JAMA Oncology | Original Investigation

Rechallenge for Patients With *RAS* and *BRAF* Wild-Type Metastatic Colorectal Cancer With Acquired Resistance to First-Line Cetuximab and Irinotecan

Chiara Cremolini, MD, PhD; Daniele Rossini, MD; Emanuela Dell'Aquila, MD; Sara Lonardi, MD; Elena Conca, PhD; Marzia Del Re, PharmD, PhD; Adele Busico, PhD; Filippo Pietrantonio, MD; Romano Danesi, MD, PhD; Giuseppe Aprile, MD; Emiliano Tamburini, MD; Carlo Barone, MD, PhD; Gianluca Masi, MD; Francesco Pantano, MD; Francesca Pucci, MD; Domenico C. Corsi, MD; Nicoletta Pella, MD; Francesca Bergamo, MD; Eleonora Rofi, PhD; Cecilia Barbara, MD; Alfredo Falcone, MD; Daniele Santini, MD

IMPORTANCE Based on a small retrospective study, rechallenge with cetuximab-based therapy for patients with *KRAS* wild-type metastatic colorectal cancer (mCRC) who were previously treated with the same anti–epidermal growth factor receptor–based regimen might be efficacious. Recent data suggest the role of liquid biopsy as a tool to track molecular events in circulating tumor DNA (ctDNA).

OBJECTIVE To prospectively assess the activity of cetuximab plus irinotecan as third-line treatment for patients with *RAS* and *BRAF* wild-type mCRC who were initially sensitive to and then resistant to first-line irinotecan- and cetuximab-based therapy.

DESIGN, SETTING, AND PARTICIPANTS This multicenter, phase 2, single-arm trial was conducted from January 7, 2015, to June 19, 2017. Liquid biopsies for the analysis of ctDNA were collected at baseline. Main eligibility criteria included *RAS* and *BRAF* wild-type status on tissue samples; prior first-line irinotecan- and cetuximab-based regimen with at least partial response, progression-free survival of at least 6 months with first-line therapy, and progression within 4 weeks after the last dose of cetuximab; and prior second-line oxaliplatin- and bevacizumab-based treatment.

INTERVENTIONS Biweekly cetuximab, 500 mg/m^2, plus irinotecan, 180 mg/m^2.

MAIN OUTCOMES AND MEASURES Overall response rate according to the Response Evaluation Criteria in Solid Tumors, version 1.1. Secondary end points included progression-free survival and overall survival and, as an exploratory analysis, *RAS* mutations in ctDNA.

RESULTS Twenty-eight patients (9 women and 19 men; median age, 69 years [range, 45-79 years]) were enrolled. Six partial responses (4 confirmed) and 9 disease stabilizations were reported (response rate, 21%; 95% CI, 10%-40%; disease control rate, 54%; 95% CI, 36%-70%). The primary end point was met because the lower limit of the 95% CI of the response rate was higher than 5%. *RAS* mutations were found in ctDNA collected at the rechallenge baseline in 12 of 25 evaluable patients. (48%) No *RAS* mutations were detected in samples from patients who achieved a confirmed partial response. Patients with *RAS* wild-type ctDNA had significantly longer progression-free survival than those with *RAS* mutated ctDNA (median progression-free survival, 4.0 vs 1.9 months; hazard ratio, 0.44; 95% CI, 0.18-0.98; P = .03).

CONCLUSIONS AND RELEVANCE This is the first prospective demonstration that a rechallenge strategy with cetuximab and irinotecan may be active in patients with *RAS* and *BRAF* wild-type mCRC with acquired resistance to first-line irinotecan- and cetuximab-based therapy. The evaluation of *RAS* mutational status on ctDNA might be helpful in selecting candidate patients.

JAMA Oncol. doi:10.1001/jamaoncol.2018.5080
Published online November 21, 2018.

Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Alfredo Falcone, MD, Unit of Medical Oncology 2, Department of Translational Research and New Technologies in Medicine and Surgery, Azienda Ospedaliero-Universitaria Pisana, Via Roma 67, Pisa, 56126, Italy (alfredo.falcone@med.unipi.it).

The combination of an anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibody (cetuximab or panitumumab) with a chemotherapy doublet is a first-line treatment option for patients with *RAS* (*KRAS*: OMIM, 190070; and *NRAS*: OMIM, 164790) and *BRAF* (OMIM, 164757) wild-type metastatic colorectal cancer (mCRC).[1-3] A retrospective study highlighted the potential efficacy of reintroducing cetuximab for patients with acquired resistance to a previous treatment with chemotherapy plus cetuximab, followed by at least 1 intervening line of therapy.[4] Although the study was limited by its retrospective nature, the finding is currently supported by an intriguing biological rationale. The emergence of *RAS* mutations in tumors that were initially *RAS* wild-type is a well-recognized mechanism of acquired resistance to anti-EGFR monoclonal antibodies.[5-8] It is currently unclear whether this event might be due to the late acquisition of these mutations by cellular subclones or to the progressive selection of initially undetectable mutated subclones. According to the latter hypothesis, an anti-EGFR-based therapy would be able to substantially decrease the bulk of sensitive (wild-type) cells, thus making the resistant (mutant) clones progressively predominant until the clinical evidence of disease progression. During a subsequent treatment that was not anti-EGFR based, sensitive clones would be at least partially restored, thus laying the foundation for the potential and reported activity of anti-EGFR rechallenge.[5]

