Plasma HER2 (ERBB2) Copy Number Predicts Response to HER2-targeted Therapy in Metastatic Colorectal Cancer

Giulia Siravegna1,2, Andrea Sartore-Bianchi3,4, Rebecca J. Nagy5, Kanwal Raghav6, Justin I. Odgaard5, Richard B. Lanman5, Livio Trusolino1,2, Silvia Marsoni7, Salvatore Siena3,4, and Alberto Bardelli1,2

Abstract

Purpose: ERBB2 (HER2) amplification is an emerging biomarker in colon cancer, conferring sensitivity to combination anti-HER2 therapy. Measurement of HER2 copy number is typically performed using surgical specimens, but cell-free circulating tumor DNA (ctDNA) analysis may be a noninvasive alternative. We determined the sensitivity of plasma copy number (pCN) for detecting ERBB2 amplifications and whether pCN correlated with tissue-detected copy number. We also assessed response to HER2-targeted therapy based on pCN and suggest a pCN threshold predictive of response.

Experimental Design: Forty-eight pretreatment and progression plasma samples from 29 HER2-positive patients in the HERACLES A clinical trial were tested using the Guardant360 cfDNA assay. We correlated ERBB2 pCN with progression-free survival (PFS) and best objective response (BOR) and applied an adjustment method based on tumor DNA shedding using the maximum mutant allele fraction as a surrogate for tumor content to accurately determine the pCN threshold predictive of response.

Results: Forty-seven of 48 samples had detectable ctDNA, and 46 of 47 samples were ERBB2-amplified on the basis of ctDNA [2.55–122 copies; 97.9% sensitivity (95% confidence interval, 87.2%–99.8%)]. An adjusted ERBB2 pCN of ≥25.82 copies correlated with BOR and PFS (P = 0.0347).

Conclusions: cfDNA is a viable alternative to tissue-based genotyping in the metastatic setting. The cfDNA platform utilized correctly identified 28 of 29 (96.6%) of pretreatment samples as ERBB2-amplified and predicted benefit from HER2-targeted therapy. In this study, an observed pCN of 2.4 and an adjusted pCN of 25.82 copies of ERBB2 are proposed to select patients who will benefit from HER2-targeted therapy.

Introduction

Colon cancer is the third most common cancer worldwide, and approximately 20% of patients present with metastatic disease (metastatic colorectal cancer; mCRC), which is associated with a poor prognosis and median overall survival (OS) of 24–30 months (1). Use of the anti-EGFR mAbs cetuximab and panitumimab has improved progression-free survival (PFS) and OS in patients who are negative for KRAS, NRAS, and BRAF mutations; however, these therapies are inevitably followed by disease progression (2).

Very few effective therapies remain for the majority of patients with mCRC tumors that have become resistant to cetuximab or panitumimab (3). Amplification of ERBB2 (HER2) is an emerging biomarker present in 3%–5% of genetically unselected mCRC and is enriched in RAS/RAF/PIK3CA-wild-type tumors (4, 5). Several preclinical studies have also suggested that ERBB2 copy number gain is a negative predictor of response to anti-EGFR therapy (4, 6–8).

Two studies have assessed the feasibility of targeting ERBB2 amplification in patients with mCRC. HERACLES A was an open-label, phase II trial of trastuzumab and lapatinib in chemotherapy and EGFR antibody-refractory, HER2-positive patients and showed an objective response rate (ORR) of 30% (95% confidence interval [CI], 14%–50%) and a disease control rate (DCR) of 59% (95% CI, 39%–78%) compared with a 41.9% DCR associated with standard-of-care therapy (9–11). More recently, the MyPathway open-label phase II basket trial showed a 38% (95% CI, 23%–55%) ORR using pertuzumab and trastuzumab in the same population (12). These studies confirmed HER2 as an important driver of mCRC and a successful therapeutic target in EGFR antibody- and chemotherapy-refractory disease.
HER2 overexpression and/or amplification can be assessed using a variety of tissue-based approaches, including IHC, FISH, PCR, or next-generation sequencing (NGS). Noninvasive methods are also possible and include assessment of HER2 overexpression in circulating tumor cells (CTC) or measurement of ERBB2 copy number via NGS of cell-free DNA (cfDNA). Although tissue copy number (tCN) appears to predict benefit from HER2 inhibition (10), no data exist regarding use of cfDNA to predict this benefit in mCRC.

