

Update On Gastric Cancer: Molecular Pathology and Targeted Therapies

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Gastric carcinoma is the fourth most frequent cancer worldwide, representing the second most common cause of death from cancer (approximately 700,000/year)¹. In the United States 21,259 new cases of stomach cancer were estimated in 2007, remaining stable at 21,130 new cases in 2009². The incidence of gastric cancers (GC) involving the distal stomach, body and fundus, which have been associated with Helicobacter infection have declined over past decades, while adenocarcinomas of the cardia and gastroesophageal GE-junction (GEJ) is increasing. Combined figures for GE junction and gastric cancer indicate 1.4 million new cases diagnosed annually with 1.1 million attributed deaths³.

Clinical trials of targeted therapies for advanced gastric cancer have generally included gastric and gastroesophageal junction adenocarcinomas. Additionally, some trials used the classification of GE junction adenocarcinomas as described by Siewert, classifying GE junction carcinomas into types I, II and III depending on the relative extent of involvement of the esophagus and stomach⁴.

Histopathologically and genetically, gastric and gastro-esophageal junction cancers are heterogeneous and are influenced by gene-environment interactions resulting in activation of multiple molecular pathways. The molecular subtypes of gastric cancer include three main groups of tumors characterized by either the chromosomal instability pathway (CIN), the microsatellite instability pathway (MSI), and the CpG island methylator phenotype pathway (reviewed in⁵). Currently, it is not clear whether and how these subtypes of gastric and GEJ carcinomas can be useful in clinical practice to predict specific pathways with mutational and regulatory alterations that may interfere with targeted therapies.

Surgical resection is the only potentially curative option for gastric cancer and is recommended for stages Tis-T3N0-N2M0 or T4N0M0⁶. For tumors not amenable to surgical curative resection, including locally advanced, recurrent, or metastatic cancers, a number of chemotherapy regimens can be used, albeit with limited success, such that 5 year survival rates for advanced gastric and GE junction cancers remain extremely poor at 20-50% for stages II-III and 5-10% for stage IV tumors. Recently, a number of agents that target specific molecules in cancer related pathways have become available and are being tested in patients with gastric and GE junction carcinomas. Here we will review the targeted therapies that have advanced to phase II or III clinical trials and offer promise in the treatment of these cancers. In addition, the specific roles of pathology and molecular testing as it relates to specific targeted therapies will be discussed.

Cell surface receptor inhibitors

EGFR Family Inhibitors

Current available therapies target the EGFR pathways through inhibition of the EGFR using two different mechanisms: 1) inhibition of the EGFR via monoclonal antibodies (i.e. cetuximab, matuzumab, panitumumab, trastuzumab) or 2) tyrosine kinase inhibitors (i.e. gefitinib, erlotinib).

The EGFR family of transmembrane receptor tyrosine kinases is composed of four members: HER1 (also known as the EGFR and erbB1), HER2 (p185, HER2/neu, ErbB-2), HER3 (also termed ErbB-3), and HER4 (also termed ErbB-4). The molecular structures of EGFRs include an extracellular ligand-binding domain, a short transmembrane domain, and an intracellular domain with tyrosine kinase (TK) activity (except HER3). The binding of different ligands, including epidermal growth factor (EGF) and TGF-alpha to the extracellular domain initiates a signal transduction cascade that contributes to neoplastic behavior including cell proliferation, apoptosis, adhesion, migration, and differentiation. Ligand binding induces EGFR homodimerization as well as heterodimerization with other types of HER proteins. HER2 does not bind to any known ligand, but it is the preferred heterodimerization partner for other members of the HER family. Ligand binding to the EGFR extracellular domain leads to EGFR activation followed by dimerization, resulting in phosphorylation of the intracellular tyrosine kinase which results in a series of intracellular signals including the activation of Ras/Raf/mitogen activated kinase (MAPK) or the AKT/mTOR pathways (reviewed in ⁷).

Table 1: Cell surface receptor inhibitors: Ongoing phase II and III trials for gastric and GEJ cancers (adapted from ⁷)

Inhibitor-type	Drug	Clinical trials phase
EGFR Antibody	Cetuximab	III
	Panitimumab	III
	Matuzumab	I-II
EGFR tyrosine kinase inhibitors	Gefitinib	II
	Erlotinib	II
HER2-R Antibody	Trastuzumab	III
EGF/HER2-R	Lapatinib	II
	BIBW 2992	II
VEGF-R antibody	Bevacizumab	III
VEGF-R tyrosine kinase inhibitors	Vatalanib	II

EGFR (HER1; erbB1) targeted therapy

Cetuximab is a recombinant chimeric IgG1 monoclonal antibody that binds specifically to the extracellular domain of EGFR and competitively inhibits the binding of EGF and other ligands such as TGF-alpha. Cetuximab also mediates antibody-dependent cell cycle toxicity. Cetuximab is currently approved for patients with advanced colorectal and head and neck cancer ⁸. Currently there are several ongoing phase III trials evaluating cetuximab in combination with other chemotherapy agents for patients with advanced gastric and GE junction cancers.