More recently, a growing amount of molecular evidence highlighted the intratumoral heterogeneity of colorectal cancer and the dynamism of clonal evolution under the pressure exerted by treatments. In particular, preliminary proof of concept results pointed out the biological relevance of circulating tumor DNA (ctDNA) as an extremely sensitive tool to document the complexity of the tumor and to potentially drive strategies of therapy adaptation.[5,9-12] The emergence of *RAS* mutations at the time of disease progression to first-line chemotherapy plus anti-EGFR monoclonal antibodies may be followed by a dwindling of the fractional abundance of acquired *RAS* mutations—even to undetectable levels—after withdrawal of EGFR blockade.[5]

The CRICKET (Cetuximab Rechallenge in Irinotecan-Pretreated mCRC, *KRAS*, *NRAS* and *BRAF* Wild-Type Treated in 1st line With Anti-EGFR Therapy) trial was designed to prospectively evaluate the activity of a rechallenge strategy with irinotecan and cetuximab as third-line treatment for patients experiencing an initial response and then progression with a first-line irinotecan- and cetuximab-containing therapy, and receiving second-line chemotherapy plus bevacizumab. The prospective collection of liquid biopsy samples from enrolled patients was planned to investigate whether the analysis of potential mechanisms of acquired resistance to anti-EGFR monoclonal antibodies in ctDNA could help determine the benefit of this strategy.

Key Points

Question Is third-line cetuximab plus irinotecan an active option for patients with *RAS* and *BRAF* wild-type metastatic colorectal cancer who have acquired resistance to first-line irinotecan- and cetuximab-based therapy?

Findings In this phase 2 single-arm clinical trial, rechallenge with cetuximab plus irinotecan was active in 21% of patients with *RAS* and *BRAF* wild-type metastatic colorectal cancer. Preplanned circulating tumor DNA profiling revealed that only patients with *RAS* and *BRAF* wild-type circulating tumor DNA at the time of rechallenge could derive benefit.

Meaning These findings lay the foundation for further evaluating the efficacy of anti-epidermal growth factor receptor rechallenge in larger studies including only patients with no mechanisms of acquired resistance detectable in circulating tumor DNA.

Methods

Patient Population

The CRICKET trial (NCT02296203) was a prospective, open-label, multicenter, single-arm phase 2 trial that recruited patients with mCRC from 9 Italian oncology units from January 7, 2015, to June 19, 2017. The main inclusion criteria were histologically confirmed colorectal adenocarcinoma; *RAS* and *BRAF* wild-type status of primary colorectal cancer and/or related metastasis; older than 18 years of age; an Eastern Cooperative Oncology Group performance status of 0 to 2; measurable metastatic disease according to the Response Evaluation Criteria in Solid Tumors, version 1.1[13]; first-line irinotecan-based cetuximab-containing therapy (FOLFIRI [fluorouracil and leucovorin combined with irinotecan] or FOLFOXIRI [fluorouracil, leucovorin, oxaliplatin, and irinotecan] plus cetuximab) producing at least a partial response; first-line progression-free survival (PFS) of 6 months or more; documentation of progression to first-line therapy within 4 weeks after the last administration of cetuximab; time between the end of first-line therapy and the start of third-line therapy of 4 months or more; and progression to a second-line oxaliplatin-based bevacizumab-containing therapy (FOLFOXIRI, FOLFOX [leucovorin, fluorouracil, and oxaliplatin], or XELOX [capecitabine and oxaliplatin] plus bevacizumab). The trial was conducted in accordance with the Declaration of Helsinki[14] and adhered to the international Good Clinical Practice guidelines. The protocol was approved by local ethics committees of participating sites (Centre of Pisa and Pontedera: Comitato Etico Area Vasta Nord Ovest and Sezione Autonoma Del Comitato Etico Regionale Per La Sperimentazione Clinica, Centre of Roma Campus Biomedico: Comitato Etico dell'Università Campus Biomedico di Roma, Centre of Parma: Comitato Etico per Parma, Centre of Padova: Comitato Etico per la Sperimentazione Clinica dell' Istituto Oncologico Veneto, Centre of Roma-Isola Tiberina: Comitato Etico Lazio 1 c/o Farmacia dell'Ospedale San Camillo, Centre of Udine: Comitato Etico Regionale Unico, Centre of Roma-Gemelli: Comitato Etico Università Cattolica del Sacro Cuore,

and Centre of Rimini: Comitato Etico di Area Vasta Romagna). All patients provided written informed consent to study procedures.

Study Treatment and Procedures

Patients were treated with intravenous cetuximab, 500 mg/m^2, and intravenous irinotecan 180, mg/m^2, repeated biweekly until disease progression, patient's refusal, unacceptable toxic effects, or withdrawal of consent. The response was evaluated according to the Response Evaluation Criteria in Solid Tumors, version 1.1. A computed tomographic scan was recommended every 8 weeks.[13] Investigator-reported measurements were subsequently centrally reviewed. Adverse events were recorded and graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events guidelines, version 4.0.[15] Patients' registration and data collection were centralized by the Gruppo Oncologico del Nord Ovest.

Molecular Analyses

Liquid biopsies were collected at the rechallenge baseline. Circulating tumor DNA was analyzed with droplet digital polymerase chain reaction for specific *RAS* and *BRAF* mutations and then by means of ultra-deep next-generation sequencing with Ion Torrent S5 XL (Thermo Fisher Scientific).