Measurement of copy number using cfDNA is challenging due to the overwhelming excess of diploid leukocyte-derived DNA relative to the very small amount of tumor DNA in the cell-free compartment, even in individuals with metastatic disease who typically have a large tumor burden. A recent study of cfDNA analysis in >21,000 individuals with metastatic solid tumors showed a median mutant allele fraction of only approximately 0.4%, which is equivalent to 4 mutant molecules for every 1,000 total (mutant and wild-type) molecules, and a median copy number for gene amplifications of 2.56 (13). Therefore, any cfDNA assay utilized for clinical genotyping must be highly sensitive while maintaining high specificity. Furthermore, if the observed copy number in the tumor is low to moderate, even a tumor shedding large amounts of cfDNA into circulation may not yield detectable elevations in plasma copy number (pCN). In this context, as the pCN in plasma is driven not only by tCN but also by the extent of tumor DNA shedding, distinguishing between genomic characteristics, tumor burden, tumor proclivity to shed DNA, and tumor volume changes during therapy remains daunting.

Finally, clinical specificity can also be a challenge, as copy number gains in tumor cells can be the result of focal gene amplification, which is often a viable target for drug therapy, or of amplification of large portions of a chromosome, which is less likely to associate with response to targeted therapy (14, 15). To effectively identify candidate treatment targets, any assay must be able to discriminate between these two scenarios. Complicating assessment of cfDNA assays, direct comparisons to commonly utilized tissue-based approaches such as FISH and IHC are difficult, as differential shedding of tumor DNA into the circulation affects the pCN.

The goals of this study were as follows: (i) to determine the sensitivity of ERBB2 amplification detection in plasma using a CLIA-certified, CAP-accredited cfDNA assay relative to standard tissue testing; (ii) to assess ERBB2 pCN as a predictor of HER2-targeted therapy benefit; and (iii) to establish a pCN threshold to enrich patients potentially responsive to HER2-targeted therapy.

Materials and Methods
HERACLES A patient cohort
HERACLES A patients had a histologically confirmed diagnosis of metastatic colorectal cancer wild-type for KRAS exon 2 (codons 12 and 13) and positive for HER2 as defined by 3+ staining in >50% of cells by IHC or 2+ staining and a HER2:CEP17 ratio >2 in >50% of cells by FISH (14). Tissue samples for HER2 testing were derived from primary tumors for 11 (39%) of 28 patients, from metastatic lesions for the remaining 17 (61%). The patient cohort is described in Table 1 and Fig. 1. All had measurable disease according to RECIST version 1.1. Patients received treatment and were assessed for objective response, PFS, best overall response, and duration of response (11). As part of the trial, patients also underwent serial plasma collection for cfDNA analysis. Pretreatment and at-progression plasma samples (N = 48; 29 pretreatment and 19 at-progression) from 29 HER2-tissue-positive patients were tested using the Guardant360 Assay (Guardant Health, Inc.), and sensitivity was calculated. We also determined the observed ERBB2 pCN cutoff that maximized the identification of the HERACLES A intent-to-treat population and best predicted response to trastuzumab plus lapatinib therapy.

Guardant360 database and historical mCRC cohort
ERBB2 amplification frequency, pCN distribution, and RAS/RAF mutation cooccurrence were determined using the Guardant Health database (accessed April 11, 2018). This cohort comprised 4,294 plasma samples from individuals with stage III/IV colorectal cancer undergoing cfDNA analysis (Guardant360) as part of their routine care.

cfDNA analysis
cfDNA NGS analysis was performed at Guardant Health, Inc. (Guardant360), a CLIA-certified, College of American Pathologists (CAP)-accredited, New York State Department of

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
</tr>
<tr>
<td>Median age at enrollment</td>
<td>63 years (range 41-86)</td>
</tr>
<tr>
<td>Primary tumor site</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>7</td>
</tr>
<tr>
<td>Proximal colon</td>
<td>5</td>
</tr>
<tr>
<td>Distal colon</td>
<td>16</td>
</tr>
<tr>
<td>HER2 IHC Score</td>
<td></td>
</tr>
<tr>
<td>2+ (FISH amplified)</td>
<td>6</td>
</tr>
<tr>
<td>3+</td>
<td>22</td>
</tr>
<tr>
<td>No. of previous lines therapy</td>
<td></td>
</tr>
<tr>
<td>&lt;3</td>
<td>7</td>
</tr>
<tr>
<td>&gt;5</td>
<td>21</td>
</tr>
</tbody>
</table>

