Identification of Actionable Fusions as an Anti-EGFR Resistance Mechanism Using a Circulating Tumor DNA Assay

Katherine Clifton, MD1; Thereasa A. Rich, MS2; Christine Parseghian, MD1; Victoria M. Raymond, MS2; Arvind Dasari, MD1; Allan Andresson Lima Pereira, MD, PhD1; Jason Willis, MD1; Jonathan M. Loree, MD1; Todd M. Bauer, MD1; Young Kwang Chae, MD, MPH, MBA1; Gary Sherrill, MD1; Paul Fanta, MD, MS1; Axel Grothey, MD1; Andrew Hendifar, MD, MPH1; David Henry, MD1; Daruka Mahadevan, MD, PhD1; Mohammad Amin Nezami, MD1;2; Benjamin Tan, MD1;2; Zev A. Wainberg, MD1;2; Richard Lanman, MD1; Scott Kopetz, MD, PhD1; and Van Morris, MD1

PURPOSE Gene fusions are established oncogenic drivers and emerging therapeutic targets in advanced colorectal cancer. This study aimed to detail the frequencies and clinicopathological features of gene fusions in colorectal cancer using a circulating tumor DNA assay.

METHODS Circulating tumor DNA samples in patients with advanced colorectal cancer were analyzed at 4,581 unique time points using a validated plasma-based multigene assay that includes assessment of fusions in FGF2, FGF3, RET, ALK, NTRK1, and ROS1. Associations between fusions and clinicopathological features were measured using Fisher's exact test. Relative frequencies of genomic alterations were compared between fusion-present and fusion-absent cases using an unpaired t test.

RESULTS Forty-four unique fusions were identified in 40 (1.1%) of the 3,808 patients with circulating tumor DNA detected: RET (n = 6; 36% of all fusions detected), FGF3 (n = 2; 27%), ALK (n = 10, 23%), NTRK1 (n = 3; 7%), ROS1 (n = 2; 5%), and FGF2 (n = 1; 2%). Relative to nonfusion variants detected, fusions were more likely to be subclonal (odds ratio, 8.2; 95% CI, 2.94 to 23.00; P < .001). Mutations associated with a previously reported anti-epidermal growth factor receptor (anti-EGFR) therapy resistance signature (subclonal RAS and EGFR mutations) were found with fusions in FGF3 (10 of 12 patients), RET (nine of 16 patients), and ALK (seven of 10 patients). For the 27 patients with available clinical histories, 21 (78%) had EGFR monoclonal antibody treatment before fusion detection.

CONCLUSION Diverse and potentially actionable fusions can be detected using a circulating tumor DNA assay in patients with advanced colorectal cancer. Distribution of coexisting subclonal mutations in EGFR, KRAS, and NRAS in a subset of the patients with fusion-present colorectal cancer suggests that these fusions may arise as a novel mechanism of resistance to anti-EGFR therapies in patients with metastatic colorectal cancer.

JCO Precis Oncol. © 2019 by American Society of Clinical Oncology
Licensed under the Creative Commons Attribution 4.0 License

INTRODUCTION

Fusions resulting in activation of proto-oncogenes lead to pathologic proliferation in a variety of malignancies and can serve as potential therapeutic targets.1,2 Although selective kinase inhibitors have become standard-of-care therapies for ALK- and ROS1-rearranged non–small-cell lung cancers (NSCLCs), no US Food and Drug Administration–approved targeted therapies for fusions in colorectal cancer (CRC) were available until the recent approval of larotrectinib for any advanced solid tumor with NTRK fusions.3 In two small series, the ALK inhibitors ceritinib and entrectinib demonstrated benefit in patients with CRC harboring ALK fusions.4,5 In addition, rearranged during transfection (RET) inhibitors have shown preclinical promise in RET-fusions both in vitro and in vivo for RET-fusion CRC.6,7 Using tissue-based assays, fusions have been reported in approximately 1% of patients with CRC but are more common in right-sided, RAS wild-type, microsatellite instability–high (MSI-H) colon cancers.8,12 However, no studies to date have comprehensively described the prevalence and genomic landscape of fusions in CRC using circulating tumor DNA (ctDNA).

When measured by ctDNA, early truncal mutations tend to occur at higher variant allele fractions (VAFs) compared with mutations acquired later in disease progression.13 ctDNA may thereby uncover the genomic evolution of mechanisms of treatment resistance, because subclonal mutations not initially detected in primary tumor specimens may become detectable after selective pressure of targeted therapies.14 For example, using ctDNA assays, KRAS, NRAS, MET, ERBB2, EGFR, FGFR1, and MAP2K1 mutations have been identified as mechanisms of resistance to anti-EGFR antibody therapy in patients
Coexisting subclonal mutations in patients with fusion-present colorectal cancer implicate fusions as a previously unreported, novel mechanism of resistance to anti–epidermal growth factor receptor therapies in patients with metastatic colorectal cancer.

This research was approved by the Quorum Institutional Review Board for the generation of de-identified data sets for research. All work was conducted in accordance with the Declaration of Helsinki. Human investigations were performed after approval by a local human investigations committee and in accordance with an assurance filed with and approved by the Department of Health and Human Services, where appropriate.

ctDNA Assay Analysis

VAF was calculated as the ratio of the number of ctDNA molecules harboring a mutation relative to the total number of molecules (variant plus wild type) for a given gene locus. To annotate a given alteration by clonality, relative VAF (rVAF) was assessed by normalizing the VAF to the maximum VAF of all aberrations detected within a given plasma sample, adjusting for copy number amplification as previously described. For the purpose of this study, clonal aberrations were defined as rVAF of 0.5 to 1, subclonal aberrations as rVAF between 0.1 and 0.5, and subclonal minor as rVAF less than 0.1.

