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Gastrointestinal Cancer

Prospective Comprehensive Genomic Profiling of Advanced Gastric Carcinoma Cases Reveals Frequent Clinically Relevant Genomic Alterations and New Routes for Targeted Therapies

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Gastric cancer • Sequencing • Targeted therapy • Mutation • Profiling • MET

Abstract

Background. Gastric cancer (GC) is a major global cancer burden and the second most common cause of global cancer-related deaths. The addition of anti-ERBB2 (HER2) targeted therapy to chemotherapy improves survival for *ERBB2*-amplified advanced GC patients; however, the majority of GC patients do not harbor this alteration and thus cannot benefit from targeted therapy under current practice paradigms.

Materials and Methods. Prospective comprehensive genomic profiling of 116 predominantly locally advanced or metastatic (90.0%) gastric cancer cases was performed to identify genomic alterations (GAs) associated with a potential response to targeted therapies approved by the U.S. Food and Drug Administration or targeted therapy-based clinical trials.

Results. Overall, 78% of GC cases harbored one clinically relevant GA or more, with the most frequent alterations being found in *TP53* (50%), *ARID1A* (24%), *KRAS* (16%), *CDH1* (15%), *CDKN2A* (14%), *CCND1* (9.5%), *ERBB2* (8.5%), *PIK3CA* (8.6%), *MLL2* (6.9%), *FGFR2* (6.0%), and *MET* (6.0%). Receptor tyrosine kinase genomic alterations were detected in 20.6% of cases, primarily *ERBB2*, *FGFR2*, and *MET* amplification, with *ERBB2* alterations evenly split between amplifications and base substitutions. Rare *BRAF* mutations (2.6%) were also observed. One *MET*-amplified GC patient responded for 5 months to crizotinib, a multitargeted ALK/ROS1/MET inhibitor.

Conclusion. Comprehensive genomic profiling of GC identifies clinically relevant GAs that suggest benefit from targeted therapy including *MET*-amplified GC and *ERBB2* base substitutions.

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Implications for Practice: Despite description of many potentially clinically relevant genomic alterations in retrospective research studies, these alterations are not regularly assessed in a comprehensive manner in clinical practice. This study demonstrates the feasibility of prospective comprehensive genomic profiling (CGP) for advanced gastric carcinoma. It also demonstrates a high frequency of genomic alterations associated with potential benefit from targeted therapies. CGP in this setting may inform therapeutic options beyond standard of care testing by identifying genomic alterations such as point mutations in the kinase domain of *ERBB2* and *MET* amplification. Genotype-directed management is highlighted by the response of a *MET*-amplified gastric carcinoma patient to crizotinib.

Introduction

Gastric cancer (GC) is the second most frequent cause of cancer-related death worldwide [1]. The majority of patients present with advanced disease, and the overall 5-year survival rate is <28%, compared with <5% for patients presenting with metastatic disease [2–4]. The clinical heterogeneity of GC is highlighted by significant worldwide geographic variations, differences in anatomic origin (proximal vs. distal), risk factors including *Helicobacter pylori* infection and dietary patterns, and a poorly understood relationship to Asian ethnicity [5–7]. Within the common intestinal histologic subtype, there are differences in *ERBB2* amplification frequencies (proximal vs. distal) and association with *H. pylori* and progression from a metaplastic background (distal vs. proximal, intestinal type) [8–10].

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Efforts to identify predictive biomarkers to guide decision making for systemic therapy have yielded inconsistent results. To date, the only validated predictive biomarker for targeted therapy is *ERBB2* amplification, which predicts benefit from the anti-ERBB2 (HER2) antibody trastuzumab in advanced disease [8, 11, 12].

Systemic therapy for metastatic, relapsed, or refractory GC is largely based on empiric 5-fluoropyrimidine and platinum combinations, and there are no definitive clinical predictors of response [13]. Although trastuzumab offers improved survival for the 7%–34% of GC patients with ERBB2 amplification, there are no approved molecularly directed therapies for the majority of patients [8, 14]. Although the recent approval of the anti-VEGFR2 antibody ramucirumab increases the GC armamentarium, there are no validated predictive biomarkers to identify patients who may derive benefit from anti-VEGFR targeted therapies [13, 15].

Large-scale retrospective whole-genome sequencing analyses have highlighted recurrent genomic alterations in gastric cancer such as *ARID1A*, *CDH1*, *RHOA*, and *FGFR2* [10, 16–18]. Prospective comprehensive genomic profiling based on a clinical next-generation sequencing (NGS) assay in the course of clinical care can identify novel and known clinically relevant genomic alterations (GAs) and increase understanding of the underlying biology and immediately inform patient management options. In this study, we present a large series of primarily relapsed and metastatic gastric carcinoma clinical specimens that underwent prospective comprehensive genomic profiling and highlight therapeutic implications.

Materials and Methods

Comprehensive genomic profiling using a clinical NGS-based assay (FoundationOne) was performed in a Clinical Laboratory Improvements Amendment-certified, College of American Pathologists-accredited laboratory (Foundation Medicine, Cambridge, MA, http://www.foundationmedicine.com) using validated methods [19]. Clinical samples were sent in from both academic and community oncologists for genomic profiling in the context of clinical care, and patient outcomes in selected cases were obtained from the primary treating physician. With the exception of three samples received as extracted DNA, a pathologist reviewed hematoxylin and eosin-stained slides to confirm diagnosis of GC and to ensure adequate formalin-fixed, paraffin-embedded (FFPE) specimen quality: sample volume >1 mm^3, nucleated cellularity $>80\%$ or $>30{,}000$ cells, and $>20\%$ tumor nuclei. When required, macrodissection was performed to enrich samples with $<20\%$ tumor nuclei.

