Review Article

Gastrointestinal cancers: current biomarkers in esophageal and gastric adenocarcinoma

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Abstract: Esophageal and gastric adenocarcinomas are frequently diagnosed at an advanced stage and have a dismal prognosis. Even in patients with potentially curative cancer, nearly 50% will develop recurrent disease despite aggressive treatments. A number of biomarkers currently guide treatment decisions for patients with esophageal and gastric adenocarcinoma and include human epidermal growth factor receptor 2 (HER2) amplification, mismatch repair deficiency/microsatellite instability (dMMR/MSI-H) and program death-ligand 1 (PD-L1) expression. This review will focus on the function, testing and FDA-approved targeted therapies for HER2, dMMR/MSI-H and PD-L1. In addition, a number of novel targets in esophageal and gastric cancer are being studied in clinical trials. Neurotrophic-tropomyosin receptor kinase (NTRK), claudin-18 (CLDN18)/Rho GTPase activating protein 26 (*ARHGAP26*) gene fusion, fibroblast growth factor receptor (FGFR), lymphocyte-activation gene 3 (LAG3) and T cell immunoglobulin and mucin-domain containing-3 (TIM3) will be briefly reviewed. Despite several biomarkers used in the selection of treatment therapies, treatment outcomes remain poor. Future research efforts will focus on the identification of new biomarkers, moving existing biomarkers into earlier lines of therapy, and evaluating new combinations of existing biomarkers and therapies.

Keywords: Human epidermal growth factor receptor 2 (HER2); microsatellite instability (MSI); program death-ligand 1 (PD-L1)

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Introduction

Esophageal and gastric adenocarcinomas are estimated to represent over 45,000 cases in the USA in 2019 and rank as the 15th (stomach) and 18th (esophageal) most common malignancies in the United States (1). Many cases are diagnosed at an advanced stage and as such, have a dismal prognosis. Early stage carcinomas are typically treated with multimodality therapy, which results in improved survival rates compared to surgery alone. Despite aggressive treatments of potentially curative esophageal cancer, about 50% of patients ultimately developed recurrent disease. Cure in the setting of locally advanced or metastatic disease is exceedingly rare. Systemic therapy is the cornerstone of treatment in the advanced setting. A number of biomarkers that can guide treatment options for patients with esophagogastric adenocarcinoma (EGA) have been identified and these biomarkers will be the focus of this review.

Human epidermal growth factor receptor 2 (HER2/ERBB2)

Human epidermal growth factor receptor (HER) represents a family of receptors, of which HER2 is a member. These proteins are a group of tyrosine kinase receptors that are essential in regulating cellular proliferation, differentiation and survival, but also play a pivotal role in the pathogenesis of several human cancers. The family includes HER1 (ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4) (2). All four receptors have common structural features, which include a extracellular ligand-binding domain that is rich in cysteine residues, a lipophilic transmembrane domain and tyrosine kinase catalytic activity present within the intracellular protein domain (3). HER receptors are located on the cell surface as monomers and undergo dimerization and transphosphorylation of intracellular domains when ligand binds to the extracellular domain. HER2 is an exception in that it does not have a ligand. Activation of HER2-mediated signaling occurs by either by homodimerization (when in high concentrations) or heterodimerization with ligand-activated EGFR or HER3. Dimerization of the receptors results in the tyrosine residues within the cytoplasmic domain to become autophosphorylated with the activation of downstream pathways including protein kinase C (PKC), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), Janus kinase/signal transducer and activator of transcription (JAK/STAT), and mitogen-activated protein kinase (MAPK) which leads to cell proliferation, differentiation, invasion, and angiogenesis (4,5). HER2 containing heterodimers have the greatest mitogenic potential among the HER complexes. The heterodimer pair of HER2-HER3 in particular is the most powerful stimulator of the PI3K signaling cascade, which regulates cell growth and survival (6,7).

Abnormal HER2 signaling has been identified in a number of different cancers, and overexpression and/or amplification of HER2 is a poor prognostic marker in several malignancies (8). In the context of esophageal and gastric adenocarcinoma, prognostic data are conflicting, and large studies fail to show correlation with overall survival (OS) (9-13).

Overexpression

HER2 overexpression is seen in up to ~20% of esophageal and gastric cancers (14,15). Moreover, overexpression in the stomach varies with histologic subtype, with HER2 being more frequently noted in intestinal-type carcinomas compared to diffuse-type (or poorly cohesive) carcinomas and in tumors that are well to moderately-differentiated compared to those that are poorly-differentiated (15-17). In contrast to breast cancer, the expression of HER2 in esophageal and gastric cancers is quite heterogeneous (13,18).