Associations between the presence of fusions and clinicopathological features were evaluated using a Fisher’s exact test (SPSS, version 24.0; La Jolla, CA). Relative frequencies of genomic alterations (point mutations, indels, and splice variants) were compared between fusion-present and fusion-absent cases using an unpaired t test.

RESULTS

Occurrence of Fusions in a ctDNA Assay

The median age at time of ctDNA testing was 59 years (interquartile range, 50-69 years). A total of 1,909 patients (44.5%) were female. Of the 3,808 patients with detectable alterations at any time point (Fig 1A), 44 unique fusions were reported in 40 patients (1.1% prevalence). These fusions detected RET (n = 16; 36% of all fusions detected), FGFR3 (n = 12; 27%), ALK (n = 10; 23%), NTRK1 (n = 3; 7%), ROS1 (n = 2; 5%), and FGFR2 (n = 1; 2%). When examining the prevalence of fusions by rearrangement
partner, the most commonly detected fusions were the FGFR3-TACC3 \((n = 12)\) and NCOA4-RET \((n = 9)\) fusions (Appendix Table A2). Co-occurring fusions were found in three of 40 patients (Fig 1B).

The prevalences of ALK and FGFR3 fusions were significantly higher in this ctDNA cohort compared with a previously reported cohort of 4,422 CRC tissue specimens undergoing comprehensive NGS genomic profiling \((P = .04, P = .01, \text{respectively})\). There was no difference in frequencies of RET or NTRK fusions between ctDNA and tissue assays (Fig 1B; Table 1).

**Genomic Profiling of Fusion-Positive Patients**

Clinicopathology history was available for a subset of patients (Table 2; Appendix Table A3). Because this was a retrospective review of clinically treated patients, tissue testing methodology varied over time and across different practices. At least some of the molecular data from tissue testing collected at the time of initial diagnosis was available for 24 of 40 ctDNA fusion-positive patients, eight of whom had comprehensive NGS in which the presence of fusions was assessed. The median time between tissue testing and ctDNA collection was 24.1 months (range, 0.67 to 92 months; \(n = 22\)). From the available clinical and tissue data, nine of 27 (33%) were right-sided, tumors were predominantly KRAS wild type \((n = 23 \text{ of } 24; 96\%)\), with no concurrent NRAS or BRAFV600E mutations, and three of 22 (14%) were MSI-H (Table 2; Table A3). Interestingly, in 11 of the 23 patients with tissue \(RAS\) wild-type status, a \(RAS\) mutation was detected in ctDNA. Similarly, in two of the

---

**TABLE 1.** Fusion Prevalence in ctDNA-Based Assay Compared With Tissue-Based Assay

| Assay | RET | ALK | FGFR3 | NTRK |
|-------|-----|-----|-------|------|
| Tissue: No. of fusions present | 8 | 3 | 1 | 2 |
| Tissue: No. of samples tested | 4,422 | 4,422 | 4,422 | 4,422 |
| ctDNA: No. of fusions present | 16 | 10 | 12 | 3 |
| ctDNA: No. of samples tested | 3,808 | 3,808 | 3,808 | 3,808 |

Abbreviation: ctDNA, circulating tumor DNA.
16 patients with \textit{BRAF}\textsuperscript{V600E} tissue wild-type status, \textit{BRAF}\textsuperscript{V600E} was detected in ctDNA. Among the eight patients with tissue NGS available, only two had the matched fusion detected. Cumulatively, the data suggest that a sizable proportion of the ctDNA fusion-positive population may have had \textit{RAS}/\textit{RAF} mutations and/or the fusion present at levels below the limit of detection in tissue or in a subclone of the tumor tissue that was not sampled for testing.

The frequency of amplifications, indels, and SNVs in clinically relevant cancer genes detectable using the blood-based NGS assay were compared between fusion-positive and fusion-negative samples (Fig 2A). There was no association between the presence of a fusion and coexisting mutation in \textit{KRAS}, \textit{NRAS}, or \textit{BRAF}. Furthermore, co-occurring mutations were more likely in \textit{EGFR} (odds ratio [OR], 3.66; 95% CI, 1.97 to 6.84; \(P < .001\)), \textit{MET} (OR, 2.56; 95% CI, 1.30 to 5.04; \(P < .01\)), and \textit{FGFR1} (OR, 2.46; 95% CI, 1.20 to 5.06; \(P = .01\)) for specimens with fusions, when compared with nonfusion cases (Fig 2A).