DNA was extracted from unstained FFPE specimens using the Promega Maxwell 16 Tissue LEV DNA kit (Promega, Madison, WI, http://www.promega.com) and quantified using an Invitrogen PicoGreen fluorescence assay (Thermo Fisher Scientific, Waltham, MA, http://www.thermofisher.com). Library construction was performed with 50–200 ng of DNA sheared by sonication (E210; Covaris, Woburn, MA, http://covarisinc.com) to ~100–400 base pairs before end repair, dA addition, and ligation of indexed Illumina sequencing adaptors (Illumina, San Diego, CA, http://www.illumina.com). Prior to hybrid selection and sequencing, libraries were amplified with polymerase chain reaction (PCR) for 10 cycles using KAPA HiFi (Kapa Biosystems, Wilmington, MA, http://www.kapabiosystems.com). Solution-based hybrid selection was performed with a custom bait set of 120-bp biotinylated DNA oligonucleotides (Integrated DNA Technology, Coralville, IA, http://www.idtdna.com) covering 3,769 exons of 236 cancer-related genes and 47 introns of 19 genes frequently rearranged in cancer. The Illumina HiSeq 2000 and Illumina HiSeq 2500 platforms were used to perform 49×49 paired-end sequencing. Sequence alignment, PCR duplicate-read removal, and local alignment optimization were performed using Burrows-Wheeler aligner bwa-0.5.9 (SourceForge; Slashdot Media, San Francisco, CA, http://slashdotmedia.com), Picard 1.47 (Broad Institute, Cambridge, MA, http://broadinstitute.github.io/picard/), SAM Tools samtools-0.1.12a (SourceForge; Slashdot Media), and GATK 1.0.4705 (Broad Institute).

Variant calling was performed using custom tools. Base substitutions were called using a Bayesian methodology, and short insertions-deletions (indels) were called using local assembly. Somatic variants were annotated using COSMIC, and germline variants were removed using dbSNP. Rearrangements were called using chimeric read pairs clustered by genomic position. Copy number alterations (CNAs) were detected by fitting a statistical copy-number model to normalized coverage and allele frequencies at all exons and ~3,500 genomewide single nucleotide polymorphisms and accounting for stromal admixture. An extensive validation was performed for base substitutions, short indels, and CNAs. To validate CNA detection, seven tumor cell lines bearing 19 focal gene amplifications (6–15 copies, 15 genes) and 9 homozygous gene deletions (6 genes) with their matched normal cell lines (thereby maintaining consistent genotypes) were pooled to create five ratios ranging from low to high tumor content (20%–75%), creating a total test set of 210 CNAs.

High performance was achieved for both high-level amplifications (copy number ≥ 8) and homozygous deletions when tumor purity was as low as 30%: sensitivity was 99% (91 of 92) with positive predictive value $>99\%$ (127 of 127). Performance was reduced for lower CNAs (6–7 copies) and at lower sample purities (20%–30%), with overall sensitivity $>80\%$. Cancer-related alterations were defined as those that are known sites of somatic mutation, truncations or homozygous deletions of known tumor suppressor genes, and amplifications of oncogenes and fusions of genes known to be rearranged in solid tumors.

Clinically relevant GAs were defined as those that suggested potential response to targeted therapies approved in gastric carcinoma or in other tumor types or that suggested benefit from targeted therapy under development and being administered in the context of a clinical trial. The two-tailed Fisher's exact test was used to determine statistical significance of all group comparisons. Local site permissions were used to study these samples.

Results

Comprehensive genomic profiling was performed on 116 GC cases. The median patient age at time of testing was 62 years (range: 26–87 years) (Table 1). Sixty-five (56%) specimens were from male patients. The stage distribution is shown in Table 1. Of the samples, 69% ($n = 80$) were from the primary GC and 31% ($n = 36$) were from metastatic sites including ovary ($n = 7$), peritoneum ($n = 4$), omentum ($n = 3$), colon ($n = 3$), bone ($n = 3$), pleural fluid ($n = 3$), lymph node ($n = 3$), ascites ($n = 2$), esophagus ($n = 2$), small intestine ($n = 2$), mesentery ($n = 1$), liver ($n = 1$), pelvis ($n = 1$), and soft tissue ($n = 1$).

Table 1. Clinicopathological and genomic characteristics of 116 gastric cancer cases prospectively assayed by a comprehensive genomic profiling assay

Characteristic	Result
Patient age, average, years	59.5
Sex, %	
Male	44
Female	56
Histology, *n* (%)	
Diffuse type	24 (20.7)
Intestinal type	12 (10.3)
Gastric carcinoma NOS	80 (69.0)
Histologic grade, *n* (%)	
1	2 (1.7)
2	24 (20.7)
3	90 (77.6)
Stage, *n* (%)	
I	4 (3.4)
II	7 (6.0)
III	21 (18.1)
IV	84 (72.4)
Site of tumor, *n* (%)	
Primary site	80 (69)
Metastatic sites[a]	36 (31)
GA	
Total alterations	501
Average per sample	4.3
Clinically relevant GA	201
Clinically relevant GA per sample	1.8

[a]Including ovary ($n = 7$), peritoneum ($n = 4$), omentum ($n = 3$), colon ($n = 3$), bone ($n = 3$), pleural fluid ($n = 3$), lymph node ($n = 3$), ascites ($n = 2$), esophagus ($n = 2$), small intestine ($n = 2$), mesentery ($n = 1$), liver ($n = 1$), pelvis ($n = 1$), and soft tissue ($n = 1$).
Abbreviations: GA, genomic alteration; NOS, not otherwise specified.