Downloaded from clincancerres.aacrjournals.org on August 13, 2019. © 2019 American Association for Cancer Research.
Health–approved laboratory (13, 16). The Guardant360 assay detects single-nucleotide variants (SNV), indels, fusions, and copy number alterations in 73 genes with a reportable range of 0.04%, 0.02%, and 2.12 copies, respectively, as well as microsatellite instability (Supplementary Fig. S1A). For the HERACLES A trial, 10 mL of whole blood was collected in EDTA tubes. Plasma was separated within 5 hours of collection using two different centrifugation steps. Plasma was stored at 8°C until cfDNA extraction. cfDNA was extracted from 1 to 2 mL of plasma (QiAmp Circulating Nucleic Acid Kit, Qiagen, Inc.), labeled with nonrandom oligonucleotide barcodes (IDT, Inc.) and used to prepare sequencing libraries, which were then enriched by hybrid capture (Agilent Technologies, Inc.), pooled, and sequenced by paired-end synthesis (NextSeq 500 and/or HiSeq 2500, Illumina, Inc.). Separate sequencing controls were utilized for SNVs and CNs/fusions/indels (CFI; Supplementary Fig. S1B).

Bioinformatic analysis and observed copy number determination

As described previously, base call files generated by Illumina’s RTA software (v2.12) were demultiplexed using bcl2fastq (v2.19) and processed with a custom pipeline for molecule barcode detection, sequencing adapter trimming, and base quality trimming (discarding bases below Q20 at the ends of the reads, ref. 13). Processed reads were then aligned to hg19 using BWA-MEM (arXiv:1303.3997v2) and used to build double-stranded consensus representations of original unique cfDNA molecules using both inferred molecular barcodes and read start/stop positions. To detect copy number amplification, probe-level unique molecule coverage was normalized for overall unique molecule throughput, probe efficiency, GC content, and signal saturation and robustly summarized at the gene level. pCN determinations were based on training set-established decision thresholds for both observed copy number deviation from per-sample diploid baseline and deviation from the baseline variation of probe-level normalized signal in the context of background variation within each sample’s own diploid baseline. Per-sample relative tumor burden was determined by normalization to the mutational burden expected for tumor type and cell-free circulating tumor DNA (ctDNA) fraction and reported as a z-score. Observed ERBB2 pCN values representing the lower 50th, 50th–90th, and the top 10th percentiles across all amplified samples in the Guardant Health database were calculated (13).

Correlation between ISH, tCN, and calculation of an adjusted pCN

tCN in the HERACLES A trial was centrally determined using several methodologies including IHC, FISH, and qRT-PCR. Observed pCN using cfDNA NGS was compared with FISH and qRT-PCR methods, and Spearman correlations were calculated. To correct for variation in plasma tumor fraction between samples that can affect the tumor contribution to the circulating DNA pool and consequently pCN, we adjusted the observed pCN to the proportion of tumor DNA in each cfDNA sample. To do this, we used the maximum mutant allele fraction (MAF%/100) observed in each individual sample as a surrogate for plasma tumor fraction (T%), as this typically represents the earliest initiating mutation shared by all tumor clones. Genes with the highest MAF were TP53 (n = 15), APC (n = 11), PTEN (n = 1), and RAF1 (n = 1; Supplementary Table S2). We then calculated an adjusted pCN (ApCN); Adjusted pCN = [Observed pCN – 2*(1-T%)]/T% where T% = 2 X

Figure 1.

Consort diagram showing the HERACLES A cohort and the subset of patients included in this study.
Results
ERBB2 pCN and RAS/RAF status in the HERACLES A cohort
Forty-eight banked plasma samples from 29 patients were available for analysis, including 29 pretreatment and 19 at-progression plasma samples. One at-progression sample had no detectable ctDNA, leaving 47 (29 pretreatment and 18 posttreatment) evaluable samples. ERBB2 amplification was identified in 46 of 47 plasma samples (28/29 pretreatment and 18/18 at-progression) for a sensitivity of 97.9% (95% CI, 87.2%–99.8%). The mean pCN in pretreatment samples was 23.1 copies (median = 9.28; range = 2.6–121.7 copies) and 16.76 (median = 8.48; range = 2.13–82.17) in at-progression samples.

To assess focal versus nonfocal amplification, we examined the copy number of genes neighboring ERBB2 on chromosome 17 to differentiate aneuploidy or large (e.g., arm-level) events. Only a single sample in this study demonstrated a pattern suggestive of a large-scale chromosome 17 amplification event (Supplementary Fig. S2A). This patient had stable disease and progressed after 6 months of therapy. The remainder of samples showed patterns most compatible with focal amplification (Supplementary Fig. S2B).

Ten KRAS, NRAS, and BRAF mutations were identified in pretreatment samples from 9 patients. There were 3 RAS codon 12 of 13 mutations, 5 noncodon 12 of 13 mutations (4 KRAS and 1 NRAS), and 3 BRAF mutations (V600E, G469A, and G596R). BRAF G469A and KRAS Q61H cooccurred in one sample. Three patients with primary resistance to therapy (n = 1) or radiographic progression (n = 2) had clonal RAS/RAF driver mutations as defined by a RAS/RAF maximum allelic fraction ratio of >0.3 (Fig. 2; ref. 17).

ERBB2 amplification frequency in a historical ctDNA cohort
There were 4,294 patients with mCRC in the Guardant Health database tested between February 5, 2015 and April 11, 2018. Centiles of ERBB2 pCN were as follows: copy number 2.4, 50th percentile; copy number 4, 90th percentile (Supplementary Fig. S3). Of the 4,294 patients, 247 (5.8%) had detectable ERBB2 amplification, which is compatible with previous reports of prevalence (4, 18, 19). An ERBB2 pCN cutoff of ≥2.4 copies in the historical cohort allowed for exclusion of 84% of all KRAS, NRAS, and BRAF driver mutations in the historical cohort (Supplementary Fig. S4) and suggests that samples with pCN above this threshold represent those for which ERBB2 amplification is the primary driver of malignancy. An observed pCN cutoff of 2.4 accurately identified 100% of the intent-to-treat HERACLES A population (Fig. 2).

Correlation between tCN and observed and ApCN
To determine the correlation between tCN and pCN, we compared ERBB2 observed pCN values with ISH and with tCN as measured by qRT-PCR. There was modest correlation between observed pCN and ISH (Spearman r = 0.49; Fig. 3A). As observed pCN and qRT-PCR tCN (Spearman r = 0.52; Fig. 3C) compared with observed pCN, ApCN showed stronger correlation with ISH and ERBB2 pCN by qRT-PCR (Fig. 3B and D) with a Spearman R of 0.77 and 0.86, respectively.

Response to therapy based on ApCN and cooccurring alterations
Radiographic response was assessed in 26 patients with ERBB2 amplification detected in plasma to determine whether ApCN correlated with BOR (Fig. 4). Two patients had early clinical progression and were not imaged. We determined an ERBB2 ApCN cut-off value of 25.82 for optimal segregation of responders versus nonresponders using ROC analysis (Supplementary Table S2). There were 6 patients with RECIST-defined progressive disease (PD) and 2 patients with clinical evidence of primary resistance to therapy for whom imaging was not available at the time of progression. Of these 8 cases, 6 had an ApCN below 25.82 and 2 had a pCN ≥ 25.82 (Supplementary Table S2). In addition to a pCN below 25.83, 3 patients with PD had clonal KRAS or BRAF mutations (KRAS G12V, G12D, and BRAF V600E) identified in plasma (Supplementary Table S2). Twenty patients (20/28, 71%) had some degree of clinical benefit, including 13 with stable disease, 6 partial response, and 1 complete response. Thirteen of these 20 patients had a ApCN ≥ 25.82. Of these 13 patients with a ApCN ≥25.82, 7 had their disease controlled by anti-HER2 treatment: 1 achieved a partial response and the remaining 6 had stable disease according to RECIST. We also assessed the correlation between ApCN and PFS. The median PFS in individuals with a ApCN < 25.82 was 14.8 weeks, as compared with 22.5 weeks in those with a ApCN ≥ 25.82 (Mantel Cox P = 0.0347, Fig. 5).
Discussion

Noninvasive methods of comprehensive genomic profiling are becoming standard clinical practice because they provide rapid and accurate identification of clonal driver alterations and selection of appropriate targeted therapy and allow for serial assessment of clonal tumor dynamics (20). In mCRC, HER2-targeted therapy is emerging as an active therapy, but it has not entered widespread use, in part, due to difficulties in identifying patients with HER2-positive mCRC who are likely to experience clinical benefit (17). In this context, not all cfDNA assays are well validated, and many have limitations around the types of alterations they can detect with high sensitivity and specificity (21). Similarly, attempts in mCRC to isolate circulating tumor cells have resulted in limited and mixed success (22). Therefore, we utilized a cfDNA NGS assay that has been extensively validated for all four major types of genomic alterations and microsatellite instability (13). Copy number amplification is a challenging alteration type to detect in cfDNA due to the high ratio of diploid leukocyte DNA to tumor DNA in circulation, which dilutes tumor copy number signals. In our analysis, the cfDNA assay utilized detected ERBB2 amplification in 28 of 29 pretreatment and 18 of 18 evaluable at-progression plasma samples from the HERACLES A cohort. In this series, we observed weak correlation between observed pCN and tCN using a variety of methods, including IHC, ISH, and qRT-PCR. This is, in part, due to the fact that different methodologies are measuring different analytes (protein overexpression vs. number of gene copies) and are not all quantitative (IHC, ISH), which can make direct comparisons difficult. However, the major confounding factor when comparing blood and tissue methods of copy number assessment is the variation in the amount of tumor DNA shed into the bloodstream. Concordance between tissue and plasma is often high when two criteria are met: (i) the observed copy number in the tumor is high and (ii) there is ample shedding of DNA into circulation, that is, the tumor fraction in circulation is high. In addition, pCN represents a summary of all amplified lesions that may be shedding DNA into circulation. As a result, pCN may be impacted by the heterogeneity of actual copy number across tumor sites. These factors may limit the sensitivity of cfDNA assessment, and make comparison of copy number between tissue and plasma challenging. Furthermore, observed pCN is often misleadingly low in samples with low tumor fraction, despite high tCN, which could prevent patients with true oncogenic driver amplifications from receiving appropriate targeted therapy. To overcome some of these technical barriers, we adjusted the observed pCN for the amount of tumor DNA shedding using the maximum mutant allele fraction as a
surrogate for tumor content. The resulting ApCN correlated with ICN and response to therapy in our analysis.

Our results demonstrate a correlation between PFS/BOR and level of ERBB2 amplification; however, not all patients with high ERBB2 pCN responded (Fig. 4). One patient (121024) showing PD had ERBB2 ApCN of 27.38, but there were no cooccurring mutations present in the baseline sample to explain the lack of response. There were also 5 patients with stable disease, 1 with an ApCN of <25.82, 2 patients with primary clinical progression who did not undergo radiographic imaging.

Figure 4.
Best overall response (BOR) based on ApCN in baseline HERACLES A samples. Bars, change in target lesion size from baseline to first progression. Horizontal dotted lines correspond to a 20% increase in target lesion size from baseline (top line) and 30% decrease in target lesion size from baseline (bottom line). Red bars, samples with an ApCN ≥ 25.82 and blue bars an ApCN of <25.82. Patients with clonal RAS/RAF mutation in baseline plasma samples. Arrows, 2 patients with primary clinical progression who did not respond. There were also 5 patients with stable disease, 1 with an ERBB2 CN mutation present in the baseline sample to explain the lack of PFS by ApCN. Red line, patients with an ApCN > 25.82.

Figure 5.
PFS by ApCN. Red line, patients with an ApCN ≥ 25.82; blue line, patients with an ApCN of <25.82.

ctDNA Copy Number Predicts HER2-Targeted Therapy Response

CT DNA Copy Number Predicts HER2-Targeted Therapy Response

Interestingly, 3 patients with either PD or primary clinical progression harbored clonal KRAS (n = 2) BRAF (n = 1) mutations, as assessed by ddPCR analysis. Although the HERACLES A trial excluded patients with KRAS mutations in their diagnostic tissue in most cases (the treatment-naive primary tumor), these RAS/RAF mutations presumably developed during the prior course of anti-EGFR therapy and dominated the original KRAS WT clonal populations under selective pressure. Screening for BRAF mutations was not required for entry into HERACLES A but is now part of the NCCN guidelines, given the 4%–5% mutation frequency in mCRC and associated lack of response to anti-EGFR therapy (3).

Several studies have discussed the utility of ctDNA in assessing copy number amplification. Liang and colleagues showed perfect concordance (n = 7/7 patients) between tissue and plasma-identified ERBB2 CN in patients with metastatic breast cancer (23). All 7 patients were given anti-HER2 therapy, and 6 of the 7 had a clinical response to therapy, underscoring the therapeutic relevance of ERBB2 pCN assessment. Similarly, in two separate studies of patients with untreated gastric/gastroesophageal cancer, high ERBB2 pCN was a positive predictor of patient response and plasma was able to capture copy number changes present in both the primary tumor and the metastases (24). These papers highlight the fact that pCN measurements, unlike tissue measurements, are often a summary of all shedding lesions and can be influenced by tissue heterogeneity. The HERACLES A study further highlights the benefits of ctDNA copy number analysis and underscores the ability of ctDNA to capture tumor heterogeneity in patients with mCRC. This latter capability may be similarly important in mCRC as, despite high concordance for somatic mutations between primary tumors and metastases, there is significant discordance (6%–15%) for tissue-assessed ERBB2 copy number amplifications (25, 26).

Limitations of this study include the small sample size and the lack of HER2-negative patients in the HERACLES A cohort. While the latter prohibits calculation of specificity, positive predictive value, and negative predictive value in this cohort, the specificity and positive predictive value of the copy number assessment method utilized here have been described elsewhere (13). In that study, analytic specificity was ≥99.9% and PPV was 100% when compared with ddPCR of cell lines with known gene copy number status. Another possible limitation of the methods employed here is distinction between focal copy number amplification, which in the case of ERBB2 in mCRC, is a druggable target, as compared with aneuploidy, which may not always result in protein overexpression and thus may not always respond to targeted agents. Despite the clear pattern of focal amplification in this study and others (19), these results cannot be considered representative of focal versus nonfocal amplification rates in other contexts as these vary widely by chromosome, cancer type, and treatment context. Another limitation of the study is the lack of detailed clinical information on the historical ctDNA cohort. The ctDNA test utilized here is performed in a large reference laboratory where details such as previous therapy and current therapy at the time of the blood draw, histology, stage of disease at diagnosis, previous genomic testing results, etc. are not typically provided by the ordering clinician. Therefore, although the historical cohort represents patients with later stage mCRC, the cohort is likely heterogeneous in terms of previous and current therapy status and direct comparisons between this cohort and the HERACLES A cohort must be done with caution. Finally, the pCN adjustment method
utilized here remains exploratory in nature and has not been validated in a separate cohort. Although the correlation of ApCN with both FISH and iCN as determined by qT-PCR and PFS/BOR suggests that this is a robust correction method, further validation of the model is necessary in additional larger cohorts. Furthermore, the current adjustment method does not consider copy number amplification or loss of heterozygosity of the gene comprising the mutation with the maximum mutant allele fraction from which tumor fraction is inferred. In particular, loss of heterozygosity in TP53 or APC, which are often the mutations with the highest mutant allele fraction in mCRC samples, could result in an over-representation of mutant alleles in the cfDNA sample and therefore result in an overestimation of tumor fraction. Correction for this may provide more accurate estimates of iCN as reflected in the plasma and should be explored.

**ERBB2** amplification is an emerging therapeutic target in the mCRC setting and may also be a negative predictor of response to anti-EGFR therapy (4, 7). In this series, comprehensive cfDNA NGS accurately identified ERBB2 amplification in 96.6% (28/29) of the intent-to-treat population, suggesting that cfDNA can be used as a surrogate for tissue especially in cases when archival tissue cannot be obtained in a timely manner and rebiopsy is not preferred. In an independent study (20), ERBB2 amplification was detected in 2 patients with mCRC by both cfDNA profiling and chromogenic in situ hybridization, further attesting to the value of cfDNA analysis in capturing the tissue genomic make-up. Additional investigation is needed to determine whether pCN can replace tCN assessment. In addition, as described previously, the assay identified cooccurring mutations in KRAS, BRAF, and ERBB2 that were predictive of resistance to therapy (27). Furthermore, we describe an ApCN threshold above which patients are more likely to respond to targeted therapy. These results support the use of appropriately validated cfDNA tests as an alternative to tissue biopsy to identify individuals who may benefit from anti-HER2 therapy. Additional prospective studies in larger cohorts are needed, particularly in patients with treatment-naive mCRC where targeted therapy may be most efficacious.

**Disclosure of Potential Conflicts of Interest**

A. Sartore-Bianchi reports receiving speakers honoraria from Amgen, Bayer, and Sanofi, and is a consultant/advisory board member for Amgen, Bayer, and Sanofi. R.J. Nagy has ownership interests (including patents) at Guardant Health. J.I. Odegaard is an employee of and has ownership interests (including patents) at Guardant Health. R.B. Lanman is an employee of and has ownership interests (including patents) at Guardant Health and BioIsle Inc., and has ownership interests (including patents) at and is a consultant/advisory board member for Forward Medical Inc. L. Trusolino reports receiving speakers bureau honoraria from Eli Lilly, AstraZeneca, and Merck, and reports receiving commercial research grants from Symphogen, Merus, Pfizer, and Servier. S. Siena is a consultant/advisory board member for Amgen, Bayer, Bristol-Myers Squibb, CheckMate, Incyte, Merck, Novartis, Roche, and Seattle Genetics. A. Bardelli is a consultant/advisory board member for SAB Guardant Health. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

**Conception and design:** G. Siravegna, R.J. Nagy, R.B. Lanman, L. Trusolino, S. Marsoni, S. Siena, A. Bardelli

**Development of methodology:** R.J. Nagy, R. Kaghav, J.I. Odegaard, R.B. Lanman

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** G. Siravegna, A. Sartore-Bianchi, R.J. Nagy, R.B. Lanman, S. Siena

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** G. Siravegna, R.J. Nagy, K. Raghav, J.I. Odegaard, R.B. Lanman, L. Trusolino, S. Siena, A. Bardelli

**Writing, review, and/or revision of the manuscript:** G. Siravegna, A. Sartore-Bianchi, R.J. Nagy, K. Raghav, J.I. Odegaard, R.B. Lanman, L. Trusolino, S. Siena, A. Bardelli

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** R.J. Nagy, J.I. Odegaard

**Study supervision:** L. Trusolino, S. Marsoni, S. Siena, A. Bardelli

**Others (study chairman of HERACLES clinical trials that support this study):** S. Siena

**Acknowledgments**

This work was supported by European Community’s Seventh Framework Programme under grant agreement no. 602901 MerCurRC (to A. Bardelli), H2020 grant agreement no. 635342-MoTiColor (to A. Bardelli and S. Siena); ICB contract no. 115749 CANCER-ID (to A. Bardelli); AICG IG no. 16788 (to A. Bardelli); Fondazione Piemontese per la Ricerca sul Cancro-ONLUS 5 per mille 2014 e 2015 Ministero della Salute (to A. Bardelli, S. Siena, L. Trusolino, and S. Marsoni); AICG IG no. 18532 (to L. Trusolino); Transcan, TACTIC (to L. Trusolino); AICR Special Program 5 per mille metastases Project n. 21091 (to A. Bardelli, S. Marsoni, S. Siena, and L. Trusolino); AICR 3-year fellowship and Roche per la ricerca grant 2017 (to G. Siravegna); Terapia Molecolare Tumori by Fondazione Oncologia Niguarda Onlus (to A. Sartore-Bianchi and S. Siena); and Genomic-Based Triage for Target Therapy in Colorectal Cancer Ministero della Salute, project no. NET 02352137 (to A. Sartore-Bianchi, S. Siena, and S. Marsoni).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 20, 2018; revised December 10, 2018; accepted February 4, 2019, published first February 26, 2019.

**References**


Plasma HER2 (ERBB2) Copy Number Predicts Response to HER2-targeted Therapy in Metastatic Colorectal Cancer


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-18-3389

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2019/02/09/1078-0432.CCR-18-3389.DC1

Cited articles
This article cites 27 articles, 8 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/25/10/3046.full#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/25/10/3046. Click on "Request Permissions" which will take you to the Copyright Clearance Center’s (CCC) Rightslink site.