

RESEARCH ARTICLE

<https://doi.org/10.1158/2767-9764.CRC-25-0002>

OPEN ACCESS



Clinical Validity of FoundationOne Liquid CDx for Detection of *BRAF*^{V600E} in Colorectal Cancer

Rona Yaeger¹, Jean-François Martini², Lincoln Pasquina³, Brian Tunkist⁴, Xiaosong Zhang⁵, Fatima Kaiser⁶, Norberto Pantoja Galicia⁷, Shijing Deng², Siliang Gong⁶, Cui Guo⁷, Jimmy Kiely⁷, Ta-Chou Vincent Ng⁷, Graham Ferrier⁸, Josep Tabernero⁹, and Scott Kopetz¹⁰

ABSTRACT

Purpose: The BRAF inhibitor encorafenib (Enco) plus the anti-EGFR antibody cetuximab (Cetux) improved overall survival, objective response rate, and progression-free survival in previously treated *BRAF*^{V600E}-mutant metastatic colorectal cancer in BEACON, a phase III randomized trial, leading to regulatory approval for this indication. To support rapid, plasma-based testing for *BRAF*^{V600E} identification, clinical validity of a ctDNA-based assay, FoundationOneLiquid CDx (F1LCDx), was assessed against the reference tumor-based clinical trial assay (CTA) in liquid biopsy-evaluable samples from BEACON and commercially obtained tissue-matched plasma samples.

Patients and Methods: Pretreatment tissue samples were collected in BEACON to confirm *BRAF* mutational status using the central single gene PCR assay. Concordance between the CTA and liquid biopsy tests was assessed, and clinical validity of liquid biopsy testing was examined using clinical outcomes from BEACON.

Results: Of the 523 evaluable patients, 433 with matched tissue and plasma samples had CTA and F1LCDx results available (BEACON, *n* = 328; commercial, *n* = 105). A strong concordance in detecting

BRAF^{V600E} was found between F1LCDx and CTA, with a positive percent agreement of 87.2% and negative percent agreement of 97.1%. Among 42 F1LCDx-CTA+ samples, 41 (97.6%) had ctDNA tumor fraction <1%. Among samples with ctDNA tumor fraction >1%, the positive percent agreement was 99.4% and negative percent agreement was 86.7%. Clinical outcomes with Enco plus Cetux were similar between those identified as F1LCDx+/CTA+ and CTA+ overall.

Conclusions: This study supports using liquid biopsies as a clinically valid assay for identifying *BRAF*^{V600E} alterations in patients with metastatic colorectal cancer, particularly when ctDNA tumor fraction was >1%.

Significance: In the phase III BEACON trial, which established Enco plus Cetux as a standard of care for previously treated *BRAF*^{V600E}-mutant metastatic colorectal cancer, mutational status was confirmed through testing of tumor tissue. To support rapid, less invasive testing for *BRAF*^{V600E} in plasma, this retrospective study assessed a ctDNA-based assay and found strong concordance between the liquid biopsy test and the tumor-based assay in detecting *BRAF*^{V600E}.

Introduction

Advances in detection of ctDNA have made liquid biopsy (LBx) an increasingly utilized method for tumor profiling (1). ctDNA-based tumor profiling is less invasive and has a shorter turnaround time than tissue-based DNA profiling, which is critical for patient diagnosis and management. In addition, LBx allows for multiple serial sample collections, which can be useful for reflecting real-time tumor information, detecting disease progression, monitoring treatment response, and identifying potential biomarkers of resistance or sensitivity (2, 3). In gastrointestinal cancer trials, ctDNA genotyping has resulted in significantly reduced screening time and improved trial enrollment without compromising treatment efficacy compared with tissue genotyping (4). In recent years, several ctDNA-based companion diagnostic assays, including FoundationOne Liquid CDx (F1LCDx), cobas EGFR Mutation Test v2, and Guardant360 CDx, were approved by the US FDA to detect biomarkers in patients with non-small cell lung cancer (5). Although the treatment of metastatic colorectal cancer (mCRC) often involves resection which allows tissue availability, detection of targetable

¹Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York. ²Translational Science Operations, Pfizer, Inc., La Jolla, California. ³Clinical Development, Foundation Medicine Inc., Boston, Massachusetts. ⁴Formerly Pfizer, Inc., New York, New York. ⁵Global Development, Pfizer, Inc., South San Francisco, California. ⁶Formerly Foundation Medicine Inc., Boston, Massachusetts. ⁷Biometrics, Foundation Medicine Inc., Boston, Massachusetts. ⁸Global Medical, Pfizer, Inc., New York, New York. ⁹Department of Medical Oncology, Vall d'Hebron Hospital Campus and Vall d'Hebron Institute of Oncology (VHIO), University of Vic – Central University of Catalonia, Barcelona, Spain. ¹⁰Department of Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, Texas.

Corresponding Author: Jean-François Martini, Formerly Translational Science Operations, Pfizer, Inc., La Jolla, 10770 Science Center Drive, San Diego, CA 92121. E-mail: jf_martini@yahoo.com

doi: 10.1158/2767-9764.CRC-25-0002

This open access article is distributed under the Creative Commons Attribution 4.0 International (CC BY 4.0) license.

©2025 The Authors; Published by the American Association for Cancer Research

alterations by LBx can be a clinically desirable and less invasive disease-monitoring option after first-line treatment (6). Although archived tissue biopsy only reflects static tumor information, LBx can reflect real-time tumor information and allows dynamic monitoring of mutations during disease progression (3). mCRC is among the most common tumor types for detectability of ctDNA (7, 8), making LBx a particularly attractive option for tumor profiling. However, ctDNA-based assays vary in genes tested, methodology, and sensitivity (9).