Testing

Immunohistochemistry (IHC) and *in situ* hybridization (ISH) techniques are appropriate methods to assess HER2 overexpression according to the guidelines outlined by the College of American Pathologists (CAP), American Society of Clinical Oncology (ASCO) and the American Society for Clinical Pathology (ASCP) (19). Resection and biopsy specimens from patients with esophageal or gastric tumors should be placed in 10% neutral buffered formalin within 1 hours of collection (cold ischemia time) and allowed to fix to a minimum of 6 and a maximum of 72 hours. IHC should be used as the first testing modality to assessment HER2 expression. HER2 IHC evaluates membranous protein expression in the cancer cells. A four tiered scoring system for HER2 was developed by Hofmann *et al.*, using an assessment area containing at least 10% expression within tumor cells in a resection specimen while a single small cluster of cells (containing at least 5 neoplastic cells) was required for biopsy samples (20). The "completeness" or full membranous staining that is a requirement in HER2 assessment in breast cancers is not seen in gastric or esophageal adenocarcinomas; these tumors typically have a basolateral staining pattern. Therefore, intensity and percentage of HER2 expression on the tumor cells is assessed using a scale from 0 to 3+ (see *Table 1*). HER2-positive esophageal and gastric cancer is defined as tumors with an IHC score of 3+ (see *Figure 1*). Scores of 0 or 1+ are considered HER2 negative. For cases exhibiting a HER2 score of 2+ (i.e., equivocal), additional testing using FISH or other ISH techniques should be performed to confirm HER2 status.

A wide array of ISH techniques can be used for detection of *HER2* gene amplification. Some of these are fluorescence *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH), silver-enhanced *in situ* hybridization (SISH), and dual *in situ* hybridization (DISH). While a single HER2(ERBB2) probe may be used for the detection of gene copy number, most assays typically include a chromosome enumeration probe to more accurately calculate the ratio of HER2(ERBB2) signals to copies of chromosome 17 (CEP17) (see *Figure 2*). A minimum of

Table 1 Immunohistochemistry scoring for HER2 in gastric and gastro-oesophageal junction cancer, by type of diagnostic specimen

Score	Surgical specimen staining pattern	Biopsy specimen staining pattern	HER2 overexpression assessment
0	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
1+	Faint or barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal
3+	Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Positive

Copy with the approval from Elsevier. HER2, human epidermal growth factor receptor 2 (also known as ERBB2).

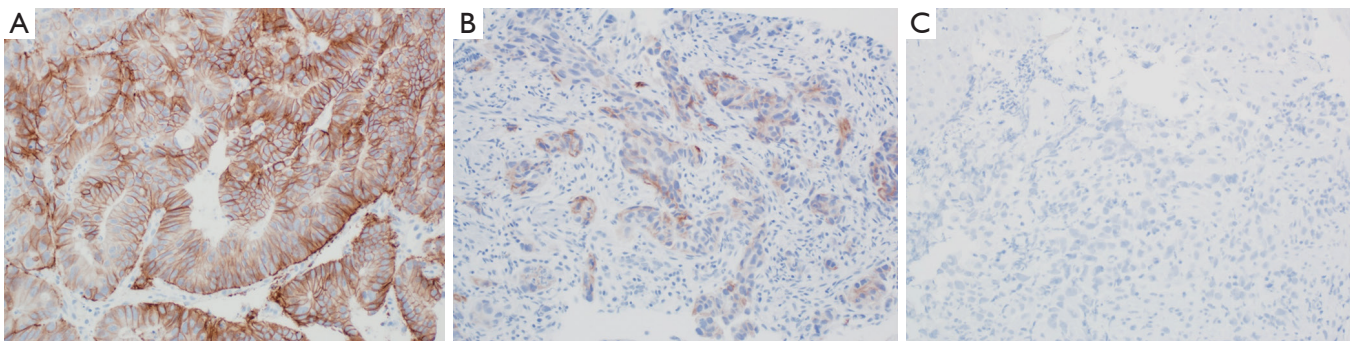

Figure 1 Immunohistochemical expression of human epidermal growth factor receptor 2 (HER2) in esophageal adenocarcinoma. (A) HER2-positive (3+) expression with strong basolateral membranous immunostaining. (B) An equivocal case of HER2 expression (2+) with moderate, basolateral membranous staining. (C) An example of a HER2-negative (0) case with no expression noted in cancer cells. All images are at 200× magnification.

20 discrete tumor nuclei are evaluated for the HER2 and CEP17 probe signals. According to the 2016 CAP/ASCP/ASCO guidelines, HER2(ERBB2) amplification is defined as HER2 (ERBB2):CEP17 ratio of ≥2. In cases where the IHC score is 2+ (equivocal), an average of 3 or more CEP17 signals are present with a ratio ≤2, then the presence of more than 6 HER2 signals is interpreted as positive for HER2 amplification by ISH/FISH. In cases where ≤4 HER2 signals are present, the findings are interpreted as negative for HER2 amplification. For cases where a range of 4–6 signals are identified, it is recommended that an additional 20 cells be scored in a separate area or another sample be submitted for evaluation (19).