Whether the molecular testing considerations regarding mutations in the Kras pathway will be used for treatment of gastric and GE junction cancers with cetuximab, as they are for colorectal cancer is not established ^{9,10}. A recent study ¹¹ presented at the 2009 ASCO annual meeting assessed whether the mutational profile of KRAS and BRAF genes affected the response to cetuximab combination therapy in GC. In this study, the frequency of mutations in KRAS and

BRAF was lower as compared to colorectal cancer, and the mutational status of KRAS and BRAF genes did not correlate with the response to cetuximab-based therapy in advanced gastric cancer patients. Forty four tumor samples were collected from patients with locally advanced or metastatic GC undergoing cetuximab combination therapy as first-line treatment in two consecutive phase II studies, FOLCETUX Study and DOCETUX Study. The mutational status of KRAS (exon 2) and BRAF (exon 15) was detected by PCR amplification followed by direct sequencing. KRAS and BRAF mutations were detected in 5 (11.4%) and 1 (2.3%), respectively, of the 44 tumors analyzed. These frequencies are consistent with those previously reported in GC. The only BRAF mutation found in 1 sample was the classic V600E substitution. As a whole, 13.6 % of the analyzed tumors carried a mutation in either KRAS or BRAF genes. KRAS and BRAF mutations were, as expected, mutually exclusive. As this study examined a small cohort, additional studies are warranted before clinical guidelines can be established.

Panitumumab is the first fully human monoclonal antibody (IgG2) specific to EGFR. It has been used successfully in patients with advanced colorectal cancer who failed standard therapies¹². A phase III trial (REAL III) is due to start investigating the role of panitumumab in combination therapy for locally advanced or metastatic gastric or GEJ cancer.

The EGFR tyrosine kinase inhibitors have proved minimal evidence of efficacy in gastric carcinomas, but studies are ongoing⁷.

HER2 (HER2/neu, ErbB-2) targeted therapies

HER2 has no known ligand (orphan receptor), and preferentially heterodimerizes with HER3 which lacks intrinsic tyrosine kinase activity. The HER2, and the HER2/HER3 heterodimer is likely to be the most effective complex for activating pathways downstream of EGF receptors^{13, 14}.

Overexpression and amplification of HER2 has been described in 6-35% of gastric and GEJ adenocarcinomas^{15;16} (Table 2).

Trastuzumab (Herceptin) is a monoclonal antibody which specifically targets HER2 protein by directly binding the extracellular domain of the receptor. Trastuzumab enhances survival rates in both primary and metastatic HER2-positive breast cancer patients. The efficacy of trastuzumab in breast cancer patients has led to investigate its antitumor activity in patients with HER2-positive cancers, including gastric adenocarcinomas.

HER2/neu positivity rates have been reported to be more frequent in intestinal type gastric cancer (21.5%) than in diffuse gastric cancer (2%) or mixed types (5%)¹⁵; these findings were confirmed in the ToGA trial with HER2 positivity being more frequent in intestinal than diffuse/mixed cancer (32.2% vs 6.1%/20.4%), respectively¹⁷. In addition, HER-2/neu amplification in gastric carcinoma is associated with poor outcome¹⁵ and has been shown to be an independent prognostic factor¹⁸.

Results from the largest study to date (ToGA trial) evaluating the addition of trastuzumab (Herceptin) to chemotherapy in HER2-positive advanced gastric cancer were reported at the 2009 ASCO meeting^{17, 19}. The ToGA trial is the first randomized Phase III trial providing prospective information on HER2-positivity rates in GC (Table 2). The trial enrolled 3,883 patients from 24 countries. A HER2-scoring system modified from the protocol in breast cancer was used: a score of IHC 3+ and/or FISH positive was defined as HER2 positive. The modified HER2-scoring system showed concordance between IHC and FISH results of 87.5%. In breast cancer most IHC 0/1 samples are FISH negative but, in the ToGA cohort, the frequency of IHC

0/1 samples testing FISH positive was almost as high as IHC 2/FISH-positive samples (23% vs. 26%). The study reported an overall HER2-positivity rate of 22.1% evaluated from 3807 patients¹⁷.