The BEACON trial was a randomized, open-label, multicenter, three-arm, phase III study that compared the efficacy and safety of encorafenib (Enco; Braftovi) + binimetinib (Mektovi) + cetuximab (Cetux; Erbitux) and the Enco + Cetux arm with irinotecan/Cetux or FOLFIRI/Cetux (control arm) in patients with a $BRAF^{V600E}$ alteration in colorectal cancer whose disease had progressed after one or two prior regimens in the metastatic setting (10). The clinical trial tested the efficacy of therapy, with enrollment based on tissue-based PCR and next-generation sequencing (NGS)-based local assay results with confirmation by clinical trial assay [CTA; the investigational Qiagen Therascreen $BRAF^{V600E}$ RGQ PCR Kit (Qiagen)] or enrollment through CTA to select patients with colorectal cancer whose tumors harbor the $BRAF^{V600E}$ alteration. Enco in combination with Cetux is the first and only FDA-approved targeted therapy regimen for the treatment of adult patients with mCRC with a $BRAF^{V600E}$ mutation after prior therapy based on the efficacy population of the Enco + Cetux arm ($n = 220$) versus the control arm ($n = 221$). Overall survival (OS) and objective response rate (ORR) by RECIST version 1.1 were the major efficacy outcomes, and the median progression-free survival (PFS) and duration of response were additional efficacy outcome measures. For patients treated in the Enco + Cetux arm of the study, the median OS was 8.4 months [95% confidence interval (CI), 7.5–11.0] and the median PFS was 4.2 months (95% CI, 3.7–5.4). ORR (20%; 95% CI, 13%–29%) and duration of response (6.1 months; 95% CI, 4.1–8.3) were both assessed in the subset of the first 220 patients who were included in the randomized portion of the Enco + Cetux ($n = 113$) and control ($n = 107$) arms.

The Qiagen Therascreen assay is a real-time PCR *in vitro* diagnostic device for the qualitative detection of the $BRAF^{V600E}$ alteration in patients with mCRC. The assay detects the V600E alteration in the $BRAF$ gene using genomic DNA extracted from formalin-fixed, paraffin-embedded colorectal cancer tissue. The FDA granted pre-market approval to the Qiagen Therascreen for use as a companion diagnostic to identify patients with mCRC whose tumors harbor the $BRAF^{V600E}$ alteration to be treated with Enco in combination with Cetux (11). FILCDx is a qualitative NGS-based *in vitro* diagnostic test that uses targeted high-throughput hybridization-based capture technology to detect and report substitutions, insertions, and deletions in 311 genes. FILCDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood collected from patients with cancer and is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies (12). The FILCDx test is the first LBx companion diagnostic assay approved by the FDA that offers comprehensive tumor profiling to identify $BRAF^{V600E}$ alterations in patients with mCRC who are eligible for treatment with Enco in combination with Cetux (13).

This clinical bridging study evaluated the concordance between CTA and FILCDx and the clinical efficacy of Enco in combination with Cetux in patients with mCRC harboring the $BRAF^{V600E}$ mutation identified by FILCDx, through retrospective analysis of banked plasma samples from patients enrolled in the BEACON study. In addition, in a *post hoc* exploratory analysis not prespecified in the clinical bridging statistical analysis plan, we further investigated the sensitivity of FILCDx as a function of ctDNA content as measured by ctDNA tumor fraction (TF).

Materials and Methods

BEACON ethical statement

The BEACON trial was approved by the institutional review board or independent ethics committee at each center and was conducted in accordance with the requirements of the regulatory authorities of each country and with the provisions of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Council on Harmonization (10). All patients provided written informed consent.

Clinical bridging study design and samples

The BEACON CTA was performed on tumor tissue collected any time prior to trial enrollment using the Qiagen Therascreen $BRAF^{V600E}$ RGQ PCR Kit. This study was conducted to evaluate the concordance between the CTA and a CTA based on FILCDx (LBx test) in identifying patients with mCRC harboring $BRAF^{V600E}$ from the Enco + Cetux and control arms. Based on this, the efficacy in the Enco + Cetux arm versus the control arm in patients identified as $BRAF^{V600E}$ -positive by the LBx test was estimated, and the robustness of concordance analysis and efficacy analysis was assessed with a sensitivity analysis that accounts for the uncertainty due to missing data for the $BRAF^{V600E}$ alteration status as defined by the LBx test.

Pretreatment plasma samples were collected at the time of trial screening from participants in the BEACON trial by the therapeutic investigational sites per the study protocol and shipped to the College of American Pathologists–Clinical Laboratory Improvement Amendments–certified central testing laboratories. LBx testing was performed on available plasma samples from patients in the Enco + Cetux and control arms in the BEACON clinical trial who tested positive for $BRAF^{V600E}$ by CTA (CTA+). Additionally, commercially procured $BRAF^{V600E}$ -negative tissue samples from patients with colorectal cancer with matched plasma were tested. The LBx test clinical bridging study involved retrospective testing of plasma samples from the BEACON clinical trial; as such, no additional patient follow-up was conducted.

Assessment of detection on FILCDx was performed without knowledge of the CTA results. The analysis plan was created without visibility into clinical information. Assessment of concordance was performed as pre-specified, independent of the clinical information. Clinical information was available during the assessment of clinical validity. For the Qiagen Therascreen test, the limit of detection (LoD) for the $BRAF^{V600E}$ system is 7.8% [mutant allele frequency (MAF)], which represents the highest LoD value observed across all DNA inputs; however, with high DNA input, the LoD is 2% MAF (14). For FILCDx, the LoD (the lowest MAF at which 95% of target variants are detected) for $BRAF^{V600E}$ in colorectal cancer samples is 0.33% MAF (15).

Inclusion criteria for samples included in this clinical bridging study were specimens that were in frozen plasma, met the minimum criteria for LBx operational testing requirements, and were obtained with appropriate consent and institutional review board oversight to allow clinical bridging. Samples must have cfDNA input for LBx testing ≥ 20 ng DNA for primary analysis, as assessed by the TapeStation assay (RRID: SCR_018435). Exclusion criteria included tissue and other liquid samples, samples that do not meet minimum LBx test operational testing requirements, and samples not obtained with appropriate consent and institutional review board oversight.

to allow clinical bridging. Samples with <20 ng DNA as assessed by the TapeStation assay were excluded from LBx testing.