Overall, 501 cancer-related genomic alterations were identified in 116 cases, yielding an average of 4.32 alterations per sample (Table 1; Fig. 1). Of 501 GAs identified, 210 (41%) were clinically relevant alterations, yielding an average of 1.8 clinically relevant GAs per case (Table 1). Moreover, 78% of GC cases harbored at least 1 clinically relevant variant associated with targeted therapies approved by the U.S. Food and Drug Administration (FDA) or mechanism-based trials (Table 2). The most common clinically relevant GAs were *KRAS*, *CDKN2A*, *CCND1*, *ERBB2*, *PIK3CA*, *MLL2*, *MET*, *PTEN*, *ATM*, *DNMT3A*, *NF1*, *NRAS*, and *MDM2* (Table 2). The most common GAs in the 116 cases were *TP53* (58 cases, 50%), *ARID1A* (28 cases, 24%), and *CDH1* (17 cases, 15%).

Twenty-eight cases (24%) harbored loss-of-function *ARID1A* alterations (Fig. 2). *ARID1A*-altered samples harbored an increased frequency of *CREBBP* variants ($p < .0005$), *PIK3CA* variants ($p = .0017$), and *MLL2* variants ($p = .019$) and were less likely to harbor *TP53* variants ($p < .050$). In this series, tumors with *ARID1A* alterations had less frequent amplifications of cancer-related genes compared with cases with wild-type *ARID1A*. This finding was statistically significant but was not replicated in an independent set of 192 gastric carcinomas (data not shown). All other *ARID1A* findings remained significant with a type I error rate of $<.05$ with a multiple hypothesis correction applied.

Somatic *CDH1* mutations were found in 6 of 24 (25%) diffuse GC cases compared with 11 of 92 (12%) nondiffuse GC cases ($p = .12$). Matched normal tissue was not available to investigate germline *CDH1* status. Enrichment of *APC*, *CREBBP*, and *MLL2* alterations was observed in intestinal GC; 3 of 12 cases (25%) contained *APC* mutations compared with 3 of 104 cases (2.9%) of nonintestinal GC ($p = .014$). *APC* variants were not observed in any of the 24 diffuse GC cases. *CREBBP* was altered in 4 of 12 (33%) intestinal cases compared with 2 of 104 (1.9%) nonintestinal cases ($p < .001$), and *MLL2* was altered in 4 of 12 (33%) intestinal cases compared with 4 of 104 (3.8%) nonintestinal cases ($p = .0038$).

Alterations of genes involved in mismatch repair were observed in this series at a frequency of 2.6% for *MLH1*, 0.8% for *MSH2*, and 0.8% for *MSH6*. Three of these five cases harbored truncating alterations that are predicted to cause loss of function, and no single case contained more than one alteration in this pathway. No information on microsatellite stability assessed by PCR testing was available.

Alterations in receptor tyrosine kinases (RTKs) were harbored by 24 cases (20.6%). Ten samples (8.6%) harbored *ERBB2* alterations, 5 contained somatic base substitutions and 5 harbored amplifications (6–24 copies), with these events being mutually exclusive. *ERBB2* base substitutions in this series consisted of R678Q (two cases), S310F (one case), L755S (one case), and V842I (one case). One case harbored both *ERBB2* R678Q and *MET* amplification. *CDH1* alteration was not associated with *ERBB2* alteration in our GC sample (data not shown).

Seven cases (6%) harbored *MET* amplifications (7–30 copies) and seven cases (6%) had *FGFR2* amplifications (12–32 copies) (Table 2). One patient with *MET*-amplified gastric carcinoma received crizotinib and achieved disease control (Fig. 3).

One *FGFR2*-altered case also harbored a coexisting *ARID1A* alteration. *EGFR* alterations were detected in four cases (3.4%) consisting of two amplifications, one point mutation (F795V), and one case with a deletion of exons 2–7 (EGFR viii). Rare RTK alterations identified included *FLT3* (amplification; one case), *KIT* (D716N; one case), and *FGFR3* (amplification; one case) (supplemental online Table 1). *EGFR* amplifications were not exclusive of other RTK alterations because they coexisted with *FGFR2* amplification (one case) and both *MET* and *ERBB2* amplifications (one case). No *ROS1* alterations, including fusions, were detected. Among clinically relevant alterations in other kinases, *BRAF* alterations occurred at a frequency of 2.6%, two cases harbored D594X and one case harbored G469V (supplemental online Table 1). Alterations in vascular endothelial growth factor receptors 1–3 (VEGFR1–3) were limited to *KDR* (*VEGFR2*) R275* and *FLT4* (*VEGFR3*) S637R in all 116 GC cases, and neither alteration has been linked to benefit from ramucirumab [13, 15].