Six milliliters of blood were collected in EDTA tubes and centrifuged at 4°C for 10 minutes at 3000 rpm within 2 hours after blood collection. Plasma samples were stored at −80°C until analysis. Circulating tumor DNA was extracted using a QIAmp Circulating nucleic acid Kit (Qiagen) from 2 to 3 mL of plasma following the manufacturer's protocol, and the ctDNA was eluted in 50 µL of elution buffer. The analysis was performed with a droplet digital polymerase chain reaction *KRAS* and *NRAS* Screening Multiplex Kit, and the results were confirmed using single mutation assays (BioRad). *BRAF* analysis for the V600E mutation was conducted using the droplet digital polymerase chain reaction *BRAF* V600E Mutation Assay (BioRad). The droplet reader (BioRad) was used for the fluorescence signal quantification, the QuantaSoft (BioRad) software was used to measure the number of positive vs negative droplets for both fluorophores (5(6)-carboxyfluorescein and 6-carboxy-2′,4,4′,5′,7,7′-hexachlorofluorescein [FAM/HEX]), and their ratio was fitted to a Poisson distribution to determine the copy number per milliliter of the target molecule in the input reaction. A fluorescence intensity threshold of 3000 was set as a cutoff point, and all droplets above this threshold were scored as positive for *RAS* and *BRAF* mutations.

The next-generation sequencing was performed using the Ion AmpliSeq Cancer Hotspot Panel (Thermo Fisher); this panel is designed to amplify 207 amplicons covering approximately 2800 COSMIC (Catalogue Of Somatic Mutations In Cancer) mutations from 50 oncogenes and tumor suppressor genes commonly mutated in human cancers, including *RAS* and *BRAF* mutations (eTable 1 in the Supplement). The libraries were prepared by IonAmpliSeq Library kit 2.0 (Thermo Fisher).[16] Emulsion polymerase chain reaction and chip loading were performed on the IonChef System (Thermo Fisher), according to the manufacturer's instructions. Sequencing was performed on the ION S5 XL System (Thermo Fisher) using Ion 540 Chips and Ion 540 Kit-Chef according to the manufacturer's instructions (MAN0010846). Data were processed by using Torrent Suite (Thermo Fisher); the variant calling from sequencing data was generated by using the Variant Caller plugin. The resulting variants were annotated using the Ensemble Variant Effect Predictor pipeline, Ion Reporter analysis software, the COSMIC database,[17] the dbSNP database,[18] and the ClinVar database of the National Center for Biotechnology Information.[19] The filtered variants were examined using the Integrative Genomic Viewer IGV tool (Broad Institute) to check their quality level and confirm the variant's presence on both the positive and the negative strand.[20] For all plasma ctDNA samples, the coverage depth of most amplicons was over 5000×.

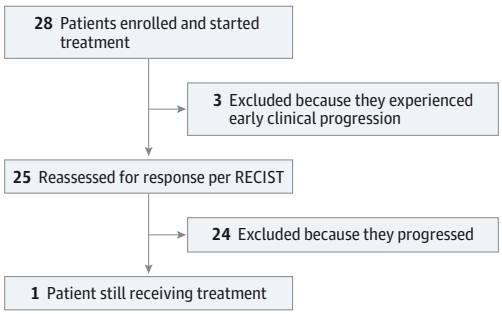
Statistical Analysis

The primary end point of the study was the overall response rate, defined as the proportion of patients achieving complete response or partial response. Patients whose disease was not reassessed and those who were unavailable for follow-up or who were dead before disease reassessment were considered not to be responders for the purpose of the primary endpoint analysis. According to the Fleming single-stage design, selecting $P = .05$ (overall response rate in the null hypothesis) based on results with second-line irinotecan-based therapies[21,22] and selecting $P = .20$ (overall response rate in the alternative hypothesis) as a potential target of interest for future studies, with α (1-sided) errors of .05 and β errors of .20, we found that a total of 27 patients were required. The null hypothesis would have been rejected if at least 4 patients had achieved a response.[23,24] Secondary end points included PFS, overall survival (OS) calculated from the start of treatment, the toxicity profile, and the results of translational analyses. Progression-free survival and OS were summarized using the Kaplan-Meier method; hazard ratios and corresponding 95% CIs were estimated with the Cox proportional hazards regression model. Patients not experiencing disease progression or death were censored at the date of the last follow-up visit. The median period of follow-up was calculated for the entire study cohort according to the reverse Kaplan-Meier method. Statistical analyses were performed by using MedCalc Statistical Software, version 14.8.1 (MedCalc Software) and GraphPad Prism, version 6.00 for Windows (GraphPad Software).

Results

From January 7, 2015, to June 19, 2017, 28 patients were enrolled in 9 Italian oncology units (**Figure 1**). The patients' main characteristics are reported in eTable 2 in the Supplement: the median age was 69 years (range, 45-79 years), 18 patients (64%) had an Eastern Cooperative Oncology Group performance status of 0, metastases' presentation was synchronous in 20 cases (71%), 25 patients (89%) had undergone resection of the primary tumor, and 21 patients (75%) had multiple sites of metastases, while metastases were still limited to the liver in 5 pa-

Figure 1. Study CONSORT Diagram



RECIST indicates Response Evaluation Criteria in Solid Tumors.

Table. Data on Responses of Patients

Type of Response	No. (%) (N = 28)
Complete response	0
Partial response	6 (21)
Confirmed	4 (14)
Unconfirmed	2 (7)
Stable disease	9 (32)
Progressive disease	10 (36)
Not evaluable	3 (11)
Objective response rate[a] (95% CI)	6 (21) (10-40)
Disease control rate[b] (95% CI)	15 (54) (36-70)

[a] Complete response or partial response.

