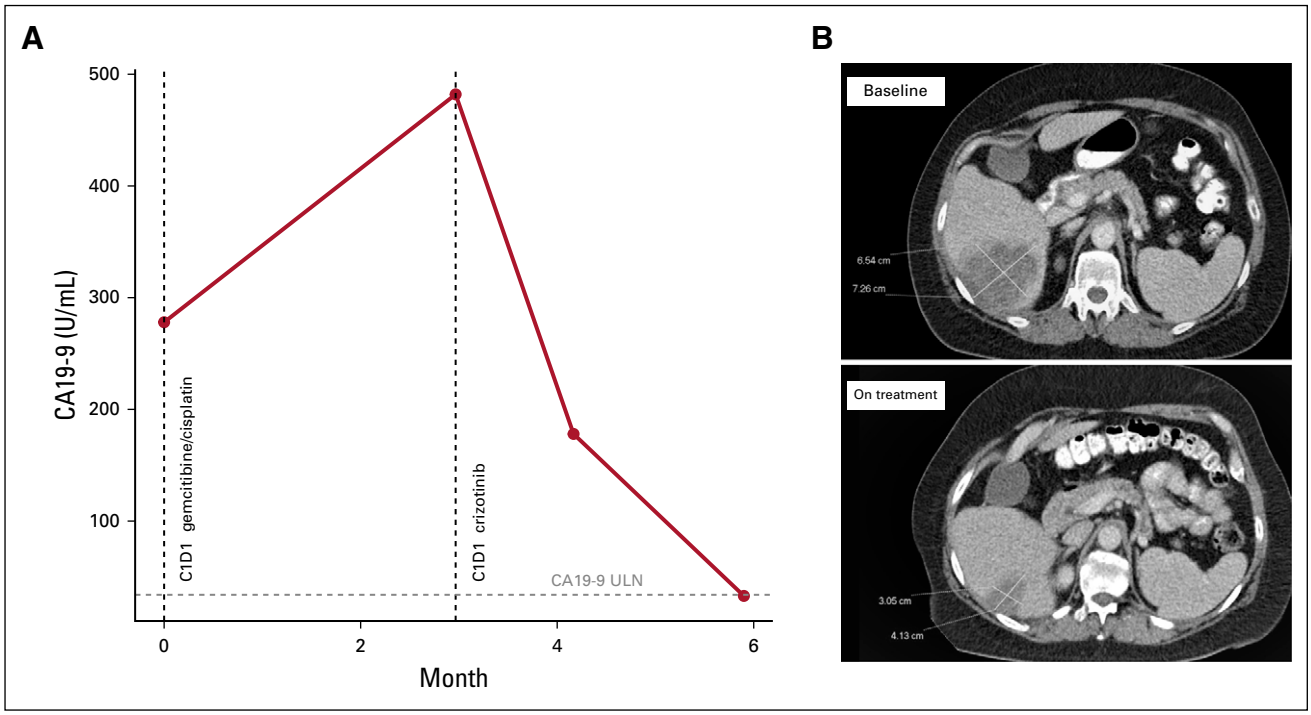

FIG 1. (A) Carbohydrate antigen 19-9 (CA19-9) at baseline, in response to chemotherapy, and then in response to crizotinib. (B) Target lesion measurements before and after crizotinib treatment. ULN, upper limit of normal (36.0 U/mL).

cholangiocarcinoma is approximately 1 year. Recently, actionable mutations in cholangiocarcinoma (particularly ICC) have been described, including FGFR fusion rearrangements and IDH1 mutations.[11,12] Several reports have described apparent *ROS1* translocations in cholangiocarcinoma. The Catalogue of Somatic Mutations in Cancer (COSMIC) database reports *ROS1* fusions in less than 1% of BTCs and 1% of gallbladder adenocarcinomas (GBCs). *ROS1* fusions have been reported in ICC, extrahepatic cholangiocarcinoma, and GBC with varying frequencies (Table 1).