Discussion

The phase III ToGA trial demonstrated the power of molecular testing to prospectively identify a molecularly defined subgroup of patients who are likely to benefit from anti-ERBB2 (HER2)-directed therapy; the addition of trastuzumab to

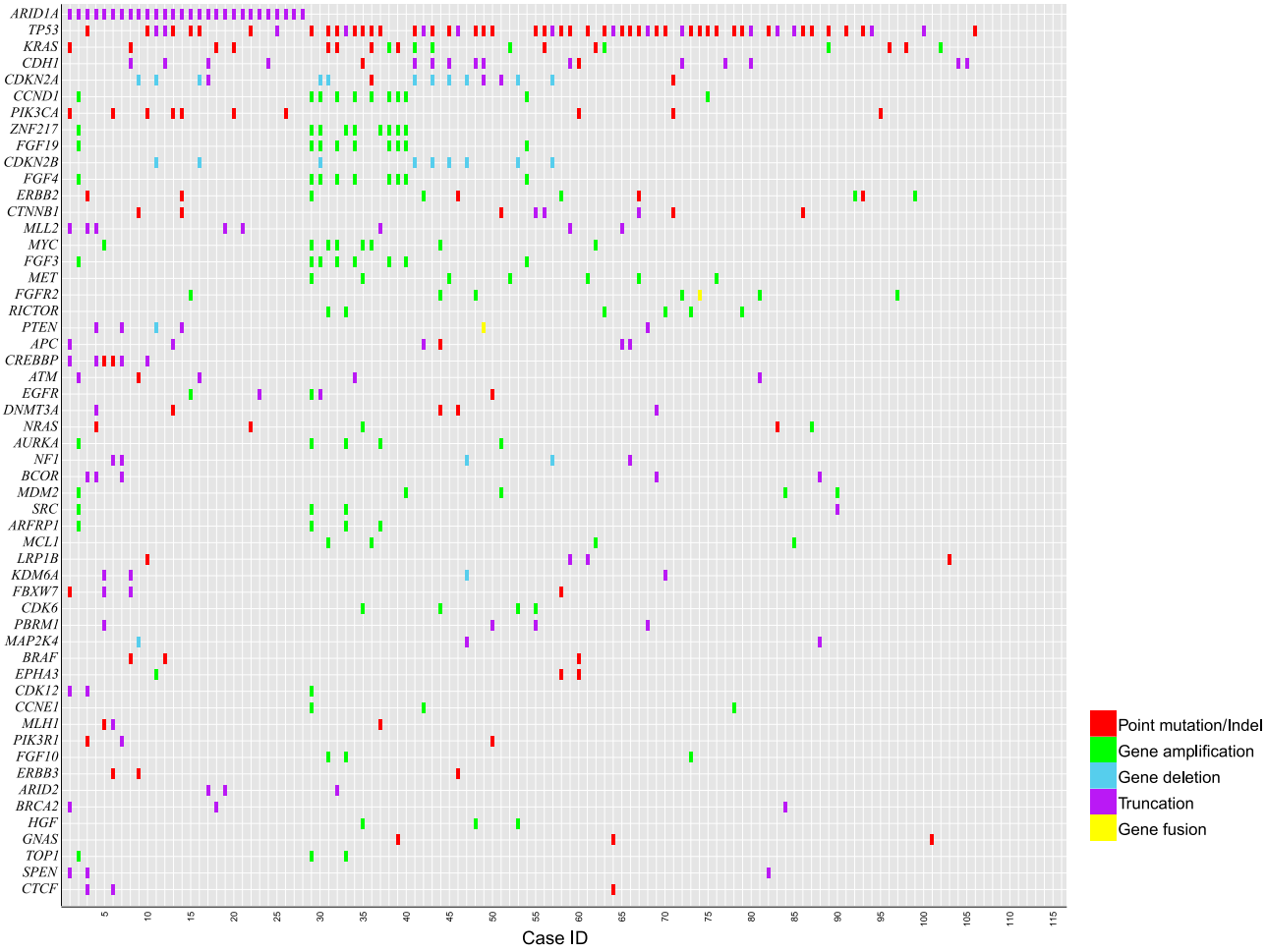

Figure 1. Tile plot of genomic alterations in 116 consecutive gastric cancer cases.

standard 5-fluorouracil/platinum chemotherapy led to a statistically significant 2.5-month improvement in overall survival for *ERBB2*-amplified gastric cancer [8]. The methods of molecular testing, however, are also important, as demonstrated by the negative results of the TyTAN trial. In that trial, the addition of lapatinib (an oral HER2 inhibitor) to paclitaxel did not lead to significant improvement in progression-free survival or overall survival (OS) when compared with single-agent paclitaxel as second-line treatment in *EBRR2*-amplified GC, as determined by fluorescence in situ hybridization (FISH) alone [20]. Subgroup analysis indicated that *ERBB2* amplification and immunohistochemistry (IHC) 3+ derived a significant 6.4-month improvement in overall survival. Similarly, early reporting from the LoGIC trial demonstrated OS improvements in Asian patients and those aged <60 years but failed to demonstrate an association between OS and IHC score [21]. Neither of these trials included patients with *ERBB2* mutations.

In contrast, the phase III EXPAND and REAL3 trials in an unselected gastric and esophageal cancer population failed to demonstrate improvement in outcomes with the addition of the EGFR-targeted therapies cetuximab or panitumumab to chemotherapy [22, 23]. Retrospective analysis of this trial has not identified any predictive response biomarkers to date [13]. Similar negative results have also been observed in the phase III GRANITE-1 trial of everolimus in unselected advanced GC patients, and few data support empiric use of targeted and biologic agents in advanced GC [24].

