Oncogenic ALK Fusion in Rare and Aggressive Subtype of Colorectal Adenocarcinoma as a Potential Therapeutic Target

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Running title: ALK Fusion in Colorectal Carcinoma

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Conflict of Interest: Kai Wang, Ph.D., Kyle Gowen, B.S., James Sun, Ph.D., Vincent A. Miller M.D., Philip J. Stephens Ph.D., Siraj M. Ali M.D., Ph.D., and Jeffrey S Ross, M.D. are employed by and hold ownership interest (including patents) in Foundation Medicine. No potential conflicts of interest are disclosed by the other authors.
Translational Relevance

*ALK* rearrangement defines a rare molecular subtype of CRC with distinct clinical and pathological features, including advanced stage, proximal colon location, often microsatellite instability, and mucinous phenotype. Although *ALK* fusions occur in 0.1-0.2% of patients with CRC, this equates to approximately 265 new cases of CRC per year in the United States and approximately 2,800 new cases of CRC per year worldwide. We provided first clinical evidence that patients with *ALK*-rearranged CRC may achieve an exceptional clinical benefit from targeted monotherapy with *ALK* inhibitor ceritinib. These data support further evaluation of patients with CRC harboring *ALK* fusions in early-phase clinical trials and also suggest that such patients today may benefit from already approved *ALK* inhibitors as well as investigational agents.
ABSTRACT

Purpose: Chromosomal translocations in the anaplastic lymphoma kinase (ALK) gene have been identified as oncogenic drivers in lung adenocarcinomas and other tumors, recently including rare cases of colorectal carcinoma (CRC). We identified a patient with refractory metastatic CRC harboring a STRN–ALK gene fusion who achieved an exceptional clinical benefit to the ALK inhibitor ceritinib. Our goal was to further define the clinicopathologic features of ALK-rearranged CRC in a large cohort.

Experimental Design: Clinical cases of CRC evaluated by comprehensive genomic profiling (CGP) or by ALK immunohistochemistry (IHC) were reviewed retrospectively. FISH and MSI analyses were performed.

Results: Nine CRC cases harbored ALK gene fusions. Six cases were identified by CGP of 3,157 CRC (0.2%), and 3 by IHC of 2,980 CRC (0.1%). The ALK fusions involved known ALK partners EML4, C2orf44, CAD, and the novel STRN, PPP1R21, SENPF, MAPRE3, and PRKAP1B partners. These advanced stage CRC lacked mutations in other oncogenic drivers, predominantly involved the proximal colon, and often exhibited microsatellite instability and mucinous phenotype. The index patient was treated with the ALK inhibitor ceritinib resulting in a marked decrease in size of a skin metastasis, and resolution by computerized tomography of all contrast enhancing tumor. After 9 months of treatment, biopsy of progressive disease demonstrated a KRAS mutation, consistent with acquired resistance to ceretinib.

Conclusions: CRC harboring ALK fusions represent a rare aggressive subtype
of CRC with distinct clinicopathologic features. This report provides first clinical evidence that such patients may benefit from targeted monotherapy with ALK inhibitors.
INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer diagnosed in both men and women in the United States with estimated 132,700 new cases in 2015 (1). Approximately 20% to 25% of CRC patients present with hepatic metastases on initial diagnosis and a further 40% to 50% of patients will develop hepatic metastases within 3 years of their primary surgery (2). With recent advances in chemotherapy, the median survival for patients with metastatic colorectal carcinoma is approximately 25-30 months (3,4). Recent genome-scale molecular analyses have uncovered several potential molecular targets in this disease, including ERBB2 amplifications and sequence mutations as well as recurrent gene fusions that lead to oncogenic tyrosine kinase activation including those involving ALK, RET, ROS1, and NTRK (5-7). Given the poor prognosis of advanced CRC, investigation of the druggability of these altered kinases is important to address this unmet clinical need.

Gene rearrangements involving ALK receptor tyrosine kinase were first discovered in anaplastic lymphoma, and later found in several tumor types from different lineages, including hematolymphoid, mesenchymal, neural, and epithelial (8,9). The frequency of ALK fusions varies according to the tumor type. Among the epithelial cancers they are most common in non-small cell lung cancer (NSCLC), comprising approximately 3-7% of lung adenocarcinoma (10), while only 9 cases of colorectal carcinoma harboring ALK fusions have been described in 7 previous studies (6,7,11-15, Supplementary Table 1). Tumors with ALK rearrangements express activated fusion kinases composed of the intact ALK tyrosine kinase domain
fused with different unrelated gene partners. The resulting ALK gene fusion creates a constitutively active kinase that drives tumor cell growth through activation of MAP kinase, STAT3, and phosphoinositide 3-kinase (PI3K) pathways among others (9).