Prior treatment histories were available for only 27 patients, the majority (\(n = 21; 78\%\)) of whom did have prior exposure to one or more EGFR monoclonal antibodies as treatment of metastatic CRC at the time of ctDNA collection (Appendix Table A4; Appendix Fig A1). Therefore, we next explored if fusions were associated with a previously validated genomic signature associated with CRC progression on prior anti-EGFR therapies, because treatment histories were not available for the entire fusion cohort.\textsuperscript{16,29}

### Anti-EGFR Signature

cDNA genomic features of progression on prior cetuximab or panitumumab include the presence of subclonal \textit{RAS} mutation (VAF < 50% of the maximum VAF in the sample), multiple concurrent \textit{RAS} mutations, and/or \textit{EGFR} mutations.\textsuperscript{29}

### TABLE 2. Tissue Molecular and MSI Testing at Time of Metastatic CRC Diagnosis

| Molecular Mutation and MSI Status | Positive (or MSI-H) | WT (or MSS) | Not Tested | Unknown | Sum | Total Known | Alteration/All Tested Cases |
|-----------------------------------|---------------------|-------------|------------|---------|-----|-------------|-----------------------------|
| **KRAS**                          |                     |             |            |         |     |             |                             |
| Overall                           | 1                    | 23          | 0          | 16      | 40  | 24          | 0.0416                      |
| Expanded (codons 12, 13, 59, 61, 146; eg, exons 2-4) | 9                    |             |            |         |     |             |                             |
| Limited (known codons)            |                     |             |            |         |     |             |                             |
| Codon 12                          | 6                    |             |            |         |     |             |                             |
| Codon 13                          | 6                    |             |            |         |     |             |                             |
| Codon 61                          | 5                    |             |            |         |     |             |                             |
| Limited NOS                       | 3                    |             |            |         |     |             |                             |
| Unknown                           | 5                    |             |            |         |     |             |                             |
| **NRAS**                          |                     |             |            |         |     |             |                             |
| Overall                           | 0                    | 17          | 5          | 18      | 40  | 17          | 0                           |
| Expanded (codons 12, 13, 59, 61, 117, 146; eg, exons 2-4) | 9                    |             |            |         |     |             |                             |
| Limited (known codons)            |                     |             |            |         |     |             |                             |
| Codon 12                          | 0                    |             |            |         |     |             |                             |
| Codon 13                          | 0                    |             |            |         |     |             |                             |
| Codon 61                          | 1                    |             |            |         |     |             |                             |
| Limited NOS                       | 1                    |             |            |         |     |             |                             |
| Unknown                           | 6                    |             |            |         |     |             |                             |
| **BRAF V600E**                    |                     |             |            |         |     |             |                             |
| Overall                           | 0                    | 16          | 6          | 18      | 40  | 16          | 0                           |
| **Fusion**                        |                     |             |            |         |     |             |                             |
| Overall                           | 2                    | 5           | 11         | 21      | 40  | 7           | 0.286                       |
| **MSI**                           |                     |             |            |         |     |             |                             |
| Overall                           | 3                    | 19          | 2          | 16      | 40  | 22          | 0.1363                      |
| Loss of MLH1/PMS2                 | 2                    |             |            |         |     |             |                             |
| Loss of PMS2                      | 1                    |             |            |         |     |             |                             |

Abbreviations: MSI, microsatellite instability; MSI-H, MSI-high; MSS, microsatellite stable; NOS, not otherwise specified; WT, wild type.
(84%) patients were known treatment histories (Fig A1). Of these 19 patients, 16 with this anti-EGFR therapy resistance signature, 19 had the 24 fusion-positive patients with mutations associated with one of these variables was highly predictive of RAS mutations. Fifteen of 40 (38%) had two or more of these. Mutations associated with this anti-EGFR therapy resistance signature were found with fusions in FGFR3 (10 of 12 patients), RET (nine of 16 patients), and ALK (seven of 10 patients), including two of the patients with multiple fusions (Fig 2B). Among the 24 fusion-positive patients with mutations associated with this anti-EGFR therapy resistance signature, 19 had known treatment histories (Fig A1). Of these 19 patients, 16 (84%) patients were confirmed to have prior exposure to anti-EGFR therapy. The median duration of exposure to treatment with an anti-EGFR agent was 8.5 months (range, 2 to 17 months; Appendix Tables A4 and A5).

The presence of an anti-EGFR signature was associated with fusions occurring at lower rVAF (median, 0.01 v 0.19; P = .036; Fig 3). Furthermore, the low rVAFs of co-occurring RAS, EGFR, and BRAF mutations were consistent with subclonal genomic events occurring later in tumorigenesis (Appendix Fig A2). Among the six patients with an anti-EGFR resistance signature who had comprehensive genomic profiling results available from tissue, four were wild type at the time of initial diagnosis of CRC for the corresponding fusion and/or RAS/RAF alterations that were later detected in ctDNA, consistent with these genomic events being acquired later in tumorigenesis.

DISCUSSION

To our knowledge, this is the largest case series describing fusion-positive cases in CRC (whether in tissue or plasma) and demonstrates that fusions in patients with CRC can be identified using a ctDNA assay. Here, fusions were detected at a prevalence of approximately 1% in patients with advanced CRC, similar to fusion prevalence using orthogonal tissue-based assays in separate series of patients with CRC. All fusions identified are potentially actionable with available targeted drugs. Thus, this ctDNA approach has the potential to allow clinicians to consider additional studies with novel therapeutic combinations for patients with metastatic CRC in future trial settings.