Prior reports characterizing the genomic landscape of gastric carcinoma have relied on banked, therapy-naive tissue from primary resections [10, 16–18, 25, 26]. Recent work from the Cancer Genome Atlas has defined four groups of gastric carcinomas, each harboring positivity for Epstein-Barr virus (EBV), microsatellite instability, or multiple copy number amplifications while genomically stable or characterized by chromosomal instability [27]. Characteristic single-gene alterations were often but not perfectly associated with each group; EBV-positive gastric carcinomas often harbored *PIK3CA* alterations, and the genomically stable group often harbored *RHOA* alterations [27]. These groups offer insight into common etiologies but do not currently direct therapeutic decision making.

In contrast, the prospective series presented in this study reflects samples characteristic of clinical practice because the series is composed of cases from patients typically with advanced gastric carcinoma. A high percentage of cases (78%) harbor clinically relevant genomic alterations, including 1 in 5 cases (20.6%) with alterations in RTKs, suggesting the utility of comprehensive genomic profiling to match patients with targeted therapies of specific potential benefit in clinical trials (Table 2). For the common genomic alterations *KRAS*, *ARID1A*, and *TP53*, their clinical relevance is best linked to possible benefit from clinical trials with targeted agents. The recent FDA approval of trametinib as a MEK pathway inhibitor for melanoma has resulted in the anecdotal use of trametinib in other tumors types and assessment in clinical trials for other indications [28].

Table 2. Therapeutic implications of recurrent somatic genomic alterations in 116 clinical gastric cancer cases analyzed by prospective genomic profiling

Gene	Type of alteration	Frequency (%)	Approved anticancer drugs	Novel targeted therapies under clinical investigation
TP53	Sub/indel	50	None	None
ARID1A	Sub/indel	24	None	None
KRAS	Sub	16	None	Trametinib
CDH1	Sub/indel	15	None	None
CDKN2A	Sub/indel	14	None	LEE011
CCND1	Amp	9.5	None	LEE011, palbociclib
ERBB2	Amp, sub	8.6	Pertuzumab, trastuzumab, lapatanib	Afatinib, neratinib
PIK3CA	Amp, sub	8.6	Everolimus, temsirolimus	BYL719, BKM120
MLL2	Sub	6.9	None	None
FGFR2	Amp	6.0	Pazopanib, ponatinib	Dovitinib, AZD4547
MET	Amp	6.0	Crizotinib, cabozantinib	Rilotumumab, AMG337
PTEN	Sub/indel	5.2	Everolimus, temsirolimus	None
ATM	Sub	4.3	None	Olaparib
DNMT3A	Sub	4.3	None	Decitabine, 5-azacitidine
NF1	Sub/indel	4.3	None	Trametinib, everolimus, temsirolimus
NRAS	Sub	4.3	None	Trametinib
MDM2	Amp	4.3	None	None
BRAF	Mut	2.5	Vemurafenib; dabrafenib or trametinib	MEK162, LGX818

Only representative examples of investigational compounds are shown because of space constraints.
Abbreviations: Amp, amplifications; Indel, small insertions and/or deletions; Sub, base substitutions.

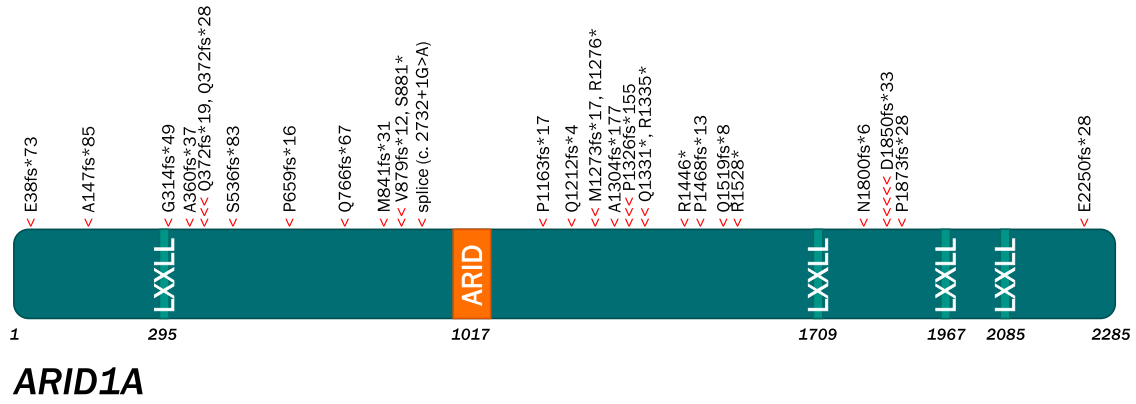

Figure 2. Lollipop plot graphically depicting the location of *ARID1A* genomic alterations in the 28 *ARID1A*-altered gastric cancer cases (one arrowhead per genomic alteration [GA] in this series, with some cases harboring several *ARID1A* GAs).

Similarly for *TP53* and *ARID1A*, targeted therapies are in clinical development, such kevetrin (NCT01664000), and inhibitors of chromatin remodeling. A recent development is the paradigm of master trials, such as the Novartis Signature Trial, with multiple agents and genomically defined entry criteria for advanced cancers rather than restriction to a tumor type. Such a trial design can accommodate the addition of future therapies to be developed, and genomic profiling can provide the rationale for entering patients.

We identified five GC cases with *ERBB2*-activating base substitutions, which cannot be detected by IHC or FISH [29].

The recent description of somatic *ERBB2* base substitutions in breast carcinoma and micropapillary urothelial carcinoma suggests that such alterations may also be oncogenic drivers in GC, and at least one such breast carcinoma patient has responded to anti-ERBB2 (HER2)-targeted therapy [29–32]. Both *ERBB2* amplifications and base substitutions were observed in our patient series but were mutually exclusive, consistent with observations in breast carcinoma [33]. The low frequency in this series (4.3%) of *ERBB2* amplification in contrast with the 20% frequency observed in previous studies is likely is due to a selection bias, that is, cases submitted for genomic profiling were previously tested for *ERBB2* amplification and were negative for *ERBB2*, prompting a search for therapeutic alternatives [34].

