Genomics and Targeted Therapies in Gastroesophageal Adenocarcinoma

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ABSTRACT

Gastroesophageal adenocarcinomas (GEA) are devastating diseases with stark global presence. Over the past 10 years, there have been minimal improvements in treatment approach despite numerous clinical trials. Here, we review recent progress toward understanding the molecular features of these cancers and the diagnostic and therapeutic challenges posed by their intrinsic genomic instability and heterogeneity. We highlight the potential of genomic heterogeneity to influence clinical trial outcomes for targeted therapies and emphasize the need for comprehensive molecular profiling to guide treatment selection and adapt treatment to resistance and genomic evolution. Revising our clinical approach to GEA by leveraging genomic advances will be integral to the success of current and future treatments, especially as novel targets become therapeutically tractable.

Significance: GEA are deadly cancers with few treatment options. Characterization of the genomic landscape of these cancers has revealed considerable genetic diversity and spatial heterogeneity. Understanding these fundamental properties of GEA will be critical for overcoming barriers to the development of novel, more effective therapeutic strategies.

INTRODUCTION

Stomach and esophageal cancers are the third and sixth most common causes of cancer mortality worldwide, respectively, and together are responsible for more than 1.2 million deaths (1). These two cancers were once considered distinct diseases plainly separated into adenocarcinomas in the stomach and squamous cell carcinomas in the esophagus. However, recent decades have witnessed a shift in the epidemiologic and anatomic patterns of these cancers, contributing to a revised and evolving understanding of their classification and pathogenesis. The worldwide incidence of gastric cancer has been declining for at least 40 years (2). Nevertheless, there are more than one million new cases annually, with the majority occurring in Eastern Asia (1). In North America and Western Europe, cancers of the distal stomach, typically associated with Helicobacter pylori (H. pylori) infection, have decreased dramatically (3). In contrast, there is a rising incidence of cancers of the proximal stomach, directly adjacent to the esophagus (3). This rise in the stomach parallels an alarming increase in adenocarcinomas of the lower esophagus and gastroesophageal junction (GEJ). Key risk factors include gastroesophageal reflux disease and obesity. Both gastric and esophageal adenocarcinomas commonly emerge with intestinal metaplasia, which can result from chronic inflammatory stimuli. The shared epidemiology, pathology, and genomic and molecular features of these adenocarcinomas suggest the common pathophysiology of esophageal and proximal gastric adenocarcinomas (3, 4).

Indeed, The Cancer Genome Atlas (TCGA) has revealed definitive genomic overlap between gastric and esophageal adenocarcinomas, and absolute molecular distinction from squamous cell carcinomas of the upper and mid-esophagus (5, 6). This review will focus on gastroesophageal adenocarcinomas (GEA); additional information on the genomics of esophageal squamous cell carcinomas (ESCC) can be found in ref. 6.

In addition to the rising incidence of esophageal, GEJ, and proximal gastric adenocarcinomas, another epidemiologic trend involves an increase in cancers of the gastric corpus or body (and fundus, to a lesser extent), predominantly in non-Hispanic white women younger than 50 years old, and restricted to areas with less than 20% poverty (7–9). Whereas the current gastric cancer male:female incidence rate ratio for patients ages 60 to 74 years is 2.5, the ratio is 1.0 for patients ages 25 to 29 years (7). It has been estimated that if the upward trend in early-onset disease continues, by 2030 overall gastric cancer incidence will be increasing, and female incidence will surpass male incidence (7). The histologic and molecular subtypes of these “CYF” (corpus-dominant, young...
age-dominant, female-dominant) gastric cancers have not been reported, and their risk factors are unknown, though they may be rooted in the evolving gastric microbiome in the wake of \textit{H. pylori} decline, and/or linked to autoimmunity and reproductive factors (7, 8).

GEAs have dismal outcomes, with cumulative five-year relative survival of 21% to 31% in the United States (10, 11). Five-year relative survival for those with locoregional gastric cancer (31%–67%) is far inferior to that for colorectal cancer (70%–91%; ref. 10), indicating that later diagnosis alone does not account for these poor outcomes. GEAs also have substantial propensity for early spread of disease, and systemic therapy for disseminated disease remains woefully inadequate, with 5-year relative survival of 5% (10). The convergence of a new molecularly based classification, recent genomic insight into drivers of GEA pathogenesis, and an imperative clinical need make this an opportune time to address how our emerging understanding of GEA can ultimately be translated into new therapeutic strategies.

**DISEASE CLASSIFICATION IN THE PREGENOMIC ERA**

Fifty years ago, the Lauren classification subtyped gastric cancers into intestinal, diffuse, and indeterminate/mixed histologies (12). Intestinal-type tumors are most common, consisting of cohesive cells in glandular formations, often associated with intestinal metaplasia and \textit{H. pylori} infection. Diffuse-type tumors have noncohesive scattered cells, sometimes with signet-ring features, that tend toward peritoneal dissemination due to locally invasive properties. The World Health Organization (WHO) further refines the histologic classification of gastric cancer into tubular, papillary, mucinous, poorly cohesive (including Lauren diffuse type), and mixed variants. Most esophageal adenocarcinomas (EAC) resemble intestinal-type gastric cancer, especially given their evolution from intestinal metaplasia. To date, these histologic classifications have not appreciably affected clinical care, including the selection of systemic agents. Another classification, the Siewert classification, is focused on refined anatomic staging of GEJ adenocarcinomas to guide surgical approaches (13). Beyond histologic and anatomic types, it is also worth noting that there have been long-standing debates regarding the comparative features of GEA in the Asian and Western populations. Asian patients, beyond having higher rates of gastric cancer, tend to have more distal gastric tumors associated with \textit{H. pylori}, and less predilection toward GEJ disease and adenocarcinomas of the tubular esophagus, or its antecedent, Barrett’s esophagus (14–16). Although extensive discussion of the comparisons of Eastern/Western tumors is beyond the scope of this review, early data have pointed to the genomic similarity of these tumors (17), but potential distinctions in their tumor–immune microenvironment (18).

