Title

Analysis of Circulating Tumor DNA and Clinical Correlates in Patients with Esophageal, Gastroesophageal Junction, and Gastric Adenocarcinoma

Journal

Clinical Cancer Research, 24(24)

ISSN

1078-0432

Authors

Kato, Shumei
Okamura, Ryosuke
Baumgartner, Joel M
et al.

Publication Date

2018-12-15

DOI

10.1158/1078-0432.ccr-18-1128

Peer reviewed

HHS Public Access

Author manuscript

Clin Cancer Res. Author manuscript; available in PMC 2019 December 15.

Published in final edited form as:

Clin Cancer Res. 2018 December 15; 24(24): 6248–6256. doi:10.1158/1078-0432.CCR-18-1128.

Analysis of Circulating Tumor DNA and Clinical Correlates in Patients with Esophageal, Gastroesophageal Junction and Gastric Adenocarcinoma

Shumei Kato, M.D.[*,1], **Ryosuke Okamura, M.D.**[*,1], **Joel M. Baumgartner, M.D.**[2], **Hitendra Patel, M.D.**[1], **Lawrence Leichman, M.D.**[1], **Kaitlyn Kelly, M.D.**[2], **Jason K. Sicklick, M.D.**[2], **Paul T Fanta, M.D.**[1], **Scott M Lippman, M.D.**[¶,1], and **Razelle Kurzrock, M.D.**[¶,1]

[1]Center for Personalized Cancer Therapy and Division of Hematology and Oncology, Department of Medicine, UC San Diego Moores Cancer Center, La Jolla, CA, USA

[2]Division of Surgical Oncology, Department of Surgery, UC San Diego Moores Cancer Center, La Jolla, CA, USA

Abstract

Purpose: Esophageal, gastro-esophageal junction and gastric adenocarcinoma (herein gastroesophageal adenocarcinomas) are associated with poor prognosis and limited systemic treatment options. To further understand the genomic landscape of gastroesophageal cancers and its clinical correlations, circulating tumor DNA (ctDNA) from patients' plasma was evaluated using next-generation sequencing (NGS).

Methods: We analyzed genomic alterations of 55 patients (mostly advanced disease; nine, surgically resectable) with gastroesophageal adenocarcinomas using clinical-grade NGS performed on plasma-derived ctDNA (54-73 gene panel). The test detects single nucleotide variants, as well as copy number amplifications, fusions and indels in selected genes.

Results: Seventy-six percent of patients (42/55) had ≥1 genomic alteration (including variants of unknown significance [VUSs]) and 69.1% (38/55) had ≥1 characterized alteration (excluding VUSs). The median number of alterations per patient was 2 (range, 0–15). *TP53* (50.9%, 28/55), *PIK3CA* (16.4%, 9/55), *ERBB2* (14.5%, 8/55) and *KRAS* (14.5%, 8/55) genes were most frequently affected characterized alterations. Thirty-one patients also had tissue NGS. Concordance between tissue and ctDNA ranged from 61.3% (*TP53* alterations) to 87.1% (*KRAS* alterations). *ERBB2* alterations were significantly associated with poor overall survival (HR: 14.06, 95% CI: 2.44 – 81.03, P=0.003 multivariate analysis). Among patients with ≥1 alteration, no two patients had identical molecular portfolios. All patients with ≥1 characterized alteration

Corresponding author's contact information: Shumei Kato, M.D., Center for Personalized Cancer Therapy and Division of Hematology and Oncology, Department of Medicine, UC San Diego Moores Cancer Center, 3855 Health Sciences Drive, La Jolla, CA 92093, smkato@ucsd.edu, Phone: 858-822-2372, FAX: 858-822-6186.

*Contributed equally

¶Contributed equally

Conflict of Interest Disclosures: Dr. Kurzrock has research funding from Incyte, Genentech, Merck Serono, Pfizer, Sequenom, Foundation Medicine, and Guardant Health, as well as consultant fees from X-Biotech, Loxo, and Actuate Therapeutics, speaker fees from Roche, and has an ownership interest in Curematch, Inc. Dr. Sicklick has research funding from Foundation Medicine and Novartis, as well as consultant fees from Loxo and Grand Rounds.

had theoretically targetable alterations by an FDA-approved agent (on- or off-label). Illustrative case treated with cognate agent is presented.

Conclusions: Evaluation of ctDNA by NGS among gastroesophageal adenocarcinoma patients is feasible. Patients harbored heterogeneous patterns of genomics, with most having alterations that are potentially pharmacologically tractable.

Keywords

Circulating tumor DNA; liquid biopsy; next-generation sequencing; esophageal cancer; gastro-esophageal cancer; gastric cancer

INTRODUCTION

Globally, gastric and esophageal malignancies (herein gastroesophageal cancers) are one of the most frequent types of cancers with approximately 1.5 million patients diagnosed each year. They are associated with high mortality and 1.1 million patients are estimated to die each year (1). At the time of diagnosis, about 50–60% of patients have regional lymph node involvement or distant metastatic disease (2). Combinations of systemic chemotherapies are generally used for the management of metastatic gastroesophageal cancers. Systemic therapies with anti-metabolite (5-fluorouracil or capecitabine) and platinum (oxaliplatin or cisplatin) based therapies as well as taxanes are widely used; however, median survival remains poor (9–11 months) (3, 4). Thus, there is an urgent need to better understand the molecular biology of these neoplasms.

Along with the rapid advances in next-generation sequencing (NGS) technology, the molecular nosology of gastroesophageal cancers is now better understood. The Cancer Genome Atlas Research Network categorized gastric cancer patients into four different subtypes: (i) Epstein-Barr virus-related group, which are associated with *PIK3CA* mutations, PD-L1/2 overexpression, and *CDKN2A* silencing; (ii) microsatellite unstable group, associated with hypermutation and *MLH1* silencing; (iii) chromosomal unstable group associated with receptor tyrosine kinase and RAS activation; and (iv) genomically stable subtypes (5).

Clinically, several genomic and/or protein markers are now being used to guide treatment decisions for patients with gastroesophageal cancers. Examples include targeting of HER2 overexpressed/amplified gastric and gastroesophageal junction cancers with trastuzumab (anti-HER2 antibody) (6) and PD-L1 overexpressed cases with pembrolizumab (anti-PD-1 antibody) (7, 8), which are now Food and Drug Administration (FDA) approved. Although some achievements were seen with the aforementioned targeted therapy approaches, clinical benefit has been modest (trastuzumab: 2.7 months of survival gain when added to chemotherapy (6); pembrolizumab response rate [RR] of ~20% (7)). Moreover, targeting other markers including *FGFR* amplification or MET overexpression, has not been able to demonstrate clinical benefit, at least as monotherapies, in the setting of gastroesophageal cancers (9, 10).

One of the major challenges to addressing genomic alterations in the clinic is tumor heterogeneity. Pectasides *et al*, compared genomic alterations between primary and metastatic lesions in 26 patients with gastroesophageal adenocarcinomas and revealed extensive differences (11). Kim *et al.*, reported that more than half of the *ERBB2*-amplified gastroesophageal adenocarcinomas had additional oncogenic alterations, with each patient harboring unique molecular patterns that could explain the reason for the modest effects achieved with *ERBB2*-targeting agents (12). Moreover, dynamic change in underlying genomic alterations can evolve along with tumor progression and therapeutic pressure, which further confounds targeted therapy approaches (13).