IHC and FISH have been studied extensively, while next generation sequencing (NGS) for the identification of HER2 amplification is still in its early stages. NGS is often utilized when tissue samples are limited and patients are unable to undergo additional sampling. NGS testing allows for HER2 copy number and HER2 mutations to be assessed at the same time. At present, there are limited data assessing copy number variation (CNV) compared to the current gold-standard methods of IHC and ISH. Unlike in breast cancer, the concordance of HER2 copy number via NGS in gastric cancer was only moderate compared to IHC and ISH (21). The larger discordance in gastric cancer may be a function of underlying biological mechanisms and further studies are warranted. HER2 copy number in liquid biopsies or circulating tumor deoxyribonucleic acid (ctDNA) is

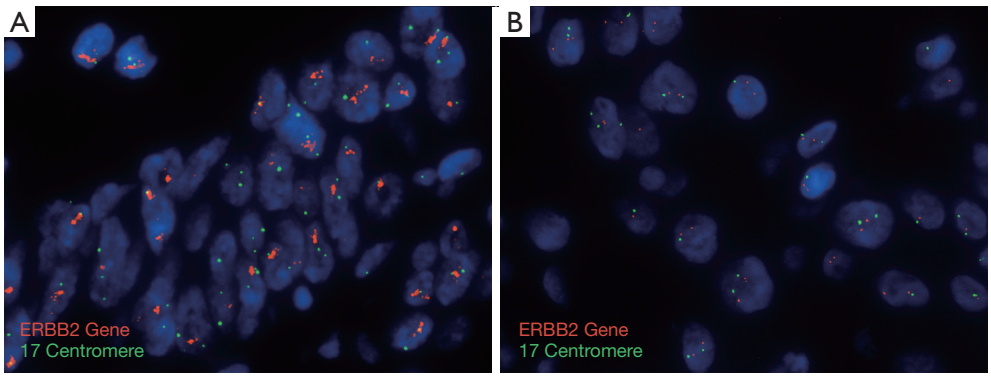

Figure 2 Human epidermal growth factor receptor 2 (HER2) expression by fluorescence *in situ* hybridization (FISH) in gastroesophageal junctional adenocarcinoma. The HER2 (ERBB2) probe is shown in red, while the chromosome 17 enumeration probe (CEP17) is noted in green. (A) A tumor with HER2 amplification as demonstrated by a HER2(ERBB2):CEP17 ratio of ≥2. (B) HER2 non-amplified tumor exhibiting a HER2(ERBB2):CEP17 ratio of ≤2. Images are acquired at 400× magnification.

being examined for disease burden and capability to predict trastuzumab efficacy in patients with gastric cancer (22). Tumor tissue and ctDNA samples had a high HER2 amplification concordance, signifying that ctDNA may serve as an alternative method for screening HER2-targeted populations, however additional studies are needed.

Targeting therapy

Trastuzumab is a monoclonal antibody that binds to the extracellular domain of the HER2 receptor and prevents the activation of its intracellular tyrosine kinase. In the US, trastuzumab combined with chemotherapy is approved for the 1st line treatment of HER2 amplified esophageal and gastric adenocarcinomas. This approval is based on the positive results from the Trastuzumab for Gastric Cancer (ToGA) trial, which was an open-label, international, phase 3 randomized controlled trial using the anti-HER2 humanized monoclonal antibody trastuzumab. This study demonstrated a median survival of 13.1 months for patients who received trastuzumab and chemotherapy and 11.7 months for patients who were treated with chemotherapy alone (15). Trastuzumab was most effective in HER2-positive tumors. As such, trastuzumab is recommended for tumors with IHC score of 3+ and those with an IHC score of 2+ with evidence of HER2(ERBB2) amplification by ISH. In patients who were found to be positive by ISH, but had IHC scores of 0 or 1+, there was no significant survival benefit seen for these patients and as such trastuzumab is not recommended in this setting (15,23,24).

DNA mismatch repair (MMR)/microsatellite instability (MSI)

DNA MMR is a decidedly preserved mechanism, which exists in organisms from prokaryotes to eukaryotes. MMR is involved in genomic fidelity by repairing DNA after mismatching inaccuracies occur during replication and recombination (25,26). MMR has also been shown to be involved in cell cycle regulation and apoptotic response to a variety of DNA damage (27,28).