Our data provide new evidence that fusions, particularly involving FGFR3 or RET, may contribute to anti-EGFR therapy resistance in CRC. Here, the majority of the fusions were subclonal. On the basis of previously validated genomic signatures in this setting, we hypothesize that fusions may arise as a novel, unreported mechanism with anti-EGFR therapy resistance, given the clinicopathologic data and frequent co-occurrence with subclonal RAS and EGFR mutations in ctDNA. The profile of concomitant EGFR mutations and subclonal RAS mutations mirrors prior studies that have shown associations between these mutations and post-EGFR resistance. Interestingly, prior series performed in tissue have associated fusions with RAS wild-type CRC tumors. In our series, 23 of 24 (96%) of the ctDNA fusion-positive patients with tissue testing available for RAS mutational status were RAS wild type, whereas 25 of 44 (57%) of fusion-positive ctDNA samples in our series had one or more RAS mutations. We reconcile these findings on the basis of the greater sensitivity to detect low allele frequency often not detectable with tissue-based assays. Furthermore, tissue specimens are often obtained at surgical resections, before a multiple number of sequential lines of systemic therapy, and therefore before exposure to selective pressures that mediate acquisition of resistance mechanisms. The majority of blood samples obtained in this cohort of patients with CRC came from treatment-refractory individuals seeking clinical trial options who frequently had been exposed to anti-EGFR therapies. Thus, the occurrence of subclonal resistance alterations in ctDNA accounted for differences in the tumor genomic profiles of advanced, typically heavily pretreated cancers, relative to the less-mutated genomic profiles of the tumor taken before therapy initiation. In this series, only one patient had a fusion detected in pretreatment tumor tissue and subsequently had anti-EGFR therapy but did not have a clinical response.
Additional investigation into whether fusions also cause primary resistance to anti-EGFR therapy is warranted.

To lend additional support to this association between fusions and resistance to anti-EGFR antibodies, we confirmed the clinical histories of patients with fusion-detected CRC. In those patients with prior treatment data available, 21 of 27 (78%) had previous exposure and progression on anti-EGFR antibodies. Thus, these data further support the notion that subclonal fusions, here identified by ctDNA, may arise after treatment with anti-EGFR antibodies and may represent a novel mechanism of resistance in CRC to these agents.

Our findings in CRC are consistent with previously reported series linking activating fusions as mechanisms of acquired resistance to targeted therapies in other malignancies.21-24 For example, RET fusions were found in patients with NSCLC after the EGFR tyrosine kinase inhibitor (TKI) osimertinib.23 Previous studies have shown that FGFR3...
Fusions may substitute for EGFR signaling, which provides a hypothesized rationale for a mechanism of acquired resistance to anti-EGFR therapy. All of the fusions detected in this series are predicted to lead to the generation of a chimeric protein involving fusion of a tyrosine kinase domain with a partner protein that enhances its activation, thereby promoting downstream signaling of the mitogen-activated protein kinase (MAPK) pathway. Activation of this alternative MAPK signaling pathway bypasses the reduction in MAPK signaling afforded by anti-EGFR antibodies, thus providing plausible biologic rationale for the association of fusion anti-EGFR therapy resistance.

Furthermore, alterations in MET and FGFR1 were also observed more commonly in patients with fusions. Such alterations have been previously reported as acquired mechanisms of resistance to anti-EGFR therapies in CRC. Collectively these and our data point to a diverse, heterogeneous landscape of potential resistance mechanisms adapted by RAS wild-type CRC tumors to overcome EGFR blockade.

Fusions represent a potentially actionable therapeutic target in the anti-EGFR resistance setting. Dual pathway suppression with the RET inhibitor BLU-667 and an EGFR TKI demonstrated antitumor activity both in cell lines and clinically in patients with EGFR-mutant NSCLC who had RET fusions after disease progression while receiving TKIs. Importantly, fusions were often seen co-occurring with multiple other known acquired mechanisms of resistance to anti-EGFR therapy in this series, which points to a diverse, heterogeneous landscape of potential resistance mechanisms adapted by RAS wild-type CRC tumors to overcome EGFR blockade. Therefore, although targeting subclonal fusions alone may be only partially successful, multipathway suppression may be a promising avenue of additional investigation, possibly in combination with anti-EGFR therapies. Such strategies would need to be highly individualized, given the diversity of resistance mechanisms, and could be informed by comprehensive ctDNA testing, especially because serial tissue biopsies are less feasible in patients with advanced cancer.

In several previous data sets using tissue-based assays, fusions in patients with CRC were associated with MSI-H cancers. Although rates of MSI-H and right-sided tumors in our data set were similar to average rates reported in advanced CRCs, a proportion of the fusion-positive patients in this series are suspected to have acquired the fusion after selective pressure from anti-EGFR therapy, and therefore the fusion may have been present in the primary tumor at levels too low to be associated with MSI-H status. In both cases in
this series where the fusion was tested for and detected in tissue, the tumors were found to be MSI-H.

One of the limitations of this data analysis is that complete clinicopathologic features were not available for all patients, given the retrospective nature of the study, and therefore we were unable to obtain clinical histories from all patients with fusions. However, using a previously validated method, the majority of fusion-positive patients had at least one variable, which was highly predictive of prior anti-EGFR exposure. In addition, among the patients with known treatment history and this signature, the majority were indeed confirmed to have prior anti-EGFR therapy, thus internally validating the efficacy of this genomics-first strategy to identify likely resistance cases.

We also did not have matched pre- and post-treatment tissue and plasma for orthogonal and serial profiling to confirm which fusions and other co-occurring mutations were acquired/selected for after anti-EGFR therapy versus those present as truncal/clonal events. For the majority of our patients, we do not have access to the tissue or pretreatment plasma for additional NGS analysis based on the retrospective nature of study. However, genomic events that are acquired during cancer progression tend to have lower relative VAF in ctDNA than do early truncal mutations, such as those in tumor suppressor genes or clonal RAS mutations. In our series, fusions occurring at low rVAF tended to be found in samples containing other genomic mechanisms of anti-EGFR therapy resistance, which is consistent with our hypothesis that some fusions in CRC occur at subclonal levels that are undetectable in pre-treatment tissue but are selected for and become detectable in ctDNA after anti-EGFR therapy resistance. The quantitative nature of ctDNA can therefore not only characterize the fusion identity but also provide insight into the clonal contribution via a single blood draw. Another limitation is that the VAF may be affected by biologic factors, such as the degree of tumor shedding, as well as technical factors, including that fusions are more difficult to detect by NGS and in ctDNA samples than SNVs. Taken together, the fusion prevalences and VAFs observed in this study may be lower than actual because of these technical reasons.