Specimens included in the clinical bridging study were tested according to the standard testing protocol for the LBx test with recommended cfDNA input of ≥20 ng for the library construction step. Samples for the study were selected from both plasma samples from patients enrolled in the BEACON trial and commercially procured plasma samples from patients with colorectal cancer with matched tissue who were *BRAF*^{V600E}-negative. Figure 1 shows the sample disposition for the 694 samples from the 665 patients from the BEACON trial. Samples from the patients randomized to the Enco + Cetux (220 samples) and control (221 samples) arms were selected for analysis; 37 samples from the safety lead-in, 212 samples from the Enco + binimetinib + Cetux arm, three samples from on-treatment blood draws, and one sample from a screen failure were excluded. An additional 51 samples were unavailable for sequencing, and 35 samples with assay failures were excluded, resulting in a total of 355 clinical trial samples (170 control + 185 Enco + Cetux) processed in this bridging study. There were 441 patients with plasma samples from the control and Enco + Cetux arms, consisting of 402 CTA+, 31 CTA unevaluable, and eight CTA- samples. In addition, *BRAF*^{V600E}-negative commercial tissue-matched plasma samples from 120 patients not enrolled in the BEACON study were also tested. To mimic the BEACON study enrollment, the 120 commercial tissue samples were tested with the CTA, and the matched plasma samples were subsequently tested with the LBx test. LBx test results for plasma testing were observed for tissue samples that had a negative CTA result.

Concordance analysis

The concordance between the CTA and the FILCDx tests was evaluated by the positive percent agreement (PPA) and negative percent agreement (NPA), as well as their two-sided 95% Wilson score CIs. PPA for FILCDx+/CTA+ was calculated using the formula $PPA = 100 \times \frac{n11}{(n11+n10)}\%$ and NPA for FILCDx-/CTA- was calculated using the formula $NPA = 100 \times \frac{n00}{(n00+n01)}\%$, in which n11 is defined as the number of FILCDx+/CTA+ samples, n10 is defined as the number of FILCDx-/CTA+ samples, n00 is defined as the number of FILCDx-/CTA- samples, and n01 is defined as the number of FILCDx+/CTA- samples. The prevalence-adjusted positive predictive values (PPV), negative predictive values, and their two-sided 95% bootstrapping CIs were calculated by adjusting for the prevalence of *BRAF*^{V600E} mutations among the intention-to-treat population, with 5% and 10% as the estimated prevalence. The ctDNA TF was quantified using a combination of aneuploidy and variant allele frequencies of genomic alterations, whereas mutations and aneuploidy derived from clonal hematopoiesis were excluded using fragmentomic signal from cfDNA (16, 17).

Clinical validation

A description of the BEACON study endpoints was previously published (10); details are described in the Supplementary Materials. In the present analysis, the clinical validity of the LBx test was evaluated by assessing clinical efficacy in the FILCDx *BRAF*^{V600E}-positive population (FILCDx+) based on the ORR difference between the Enco + Cetux and control arms, as well as the log HR between the two arms from the Cox regression model.

Sensitivity analysis

To assess the robustness of the results subject to missing LBx test results, a sensitivity analysis was performed. Multiple imputation plus bootstrapping were used to impute the FILCDx *BRAF*^{V600E} status in the LBx-unevaluable population (i.e., no sample was available for testing or the assay failed). The concordance analysis and the clinical efficacy for FILCDx+ patients were updated by accounting for the imputed data. Multiple imputation was conducted in the original dataset and a total of 50 imputation datasets were generated. The PPA and PPV estimates were computed for each of the 50 imputed complete datasets.

Statistical analysis

For the concordance analysis results, 95% two-sided CIs were calculated using the Wilson score method for PPA and NPA, whereas they were calculated using the bootstrap method for the adjusted PPV and negative predictive value. For the efficacy results, log HR between the Enco + Cetux and control arms was estimated using the Cox proportional hazards model. For the ORR difference between the Enco + Cetux and control arms, the corresponding 95% two-sided CI was calculated using the Newcombe method.

Data availability

Upon request, and subject to review, Pfizer will provide the data that support the findings of this study. Subject to certain criteria, conditions, and exceptions, Pfizer may also provide access to the related individual de-identified participant data. See <https://www.pfizer.com/science/clinical-trials/trial-data-and-results> for more information.

Results

Baseline characteristics

Overall, the baseline patient demographics and disease characteristics were similar between patient samples from the LBx-evaluable/CTA+ and LBx-unevaluable/CTA+ subsets (Table 1) and were reflective of the intention-to-treat population in BEACON (10). The representativeness of the analysis population is described in Supplementary Table S1. The median age of patients was 61.0 and 59.5 years in the LBx-evaluable/CTA+ and LBx-unevaluable/CTA+ subsets, respectively. Most patients had Eastern Cooperative Oncology Group performance status of 0 or 1 in both subsets (99.1% in LBx evaluable/CTA+ and 98.7% in LBx unevaluable/CTA+). Most patients had one previous line of therapy (65.9% in LBx evaluable/CTA+ and 64.9% in LBx unevaluable/CTA+).