Four genes regulate the MMR mechanism: mutL homologue 1 (*MLH1*), postmeiotic segregation increased 2 (*PMS2*), and mutS homologue 6 (*MSH6*). The proteins form heterodimers, namely MLH1/PMS2 and MSH2/MSH6. The MSH2/MSH6 heterodimer is responsible for recognizing base mismatches and insertion-deletion loops, while successive enlistment of the MLH1/PMS2 heterodimer excises the altered stretch of base pairs and resynthesizes the corrected DNA bases in this mismatch site.

The biallelic inactivation of one of the *MMR* genes can result from mutations (either somatic or germline) or from epigenetic silencing (25,29). When one or more of the MMR proteins are not expressed, the result is referred to as deficient mismatch repair (dMMR); while when all proteins are intact, the status is considered proficient mismatch repair (pMMR). The loss of MLH1 expression leads to inactivation of the MLH1/PMS2 heterodimer and degradation of PMS2, while loss of MSH2 expression leads to inactivation of the MSH2/MSH6 heterodimer and loss of MSH6.

dMMR was identified as a germline mutation in Lynch syndrome patients in 1993 (30). Patients with Lynch syndrome were found to have increased number of microsatellites, or short tandem repeats, in their DNA. Microsatellites are repeating DNA sequences ranging in length from one nucleotide and up to six nucleotides which can be identified within both coding and non-coding regions of the genome (31). These repeating DNA sequences are sensitive to mismatch errors; when MMR systems are deficient (dMMR), there is an accumulation of mutations defined as MSI that can be detected when analyzing polyA microsatellites by polymerase chain reaction. Both MSI and chromosomal instability represent a mutator phenotype; while MSI is a marker of dMMR, not all hypermutated cells will be dMMR/MSI-H (32).

MSI can be seen in up to 5% of esophageal adenocarcinoma. Similar to its colorectal counterpart, MSI-H esophageal adenocarcinomas are histologically distinct in that they have increased tumor infiltrating lymphocytes and high-grade histology, such as medullary, mucinous or signet-ring cell subtype (33). MSI in gastric adenocarcinomas is noted in about 6–9% of cases and is observed in older females, tumors located in the antrum, tumors with intestinal subtype, early stage and are associated with a better prognosis (34-36). Lynch syndrome associated esophageal and gastric adenocarcinoma is rare. The frequency of gastric cancer in Lynch syndrome patients is estimated to be ~1.6% (37), while esophageal adenocarcinoma has not been associated with Lynch syndrome. It should be noted that although 3–5% of esophageal carcinoma cases appears to have MMR deficiency, this is due to somatic and not germline mutations (38).

Testing

Testing to assess the functionality of the MMR system is becoming increasingly common as it has important implications for the screening of Lynch syndrome. MMR status is also prognostic and predictive of treatment response to immune checkpoint inhibitors. Therefore, MMR deficiency should be assessed in patients with locally advanced, recurrent or metastatic esophageal or gastric adenocarcinoma who are candidates for treatment with PD-1 inhibitors. Two clinically utilized testing platforms are used to detect a deficient MMR system in cancer. MSI testing by polymerase chain reaction (PCR) is used to detect instability in microsatellite repeats, while IHC is used to detect the presence or absence of nuclear expression of one or more of the MMR proteins.

IHC

There are commercially available antibodies directed against the MMR proteins MLH1, MSH2, MSH6, and PMS2. IHC detection systems are used to identify the presence or absence of nuclear protein expression. Testing can be performed on formalin-fixed, paraffin-embedded (FFPE) tumor tissue from a biopsy or the resected surgical specimen (see *Figure 3*). Loss of nuclear expression in one or more the MMR proteins has good correlation with DNA-based MSI testing (39). IHC is widely available, routinely used, and results reported within 48 hours in most pathology laboratories.

MSI testing by PCR

Molecular analysis can be conducted on DNA extracted from fresh, frozen, or FFPE tumor tissue using a PCR-based assay for the detection of MSI. In order to minimize the variability between different MSI tests, the National Cancer Institute (NCI) in 1997 recommended a panel of five microsatellite markers for testing termed the NCI/Bethesda panel. This panel consisted of two mononucleotides loci [big adenine tract (BAT) 25 and BAT26] along with three dinucleotide repeats (D2S123, D5S346 and D17S250) (40). A 2004 NCI workshop recognized the limitations of the original Bethesda panel and recommended the use of a panel of five microsatellite markers, including mononucleotide repeats (BAT25, BAT26, NR21, NR22, and NR 24 or NR27) and dinucleotide repeats (D2S123, D5S346 and D17S250) (Umar 2004, Umar 2004). The NCI panel also provided guidelines for interpretation of MSI-high (MSI-H), MSI-low (MSI-L), and microsatellite stable (MSS) (40). Tumors are classified as MSI-H when there is a documented shift in at least 2 of five tumor loci when compared to non-neoplastic tissue (see *Figure 4*). If a larger panel is used, then a shift of >30% of loci is required for MSI-H. When there is a shift in one locus (<30% of loci in larger panels), the tumor is classified as MSI-L. When there is no instability detected (<10% loci in larger panels), the tumor is considered MSS. Notably, subsequent studies discovered that a panel of mononucleotide markers, which are often more unstable than dinucleotide markers, was more sensitive than the original NCI panel (41-44).