The discovery of ALK rearrangements in NSCLC has initiated a new era of targeted therapy in lung cancer (16). The ALK inhibitors crizotinib and ceritinib have become standard of care for ALK-driven lung cancer, with superior efficacy and improved tolerability, in comparison with cytotoxic drugs (17,18). In two preclinical studies, the growth of CRC cell lines harboring EML4-ALK fusions was inhibited by the ALK inhibitors crizotinib and entrectinib (7,15).

Herein, we describe the first patient with advanced metastatic CRC harboring a STRN-ALK gene fusion identified by comprehensive genomic profiling (CGP) after failing standard therapies, who achieved an exceptional clinical response to ceretinib, a second generation ALK inhibitor. In a retrospective analysis, we identified 8 additional cases of CRC with ALK fusions and present a detailed analysis of the molecular, clinical, histopathologic features, and biologic behavior of this rare aggressive subtype of CRC.
PATIENTS AND METHODS

Clinical Trial

The index patient was enrolled in the Novartis Signature Trial within the arm “Ceritinib (LDK378) for Patients Who Have Tumors with ALK or ROS1 Gene Alterations (Signature)” (ClinicalTrials.gov Identifier: NCT02186821). Comprehensive genomic profiling was performed after written informed consent for participation in the Rhode Island Hospital Institutional Review Board (IRB) protocol “Molecular Testing of Cancer by Integrated Genomic, Transcriptomic, and Proteomic Analysis” (ClinicalTrials.gov Identifier: NCT02213822). Response to ceritinib was classified by Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 (19).

Study Population

A database of 3,117 clinical cases of CRC evaluated by CGP in the course of clinical care at Foundation Medicine (Cambridge, MA) was reviewed retrospectively to identify cases harboring ALK gene fusions, somatic mutations, and copy number alterations (amplifications and deletions). All cases were clinically advanced stage III or IV tumors at the time of sequencing. Local site permissions to use clinical samples were obtained for this study. Four previously reported ALK-rearranged CRC cases (12,14,15) were also included for the analysis. One of these cases was identified by comprehensive genomic profiling of 40 CRC cases (12), another case by immunohistochemical (IHC) screening of 1,889 CRC cases at the Department of Anatomical Pathology, Royal North Shore Hospital, Sydney, Australia (14), and two...
cases by IHC screening of 222 CRC cases at the Department of Pathology and Translational Genomics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea (15). For this study, the database of Australian cases was updated by IHC screening of 719 additional CRC cases, and 150 cases were screened at Rhode Island and Miriam Hospitals, Providence, RI. The presence of ALK fusions in all 3 cases identified by IHC was further confirmed by CGP.

**Comprehensive Genomic Profiling**

Hybrid capture-based NGS was performed using DNA extracted from formalin-fixed paraffin-embedded sections cut at 10 µm thickness in a CLIA-certified laboratory (Foundation Medicine). The sample submitted for DNA extraction contained a minimum of 20% DNA derived from tumor cells. DNA sequencing was performed for the entire coding region of 315 cancer-related genes plus introns from 28 genes frequently rearranged in cancer on an indexed, adaptor-ligated, hybridization-captured library and fully sequenced using 49 bp reads (Illumina HiSeq 2500, Hayward, CA) to a median exon coverage of 555X for this specific case. These 315 genes included key genes involved in colorectal carcinogenesis, such as KRAS, BRAF, PIK3CA, PTEN, CTNNB1, APC, MLH1, PMS2, MSH2, MSH6, HER2, ALK, ROS1, and NTRK (full list is available at foundationone.com). Sequence reads were mapped to the reference human genome (hg19) and analyzed for genomic alterations including base substitutions, small insertions and deletions (indels), copy number alterations (amplifications and homozygous deletions), and select gene fusions/rearrangements as previously described (20).
Assessment of MSI by NGS-Based Approach

A novel computational method was developed to assess a sample’s MSI status by examining indel characteristics at 114 homopolymer repeat loci in or near the targeted gene regions of the FoundationOne gene panel. This method utilizes the same CGP sequencing data and eliminates the need for standard pentaplex PCR or IHC thus conserving the tissue. This novel methodology has high sensitivity (95%) and specificity (98%) when compared to standard PCR or IHC and is 97% concordant with these techniques (21).

The 114 loci were selected from a total set of 1,897 microsatellites that have adequate sequence coverage and maximized variability between samples. Each chosen locus was intronic and had a hg19 reference repeat length of 10-20bp. This range of repeat lengths was chosen such that the microsatellites are long enough to produce a high rate of DNA polymerase slippage, while short enough to be well within the 49bp read length of NGS to facilitate alignment to the human reference genome. Using the 114 loci, the repeat length in each read that spans the locus was calculated for each sample. The means and variances of repeat lengths across the reads were recorded, forming 228 data points per sample. In a large training set of data from clinical specimens, the principal components analysis (PCA) was applied to project the 228-dimension data onto a single dimension (the first principal component) that maximizes the data separation, producing an NGS-based “MSI score”. Ranges of the MSI score were assigned as MSI-high (MSI-H), MSI-ambiguous, or microsatellite stable (MSS) by manual unsupervised clustering of specimens for which MSI status was previously assessed either by IHC if available or approximated
by the number of homopolymer indel mutations detected by standard pipeline.

**Histologic Assessment**

The tumor histology in all cases with *ALK* translocations was reviewed by two pathologists (S.M. Ali and E. Yakirevich). In five cases the H&E stained sections were available for microscopic analysis. Digital images of the tumors were reviewed in 4 cases in which the tissue was not available. The histological grade of the tumors was determined according to the WHO classification as well differentiated (grade 1) for those with 5% or less of solid growth, moderately differentiated (grade 2) for those with 6-50% of solid growth and poorly differentiated (grade 3) for those with more than 50% solid growth (22). The proportion of the mucinous component was determined by quantitation of percentage of extracellular mucin in H&E stained slides and calculation of the mean.

**Immunohistochemistry**

Immunohistochemistry was performed on 4-μm formalin-fixed paraffin-embedded tissues sections using a BenchMark Ultra autostainer, version 12.3 (Ventana Medical Systems, Tucson, AZ). Tissue sections were deparaffinized and pretreated in CC1 solution (EDTA, pH9), extended regime. The primary rabbit monoclonal anti-ALK antibody clone D5F3 (Cell Signaling Technology, Beverly, MA) was applied at a 1:50 dilution and the slides were incubated at 37°C for 60 minutes. The UltraView DAB detection kit was used with the amplification kit. Immunohistochemical staining for CDX2 and TTF1 was performed with the Dako Autostainer Link 48 (Dako,
Carpinteria, CA, USA) and EnVision Flex detection system (Dako) with DAB (Dako). The anti-CDX2 mouse monoclonal antibody, clone CDX2-88 (Biogenex, San Ramon, CA, USA), was used at 1:50 dilution. The anti-TTF1 mouse monoclonal antibody, clone SPT24 (Vector Laboratories, Burlingame, CA, USA), was used at a 1:1000 dilution. All cases were evaluated and scored as either positive or negative by two pathologists (S. Mangray and E. Yakirevich).

**Fluorescence in situ hybridization (FISH)**

Fluorescence in situ hybridization (FISH) was performed on 5-μm paraffin sections using the Vysis LSI ALK Dual Color Break Apart rearrangement probe (Abbott Molecular, Abbott Park, IL). FISH analysis was considered positive for ALK rearrangement if >15% of tumor cells (>50 tumor cells counted) showed isolated red signals and/or split red and green signals.
RESULTS

Index Patient Presentation

The patient is an 87-year old woman without significant past medical history who presented three years ago with fatigue, hemoglobin of 8.6 gm/dL and iron deficiency. Colonoscopy revealed a cecal mass and the patient underwent a right hemicolecctomy. Pathologic examination of the specimen revealed a 5.1 cm moderately differentiated adenocarcinoma with a prominent mucinous component that penetrated to the visceral peritoneal surface and involved 7 of 28 lymph nodes (pathologic stage pT4aN2b) (Fig. 1A, B). Analysis of mismatch repair proteins by immunohistochemistry revealed loss of expression of MLH1 and PMS2 with retained expression of MSH2 and MSH6. Staging chest computed tomography (CT) revealed small bilateral pulmonary nodules suspicious for metastatic disease. The patient’s disease was staged clinically as T4N2M1 (stage IV).

The patient received 10 cycles of mFOLFOX6 with resolution of pulmonary nodules. She was without evidence of disease until she presented with peritoneal carcinomatosis 18 months later. The patient subsequently received 8 treatments of FOLFIRI followed by maintenance fluorouracil and leucovorin. She then experienced further peritoneal progression including a metastasis to a periumbilical area that extended through the skin obliterating the umbilicus. mFOLFOX was reinstiuted but after an initial response to 6 cycles of treatment there was further tumor growth. To identify avenues for potential benefit from targeted therapy, archival formalin-fixed paraffin embedded tumor tissue obtained from the colectomy specimen was submitted for hybrid-capture based comprehensive genomic profiling.
utilizing the FoundationOne (Foundation Medicine, Cambridge, MA) assay platform.

**Comprehensive Genomic Profiling, Immunohistochemistry, and FISH**

CGP of the primary tumor sample identified 14 genomic alterations: a *STRN-ALK* fusion (Fig. 1C) and mutations in *KRAS* R164Q, *STK11*, *TP53*, *AEID1A*, *BCOR*, *CDH2*, *MLL2*, *MLL3*, *PAX5*, and *RAD50* genes. Although no *BRAF* mutations were identified, genomic alterations were not detected in genes encoding the mismatch repair proteins *MLH1*, *PMS2*, *MSH2*, and *MSH6*, consistent with a sporadic microsatellite instable (MSI) colorectal cancer. The *KRAS* R164Q mutation present in this case was reported as non-activating and essentially equivalent to wild type *KRAS* status (23).

A *STRN-ALK* fusion involved the intrachromosomal translocation of exons 1-3 of *STRN* to exons 20-29 of *ALK* (Fig. 1D) within the short arm of chromosome 2 (2p22.2 and 2p23, separated by ~ 7.69 Mb), consistent with an intrachromosomal paracentric rearrangement. The predicted fusion protein does not have a calmodulin-binding domain and the WD-repeats, but retains the N-terminal caveolin-binding and the coiled-coil binding domains of *STRN*, fused to the intracellular juxtamembrane region of *ALK*, containing the kinase domain (Fig. 1D).

Immunohistochemical staining of the primary tumor demonstrated diffuse intracytoplasmic immunoreactivity with the anti-ALK antibody (Fig. 1E). This staining pattern confirmed the loss of ALK extracellular and intramembrane domains as detected by sequencing, and thus the positioning of the fusion protein in the intracellular compartment. FISH analysis also showed the presence of *ALK* gene rearrangement in 74% of the tumor cells (Fig. 1F).
Clinical Response of the Patient with *STRN-ALK* Fusion and Development of Resistance to Ceritinib

Based on the *STRN-ALK* fusion, the patient was treated with ceritinib, an orally available ALK inhibitor, 750 mg/day, on the Novartis Signature Trial. The tumor metastasis involving periumbilical skin before ceritinib therapy is shown in Fig 2A. This periumbilical cutaneous metastasis significantly decreased in size within 7 weeks of treatment (Fig. 2B). After an additional 2 weeks of treatment there was marked decrease in periumbilical thickening (Fig. 2C). At 6 months follow-up the mass protruding through the skin could no longer be seen (Fig. 2D). Although the size of the pelvic tumor by CT scan did not change (deemed stable disease by RECIST), there has been resolution of all contrast enhancing tumor by CT scan (Fig. 2E-F). The treatment was accompanied by a clinical benefit with the disappearance of tumor-related abdominal and periumbilical pain. However, after 9 months of treatment the disease progressed with increase in size of existing masses and the development of new contrast enhancing peritoneal implants and ascites consistent with progressive peritoneal carcinomatosis (Fig. 2G-H). At that time, the patient underwent a biopsy of a periumbilical metastasis, and post-targeted therapy CGP was performed which now identified a new *KRAS* G13D mutation in addition to the *STRN-ALK* fusion.

Molecular and Clinicopathologic Features of a Subset of Colorectal Carcinoma with *ALK* Fusions
To further define the frequency and molecular and clinicopathologic features of CRC harboring ALK fusions, we identified 5 additional ALK-rearranged CRC, including 4 previously unreported cases identified by CGP in a retrospective analysis of 3,157 relapsed and refractory clinical cases of CRC originally evaluated at Foundation Medicine (estimated incidence 0.2%). Three previously published cases (14,15) that were identified by IHC (estimated incidence 0.1%) and further confirmed by CGP were also included in the analysis. The colorectal origin of the tumors was confirmed by an immunohistochemical staining pattern with positivity for CDX2, a marker of intestinal differentiation, and negative staining for TTF-1, a marker of pulmonary differentiation.

The ALK gene rearrangements in the series of 9 cases, including the current case involved known ALK fusion partners, such as EML4 (in two cases), C2orf44, CAD, as well as the novel STRN, PPP1R21, SENPF, MAPRE3, and PRKAP1B fusion partners reported here for the first time (Table 1, Figure 3). All ALK fusions were in-frame starting at the canonical exon 20 recombination site as has been previously reported in other cancers (10). Intrachromosomal paracentric translocations involving fusion partners EML4, STRN, C2orf44, CAD, PPP1R21, and MAPRE3 were the most common type of chromosomal rearrangements. In two cases, interchromosomal reciprocal translocations involved CENPF gene on chromosome 1, and PRKAR1B gene on chromosome 7. None of the ALK-rearranged tumors harbored mutations in other oncogenic drivers, including EGFR, KRAS, BRAF, and ERBB2 as well as in other kinase fusions such as ROS1, RET, and NTRK (Table 1).

This cohort of ALK-driven CRC included 6 females and 3 males with a mean
age of 61 (range 43 to 87) (Table 2). Eight patients had stage IV disease with metastases to liver and lung, and one patient had locally advanced tumor at the time of CGP. Eight (89%) of the \textit{ALK} fusion positive CRC had a site of origin in the proximal colon, while one (11%) originated in the rectum. Three (33%) of the 9 cases tested positively for MSI with loss of MLH1 and PMS2 mismatch repair proteins by immunohistochemistry. The MSI status for these 3 cases was further confirmed by an NGS-based test at 114 microsatellite loci.

Four tumors, including 2 with MSI exhibited mucinous differentiation, one MSI case had medullary carcinoma features (patient 8), one had signet ring cell features (patient 9), and the remainder were moderately differentiated adenocarcinomas, with morphology typical of primary CRC. Four of 5 (80%) \textit{ALK} rearranged CRCs with tissue available for further assays were ALK positive by IHC, while all 5 cases (100%) were \textit{ALK} rearranged by FISH.

**Non-Fusion \textit{ALK} Genomic Alterations in Colorectal Carcinoma**

In addition to \textit{ALK} fusions, we retrospectively analyzed a database of 3,117 clinical cases of CRC evaluated by CGP at Foundation Medicine for other \textit{ALK} genomic alterations including point mutations and copy number alterations.

Somatic point mutations in \textit{ALK} were identified in 10 cases (0.3%) (Supplementary Table 2). All mutations were non-synonymous base substitutions. Three mutations (T1151M, A1200V, and R1209Q) involved the intracellular tyrosine kinase domain. One recurrent mutation R401* involving the ligand-binding
domain was identified in 3 cases. The remaining two mutations (recurrent A585T and V757M) did not involve critical domains.

Gene amplifications at the $ALK$ locus were observed in 2 cases (0.06%) (Supplementary Table 2). Twenty five of the 29 $ALK$ exons gained 8 gene copies. No $ALK$ deletions were present.
DISCUSSION

This is the first report of treatment and response to an ALK inhibitor in a patient with ALK-rearranged CRC and a detailed analysis of molecular and clinicopathologic characteristics of the largest series of CRC harboring ALK fusions to date.

The ALK gene encodes a receptor tyrosine kinase protein with a crucial role in the pathogenesis of various hematologic malignancies and solid tumors. Since discovery of ALK as a gene target of the t(2;5) chromosomal translocation in anaplastic large cell lymphoma in 1994, ALK gene alterations, including gene fusions involving the ALK locus at 2p23, point mutations, and gene amplifications have been described in 18 different tumor types (8,9). The term “ALKoma” was suggested in order to unify these various tumor types arising in different organs but sharing ALK oncogenic activation as an essential tumor growth driver (24). Among epithelial tumors, ALK gene rearrangements are most common in lung carcinomas, varying from 4 to 7%, and rare in kidney, breast, colorectal, esophageal, thyroid, ovarian, and bladder carcinomas (8,9).

Our findings confirm that ALK fusions are rare in CRC patients with a frequency of only 0.2% in a large retrospective series of CRC assayed by CGP. Lower detection rates were observed by immunohistochemical screening of CRC (0.1%) indicative of a higher sensitivity of CGP in identification of ALK fusions in CRC. Although the frequency of ALK fusions in CRC is low, the incidence of 0.2% translates into approximately 265 patients in the United States each year and approximately 2,800 patients worldwide each year. The relatively broad range of ALK fusion frequencies in colorectal cancer varying from 0.13% to 2.5% described
in previous studies (Supplementary Table 1) may be related to different patient cohorts analyzed, in some studies limited to 40 cases, and different testing methodologies including IHC, FISH, real-time PCR, exon array, comprehensive genomic profiling, and transcriptome analysis (6,7,11-15). In contrast, in this study ALK rearrangements were identified in a cohort of 3,157 CRC patients employing CGP and cohort of 2,980 CRC patients by IHC. It has yet to be determined what screening test should be employed clinically in order to identify potential candidates for treatment with ALK inhibitors. Currently, detection of ALK rearrangements is established as a routine clinical test in cases of NSCLC. Screening for ALK fusions or ALK protein will help to identify potential candidates for ALK inhibitor therapy in CRC.

In addition to ALK rearrangements we found somatic ALK point mutations in 0.3% of cases and very rare ALK amplifications in 0.06% of CRC cases. Three of these mutations were located within the tyrosine kinase domain and potentially may alter tyrosine kinase activity of ALK. Of note, one of these mutations, T1151M, was previously reported in neuroblastoma and was associated with modest constitutive activation of the tyrosine kinase domain (25,26). Based on the data available from large scale cancer genomics datasets, including TCGA, Genentech, and MSKCC, the rate of ALK mutations in CRC cases is estimated as 5.5%, 11.1%, and 2.2%, respectively (5,27,28). Although these frequencies are higher than in this report, ALK mutations involving the tyrosine kinase domain were present in only one CRC case from the TCGA dataset (0.4%), and none of the Genentech or MSKCC cases. Therefore, the rate of clinically significant ALK mutations in CRC is unclear
and needs to be determined in additional studies.

Our data provide additional detailed molecular characterization of ALK-rearranged CRC. In contrast to previous reports addressing ALK alterations only, we sequenced all exons of a broad panel of 315 cancer-related genes and 28 genes frequently rearranged in cancer (20). The lack of mutations in known oncogenic drivers, including KRAS, BRAF, EGFR, and ERBB2 indicate that ALK fusions are indeed driving oncogenic events in CRC and support the study of monotherapy with ALK inhibitors targeting ALK fusion products in CRC.

Ceritinib (LDK378, Zykadia, Novartis Pharmaceuticals) is an orally administered, small-molecule, ATP-competitive, second generation ALK inhibitor (18). In the preclinical setting, ceritinib is 20 times as potent as crizotinib against ALK (18). In addition to ALK inhibitor activity, both crizotinib and ceritinib inhibit ROS1. In contrast to crizotinib, ceritinib does not inhibit kinase activity of MET, a tyrosine kinase that can be activated in NSCLC but does inhibit insulin-like growth factor 1 receptor (IGF-1R), and insulin receptor (InsR) (18). In a phase 1 study ceritinib was highly effective in patients with advanced ALK-rearranged NSCLC, with an overall response rate of 58% (29). Moreover, responses were observed in patients with various resistance mutations in ALK acquired during crizotinib treatment (29).

The patient’s clinical protocol did not include a tumor biopsy which would have been required to demonstrate loss of ALK related activity within the tumor following the treatment with ceritinib. Since ceritinib may also target IGF-1R, it is possible that some of the clinical benefit could have been related to IGF-1R.
inhibition. Patel et al have observed that colon cancer cells surviving chemotherapy are enriched in colon cancer stem cells that express increased levels of IGF-1R (30). However in vitro effects of IGF-1R agents appear to have, at best, static effects in colon cancer cell lines as reported by Garcia-Carbonero et al (31). Furthermore as reported by Cutsem et al, the addition of an IGF-1R inhibitor ganitumab to the EGFR inhibitor panitumumab did not increase response rate in patients with advanced colon cancer as compared to panitumumab alone (32). Therefore, it is unlikely the marked clinical effect observed in the case was due to ceritinib inhibition of IGF-1R. Further confirmation of the driving oncogenic properties of the ALK fusion observed in this case is the development of resistance to ceritinib accompanied by acquisition of a KRAS oncogenic mutation. The early clinical and radiographic response to ceritinib in this case is similar to rapid responses observed to ceritinib therapy in lung cancer as is the chronology of acquired resistance (18,29). This effect represents a major limitation of its long term clinical benefit and requires development of new therapeutic strategies to overcome the resistance.

Importantly, this finding is one of the few, initial descriptions of benefit from targeted monotherapy for advanced CRC. The failure of vemurafenib treatment for BRAF V600E CRC has been held up as a counter-example to the success of targeted therapy (33). Recent data indicating benefit from dabrafenib and trametinib for these patients highlights the potential benefit of combination targeted therapy for CRC (34). However, the index case presented here suggests a sufficiently potent targeted therapy may be effective as monotherapy in advanced CRC with a targetable oncogenic driver, such as a kinase fusion. This is borne out by
the recent report of an NTRK fusion expressing CRC responding to entrectinib, a specific NTRK inhibitor (35), and hinted at in a report of a RET fusion CRC (36).

In this case an ALK fusion event involving the STRN gene was detected for the first time in CRC. This fusion leads to constitutive activation of ALK kinase via dimerization mediated by the coiled-coil domain of STRN, transforms cells in vitro, and induces tumor formation in nude mice (37). In addition, STRN-ALK driven cells are sensitive to ALK inhibitors (37). STRN-ALK fusions have been reported most frequently in thyroid carcinoma patients, but were also identified in lung carcinoma and papillary renal cell carcinoma (6,37). Interestingly, similar to this case, thyroid tumors harboring STRN-ALK present with aggressive features, such as extrathyroidal extension and lymph node metastasis (37,38). In this study seven additional ALK fusion partners were identified, including 4 novel ALK fusion partners PPP1R21, SENPF, MAPRE3, and PRKAP1B, which add to the growing list of ALK fusion partners in CRC.

This study provides important information on distinct clinical features of ALK-rearranged subtype of CRC. All of these tumors are high-stage adenocarcinomas, most with distant metastases to liver and lung. This subtype has predilection to right colon, and often shows mucinous differentiation and MSI phenotype, as seen also in the index case. MSI CRC are found in 10-15% and usually exhibit an indolent clinical behavior and are less prevalent in more advanced stages of the disease, accounting for <5% in the metastatic setting (5,39). Identification of ALK-rearranged subtype of MSI CRC may define a distinct aggressive subtype of MSI CRC, which may benefit from targeted therapy with ALK inhibitors. Of note, analysis
of 151 cell lines identified one MSI cell line harboring *EML4-ALK* fusion gene (7). In a preclinical study cell proliferation of this cell line was inhibited by RNAi knockdown and ALK inhibitor crizotinib. Moreover, crizotinib treatment downregulated MAPK and PI3K pathways in this cell line (7). This observation also raises the possibility that combination treatment with an ALK inhibitor and a checkpoint inhibitor in this subset of CRC might results in more durable benefit (40).

In summary, this is the first case of a patient with advanced metastatic colorectal cancer harboring an oncogenic *ALK* gene fusion who achieved durable benefit following treatment with a tyrosine kinase inhibitor ceritinib. CRC harboring *ALK* fusions represent a rare aggressive subtype of CRC that may be responsive to ALK inhibitors.
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Table 1. Molecular alterations in clinical cases of CRC harboring ALK fusions

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<th>ALK fusion partner</th>
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<td>PRKAR1B</td>
<td>CTNNB1</td>
<td>GNAS (7), AURKA (7), ZNF217 (7), BCL2L1 (7), TOP1 (7)</td>
</tr>
<tr>
<td>6</td>
<td>609</td>
<td>23</td>
<td>PPP1R21</td>
<td>CTNNB1</td>
<td>GATA6</td>
</tr>
<tr>
<td>7</td>
<td>453</td>
<td>7</td>
<td>CAD</td>
<td>CTNNB1</td>
<td>GATA6</td>
</tr>
<tr>
<td>8</td>
<td>507</td>
<td>3</td>
<td>EML4</td>
<td>CTNNB1</td>
<td>GATA6</td>
</tr>
</tbody>
</table>

Table 2. Clinicopathologic characteristics of CRC patients harboring ALK fusions

<table>
<thead>
<tr>
<th>Patient</th>
<th>ALK Fusion partner</th>
<th>Age</th>
<th>Sex</th>
<th>Primary site</th>
<th>Tumor size, cm</th>
<th>Grade</th>
<th>Stage*</th>
<th>Metastases</th>
<th>MSI</th>
<th>Mucinous</th>
<th>ALK IHC</th>
<th>ALK FISH</th>
<th>RFS (mo)</th>
<th>OS (mo)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>STRN</td>
<td>84</td>
<td>F</td>
<td>Cecum</td>
<td>5.1</td>
<td>2</td>
<td>IV</td>
<td>Lung, Umbilicus Liver</td>
<td>MSI 30%</td>
<td>Pos (74%)</td>
<td>NA</td>
<td>18</td>
<td>AWD 39</td>
<td>NA</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>SENPF</td>
<td>62</td>
<td>M</td>
<td>Proximal transverse Ascending</td>
<td>7.0</td>
<td>2</td>
<td>IV</td>
<td>Lung, Umbilicus Liver</td>
<td>MSI 0%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>MAPRE3</td>
<td>43</td>
<td>F</td>
<td>Ascending</td>
<td>3.5</td>
<td>2</td>
<td>IV</td>
<td>Lung</td>
<td>MSI 0%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>EML4</td>
<td>53</td>
<td>F</td>
<td>Right colon</td>
<td>NA</td>
<td>2</td>
<td>IV</td>
<td>Omentum, peritoneum Liver</td>
<td>MSI 60%</td>
<td>Neg (69.5%)</td>
<td>Pos</td>
<td>0</td>
<td>DOD 3</td>
<td>17.6</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>PRKAR1B</td>
<td>55</td>
<td>M</td>
<td>Right colon</td>
<td>NA</td>
<td>2</td>
<td>IV</td>
<td>Omentum, peritoneum Liver</td>
<td>MSI 70%</td>
<td>Pos (64%)</td>
<td>Pos (66%)</td>
<td>8</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>C2orf44</td>
<td>58</td>
<td>F</td>
<td>Ascending</td>
<td>6.1</td>
<td>2</td>
<td>IV</td>
<td>Omentum, peritoneum Liver</td>
<td>MSI 70%</td>
<td>Pos (64%)</td>
<td>Pos (66%)</td>
<td>1</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>PPP1R21</td>
<td>87</td>
<td>F</td>
<td>Distal transverse Rectum</td>
<td>4.8</td>
<td>2</td>
<td>II</td>
<td>No</td>
<td>MSI 70%</td>
<td>Pos (64%)</td>
<td>Pos (66%)</td>
<td>1</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>CAD</td>
<td>46</td>
<td>F</td>
<td>Ascending</td>
<td>6.5</td>
<td>3</td>
<td>IV</td>
<td>Lung, pericardium Liver</td>
<td>MSI 40%</td>
<td>Pos (66%)</td>
<td>Pos (66%)</td>
<td>1</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>EML4</td>
<td>65</td>
<td>M</td>
<td>Ascending</td>
<td>4.0</td>
<td>3</td>
<td>IV</td>
<td>Lung, pericardium Liver</td>
<td>MSI 40%</td>
<td>Pos (66%)</td>
<td>Pos (66%)</td>
<td>1</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MSI, microsatellite instable, MSS, microsatellite stable; IHC, immunohistochemistry; FISH, fluorescence in-situ hybridization; Pos, positive; Neg, negative; NA, not
available; RFS, relapse free survival; OS, overall survival; mo, months; AWD, alive
with disease; DOD, death of disease, LTF, lost to follow up
*Previously reported cases with material available for pathologic review and
additional testing
**Stage is provided at the time of comprehensive genomic profiling
FIGURE LEGENDS

Figure 1. Histologic, Molecular, and Immunohistochemical Analyses of the Tumor. 
A, Gross image of a 15.1 cm cecal tumor. B, A Representative histologic section of the tumor shows moderately differentiated adenocarcinoma containing glands exhibiting prominent infoldings, pseudopapillary structures, and a prominent mucinous component (hematoxylin and eosin). C, Screenshot of Integrative Genomics Viewer demonstrating uniquely mapped paired-end reads in the intronic regions of STRN (Intron 3) and ALK (Intron 19) on chromosome 2. D, Schematic representation of the STRN-ALK fusion of the N-terminal portion of STRN and C-terminal portion of ALK. Exons 1-3 of STRN containing the caveolin-binding domain (CB) and coiled-coil domain (CC) are shown in blue. Exons 20-29 of ALK containing the tyrosine kinase domain (TK) are shown in orange. Both fusion partners are located on the short arm of chromosome 2 (2p22.2 and 2p23, separated by ~ 7.69 Mb), indicating that the fusion is a result of intrachromosomal paracentric rearrangement. The coiled-coil STRN domain may act as a dimerization motif that could constitutively activate ALK tyrosine kinase. E, Immunohistochemistry with the D5F3 anti-ALK antibody showing strong diffuse intracytoplasmic immunoreactivity confirming ALK overexpression as a result of STRN-ALK fusion. The loss of ALK extracellular and intramembrane domains detected by sequencing is associated with intracellular localization of the fusion protein. F, Dual-color break-apart fluorescence in situ hybridization (FISH) analysis of the tumor cells performed with a 5’ ALK probe (green) and 3’ ALK probe (red). Single isolated red
probe signals (arrow) indicate the ALK chromosomal rearrangement. An unsplit red and green probe signals indicate the non-rearranged wild type ALK locus (arrowhead).

**Figure 2.** Umbilical Skin Metastasis and Imaging Studies Before and After Ceritinib Treatment. **A,** A pretreatment photograph shows the tumor metastasis involving periumbilical skin. **B,** Skin metastasis significantly decreased within 7 weeks of treatment with ceritinib. **C,** After an additional 2 weeks of treatment there is marked decrease in periumbilical thickening. **D,** At 6 months follow-up the mass protruding through the skin resolved, leaving a scar. **E,** Pretreatment CT scan shows large pelvic tumor mass. **F,** Six months after ceritinib therapy a CT scan demonstrates resolution of all contrast-enhancing tumor. **G,** Nine months after ceritinib therapy the periumbilical metastasis increased in size. **H,** CT scan at 9 months revealing peritoneal carcinomatosis and enhancement of the tumor.

**Figure 3.** Putative ALK fusion diagrams for each patient based on DNA sequencing data; all fusions include the ALK tyrosine kinase domain. Genomic partner is on the left and ALK is on the right. Inter chromosomal partners are displayed in orange, intra chromosomal partners in yellow, ALK in green, protein domains in purple. Arrows indicate the direction of transcription for each gene. Previously reported cases are indicated by asterisks. 1) *STRN-ALK* fusion containing a portion of the *STRN* Striatin domain. 2) *CENPF-ALK* fusion. 3) *MAPRE3-ALK* fusion containing the MAPRE3 Calponin homology domain and EB1-like C-terminal motif. 4) *EML4-ALK* fusion. 5) *PRKAR1B-ALK* fusion containing the PRKAR1B Regulatory subunit of type
II PKA R-subunit (RIIa). 6) C2orf44-ALK fusion. 7) PPP1R21-ALK fusion. 8) CAD-ALK fusion. 9) EML4-ALK fusion containing the EML4 Hydrophobic EMAP-Like Protein motif and a portion of the WD40 domain.
Oncogenic \textit{ALK} Fusion in Rare and Aggressive Subtype of Colorectal Adenocarcinoma as a Potential Therapeutic Target

Evgeny Yakirevich, Murray B Resnick, Shamlal Mangray, et al.

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