NGS of plasma-derived circulating tumor DNA (ctDNA) (also known as cell-free tumor DNA) has recently been evaluated in several tumor types (14–17). Detection of tumor-specific mutations that are shed into the blood from cancer cells can be performed on a small vial of blood, enabling the characterization of genomic alterations of tumors in a timely fashion. Although analysis of ctDNA among gastroesophageal cancers has been investigated (18), clinical application of the results are not well described. Here we investigated clinical characteristics and therapeutic outcomes among patients with gastroesophageal adenocarcinomas whose ctDNA was interrogated by clinical-grade NGS and compared the results to tissue NGS results in those patients in whom both tests were performed.

MATERIALS AND METHODS

Patients

We investigated the genomic alteration status and clinical outcomes of 55 patients with gastroesophageal adenocarcinomas seen at the UC San Diego Moores Cancer Center (January 2014 to July 2017). Blood samples were evaluated at the clinical laboratory improvement amendments (CLIA)-licensed and College of American Pathologist (CAP)-accredited clinical laboratories--*Guardant Health, Inc.* (http://www.guardanthealth.com) or *Foundation Medicine* (https://www.foundationmedicine.com) for ctDNA analysis. Tissue samples were sent to *Foundation Medicine* for tissue DNA testing using NGS. All investigations followed the guidelines of the UCSD Internal Review Board for data collection (NCT02478931) and for any experimental therapeutic trials for which consents were obtained.

Next-generation sequencing

Most ctDNA analyses were done through Guardant Health, Inc (N=49/55). ctDNA was extracted from whole blood collected in 10mL Streck tubes, and 5 to 30ng of ctDNA was prepared for sequencing as previously described (19). All ctDNA was sequenced, including the somatic ctDNA and the germline ctDNA that is derived from natural leukocyte lysis. Germline alterations were filtered out and not reported. The fractional concentration or variant allele fraction for a given somatic mutation is calculated as the fraction of ctDNA harboring that mutation in a background of wild-type ctDNA fragments at the same nucleotide position. The analytic sensitivity reaches detection of 1–2 single mutant fragments from a 10 ml blood sample (0.1% limit of detection) and analytic specificity is greater than 99.9999%. Throughout the timeframe of this study, the ctDNA assay performed

by Guardant Health, Inc. expanded from 54 to 68 to 70 to 73 genes (N=49, Table 1 and Supplemental Table 1). Degree of copy number alterations were reported as follows: 1+, 2.13–2.40, which is the 10^{th} to 50^{th} percentile; 2+, 2.41–4.00, which is >50^{th} to 90^{th} percentile; and 3+, greater than 4.0 copy numbers, which is >90^{th} percentile. The ctDNA assay performed by Foundation Medicine interrogated 67 genes (N=6, Table 1 and Supplemental Table 2) which was previously described (20). Only non-synonymous alterations were analyzed in this study. The variant allele fraction or fractional concentration for a given somatic mutation is derived from the fraction of ctDNA harboring that mutation in a background of wild-type ctDNA fragments at the same nucleotide position (19). When patients had multiple ctDNA evaluated at different time points, the results of ctDNA at the earlier time point was used for the analysis.

Thirty-one patients had both ctDNA and tissue DNA analysis. All tissue DNA analyses were performed by Foundation Medicine as previously described (21, 22). The assay for tissue DNA was designed to include all genes known to be somatically altered in human solid tumors that are validated targets for therapy and interrogated 236 genes as well as 47 introns of 19 genes commonly rearranged in cancer (n=2) and 315 genes as well as introns of 28 genes commonly rearranged in cancer (N=29).

Endpoints, statistical methods, and case studies

Descriptive statistics were used to summarize the genomic alterations identified in this study. The Mann-Whitney U test was used for continuous data and Fisher's exact test was used for categorical data. Concordances of ctDNA and tissue DNA were quantified by concordance percentage and Kappa value with the standard error. Kappa values are interpreted by commonly used agreement categories: $\kappa = 1$ (perfect agreement) to $\kappa = 0$ (no agreement other than would be expected by chance). Overall survival was calculated from the time of ctDNA analysis to last follow up. Survival analyses were assessed by Kaplan-Meier analysis and Cox's proportional hazard model was used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs). For multivariate analysis, variables with P-values < 0.20 in univariate analysis were included in the multivariate regression model.

RESULTS

Patient characteristics among patients with gastroesophageal cancer evaluated for ctDNA

A total of 55 patients with gastroesophageal adenocarcinomas were evaluated for ctDNA. Tumors located in esophagus, gastro-esophageal junction, and stomach were represented as follows: 20.0% (N=11), 30.9% (N=17), and 49.1% (n=27), respectively (Table 1) (Figure 1). Amongst all patients with gastroesophageal adenocarcinomas (N=55), the median age at the time of diagnosis was 62.6 years old (range, 23.5–91.5), and 63.6% (N=35) were men. ctDNA tests were performed prior to surgical resection in nine patients (16.4%). Among patients who had ctDNA analysis, tissue NGS was performed in 31 patients (56.4%) (Table 1).

Amongst these 55 patients evaluated for ctDNA, 76.4% (N=42) had at least one genomic alteration (includes characterized alterations and variants of unknown significance [VUSs])

and 69.1% (N=38) had at least one characterized alteration. The median number of alterations per patient was 2 (range: 0–15); the median number of characterized alterations per patient was 1 (range: 0–7). The most common characterized alteration was *TP53* (50.9%, N=28) followed by *PIK3CA* (16.4%, N=9), *ERBB2* (14.5%, N=8) and *KRAS* (14.5%, N=8) (Figure 2, Supplemental Table 3). Thirteen patients (23.6%) had ≥5% of allele frequency and the median percentage of highest allele frequency was 0.8% (range: 0–50.7%) (Table 1).

Number of alterations with possible cognate targeted therapies

Amongst 55 cases with gastroesophageal cancers, a total of 159 alterations were identified (including VUSs). Among those alterations, 68.6% (109/159) were characterized alterations, including substitutions (51.6% [82/159]) and amplifications (17.0% [27/159]). Among the 109 characterized alterations, 89.9% (98/109) were potentially targetable with FDA-approved agents as on- or off-label use, and an additional 8.2% (8/109) were theoretically targetable with therapies that are currently in clinical trials. Altogether, among all characterized alterations, 97.2% (106/109) were theoretically actionable either with agents that are approved by the FDA (including off-label) or with agents that are in clinical trials. Among all 55 patients with gastroesophageal cancers, 69.1% (N=38) (all patients with ≥1 characterized alteration) had ≥1 theoretically actionable alterations by an FDA-approved agent (on- or off-label). Patients who did not have targetable alterations were those who only had VUSs (7.3%, N=4) or patients without detectable alteration (23.6%, N=13).

Distinctness of genomic alterations among 55 patients with gastroesophageal cancers

Among the 38 patients who had ≥1 characterized alteration, no two patients had identical molecular portfolios (e.g. *TP53* R273H and *TP53* R175H considered as molecularly distinct), while 8 patients had identical genomic portfolios (e.g. *TP53* R273H and *TP53* R175H considered as genomically identical) [ID#15, 27, 29, 31, 40, 48, 51] (Supplemental Table 4).