In conclusion, actionable fusions were able to be detected at low frequencies but at similar frequencies to the historical tissue-based NGS approach in a large series of patients with CRC using a ctDNA assay. The distribution of coexisting subclonal mutations in EGFR, KRAS, and NRAS in fusion-present CRC cases matches genomic profiles of CRC tumors after progression on prior anti-EGFR therapy in tumors initially identified as RAS wild type using a less-sensitive tissue-based assay. Actionable fusions may therefore represent a newly reported mechanism of acquired resistance after anti-EGFR therapies. Testing ctDNA in patients to detect fusions as targetable drivers and/or resistance biomarkers is warranted and may carry important implications for the treating oncologist to identify novel therapeutic approaches.

AFFILIATIONS
1The University of Texas MD Anderson Cancer Center, Houston, TX
2GuardianHealth, Redwood City, CA
3BC Cancer, Vancouver, British Columbia, Canada
4Tennessee Oncology Sarah Cannon Research Institute, Nashville, TN
5Northwestern University Feinberg School of Medicine, Chicago, IL
6Cone Health Cancer Center, Greensboro, NC
7University of San Diego Moores Cancer Center, La Jolla, CA
8The University of Tennessee West Cancer Center, Memphis, TN
9Cedars-Sinai Medical Center, Los Angeles, CA
10University of Pennsylvania, Philadelphia, PA
11The University of Arizona Cancer Center, Tucson, AZ
12Pacific Medical Center of Hope, Fresno, CA
13Washington University School of Medicine, St Louis, MO
14University of California Los Angeles, Los Angeles, CA

CORRESPONDING AUTHOR
Van Morris, MD, Department of Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center, 1400 Holcombe Blvd, Unit 426, Houston, TX 77030; Twitter: @VanMorrisMD; e-mail: vkmorris@mdanderson.org.

EQUAL CONTRIBUTION
K.C. and T.A.R. contributed equally to this work.

PRIOR PRESENTATION
Presented at the 2018 annual ASCO meeting, Chicago, IL, June 1-5, 2018.

SUPPORT
Supported by funding support from the National Cancer Institute T32 Grant No. CA009666 (K.C.) and K12 Grant No. CA088084 (V.K.M.).

AUTHOR CONTRIBUTIONS
Conception and design: Katherine Clifton, Thereasa A. Rich, Victoria M. Raymond, Allandrasson Lima Pereira, Jonathan M. Loree, Axel Grothey, Mohammad Amin Nezami, Richard Lanman, Scott Kopetz, Van Morris
Financial support: Van Morris
Administrative support: Victoria M. Raymond, Mohammad Amin Nezami, Scott Kopetz
Provision of study material or patients: Victoria M. Raymond, Todd M. Bauer, Young Kwang Chae, Paul Fanta, Axel Grothey, Andrew Hendifar, Daruka Mahadevan, Benjamin Tan, Zev A. Wainberg
Collection and assembly of data: Thereasa A. Rich, Victoria M. Raymond, Todd M. Bauer, Young Kwang Chae, Paul Fanta, Andrew Hendifar, Daruka Mahadevan, Benjamin Tan, Zev A. Wainberg, Richard Lanman, Van Morris
Data analysis and interpretation: Thereasa A. Rich, Christine Parseghian, Victoria M. Raymond, Arvind Dasari, Jason Willis, Jonathan M. Loree, Todd M. Bauer, Young Kwang Chae, Paul Fanta, Andrew Hendifar, David Henry, Daruka Mahadevan, Zev A. Wainberg, Richard Lanman
Manuscript writing: All authors
Final approval of manuscript: All authors
AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Katherine Clifton
Employment: WellCare (I)
Stock and Other Ownership Interests: WellCare (I)

Theresa A. Rich
Employment: Guardant Health
Stock and Other Ownership Interests: Guardant Health, Myriad Genetics

Victoria M. Raymond
Employment: TrovaGene, Guardant Health
Stock and Other Ownership Interests: TrovaGene, Guardant Health

Arvind Dasari
Consulting or Advisory Role: Ipsen, AbbVie/Stemcentrx, Novartis, Voluntis, Lexicon
Research Funding: Novartis, eFFCTOR Therapeutics, Eisai, Hutchison MediPharma, Merck, Guardant Health

Jonathan M. Loree
Consulting or Advisory Role: Taiho Pharma Canada, Ipsen, Novartis, Bayer, Amgen