Targeting therapy

In 2017, the FDA approved pembrolizumab for patients with dMMR/MSI-H solid tumors that are deemed unresectable or metastatic, irrespective of tumor type or

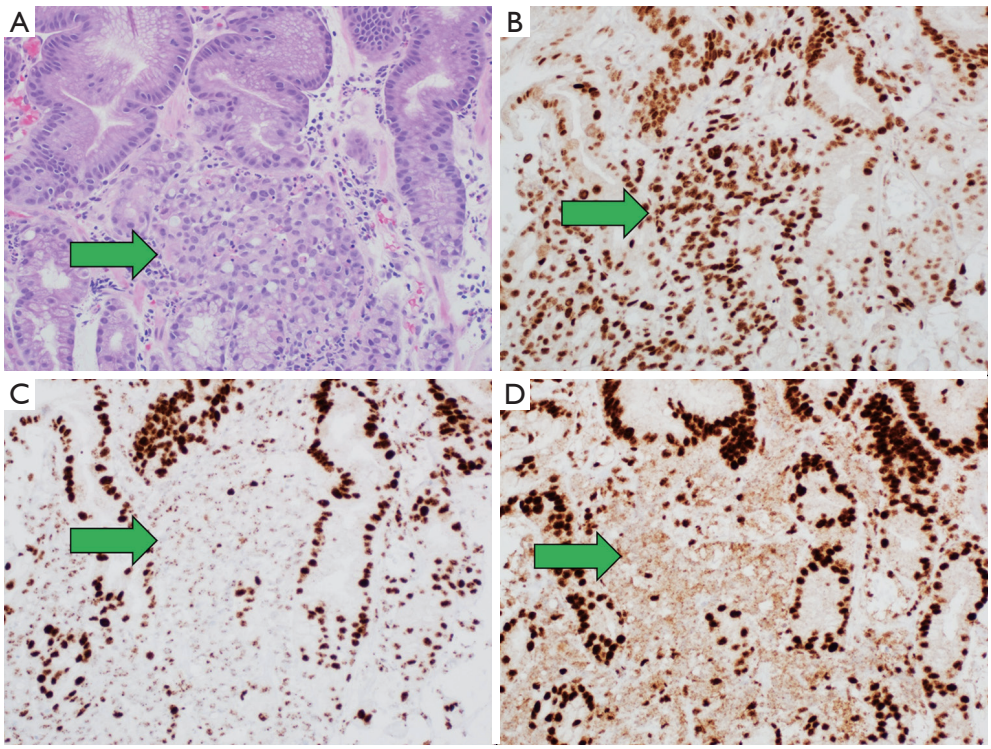

Figure 3 Mismatch repair (MMR) by immunohistochemistry in gastric adenocarcinoma. (A) Hematoxylin & eosin (H&E) stained slide exhibiting normal gastric foveolar glands and in the center of the image (green arrow) is a focus of adenocarcinoma with loss of gland formation. (B) Immunohistochemistry for MSH6 shows intact nuclear expression in normal and tumor nuclei. (C) MLH1 immunoexpression shows a faint, dot-like peri-Gogli staining pattern that is interpreted as loss of nuclear expression in the tumor cells. (D) PMS2 nuclear staining is also lost in the tumor cells. Images acquired at 200× magnification.

site. These patients have progressed on at least one line of prior therapy. This approval is notable as it is the first biomarker-based, disease agnostic approval in oncology. The FDA's approval of pembrolizumab was based on data from five multicenter, single cohort clinical trials, which enrolled a total of 149 patients with dMMR/MSI-H tumors. Objective responses were seen in 39.6% of subjects, and lasted for 6 months in 78% of the responders. The overall response rate was similar across different tumor types (45).

Programmed death-ligand 1 (PD-L1)

The immune system plays an important role in homeostasis. T-lymphocytes can selectively identify and kill pathogens and tumor cells by coordinating responses by the innate and adaptive immune systems. There are numerous checks and balances so that the immune system does not mistakenly destroy healthy cells during their response. Cancer cells frequently exploit these immune checkpoints in order to evade detection.