In the ToGA trial, patients with HER2-positive gastroesophageal and gastric adenocarcinoma (locally advanced, recurrent, or metastatic) were randomized to receive Trastuzumab (H; Herceptin) H+CT (5-fluorouracil or capecitabine and cisplatin) q3w for 6 cycles or CT alone. The primary end point was overall survival (OS); secondary end points included overall response rate (ORR), progression-free survival, time to progression, duration of response, and safety. Median OS was significantly improved with H+CT compared to CT alone: 13.5 vs. 11.1 months, respectively (p=0.0048; HR 0.74; 95% CI 0.60, 0.91). ORR was 47.3% in the H+CT arm and 34.5% in the CT arm (p=0.0017). This first randomized trial investigating anti-HER2 therapy in advanced GC showed that H+CT is superior to CT alone. The OS benefit indicates that H is a new, effective, and well-tolerated treatment for HER2-positive GC¹⁹.

Authors	Number	Region	Over-expression (%)	IHC method	Amplification (%)	Method
Yano et al. ²⁰	200	Japan	23	HercepTest	27	FISH
Gravalos et al. ¹⁶	166	Europe	13	HercepTest	if IHC 2+	FISH
Allgayer et al.	203	Europe	91	Elite kit	-	-
Park et al. ¹⁸	Gast: 182	Korea	16	HercepTest	7 patients	FISH/ CISH
Lordick et al.	1527	Europe; Asia; Latin America	22*	HercepTest	-	FISH
Tanner et al. ¹⁵	Gast: 131 GEJ:100	Europe	-	-	12.2 24	CISH
Bang et al. ¹⁷	Gast; GEJ	14 countries	Gast: 20.9 GEJ: 32.2	HercepTest		FISH**

Table 2. HER2 expression and amplification in gastric and GEJ cancers (modified from Gravalos et al.¹⁶) * HER2 overexpression by IHC or FISH** (PharmDx). IHC (immunohistochemistry); CISH (chromogenic in situ hybridization).

Antiangiogenic agents

The current status of anti-angiogenic agents for gastric and GE junction tumors has been recently reviewed⁷. Tumor associated angiogenesis requires a number of pro-angiogenic factors, among which vascular endothelial growth factors (VEGF family A-D) play a key role in vasculogenesis and angiogenesis. A number of anti-angiogenic agents has been investigated or are undergoing clinical trials.

Bevacizumab is a chimeric monoclonal antibody that binds the VEGF-receptor and prevents the interaction of VEGF to its receptors (Flt1 and KDR) on the surface of endothelial cells. Clinical data of bevacizumab therapy in patients with advanced gastric or GEJ cancer is promising but is limited to phase II trials.

Another approach to inhibit the VEGF pathway uses tyrosine kinase inhibitors directed against the receptors of VEGF (Flt1 and KDR). There are several compounds available, some specifically targeting VEGF receptors, such as PTK787/ZK222584 (Vatalanib) and others that inhibit both the VEGF receptors and other tyrosine kinase receptors such as sunitinib and sorafenib. Phase II results are available for sorafenib, in which 44 patients with advanced gastric or GE junction cancers were treated with a combination of docetaxel, cisplatin and sorafenib, with an objective response rate of 38.6%. The median PFS was 5.8 months and the median OS was 14.9 months.

Other targeted therapies for gastric and GEJ cancers

A large number of specific inhibitors of molecular targets in gastric and GE junction cancer pathways are in the early stages of clinical trials, while supporting data for others is limited to pre-clinical studies. Among these agents are the insulin-like growth factor-I (IGF-IR) receptor inhibitors, fibroblast growth factor (FGF) receptor inhibitors and c-Met signaling pathway inhibitors. A number of cell cycle associated drug targets including Aurora kinase inhibitors, polo-like kinase inhibitors and cyclin dependent kinase (cdk) inhibitors are being tested.

Another approach has involved the use of inhibitors of cancer associated epigenetic changes. Histone deacetylase (HDAC) inhibitors are under considerable research given the potential to re-express tumor suppressor genes silenced by hypermethylation in cancer cells. A phase II clinical trial is ongoing.

Other agents under current trials include heat shock protein 90 (HS90) inhibitors, ubiquitin-proteasome pathway inhibitors, PI3K/Akt/mTOR pathway inhibitors and matrix metalloproteinase (MMP) inhibitors.

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