Todd M. Bauer
Employment: Tennessee Oncology
Consulting or Advisory Role: Ignyta (Inst), Guardant Health, Loxo, Pfizer, Moderna Therapeutics (Inst), Pfizer (Inst), Exelixis
Speakers’ Bureau: Bayer
Research Funding: Daiichi Sankyo (Inst), MedPacto (Inst), Incyte (Inst), Mirati Therapeutics (Inst), MedImmune (Inst), AbbVie (Inst), AstraZeneca (Inst), Leap Therapeutics (Inst), MabVax Therapeutics (Inst), Stemline Therapeutics (Inst), Merck (Inst), Eli Lilly (Inst), GlaxoSmithKline (Inst), Novartis (Inst), Pfizer (Inst), Genentech (Inst), Deciphera Pharmaceuticals (Inst), Merrimack (Inst), Immunogen (Inst), Millennium (Inst), Ignyta (Inst), Calithera Biosciences (Inst), Kollta Pharmaceutical (Inst), Principia Biopharma (Inst), Peleton (Inst), Immunocore (Inst), Roche (Inst), Aileron Therapeutics (Inst), Bristol-Myers Squibb (Inst), Amgen (Inst), Moderna Therapeutics (Inst), Sanofi (Inst), Boehringer Ingelheim (Inst), Astellas Pharma (Inst), Five Prime Therapeutics (Inst), Jacobio Pharmaceuticals (Inst), Top Alliance BioSciences (Inst), Loxo (Inst), Janssen (Inst), Clovis Oncology (Inst), Takeda (Inst), Karyopharm Therapeutics (Inst), Onyx (Inst), Phosplatin Therapeutics (Inst), Foundation Medicine (Inst), ARMO BioSciences (Inst)
Travel, Accommodations, Expenses: Astellas Pharma, AstraZeneca, Celgene, Clovis Oncology, EMD Serono, Genentech, Eli Lilly, Merck, Novartis, Pharmacyclics, Sysmex

Young Kwang Chae
Consulting or Advisory Role: Foundation Medicine, Boehringer Ingelheim, Biodexis, Counsyl, AstraZeneca, Guardant Health, Takeda, Genentech, ImmuneOncia, Hanmi
Speakers’ Bureau: Genentech, Merck, AstraZeneca, Eli Lilly
Research Funding: AbbVie, Bristol-Myers Squibb, Lexent Bio, Freenome, Biodexis
Travel, Accommodations, Expenses: Hanmi

Axel Grotthey
Consulting or Advisory Role: Genentech (Inst), Bayer (Inst), Bristol-Myers Squibb (Inst), Eli Lilly (Inst), Boston Biomedical (Inst), Amgen (Inst), Array BioPharma (Inst), Guardant Health (Inst), Daiichi Sankyo (Inst)
Research Funding, Genentech (Inst), Bayer (Inst), Pfizer (Inst), Eisai (Inst), Eli Lilly (Inst), Boston Biomedical (Inst), Daiichi Sankyo (Inst), Array BioPharma (Inst)
Travel, Accommodations, Expenses: Genentech, Bayer, Bristol-Myers Squibb, Boston Biomedical, Amgen, Boehringer Ingelheim, Merck Sharp & Dohme

Andrew Hendifar
Consulting or Advisory Role: Novartis, Ipsen, Perthera, Celgene, AbbVie
Research Funding: Ipsen
Travel, Accommodations, Expenses: Halozyme

David Henry
 Honoraria: Amgen, AMAG Pharmaceuticals
Consulting or Advisory Role: AMAG Pharmaceuticals, Amgen
Research Funding: Amgen
Travel, Accommodations, Expenses: Amgen, AMAG Pharmaceuticals

Daruka Mahadevan
Honors: Guardant Health, Caris Life Sciences
Speakers’ Bureau: Guardant Health, Caris Life Sciences
Travel, Accommodations, Expenses: Guardant Health, Caris Life Sciences

Mohammad Nezami
Employment: Orange Coast Medical Center of Hope
Stock and Other Ownership Interests: Sahel Oncology

Benjamin Tan
Consulting or Advisory Role: Genentech
Research Funding: Genentech, Eli Lilly, Pfizer, Bayer, Eisai, Exelixis, Merck Serono, Tyrogenex, SillaJen, Boehringer Ingelheim

Zev A. Wainberg
Consulting or Advisory Role: Array BioPharma, Five Prime Therapeutics, Novartis, Eli Lilly, Merck, Merck KGaA, Bristol-Myers Squibb, Genentech, Bayer
Research Funding: Novartis (Inst), Plexxikon (Inst), Pfizer (Inst), Merck (Inst), Five Prime Therapeutics (Inst)
Travel, Accommodations, Expenses: Genentech

Richard Lannan
Employment: Guardant Health, Veracyte
Leadership: Guardant Health, Biolase
Stock and Other Ownership Interests: MolecularMatch, Navire
Research Funding: Guardant Health

Scott Kopetz
Stock and Other Ownership Interests: MolecularMatch, Navire
Consulting or Advisory Role: Roche, Genentech, EMD Serono, Merck, Karyopharm Therapeutics, Amal Therapeutics, Navire Pharma, Symphogen, Holy Stone, Biocartis, Amgen, Novartis, Eli Lilly, Boehringer Ingelheim, Boston Biomedical, AstraZeneca/MedImmune, Bayer Health, Pierre Fabre, EMD Serono
Research Funding: Amgen (Inst), Sanofi (Inst), Biocartis (Inst), Guardant Health (Inst), Array BioPharma (Inst), Genentech (Inst), EMD Serono (Inst), MedImmune (Inst), Novartis (Inst), Eli Lilly (Inst)
REFERENCES