Concordance between the CTA and LBx test

To assess the concordance between the CTA and LBx test in detecting *BRAF*^{V600E}, a total of 523 CTA-evaluated specimens (402 CTA+ and 121 CTA-) from patients enrolled in the BEACON trial and commercially procured plasma samples were further evaluated. Among the 523 specimens, 481 (360 CTA+ and 121 CTA-) had available plasma samples for LBx testing (Supplementary Table S2). Among the 481 samples analyzed retrospectively by the LBx test, 433 samples (90%) yielded valid FILCDx testing results. Of the total 402 CTA+ samples, 328 samples were evaluable for the LBx test, with 286 FILCDx+ and 42 negative for *BRAF*^{V600E} by FILCDx (FILCDx-; Table 2). The resulting PPA was 87.2% (95% two-sided Wilson

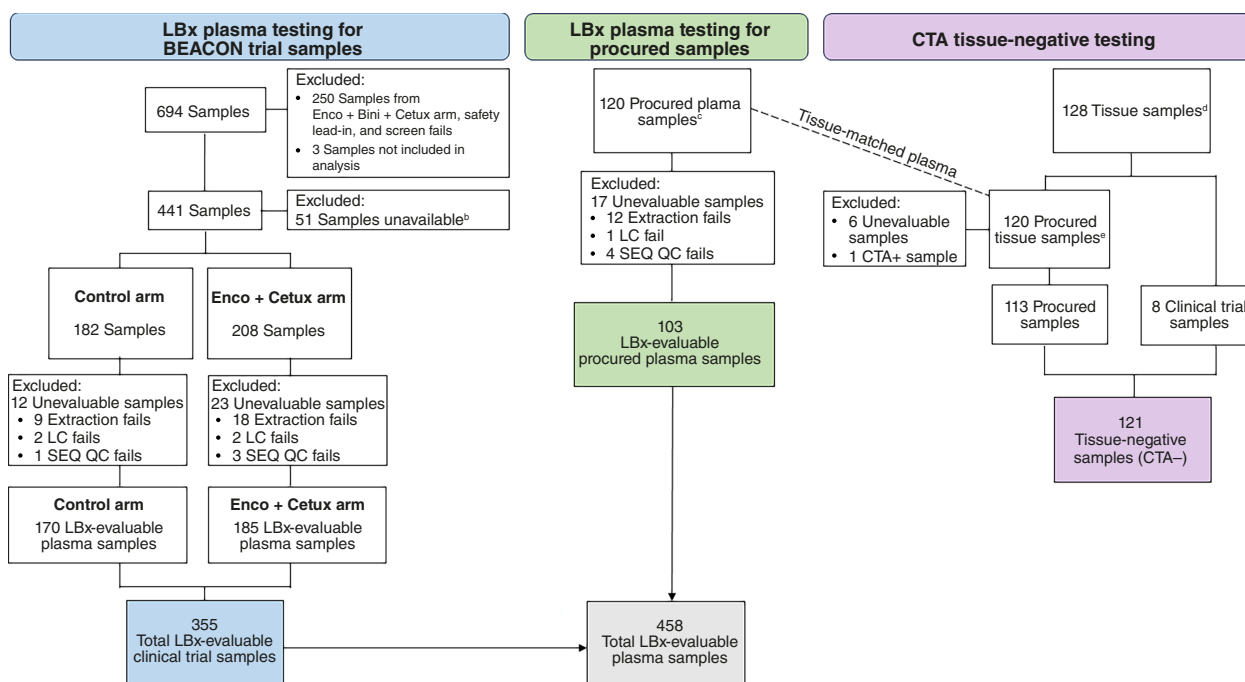


FIGURE 1 Sample processing flow chart. LC, library construction; QC, quality control; SEQ, sequencing. ^aData from these samples are not included in this study. ^bPlasma sample was not available for testing with LBx. ^cMatching plasma comes from clinical trial samples. ^dTested externally by the Qiagen Therascreen *BRAF* V600E RGQ PCR Kit. ^eCommercially procured tissue-matched plasma samples that were *BRAF* wild-type by tissue testing and were used for concordance testing.

score CI, 83.1%–90.4%; Table 3). Of the 42 cases that were positive for *BRAF*^{V600E} by the tissue-based CTA but not FILCDx (FILCDx–/CTA+), 41 (97.6%) had ctDNA TF <1% (Supplementary Table S3). Among samples with ctDNA TF >1%, PPA was 99.4% (95% two-sided Wilson score CI, 96.7%–99.9%) and NPA was 86.7% (95% two-sided Wilson score CI, 62.1%–96.3%; Table 3). Among the patients screened, 105 patients who were negative for *BRAF*^{V600E} by the CTA (CTA–) were included in the concordance analysis (Table 3). Of those, 102 were also found to be *BRAF*^{V600E} negative by the LBx test (FILCDx–/CTA–) and three were *BRAF*^{V600E} positive by the LBx test (FILCDx+/CTA–). The resulting NPA was 97.1% (95% two-sided Wilson score CI, 91.9%–99.0%). Among the FILCDx+/CTA– samples, one (33.3%) had ctDNA TF <1% (Supplementary Table S3).

Clinical validity of the LBx test

We next evaluated the clinical outcomes of patients from the BEACON trial who would have been identified by LBx testing. The median OS of patients who were FILCDx+/CTA+ (7.6 and 5.4 months in the Enco + Cetux and control arms, respectively; log HR = –0.5) was similar to that of the CTA+ cohort (9.5 and 5.9 months in the Enco + Cetux and control arms, respectively; log HR = –0.5; Table 4). The ORR was similar between patients who were FILCDx+/CTA+ (18.5% and 1.4% in the Enco + Cetux and control arms, respectively) and in the CTA+ cohort (19.9% and 1.5% in the Enco + Cetux and control arms, respectively; Table 4). The ORR difference (17.1%) between the Enco + Cetux and control arms and the log HR (–0.5) in the FILCDx+/CTA+ group were similar to those in the CTA+ group (ORR difference, 18.4%; log HR = –0.5). There were 16 responders with complete

or partial response who were unevaluable by LBx, resulting in a bigger ORR difference (25.7%) in the FILCDx-unevaluable/CTA+ population. After imputing the CDx detection status for the LBx-unevaluable samples, the ORR of patients treated with Enco + Cetux improved for the FILCDx+/CTA+ cohort (mean ORR difference of 18.9% and mean log HR = –0.5) compared with the CTA+ cohort (ORR difference of 18.4%; log HR = –0.5).