Some of these immune checkpoint proteins include programmed cell death protein 1 (PD-1) and PD-L1 that act as co-inhibitory factors that can stop or minimize T-cell responses. PD-1 was originally cloned in 1992 and was felt to be responsible for the process of apoptosis (46). PD-1 is a type I transmembrane protein located on chromosome 2q37, coded by the *PDCD1* gene. PD-1 is known to be present on the surface of B- and T-lymphocytes, macrophages and some dendritic cells (47,48). PD-L1 is encoded by the *CD274* gene located on chromosome 9p24.2 that results in a 40-kDa transmembrane protein expressed on macrophages and dendritic cells that binds to PD-1 (49). PD-L1 is known to be expressed in a wide variety of non-inflammatory cells including pneumocytes, hepatocytes, endothelial cells, etc. (48,50,51). Moreover, PD-L1 has been shown to have high expression levels in several tumor types including breast carcinoma, hepatocellular carcinoma, squamous cell carcinomas of the head and neck, and lung

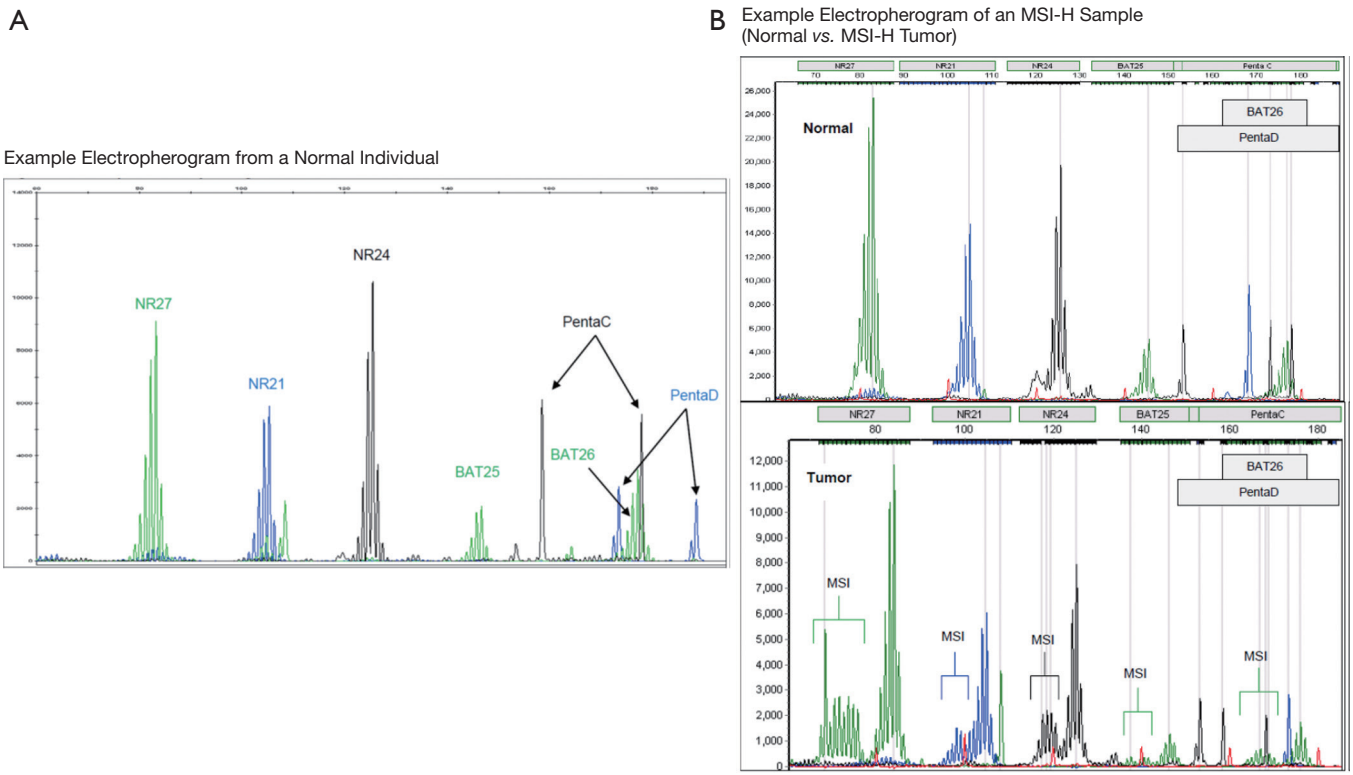

Figure 4 Electropherogram for assessment of microsatellite instability using a panel of seven markers. (A) Microsatellite stable (MSS) case from a patient without underlying malignancy. (B) Case with microsatellite instability with normal tissue (top panel) and tumor tissue (bottom panel); there is a shift in 5 out of the seven loci, consistent with MSI-H. MSI, microsatellite instability.

adenocarcinomas (52-57). Several studies, including those for esophageal and gastric adenocarcinoma, have identified PD-L1 overexpression as a predictive biomarker for response to immune checkpoint inhibitors (55,58).

Testing

There are commercially available antibodies directed against various clones of PD-L1 that can detect the presence of membranous protein expression. PD-L1 expression is seen in about 40% of gastroesophageal adenocarcinomas (59,60). PD-L1 expression on tumor cell themselves is infrequent, and most of PD-L1 staining is seen in immune cells within the stroma of the tumor, specifically lymphocytes and macrophages (60). As such, results from PD-L1 testing in upper gastrointestinal cancers is reported as combined positive score (CPS) to account for all the cell types that stain positively, in contrast to total positive score (TPS), which is used to analyze lung cancer specimens.

Prior to immunostaining, it should be confirmed that at least 100 viable tumor cells are present within the sample. Immunohistochemistry slides are examined at low magnification to assess the extent of membrane staining of cells within the tumor. At higher magnification, the number of PD-L1 positive cells (tumor cells, lymphocytes and macrophages) relative to total number of viable tumor cells is determined. It should be noted that necrotic debris and stromal cells within the tumor should not be included within the calculation. The results are reported in the setting of esophageal and gastric carcinomas as CPS utilizing the formula:

CPS = [# positive PD-L1 cells (tumor cells, lymphocytes, macrophages)/# PD-L1 positive and PD-L1 negative tumor cells] ×100%

CPS is reported as a single number with a maximum score of 100. CPS is considered positive with a score ≥1, while CPS ≤1 is considered negative (see *Figure 5*).

Targeting therapy

Pembrolizumab is an anti PD-1 antibody that was approved

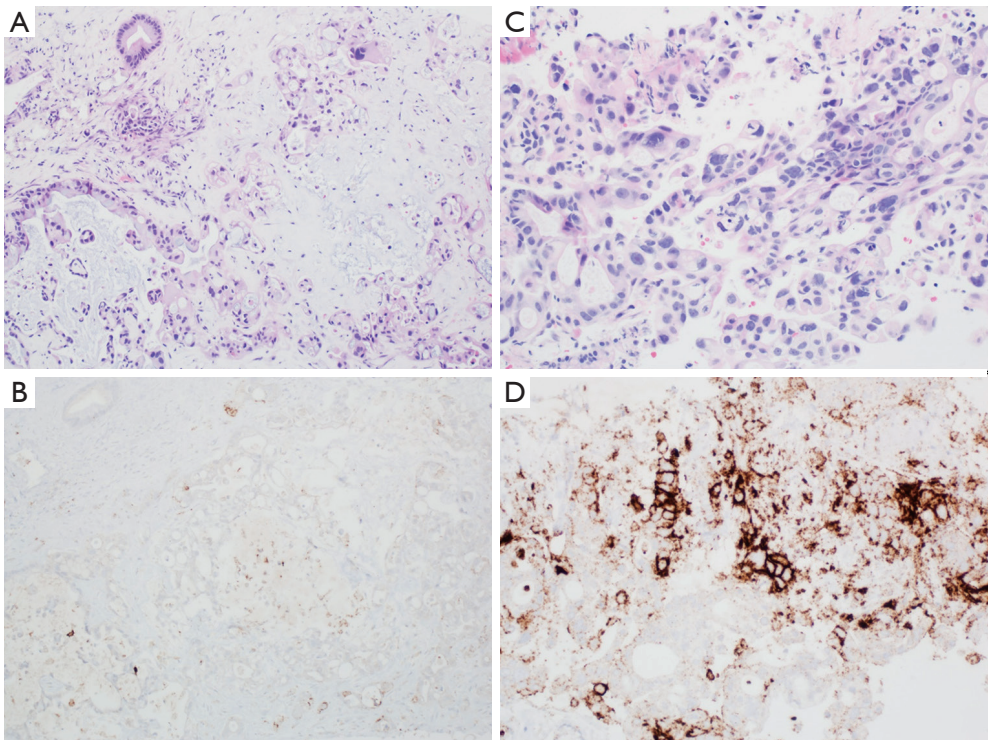

Figure 5 PD-L1 (clone 22C3) expression in esophageal adenocarcinoma by immunohistochemistry. (A) Hematoxylin and eosin stained tumor section confirming presence of at least 100 tumor nuclei. (B) Rare, PD-L1 positive, membranous expression is noted and thus has a CPS ≤1 (negative). There is no evidence of PD-L1 (C) Hematoxylin and eosin stained section from a different tumor, again confirming at least 100 tumor nuclei. (D) Strong PD-L1 positive, membranous expression is noted within the tumor cells and in lymphocytes and thus has a CPS of 20–25 (positive). PD-L1, program death-ligand 1; CPS, combined positive score. Images (A and B) acquired at 40× magnification, while images (C and D) are acquired at 100× magnification.