Concordance between ctDNA and tissue DNA testing

Of 31 patients who had both ctDNA and tissue DNA analyses, the median time interval between tissue and ctDNA collection was 1.3 months (range 0 – 47.1 months). The overall concordance rate was 61.3% for *TP53*, 83.9% for *ERBB2*, 74.2% for *PIK3CA* and 87.1% for *KRAS* alterations (statistically significant concordance only seen with *ERBB2* alterations [P = 0.048]) (Supplemental Table 5a). When overall concordance was compared between ctDNA and tissue NGS, one patient (3.2% [1/31]) had complete concordance (*TP53* R342*, *PTEN* H75fs*2 and *KRAS* amplification were found in both ctDNA and tissue [Supplemental Table 4, ID: 43]). Partial concordance (e.g. *ERBB2* amplification found in both ctDNA and tissue NGS, but *TP53* alteration only found in tissue NGS) was seen in 54.8% (17/31) of cases. There was no concordance seen in 41.9% (13/31) of cases (including 10 patients without detectable characterized alterations from ctDNA) (Supplemental Table 4). Similar concordance was observed when the interval between blood draw and tissue biopsy was ≤ 6 months (N = 19 patients) vs. > 6 months (N = 12 patients), but the small number of individuals in each group precludes definitive conclusions (Supplemental Table 5b). When concordance was evaluated depending on the site of biopsies (primary site [N = 23] vs. metastatic site [N = 8]), higher concordance was

observed between cfDNA and metastatic sites for *TP53* and *ERBB2* alterations (concordance rate between cfDNA and primary site vs. cfDNA and metastatic site was as follows: *TP53*: 52.2% vs. 87.5%, *ERBB2*: 78.3% vs. 100%); however, these differences were not statistically significant (Supplemental Table 5c). Since treatment can affect ctDNA levels, we ran the concordance analysis of the 20 patients who had not received systemic therapy or were off treatment for at least four weeks. We found similar concordance rates to those in the analysis of all patients (Supplemental Table 5d).

Survival analysis among 46 patients with locally advanced, metastatic or recurrent gastroesophageal cancer

Patients with locally advanced, metastatic or recurrent gastroesophageal cancers (N=46) were included in the survival analysis. Characteristics with at least five patients affected were included. When overall survival was evaluated from the time of ctDNA analysis to last follow up, in the univariate analysis, gender, *PIK3CA* and *KRAS* alteration*s* as well as lines of systemic therapies (≥ 2 lines) before the ctDNA analysis were not statistically associated with the overall survival (OS) outcome. On the other hand, patients with *TP53* alterations, highest %ctDNA ≥ 1.65% and cumulative total %ctDNA ≥ 2.3% had a trend towards worse OS (HR: 2.28, 95% CI: 0.72 – 7.29, P=0.154, HR: 2.49, 95% CI: 0.78 – 8.00, P=0.115 and HR: 2.50, 95% CI: 0.75 – 8.28, P=0.122 respectively) (Cut off of %ctDNA ≥ 1.65% and ≥ 2.3% was chosen because it was the median %ctDNA). Patients whose ctDNA was obtained ≥ 6.8 months from the time of metastatic/recurrent disease had a trend towards better OS (HR: 0.48, 95% CI: 0.15 – 1.46, P=0.183) (cut off of 6.8 months was chosen since it was the median). *ERBB2* alterations were significantly associated with worse OS (HR: 8.02, 95% CI: 2.41 – 26.69, P<0.001) in univariate analysis (Table 2 and Figure 3). After the multivariate analysis, *ERBB2* alterations continued to be an independent factor associated with poor OS (HR: 14.06, 95% CI: 2.44 – 81.03, P=0.003). Patients whose ctDNA were obtained ≥ 6.8 months from the time of metastatic/recurrent disease was associated with better OS (HR: 0.18, 95% CI: 0.04 – 0.82, P=0.026) (Table 2).

Detection of ctDNA among patients with early stage and advanced stage gastroesophageal cancers

Among 9 patients whose ctDNA was examined prior to resection, 3 (33.3%) had detectable characterized alterations, while 76.1% (35/46) of patients with metastatic, recurrent, or locally advanced diseases had detectable alterations (P=0.019). Among patients who underwent surgery (N=9), the median number of alteration (0 vs. 3, P=0.009) and median value of highest allele frequency of %ctDNA (0 vs. 1.7, P=0.001) were significantly lower than that of patients with metastatic, recurrent, or locally advanced diseases (Supplemental Table 6).

Representative case among patient who received matched targeted therapies based on ctDNA results

Among 55 patients evaluated for ctDNA, 24 patients received systemic therapy after the ctDNA analysis. Among those 24 patients, 70.8% (17/24) had at least one actionable genomic alteration (the remaining 7 patients had no characterized alterations). However only

1 of 24 patients (4.2%) were treated with matched targeted therapy approach (Figure 1 and Supplemental Table 7).

The summary of the patient who received matched targeted therapy is given in Supplemental Table 7.

Patient ID.42: This is a 68-year-old woman with gastroesophageal junction adenocarcinoma who was found to have multiple liver metastases and lymphadenopathy at diagnosis. Both ctDNA and tissue NGS revealed *EGFR* amplification. The patient was started on dual anti-*EGFR* therapy (23) (cetuximab and erlotinib) and achieved partial response (67% decrease by RECIST 1.1, PFS of 18 months) (17). Along with radiographic response (Figure 4.A.), serial ctDNA analysis four and twelve months after the first ctDNA analysis showed normalization of *EGFR* copy number in plasma (pre-treatment *EGFR* copy number = 143, down to reference range) (Figure 4.B.).

DISCUSSION

Patients with advanced gastroesophageal cancers have a poor prognosis with a median survival of 9 to 11 months (3, 4). Although molecular characteristics of gastroesophageal cancers have been investigated (5), to date, understanding clinical correlates of genomic data and capitalizing on this information in the patient care setting has been limited (6, 7, 9, 10). Herein, we report the biologic and clinical correlates of genomic alterations among 55 patients with mostly advanced-stage gastroesophageal cancers using blood-derived ctDNA interrogated by clinical-grade NGS.

Altogether, 76.4% (42/55) of patients had at least one non-synonymous alteration detected from ctDNA and 69.1% (38/55) had at least one characterized alteration (VUSs excluded). The most frequent characterized alterations were in the *TP53* gene (50.9%, 28/55) followed by the *PIK3CA* (16.4%, 9/55), *ERBB2* (14.5%, 8/55) and *KRAS* genes (14.5%, 8/55) (Figure 2, Supplemental Table 3), which is consistent with a previous report (18). Among frequently altered genes, concordance rate between tissue DNA and ctDNA varied from 61% −87% depending on the alterations (Supplemental Table 5a). This observation is consistent with a previous report by Pectasides *et al.*, which showed discordance between tumor DNA and ctDNA in gastroesophageal cancers (11). This may not be surprising since genomic alterations from the areas of cancer that were not biopsied (e.g. distant metastases) or intratumoral heterogeneity are likely being uncovered by ctDNA analysis. Indeed, Pectasides and colleagues noted discordance in findings between primary tumors and metastases in gastroesophageal cancers (11). In our study, comparison between the blood draw and tissue biopsy ≤ 6 months apart (N=19) vs. > 6 months apart (N=12) did not reveal a difference in concordance rate among frequently altered genes (Supplemental Table 5b). These observations differ from previous reports in non-gastric cancers that showed high concordance between tissue DNA and blood-derived ctDNA when the interval between two tests was short (24, 25). The relatively small number of patients in our study and in previous work (11) may have confounded the results and/or the concordance in gastric cancers may be lower than in other types of malignancies. Technical differences between the tissue and blood ctDNA assays cannot be ruled out.

Interestingly, patients with early stage/surgically resectable disease had a significantly lower number of alterations detected from ctDNA as well as lower variant allele fraction of ctDNA when compared to patients with metastatic/ advanced disease (Supplemental Table 6). This observation is consistent with previous reports that showed that the level of ctDNA was associated with underlying tumor burden and can demonstrate dynamic changes along with the therapeutic course (24, 26). Moreover, among patients with early-stage colon cancer, postsurgical detection of ctDNA was strongly associated with tumor recurrence suggesting that ctDNA may be used as a surrogate for minimal residual disease after surgery (27). Further investigation in this respect is necessary in patients with gastroesophageal cancers. In regard to outcome, prior reports have shown an association between high %ctDNA and survival (24, 25). In the current report, we found that high %ctDNA (evaluated via the ctDNA alteration presenting the highest variant allele fraction as well as by calculating the cumulative percentages of all ctDNAs in each patient) showed a trend towards correlation with OS in univariate, but not in multivariate, analysis (Table 2).