Forty-two patients were found to be FILCDx–/CTA+. The median OS for the FILCDx–/CTA+ cohort was not available in the Enco + Cetux arm because of the small number of events and 12.2 months in the control arm (log HR = –2.7). The ORR in this cohort was 17.4% and 0.0% in the Enco + Cetux and control arms, respectively. Furthermore, 74 patients were FILCDx-unevaluable/CTA+. The median OS for this cohort was 18.9 and 7.2 months in the Enco + Cetux and control arms, respectively (log HR = –0.4). The ORR was 28.1% and 2.4% in the Enco + Cetux and control arms, respectively.

Sensitivity analysis

To assess the robustness of the concordance analysis and efficacy analysis, a sensitivity analysis was performed to account for the missing data for the *BRAF*^{V600E} alteration status in the LBx-unevaluable population. After including imputed data, the estimated median PPA was 85.5%, median PPV (10% prevalence parameter) was 76.9%, and median PPV (15% prevalence parameter) was 84.1% (Supplementary Table S4). For the FILCDx+/CTA+ population, the median ORR difference after including imputed data was 19.0 (log HR = –0.53; Supplementary Table S5). The estimated efficacy results for the FILCDx+ population were comparable with those for the

TABLE 1 Demographics and clinical characteristics in the LBx test-evaluable and -unevaluable subsets

Covariate	CTA+ ^a (n = 402)	LBx evaluable/CTA+ (n = 328)	LBx unevaluable/CTA+ (n = 74)
Age			
Median (range), years	61 (27–91)	61 (27–91)	60 (29–80)
Sex, n (%)			
Female	212 (52.7)	173 (52.7)	39 (52.7)
Male	190 (47.3)	155 (47.3)	35 (47.3)
ECOG PS, n (%)			
0	197 (49.0)	154 (47.0)	43 (58.1)
1	201 (50.0)	171 (52.1)	30 (40.5)
2	4 (1.0)	3 (0.9)	1 (1.4)
Location of primary tumor, n (%)			
Both sides	28 (7.0)	24 (7.3)	4 (5.4)
Left colon	137 (34.1)	109 (33.2)	28 (37.8)
Right colon	213 (53.0)	178 (54.3)	35 (47.3)
Unknown colon	24 (6.0)	17 (5.2)	7 (9.5)
Involvement of ≥3 organs, n (%)	185 (46.0)	157 (47.9)	28 (37.8)
Presence of liver metastases, n (%)	232 (57.7)	194 (59.2)	38 (51.4)
Primary tumor removed, n (%)			
Completely resected	228 (56.7)	190 (57.9)	38 (51.4)
Partially resected or unresected	174 (43.3)	138 (42.1)	36 (48.7)
Previous lines of therapy, n (%)			
1	264 (65.7)	216 (65.9)	48 (64.9)
2 ^b	137 (34.1)	111 (33.8)	26 (35.1)
High microsatellite instability ^c , n (%)	31 (9.5)	25 (8.7)	6 (15.8)
Baseline carcinoembryonic antigen level >5 µg/L, n (%)	301 (75.1)	256 (78.1)	45 (61.6)
Baseline C-reactive protein level >10 mg/L, n (%)	152 (38.4)	126 (39.0)	26 (35.6)
Irinotecan status, n (%)	206 (51.2)	172 (52.4)	34 (46.0)

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status.

^aOf the 441 patients in the control and Enco + Cetux arms, 31 were CTA not evaluable and eight were CTA–; therefore, these were excluded, resulting in 402 samples.

^bOne patient in the control arm received more than two previous lines of therapy.

^cHigh microsatellite instability was determined by PCR.

CTA+ population (and were better when $c = 100\%$; Supplementary Table S6).

Discussion

Overall, we found a high concordance between the LBx test and the CTA in detecting $BRAF^{V600E}$ in samples prospectively collected from patients in the

TABLE 2 Contingency table comparing $BRAF^{V600E}$ status between the CTA and F1LCDx

	CTA+	CTA–	Total
F1LCDx+	286	3	289
F1LCDx–	42	102	144
F1LCDx unevaluable	74	16	90
Total	402	121	523

phase III BEACON study, with PPA and NPA values similar to previous concordance analyses between tissue and LBx testing (12, 18–20). The clinical validity of the F1LCDx test in identifying patients with $BRAF^{V600E}$ was comparable with that observed in the CTA group, which supports the reasonable assurance of effectiveness of the LBx test. The discordance observed in the F1LCDx–/CTA+ and F1LCDx+/CTA– samples may be attributed to the low ctDNA TF (<1%) among these samples. For samples with low ctDNA TF, concurrent tissue and ctDNA testing should be considered to improve the detection of $BRAF^{V600E}$ mutations (18).

The clinical outcomes of patients treated with Enco + Cetux were similar between those in the F1LCDx+/CTA+ cohort and those in the CTA+ cohort. Of note, after imputing the CDx detection status for F1LCDx-unevaluable samples, efficacy for the F1LCDx+/CTA+ population treated with Enco + Cetux improved compared with that of the CTA+ population. These results support the utility of LBx testing for identifying patients with mCRC who have a qualifying $BRAF^{V600E}$ mutation and may benefit from treatment with Enco in combination with Cetux.

TABLE 3 Concordance analysis results

	Prevalence, %	Concordant result with CTA and F1LCDx test	Denominator ^a	Point estimate, (95% two-sided CI ^b), %
PPA	NA	286	328	87.2 (83.1–90.4)
NPA	NA	102	105	97.1 (91.9–99.0)
Adjusted PPV	5	NA	NA	61.6 (40.9–100.0)
Adjusted NPV	5	NA	NA	99.3 (99.1–99.5)
Adjusted PPV	10	NA	NA	77.2 (59.4–100.0)
Adjusted NPV	10	NA	NA	98.6 (98.2–98.9)
ctDNA TF >1%				
PPA	NA	169	170	99.4 (96.7–99.9)
NPA	NA	13	15	86.7 (62.1–96.3)
Adjusted PPV	5	NA	NA	28.2 (13.6–100.0)
Adjusted NPV	5	NA	NA	100.0 (99.9–100.0)
Adjusted PPV	10	NA	NA	45.3 (24.9–100.0)
Adjusted NPV	10	NA	NA	99.9 (99.7–100.0)

Abbreviations: NA, not available; NPV, negative predictive values.