Among *ERBB2* base substitutions in this series, some have been functionally characterized as activating and sensitive to lapatinib (S310F, V842I) or resistant to lapatinib (L755S) [30]. The frequency of 4.3% was very similar to the frequency reported in other series, confirming that no selection bias was present because standard of care testing does not detect these clinically relevant alterations [34, 35]. Although *ERBB2* R678Q was not found to be activating or to confer resistance to anti-ERBB2 (HER2)-targeted therapy, it has been observed multiple times in the context of cancer, which may indicate biologic

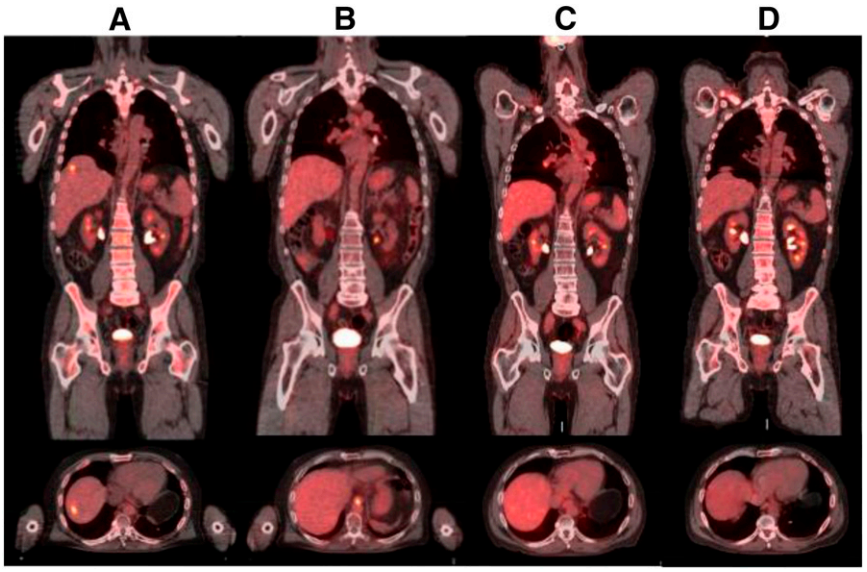

Figure 3. Response to crizotinib in a patient with *MET*-amplified gastric cancer identified by prospective comprehensive genomic profiling. **(A):** Precrizotinib FDG-avid hepatic metastasis. **(B–D):** Ongoing response up to 5 months after crizotinib initiation.

significance. Activating *ERBB2* base substitutions appear to be sensitive to neratinib, suggesting a possible pathway to clinical treatment, and genomically selected basket trials of neratinib (NCT01953926) are ongoing for *ERBB2*-altered tumors [30].

Previous studies have strongly associated *ERBB2*-amplified gastric carcinomas with intestinal type histology and proximal gastric location [8, 12]. All five *ERBB2*-amplified GC cases in this series had histology diagnosed as or at least suggestive of the intestinal subtype but were approximately evenly distributed in site of origin between the proximal and distal stomach (supplemental online Table 1). In contrast, *ERBB2* base substitutions were associated with signet ring features in three of four cases with histology available for review. The differing histology of these cases may suggest differing clinicopathologic characteristics of *ERBB2*-amplified GC compared with *ERBB2* base-substituted gastric carcinomas, but this awaits independent confirmation in a larger series.

Alterations in the *FGFR* family are well recognized as oncogenic drivers [36, 37]. *FGFR2* was amplified at 6% in this GC series, similar to a previous study [18, 38]. Limited clinical studies have shown that *FGFR2*-amplified breast carcinoma patients responded favorably to dovitinib, a multikinase inhibitor that inhibits FGFR family members [39]. For *FGFR2*-amplified GC, preclinical evidence suggests such tumors are sensitive to FGFR-targeted therapy, and molecularly stratified clinical trials are ongoing (NCT01719549) [40].

Amplification of *MET* is a known driver of gastroesophageal and lung carcinomas and other tumor types [38, 41, 42]. We identified *MET* amplification ($>$6 copies) in 6% of GC cases in this series. Based on the genomic profile, one of the patients with *MET* amplification (12 copies) was treated with crizotinib and had regression of liver metastasis and disease control for 5 months (Fig. 3). This finding is consistent with previous results for phase I trials for crizotinib in which two advanced gastroesophageal carcinoma patients with *MET* amplifications (FISH *MET/CEN7P* ratio of $>$2.2) had partial response and stable disease with time to progression of 3–4 months [41]. The comprehensive genomic profiling assay in this series used a process-matched normal control to quantitatively estimate the absolute copies of *MET* while controlling for ploidy. The threshold of six copies for the designation of *MET* amplification by FoundationOne in cases with a diploid genome can be translated as exceeding a *MET/CEP7* ratio of 2.2 (Fig. 4).