Notably, among several alterations that were identified, presence of an *ERBB2* alteration was significantly associated with poor OS (HR: 14.06, 95% CI: 2.44 – 81.03, P=0.003 by multivariate analysis; *ERBB2* altered versus not) (Table 2 and Figure 3). Trastuzumab is approved for gastroesophageal cancers with HER2 overexpression or amplification; addition of trastuzumab to chemotherapy demonstrated clinical benefit (trastuzumab plus chemotherapy vs. chemotherapy alone: median OS: 13.8 vs. 11.1 months; ToGA trial) (p = 0.0046) (6). The survival in our patients with *ERBB2*-altered malignancies was considerably shorter than in the ToGA trial, perhaps because the ToGA trial included only patients who had not had prior therapy in the metastatic setting. Also none of our patients were treated with ErbB2-targeting agents after the ctDNA analysis. Additionally, detection of ERBB2 overexpression or amplification can differ depending on the various methodologies used (Supplemental Table 8), which can confound the clinical outcome. Previous reports indicate that gastroesophageal cancers with *ERBB2* alterations commonly harbor genomic co-alterations that can potentially drive therapeutic resistance to anti-HER2 directed therapies (12). In the current study, we have also observed that all patients with *ERBB2* alterations (N=8) harbored at least one co-alteration, including anomalies in *FGFR2*, *RAF1*, *PIK3CA* and *KRAS* that can be associated with resistance to anti-HER2 regimens (Supplemental Table 4, patient ID: 1, 4, 17, 30, 32, 33, 47 and 53). These observations suggest that co-targeting of resistance signals may enhance the efficacy of Erbb2-antagonist agents. Furthermore, among patients whose tumors harbored ≥1 characterized alteration, no two patients had identical molecular portfolios (Supplemental Table 4). Studies with customized combination regimens have been initiated (28).

Although there is growing evidence that matched targeted therapy is potentially promising as a therapeutic approach (29–32), one of the realistic challenges to this strategy has been the low rate of patients receiving matched treatments (approximately 5–20% in many studies) (29, 33–35). Low target-drug matching rate is often due to a number of reasons, including lack of drug accessibility, deterioration of patient's condition at the time of matching, or lack of actionable targets (36). In the current report, we have also observed that molecularly matched therapies based upon profiling with ctDNA was underutilized in the clinic, as only 1.8% (1/55) of patients were treated in this manner (Figure 1 and Supplemental Table 7)

despite the fact 69.1% (38/55) of patients had theoretically tractable targets (Supplemental Table 4). Although there was only one patient who received matched cognate agents, this individual showed clinical benefit, with a partial response (67% regression) and PFS of 18 months in a patient with *EGFR* amplification who was given dual anti-EGFR therapies (cetuximab and erlotinib) (Figure 4 and Supplemental Table 7). Further clinical investigation is warranted.

There were several limitations to the current study. First, the study was performed retrospectively with a small sample size at a single institution. Second, not all patients had tissue NGS as a comparator. Third, it is conceivable that using different ctDNA assays may provide different results, even if the assays are clinical grade. Fourth, the number of genes evaluated changed over time (from 54 to 68 to 70 to 73 gene panels for the Guardant assay), which makes direct comparison challenging; however, it should be noted that only the genes found in both the tissue and the ctDNA panel were compared for concordance. Fifth, some of the concordance was driven by the wild-type cases (where both ctDNA and tissue would be negative for an alteration); hence, the P-values might be misleading for positive concordance. Sixth, different systemic therapies at the time of ctDNA analysis can affect the level of variant allele fractions, which may have confounded the survival analysis. Finally, results of germline alterations were filtered out and not reported in this study. To comprehensively understand the biology of cancer in each patient, evaluation of both germline as well as somatic alterations may be required. Thus, the current findings require further validation with larger numbers of patients in the setting of prospective studies using newer methodologies, as they become available, since the capability to detect alterations is improving with time.

In conclusion, we have evaluated 55 patients with gastroesophageal cancers who had blood-derived ctDNA analysis by clinical-grade NGS. The most frequent alterations were in *TP53* (50.9%) followed by *PIK3CA* (16.4%), *ERBB2* (14.5%) and *KRAS* genes (14.5%). At least one alteration was identified in 76.4% of patients. Concordance between ctDNA and tissue DNA among commonly altered genes ranged from 61.3% to 87.1%. Discordant results may be due to the dynamic changes in ctDNA after treatment or tumor progression, tumor heterogeneity, or from disparities in sensitivity between tissue and ctDNA analysis. Technical issues cannot be ruled out. *ERBB2* alterations were associated with significantly worse OS (median OS = 1.7 versus 20.2 months). Importantly, among patients who had at least one characterized alteration, no two patients had identical molecular portfolios, suggesting that customized therapy may be necessary. Although the number of patient who received therapy that matched ctDNA analysis was small, the patient showed benefit. Further investigations of the clinical utility of blood-derived ctDNA among patients with gastroesophageal cancers are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment:

Funded in part by the Joan and Irwin Jacobs Fund (RK) and the Jon Schneider Memorial Cancer Research Fund (JKS, PTF), as well as by National Cancer Institute grants P30 CA023100 (RK), K08 CA168999 (JKS) and R21 CA192072 (JKS).

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90. [PubMed: 21296855]
2. Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Kosary CL, et al. SEER Cancer Statistics Review, 1975–2014, National Cancer Institute Bethesda, MD, https://seer.cancer.gov/csr/1975_2014/, based on November 2016 SEER data submission, posted to the SEER web site, April 2017.
3. Cunningham D, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, et al. Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med 2008;358:36–46. [PubMed: 18172173]
4. Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, et al. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. J Clin Oncol 2006;24:4991–7. [PubMed: 17075117]
5. Cancer Genome Atlas Research N. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014;513:202–9. [PubMed: 25079317]
6. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010;376:687–97. [PubMed: 20728210]
7. Fuchs CS, Doi T, Jang RW-J, Muro K, Satoh T, Machado M, et al. KEYNOTE-059 cohort 1: Efficacy and safety of pembrolizumab (pembro) monotherapy in patients with previously treated advanced gastric cancer. American Society of Clinical Oncology; 2017.
8. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Mol Cancer Ther 2015;14:847–56. [PubMed: 25695955]
9. Catenacci DVT, Tebbutt NC, Davidenko I, Murad AM, Al-Batran SE, Ilson DH, et al. Rilotumumab plus epirubicin, cisplatin, and capecitabine as first-line therapy in advanced MET-positive gastric or gastro-oesophageal junction cancer (RILOMET-1): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2017;18:1467–82. [PubMed: 28958504]
10. Van Cutsem E, Bang YJ, Mansoor W, Petty RD, Chao Y, Cunningham D, et al. A randomized, open-label study of the efficacy and safety of AZD4547 monotherapy versus paclitaxel for the treatment of advanced gastric adenocarcinoma with FGFR2 polysomy or gene amplification. Ann Oncol 2017;28:1316–24. [PubMed: 29177434]
11. Pectasides E, Stachler MD, Derks S, Liu Y, Maron S, Islam M, et al. Genomic Heterogeneity as a Barrier to Precision Medicine in Gastroesophageal Adenocarcinoma. Cancer Discov 2018;8:37–48. [PubMed: 28978556]
12. Kim J, Fox C, Peng S, Pusung M, Pectasides E, Matthee E, et al. Preexisting oncogenic events impact trastuzumab sensitivity in ERBB2-amplified gastroesophageal adenocarcinoma. J Clin Invest 2014;124:5145–58. [PubMed: 25401468]
13. Hiley C, de Bruin EC, McGranahan N, Swanton C. Deciphering intratumor heterogeneity and temporal acquisition of driver events to refine precision medicine. Genome Biol 2014;15:453. [PubMed: 25222836]
14. Kato S, Krishnamurthy N, Banks KC, De P, Williams K, Williams C, et al. Utility of Genomic Analysis In Circulating Tumor DNA from Patients with Carcinoma of Unknown Primary. Cancer Res 2017;77:4238–46. [PubMed: 28642281]
15. Riviere P, Fanta PT, Ikeda S, Baumgartner J, Heestand GM, Kurzrock R. The Mutational Landscape of Gastrointestinal Malignancies as Reflected by Circulating Tumor DNA. Mol Cancer Ther. 2017.