**MOLECULAR CLASSIFICATION IN THE GENOMICS ERA**

The Lauren and WHO classifications are basic descriptors for the microscopic organization of gastric tumors. However, they offer no immediate scientific insight into the inner workings of a cancer cell, nor clinical insight into rational systemic therapeutic strategies. In recent years, new genomic technologies have enabled groundbreaking studies that have revolutionized our understanding of the genomic composition of GEA, ultimately providing a framework for molecular classification and new leads for therapeutic development. Pivotal studies have been led by TCGA, the Asian Cancer Research Group (ACRG), and the Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium (5, 6, 19, 20). Among these, ACRG focused on gastric adenocarcinoma at a South Korean medical center, OCCAMS focused on EAC in a Western European population, and the TCGA studied both adenocarcinoma and squamous cell carcinoma spanning the stomach and esophagus using a diverse internationally derived sample collection.

Within TCGA, the first published analysis included 295 gastric adenocarcinomas, inclusive of GEJ tumors, using the tools standard to TCGA-led studies: array-based somatic copy-number analysis, whole-exome sequencing, array-based DNA methylation profiling, mRNA sequencing, miRNA sequencing, and reverse-phase protein array (5). Four distinct molecular subtypes of gastric cancer emerged from integration of these data: Epstein–Barr virus (EBV)–positive (8.8%), microsatellite-unstable (MSI; 21.7%), genomically stable (GS; 19.7%, enriched for Lauren diffuse type), and tumors with chromosomal instability (CIN; 49.8%; Fig. 1). In comparison with the TCGA classification, where underlying somatic genomic processes were central to development of the classifier, ACRG developed a classification into four groups based on array-based gene-expression profiling of 300 gastric adenocarcinomas: MSI (22.7%), microsatellite stable with epithelial-to-mesenchymal transition (MSS/EMT; 15.3%), MSS/TP53+ (26.3%), and MSS/TP53− (35.7%; ref. 19). Applying the TCGA classifiers to the ACRG tumors showed the expected concordance in MSI tumors. The MSS/EMT class was enriched in diffuse-type tumors classified as GS by TCGA, and MSS/TP53+ was enriched for EBV+ tumors. A key difference was that all four ACRG subtypes had CIN tumors, with the highest proportion of CIN tumors in MSS/TP53+. The ACRG analysis demonstrated inferior survival among the MSS/EMT+ group. Although no survival associations were seen in the TCGA study, potentially owing to the therapeutic heterogeneity of the internationally diverse sample set, subsequent studies applying the TCGA classes to two large independent cohorts demonstrated that EBV+ tumors have superior survival and GS tumors have inferior survival (21). Major genomic features of these molecular subtypes are discussed below.

Although the second TCGA study was nominally an analysis of esophageal cancer, this study evaluated both ESCC and EAC with the entire gastric cancer dataset in an effort to address debates regarding the comparative features of EAC, GEJ, and gastric tumors (6). As would be predicted by studies in other cancer types, the TCGA analysis identified systematic differences between squamous cell carcinoma and adenocarcinoma across all molecular platforms. Indeed, these data robustly argued that high-level histologic classifications transcended the organ of origin. Whereas ESCCs clustered more strongly with head and neck SCCs than with EACs, EACs were more similar to gastric tumors than...
to ESCCs. More subtle analysis refined the comparison of EAC with gastric cancer, noting that EAC showed the most similarity to the CIN class of gastric cancer. Furthermore, a joint analysis of GEA showed an anatomic gradient, with the proportion of CIN tumors increasing proximally such that 71 of 72 tumors categorized as definitively esophageal based on anatomic location and presence of Barrett’s esophagus were CIN tumors. Although specific methylation patterns were enriched in EACs relative to distal gastric cancers, multiple analyses attempting to differentiate the molecular features of CIN tumors of the stomach and esophagus failed to dichotomize these cancers. Still, several somatic features were differentially distributed between EACs and gastric cancers. EACs have fewer APC mutations, suggesting less WNT dependence or alternate means of activating WNT signaling, and higher frequency of RUNX1 deletion and VEGFA and MYC amplifications. Intriguingly, new studies from the OCCAMS Consortium found that patients with EAC with synchronous Barrett’s esophagus had better survival compared with patients without evident Barrett’s esophagus (22), suggesting that the most aggressive tumors might outstrip the potential for Barrett’s esophagus to take hold in the lower esophagus. Although the TCGA study did not define subgroups within EAC, whole-genome sequencing by OCCAMS suggests that mutational signatures may inform targeted therapy (23).

Together, these studies directly inform our broader view of gastroesophageal cancer. The dramatic and consistent separation of adenocarcinoma and squamous cell carcinoma clearly challenge prior notions of treating esophageal cancer as a single entity. Although still debated, these data also suggest the field should transition to viewing GEA more holistically, especially for the CIN class of tumors. These data also indicate that any previous effort to compare gastric adenocarcinoma and EAC, or to compare Eastern and Western gastric...
cancers, had an unrecognized confounding by subtype, as CIN tumors are dominant in the esophagus and GEJ (and more prevalent in the West) compared with distal stomach tumors (more prevalent in the East) where GS/diffuse, MSI, and EBV+ are more common. Although the anatomic gradients of molecular subtypes and molecular features could be used to argue firmer separation of cancers in the anatomic esophagus or stomach, it is notable that such gradients are present within analogous diseases such as colorectal cancer, where methylation and MSI are more prevalent in the ascending colon and CIN more common in the distal colon and rectum. For the purpose of this review and the discussion of biologically driven therapies, we will treat CIN GEA as a single category given the consistent overlap in oncogenic drivers and other molecular features.

CIN

CIN tumors are the predominant form of GEA. Half of gastric antrum/pylorus tumors classify as CIN, and this proportion increases proximally according to the aforementioned gradient such that nearly all EACs are CIN (6). Although a firm definition of CIN has yet to be established, CIN tumors are characterized by extensive aneuploidy and lack the somatic hypermutation seen in MSI tumors. Although the term CIN has been applied to many cancers to describe the state of harboring frequent gross structural chromosomal aberrations and aneuploidy, the CIN present within GEAs is overwhelmingly associated with widespread focal events (i.e., less than half the chromosome arm length), especially recurrent focal amplifications at numerous oncogenic loci (24, 25).

A meta-analysis of TCGA data spanning GEA and colorectal cancer detailed the differential features of CIN in these diseases (25). In this report, a score was developed to subclassify CIN tumors based on high-amplitude focal amplifications (CIN-F) or low-amplitude broad amplifications (CIN-B). This analysis revealed a dramatic distinction between GEA and colorectal cancer, as 75% of CIN tumors are CIN-F, in contrast to only 25% of CIN colorectal cancer. These data correspond to the discrepant patterns of oncogene activation in these tumors, with amplifications predominant in GEA, and mutations, chiefly RAS, prevalent in colorectal cancer.

Within GEA, CIN-B tumors sustain more frequent somatic activating mutations of receptor tyrosine kinases (RTK) and RAS oncogenes, and inactivation of tumor suppressors in cell-cycle, WNT, and TGFβ pathways. CIN-F GEAs frequently harbor amplifications of RTKs, RAS, and cell-cycle genes. TPS3 mutations are more common in CIN-F tumors (76% as compared with 54% of CIN-B tumors), as is whole-genome doubling (68% vs. 42%). These somatic features of the more prevalent CIN-F GEAs suggest distinct pathogenesis whereby a TPS3-null cell may undergo genome doubling to an unstable tetraploid intermediate, which is then prone to catastrophic genomic events leading to transformation, as has been posited from studies of the evolution of Barrett’s esophagus (26, 27). Indeed, this pattern is consistent with what has been observed via whole-genome sequencing of these cancers (28).

The etiologies for the distinct genomic pathways of cancer development and the marked differences of the CIN phenotype between GEA and colorectal cancer have not been established. A specific mutational signature of A>C transversions at AA dinucleotides has been observed in GEAs (20, 29) and is associated with the CIN-F phenotype (25). Although the etiology of this signature remains unresolved, it has been posited to be caused by oxidative damage (30) and associated with greater inflammatory infiltrates in EAC (23). Intriguingly, this signature has been reported in colorectal cancer associated with inflammatory bowel disease (IBD; ref. 31). As IBD-associated colorectal cancers also resemble CIN GEA in their relative paucity of APC mutations and greater prevalence of oncogene activation via amplification, these data raise the hypothesis that inflammation may contribute to the patterns of genomic evolution in GEA.

As explored in greater depth below, the pattern and putative mechanisms of the predominantly focal CIN seen in GEA have definite implications for optimal therapy. Beyond the need to evaluate and target amplified rather than mutant oncoproteins, numerous studies have documented the genomic complexity and heterogeneity of oncogene profiles in GEA (23, 32–35). These features include both transformation leading to cancer cells with multiple driving mitogenic oncogenes (e.g., cancer cells with coamplification of ERBB2 and MET) as well as heterogeneous tumors where distinct driving amplified oncogenes are present in distinct subclones of tumors. Either case poses a clear challenge to simple targeted therapy approaches, likely contributing to the failure of many such therapies in GEA.

GS

GS tumors are most prevalent in the antrum/pylorus and fundus/body (5). The origin of the GS nomenclature in TCGA followed their lack of CIN, MSI/hypermutation, and the hypermethylation found in EBV+ tumors (5). Seventy percent are Lauren diffuse type, a histology that is enriched in the estimated 10% to 15% of patients who develop gastric cancer prior to 45 years of age (36, 37). Diffuse gastric cancers are also enriched in Alaska Natives and Hispanics compared with non-Hispanic whites (38, 39). The most characteristic somatic features of GS tumors are CDH1 (E-cadherin) loss-of-function mutations in 30% (25); notably, this gene is also responsible for hereditary diffuse gastric cancer when altered in the germline (40). In families without altered CDH1, mutations have been described in CTNN1, which functions in the same complex as E-cadherin; in DNA-repair genes such as PALB2, BRCA1, BRCA2, RAD51C, and ATM; and in MSR1 and STK11 (40–42). A proteomic analysis of 84 pairs of diffuse gastric cancer and adjacent normal tissue identified three subtypes, in order of worsening overall survival (OS): PX1, characterized by cell-cycle dysregulation; PX2, enriched in EMT pathways; and PX3, comprised of immune response proteins (43). It remains to be seen whether this classification translates clinically beyond prognostic stratification.

Beyond CDH1, the most notable constellation of genomic findings in these cancers relates to the RHO pathway. Highly recurrent missense mutations in the RHOA GTPase were identified contemporaneously by three groups in 2014 (5, 44, 45). Although missense mutations cluster in the aminoterminal GTPase domain, studies on the activity of these
mutations are mixed, with data supporting a mechanistic gain-of-function but also attenuation of the GTP loading of RHOA, a proxy for its activation. Intriguingly, the most recurrent RHOA mutation is in the highly conserved core effector region at codon Y42. This residue is analogous to HRAS Y40, where mutations selectively modulate specific downstream effects of HRAS (46). Aside from RHOA, recurrent fusion genes have been identified which include nearly the entire coding sequence of the transmembrane tight junction protein CLDN18 fused by its cytoplasmic tail to the RHO GTPase–activating proteins ARHGAP26 or ARHGAP6 (5, 47). Intriguingly, the CLDN18–RHOGAP fusions are mutually exclusive with both CDH1 and RHOA alterations, suggesting this fusion may modulate both cellular adhesion and the RHO pathway in gastric cancer pathogenesis. Moreover, given the roles of these genes in cell adhesion and motility, their unique recurrence in this disease suggests clear connection with a tumor phenotype characterized by noncohesion, invasion, and dissemination.

From a therapeutic perspective, the GS/diffuse gastric cancer population remains a vexing problem. As suggested by the more mesenchymal nature of these tumors, standard cytotoxic chemotherapeutic agents may be less active in these patients (21, 48). Coupled to the paucity of classic targetable oncogenes and the propensity for invasion and metastasis, this disease carries a dismal prognosis, as documented by the ACRG study (19). Deeper mechanistic inquiry aiming to define vulnerabilities of these cancers is a substantial priority within the GEA field.

MSI

MSI tumors arise in the distal stomach in the antrum/pylorus and fundus/body. Within the TCGA project, MSI tumors were not observed in the esophageal lineage despite the enrichment of hypermethylation in EAC (6). Clinically, MSI is a favorable prognostic factor in resectable primary gastric cancer (49). Post hoc exploratory analyses of the MAGIC and CLASSIC trials suggest that in contrast to MSS patients, who benefit from the addition of adjuvant or perioperative chemotherapy, MSI patients demonstrate better outcomes with surgery alone, and the addition of chemotherapy may have a detrimental effect in MSI patients (50–52). These data suggest that MSI tumors may be intrinsically chemoresistant, or that chemotherapy blunts the robust immune response stimulated by these hypermutated tumors. However, these observations have yet to be validated through prospective clinical trials.

As in colorectal cancer, MSI gastric adenocarcinomas typically occur following MLH1 silencing in the presence of a DNA hypermethylation phenotype, or less commonly MLH1 or MSH2 mutations, leading to defective DNA mismatch repair resulting in insertion/deletion events and single-nucleotide variants (SNV). Numerous other genomic features of MSI gastric cancers resemble MSI colorectal cancer, including ARID1A, RNFL4, PIK3CA, and KRAS mutations (25). Strikingly, however, the classic BRAFV600E mutations that frequently occur in sporadic MSI colorectal cancer with DNA hypermethylation are notably absent in MSI gastric cancer despite otherwise highly similar DNA methylation patterns (5, 25). Perhaps most importantly, MSI tumors exhibit high gene-expression scores for IFNγ signaling, CD8+ T cells, and M1 macrophages, second only to EBV+ tumors, highlighting their immunogenicity (25, 53). As in other tumors with MSI, immune checkpoint blockade has demonstrated efficacy in this population (54, 55). Beyond MSI, there are also rare tumors with hypermutation not due to mismatch repair but secondary to POLE mutations. These tumors demonstrate a high frequency of SNVs, yet without the insertion/deletion events observed in MSI cancers (25). Unlike MSI tumors, these hypermutated SNV tumors do not display elevated immune signatures (25).

EBV+

EBV+ tumors localize to the fundus/body and proximal stomach and are notable for their marked DNA hypermethylation. Indeed, these tumors harbor more DNA hypermethylation than any other class of cancer in the TCGA collection (5). EBV+ gastric tumors epigenetically silence CDKN2A, similar to many CIN GEAs, but infrequently harbor TP53 mutation or CIN (5, 25). EBV+ gastric cancers have long been recognized to have strong lymphoid infiltration, leading to the term “lymphoepithelioma-like carcinoma” on histologic analysis. Accordingly, focused molecular analysis of these tumors noted prominent immune signatures (5, 53). Genomic analysis also identified recurrent 9p24.1 amplification at the loci of genes encoding PD-L1, PD-L2, and JAK2, consistent with elevated expression of these genes (5), an event that parallels the presence of this amplification recurrently in EBV-associated lymphoid cancers (56). JAK2 transcriptionally activates both PD-1 ligands in lymphoma models (56). Moreover, EBV infection alone can induce PD-L1 expression independent of 9p24.1 amplification (57). This confluence of immune features imparts sensitivity to immune checkpoint blockade, as observed by the impressive response rates in lymphomas with 9p24.1 amplification and in EBV+ lymphomas (58), and more recently, in EBV+ gastric cancers (55). Beyond immunotherapy, somatic analysis has also revealed highly recurrent PIK3CA-activating mutations and mutations in ARID1A and BCOR (5, 25). Thus, EBV status provides key guidance for prioritizing an immunotherapeutic approach, and raises intriguing possibilities about the utility of PI3K pathway inhibitors and DNA-demethylating agents in these cancers.

TARGETED THERAPY IN THE PRECISION MEDICINE ERA: ERBB2 AS A CAUTIONARY TALE

Given the paucity of targets in GS/diffuse gastric cancer and the exciting results with immunotherapy in MSI/EBV+ disease, the bulk of this review will discuss issues revolving around targeted molecular therapy for the most common subtype, CIN GEA, especially in the Western-predominant proximal gastric/GEJ/EAC cancers. The genomic characterization of CIN GEA has unveiled a multitude of candidate oncogenic drivers that may be potential biomarkers for targeted therapies, most prominently RTKs. The poster child for this paradigm shift to precision medicine in GEA was amplification of ERBB2, which occurs in 15% to 20% of GEA (5, 6, 25). The ToGa trial
Genomics and Targeted Therapies in GEA

Genomics and Targeted Therapies in GEA

antibodies that direct a payload to ERBB2 efficacy in early-phase clinical trials. These new drugs include approaches for GEA with amplification. biomarker positivity, and designing more effective targeted of refining patient selection, fine-tuning the definition of had significantly improved OS, highlighting the importance + in Asian patients and in younger patients, and in the TyTAN analysis in the LOGiC study did show a significant OS benefit in first-line treatment, respectively (66, 67). A preplanned subgroup analysis in the LOGiC study did show a significant OS benefit in Asian patients and in younger patients, and in the TyTAN study, patients with ERBB2 immunohistochemistry (IHC) 3+ had significantly improved OS, highlighting the importance of refining patient selection, fine-tuning the definition of biomarker positivity, and designing more effective targeted approaches for GEA with ERBB2 amplification.

Several emerging ERBB2 agents have shown encouraging efficacy in early-phase clinical trials. These new drugs include antibodies that direct a payload to ERBB2+ cells and those that are optimized to enhance immunologic attack against the cancer cell following binding of the antibody to ERBB2. A phase I study of trastuzumab deruxtecan (DS-8201), a humanized anti-ERBB2 antibody conjugated to a topoisomerase I inhibitor payload, demonstrated objective responses in 19 of 44 patients with advanced unresectable or metastatic ERBB2+ gastric/GEJ tumors, all of whom were previously treated with trastuzumab (68). Margetuximab (MGAH22) is a monoclonal antibody that binds the same epitope of ERBB2 as trastuzumab, but with an altered Fc domain that enhances binding to the CD16A receptor on natural killer cells and macrophages to boost antibody-dependent cell-mediated cytotoxicity. A phase I study showed good tolerability and cytotoxic activity against ERBB2+ advanced solid tumors, including partial responses in 2 of 20 patients with GEA (69). A phase I/II study of margetuximab plus pembrolizumab for the second-line treatment of patients with ERBB2+ GEA (post-trastuzumab) is ongoing (NCT02689284; ref. 70).

Although these newer agents offer some optimism, it is critical to assess the potential reasons why patients progress on first-line trastuzumab-based therapy and to understand why many agents that have been successful in breast cancer fail in GEA. Potential differential features of GEA and breast cancer may include fundamental lineage-related cancer cell pathophysiology, as well as more clearly delineated distinctions in somatic genomic patterns such as the extent of spatial heterogeneity (Fig. 2). In intraleision heterogeneity, individual oncogenes may be uniquely amplified in distinct subclones of the same tumor, or different oncogenes may be co-amplified within the same cancer cell. Compared with breast cancers, gastroesophageal cancers have more intraleision heterogeneity of ERBB2 expression and amplification as observed by IHC and multiregion next-generation sequencing (NGS) of primary tumors (35, 71). This intraleision heterogeneity has direct therapeutic implications. First, the degree of ERBB2 amplification by NGS correlates with the benefit from trastuzumab (34), possibly serving as a measure of ERBB2 dependence in the tumor. Second, trastuzumab resistance has been observed to be commonly mediated by the rise of a non–ERBB2-amplified clone (34, 72). Although it is often referred to as “loss” of ERBB2, this situation more likely reflects preexisting heterogeneity and the selection of ERBB2-negative subclones during trastuzumab therapy. This phenomenon might have contributed to the failure of second-line T-DM1 in the GAVSBY trial, where more than 75% of patients had already progressed on trastuzumab and patients were not rebiopsied to evaluate ERBB2 status prior to randomization (65, 73).

Genomic analysis has demonstrated that more than half of ERBB2-amplified GEA tumors have concomitant baseline genomic alterations that may contribute to intrinsic trastuzumab resistance, including activating mutations in the PI3K pathway and amplifications of CCNE1, CDK6, EGFR, and MET, in line with the focal CIN pattern characteristic of these tumors (32, 34, 74, 75). As observed by the OCCAMS Consortium, this phenomenon of genomic co-amplification in GEA holds not only for ERBB2 but for other RTKs too (23). Moreover, genomic analysis demonstrated that patients with GEA with ERBB2 amplification and co-occurring lesions had inferior survival, especially when compared with those with the highest level of ERBB2 amplification by NGS (34). Preclinical studies suggest that dual blockade of multiple kinases present in GEA can overcome resistance to single-drug therapy, results confirmed clinically (23, 32, 33, 76). For example, a phase II study of afatinib in trastuzumab-resistant ERBB2+ GEA demonstrated an association between afatinib response and tumors harboring dual amplification of ERBB2 and EGFR (76), suggesting that combined targeting of ERBB2 and specific co-occurring genomic alterations may be essential for therapeutic efficacy.

Perhaps even more striking than evolution of the tumor to an ERBB2-negative state upon trastuzumab therapy, gastroesophageal cancers also demonstrate extensive baseline discordance in ERBB2 status between synchronous primary tumors and metastases prior to systemic therapy, in contrast to breast cancers that are ERBB2 concordant in more than 90% of cases (35). This interlesion heterogeneity suggests that the field should revisit the guidelines for assessment of ERBB2 status in GEA, which recommend testing of only a
Table 1. Key clinical trials for GEA

<table>
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<th>Target</th>
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### Table 1. Key clinical trials for GEA (Continued)

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<th>Trial (reference)</th>
<th>Phase</th>
<th>Line of therapy</th>
<th>Treatment</th>
<th>ORR (%)</th>
<th>Median OS (months)</th>
<th>Median PFS (months)</th>
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<td>Cisplatin/fluoropyrimidine + ramucirumab</td>
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<td>16</td>
<td>6.9</td>
<td>3.2</td>
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NOTE: Primary endpoint is median OS unless otherwise specified.
Abbreviations: CapeOx, capecitabine/oxaliplatin; CPS, PD-L1 combined positive score; ECX, epirubicin/cisplatin/capecitabine; EOC, epirubicin/oxaliplatin/capecitabine; mFOLFOX6, leucovorin/fluorouracil/oxaliplatin; NS, not significant; ORR, objective response rate.

in patients with ERBB2 somatic mutations (78). Although caveats should be drawn to the differences between mutation versus amplification, and small-molecule kinase inhibitor versus antibody, the results were notable for robust responses in ERBB2-mutant breast cancer but remarkable lack of efficacy in GEA or colorectal cancer (78). These results evoke memories of the failure to reproduce the stunning albeit transient effects of BRAF inhibition from V600E-mutant melanoma in colorectal cancers also harboring the identical mutation (79, 80). Key lineage-dependent differences in the response to a targeted agent can have far-reaching consequences on therapeutic efficacy. As many approaches being evaluated in ERBB2+ GEA originated with clinical success in breast cancer, these results are a reminder of the importance of focused biological study of GEA.

**BEYOND ERBB2: PARALLEL LESSONS FROM OTHER TARGETED THERAPIES**

**EGFR**

Phase III trials of other targeted therapies in gastroesophageal cancer have fallen short of the mark left by trastuzumab (Table 1). EGFR is amplified in 5% of GEA and the protein is overexpressed in 30% to 50% of GEA (81–84). Neither the addition of cetuximab (EXPAND trial) nor panitumumab (REAL3 trial), both anti-EGFR mAbs, to chemotherapy improved progression-free survival (PFS) or OS (85, 86). In REAL3, patients in the panitumumab plus chemotherapy arm actually had worse OS, with the important caveat that this combination arm also received less chemotherapy because of overlapping toxicity with panitumumab (86). In the second-line or higher setting, the EGFR tyrosine kinase inhibitor (TKI) gefitinib had no benefit compared with placebo (COG trial; ref. 87). However, it is critical to note that these negative EGFR trials were all conducted in unselected patient populations, irrespective of EGFR status. Indeed, subset analysis of the COG data showed a statistically significant OS benefit in EGFR-amplified patients (88), and a similar trend was observed in the EXPAND trial for tumors with high EGFR IHC expression (89), again emphasizing the simple yet crucial lesson of testing targeted therapies in biomarker-positive patient populations. More recent data highlight the
potential efficacy of EGFR-directed therapy both with and without chemotherapy in patients with bona fide EGFR-amplified GEA (81).

**MET**

Clinical trials using MET-directed agents in GEA have also been disappointing. Support for targeting MET followed marked MET inhibitor sensitivity against kinase inhibitors in MET-amplified GEA cell lines (90). Whereas MET amplifications occur in 6% of GEA, MET overexpression has been reported in 25% to 60% of GEA (5, 91–93). The METGastric study examined chemotherapy with onartuzumab, a mAb against the MET extracellular domain that blocks interaction with the MET ligand HGF (94). This trial was stopped early because no predictive cutoff for MET IHC could be identified in a parallel phase II study (95). Even MET IHC 2/3+ patients in the phase III trial had no OS benefit (11 vs. 9.7 months, P = 0.06), although statistical significance may have been thwarted by decreased power from premature termination (94). The RILOMET-1 study was also discontinued early, in this case because of decreased survival in the experimental arm of chemotherapy plus rilotumumab, a mAb against HGF (96). As compared with METGastric, which required MET IHC ≥1+ in at least 50% of cells, RILOMET-1 had looser inclusion criteria (≥1+, ≥25% cells), included fewer IHC 2/3+ patients, and had no trend toward an interaction between MET expression and outcomes. Also, there were very few MET-amplified patients in RILOMET-1, and FISH ratios were not reported in METGastric.

In contrast to these studies, MET amplification was used as the inclusion criteria for a single-arm phase II study of the MET TKI AMG 337 in heavily pretreated patients with advanced-stage tumors (97). Disappointingly, this trial was also stopped early because the primary endpoint of objective response rate (ORR; 18%) was lower than expected compared with phase I data (30%; ref. 98). A key limitation of the AMG 337 studies was that MET status was derived from archival tumor specimens, generally obtained prior to first-line cytotoxic therapy. In light of the above discussion regarding ERBB2 heterogeneity, this raises the hypothesis that some patients may not have been truly MET-amplified at enrollment. Indeed, similar to ERBB2, MET amplification is heterogeneous between primary tumors and metastases, and MET inhibition permits outgrowth of non–MET-amplified clones (33). Moreover, in one study, 10 of 21 MET-amplified GEsAs harbored coamplification of ERBB2 and/or EGFR with MET in the same tumor cells, conferring de novo resistance to MET inhibition (33). These results again emphasize the inadequacy of simple biomarker assessment in CIN GEA, and suggest we have rejected targeted therapies that might be effective if used in optimal patients or in optimal combinations.

**mTOR**

The PI3K–AKT–mTOR pathway is frequently activated in GEA, particularly in the EBV+ subtype, through PIK3CA-activating mutations and amplifications, and PTEN truncating mutations and deletions (5, 6). The GRANITE-1 study enrolled unselected patients with gastric cancer to evaluate second- and third-line treatment with everolimus compared with placebo, unfortunately without improvement in OS (99). Biomarker analysis for this trial has not been published; however, an earlier phase II study identified phosphorylated S6, a downstream effector of mTOR, as a potential predictive factor that remains to be validated (100).

**FGFR2**

FGFR2 amplifications occur in 5% of GEA, with enrichment in the CIN and GS subtypes (5, 25). As with MET, cell line studies in GEsA with FGFR2 amplification show robust responses to kinase inhibition (101, 102). Mechanistic studies suggest that excess FGFR2 activates PI3K signaling indirectly through transactivation of alternative RTKs such as ERBB3 and IGF1R (102). However, no differences in PFS were observed in the randomized phase II SHINE study comparing the FGFR1/2/3 TKI AZD4547 to paclitaxel for the second-line treatment of gastric cancers with FGFR2 polysomy or amplification as determined by FISH on archival tumor specimens (103). A limitation of this study was the considerable intratumor heterogeneity of FGFR2 status and lack of correlation of FGFR2 genomic status with FGFR2 mRNA expression, possibly explaining the negative results (103). Indeed, another translational clinical trial demonstrated that response to AZD4547 can be predicted by high-level homogeneous FGFR2 amplification as opposed to subclonal amplification (102), again highlighting the critical importance of identifying an effective biomarker threshold and considering heterogeneity.

**Antiangiogenic Therapies**

Although antiangiogenic therapies are currently standard of care and are distinct from the targeted oncogene approaches described above, clinical trials of these agents in unselected patients with GEA have had mixed efficacy, suggesting there may be room for improvement through investigation of biomarkers and effective combination strategies. The anti-VEGFR2 antibody ramucirumab demonstrated significant albeit modest OS benefit in the second-line setting both as monotherapy (vs. placebo in the REGARD trial) and combined with chemotherapy (vs. chemotherapy alone in the RAINBOW trial; refs. 104, 105). On the other hand, in the first-line setting, there was no improved OS or PFS (by central independent review) with the addition of ramucirumab to chemotherapy (RAINFALL trial; ref. 106). Similarly, the anti-VEGFA antibody bevacizumab improved overall response rate and PFS, but not OS, when added to chemotherapy for the first-line treatment of advanced gastric cancer (AVAGAST trial; ref. 107). Interestingly, biomarker analysis pinpointed high baseline plasma VEGFA levels (but not tumor VEGFA) and low tumor protein expression of neuropilin 1 (a VEGF factor that remains to be validated (100).

**Immune Checkpoint Blockade**

The role of immune checkpoint blockade for GEA is reviewed elsewhere (110) and will be addressed only briefly. Recent work
has demonstrated clear enrichment of responses in patients with MSI and EBV+ gastric cancer (55), again emphasizing the importance of molecular stratification in the application of immunotherapy for GEA. Pembrolizumab is approved for the second-line treatment of MSI-high/mismatch repair-deficient gastric cancer. Notably, unlike in colorectal cancer, there are also responses to immune checkpoint inhibition in non-MSI and non-EBV+ GEA. The phase III KEYNOTE-062 trial demonstrated noninferior OS and a more favorable safety profile for pembrolizumab compared with chemotherapy as first-line treatment for patients with PD-L1+/ERBB2+ advanced gastric/GEJ adenocarcinoma (NCT02494583; ref. 111). In addition, pembrolizumab prolonged OS in the subset of patients with tumors expressing high levels of PD-L1 (17.4 vs. 10.8 months). Although the combination of pembrolizumab plus chemotherapy improved ORR, it did not improve OS or PFS compared with chemotherapy alone. The phase II KEYNOTE-059 trial led to FDA approval of pembrolizumab for patients with PD-L1+ advanced gastric/GEJ adenocarcinoma who have received two or more lines of therapy (112). In an Asian population of relapsed/refractory gastric/GEJ cancers, the phase III ATTRACTION-2 study showed a modest median OS benefit for nivolumab compared with placebo (5.3 vs. 4.1 months, \( P < 0.0001 \)), and a substantial benefit in 12-month OS rate (26.2% vs. 10.9%; ref. 113).

However, other studies have tempered expectations from these agents and emphasize the need for additional biomarkers beyond PD-L1. KEYNOTE-061, a phase III trial of pembrolizumab compared with paclitaxel for the second-line treatment of PD-L1+ gastric/GEJ tumors, failed to show a survival benefit for immune checkpoint blockade (114). The phase II single-arm KEYNOTE-180 trial of pembrolizumab for advanced, metastatic esophageal cancer progressing after two or more lines of therapy demonstrated an ORR of only 5.2% in patients with EAC/GEJ, in contrast to 14.3% in patients with ESCC (115). The phase III KEYNOTE-181 study compared pembrolizumab to chemotherapy in patients with advanced or metastatic ESCC or EAC/GEJ after first-line standard therapy (NCT02564263; ref. 116). Despite favorable trends and a better safety profile, pembrolizumab did not significantly improve OS in the intention-to-treat population. Pembrolizumab did improve OS in a prespecified subgroup analysis of patients with tumors expressing high levels of PD-L1 (9.3 vs. 6.7 months); however, this benefit was restricted to patients with ESCC (10.3 vs. 6.7 months) and not observed in patients with EAC/GEJ (6.3 vs. 6.9 months). Whereas these trials led to FDA approval of pembrolizumab in the ESCC population, numerous ongoing studies will address pivotal questions about how to enhance the efficacy of immunotherapy in GEA and identify combinations with other targeted and conventional therapies.

MOVING FORWARD: MAXIMIZING OPPORTUNITIES FOR THERAPEUTIC EFFICACY

As a field, we are now poised to leverage the failures of targeted therapy to revise our clinical approach to GEA. The prevalence of concomitant genomic alterations, especially RTK coamplifications that confer resistance to single-agent therapy, underscores the need for comprehensive molecular profiling to guide treatment selection, including combination strategies. Furthermore, the breadth of interlesion tumor heterogeneity within an individual patient means that biopsy of a single lesion incompletely represents the clinically relevant genomic composition of a patient’s cancer burden.

To bypass the impracticality of taking multiple tissue biopsies, including synchronous primary and metastatic biopsies, emerging data suggest the clear potential of minimally invasive liquid biopsies for the isolation and sequencing of cell-free circulating tumor DNA (ctDNA) in peripheral blood (reviewed in ref. 117). Although systematic validation of the accuracy, reliability, and reproducibility of ctDNA assays is a work in progress, clinical utility has been demonstrated in several studies of GEA with amplifications of ERBB2, EGFR, MET, and FGFR2 (33, 35, 72, 76, 81, 102). In an umbrella trial of targeted therapy in GEA, approximately one third of patients had discordant oncogene amplification between synchronous primary tumor and metastatic biopsies, leading to treatment reassignment (35, 118). Biomarker concordance between metastases and ctDNA surpasses correlation between metastases and primary tumor, raising the hypothesis that ctDNA may complement traditional tissue-based genomic profiling to guide treatment in the metastatic setting. Beyond initial treatment selection, ctDNA may be sampled longitudinally during the course of treatment to trace the evolution of clonal heterogeneity, including identifying drivers of intrinsic and acquired resistance. Biomarker detection and validation might also improve with ctDNA analysis; for example, higher EGFR copy number in ctDNA correlated with response to anti-EGFR therapy (81), and a similar finding was reported for FGFR2 amplification and response to an FGFR TK1 (102). In addition, ctDNA mutational load predicted response to pembrolizumab in MSI-high metastatic gastric cancer, and lower ctDNA levels at 6 weeks post-treatment predicted improved outcomes (55).

Integrating NGS and ctDNA technologies into clinical trials will enhance patient and treatment selection, especially for innovative trial designs like basket and umbrella studies. PANGEA (NCT02213289; ref. 118) and VIKTORY (NCT02299648; ref. 119) are both biomarker-based umbrella trials for metastatic GEA or gastric cancer, respectively, that prospectively use tumor genomic profiling to assign patients to targeted therapy treatment arms spanning key RTK pathway amplifications and mutations. Whereas VIKTORY focuses on primary tumor specimens, PANGEA analyzes both primary and metastatic lesions at baseline and at progressive disease, and both trials perform ctDNA sequencing for correlative studies. Outcomes from PANGEA are pending; however, the biomarker-driven treatment group in VIKTORY demonstrated better OS (9.8 vs. 6.9 months, \( P < 0.001 \)) and PFS (5.7 vs. 3.8 months, \( P < 0.0001 \)) compared with patients who received conventional second-line treatment (119). More adaptive trials such as these are needed to address the intricate, heterogeneous genomes of patients with GEA. Although these trials will certainly be more complex, recent experience has demonstrated that unless we change our approach, we will continue to conduct negative studies that fail to identify the efficacy of drugs that may be beneficial in a more tailored patient population. These refined clinical trial approaches
will pave the way for exploring and ultimately translating new candidate genetic dependencies and therapeutic vulnerabilities of GEA, as described below.

**UNCHARTED TERRITORY: NOVEL TARGETS AND TREATMENTS ON THE HORIZON**

The diversity of oncogenic amplifications in the CIN subtype of GEA have made it challenging to identify a unifying vulnerability of these tumors. The common thread is their underlying genomic instability and aneuploidy. Long-term therapeutic advancements may require a deeper understanding of the causes and consequences of CIN, and the vulnerabilities stemming from CIN. This not only includes targeting oncogenes that are activated in the presence of genomic instability, but also defining vulnerabilities inherent to the underlying state of a cell with such marked genomic disruption.

Meanwhile, the focus of targeted therapy efforts for CIN GEA has been RTK amplifications. However, there are a host of other targets worthy of evaluation. One example followed the recognition that CIN GEEs frequently activate the KRAS oncogene via canonical mutations seen in many other cancers but through focal high-level genomic amplification comprising 14% of CIN GEA and causing as much as several hundred-fold overexpression of wild-type KRAS protein (25, 120). Laboratory-based evaluation of the intrinsic wiring of these tumors demonstrated that MEK inhibitors, which form the backbone of many current approaches for developing combination therapy for RAS-driven cancers, led to marked adaptive KRAS activation owing to the massive KRAS expression. This adaptive response could be overcome by dual inhibition of MEK and the protein tyrosine phosphatase SHP2, also known as PTPN11, leading to tumor regression in xenograft models (120). Phase I trials are under way to evaluate the safety and tolerability of the SHP2 inhibitors TNO155 (NCT03114319) and RMC-4630 (NCT03634982) in advanced solid tumors.

Beyond RTK/MAPK pathways, molecular dissection of CIN GEA also revealed frequent alterations in cell-cycle regulators (NCT03634982) in advanced solid tumors.

For CCNE1-amplified GEA, preclinical data from gastric cancer patient-derived xenografts and from high-grade serous ovarian cancer (HGSOC) demonstrate that targeting CDK2, the partner kinase of CCNE1, may be effective (123, 124). Notably, CCNE1 amplifications are mutually exclusive and synthetic lethal with BRCA1 loss in HGSOC (125), suggesting the necessity of homologous recombination (HR) DNA repair in these cancers secondary to CCNE1-induced replication stress. Thus, targeting the DNA-damage response may be a key therapeutic approach that is further supported by enrichment of an HR deficiency (HRD) mutational signature (also known as COSMIC signature 3, or the BRCA signature) in the CIN subtype, specifically associated with focal somatic copy-number alteration events (25, 126). The HRD signature correlates with response to platinum- and PARP inhibitor–based therapies in breast and ovarian cancers (127), suggesting there may be a subset of CIN GEA harboring similar susceptibility. Indeed, a retrospective analysis of patients with South Korean gastric cancer showed that those with CIN tumors yielded the greatest benefit from adjuvant chemotherapy (21), perhaps hinting at a connection between CIN, HRD, and platinum sensitivity in gastric cancer.

PARP inhibitors have been clinically evaluated in GEA. Despite promising phase II data (128), the phase III GOLD trial failed to show benefit from adding the PARP inhibitor olaparib to paclitaxel in the second-line setting (129). These negative results in the overall patient population might be understandable because sensitivity to PARP inhibition typically depends on HRD. Unexpectedly, however, the subgroup of patients with loss of ATM expression by IHC also did not benefit from olaparib. Potential explanations include immaturity follow-up of the ATM-negative subgroup, and confounding of treatment by favorable prognostic factors enriched in ATM-low tumors, such as PD-L1 expression (129, 130). The data also suggest that additional genomic and functional measures of HRD and replication stress should be explored as predictive biomarkers of response to PARP inhibition. Platinum sensitivity may itself be a predictive biomarker, a concept that has led to a phase III trial of PARP inhibition versus placebo as maintenance therapy in inoperable, locally advanced, or metastatic gastric cancer that responded to platinum-based first-line chemotherapy (NCT03427814; ref. 131). More broadly, there is a widening spectrum of therapeutic agents in the DNA-damage sphere, for example, inhibitors of CHEK1, ATR, and WEE1, which may be combined with PARP inhibition to attack cancer cells with high basal replication stress, even when HR remains intact (132). Evaluation of these agents in GEA, both preclinically and clinically, may be a critical path for the future.

The development of targeted therapies for diffuse gastric cancer is a pressing unmet need. Although these tumors are generally characterized by altered cell adhesion and motility, up to 75% retain strong protein expression of the tight junction molecule claudin 18 isoform 2 (CLDN18.2), a lineage marker highly specific to differentiated gastric mucosal epithelial cells (133). CLDN18.2 is also expressed at lower frequency in intestinal-type gastric cancers and ectopically activated in EAC (133). The phase II FAST trial randomized patients with advanced/recurrent gastric and GEJ cancers, including 45% with diffuse type histology, to first-line
chemistry with or without IMAB362 (also known as claudixin or zolbetuximab), a chimeric mAb against CLDN18.2 that functions not via inhibition of CLDN18.2 but instead by using CLDN18.2 as a specific marker for antibody binding to elicit immune activation (NCT01630083; refs. 134–136). Inclusion criteria required CLDN18.2 IHC ≥+ in at least 40% of tumor cells. The addition of IMAB362 improved ORR (39% vs. 25%, P = 0.022), the primary endpoint of PFS (7.5 vs. 5.3 months, P < 0.0005), and OS (13.0 vs. 8.4 months, P = 0.0008), with even greater benefit in the subgroup with CLDN18.2 IHC ≥+ in at least 70% of cells (135). These encouraging results have spurred two phase III trials for further evaluation of IMAB362 (NCT03350439; NCT03653507). Other approaches to target CLDN18.2* cells using antibody-drug conjugates for delivery of a cytotoxic payload, CD3 bispecific antibodies to induce T cell–mediated killing of tumor cells, and CLDN18.2–specific chimeric antigen receptor T cells are under preclinical investigation (137, 138).

CONCLUSION

Our understanding of gastroesophageal cancer has rapidly evolved over the past five years. Delving into the genomic landscape has revealed strong similarities between adenocarcinomas of the stomach and esophagus, and their definitive distinction from squamous cell carcinomas of the esophagus. Moreover, these genomic blueprints have enabled classification into molecular subtypes that elucidate the diverse, complex oncogenic pathways underlying GEA pathogenesis. These achievements are essential stepping stones on the path toward developing more effective therapeutic strategies. Unfortunately, however, the Occam’s razor “single target, single drug” rationale has proved too simplistic for GEA because of the intrinsic genomic instability and heterogeneity of this disease. To address these formidable obstacles, comprehensive and repeated molecular profiling will be integral to patient and treatment selection, and to the success of future translational clinical trials in GEA. These changes in our clinical approach to GEA will be complemented and fueled by ongoing laboratory investigation into novel genetic dependencies and vulnerabilities of GEA. Ultimately, although considerable challenges remain, the coming years hold the promise of a turning point in the battle against these deadly cancers.

Disclosure of Potential Conflicts of Interest

A.J. Bass is a consultant at Lilly and Glenmark, reports receiving commercial research grants from Novartis, Merck, Sanofi, and Bayer, and has ownership interest (including patents) in Earli, Helix Nano, and Signet Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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