Notably, the comprehensive genomic profiling assay used in this study (FoundationOne) provides quantitative estimates of copy number amplifications (Fig. 4). Copy number estimates made by the genomic profiling assay used do not directly translate to a FISH ratio per se but, as shown by our patient response, provide clinically relevant information that can guide use of targeted therapy (Fig. 3). Comprehensive genomic profiling provides the advantage of simultaneous assessment of many possible clinically relevant copy number amplifications including *MET*, *FGFR2*, and *ERBB2* while minimizing consumption of the specimen [19]. In contrast, other forms of molecular testing are hypothesis driven, that is, a "hotspot" exon examines only specific exons of genes of interest and is often combined with FISH to assay for amplifications (i.e., *ERBB2* and *MET*). A focused approach offers conceptual simplicity, but for those cases harboring relevant genomic alterations outside the scope of such hotspot testing, genomic profiling could be done to identify potential benefits of targeted therapy instead of expending both time and resources on hotspot testing that might not yield information to guide treatment.

In one of the largest screening studies for *KRAS* mutations involving GC samples from U.K., Japan, and Singapore, *KRAS* mutations were found in 29 of 710 GC samples (4.1%). The frequency of *KRAS* mutations was 5.8% among U.K. patients, 4.0% among Japanese patients, and 1.5% among Singapore Chinese patients. The role of *KRAS* mutation in GC is unknown, but in this series, *KRAS* mutations were identified in 16% of GC cases. The most common alterations were G12V (3.4%) and G12D (2.5%), which are both transversions. This most likely reflects a selection bias in this sample population, with patients sent for genomic profiling having poor prognosis and possible *KRAS* enrichment.

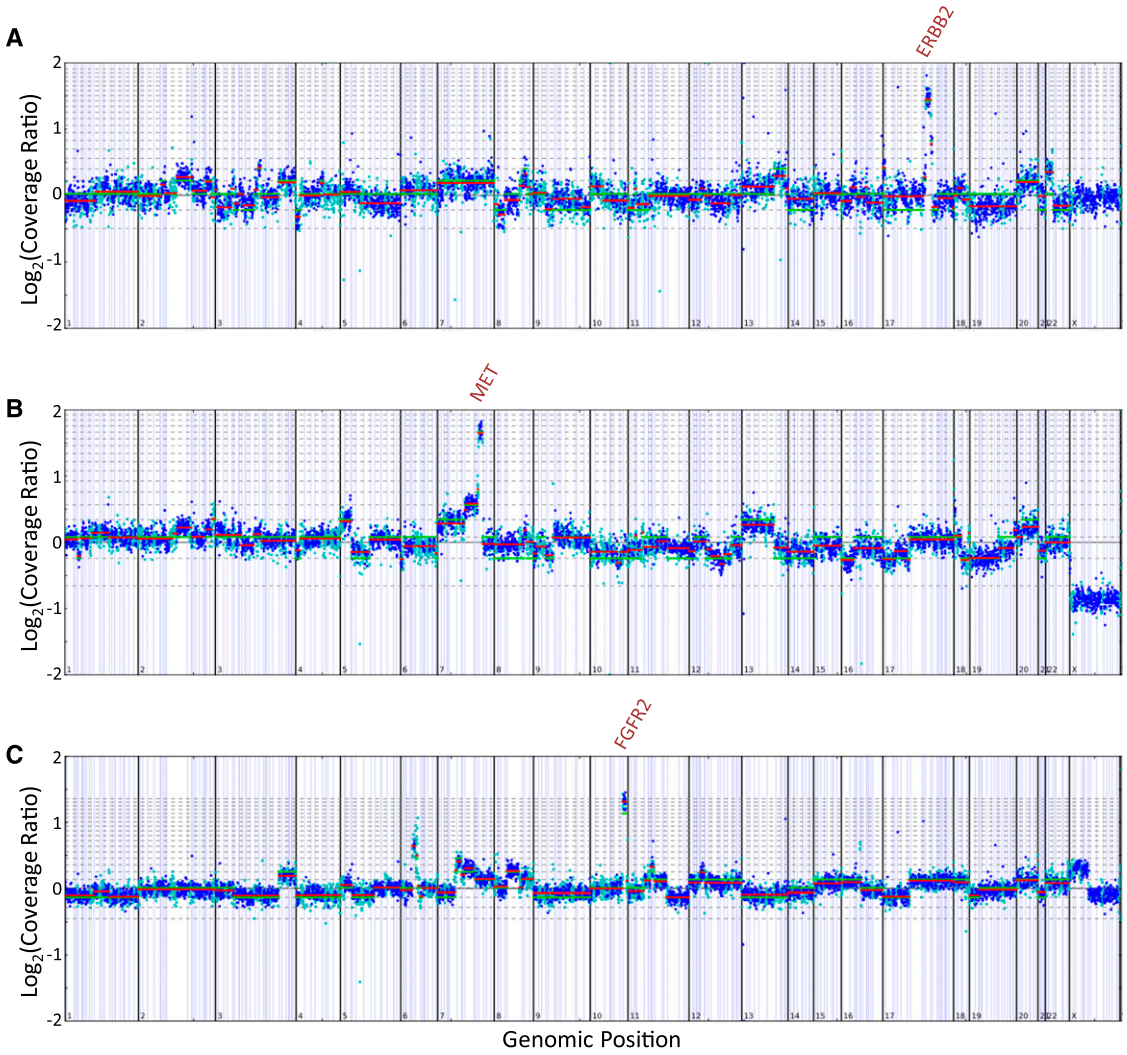

Figure 4. Copy number alteration plots for several cases harboring receptor tyrosine kinase amplifications.