[b] Complete response, partial response, or stable disease.

tients (18%). The median time from the diagnosis of metastatic disease to study entry was 24.4 months (95% CI, 20.2-31.7 months).

At the time of data cutoff on March 1, 2018, the median follow-up was 15.4 months (interquartile range, 4.35-13.25 months). Six patients (21%) achieved a partial response that was confirmed in 4 patients at the subsequent computed tomographic scan assessment (**Table**). The overall response rate was 21% (95% CI, 10%-40%). Nine patients (32%) reported stable disease, for a disease control rate of 54% (95% CI, 36%-70%). Thirteen of 25 patients (52%) assessed for a radiologic response had tumor shrinkage (**Figure 2**A). Three patients (11%) had clinically detectable disease progression before undergoing the first computed tomographic scan reassessment. The median duration of disease control was 9.9 weeks (95% CI, 8.1-23.1 weeks) (Figure 2B). The median PFS was 3.4 months (95% CI, 1.9-3.8 months), and the median OS was 9.8 months (95% CI, 5.2-13.10 months) (eFigure in the Supplement).

eTable 3 in the Supplement shows treatment-related grade 3 or higher adverse events. The most common adverse events were diarrhea (5 [18%]), acneiform skin rash (4 [14%]), neutropenia (4 [14%]), and hand-foot syndrome (2 [7%]). No grade 5 adverse events occurred. No patient interrupted treatment because of adverse events, and treatment withdrawal was not requested by any patient.

A total of 202 cycles (median, 4.5 cycles per patient) were administered. Treatment was delayed for any reason in 29 cycles (14.4%) or because of an adverse event in 11 cycles (5.4%) and was administered with reduced dosage in 35 cycles (17.3%). The mean (SD) relative dose intensities were 87.3% (20.8%) for irinotecan and 94.8% (13.4%) for cetuximab.

As described in eTable 4 in the Supplement, *RAS* mutations were found in liquid biopsy samples collected at the rechallenge baseline in 12 (48%) of 25 patients reassessed by computed tomographic scan (6 *KRAS* G12D, 5 *KRAS* G12V with 1 also harboring a Q61H mutation, and 1 *NRAS* Q61L). No *BRAF* or *PIK3CA* (OMIM 171834) mutations were found.

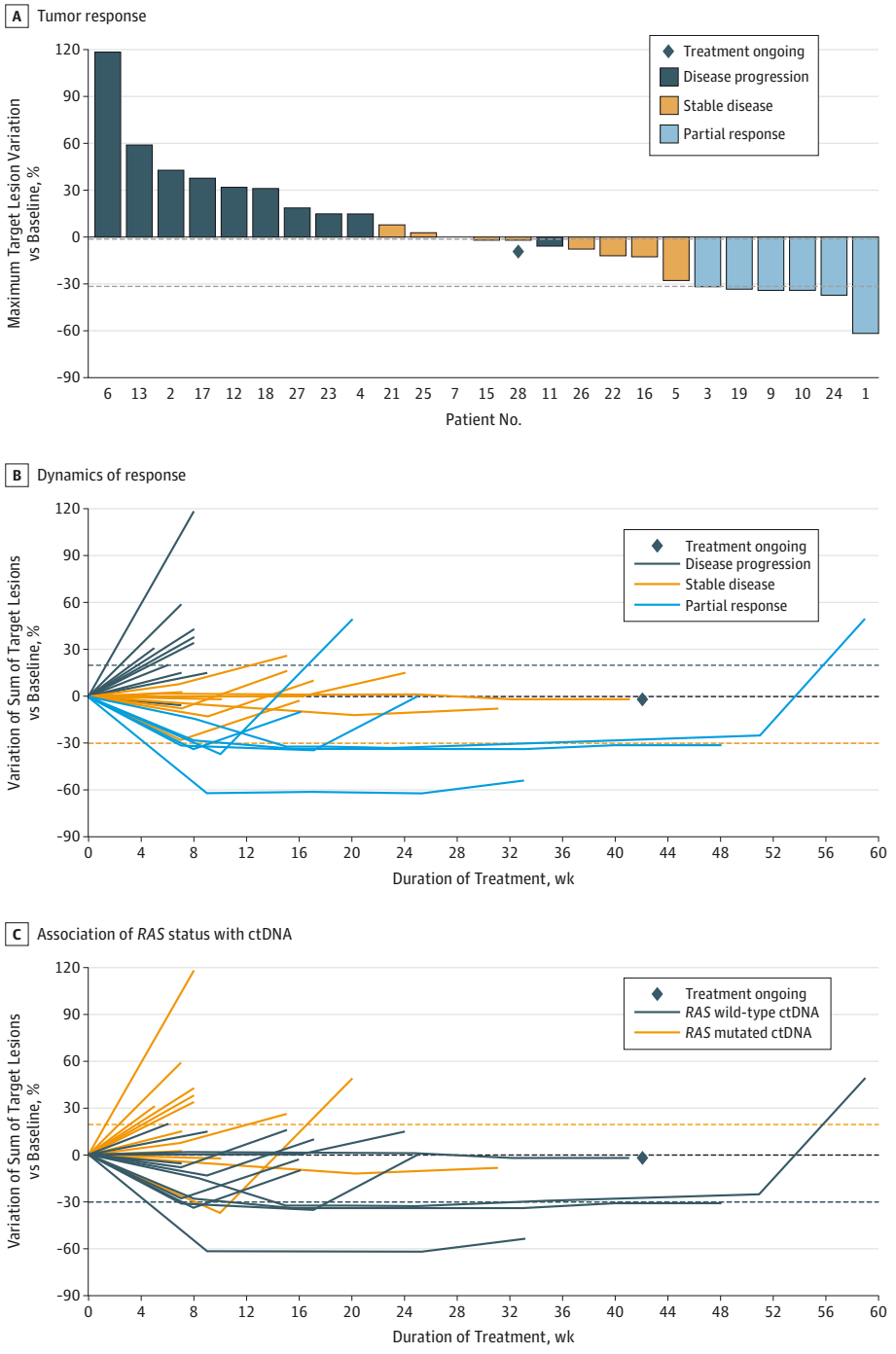
No *RAS* mutations were detected in ctDNA from patients who achieved a confirmed partial response compared with 12 (57%) of 21 patients who did not achieve a partial response ($P = .10$ determined by the Fisher exact test). Patients with *RAS* wild-type ctDNA (n = 13) had significantly longer PFS than did those with *RAS* mutated ctDNA (n = 12), with a median PFS of 4.0 vs 1.9 months (hazard ratio, 0.44; 95% CI, 0.18-0.98; $P = .03$) (**Figure 3**A), while no significant differences were reported in terms of OS (median OS, 12.5 vs 5.2 months; hazard ratio, 0.58; 95% CI, 0.22-1.52; $P = .24$) (Figure 3B). The percentage variation of the sum of target lesions at different time points according to ctDNA *RAS* status at rechallenge baseline is shown in Figure 2C.