1. Medves S, Demoulin JB: Tyrosine kinase gene fusions in cancer: Translating mechanisms into targeted therapies. J Cell Mol Med 16:237-248, 2012
2. Schram AM, Chang MT, Jonsson P, et al: Fusions in solid tumours: Diagnostic strategies, targeted therapy, and acquired resistance. Nat Rev Clin Oncol 14:735-748, 2017
3. Dritlo A, Laetsch TW, Kummar S, et al: Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. N Engl J Med 378:731-739, 2018
4. Yakirevich E, Resnick MB, Mangray S, et al: Oncogenic ALK fusion in rare and aggressive subtype of colorectal adenocarcinoma as a potential therapeutic target. Clin Cancer Res 22:3831-3840, 2016
5. Armutlu A, Somaschini A, Cerea G, et al: Novel CAD-ALK gene rearrangement is drugable by entrectinib in colorectal cancer. Br J Cancer 113:1730-1734, 2015
6. Subbiah V, Gainor JJ, Rahal R, et al: Precision targeted therapy with BLU-667 for RET-driven cancers. Cancer Discov 8:836-849, 2018
7. Subbiah V, Velcheti V, Tuch BB, et al: Selective RET kinase inhibition for patients with RET-altered cancers. Ann Oncol 29:1869-1876, 2018
8. Rankin A, Klempner SJ, Erlich R, et al: Broad detection of alterations predicted to confer lack of benefit from EGFR antibodies or sensitivity to targeted therapy in advanced colorectal cancer. Oncologist 23:1163-1171, 2018
9. Zill OA, Banks KC, Fairclough SR, et al: The landscape of actionable genomic alterations in cell-free circulating tumor DNA from 21,807 advanced cancer patients. Clin Cancer Res 24:3528-3538, 2018
10. Diaz LA Jr, Sausen M, Fisher GA, et al: Insights into therapeutic resistance from whole-genome analyses of circulating tumor DNA. Oncotarget 4:1856-1857, 2013
11. Misea S, Yagami R, Hobor S et al: Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 486:532-536, 2012
12. Stirkler JH, Loree JM, Ahronian LG, et al: Genomic landscape of cell-free DNA in patients with colorectal cancer. Cancer Discov 8:164-173, 2018
13. Diaz LA Jr, Williams RT, Wu J, et al: The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 486:537-540, 2012
14. Bardelli A, Corso S, Bertotti A, et al: Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov 3:658-673, 2013
15. Siravagga G, Mussolin B, Buscarino M, et al: Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat Med 21:795-801, 2015 [Erratum: Nat Med 21:827, 2015]
16. Bertotti A, Papp E, Jones S, et al: The genomic landscape of response to EGFR blockade in colorectal cancer. Nature 526:263-267, 2015
17. Daly C, Castanoa C, Zhang W, et al: FGFR3-TACC3 fusion proteins act as naturally occurring drivers of tumor resistance by functionally substituting for EGFR/ERK signaling. Oncogene 36:471-481, 2017
18. McCaughan CE, Blakeley CM, Banks KC, et al: Clinical utility of cell-free DNA for the detection of ALK fusions and genomic mechanisms of ALK inhibitor resistance in non-small cell lung cancer. Clin Cancer Res 24:2758-2770, 2018
19. Piotrowska Z, Isozaki H, Lennert J, et al: Landscape of acquired resistance to osimertinib in EGFR-mutant NSCLC and clinical validation of combined EGFR and RET inhibition with osimertinib and BLU-667 for acquired RET fusion. Cancer Discov 8:1529-1539, 2018
20. Wang J, He Q, Chen Y et al: Metastatic EML4-ALK fusion detected by circulating DNA genotyping in an EGFR-mutated NSCLC patient and successful management by adding ALK inhibitors: A case report. BMC Cancer 16:62, 2016
21. Lanman RB, Mortimer SA, Zill OA, et al: Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA. PLoS One 10:e0140712, 2015
22. Odgaard J, Vincent J, Mortimer S, et al: Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies. Clin Cancer Res 24:3539-3549, 2018
23. Hu Y, Ulrich BC, Supplee J, et al: False-positive plasma genotyping due to clonal hematopoiesis. Clin Cancer Res 24:4437-4443, 2018
24. Rankin A, Klempner SJ, Erlich R, et al: Broad detection of alterations predicted to confer lack of benefit from EGFR antibodies or sensitivity to targeted therapy in advanced colorectal cancer. Oncologist 21:1306-1314, 2016
25. Parsheghian CM, Loree JM, Morris VK, et al: Anti-EGFR-resistant clones decay exponentially after progression: Implications for anti-EGFR re-challenge. Ann Oncol 30:243-249, 2019
26. Chae YK, Ranganath K, Hammerman PS, et al: Inhibition of the fibroblast growth factor receptor (FGFR) pathway: The current landscape and barriers to clinical application. Oncotarget 8:16052-16074, 2017
27. Bunone G, Uggeri M, Mondellini P, et al: RET receptor expression in thyroid follicular epithelial cell-derived tumors. Cancer Res 60:2845-2849, 2000
28. Oda K, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. Mol Syst Biol 1:1-17, 2005
29. Raghav K, Morris V, Tang C, et al: MET amplification in metastatic colorectal cancer: An acquired response to EGFR inhibition, not a de novo phenomenon. Oncotarget 7:54627-54631, 2016
FIG A1. Consort diagram detailing fusion history and associated anti–epidermal growth factor receptor (anti-EGFR) therapy resistance signature.
FIG A2. Relative variant allele fraction (rVAF) for 21 fusion-positive samples with RAS mutations and co-occurring EGFR extracellular domain (ECD) and BRAF^{V600E} mutations.