The specific fusion in this patient detected by FoundationOne CDx was an *RDX-ROS1* fusion. The *RDX* gene (NM_002906.3) encodes the protein radixin, a cytoskeleton protein involved in linking actin to the plasma membrane.[13] This is the first time this fusion partner has been seen in cholangiocarcinoma and potentially across solid tumors either partnered with *ROS1* or another oncogene on review of the COSMIC database.[1,14]

Additional studies highlight the additional molecular and clinical importance of *ROS1* in BTC. A preclinical study described the FIG-ROS1 fusion that induces ICC oncogenic development in an orthotopic allograft mouse model in cooperation with *Kras* and *p53*. Other conditional expression experiments using the models suggested oncogenic addiction to ROS1 signaling despite additional mutations, including *Kras*, which predicted the clinical efficacy of crizotinib described in this case report.[15] In another report, expression levels of ROS1 measured by IHC were correlated with response to gemcitabine and oxaliplatin combined with cetuximab in advanced BTC.[16] ROS1-expressing BTC tumors from patients with advanced disease were grouped with ALK- and c-MET–expressing BTC tumors because the 3 genes have been previously implicated in anti-EGFR resistance in NSCLC.[17,18] ROS1, ALK, or c-MET (RAM) high-expressing tumors were all ICC tumors and had worse outcomes compared with RAM low expressers. RAM low expressers were also found to benefit from the addition of cetuximab to gemcitabine and oxaliplatin chemotherapy. The authors concluded that RAM expression may be site-specific (more common in ICC), and RAM expression should potentially be used in treatment decisions, specifically anti-EGFR therapy. In contradiction to this study, ROS1 IHC expression levels were associated with well-differentiated histology and better survival in a series of ICC patients who underwent curative resection.[19]

The current evidence suggests that ROS1 fusions are rare events in BTC, but the low incidence may partly be a result of the challenge in detecting them. FISH is a gold standard for *ROS1* detection because it has the ability to detect translocations of *ROS1* with any fusion partner; however, there can be multiple FISH patterns in addition to the classic split pattern.[20,21] FISH and RT-PCR can also be technically challenging, time-consuming, costly, and limited by tissue quality and availably. Performing IHC studies as a prescreen is a consideration, and in lung cancer, ROS1 IHC has good concordance with FISH and RT-PCR methods and is used as a screening biomarker.[22] This

TABLE 1. *ROS1* Fusion Prevalence in Cholangiocarcinoma

First Author	Population	*ROS1* Fusion Investigated	Technique	Total No. of BTC Samples Analyzed	% Positive	No. Positive
Neia[24]	Italian	*FIG-ROS1*	DNA PCR	65	9	GBC (2 [14%] of 14 samples); ECC (4 [16%] of 25 samples); ICC (0 [0%] of 26 samples)
Gu[25]	Chinese	*FIG-ROS1*	RT-PCR	23	9	2 of 23
Graham[26]	American	—	FISH	100	1	1 of 100
Lee[19]	Korean	—	FISH	102 (all ICC)	0	0 of 102
Liu[27]	Chinese	*FIG-ROS1*, *SLC34A2-ROS1*, or *CD74-ROS1*	RT-PCR	56	0	0 of 56
Sun[28]	Korean, Singaporean	—	FISH	261 (all ICC)	1.1	3 of 261
Zehir[29]	American	—	NGS	242	0	0 of 242

Abbreviations: BTC, biliary tract cancer; ECC, extrahepatic cholangiocarcinoma; FISH, fluorescence in situ hybridization; GBC, gallbladder cancer; ICC, intrahepatic cholangiocarcinoma; NGS, next-generation sequencing; PCR, polymerase chain reaction; RT-PCR, reverse transcription PCR.

same concordance was not seen in ICC in 1 study because the ROS1 gene rearrangement by break-apart FISH was not positive in the 72 tumors reported to have moderate to strong IHC staining for ROS1.[19] Discordance may be suggestive of *ROS1* activation through alterative means such as epigenetic mechanisms.[23] Molecular techniques need to be refined because identifying these rare drivers can be key to successful and sustained treatment options.

Although *ROS1* fusions have been described in cholangiocarcinoma, to our knowledge this is the first definitive report of a patient with *ROS1* fusion–positive cholangiocarcinoma treated with a ROS1 inhibitor. Additional information about crizotinib activity in cholangiocarcinoma is anticipated from the French National AcSé Program crizotinib basket study (ClinicalTrials.gov identifier: NCT02034981), which is enrolling a cohort of patients across multiple tumor types who harbor alterations in ALK, MET, or ROS1. An initial report from this study indicated that crizotinib has clinical activity in biomarker-selected patients across various tumor types, but it did not explicitly report the response rate of crizotinib in cholangiocarcinoma.[5] Other basket trials are enrolling patients with solid tumors, including cholangiocarcinoma with *ROS1* gene fusions (ClinicalTrials.gov identifier: NCT02568267). Our report highlights the potential value of molecular profiling in cholangiocarcinoma and the significance of *ROS1* in this disease. It also adds to the growing list of tyrosine kinase inhibitor responses to *ROS1* fusions across solid tumors, and these reports collectively provide support for a tumor agnostic approach to targeting *ROS1* fusions outside NSCLC.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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