Discussion

The therapeutic landscape of later lines of treatment of mCRC has recently become more complex owing to the availability of 2 drugs with demonstrated efficacy in prolonging survival of chemorefractory patients when compared with placebo: the multitarget tyrosine kinase inhibitor regorafenib[25,26] and the novel fluoropyrimidine trifluridine-tipiracil, also known as TAS-102.[27,28] The magnitude of OS benefit provided by both these agents is limited because half of treated patients are unable to derive any advantage in terms of PFS. Moreover, no molecular or clinical factors associated with benefit from these drugs have been identified so far, making their cost-benefit ratio quite narrow. Alternatively, data from uncontrolled studies support other therapeutic strategies for small molecular subgroups, such as dual-targeted *ERBB2/HER2* (OMIM 164870) therapy for *HER2*-positive tumors,[29] immune checkpoints for microsatellite instable tumors,[30,31] and tyrosine kinase inhibition for gene fusion–positive tumors.[32,33]

Here we provide the first prospective demonstration, to our knowledge, of the potential usefulness of another treatment option for a molecularly and clinically defined subgroup of patients with mCRC: rechallenge with cetuximab and irinotecan for patients with *RAS* and *BRAF* wild-type mCRC who experienced an initial benefit and then became resistant to a first-line cetuximab-containing regimen and received second-line oxaliplatin-based chemotherapy plus bevacizumab. Although acknowledging the intrinsic limitations of a single-arm phase 2 study, our results provide a clear signal of activity for the anti-EGFR rechallenge in the third-line setting, in patients with strict clinical and molecular criteria for defining acquired resistance to first-line treatment based on anti-EGFR monoclonal antibodies. Given the increasing amount of

Figure 2. Radiographic Response

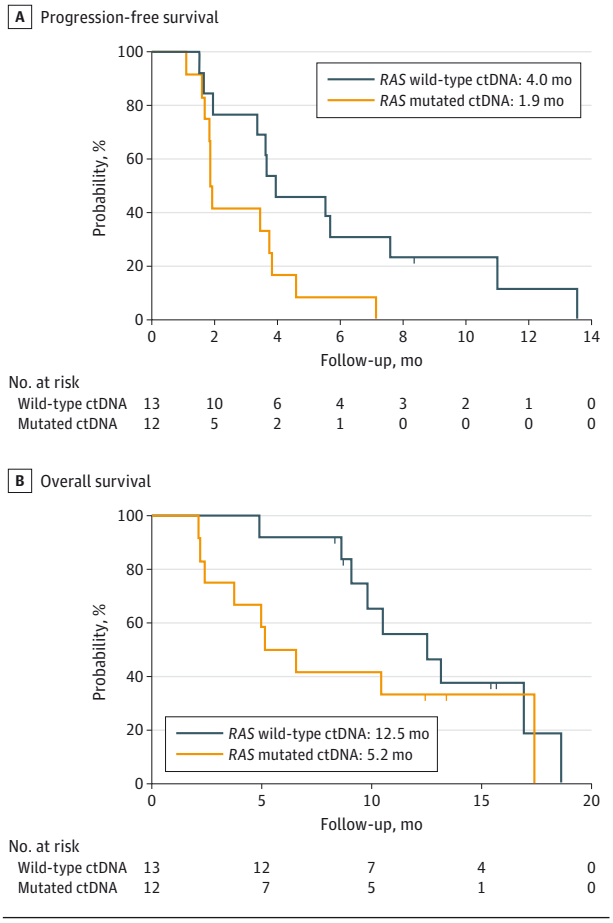


A, Tumor response in 25 evaluable patients. The bars show the best percentage change in the target lesions from baseline. Three patients progressed before the first disease assessment. The dashed horizontal line at −30 indicates the threshold value to define partial response. B, Dynamics of response according to best response in 25 evaluable patients. The individual lines represent the percentage variation of the sum of target lesions at different time points. C, Association of *RAS* status with circulating tumor DNA (ctDNA) in 25 evaluable patients. The longitudinal assessment of the sum of target lesions is shown according to *RAS* mutational status of ctDNA at rechallenge baseline.

evidence of the role of anti-EGFR-based maintenance after first-line induction regimens, the clinical scenario of anti-EGFR rechallenge might be frequently faced in future clinical practice.

