Special Report

Feasibility and clinical value of circulating tumor DNA testing in patients with gastric adenocarcinomas

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Abstract: Gastric cancer is one of the leading causes of cancer worldwide, and this trend appears to be rising. Most patients are diagnosed at an advanced stage and thus prognosis is poor. Liquid biopsy, or circulating tumor DNA (ctDNA) testing, is emerging as a promising prognostic and/or predictive biomarker for patients with various types of malignancies. Its value and utility for patients with gastrointestinal malignancies, particularly gastric cancer is still being explored. There is ongoing research in other tumor types to suggest that ctDNA testing can be potentially used to identify tumor specific genomic alterations, predict tumor mutation burden, as well as help assess clinical response. We report on the feasibility and clinical value of ctDNA testing in patients with gastric cancers in a real time clinical context by reporting data on cohort of patients with gastric cancers (including those with gastroesophageal junction adenocarcinomas) treated at our institution.

Keywords: Gastric adenocarcinoma; circulating tumor DNA (ctDNA); liquid biopsy; biomarker; heterogeneity

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Introduction

Gastric cancer is prevalent in various parts of the world, with an average of 1 million patients diagnosed annually. Despite recent advances, the 5-year survival rate is less than 30–40% in patients with advanced gastric cancer (1). Therefore development of biomarkers that can assist in timely detection, prognosis and in assessment of treatment response is of great importance. Circulating tumor DNA (ctDNA) has shown potential in various studies for further development as a biomarker with predictive and prognostic implications. This is supported by studies showing ctDNA levels to correlate with the tumor size, tumor stage and depth of invasion in patients with gastric, breast, lung, ovarian and pancreatic cancers (2,3). Further, studies have shown the level of ctDNA to be 2–3 times higher in the plasma of cancer patients (4,5). Correlation of ctDNA with treatment response has also been noted, implicating a possible role in monitoring treatment response (2). Over 80% of patients with advanced gastroesophageal cancers have been shown to have genetic aberrations detected by ctDNA (6) However, limited literature is available elucidating the feasibility of ctDNA testing in a real-time clinical setting in patients with gastric cancer including those with gastroesophageal junction cancer. Herein, we describe a cohort of such patients characterized using a clinically available ctDNA assay.

Methods

During the period extending from January 2015 to June 2018, a total of 46 patients with gastric cancer including those with gastroesophageal junction malignancies underwent ctDNA testing by Guardant Health (Redwood City, CA, USA). All patients were seen at Mayo Clinic Cancer Center. Of the 46 patients, 55 samples were obtained, with 7 patients having serial testing. Demographic information and date of blood collection were available for all patients. The analysis of data from patients in this cohort was approved by the Mayo Clinic institutional review board.

Cell-free DNA (ctDNA) isolation

ctDNA was isolated as previously described (7,8). Briefly, blood samples were collected in Streck™ tubes. Samples were shipped at room temperature overnight to Guardant Health (Redwood City, CA, USA). On receipt, 10 mL of blood was processed by centrifugation by 1,600 g for 10 minutes at 4 ℃ to isolate plasma. ctDNA was then extracted from 1mL aliquots of plasma using the QIAamp circulating nucleic acid kit (Qiagen), concentrated and size selected using Agencourt Ampure XP beads (Beckman Coulter), and quantified by Qubit fluorometer (Life Technologies, Carlsbad, CA, USA).

ctDNA sequencing

The G360 panel is a CLIA-certified, College of American Pathologists (CAP)-accredited, New York State Department of Health (NYSDOH)-approved test that detects single nucleotide variants (SNV) in 73 genes as well as copy number amplifications (CNAs) in 18 genes, fusions in 6 genes, and insertions or deletions (indels) in 23 genes. Following ctDNA isolation, 5–30 ng of DNA underwent oligonucleotide barcoding for digital sequencing library preparation. This library was amplified and enriched for the target genes using biotinylated custom baits. Each of the cancer-related genes was pair-end sequenced on an Illumina HiSeq 2500. Each base pair had a 15,000× average coverage depth. After sequencing, algorithmic reconstruction of the digitized sequencing signals was used to reconstruct the ctDNA fragments. Analytic and clinical validation has been previously reported (7).

The Illumina sequencing reads were mapped to the hg19/GRCh37, and ctDNA genomic alterations were identified from the sequencing data by Guardant Health's proprietary bioinformatics algorithms. The absolute number of unique DNA fragments at a given nucleotide position is quantified, enabling a quantitative measurement of ctDNA as a percentage of the total ctDNA. The variant allele frequency (VAF) for a given somatic alteration is calculated as the fraction of ctDNA molecules harboring the variant of interest divided by the total number of unique ctDNA molecules mapping to the variant position. The reportable range for SNV, indels, fusions, and CNAs in ctDNA by the G360 assay is cs in cportable range for SNV, y the total numctively. Plasma copy number of is reported by centiles with 2+ being between the 50th to 90th percentile in the Guardant Health database and 3+ being greater than the 90th percentile.

Over the course of the study, the gene panel composition expanded from 54 to 68 to 70 to 73 genes. The currently utilized 73 gene panel includes the addition of 5 genes to and removal of 2 genes from the prior list. The vast majority of samples in this study were tested under the 73-gene panel.

Results

Data from a total of 46 patients was included in this study. A total of 55 samples were obtained from these patients including variants of undetermined significance (VUS) with six patients undergoing testing twice and one patient undergoing testing four times. Eleven samples were noted to have no alterations. After excluding VUS we had a total of 43 samples from 34 patients. Eighteen patients were diagnosed with gastric adenocarcinoma (53%) and 16 patients were diagnosed as gastroesophageal junction adenocarcinoma (47%). Nine patients were female (26%) and twenty five were male (73%). The median age at the time of first test was at 65 years with a minimum age of 33 years and a maximum of 98 years in our cohort of patients.

Among all samples the median number of alterations, excluding VUS, per sample was 1 (range, 1–52) with an average of 4.39. The median minor allele frequency (MAF), excluding VUS, per sample was 1.2 (range, 0.03–52) with an average of 3.7.

A total of 136 genetic alterations excluding VUS or synonymous alterations were seen. The profile of genetic alterations observed was broad as shown in *Table 1*. The top 11 genes altered in this cohort of patients, excluding VUS or synonymous alterations, were *TP53* (38.2%), *KRAS* (8.1%), *PIK3CA* (7.4%), *ARID1A* (5.96%), *EGFR* (4.4%), *APC* (3.7%), *ERBB2/HER2* (3.7%), *CDK6* (2.9%), *MET* (2.9%), *PTEN* (2.9%) and *MYC* (2.2%). This illustrates that only a few genes are altered in the majority of the patients. Regarding the type of alterations, the majority (63.2%) were single nucleotide variations followed by copy number variations (31%) and indels (20%) (*Table 2*). Currently the only approved treatment by FDA that is targeted against a genetic aberration in patients with gastric cancer/GE-junction adenocarcinoma and HER-2 positivity is trastuzumab however there are approved treatments for multiple targets in various other malignancies along with the availability of experimental therapeutics (*Table 3*). *HER2/Neu* amplification/alteration was only detected in 5 out of the 46 patients included in this study. Data regarding

Table 1 Landscape of alterations

Gene	# of alterations	Frequency of alterations
AKT1	1	0.735294118
APC	5	3.676470588
ARID1A	8	5.882352941
BRAF	1	0.735294118
BRCA2	1	0.735294118
CCND1	1	0.735294118
CCND2	1	0.735294118
CCNE1	2	1.470588235
CDH1	1	0.735294118
CDK6	4	2.941176471
CDKN2A	2	1.470588235
CTNNB1	2	1.470588235
EGFR	6	4.411764706
ERBB2	5	3.676470588
ESR1	1	0.735294118
FGFR1	1	0.735294118
GNAS	1	0.735294118
JAK2	1	0.735294118
KIT	2	1.470588235
KRAS	11	8.088235294
MET	4	2.941176471
MYC	3	2.205882353
NF1	1	0.735294118
PDGFRA	1	0.735294118
PIK3CA	10	7.352941176
PTEN	4	2.941176471
RAF1	1	0.735294118
RB1	1	0.735294118
RHOA	1	0.735294118
SMAD4	1	0.735294118
TP53	52	38.23529412

Table 2 Types of alterations

Gene	SNV	Indel	CNV
AKT1	1	0	0
APC	2	3	0
ARID1A	4	4	0
BRAF	1	0	0
BRCA2	1	0	0
CCND1	0	0	1
CCND2	1	0	0
CCNE1	0	0	2
CDH1	0	1	0
CDK6	0	0	4
CDKN2A	0	2	0
CTNNB1	2	0	0
EGFR	1	0	5
ERBB2	1	0	4
ESR1	1	0	0
FGFR1	0	0	1
GNAS	1	0	0
JAK2	1	0	0
KIT	0	1	1
KRAS	10	0	1
MET	0	0	4
MYC	0	0	3
NF1	0	1	0
PDGFRA	0	0	1
PIK3CA	9	0	1
PTEN	2	2	0
RAF1	0	0	1
RB1	1	0	0
RHOA	1	0	0
SMAD4	1	0	0
TP53	45	7	0

HER2/Neu testing on tissue samples was available in 3 out of 5 patients at the time of consultation. All three of these patients were found to be positive for *HER2/Neu* via IHC testing on tissue samples. Serial testing done in patients at the time of disease progression correlated with increase in somatic mutation burden. This is shown in *Figure 1* which shows ctDNA tumor response in a patient with metastatic GE-junction adenocarcinoma who underwent serial

Table 3 Targeted treatments both FDA approved and experimental

Gene	Therapies approved in G/GE cancers	Therapies approved in other cancers	Clinical trial availability
AKT1	N/A	Temsirolimus, everolimus	Yes
APC	N/A	Celecoxib	Yes
ARID1A	N/A	N/A	Yes
BRAF	N/A	Sorafenib, regorafenib, trametinib, cobimetinib	Yes
BRCA2	N/A	Olaparib, niraparib, rucaparib	Yes
CCND1	N/A	Palbociclib, ribociclib, abemaciclib total	Yes
CCND2	N/A	N/A	N/A
CCNE1	N/A	N/A	Yes
CDH1	N/A	N/A	N/A
CDK6	N/A	Palbociclib, abemaciclib, ribociclib	Yes
CDKN2A	N/A	Ribociclib, palbociclib, abemaciclib	Yes
CTNNB1	N/A	Celecoxib	Yes
EGFR	N/A	Neratinib, cetuximab, panitumumab, afatinib	Yes
ERBB2	Trastuzumab	Lapatinib, ado-trastuzumab emtansine, pertuzumab, afatinib	Yes
ESR1	N/A	Fulvestrant	Yes
FGFR1	N/A	Pazopanib, ponatinib, nintedanib, lenvatinib	Yes
GNAS	N/A	Trametinib, cobimetinib	Yes
JAK2	N/A	Ruxolitinib	N/A
KIT	N/A	N/A	Yes
KRAS	N/A	N/A	Yes
MET	N/A	Crizotinib, cabozantinib	Yes
MYC	N/A	N/A	Yes
NF1	N/A	Temsirolimus, trametinib, everolimus, cobimetinib	Yes
PDGFRA	N/A	Pazopanib, sorafenib, sunitinib, nilotinib, regorafenib olaratumab, lenvatinib, dasatinib, imatinib, ponatinib	Yes
PIK3CA	N/A	N/A	Yes
PTEN	N/A	Temsirolimus, copanlisib, everolimus	Yes
RAF1	N/A	Regorafenib, sorafenib, trametinib, cobimetinib	N/A
RB1	N/A	N/A	N/A
RHOA	N/A	N/A	N/A
SMAD4	N/A	N/A	N/A
TP53	N/A	N/A	Yes

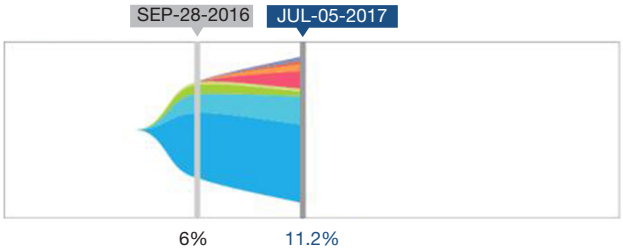

Figure 1 Serial ctDNA testing showing co-relation between somatic mutation burden and radiographic disease progression.

testing. Somatic mutation burden was at 6% upon initial consultation; subsequently patient underwent repeat testing upon radiographic disease progression and was found to have increase in somatic mutation burden along with emergence of new alterations.

Since this analysis we have identified two patients with GE junction cancer who where noted to be have a *BRCA* mutation via ctDNA testing. *BRCA* mutation is uncommon in patients with GE junction cancer. One of the patients was identified as germ line *BRCA* mutation while the other patient was identified as somatic *BRCA* mutation. Interestingly the patient who was found to have somatic *BRCA* mutation was also positive for *HE2/Neu* (data not shown).

Discussion

Circulating DNA was first reported in 1948 in healthy humans (9) which was followed by detection of ctDNA in cancer patients in 1977 (10). Some of the earlier work was on patients with myelodysplastic syndrome and pancreatic cancer, whereby aberrations in the RAS-pathway were detected in the blood (11). These initial observations paved way to the development of ctDNA as a biomarker. ctDNA offers a unique non-invasive approach for studying tumor genomics, potential targets for treatment, and disease prognosis without the hazards and difficulties usually encountered in obtaining a surgical specimen.