^aThe denominator for PPA is the total number of CTA+ samples among the F1LCDx-evaluable samples. The denominator for NPA is the total number of CTA– samples among the F1LCDx-evaluable samples.

^bCI was calculated using the Wilson score method for PPA and NPA, whereas for the adjusted PPV and NPV, the bootstrap method was used.

Patients who are not able to provide evaluable tumor tissue for genomic testing or who face significant health risks from the procedures required to obtain a biopsy may have limited options, and a well-validated and reliable LBx test could provide a pragmatic alternative to tissue biopsy for identifying patients with mCRC who might benefit from Enco in combination with Cetux. The use of multi-gene panels such as F1LCDx enables identification of other alterations within colorectal cancer (e.g., KRAS) or across tumor types (e.g., NTRK1/2/3) which are relevant to diagnosis and potential treatment options for patients (21, 22). In addition to supporting the identification of patients with BRAF^{V600E} mutations who may benefit from treatment with Enco in combination with Cetux, a comprehensive genomic profiling test such as F1LCDx may provide an opportunity for monitoring genomic alterations associated with acquired resistance alterations (23). Furthermore, colorectal cancer is mainly treated in the community, and

patients may not be followed systemically in the same center throughout the patient journey. This may require that tissue samples be requested from other institutions, which can be challenging and often lead to delays in testing. LBx offers a more straightforward approach as blood samples can be collected easily and test results can be available to physicians within 2 weeks, leading to appropriate, informed, and personalized treatment strategies for patients. Rapid genomic testing has become increasingly important given the recent approval of Enco, Cetux, and mFOLFOX6, including in the first-line setting, for BRAF^{V600E} mCRC.

Although interpretation is limited by the smaller size of the F1LCDx–/CTA+ cohort, a numerically reduced estimated efficacy of Enco + Cetux in the F1LCDx–/CTA+ cohort compared with ORR outcomes in the CTA+ cohort was observed. Additionally, among patients in the F1LCDx–/CTA+

TABLE 4 Primary efficacy in the bridging study subpopulations

	CTA+ (n = 402)	F1LCDx+/CTA+ (n = 286)	F1LCDx–/CTA+ (n = 42)	F1LCDx unevaluable/CTA+ (n = 74)
ORR for Enco + Cetux arm, %	19.9	18.5	17.4	28.1
ORR for control arm, %	1.5	1.4	0	2.4
ORR difference (95% two-sided CI), ^a %	18.4 (12.7–24.6)	17.1 (10.5–24.2)	17.4 (–2.4 to 37.1)	25.7 (9.7–43.1)
Median OS for Enco + Cetux arm, months	9.5	7.6	NA ^b	18.9
Median OS for control arm, months	5.9	5.4	12.2	7.2
Log HR (95% two-sided CI)	–0.5 (–0.8 to –0.3)	–0.5 (–0.8 to –0.2)	–2.7 (–4.7 to –0.7)	–0.4 (–1.2 to 0.3)

Abbreviation: NA, not available.

^aCI was calculated using the Newcombe method.

^bThe estimated median OS is not available because of the small number of events in this group (three events).

cohort, a numerically improved OS compared with patients in the CTA+ cohort and F1LCDx+/CTA+ cohort was observed. This may be due to a larger tumor bulk needed for detectable ctDNA shedding. Patients with smaller tumor volumes could have better disease prognosis and may thus contribute to the better survival outcomes in the F1LCDx+/CTA+ cohort. This is consistent with the association observed between undetectable or low levels of ctDNA at baseline and/or on treatment and improved PFS and OS across different treatment regimens and tumor types (24–28). To avoid missed detection of *BRAF*^{V600E} mutations, additional tissue testing should be considered if no alterations are detected by the LBx test or if samples have a low ctDNA TF <1% (16, 29, 30). Further investigation is warranted to explore the tumor burden of the disease at baseline in the F1LCDx+/CTA+ and F1LCDx+/CTA+ subsets.

In this retrospective bridging study, the main diagnostic was a tumor tissue-based single gene PCR assay (Qiagen Therascreen), whereas the F1LCDx is an NGS-based comprehensive genomic profiling assay performed on ctDNA extracted from blood. This retrospective bridging was therefore only possible for a subset of participants who had available plasma samples. Additionally, this analysis was not statistically powered.

Overall, this clinical bridging study supports using the LBx test as a clinically valid assay for identifying *BRAF*^{V600E} alterations in patients with mCRC who may be eligible for treatment with Enco in combination with Cetux.

Authors' Disclosures

R. Yaeger reports grants and personal fees from Mirati Therapeutics, Revolution Medicines, Eli Lilly, and Company, and Parabilis Medicine; personal fees from Erasca, Merck, and Bayer; grants from Pfizer, Boundless Bio, Daiichi Sankyo, and Boehringer Ingelheim; and other support from CytoDyn outside the submitted work. J.-F. Martini reports other support from Pfizer outside the submitted work. L. Pasquina reports other support from Foundation Medicine during the conduct of the study, as well as other support from Roche Holding AG outside the submitted work. B. Tunquist reports personal fees from Pfizer during the conduct of the study. N. Pantoja Galicia reports other support from Foundation Medicine during the conduct of the study, as well as other support from Roche Holding AG outside the submitted work. S. Deng reports other support from Pfizer during the conduct of the study, as well as other support from Pfizer outside the submitted work. J. Kiely reports other support from Foundation Medicine during the conduct of the study, as well as other support from Roche Holding AG outside the submitted work. T.-C.V. Ng reports personal fees from Foundation Medicine during the conduct of the study, as well as personal fees from Foundation Medicine and other support from Roche Holding AG outside the submitted work. G. Ferrier reports other support from Pfizer outside the submitted work. J. Tabernero reports personal fees from Accent Therapeutics, Alentis Therapeutics, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Carina Biotech, Cartography Biosciences, Chugai, Daiichi Sankyo, F. Hoffmann-La Roche, Genentech, Johnson & Johnson/Janssen, Eli Lilly and Company, Marengo Therapeutics, Menarini, Merus, MSD, Novartis, Ono Pharma USA, Peptomyc, Pfizer, Pierre Fabre, Quantro Therapeutics, Scandion Oncology, Scorpion Therapeutics, Servier, Sotio Biotech, Taiho, Takeda Oncology, and Tolremo Therapeutics and other support from Alentis Therapeutics, Oniria Therapeutics, ITRIALSP, and Pangaea Oncology outside the submitted work. S. Kopetz reports other support from Lutris (ownership interest), Frontier Medicines (ownership interest), and Navire (ownership interest); other support