The analysis of ctDNA revealed that approximately 50% of these patients still had detectable *RAS* mutations at the time of rechallenge, and it highlights the actual reliability of liquid biopsy as a tool to inform therapeutic decisions. None of the patients with *RAS* mutations in ctDNA at the start of rechallenge achieved response, thus making the choice of rechallenge inappropriate for them. Only 1 patient with a small fractional abundance of *RAS* mutation in ctDNA experienced a transient response to rechallenge. A potential explanation is that, even if *RAS* mutation was still detectable at the time of study entry, a significant drop in its frequency from the time of disease progression at first-line treatment had occurred, thus suggesting a dwindling of the mutant clones and a contemporary increasing prevalence of wild-type cells at the tumor

Figure 3. Kaplan-Meier Estimates of Progression-Free Survival and Overall Survival According to *RAS* and *BRAF* Circulating Tumor DNA (ctDNA) Status



A, Hazard ratio, 0.44 (95% CI, 0.18-0.98; *P* = .03). B, Hazard ratio, 0.58 (95% CI, 0.22-1.52; *P* = .24).

level during second-line therapy. Unfortunately, the lack of longitudinal paired samples during previous lines of therapy does not allow drawing any conclusion about this hypothesis.

Lack of *RAS* mutations in ctDNA is associated in our small series with a probability of 31% of achieving response (13 patients had no *RAS* mutations in ctDNA; 4 of these patients had a response [eTable 4 in the Supplement]). Although we did not identify any *BRAF* or *PIK3CA* mutation in analyzed samples, other mechanisms of acquired resistance to anti-EGFR monoclonal antibodies may occur or co-occur with *RAS* mutations and have been identified in liquid biopsy samples from patients with disease progression.[5,6,9,11,34-36]

However, in spite of intriguing preliminary proof-of-concept evidence about the potential role of liquid biopsy in driving therapeutic choices, the translation of these findings from the bench to the bedside will definitely need robust confirmation from biomarker-driven clinical trials. A further step forward on this route will be marked by the currently ongoing CHRONOS (Phase II Trial of Rechallenge With Panitumumab Driven by RAS Clonal-Mediated Dynamic of Resistance) study (NCT03227926), in which patients eligible for anti-EGFR rechallenge are eligible only if a decrease of at least 50% in the fractional abundance of *RAS* mutations in ctDNA is evident at the time of rechallenge when compared with the time of progression to the first-line anti-EGFR–containing therapy.

Limitations

This study has some limitations. Owing to its phase 2 single-arm design, the CRICKET trial is a proof-of-concept study, able to provide signals of activity and to generate preliminary evidence, supported by a sound translational background, to be further confirmed in a larger clinical trial. The registration of patients at the time of rechallenge (ie, after the second evidence of disease progression) prevents us from deriving any conclusion about how many patients with *RAS* and *BRAF* wild-type tumors who are receiving chemotherapy plus anti-EGFR monoclonal antibodies as initial treatment may be candidates for this third-line approach.

Conclusions

Based on results of the CRICKET trial, whereas trifluridine-tipiracil or regorafenib do still represent third-line options with the highest levels of evidence for patients with chemorefractory mCRC, anti-EGFR rechallenge could be a tailored strategy for selected patients. Although markers of EGFR dependency are still lacking, patients with *RAS* and *BRAF* wild-type left-sided tumors, possibly not showing other molecular mechanisms of intrinsic resistance to EGFR inhibition,[37] who derived clinically meaningful benefit from first-line anti-EGFR–containing therapy, and with undetectable markers of acquired resistance to anti-EGFR monoclonal antibodies in tissue and/or liquid biopsy samples at the time of retreatment, might be the optimal candidates for rechallenge.

ARTICLE INFORMATION

Accepted for Publication: August 29, 2018.

Published Online: November 21, 2018. doi:10.1001/jamaoncol.2018.5080

Author Affiliations: Unit of Medical Oncology 2, Department of Translational Research and New Technologies in Medicine and Surgery, Azienda Ospedaliera Universitaria Pisana, Pisa, Italy (Cremolini, Rossini, Masi, Falcone); Department of Medical Oncology, Campus Bio-Medico University of Rome, Rome, Italy (Dell'Aquila, Pantano, Santini); Medical Oncology Unit 1, Clinical and Experimental Oncology Department, Veneto Institute of Oncology Istituto Oncologico Veneto–Istituto di Ricovero e Cura a Carattere Scientifico, Padua, Italy (Lonardi, Bergamo); Department of Diagnostic Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy (Conca, Busico); Clinical Pharmacology and Pharmacogenetics Unit, Department of Clinical and Experimental Medicine, Pisa, Italy (Del Re, Danesi, Rofi); Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy (Pietrantonio); Department of Oncology, University and General Hospital, Udine, Italy (Aprile, Pella); Department of Oncology, General Hospital San Bortolo, Unità Locale Socio-Sanitaria 8 Berica, Vicenza, Italy (Aprile); Department of Medical Oncology, Infermi Hospital, Rimini, Italy (Tamburini); Medical Oncology Unit, Università Cattolica del Sacro Cuore, Rome, Italy (Barone); Medical Oncology Unit, University Hospital, Parma, Italy (Pucci); Medical Oncology Unit, Fatebenefratelli–Isola Tiberina Hospital,

Rome, Italy (Corsi); Medical Oncology Unit, Presidio Ospedaliero Felice Lotti, Pontedera, Italy (Barbara).

Author Contributions: Dr Falcone had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Cremolini and Rossini contributed equally to this work.
Concept and design: Cremolini, Rossini, Dell'Aquila, Masi, Falcone, Santini.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Cremolini, Rossini, Dell'Aquila, Del Re, Danesi, Rofi.
Critical revision of the manuscript for important intellectual content: Cremolini, Rossini, Lonardi, Conca, Busico, Pietrantonio, Danesi, Aprile, Tamburini, Barone, Masi, Pantano, Pucci, Corsi, Pella, Bergamo, Barbara, Falcone, Santini.
Statistical analysis: Cremolini, Rossini, Pantano, Santini.
Obtained funding: Rossini, Danesi, Falcone, Santini.
Administrative, technical, or material support: Rossini, Dell'Aquila, Conca, Busico, Pietrantonio, Danesi.
Supervision: Rossini, Lonardi, Conca, Tamburini, Barone, Masi, Falcone, Santini.

Conflict of Interest Disclosures: Dr Cremolini reported receiving personal fees from F. Hoffman–La Roche, Bayer, Sirtex, and Amgen. Dr Del Re reported receiving honoraria from Celgene, Sanofi Aventis, Ipsen, and Novartis. Dr Danesi reported receiving research funding from F. Hoffman–La Roche, Amgen, Merck Serono, Celgene, Bayer, Sanofi Aventis, Pfizer, Novartis, and Lilly. Dr Barone reported receiving research funding from Novartis and Merck-Serono; honoraria for serving in an advisory role from Eisai, Servier, Bayer, MSD, Merck-Serono, Amgen, Celgene, and Lilly; and reported serving on speakers' bureaus for Novartis, Roche, MSD, Celgene, and Merck. Dr Falcone reported receiving grants and personal fees from F. Hoffman–La Roche, Amgen, and Merck Serono and personal fees from Celgene, Bayer, and Sanofi Aventis. No other disclosures were reported.

Funding/Support: The CRICKET (Cetuximab Rechallenge in Irinotecan-Pretreated mCRC, *KRAS*, *NRAS* and *BRAF* Wild-Type Treated in 1st line With Anti-EGFR therapy) trial was funded in part by the Gruppo Oncologico Nord Ovest, the ARCO Foundation, and Merck Serono SpA in Italy (an affiliate of Merck KGaA, Darmstadt, Germany). Merck Serono SpA provided the study drug cetuximab.

Role of the Funder/Sponsor: Gruppo Oncologico del Nord Ovest investigators were responsible for the study design, data collection, data analysis, and data interpretation. Gruppo Oncologico del Nord Ovest was also in charge of writing the manuscript and approving its submission for publication. The ARCO Foundation supported molecular analyses but had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Dr Falcone had final responsibility for the decision to submit for publication. Merck Serono SpA had no role in the study's conduction, in data collection and analysis, or in data interpretation. Merck KGaA reviewed the manuscript for medical accuracy only before submission.

Disclaimer: The authors are fully responsible for the content of this manuscript, and the views and opinions described in the publication reflect solely those of the authors

Additional Contributions: We are grateful to all participating patients, their families, and their caregivers, as well as to the Gruppo Oncologico del Nord Ovest investigators from all participating oncology units in Italy.

REFERENCES

1. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology—colon cancer; version 2. https://www.nccn.org/professionals/physician_gls/default.aspx#site. Accessed March 28, 2018.

2. Van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol*. 2016;27(8):1386-1422. doi:10.1093/annonc/mdw235

3. Salvatore L, Aprile G, Arnoldi E, et al. Management of metastatic colorectal cancer patients: guidelines of the Italian Medical Oncology Association (AIOM). *ESMO Open*. 2017;2(1):e000147. doi:10.1136/esmoopen-2016-000147

4. Santini D, Vincenzi B, Addeo R, et al. Cetuximab rechallenge in metastatic colorectal cancer patients: how to come away from acquired resistance? *Ann Oncol*. 2012;23(9):2313-2318. doi:10.1093/annonc/mdr623

5. Siravegna G, Mussolin B, Buscarino M, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med*. 2015;21(7):795-801. doi:10.1038/nm.3870

6. Van Emburgh BO, Arena S, Siravegna G, et al. Acquired *RAS* or *EGFR* mutations and duration of response to EGFR blockade in colorectal cancer. *Nat Commun*. 2016;7:13665. doi:10.1038/ncomms13665

7. Misale S, Yaeger R, Hobor S, et al. Emergence of *KRAS* mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature*. 2012;486(7404):532-536. doi:10.1038/nature11156

8. Arena S, Bellosillo B, Siravegna G, et al. Emergence of multiple *EGFR* extracellular mutations during cetuximab treatment in colorectal cancer. *Clin Cancer Res*. 2015;21(9):2157-2166. doi:10.1158/1078-0432.CCR-14-2821

9. Montagut C, Argilés G, Ciardiello F, et al. Efficacy of Sym004 in patients with metastatic colorectal cancer with acquired resistance to anti-EGFR therapy and molecularly selected by circulating tumor DNA analyses: a phase 2 randomized clinical trial. *JAMA Oncol*. 2018;4(4):e175245. doi:10.1001/jamaoncol.2017.5245