Mechanisms regarding the release of ctDNA involve various physiological events that accompany tumor growth such as apoptosis, micro metastasis and necroptosis (12,13). ctDNA allows for quantification of somatic mutation burden along with detection of various cancer related genetic and epigenetic alterations including tumor mutations (14), promoter methylation (15), microsatellite instability, deletion, amplification, translocation of chromosome and loss of heterozygosity (16,17).

Patients with advanced cancers have been shown to have high rate of detection of genetic aberrations via next generation sequencing of ctDNA (6). A comparison has also been done with patients at early stages of cancers where higher level of ctDNA is detected in advanced stage versus early stage (14% in stage 1 versus 50% in stage III-IV) (3). Few studies have shown the concordance of ctDNA results with tissue based genomic sequencing. One study reported high concordance rates of 96%, 94%, 95% and 91% respectively between ctDNA and tissue biopsy in *KRAS*, *MYC*, *KRAS* G12V and *EGFR* amplification among patients with gastrointestinal malignancies (18). HER2/Neu amplification is seen in up to a quarter to a third of patients with gastric or gastroesophageal junction cancers (GE-junction) adenocarcinomas (19). This is a marker of more aggressive disease. However, in an era of anti-HER2 directed therapy, finding this aberration could be of great value. The overall survival benefit of such an approach has been already demonstrated in the ToGA trial, that lead to the approval of trastuzumab in patients with HER2 positive advanced gastric/GE junction adenocarcinomas (20).

HER2 positivity needs to be demonstrated, however, based on currently approved consensus criteria (IHC score of 3+ OR 2+ with a positive fluorescent *in situ* hybridization result). Two issues have been well documented with regards to HER2 measurement which include intratumoral heterogeneity which is more common in gastric cancer than breast cancer and therapeutic resistance (21). Overall rate of response to HER2 directed therapy in HER2 positive gastric cancer is 47% (20). Therefore due to potential therapeutic implications accurate measurement of HER2 status is becoming increasingly important. Various studies have shown the potential of liquid biopsy to detect HER2/Neu amplification along with showing concordance with routine IHC and FISH on tissue samples (22,23). Our own data shows that *HER2/Neu* amplification was detected via ctDNA testing in about 3.7% of all genetic alterations with concordance observed in 3 out of 5 available tissue samples.

Most studies have documented ctDNA levels in cancer patients against healthy individuals; however, a longitudinal approach is of more clinical relevance in terms of assessing disease response to various treatment modalities and evolving tumor dynamics. The non-invasive nature of ctDNA testing allows for repeated testing and monitoring evolution of tumors which is a clear advantage of the

technique over traditional tissue biopsies that are usually only done at diagnosis (17). In our own study sample radiographic disease progression correlated with increase in somatic mutation burden on ctDNA testing. Further, new alterations are detected on disease progression which can be indicative of disease response.

The evolution of tumor genome captured by ctDNA testing has been demonstrated by two recent reports on patients with colorectal cancer where liquid biopsy was used to demonstrate acquisition of RAS-mutations while on anti-EGFR therapies as mechanisms of resistance (24,25). Levels have shown not to correlate with traditional tumor markers like CEA and CA 19-9 confirming the lack of prognostic significance of these traditional tumor markers.

With the recent approval of immunotherapy in mismatch repair deficient (dMMR), or microsatellite instability high (MSI-H) solid malignancies knowledge of MMR/MSI-H status is becoming increasingly important. Although IHC for MMR proteins can not be assessed with liquid biopsies, the technology can provide MSI status along with determination of the burden of somatic mutations, also known as tumor mutational burden (TMB). The TMB appears to correlate with response to immunotherapy and further illustrates the clinical utility of a liquid biopsy in predicting response to immunotherapy (26,27). We have been able to identify atleast one patient with gastric cancer via ctDNA testing who was MSI-H and is currently being treated with front line immunotherapy as he was chemotherapy ineligible (data not shown here).

The recent joint review by the American society of Clinical Oncology and College of American Pathologists (5), was a comprehensive analysis of the analytical and clinical value of liquid biopsy. Liquid biopsy though is a promising area for future cancer research it is currently limited by lack of prospective larger studies and heterogeneity that is inherent in different types of cancers. Ongoing studies like the NEXT-2 trial where treatment was directed based on the aberrations detected via ctDNA testing will provide further insight for integrating ctDNA testing into clinical practice (28). The main limitation of our study was the small population size and retrospective design. However, we were able to show that liquid biopsy is a feasible test for patients with advanced gastric cancers with a quick turnaround time. The feasibility and value of ctDNA testing would have to be individually studied for different tumor types for understanding its limitations and strengths in addition to evaluating the concordance between tissue based testing and liquid biopsy.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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