Alterations in the tumor suppressors *TP53* and *ARID1A* are common in gastric cancer. *ARID1A* encodes the AT-rich interactive domain-containing protein 1A, a member of the SWI/SNF chromatin-remodeling complex. Inactivating alterations in *ARID1A* are frequent in ovarian clear cell carcinomas, neuroblastomas, and gastric carcinomas and loss of expression in other tumor types, consistent with the hypothesized tumor suppressor role of this protein [43, 44]. Alterations of *ARID1A* in this series did not cluster around a hotspot (Fig. 2). The statistically significant enrichment of *PIK3CA* and the paucity of *TP53* alterations in the set of *ARID1A*-altered GCs are consistent with previous findings in gastric carcinoma [43, 45]. In our series, *ARID1A*-altered cases were also enriched for *CREBBP* and *MLL2* alterations.

Interestingly, our series identified somatic *CDH1* mutation rates (25% diffuse, 12% nondiffuse) higher than previously reported (Fig. 5) [46]. The differences in alteration frequency may be related to the interrogation of the entire coding sequence of *CDH1* in this assay compared with the limited hotspot assessment of exon 7–10 hotspot interrogation in prior series [46, 47]. *CDH1* somatic mutation has been correlated with the shortest survival in GC, and selection bias may underlie increased *CDH1* mutation rates in this series because clinicians may be likely to reach for mutational profiling when options are limited [46]. In reporting these results, caveats for directed germline testing are included.

Neither *BRAF* V600E or V600M alterations previously described in GC were observed in this series, but three non*BRAF*V600 mutations were found in this series [13, 48–50] (supplemental online Table 1). The *BRAF* alterations in these series are variable activators of BRAF.

Interestingly, no alterations in VEGFR1–3 were identified in this series. Ramucirumab has been shown to improve OS in GC in second-line treatment as a single agent or in combination with paclitaxel. Ramucirumab is a monoclonal antibody against VEGFR2, but, like other antiangiogenic therapies, there are no clear predictive biomarkers [51, 52]. Current evidence is insufficient to examine whether VEGFR1–3 alterations serve as biomarkers for ramucirumab.

Identifying clinically relevant alterations in the course of clinical care of gastric carcinoma may drive clinical decisions making, which in turn will generate preliminary data on the efficacy of targeted therapies in gastric carcinoma and care of future patients and will support future systematic investigation through clinical trials. At present, most suggestion of benefit from targeted therapy in gastric carcinoma is guided by analogy to other tumor types. Such reasoning highlights the limitations of this approach, for example, *BRAF* V600E-mutant colorectal

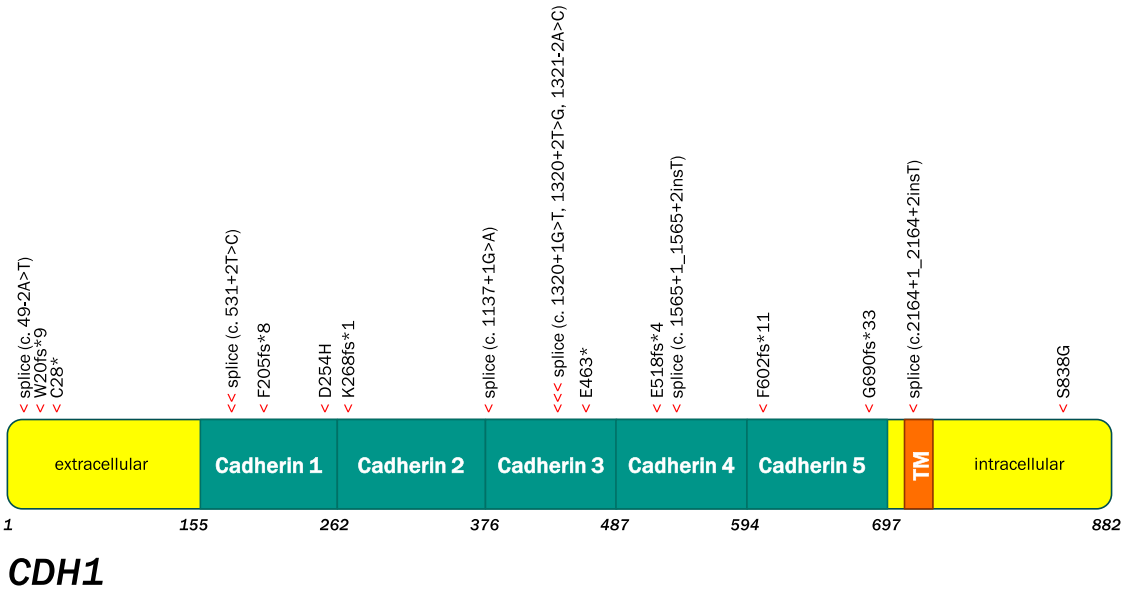

Figure 5. Lollipop plot graphically depicting the location of *CDH1* genomic alterations (GAs) in the 17 *CDH1*-altered gastric cancer cases (one arrowhead per GA in this series).

Abbreviation: TM, transmembrane.

adenocarcinoma has not suggested benefit from vemurafenib monotherapy [53, 54]. Because new approaches such as the combination of vemurafenib and erlotinib confer some benefit to similar patients, as noted, it is hoped that analogous approaches can also benefit gastric carcinoma patients [54].

Conclusion

The high frequency of clinically relevant genomic alterations in this patient population reflective of routine clinical practice is encouraging in a disease that continues to have a poor prognosis with modern chemotherapy. The clinically relevant alterations identified by this assay beyond those detected by standard of case can drive increased clinical trial participation and development (e.g., *ERBB2* base substitutions, *MET* amplifications) and clarify predictive response and resistance biomarkers.

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Disclosures

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