10. Diaz LA Jr, Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature*. 2012;486(7404):537-540. doi:10.1038/nature11219

11. Pietrantonio F, Vernieri C, Siravegna G, et al. Heterogeneity of acquired resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer. *Clin Cancer Res*. 2017;23(10):2414-2422. doi:10.1158/1078-0432.CCR-16-1863

12. Burrell RA, Swanton C. Tumour heterogeneity and the evolution of polyclonal drug resistance. *Mol Oncol*. 2014;8(6):1095-1111. doi:10.1016/j.molonc.2014.06.005

13. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247. doi:10.1016/j.ejca.2008.10.026

14. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053

15. National Cancer Institute. Common terminology criteria for adverse events, version 4.0. Bethesda, MD: May 2009.

16. Meazza C, Belfiore A, Busico A, et al. *AKT1* and *BRAF* mutations in pediatric aggressive fibromatosis. *Cancer Med*. 2016;5(6):1204-1213. doi:10.1002/cam4.669

17. Forbes SA, Beare D, Boutselakis H, et al. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res*. 2017;45(D1):D777-D783. doi:10.1093/nar/gkw1121

18. dbSNP: short genetic variations. Bethesda, MD: National Center for Biotechnology Information, National Library of Medicine. https://www.ncbi.nlm.nih.gov/SNP/. Accessed June 15, 2018.

19. ClinVar. https://www.ncbi.nlm.nih.gov/clinvar/. Accessed June 15, 2018.

20. Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform*. 2013;14(2):178-192. doi:10.1093/bib/bbs017

21. Tournigand C, André T, Achille E, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol*. 2004;22(2):229-237. doi:10.1200/JCO.2004.05.113

22. Sobrero AF, Maurel J, Fehrenbacher L, et al. EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008;26(14):2311-2319. doi:10.1200/JCO.2007.13.1193

23. Fleming TR. One-sample multiple testing procedure for phase II clinical trials. *Biometrics*. 1982;38(1):143-151. doi:10.2307/2530297

24. A'Hern RP. Sample size tables for exact single-stage phase II designs. *Stat Med*. 2001;20(6):859-866. doi:10.1002/sim.721

25. Li J, Qin S, Xu R, et al; CONCUR Investigators. Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol*. 2015;16(6):619-629. doi:10.1016/S1470-2045(15)70156-7

26. Grothey A, Van Cutsem E, Sobrero A, et al; CORRECT Study Group. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet*. 2013;381(9863):303-312. doi:10.1016/S0140-6736(12)61900-X

27. Xu J, Kim TW, Shen L, et al. Results of a randomized, double-blind, placebo-controlled, phase III trial of trifluridine/tipiracil (TAS-102) monotherapy in Asian patients with previously treated metastatic colorectal cancer: the TERRA study. *J Clin Oncol*. 2018;36(4):350-358. doi:10.1200/JCO.2017.74.3245

28. Mayer RJ, Van Cutsem E, Falcone A, et al; RECOURSE Study Group. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. *N Engl J Med*. 2015;372(20):1909-1919. doi:10.1056/NEJMoa1414325

29. Sartore-Bianchi A, Trusolino L, Martino C, et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, *KRAS* codon 12/13 wild-type, *HER2*-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2016;17(6):738-746. doi:10.1016/S1470-2045(16)00150-9

30. Overman MJ, Lonardi S, Wong KYM, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J Clin Oncol*. 2018;36(8):773-779. doi:10.1200/JCO.2017.76.9901

31. Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol*. 2017;18(9):1182-1191. doi:10.1016/S1470-2045(17)30422-9

32. Sartore-Bianchi A, Ardini E, Bosotti R, et al. Sensitivity to entrectinib associated with a novel *LMNA-NTRK1* gene fusion in metastatic colorectal cancer. *J Natl Cancer Inst*. 2015;108(1):djv306. doi:10.1093/jnci/djv306

33. Drilon A, Siena S, Ou SI, et al. Safety and antitumor activity of the multitargeted Pan-*TRK*, *ROS1*, and *ALK* inhibitor entrectinib: combined results from two phase I trials (ALKA-372-001 and STARTRK-1). *Cancer Discov*. 2017;7(4):400-409. doi:10.1158/2159-8290.CD-16-1237

34. Russo M, Siravegna G, Blaszkowsky LS, et al. Tumor heterogeneity and lesion-specific response to targeted therapy in colorectal cancer. *Cancer Discov*. 2016;6(2):147-153. doi:10.1158/2159-8290.CD-15-1283

35. Xu J-M, Wang Y, Wang Y-L, et al. *PIK3CA* mutations contribute to acquired cetuximab resistance in patients with metastatic colorectal cancer. *Clin Cancer Res*. 2017;23(16):4602-4616. doi:10.1158/1078-0432.CCR-16-2738

36. Oddo D, Siravegna G, Gloghini A, et al. Emergence of *MET* hyper-amplification at progression to *MET* and *BRAF* inhibition in colorectal cancer. *Br J Cancer*. 2017;117(3):347-352. doi:10.1038/bjc.2017.196

37. Cremolini C, Morano F, Moretto R, et al. Negative hyper-selection of metastatic colorectal cancer patients for anti-EGFR monoclonal antibodies: the PRESSING case-control study. *Ann Oncol*. 2017;28(12):3009-3014. doi:10.1093/annonc/mdx546