from Genentech (consultant), Merck (consultant), Boehringer Ingelheim (consultant), Bayer Health (consultant), Pfizer (consultant), Mirati Therapeutics (consultant), Flame Biosciences (consultant), Carina Biotech (consultant), Frontier Medicines (consultant), Replimune (consultant), Bristol Myers Squibb-Medarex (consultant), Amgen (consultant), Tempus (consultant), Harbinger Oncology (consultant), Zentalis (consultant), AVEO (consultant), Tachyon Therapeutics (consultant), Agenus (consultant), Revolution Medicines (consultant), Kestrel Therapeutics (consultant), Roche (consultant), Arcus Biosciences (consultant), AstraZeneca (consultant), BeiGene (consultant), Clasp Therapeutics (consultant), Cytovation (consultant), Dewpoint Therapeutics (consultant), Marengo Therapeutics (consultant), SageMedic (consultant), Servier (consultant), Sibylla (consultant), T-Cypher Bio (consultant), XAIRA (consultant), AmMax Bio (consultant), and Ikena (consultant); and research funding from Guardant Health, Genentech/Roche, EMD Serono, Amgen, Eli Lilly and Company, Daiichi Sankyo, Pfizer, Boehringer Ingelheim, BridgeBio, Zentalis, BioMed Valley, Johnson & Johnson, Bristol Myers Squibb, Cardiff, Jazz Pharmaceuticals, and Frontier Medicines. No disclosures were reported by the other authors.

Authors' Contributions

R. Yaeger: Investigation, resources, writing–review and editing. **J.-F. Martini:** Conceptualization, supervision, writing–original draft, project administration, writing–review and editing. **L. Pasquina:** Conceptualization, supervision, investigation, writing–original draft, writing–review and editing. **B. Tunquist:** Conceptualization, supervision, writing–original draft, project administration, writing–review and editing. **X. Zhang:** Writing–review and editing. **F. Kaiser:** Investigation, writing–review and editing. **N. Pantoja Galicia:** Conceptualization, supervision, investigation, methodology, writing–original draft, writing–review and editing. **S. Deng:** Writing–review and editing. **S. Gong:** Formal analysis, investigation, methodology, writing–review and editing. **C. Guo:** Conceptualization, formal analysis, supervision, investigation, methodology, writing–original draft, writing–review and editing. **J. Kiely:** Formal analysis, investigation, methodology, writing–review and editing. **T.-C.V. Ng:** Formal analysis, investigation, methodology, writing–review and editing. **G. Ferrier:** Conceptualization, supervision, writing–original draft, project administration, writing–review and editing. **J. Tabernero:** Investigation, resources, writing–original draft, writing–review and editing. **S. Kopetz:** Writing–original draft, writing–review and editing.

Acknowledgments

The BEACON trial was sponsored by Array BioPharma, which was acquired by Pfizer in July 2019. The authors thank the participants and their families, as well as all the staff at the participating sites. Medical writing support was provided by Blaise Low, PhD, of Nucleus Global, an Inizio Company, and was funded by Pfizer. This study was also supported by the NIH/NCI Cancer Center Support Grant P30 CA008748 to MSK (R. Yaeger).

Note

Supplementary data for this article are available at Cancer Research Communications Online (<https://aacrjournals.org/cancerrescommun/>).

Received February 04, 2025; revised May 30, 2025; accepted August 18, 2025; posted first August 20, 2025.

References

- Nikanjam M, Kato S, Kurzrock R. Liquid biopsy: current technology and clinical applications. *J Hematol Oncol* 2022;15:131.
- Ranghiero A, Frascarelli C, Cursano G, Pescia C, Ivanova M, Vacirca D, et al. Circulating tumour DNA testing in metastatic breast cancer: integration with tissue testing. *Cytopathology* 2023;34:519–29.
- Cheng F, Su L, Qian C. Circulating tumor DNA: a promising biomarker in the liquid biopsy of cancer. *Oncotarget* 2016;7:48832–41.
- Nakamura Y, Taniguchi H, Ikeda M, Bando H, Kato K, Morizane C, et al. Clinical utility of circulating tumor DNA sequencing in advanced gastrointestinal cancer: SCRUM-Japan GI-SCREEN and GOZILA studies. *Nat Med* 2020;26:1859–64.
- Vellanki PJ, Ghosh S, Pathak A, Fusco MJ, Bloomquist EW, Tang S, et al. Regulatory implications of ctDNA in immuno-oncology for solid tumors. *J Immunother Cancer* 2023;11:e005344.
- Kolenčik D, Shishido SN, Pitule P, Mason J, Hicks J, Kuhn P. Liquid biopsy in colorectal carcinoma: clinical applications and challenges. *Cancers (Basel)* 2020;12:1376.
- Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014;6:224ra24.
- Urbini M, Marisi G, Azzali I, Bartolini G, Chiadini E, Capelli L, et al. Dynamic monitoring of circulating tumor DNA in patients with metastatic colorectal cancer. *JCO Precis Oncol* 2023;7:e2200694.
- Chan HT, Chin YM, Low S-K. Circulating tumor DNA-based genomic profiling assays in adult solid tumors for precision oncology: recent advancements and future challenges. *Cancers (Basel)* 2022;14:3275.
- Kopetz S, Grothey A, Yaeger R, Van Cutsem E, Desai J, Yoshino T, et al. Encorafenib, binimetinib, and cetuximab in BRAF V600E-mutated colorectal cancer. *N Engl J Med* 2019;381:1632–43.
- QIAGEN. QIAGEN launches the screen BRAF test as companion diagnostic to a BRAF^{V600E} (encorafenib) based regimen in metastatic colorectal cancer. [cited 2024 Mar 12]. Available from: <https://corporate.qiagen.com/newsroom/press-releases/press-release-details/2020/QIAGEN-launches-the-screen-BRAF-test-as-companion-diagnostic-to-a-BRAFV600E-encorafenib-based-regimen-in-metastatic-colorectal-cancer/default.aspx>.
- Woodhouse R, Li M, Hughes J, Delfosse D, Skoletsky J, Ma P, et al. Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. *PLoS One* 2020;15:e0237802.
- NS Medical Devices. FDA approves FoundationOne liquid CDx as CDx for Pfizer's Braftovi combo in mCRC. [cited 2024 May 23]. Available from: <https://www.nsmedicaldevices.com/company-news/fda-approves-foundationone-liquid-cdx-pfizer-braftovi-combo-mcrc/?cf-view>.
- QIAGEN. the screen® BRAF V600E RGQ PCR Kit instructions for use (Handbook). Available from: https://www.accessdata.fda.gov/cdrh_docs/pdf19/P190026C.pdf.
- Foundation Medicine Inc. FoundationOne®CDx technical information. [cited 2025 May]. Available from: <https://www.foundationmedicine.com/media/273/fmi-view>.
- Rolfo CD, Madison RW, Pasquina LW, Brown DW, Huang Y, Hughes JD, et al. Measurement of ctDNA tumor fraction identifies informative negative liquid biopsy results and informs value of tissue confirmation. *Clin Cancer Res* 2024;30:2452–60.
- Chiang AC, Madison RW, Huang Y, Fine A, Jin DX, Oxnard GR, et al. Abstract 971: validation of a tumor-naïve circulating tumor DNA (ctDNA) response monitoring panel in advanced non-small cell lung cancer (aNSCLC). *Cancer Res* 2024;84(Suppl 6):971.
- Iams WT, Mackay M, Ben-Shachar R, Drews J, Manghnani K, Hockenberry AJ, et al. Concurrent tissue and circulating tumor DNA molecular profiling to detect guideline-based targeted mutations in a multicancer cohort. *JAMA Netw Open* 2024;7:e2351700.
- Schulze M, Wang X, Hamad J, Quintanilha JCF, Pasquina LW, Hopkins JF, et al. Real-world genomic landscape of colon and rectal cancer. *FEBS Open Bio* 2025;15:674–85.
- Kopetz S, Murphy DA, Pu J, Ciardiello F, Desai J, Van Cutsem E, et al. Molecular profiling of BRAF-V600E-mutant metastatic colorectal cancer in the phase 3 BEACON CRC trial. *Nat Med* 2024;30:3261–71.
- Mulki RJ, Saitova ES, Preobrazhenskaya EV, Asadulaeva KA, Bubnov MG, Otrachova EA, et al. ALK, ROS1, RET and NTRK1-3 gene fusions in colorectal and non-colorectal microsatellite-unstable cancers. *Int J Mol Sci* 2023;24:13610.
- Westphalen CB, Krebs MG, Le Tourneau C, Sokol ES, Maund SL, Wilson TR, et al. Genomic context of NTRK1/2/3 fusion-positive tumours from a large real-world population. *NPJ Precis Oncol* 2021;5:69.
- Woolston A, Barber LJ, Griffiths B, Pich O, Lopez-Bigas N, Matthews N, et al. Mutational signatures impact the evolution of anti-EGFR antibody resistance in colorectal cancer. *Nat Ecol Evol* 2021;5:1024–32.
- Zhang X, Feng R, Xu Y, Yang L, Xie F, Yang H, et al. Baseline circulating tumor DNA predicts long-term survival outcomes for patients with early breast cancer. *Gland Surg* 2024;13:684–96.
- Pascual J, Gil-Gil M, Proszek P, Zielinski C, Reay A, Ruiz-Borrego M, et al. Baseline mutations and ctDNA dynamics as prognostic and predictive factors in ER-positive/HER2-negative metastatic breast cancer patients. *Clin Cancer Res* 2023;29:4166–77.
- Song Y, Hu C, Xie Z, Wu L, Zhu Z, Rao C, et al. Circulating tumor DNA clearance predicts prognosis across treatment regimen in a large real-world longitudinally monitored advanced non-small cell lung cancer cohort. *Transl Lung Cancer Res* 2020;9:269–79.
- Zheng Y, Sun H, Cong L, Liu C, Sun Q, Wu N, et al. Prognostic value of ctDNA mutation in melanoma: a meta-analysis. *J Oncol* 2021;2021:6660571.
- Stires H, Zariffa N, Eisele M, Goren E, Espenschied CR, Guha M, et al. Changes in ctDNA levels as an early indicator of outcomes in advanced NSCLC treated with TKI: initial findings from a retrospective aggregate analysis of 8 clinical trials. *J Clin Oncol* 2023;41(Suppl 16):3030.
- Lin Z, Li Y, Tang S, Deng Q, Jiang J, Zhou C. Comparative analysis of genomic profiles between tissue-based and plasma-based next-generation sequencing in patients with non-small cell lung cancer. *Lung Cancer* 2023;182:107282.
- Paturu R, Lingaiah R, Kumari N, Singh S, Krishnani N, Srivastava S, et al. Non-small cell lung cancer: targetable variants in concurrent tissue and liquid biopsy testing in a North Indian cohort. *Asian Pac J Cancer Prev* 2023;24:3467–75.