16. Schrock AB, Pavlick D, Klempner SJ, Chung JH, Forcier B, Welsh A, et al. Hybrid Capture-Based Genomic Profiling of Circulating Tumor DNA from Patients with Advanced Cancers of the Gastrointestinal Tract or Anus. Clin Cancer Res 2018.
17. Schwaederle M, Chattopadhyay R, Kato S, Fanta PT, Banks KC, Choi IS, et al. Genomic Alterations in Circulating Tumor DNA from Diverse Cancer Patients Identified by Next-Generation Sequencing. Cancer Res 2017;77:5419–27. [PubMed: 28807936]
18. Catenacci DV, Nagy RJ, Braiteh FS, Kim S, Kwak EL, Maron SB, et al. Cell free circulating tumor DNA (ctDNA) landscape in patients with advanced gastroesophageal adenocarcinoma (GEC). American Society of Clinical Oncology; 2017.
19. Lanman RB, Mortimer SA, Zill OA, Sebisanovic D, Lopez R, Blau S, et al. Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA. PLoS One. 2015;10:e0140712. [PubMed: 26474073]
20. Ou SI, Young L, Schrock AB, Johnson A, Klempner SJ, Zhu VW, et al. Emergence of Preexisting MET Y1230C Mutation as a Resistance Mechanism to Crizotinib in NSCLC with MET Exon 14 Skipping. J Thorac Oncol 2017;12:137–40. [PubMed: 27666659]
21. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013;31:1023–31. [PubMed: 24142049]
22. Thomas RK, Nickerson E, Simons JF, Janne PA, Tengs T, Yuza Y, et al. Sensitive mutation detection in heterogeneous cancer specimens by massively parallel picoliter reactor sequencing. Nat Med 2006;12:852–5. [PubMed: 16799556]
23. Maron SB, Alpert L, Kwak HA, Lomnicki S, Chase L, Xu D, et al. Targeted therapies for targeted populations: Anti-EGFR treatment for EGFR amplified gastroesophageal adenocarcinoma. Cancer Discov. 2018.
24. Schwaederle M, Husain H, Fanta PT, Piccioni DE, Kesari S, Schwab RB, et al. Use of Liquid Biopsies in Clinical Oncology: Pilot Experience in 168 Patients. Clin Cancer Res 2016;22:5497–505. [PubMed: 27185373]
25. Schwaederle MC, Patel SP, Husain H, Ikeda M, Lanman RB, Banks KC, et al. Utility of Genomic Assessment of Blood-Derived Circulating Tumor DNA (ctDNA) in Patients with Advanced Lung Adenocarcinoma. Clin Cancer Res 2017;23:5101–11. [PubMed: 28539465]
26. Thierry A, El Messaoudi S, Mollevi C, Raoul J, Guimbaud R, Pezet D, et al. Clinical utility of circulating DNA analysis for rapid detection of actionable mutations to select metastatic colorectal patients for anti-EGFR treatment. Annals of Oncology. 2017.
27. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med 2016;8:346ra92.
28. Sicklick JK, Leyland-Jones B, Kato S, Hahn M, Williams C, De P, et al. Personalized, molecularly matched combination therapies for treatment-na. American Society of Clinical Oncology; 2017.
29. Tsimberidou AM, Iskander NG, Hong DS, Wheler JJ, Falchook GS, Fu S, et al. Personalized medicine in a phase I clinical trials program: the MD Anderson Cancer Center initiative. Clin Cancer Res 2012;18:6373–83. [PubMed: 22966018]
30. Schwaederle M, Zhao M, Lee JJ, Lazar V, Leyland-Jones B, Schilsky RL, et al. Association of Biomarker-Based Treatment Strategies With Response Rates and Progression-Free Survival in Refractory Malignant Neoplasms: A Meta-analysis. JAMA Oncol 2016;2:1452–9. [PubMed: 27273579]
31. Jardim DL, Schwaederle M, Wei C, Lee JJ, Hong DS, Eggermont AM, et al. Impact of a Biomarker-Based Strategy on Oncology Drug Development: A Meta-analysis of Clinical Trials Leading to FDA Approval. J Natl Cancer Inst 2015;107.
32. Schwaederle M, Zhao M, Lee JJ, Eggermont AM, Schilsky RL, Mendelsohn J, et al. Impact of Precision Medicine in Diverse Cancers: A Meta-Analysis of Phase II Clinical Trials. J Clin Oncol 2015;33:3817–25. [PubMed: 26304871]
33. Stockley TL, Oza AM, Berman HK, Leighl NB, Knox JJ, Shepherd FA, et al. Molecular profiling of advanced solid tumors and patient outcomes with genotype-matched clinical trials: the Princess Margaret IMPACT/COMPACT trial. Genome Med 2016;8:109. [PubMed: 27782854]

34. Lopez-Chavez A, Thomas A, Rajan A, Raffeld M, Morrow B, Kelly R, et al. Molecular profiling and targeted therapy for advanced thoracic malignancies: a biomarker-derived, multiarm, multihistology phase II basket trial. J Clin Oncol 2015;33:1000–7. [PubMed: 25667274]

35. Andre F, Bachelot T, Commo F, Campone M, Arnedos M, Dieras V, et al. Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER). Lancet Oncol 2014;15:267–74. [PubMed: 24508104]

36. Lewin J, Siu LL. Cancer genomics: the challenge of drug accessibility. Curr Opin Oncol. 2015;27:250–7. [PubMed: 25761223]

TRANSLATIONAL RELEVANCE

Gastroesophageal adenocarcinomas are associated with poor prognosis and have limited systemic treatment options. Thus, there is an unmet need for novel diagnostic tools. Investigation of ctDNA from patient plasma using clinical-grade next-generation sequencing (NGS) revealed that *TP53* (50.9%, 28/55), *PIK3CA* (16.4%, 9/55), *ERBB2* (14.5%, 8/55) and *KRAS* (14.5%, 8/55) were the most commonly altered genes. Tissue and blood-derived ctDNA results were often discordant, consistent with previous work indicating intra-patient heterogeneity in gastroesophageal cancers. Presence of *ERBB2* alterations was associated with significantly worse overall survival from time of ctDNA collection (hazard ratio: 14.06, 95% confidence interval: 2.44 – 81.03; P=0.003 by multivariate analysis). No two patients had identical molecular portfolios, suggesting that optimal targeting with customized combination strategies may be required to control gastroesophageal cancers.

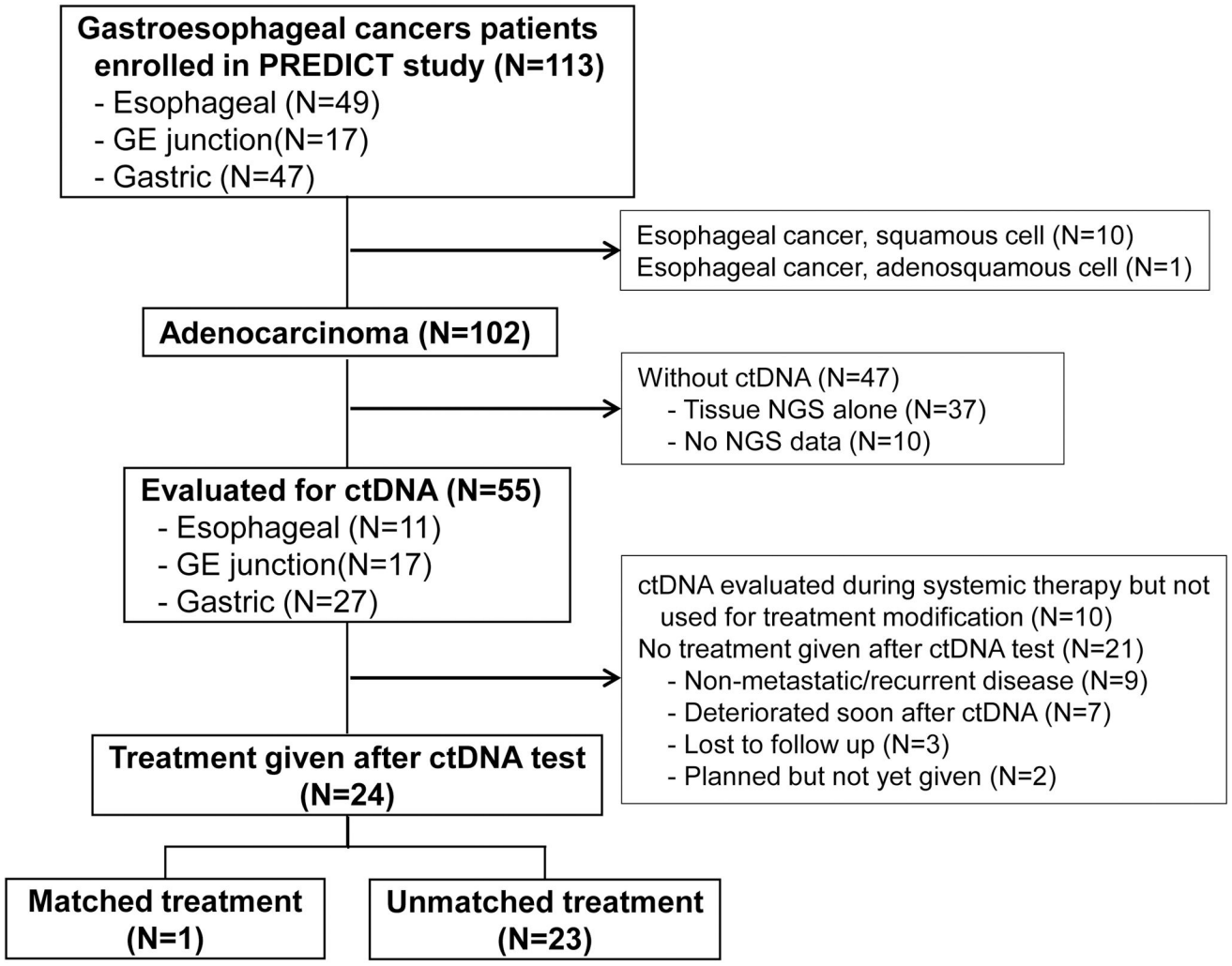

Figure 1.

Consort diagram of patients with esophageal, gastroesophageal and gastric cancer who had ctDNA analysis (N=55).

Abbreviations: ctDNA, circulating tumor DNA; GE, gastroesophageal; NGS, next-generation sequencing; PREDICT study, Profile Related Evidence Determining Individualized Cancer Therapy study.

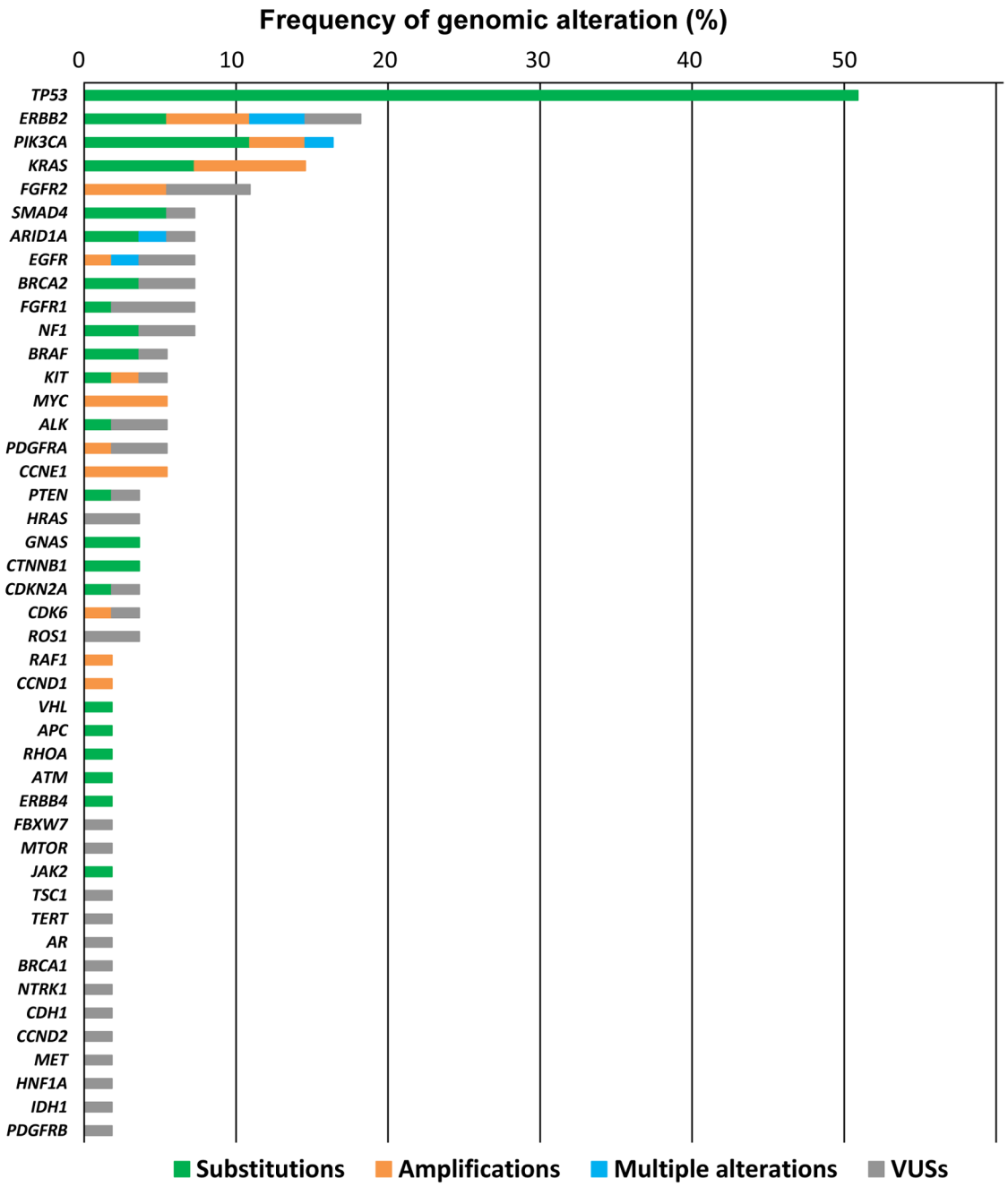

Figure 2.

Frequency of genomic alterations by ctDNA analysis amongst patients with gastroesophageal cancers (N = 55).

Frequency represents percent of patients with an alteration.

The most common alteration was in the *TP53* gene (50.9% of patients, N=28) followed by the *ERBB2* (18.1%, N=10), *PIK3CA* (16.3%, N=9) and *KRAS* genes (14.5%, N=8). See Supplemental Table 3.

Abbreviations: VUS, variants of unknown significance.

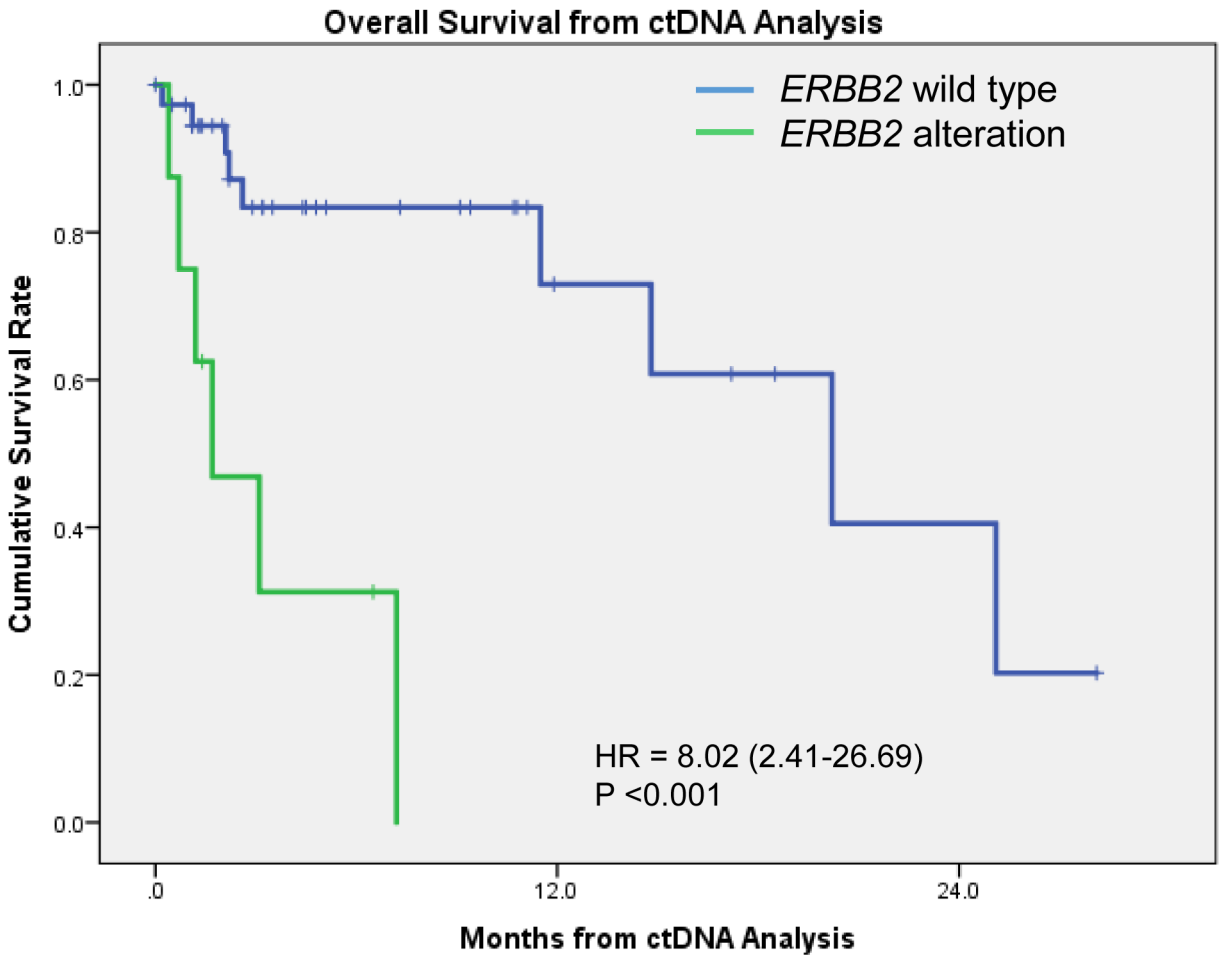

Figure 3.

Kaplan-Meier curves for overall survival from the time of ctDNA analysis to last follow up depending on the *ERBB2* alteration status (N=46).

(Nine patients were excluded because they had resectable disease for which surgery was performed).

Patients with *ERBB2* alterations had significantly worse overall survival compared to patients without *ERBB2* alteration (HR=8.02, 95%CI, 2.41 – 26.69, P <0.001 [univariate analysis]).

Abbreviations: CI, confidence interval; HR, hazard ratio.

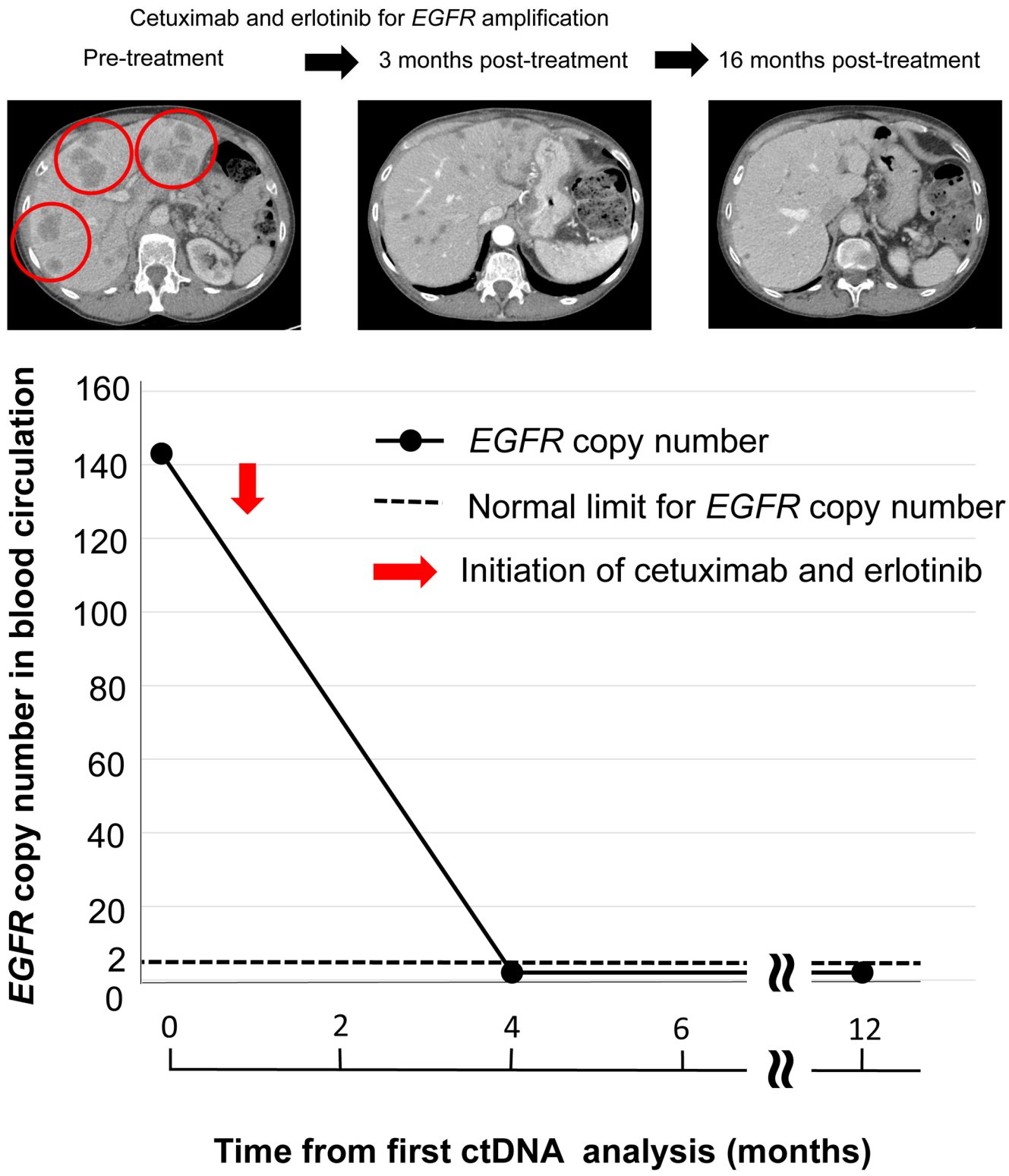

Figure 4.
Representative case of gastroesophageal cancer who was managed with matched targeted therapy approach.

Figure 4.A. and 4.B. 68-year-old female with gastroesophageal junction adenocarcinoma with multiple liver metastases and lymphadenopathy (Figure 4.A. left). ctDNA revealed *EGFR* amplification. Based on the ctDNA analysis, patient was started on dual anti-*EGFR* therapy (cetuximab and erlotinib) (23). Patient was also given nivolumab based on positive PD-L1 by immunohistochemistry (8); however, drug was held after one dose due to grade 3 rash (patient continued to receive dual anti-EGFR therapy). After 3 months of therapy, patient achieved partial response (Figure 4.A. middle) (best response = 67% decrease [Figure 4.A. right], PFS=18 months) along with the normalization of *EGFR* copy number in blood circulation four and twelve months after the first ctDNA analysis (Figure 4.B.).

Table 1.

Patient characteristics of gastroesophageal cancer patients who had ctDNA analysis (N=55)

Basic characteristics (N=55)	**N (%)**
Age, median (range) (years)	
at diagnosis	62.6 (23.5-91.5)
at the time of ctDNA analysis	63.9 (24.3-91.5)
Sex	
Male	35 (63.6%)
Female	20 (36.4%)
Ethnicity	
Caucasian	28 (50.9%)
Asian	8 (14.5%)
Hispanic	16 (29.1%)
Other	3 (5.5%)
Primary tumor location	
Esophagus (N=11)	
Upper	0 (0.0%)
Mid	3 (5.5%)
Lower	8 (14.5%)
Gastroesophageal junction (N=17)	17 (30.9%)
Stomach (N=27)	
Cardia/body/lesser and greater curvature	14 (25.5%)
Incisura/antrum/pylorus	9 (16.4%)
Unknown	4 (7.3%)
Histology	
Esophageal cancer	
Moderately differentiated	6 (10.9%)
Poorly differentiated	5 (9.1%)
Gastroesophageal junction cancer	
Moderately differentiated	3 (5.5%)
Poorly differentiated	7 (12.7%)
Signet ring cells	3 (5.5%)
Unknown	4 (7.3%)
Gastric cancer	
Moderately differentiated	3 (5.5%)
Poorly differentiated	13 (23.6%)
Signet ring cells	10 (18.2%)
Unknown	1 (1.8%)
Disease status at the time of ctDNA analysis	
Metastatic or recurrent	44 (80.0%)
Locally advanced	2 (3.6%)
Surgically resectable *	9 (16.4%)

Basic characteristics (N=55)	**N (%)**
Technique of ctDNA analysis	
Foundation Medicine (panel of 67 genes)	6 (10.9%)
Guardant, Inc.	49 (89.1%)
Panel of 54 genes	1 (1.8%)
Panel of 68 genes	8 (14.5%)
Panel of 70 genes	26 (47.3%)
Panel of 73 genes	14 (25.5%)
Median number of alterations per patient (range) **	2 (0-15)
Median number of characterized alteration per patient (range)	1 (0-7)
Number of patients with ≥ 1 alteration (including VUS)	42 (76.4%)
Number of patients with ≥ 1 characterized alteration	38 (69.1%)
Number of patients with ≥ 5% of allele frequency	13 (23.6%)
Median of highest allele frequency (range), (%)	0.8 (0-50.7)
Number of patients who also had tissue NGS	31 (56.4%)

Abbreviations: NGS, next-generation sequencing; VUS, variant of unknown significant.

* Surgically resectable cases (N=9) indicates that ctDNA analysis was done prior to surgery, except for 1 case whose analysis was done 3 weeks after the surgery. All N=9 patients had R0 resection (clear margins).

** Includes characterized alterations and VUSs.

Table 2.

Overall survival from the time of ctDNA analysis amongst patients with locally advanced, metastatic or recurrent gastroesophageal cancers (N=46).

		Univariate analysis		Multivariate analysis *****	
Characteristics	**Median OS (months)**	**HR (95%CI)**	**P-value**	**HR (95%CI)**	**P-value**
Overall survival from the time of ctDNA analysis (months)					
Age at the diagnosis					
≥ 63 (n=22) vs not (n=24)	11.5 vs 25.1	2.19 (0.74 - 6.46)	0.148	1.82 (0.53 - 6.30)	0.344
Gender					
Men (n=31) vs Women (n=15)	20.2 vs 14.8	0.64 (0.23 - 1.78)	0.392	-	-
Genomic alterations*					
TP53 (n=26) vs not (n=20)	20.2 vs 25.1	2.28 (0.72 - 7.29)	0.154	1.19 (0.21 - 6.73)	0.845
ERBB2 (n=8) vs not (n=38)	1.7 vs 20.2	8.02 (2.41 - 26.69)	**<0.001**	14.06 (2.44 - 81.03)	**0.003**
PIK3CA (n=8) vs not (n=38)	NR vs 14.8	1.71 (0.47 - 6.21)	0.413	-	-
KRAS (n=8) vs not (n=38)	14.8 vs 20.2	1.60 (0.44 - 5.83)	0.472	-	-
Highest %ctDNA**					
≥ 1.65% (n=23) vs not (n=23)	NR vs 20.2	2.49 (0.78-8.00)	0.115	1.04 (0.17 - 6.33)	0.968
Total %ctDNA * **					
≥ 2.3% (n=24) vs not (n=22)	14.8 vs 20.2	2.50 (0.75 - 8.28)	0.122	0.94 (0.10 - 9.20)	0.957
Lines of systemic therapy prior to ctDNA analysis (received ≥ 2 lines)					
Yes (n=15) vs No (n=31)	20.2 vs 14.8	0.83 (0.26 - 2.68)	0.751	-	-
Time from metastatic/recurrent disease to ctDNA collection ≥ 6.8 months ** **					
Yes (n=23) vs No (n=23)	20.2 vs 11.5	0.48 (0.15 - 1.46)	0.183	0.18 (0.04 - 0.82)	**0.026**

Abbreviations: CI, confidence interval; HR, hazard ratio; NR, not reached; OS, overall survival.

*Included only characterized alterations (no VUSs); only included characterized alterations seen in >5 patients

**Used the median of highest %ctDNA value as a cut-off.

***Total %DNA refers to the sum of allele frequency detected in each patient. Used the medial of total %ctDNA value as a cut off.

****Time point of 6.8 months was chosen since it was the median time from metastatic/recurrent disease to ctDNA collection.

*****Variables with P-values < 0.20 in univariate analysis (log-rank test) were included in multivariate analysis.