TABLE A1. Fusions Tested Using Plasma-Based Circulating Tumor DNA Next-Generation Sequencing Assay

| 68-Gene Panel (n = 727) | 70-Gene Panel (n = 1,562) | 73-Gene Panel (n = 2,293) |
|-------------------------|---------------------------|---------------------------|
| EML4-ALK                | EML4-ALK                  | EML4-ALK                  |
| STRN-ALK                | STRN-ALK                  | STRN-ALK                  |
| CCDC6-RET               | CCDC6-RET                 | CCDC6-RET                 |
| NCOA4-RET               | NCOA4-RET                 | NCOA4-RET                 |
| TRIM24-RET              | TRIM24-RET                | TRIM24-RET                |
| ERC1-ROS1               | ERC1-ROS1                 | ERC1-ROS1                 |
| SLC34A2-ROS1            | SLC34A2-ROS1              | SLC34A2-ROS1              |
| PLEKHA6-NTRK1           | PLEKHA6-NTRK1             | PLEKHA6-NTRK1             |
| TPM3-NTRK1              | TPM3-NTRK1                | TPM3-NTRK1                |
| FGFR2-TACC2             | FGFR2-TACC2               | FGFR2-TACC2               |
| FGFR3-TACC3             | FGFR3-TACC3               | FGFR3-TACC3               |
### TABLE A2. Prevalence by Rearrangement Partner

| Fusion Partner | Patients Tested | Patients With Fusion | Prevalence of Fusion (%) |
|----------------|-----------------|----------------------|--------------------------|
| ALK            |                 |                      |                          |
| EML4-ALK       | 4,289           | 5                    | 0.12                     |
| STRN-ALK       |                 | 5                    | 0.12                     |
| FGFR2-TACC2    | 3,679           | 1                    | 0.03                     |
| FGFR3-TACC3    | 3,679           | 12                   | 0.33                     |
| NTRK1          |                 |                      |                          |
| PLEKHA6-NTRK1  | 4,289           | 1                    | 0.02                     |
| TPM3-NTRK1     |                 | 2                    | 0.05                     |
| RET            |                 |                      |                          |
| CCDC6-RET      | 4,289           | 6                    | 0.14                     |
| NCOA4-RET      |                 | 9                    | 0.20                     |
| TRIM24-RET     |                 | 1                    | 0.02                     |
| ROS1           |                 |                      |                          |
| ERC1-ROS1      | 4,289           | 1                    | 0.02                     |
| SLC34A2-ROS1   |                 | 1                    | 0.02                     |

### TABLE A3. Primary Tumor Location in Fusion-Positive Patients

| Tumor Location     | No. Patients | % (of known) |
|--------------------|--------------|--------------|
| Right              | 8            | 0.30         |
| Left               | 14           | 0.52         |
| Both right and left| 1            | 0.04         |
| Transverse         | 4            | 0.15         |
| Unknown            | 13           |              |
TABLE A4. Available Clinical History of Fusion-Positive Patients

| Prior Anti-EGFR Treatment | No. of Patients | Alterations/All Tested Cases |
|---------------------------|----------------|-------------------------------|
| Yes                       | 21             | 0.78                          |
| No                        | 6              | 0.22                          |
| Unknown                   | 13             |                               |

Duration of therapy, months (n = 12 known)

|               | Median | Minimum | Maximum |
|---------------|--------|---------|---------|
|               | 8.5    | 2       | 17      |

Disease progression while receiving anti-EGFR treatment at time of G360

|               | Yes | No | Unknown | Not applicable; was never receiving anti-EGFR |
|---------------|-----|----|---------|-----------------------------------------------|
|               | 10  | 15 | 13      | 6                                             |
|               | 0.40| 0.60|         |                                               |

Time between last EGFR treatment and G360, months (n = 17 known)

|                  | >1 month | Median | Maximum |
|------------------|----------|--------|---------|
|                  | 8        | 7      | 23.5    |

Abbreviation: EGFR, epidermal growth factor receptor; G360, Guardant360.
### TABLE A5. Anti-EGFR Treatment Characteristics

| Oncoprint Number | Fusion in Pretreatment Tissue | EGFR Resistance Signature | Clinical Benefit While Receiving Anti-EGFR | Duration of Treatment (months) | Time From Treatment Stop to ctDNA (months) |
|------------------|-------------------------------|---------------------------|-------------------------------------------|-------------------------------|-------------------------------------------|
| 2                | Negative                      | No                        | Yes                                       | 7                             | 0                                         |
| 3                | Not performed                 | Yes                       | Yes                                       | 10                            | 3                                         |
| 4                | Positive                      | Yes                       | No                                        | 5                             | 3.6                                       |
| 7                | Negative                      | Yes                       | Yes                                       | 16                            | 0                                         |
| 13               | Negative                      | Yes                       | Yes                                       | 14                            | 23.5                                      |
| 16               | Not performed                 | No                        | No                                        | 2                             | 3                                         |
| 17               | Negative                      | Yes                       | Yes                                       | 10                            | 0                                         |
| 20               | Not performed                 | Yes                       | Yes                                       | 6                             | 9                                         |
| 21               | Not performed                 | Yes                       | Yes                                       | 11.5                          | 0.7                                       |
| 22               | Not performed                 | Yes                       | No                                        | 6                             | 8                                         |
| 28               | Not performed                 | Yes                       | Yes                                       | 14                            | 13                                        |
| 30               | Not performed                 | Yes                       | Yes                                       | 17                            | 7                                         |
| 32               | Negative                      | No                        | Unknown                                   | 6                             | 0                                         |
| 33               | Not performed                 | Yes                       | No                                        | 5                             | 0                                         |

Abbreviations: ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor.