



## Detection of an *ALK* Fusion in Colorectal Carcinoma by Hybrid Capture-Based Assay of Circulating Tumor DNA

ANDREA Z. LAI,<sup>a</sup> ALEXA B. SCHROCK,<sup>a</sup> RACHEL L. ERLICH,<sup>a</sup> JEFFREY S. ROSS,<sup>a</sup> VINCENT A. MILLER,<sup>a</sup> EVGENY YAKIREVICH,<sup>b</sup> SIRAJ M. ALI,<sup>a</sup> FADI BRAITEH<sup>c,d</sup>

<sup>a</sup>Foundation Medicine, Inc., Cambridge, Massachusetts, USA; <sup>b</sup>Department of Pathology, Rhode Island Hospital, Alpert Medical School, Brown University, Providence, Rhode Island, USA; <sup>c</sup>Comprehensive Cancer Centers of Nevada, Las Vegas, Nevada, USA; <sup>d</sup>University of Nevada School of Medicine, Reno, Nevada, USA

Disclosures of potential conflicts of interest may be found at the end of this article.

**Key Words.** Colorectal cancer • *ALK* fusion • Circulating tumor DNA • Genomic profiling

### ABSTRACT

*ALK* rearrangements have been observed in 0.05%–2.5% of patients with colorectal cancers (CRCs) and are predicted to be oncogenic drivers largely mutually exclusive of *KRAS*, *NRAS*, or *BRAF* alterations. Here we present the case of a patient with metastatic CRC who was treatment naïve at the time of molecular testing. Initial *ALK* immunohistochemistry (IHC) staining was negative, but parallel genomic profiling of

both circulating tumor DNA (ctDNA) and tissue using similar hybrid capture-based assays each identified an identical *STRN-ALK* fusion. Subsequent *ALK* IHC staining of the same specimens was positive, suggesting that the initial result was a false negative. This report is the first instance of an *ALK* fusion in CRC detected using a ctDNA assay. *The Oncologist* 2017;22:774–779

### KEY POINTS

- Current guidelines for colorectal cancer (CRC) only recommend genomic assessment of *KRAS*, *NRAS*, *BRAF*, and microsatellite instability (MSI) status.
- *ALK* rearrangements are rare in CRC, but patients with activating *ALK* fusions have responded to targeted therapies
- *ALK* rearrangements can be detected by genomic profiling of ctDNA from blood or tissue, and this methodology may be informative in cases where immunohistochemistry (IHC) or other standard testing is negative.

### PATIENT STORY

A 62-year old female with a previously negative screening colonoscopy in November 2013 presented in 2016 with vague pelvic pain worsening over a 2-week period and new onset constipation and colic, but with no rectal bleeding. Initial examination and blood work were nonspecific except for elevated carcinoembryonic antigen (CEA) (205 [0.5–3.0]). Pelvic examination by her gynecologist revealed a friable nodule in the vagina, which was biopsied and found to be consistent with an invasive adenocarcinoma. Computed tomography scan of the abdomen and pelvis identified a circumferential narrowing and wall thickening of the hepatic flexure. Additionally, there were suspicious metastatic liver nodules and mesenteric lymph nodes. A colonoscopy confirmed a near obstructive malignant appearing lesion of the hepatic flexure, confirmed histologically to be a colon primary. The patient's family cancer history was negative, except for her mother who had more than 20 polyps removed in her lifetime. The

patient was referred to medical oncology for management of metastatic colon cancer.

The vaginal biopsy tissue specimen was submitted for comprehensive genomic profiling (CGP) (FoundationOne; Foundation Medicine, Cambridge, MA, <http://www.foundationmedicine.com>). In parallel, a blood sample was also submitted for genomic profiling using a Clinical Laboratory Improvement Amendments (CLIA)-validated circulating tumor DNA (ctDNA) assay (FoundationACT, Foundation Medicine, Cambridge, MA, <http://www.foundationmedicine.com>). The results of these genomic profiling assays, specifically the presence of an oncogenic *ALK* fusion detected by both genomic assays, its implication as a driver in this case, and the therapeutic implications, are reviewed in this article.

Given the presence of primary right-sided disease, and despite the absence of *RAS* and *RAF* mutation, her oncologist opted for a bevacizumab-based regimen with curative intent

Correspondence: Andrea Z. Lai, Ph.D., Foundation Medicine, Inc., 150 Second Street, Cambridge, Massachusetts 02141, USA. Telephone: +1 (617) 418-2200; e-mail: [Alai@foundationmedicine.com](mailto:Alai@foundationmedicine.com) Received September 30, 2016; accepted for publication February 17, 2017; published Online First on May 15, 2017. ©AlphaMed Press 1083-7159/2017/\$20.00/0 <http://dx.doi.org/10.1634/theoncologist.2016-0376>

**Table 1.** Known *ALK* fusions in colorectal cancer

Case #	<i>ALK</i> fusion	Frequency of <i>ALK</i> rearrangement	Method of Detection	Reference
1	<i>C2orf44-ALK</i>	1/40 (2.5%)	CGP	[11]
2,3	<i>EML4-ALK</i> <i>SPTBN1-ALK</i>	2/457 (0.44%)	IHC, FISH, qPCR	[14]
4	<i>CAD-ALK</i>	1/742 (0.13%)	IHC, FISH	[15, 16]
5,6	<i>CAD-ALK</i> <i>EML4-ALK</i>	1/172 (0.6%) 1/50 (2%)	IHC, FISH, CGP	[17]
7	<i>EML4-ALK</i>	1/236 (0.4%)	FISH, RT-PCR	[18]
8,9	<i>EML4-ALK</i>	2/83 (2.4%)	RT-PCR, FISH	[19]
10	<i>SMEK2-ALK</i>	1/377 (0.26%)	RNA-Seq	[20]
11	<i>PPP1R21-ALK</i>	1/1889 (0.05%)	IHC, FISH	[12, 13]
12–16	<i>STRN-ALK</i> <i>SENPF-ALK</i> <i>MAPRE3-ALK</i> <i>EML4-ALK</i> <i>PRKAR1B-ALK</i>	5/3157 (0.2%)	CGP, IHC	[12]

Abbreviations: CGP, comprehensive genomic profiling; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; qPCR, quantitative polymerase chain reaction; RNA Seq, RNA sequencing; RT-PCR, reverse transcription polymerase chain reaction.

for this oligometastatic case. After 3 months (six cycles) of modified leucovorin calcium (folinic acid), fluorouracil, and oxaliplatin (FOXLOFOX-6) plus bevacizumab, CEA dropped from 203 ng/mL to 4 ng/mL (normal range 0–3 ng/mL), and the patient had significant pain relief and normalization of bowels. Repeat scans performed in October 2016 showed a partial response per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria with complete resolution of liver metastases. She did undergo a synchronous right hemicolectomy (with residual moderately differentiated adenocarcinoma ypT3, N0; 14 lymph nodes dissected) and right hepatic trisegmentectomy (ypT0, complete response). The vaginal cuff excision showed fibrosis with treatment effect but no residual malignancy. No other pelvic or vaginal disease was identified during intraoperative pelvic exploration. The patient completed six postoperative chemotherapy cycles for a total of 12 cycles.

At the time of publication, the patient has no evidence of disease (NED). Based on the results of genomic profiling, targeted therapy with an *ALK* kinase inhibitor may be pursued at progression.

### MOLECULAR TUMOR BOARD

Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer-related death in the U.S.; in 2016, an estimated 134,490 new cases of CRC are predicted to be diagnosed. Genome-wide molecular analyses have identified drivers of CRC, including tumors with high microsatellite instability (MSI), as well as frequently mutated oncogenes, such as *KRAS*, *PIK3CA*, *NRAS*, and *BRAF* [1, 2]. While mutation of *KRAS*, *NRAS*, and *BRAF* are associated with poor response to anti-EGFR monoclonal antibodies [3–5], approximately 40% of patients that do not respond to anti-epidermal growth factor receptor (EGFR) therapies are *KRAS*, *NRAS*, and *BRAF* wild-type [6], suggesting a role for other oncogenic drivers.

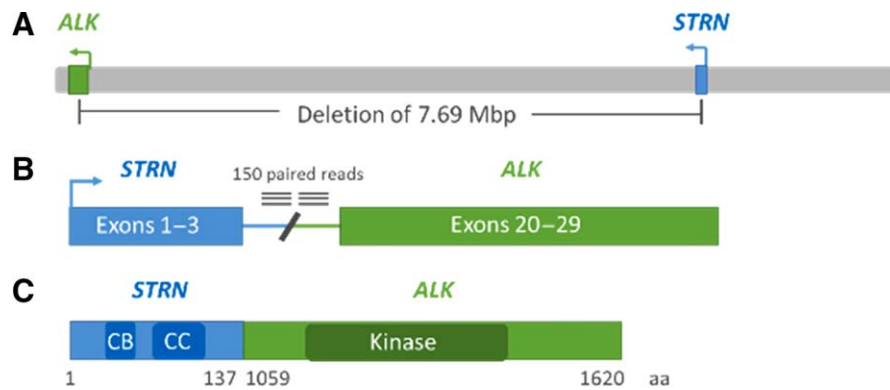
Alterations in receptor tyrosine kinases (RTKs), such as mutations, amplification, and activating rearrangements, have been reported in 2%–7% of CRCs, which suggests that these alterations may be targetable in CRC [7–10]. Rearrangements involving the *ALK* RTKs are rare in patients with CRC, with

reported frequencies of 0.05%–2.5% [11–13]. To date, *ALK* fusions have been reported in 16 cases of CRC, with various fusion partners, including *EML4*, *SPTBN1*, *CAD*, *SMEK2*, *STRN*, *SENPF*, *MAPRE3*, *PRKAR1B*, *C2orf44*, and *PPP1R21* (Table 1) [11, 12, 14–20]. Consistent with the genomics of other CRCs with RTK rearrangements [21], patients with *ALK*-rearranged CRC do not frequently exhibit co-occurring mutations in other oncogenic drivers such as *KRAS*, *BRAF*, *EGFR*, and *ERBB2* [12]; therefore, *ALK*-targeted therapies may provide clinical benefit for this patient population. Indeed, responses to *ALK*-targeted therapies have been reported in two CRC patients with *ALK* fusions [12, 15]. In this case study, we report the first instance of an *ALK* fusion in CRC detected through genomic profiling of ctDNA from a blood specimen.

### GENOTYPING RESULTS AND GENERAL INTERPRETATION

For the CLIA-validated ctDNA assay (FoundationACT), 50–100 ng ctDNA was extracted from plasma, which was isolated by a double spin protocol from two 10 mL aliquots of peripheral blood collected in cell-free DNA blood collection tubes. Adapted sequencing libraries were generated prior to hybrid capture and sample-multiplexed sequencing on an Illumina HSQ2500 (Illumina Inc, San Diego, CA, <http://www.illumina.com>). The FoundationACT ctDNA test covers 62 genes to 5000x unique coverage and utilizes proprietary algorithms to call alterations at low allele frequencies (0.1% for substitutions, 0.5% for indels and rearrangements, and 20% for copy-number amplifications). The CLIA-validated tissue-based CGP assay (FoundationOne) was performed on DNA extracted from an FFPE sample as described previously [22].

The ctDNA assay identified an intrachromosomal deletion resulting in a *STRN-ALK* fusion. The rearrangement produces a fusion of *STRN* exons 1–3, encoding the coiled-coil domain, and *ALK* exons 20–29, encompassing the kinase domain (Fig. 1). Dimerization by the *STRN* coiled-coil domain results in constitutive *ALK* kinase activation and cell transformation [23]. The initial vaginal sample was submitted for tissue-based CGP (FoundationOne) and it confirmed the presence of the *STRN-ALK* fusion (Table 2). Both tests were wild-type for *RAS* and



**Figure 1.** Schematic representation of the *STRN-ALK* fusion event detected in ctDNA. **(A):** *STRN* (2p22.2) and *ALK* (2p23.2) are both located on the short arm of chromosome 2. An intrachromosomal deletion of approximately 7.69 Mbp results in **(B)** the fusion of *STRN* exons 1–3 to *ALK* exons 20–29. There were 150 paired reads that mapped to the intronic breakpoint. **(C):** The predicted fusion protein includes the *STRN* cavaeolin binding domain (CB) and the coiled-coil domain (CC), of which the latter is predicted to promote constitutive dimerization and activation of the *ALK* tyrosine kinase domain.

**Table 2.** Results of molecular diagnostic assays

FoundationACT ctDNA assay	FoundationOne tissue assay	Additional Testing
<i>ALK STRN-ALK</i> fusion	<i>ALK STRN-ALK</i> fusion	PD-1 low positivity (tumor infiltrating lymphocytes)
<i>TP53</i> R175H	<i>TP53</i> R175H	PD-L1 low positivity (tumor cells)
<i>MYD88</i> L265P	<i>CDK6</i> amplification <i>RNF43</i> * splice site 450 + 2T>C	
Additional findings	<i>Microsatellite status</i> <sup>a</sup> MS-Stable <i>Tumor Mutational Burden</i> <sup>a</sup> TMB-intermediate (10.38 mut/Mb)	

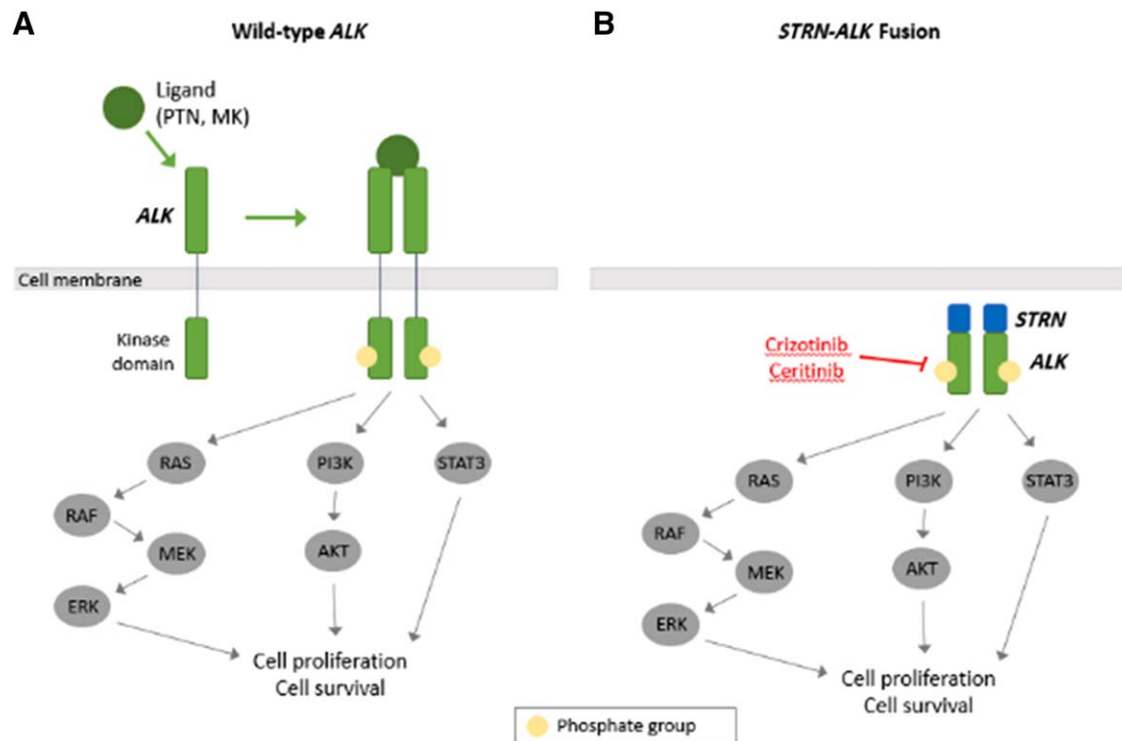
<sup>a</sup>These alterations are not assayed by FoundationACT.

*BRAF* alterations. However, initial immunohistochemistry (IHC) staining of a second vaginal biopsy collected 2 weeks later with the VENTANA *ALK* (D5F3) CDx assay (Ventana Medical Systems, Inc., Tucson, AZ, <http://www.ventana.com>) was *ALK*-negative. Both vaginal biopsies were subsequently restained by an outside lab with the same *ALK* antibody (D5F3) using Ventana BenchMark Ultra autostainer, version 12.3 (Ventana Medical Systems, Inc., Tucson, AZ, <http://www.ventana.com>). The initial biopsy, which was utilized for tissue-based CGP, was strongly diffusely positive for *ALK* (supplemental online Fig. 1A). The second biopsy was also positive (supplemental online Fig. 1B), suggesting that the initial IHC result was a false negative. A panel evaluating 17 genes associated with hereditary colorectal cancer was also performed using ColoNext (Ambry Genetics, Aliso Viejo, CA, <http://www.ambrygen.com/tests/colonext>), but it did not include *ALK* and was negative for genomic alterations. Additional findings using tissue-based CGP confirmed the presence of the *STRN-ALK* fusion and also indicated that this patient's tumor was microsatellite stable, but that it had an intermediate tumor mutational burden (10.38 mutations/megabase). Immunohistochemistry staining showed low positivity (1+ or 2+ staining intensity in 1%–24% distribution) for PD-1 in tumor infiltrating lymphocytes, using the PD-1 (NAT105) mouse monoclonal antibody (Cell Marque, Rocklin, CA, <http://www.cellmarque.com>). Low positivity (1+ or 2+ staining

intensity in 1%–24% distribution) for PD-L1 in tumor cells was also observed via IHC staining with the PD-L1/CD274 (SP142) rabbit monoclonal antibody (Spring Bioscience, Pleasanton, CA, <http://www.springbio.com>). The patient was started on mFOLFOX-6 and bevacizumab as front-line therapy, in part due to limited availability of *ALK* inhibitor trials for which the patient was eligible at presentation.

#### SIGNIFICANCE OF THE SPECIFIC MUTATION IN THE PARTICULAR CANCER

Current guidelines for patients who present with metastatic colorectal cancer recommend broad genomic assessment of *KRAS*, *NRAS*, *BRAF*, and MSI status, and first-line treatment with chemotherapeutic agents (with or without panitumumab or cetuximab, depending on *KRAS/NRAS* status) [24]. Based on a frequency of RTK alterations in CRC of 2%–7% [7, 21], approximately 2,700–9,400 newly diagnosed CRC patients in the U.S. with potentially actionable oncogenic drivers would be predicted to be missed by standard testing in 2016, including approximately 100–3,400 CRC patients with *ALK* rearrangements alone. Clinical data from CRCs with activating *ERBB2* alterations are encouraging, with clinical benefit reported in multiple patients, including a 30% overall response rate to a combination of trastuzumab and lapatinib in patients with *ERBB2* (HER2)-positive, *KRAS* wild-type tumors [9, 25]. Thus,



**Figure 2.** Targeting the ALK signaling pathway. **(A):** Upon ligand binding, dimerization of wild-type ALK leads to kinase activation and phosphorylation. ALK activation results in the initiation of a number of signaling cascades involved in promoting cell proliferation and survival. **(B):** Unlike wild-type ALK, the STRN-ALK fusion protein is constitutively dimerized and activated allowing for hyperactivation of downstream signaling pathways and oncogenic growth. Emerging clinical and preclinical data indicates that the STRN-ALK fusion protein may be sensitive to ALK inhibitors, such as crizotinib or ceritinib; therefore, ALK inhibitors may be a potential therapeutic strategy for patients with these alterations.

genomic testing of patients with CRC may identify actionable targets, including all classes of genomic alterations (base substitutions, indels, copy number alterations, and rearrangements), who could benefit from matched targeted therapies.

While *ERBB2* amplification [26] and mutation of *KRAS*, *EGFR*, and other genes [27, 28] have been identified in ctDNA samples from patients with CRC, this is the first report of an ALK rearrangement in a patient with CRC detected by ctDNA. In this case study, a *STRN-ALK* fusion was detected by genomic profiling of ctDNA and later confirmed via tissue testing. The ALK rearrangement reported here is predicted to produce a cytoplasmic fusion protein, whereby the coiled-coil domain of *STRN* promotes dimerization of the ALK kinase domain [23] (Fig. 2). In contrast to wild-type ALK, which requires ligand binding for kinase activation, the STRN-ALK fusion protein promotes constitutive activation of ALK and downstream signaling pathways [23]. ALK expression levels have also been observed at higher levels in cells with ALK fusions than cells without ALK rearrangements [23].

#### POTENTIAL STRATEGIES TO TARGET THE PATHWAY AND IMPLICATIONS FOR CLINICAL PRACTICE

Oncogenic ALK leads to the initiation of a number of downstream signaling cascades, including the STAT3 [29], AKT [30], and MAPK pathways [23] (Fig. 2). The STRN-ALK fusion has also been reported to activate downstream pathways, such as the MAPK pathway, and shown preclinically to drive cell proliferation, cell transformation, and xenograft tumor growth [23]. STRN-ALK driven cells have been shown preclinically to be

sensitive to ALK inhibitors [23], and a patient with metastatic CRC and a *STRN-ALK* fusion achieved clinical benefit with the ALK/ROS1 inhibitor ceritinib [12]. Another case study also reported an objective response to the ALK/ROS1/NTRK inhibitor entrectinib in a patient with CRC and a different ALK rearrangement [15]. Therefore, it was hypothesized that this patient may also benefit from ALK-targeted therapies. However, as most relevant clinical trials for patients with CRC currently require at least one line of systemic chemotherapy, this patient received first-line mFOLFOX-6 and bevacizumab and is now status NED postsurgery. Interestingly, patients with ALK-rearranged lung cancers treated with the chemotherapy pemetrexed have been associated with greater response (response rate of 29% vs. 12.8%) [31] and prolonged progression-free survival (9 months vs. 4 months) [32] compared with patients without ALK rearrangements [31]. Therefore, given this patient's profound response to mFOLFOX-6 plus bevacizumab, one can propose testing the hypothesis that ALK rearrangements in CRC might also predict greater sensitivity to common chemotherapeutic regimens, as compared with responses predicted in patients without these alterations.

The patient's tumor was also found to have intermediate tumor mutational burden (TMB), while being microsatellite stable, as assessed by CGP of the tumor biopsy, and low positivity for PD-L1 expression by IHC testing. While patients with CRC harboring high TMB have higher objective response rates and higher immune-related progression-free survival in response to treatment with the PD-1 inhibitor pembrolizumab, patients with intermediate TMB (6–19 mutations/Mb) experienced

lower rates of clinical benefit overall [33]. Of note, Le et al. utilized whole-exome sequencing (WES) to evaluate tumor mutational burden [33]; however, higher mutational burden load (as determined by CGP of >300 genes) has also been significantly associated with response to the PD-L1 inhibitors in other tumor types [34, 35], and the predictive accuracy of TMB determined by CGP was not statistically different from that determined by WES when gene panels of >300 cancer genes were used [35, 36]. More research with larger cohorts is needed to better understand whether patients with intermediate TMB will benefit from anti-PD-1 therapies.

## CONCLUSION

Circulating tumor DNA assays are increasingly being utilized for genomic analysis when tissue samples are unavailable. Here we illustrate a case where it was originally unclear whether the tissue availability would be sufficient for CGP. However, a ctDNA assay using a hybrid-capture platform, similarly designed to that used for the tissue-based CGP profile, was able to robustly detect the *STRN-ALK* fusion. The fusion was then also identified in tissue. Notably, initial ALK IHC testing was falsely reported negative, highlighting the inconsistencies associated with IHC scoring. These data suggest that this ctDNA assay may benefit patients in cases where a tissue sample is not readily available.

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## GLOSSARY OF GENOMIC TERMS AND NOMENCLATURE

**Circulating Tumor DNA:** cell-free tumor DNA found in the blood plasma.

**Tumor Mutational Burden:** the number of mutations in the tumor genome, often given as mutations per mega base.

## AUTHOR CONTRIBUTIONS

**Conception/Design:** Alexa B. Schrock, Siraj M. Ali

**Provision of study material or patients:** Fadi Braitheh

**Collection and/or assembly of data:** Andrea Z. Lai, Alexa B. Schrock, Fadi Braitheh, Evgeny Yakirevich

**Data analysis and interpretation:** Andrea Z. Lai, Alexa B. Schrock, Siraj M. Ali, Evgeny Yakirevich

**Manuscript writing:** Andrea Z. Lai, Alexa B. Schrock

**Final approval of manuscript:** Jeffrey S. Ross, Vincent A. Miller, Rachel L. Erlich, Siraj M. Ali, Fadi Braitheh, Evgeny Yakirevich

## DISCLOSURES

**Andrea Lai:** Foundation Medicine (E, OI); **Alexa Schrock:** Foundation Medicine (E, OI); **Rachel L. Erlich:** Foundation Medicine (E, OI); **Jeffrey Ross:** Foundation Medicine (E, OI, RF); **Vincent Miller:** Foundation Medicine (E, OI); **Siraj M. Ali:** Foundation Medicine (E, OI); **Fadi Braitheh:** Amgen, Astra Zeneca/Medimmune, Bayer, Boehringer Ingelheim, Clovis, Eli Lilly, Incyte, Ipsen, Insys, Merck, Merrimack, Pfizer, Roche/Genentech (CA, H, Other: travel expenses), Bristol Myers Squibb, Halozyme Therapeutics, Celgene (H, Other: travel expenses), Heron Therapeutics (C/A, Other: travel expenses), Biotheranostics (C/A, H), Lexicon (C/A). The other author indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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See <http://www.TheOncologist.com> for supplemental material available online.

**Editor's Note:** See the related commentary, “ALK Fusion Detection in Circulating Free DNA: Finding an Important Needle in the Haystack,” by Meghan J. Mooradian and Justin F. Gainor on pages 759–761 of this issue.