for advanced esophageal, gastric and GEJ adenocarcinoma in 2017. This approval was based on the results from multi-cohort open label nonrandomized trial (KEYNOTE-059), which enrolled 259 patients who have previously received 2 prior lines of therapy (61). Patients whose tumors were noted to be PD-L1 positive (CPS ≥1) were found to have a response rate of 15.5%, while only 6.4% of patients with PD-L1 negative tumors responded. It was encouraging to see prolonged response duration, ranging between 5.3 to 14.1 months, in this advanced setting. Activity of pembrolizumab was also evaluated in earlier lines of therapy. A phase 3 trial (KEYNOTE-061) for patients with gastric cancer and phase 3 trial (KEYNOTE-181) for patients with esophageal and Siewert type 1 gastroesophageal junction tumors evaluated activity of pembrolizumab in comparison to chemotherapy in the 2nd line setting. These trials did not improve patient OS with immunotherapy treatment. However, responses from pembrolizumab were more durable than those seen with chemotherapy, and responses were more frequent when tumors had higher CPS scores (62-64). In the 1st line setting, KEYNOTE-062, a randomized phase 3 trial examining the role of pembrolizumab alone or in combination with chemotherapy compared to chemotherapy alone failed to meet its primary endpoints (65). Therefore, at the present time, pembrolizumab remains approved for use in PD-L1 positive tumors with CPS ≥1 after progression on at least 2 prior lines of therapy, as well as for dMMR/MSI-H tumors regardless of PD-L1 expression.

Future biomarkers

A number of novel targets in esophageal and gastric cancer are being studied in clinical trials. As was discussed previously, the status of HER2 is frequently tested using FFPE material from the patient's esophageal or gastric tumor. Due to treatment modalities or upon disease

progression, it is known that the HER2 status may change. Evaluation of HER2 status by next generation sequencing repeatedly during the course of disease is essential for evaluation of secondary resistance to trastuzumab. Moreover, information about the level of *HER2* amplification, subclones lacking *HER2* amplification, deletion of *ERBB2* exon 16, and co-mutations in other signaling partners (for example RAS, MET and PI3K) may predict resistance to treatment and may uncover other clinically relevant targets (66).

Gene fusions that involve neurotrophic-tropomyosin receptor kinase (*NTRK*) genes are known drivers of oncogenesis. These fusions are rare and were observed in only 0.31% of adult tumors (67). In November 2018 and August 2019, accelerated approval was granted by the FDA to larotrectinib and entrectinib, respectively for patients age 12 years and older for *NTRK* gene fusion solid tumors that do not have a known resistance mutation. *NTRK* gene fusions are exceedingly rare in gastric and esophageal carcinoma and their response to this targeted therapy needs to be further explored.

Recent studies demonstrate a fusion between *CLDN18* and *ARHGAP26* genes in ~30% of young patients with gastric cancer. The *CLDN18* gene codes for claudin-18 that is responsible for tight junctions in epithelial cells. Rho GTPase activating protein 26 (*ARHGAP26)* gene codes for a multidomain protein that is necessary for endocytosis. The frequency of this fusion is much higher than most other reported driver mutations and druggable/targetable genes from other types of cancers, supporting its role in tumorigenesis (68-70). Nearly all CLDN18–ARHGAP26/6 fusion positive gastric carcinomas have been shown to express CLDN18.2 protein (71). IMAB362 (zolbetuximab) is a novel chimeric IgG1 antibody highly specific for CLDN18.2 and recent preclinical studies and Phase II clinical trials are showing promising results (72,73).

With recent research examining the molecular classification of gastric cancer into several subtypes, the role of fibroblast growth factor receptor (FGFR) has come to the forefront with up to 9% of gastric cancer patients being identified with FGFR2 amplifications (68,74). Due to the increased toxicity and inability to accurately predict response, the first generation tyrosine kinase inhibitors have fallen out of favor. With the release of second generation, selective pan-FGFR inhibitors, there is a renewed interest in this area and several Phase 1 trials are enrolling FGFR-aberrant tumors.

Additional biomarkers are being explored in an attempt to better define the patient population most likely to respond to immunotherapy treatments. Some of the markers currently under investigation include lymphocyte-activation gene 3 (LAG3), T-cell immunoglobulin and mucin-domain containing-3 (TIM3), and tumor mutational burden.

Conclusions

Although there have been several biomarkers identified that can aid in the selection of treatment modalities, treatment outcomes in advanced disease in esophageal and gastric adenocarcinoma remains poor. Identification of new biomarkers, moving existing biomarkers into earlier lines of therapy, and evaluating new combinations of existing biomarkers are and should be the focus of future research efforts. It is imperative to continue to identify more effective biomarkers to predict the effectiveness of therapy before treatment. It is with great hope that we look to upcoming clinical trials to shed more light on novel biomarkers and targeted therapies.

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Footnote

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

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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