



## HER2 aberrations and heterogeneity in cancers of the digestive system: Implications for pathologists and gastroenterologists

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### Abstract

Management of cancers of the digestive system has progressed rapidly into the molecular era. Despite the significant recent achievements in the diagnosis

and treatment of these patients, the number of deaths for these tumors has currently plateaued. Many investigations have assessed the role of *HER2* in tumors of the digestive system in both prognostic and therapeutic settings, with heterogeneous results. Novel testing and treatment guidelines are emerging, in particular in gastric and colorectal cancers. However, further advances are needed. In this review we provide a comprehensive overview of the current state-of-knowledge of *HER2* alterations in the most common tumors of the digestive system and discuss the operational implications of *HER2* testing.

**Key words:** *HER2*; Digestive system; Gastrointestinal tract; Gastric cancer; Colon cancer; Esophageal cancer; Gastroesophageal junction cancer; Biliary tract cancer; Gallbladder cancer; Liver cancer; Pancreas cancer

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**Core tip:** Numerous studies have broadened our understanding of *HER2* as a critical oncogene in many human cancers, including tumors of the digestive system. Due to the increasing importance of *HER2* testing in this heterogeneous group of tumors, in this review we seek to outline the current state of knowledge of *HER2* alterations in the most common malignancies occurring in the digestive system, to examine the operational implications of *HER2* testing as a biomarker and potentially targetable gene, and discuss immediate future perspectives for pathologists and gastroenterologists.

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## INTRODUCTION

The epidermal growth factor receptor 2 is a proto-oncogene that was first identified in the early 1980s in rodent neural tumor cell lines and therefore named *neu*<sup>[1]</sup>. Given the homology between the human gene and that of the rodent, adherence to appropriate nomenclature is pivotal to avoid any confusion. In this review, *HER2/neu* will refer to the gene across both species, while *HER2* and *erbB2* will be used specifically to indicate the human gene and its protein product, respectively<sup>[2]</sup>. *HER2/neu* belongs to one of the most studied growth factor receptor systems in cancer, the *erbB* tyrosine kinase family<sup>[1-4]</sup>. This family consists of four members encoding the homologous epidermal growth factor receptor proteins *erbB1*, 2, 3, 4 that are ubiquitously expressed in epithelial, mesenchymal, and neuronal normal cells and their cellular progenitors<sup>[5,6]</sup>. Each of these receptors is composed of an extracellular ligand-binding domain, a transmembrane segment and an intracellular protein kinase domain with a carboxyl terminal segment holding site of phosphorylation or tyrosine residues (Figure 1)<sup>[5,7,8]</sup>. Among the four *erbB* proteins, *erbB2* is functionally characterized by an extraordinarily strong catalytic kinase activity, representing a key oncoprotein that triggers cornerstone intracellular signaling events for cell growth and survival, ultimately leading to increased signal transduction and activation of the MAPK and PI3K/Akt pathways<sup>[4,6,9]</sup>. Importantly, *erbB2* is not involved in ligand binding of the growth factors unless is overexpressed<sup>[10]</sup>, while the other members of its family represent active receptors in basal conditions also<sup>[5,9]</sup>, as outlined in Figure 1.

Numerous preclinical and clinical studies, beginning with the intuition of Slamon and collaborators on the role of *HER2* in breast cancer, have broadened our understanding of this oncogene in many human cancers, including digestive system cancers (DSC)<sup>[8,11]</sup>. While *HER2* represents a prognostic marker of aggressive behavior in many DSC<sup>[8,12,13]</sup>, the importance of this oncogene remains closely related to its role as a potentially targetable cancer gene<sup>[4,14]</sup>. To date, anti-*HER2* antibodies such as trastuzumab, pertuzumab, the new conjugate ado-trastuzumab emtansine, and *HER2*-inhibitors (*e.g.*, lapatinib) have received the United States Food and Drug Administration (FDA) approval not only in *HER2*-positive breast cancers but also in *HER2*-positive metastatic gastric cancer (GC)<sup>[15]</sup>. Massively parallel sequencing studies have recently revealed that a substantial proportion of DSC are genetically characterized by *HER2* alterations (Figure 2)<sup>[16]</sup>. However, highly different percentages in the incidence of these molecular aberrations, ranging from 0 to 50%, have been reported even within the

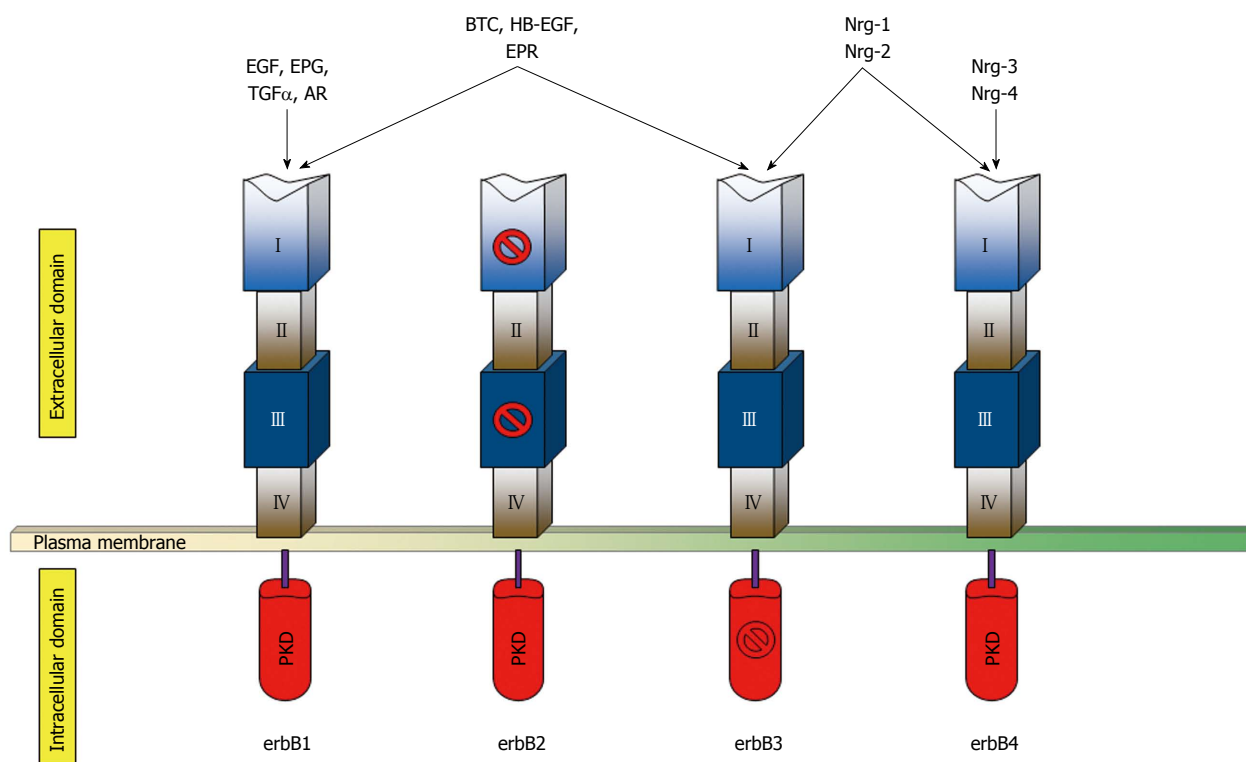
same anatomic site, such as the pancreas (Table 1)<sup>[16-19]</sup>. These partially discordant observations could have been, at least in part, responsible for the nihilistic view of *HER2* in the targeted therapeutic regimens for extra-gastric DSC. Many groups are currently establishing the role of *HER2* in DSC in both prognostic and therapeutic settings. However, targeting of tumors that overexpress *erbB2*, albeit representing the reality for advanced GC and gastroesophageal junction (GEJ) cancer, is considered a reasonable future option<sup>[8,20]</sup>. At present, the role of trastuzumab in DSC is being explored by a variety of translational research molecular pathology studies, as well as clinical trials.

Management of DSC has progressed rapidly into the molecular era<sup>[21-30]</sup>. However, the "trastuzumab-revolution" that we have experienced in the breast has yet to be realized in the digestive system tract and its accessory organs<sup>[8,31]</sup>. Due to the increasing importance of *HER2* testing in cancer and the new exciting challenges that precision medicine is providing, in this review we seek to describe the current state of knowledge of *HER2* alterations in the most common DSC, to discuss the operational issues of *HER2* testing, and to outline forthcoming clinical perspectives, in particular focusing on the cutting-edge tools available for *HER2* characterization and targeting in the digestive system.

## HER2 TESTING IN THE DIGESTIVE SYSTEM

### Esophageal cancer

Esophageal cancer (EC), excluding GEJ tumors, is among the ten most prevalent tumors worldwide and ranks fifth in cancer mortality in men and eighth in women<sup>[32]</sup>. Squamous cell carcinoma (SCC) represents the most frequent histological type<sup>[20]</sup>. The poor prognosis of EC results from the delayed diagnosis and poor efficacy of current treatments, being in most cases limited to a palliative role<sup>[23]</sup>. In the largest meta-analysis of the prognostic significance of *erbB2* overexpression and gene amplification in EC patients, 22% of tumors were *HER2*-positive, regardless of histotype<sup>[33]</sup>. However, these data are likely to be overestimated. Indeed, the overexpression of *erbB2* has been observed in 12%-17% of adenocarcinomas (ADC) in more recent studies<sup>[34]</sup>, whereas less than 4% of esophageal SCCs are *HER2*-amplified<sup>[35]</sup>. Taken together, no significant differences in survival rates have been reported in patients diagnosed with *HER2*-positive esophageal ADC compared with the *HER2*-negative cases. However, the great heterogeneity among indexed studies on *HER2* prognostic role in these malignancies demands further investigations. Interestingly, the prognostic influence of *HER2* amplification as a biomarker is slightly greater in SCC compared to ADC<sup>[33,35]</sup>. On the other hand, the small number of *HER2*-positive SCCs, the lack of large-



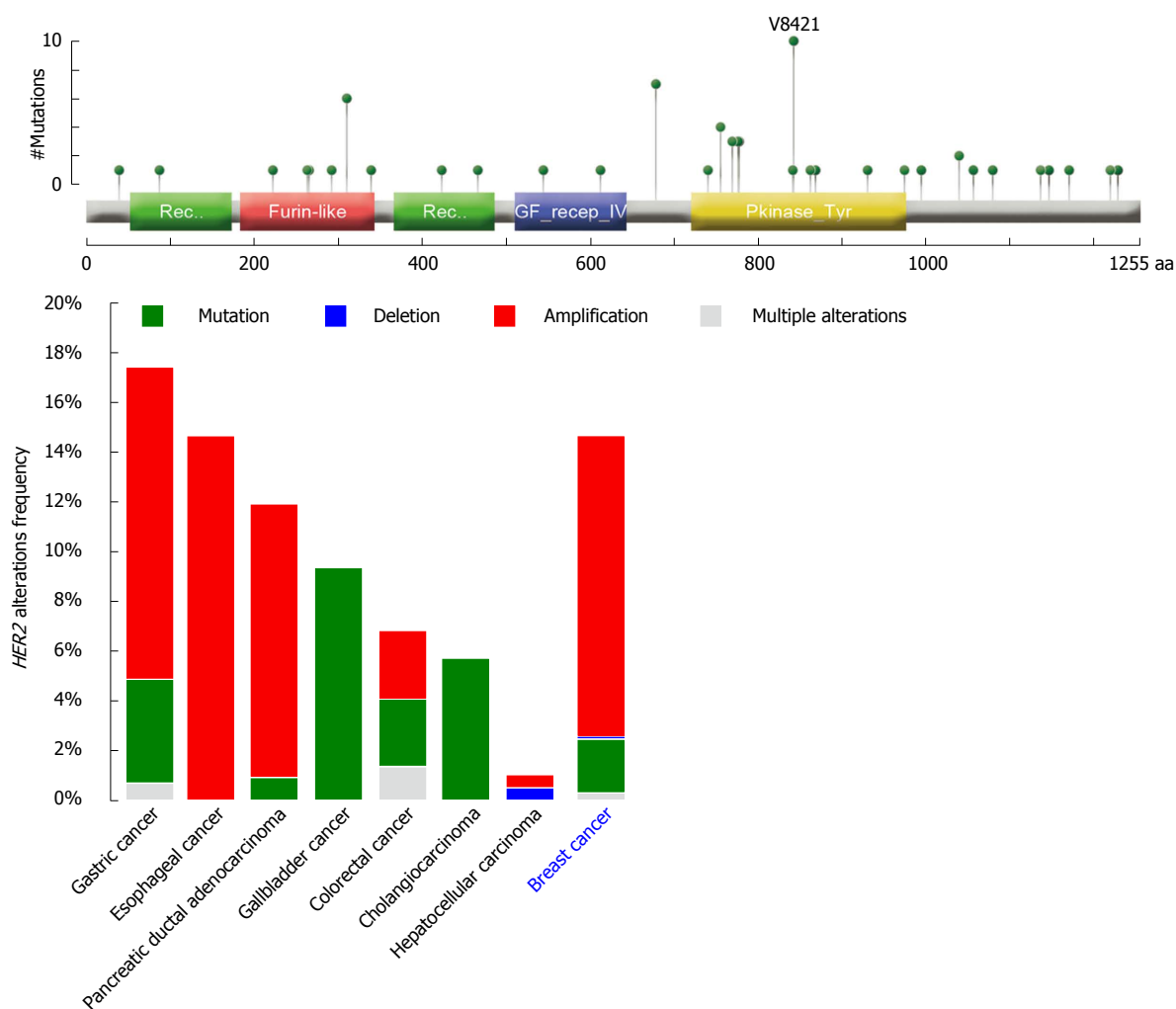
**Figure 1 Schematic representation of the human erbB receptors in basal condition.** The extracellular portion of each receptor consists of four domains (I - IV). Both domains I and III, which are related leucine-rich segments, actively participate in ligand binding, except for those of erbB2. Domains II and IV contain numerous cysteine residues and participates in dimer formation. The kinase domain of erbB3 is kinase-impaired. The growth factor groups that bind each receptor are indicated on the top. PKD: Protein kinase domain; EGF: Epidermal growth factor; EPG: Epigen; TGF $\alpha$ : Transforming growth factor- $\alpha$ ; AR: Amphiregulin; BTC: Betacellulin; HB-EGF: Heparin-binding epidermal growth-factor like growth factor; EPR: Epiregulin; Nrg-1/2/3/4: Neuregulin-1/2/3/4.

cohort studies, and the absence of standardized methods for *HER2* testing limit our knowledge of *HER2* significance in SCC of the esophagus<sup>[36]</sup>. At present, the optimal treatment for EC remains controversial. In this regard, neoadjuvant chemotherapy with subsequent surgery represent the standard approach in the United Kingdom<sup>[37,38]</sup>, whereas in Europe and United States neoadjuvant chemo-radiotherapy followed by surgery is preferred<sup>[39]</sup>. However, the individualization and optimization of therapy for EC might come across *HER2* and its epistatic interactions with other potentially actionable cancer genes. Indeed, it has recently been reported that possible alterations in epidermal growth factor receptor (*EGFR*), telomerase reverse transcriptase (*TERT*), and *HER2* are *bona fide* predictor of response to *HER2*-target therapy in EC, particularly in SCCs<sup>[35,40]</sup>. At present, RTOG 1010 (Radiation Therapy, Paclitaxel, and Carboplatin With or Without Trastuzumab in Treating Patients With EC) is the only ongoing phase III trial (<https://clinicaltrials.gov/ct2/show/study/NCT01196390>) randomizing patients with *HER2*-positive esophageal ADC to chemoradiation with or without trastuzumab.

### Gastric and gastroesophageal junction cancer

GC, including GEJ cancer, is closely related to environmental factors, reflecting its characteristic geographical distribution<sup>[32]</sup>. Although GC rates have gradually

decreased during the past decades, this tumor still represents the third leading cause of cancer-related death globally<sup>[32]</sup>. The vast majority of GCs can be divided into three distinct subtypes based on Lauren's histopathologic classification: intestinal-type, showing glandular architecture, diffuse-type, with poorly cohesive cells arranged in an infiltrative pattern, and mixed-type, bearing hybrid characteristics<sup>[20]</sup>. This morphologic heterogeneity replicates an intrinsic molecular complexity. Recently, The Cancer Genome Atlas (TCGA) network proposed a novel molecular classification of GC, dividing these tumors into four major molecular subtypes, namely tumors positive for Epstein-Barr, microsatellite unstable tumors, genomically stable tumors, and tumors with chromosomal instability<sup>[41]</sup>. Among these molecular subgroups, microsatellite unstable tumors preferentially occur in the body and antrum, and are characterized by an extraordinarily high number of mutations with the lack of targetable amplifications, including *HER2* amplification. Chromosomal instability subtype encompasses the majority of GC, has a predilection for the GEJ, is associated with intestinal-type histology, and exhibit the highest rates of *HER2* amplification among all molecular subtypes<sup>[41]</sup>. Overall, GCs overexpressing erbB2 and/or showing *HER2* amplification, range from 13% to 22% of cases<sup>[12,42]</sup>. Meta-analysis data suggest that GC harboring *HER2* amplification fares worse<sup>[43]</sup>;



**Figure 2** *HER2* alterations frequencies from public datasets accessible from cBioPortal<sup>[16]</sup> in the most common tumors occurring in the digestive system compared to breast cancer. Overall, *HER2* amplifications occur more frequently in gastric, esophageal, and pancreatic cancers, whereas gallbladder cancer and cholangiocarcinoma characteristically show *HER2* mutations (10% and 6% of cases, respectively). The domain structure of the erbB2 protein and gene alterations identified in primary carcinomas of the digestive system available from cBioPortal<sup>[16]</sup> (reported the top) show the presence of hotspot somatic mutations in the furin-like (binding site) and protein kinase domains. Mutation types are color-coded on the basis of the legend on the bottom right.

**Table 1** *erbB2* overexpression and gene amplification reported frequencies in tumors of the digestive system

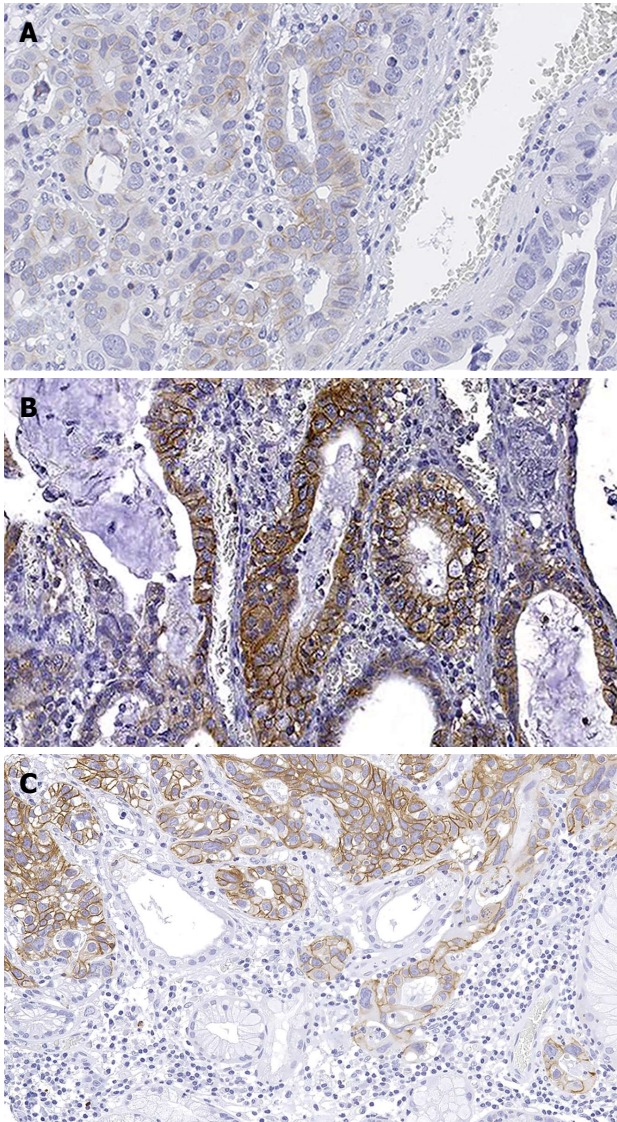
Primary tumor	<i>erbB2</i> overexpression frequencies	<i>HER2</i> amplification frequencies	Ref.
Esophageal cancer	4%-22%	4%-14%	[33-36,38,39]
Gastric cancer	13%-22%	10%-18%	[12,41-47]
Colorectal cancer	2%-11%	3%	[48-57]
Biliary tract cancer	5%-76%	1%-8%	[27,75,86,88-90]
Pancreatic cancer	0%-50%	2%-29%	[17-19,64-66,68]
Liver cancer	2%-5%	0-1%	[72-79]

however, the prognostic value of *HER2* remains controversial in the stomach<sup>[44-46]</sup>. This is probably due to the heterogeneous *HER2* status patterns in tumors arising in the stomach that basically mirror the intra-tumor morphologic and molecular heterogeneity of GC (Figure 3)<sup>[12]</sup>. Indeed, in contrast to breast carcinoma, up to 90% of *erbB2*-positive GCs are reported to harbor *erbB2* overexpression in less than 5% of tumor

cells<sup>[12,46]</sup>. From a therapeutic perspective, it is currently recommended to administer trastuzumab in combination with cytotoxic therapies in *HER2*-positive GC patients<sup>[42]</sup>. In this setting, the addition of trastuzumab to chemotherapy increased the objective response rate from 35% to 47%, improving progression-free survival from 5.5 mo to 6.7 mo and overall survival from 11.1 mo to 13.8 mo<sup>[5,42,47]</sup>. Clinical trials aiming to examine the efficacy of lapatinib in combination with paclitaxel compared with paclitaxel alone in the treatment of *HER2*-positive GC are on-going (<https://clinicaltrials.gov/ct2/show/study/NCT01705340>). Other clinical trials are currently exploring the effect of adding pertuzumab to chemotherapy (<https://clinicaltrials.gov/ct2/show/study/NCT01461057>) and comparing trastuzumab to paclitaxel or docetaxel as second-line treatment (<https://clinicaltrials.gov/ct2/show/study/NCT01641939>).

### Colorectal cancer

Colorectal cancer (CRC) is a major contributor to



**Figure 3** Representative micrographs of a gastric adenocarcinoma showing heterogeneous erbB2 immunohistochemical expression. In this paradigmatic example of *HER2* heterogeneity in gastric cancer, the tumor showed the coexistence of 2+ (A), 3+ (B, C), and negative areas (C), with the former immunohistochemical pattern involving the majority of the tumor cells. Original magnification  $\times 200$ .

cancer morbidity and mortality, with 1.36 million new cases and more than half million deaths per year worldwide<sup>[32]</sup>. Several studies assessed *HER2* status in CRC, with some authors reporting membranous erbB2 overexpression in 2%-11% and others reporting cytoplasmic overexpression in 47%-68% of cases<sup>[48-52]</sup>. However, it is currently acknowledged that the amplification of *HER2* occurs in only 3% of CRC<sup>[53]</sup>. In accordance to this notion, a recent comprehensive genomic characterization of CRC revealed a recurrent amplicon at 17q21.1 in 4% of these tumors<sup>[54]</sup>. This locus contains seven genes, including *HER2*<sup>[55]</sup>. The operational implications of *HER2* amplification in CRC, however, remain elusive, with a number of studies reporting contradictory links to prognosis<sup>[53,56,57]</sup>. How to

assess *HER2* status in CRC cancer remains a matter of debate among pathologists; however, a panel of *HER2* experts recently provided a reproducible and rigorous testing algorithm<sup>[57]</sup>. Intriguingly, no intra-tumor heterogeneity is described in CRC except for anecdotic reports<sup>[57]</sup>. Albeit *HER2* seems not to represent a reliable prognostic marker in CRC, this molecular alteration is strongly associated with wild-type status of Kirsten rat sarcoma viral oncogene homolog (*KRAS*) and amplification DNA topoisomerase 2- $\alpha$  (*TOP2A*)<sup>[16,58,59]</sup>. This observation is not trivial, raising the hypothesis that *HER2* amplification might be a *bona fide* alternative driver of Ras-Raf-MEK-ERK pathway activation in CRC. In contrast to *HER2*, intratumor heterogeneity of *KRAS* mutation status is reported and crucial in selecting patients for anti-EGFR therapy in CRC<sup>[12]</sup>. For these reasons, and given the homogeneity in erbB2 expression, it has recently been proposed that *HER2* status should be assessed as a putative biomarker of resistance to anti-EGFR therapy in *KRAS* wild-type patients and, if further studies confirm that *TOP2A* amplifications are associated with anthracycline sensitivity, as a predictor to response<sup>[57,59,60]</sup>. At present, results from phase II and III trials suggest that *HER2*-positive CRC should be treated with trastuzumab<sup>[61]</sup>.

#### **Pancreatic ductal adenocarcinoma**

Pancreatic cancer (PC) accounts for approximately 2% of new cancers, and is responsible for 7% of cancer-related death yearly worldwide<sup>[32]</sup>. The vast majority of PCs is represented by invasive ductal ADC arising in the head of the gland; 20% of cases involve the body or the tail<sup>[20]</sup>. The poor prognosis for these patients is attributed to delayed diagnosis, early metastasis, and the limited efficacy of available systemic treatments<sup>[62]</sup>. Systemic therapies are only modestly effective; however, there is emerging evidence that small groups of patients may respond well to specific treatments. Amplification of *HER2* gene and/or overexpression of its product have been implicated in the development of PC<sup>[63]</sup>. However, the reported rates of erbB2 overexpression in these neoplasms are extremely variable, ranging from 0 to 50% of cases<sup>[17-19,64]</sup>. Furthermore, the prognostic role of *HER2* amplification in PC has been investigated in numerous studies, again, with heterogeneous results<sup>[63]</sup>. As a consequence, the diagnostic criteria and prevalence of *HER2* amplification in pancreatic ductal ADC remain unclear. Preclinical studies support the potential efficacy of trastuzumab in PC<sup>[65,66]</sup>, although clinical trials have been disadvantaged by small cohorts<sup>[67,68]</sup>. In one phase II trial, 17 patients showing *HER2* amplification were treated with capecitabine combined with trastuzumab<sup>[68]</sup>. Although the therapy was well tolerated, progression-free and overall survivals were not favorable compared to standard chemotherapy. At present, there is no consensus on the treatment modalities of *HER2*-positive pancreatic ductal ADC.

### Hepatocellular carcinoma

Hepatocellular carcinoma (HCC), is the fifth most common cancer in men and the seventh most common cancer in women, resulting in approximately 700000 deaths yearly<sup>[32]</sup>. In recent years, these tumors showed increasing incidence, albeit variable throughout the world<sup>[32,69]</sup>. The asymptomatic nature of early disease and the limited use of screening protocols in high-risk individuals often lead to diagnosis in advanced stages, with subsequent requirement of systemic therapy<sup>[70,71]</sup>. To date, sorafenib (a kinase inhibitor) is the only approved drug in patients with advanced HCC, with modest effectiveness at prolonging patients' overall survival<sup>[72,73]</sup>. Given the scarce therapeutic armamentarium currently available, capturing the complexity underpinning HCC biology represents a high-priority goal<sup>[70]</sup>. In this setting, the role of *HER2* in HCC has been explored in several studies yielding, however, to extremely discrepant results due to the diverse methods used for *HER2* testing, even including cytoplasmic expression<sup>[74-76]</sup>. It is currently recognized that there is a low frequency of erbB2 strong membranous IHC overexpression in HCC<sup>[77-79]</sup>. Among these few cases, only a minority of tumors is reported to harbor *HER2* amplification<sup>[80,81]</sup>. Therefore, it is unlikely that patients with HCC would benefit from treatment with trastuzumab. In addition, recent studies suggest that there is also little indication for using *HER2* as a prognostic biomarker in these tumors<sup>[79]</sup>.

### Biliary tract cancer

Biliary tract cancer (BTC) encompasses a heterogeneous group of rare tumors originating in either the intra- or extrahepatic ducts<sup>[20]</sup>. This collection of neoplasms includes cholangiocarcinoma, gallbladder cancer (GBC), and ampulla of Vater cancer<sup>[20]</sup>. Among them, GBC is the most frequent type with an annual incidence of 2.5 cases per 100000 individuals<sup>[32]</sup>. Taken together, BTC mortality varies between geographic areas, accounting for higher mortality rates in South America and South-East Asia<sup>[69]</sup>. The current state-of-knowledge on BTC, regrettably, can be summarized briefly: complete tumor resection is the best chance of survival<sup>[82]</sup>. However, most cases are detected at an advanced stage, when surgical approaches are no longer feasible, and recurrences and distant metastases are common, with subsequent poor survival rates<sup>[82]</sup>. In addition, adjuvant chemotherapy has not shown sufficient benefit for BTC, while the efficacy of molecular targeted agents is still extremely disappointing<sup>[83,84]</sup>. To improve the prognosis of BTC, identification of prognostic markers and effective therapeutic targets is essential<sup>[85]</sup>. BTCs showing erbB2 overexpression and/or *HER2* amplification range between 5% and 76%<sup>[86]</sup>, including an estimated 13% of erbB2-positive GBC<sup>[87]</sup>. This wide range is largely dependent upon the lack of standardized methods used among different studies<sup>[27,88]</sup>. Intratumor erbB2-

expression heterogeneity has not been investigated thoroughly in BTC<sup>[75,86,88-90]</sup>. However, 51% of cases in a small cohort study showed erbB2 positivity using 50% of positive tumor cells as a threshold, suggesting the presence of heterogeneous protein expression<sup>[27]</sup>. Furthermore, in this subset of tumors, 83% of cases with heterogeneous erbB2 immunohistochemical (IHC) overexpression displayed *HER2* amplification by fluorescent *in situ* hybridization (FISH)<sup>[27]</sup>. Nevertheless, no data regarding *HER2* amplification heterogeneity have been reported in literature. The highest concordance between erbB2 expression and gene amplification was demonstrated for advanced BTC with high scores of erbB2 expression in the vast majority of tumors cells, present only in a minority of cases<sup>[68,88]</sup>. Taking into account overall and disease-free survival, *HER2* amplification seems not to have a prognostic role in BTC<sup>[27]</sup>. On the other hand, from a therapeutic standpoint, a subset of *HER2*-positive GBC responded well to trastuzumab treatment, both in monotherapy and in combination with taxane<sup>[85,90-92]</sup>. Despite these encouraging observations, an early phase clinical trial of trastuzumab for *HER2*-positive locally advanced or metastatic GBC was terminated in the United States because of the lack of participants (<https://clinicaltrials.gov/ct2/show/study/NCT00478140>). In this respect, it would be extremely beneficial to involve countries with a higher incidence of BTC in large-cohort clinical trials.

## FROM TRADITIONAL PATHOLOGY TO MOLECULAR CHARACTERIZATION

### Determination of *HER2* status and its clinical significance

These data highlight that erbB2 overexpression and/or *HER2* amplification occur more frequently in ADC of the upper gastrointestinal tract compared to the rest of the DSC. In routine diagnostic practice and in research settings, erbB2 IHC scoring, along with the assessment of other prognostic and predictive factors, remains a cornerstone in the DSC pathology<sup>[44,57,93,94]</sup>. At present, it is taking place a consensus in extending the scoring system currently adopted in gastric ADC for all other DSC<sup>[57]</sup>. Compared to the breast, erbB2 IHC in DSC has a few substantial differences not only from intra- and inter-tumor heterogeneity standpoints but also in terms of cellular staining patterns. Indeed, erbB2 expression is mainly restricted to intestinal-type, gland-forming GC, and incomplete, often basolateral or even only lateral membranous IHC staining is the rule rather than an exception for *HER2*-positive GC<sup>[93,95]</sup>. Hence, circularity of IHC staining is no longer a criterion for erbB2 IHC scoring in the digestive tract<sup>[95]</sup>. A cornerstone work on esophageal ADC aimed to compare the erbB2 scoring system routinely employed in the breast to that used in GC<sup>[96,97]</sup>. However, no similar studies have been performed on other DSCs, therefore further analyses are warranted. In this era

**Table 2** Mechanisms of resistance occurring in *erbB2*-targeting agents<sup>[107]</sup>

Type	Agent	Target	Mechanisms of resistance	Factors involved
Monoclonal antibodies	Trastuzumab	<i>erbB2</i>	Alterations in tyrosine kinase domain; overexpression of alternative <i>erbB</i> isoforms and dimerization receptors; loss of downstream checkpoints; dimerization and interaction with other receptors	p95HER2, <i>MUC4</i> , <i>EGFR</i> , <i>erbB</i> ligands (TGF $\alpha$ , EGF, HB), <i>PTEN</i> , <i>IGF1R</i> , <i>MET</i>
	Pertuzumab	<i>erbB2</i>		
	T-DM1	<i>erbB2</i>		
Tyrosine kinase inhibitors	Lapatinib	<i>erbB1</i> , <i>erbB2</i>	Alterations in tyrosine kinase domain; acquisition of <i>HER2</i> mutations; activation of further downstream signaling pathways	KIT and PDGFRA receptor signaling pathway; PI3K-AKT, mTOR
	Neratinib	<i>erbB2</i> , <i>erbB4</i>		
	Afatinib	<i>erbB1</i> , <i>erbB2</i> , <i>erbB4</i>		
	Canertinib	<i>erbB1</i> , <i>erbB2</i> , <i>erbB4</i>		
Inhibitors of the downstream targets	Everolimus	mTOR	Activation of further downstream signaling pathways	PI3K-AKT, mTOR, <i>MEK</i> , <i>MAPK</i>
	BKM120	PI3K/AKT		
	BEZ-235	PI3K/AKT/mTOR		
	GS-1101	PI3K		
	NVP-BKM120	PI3K		
	GDC-0941	PI3K		
	GSK458	PI3K/mTOR		
	GDC-0980	PI3K/mTOR		
	PI-103	PI3K/mTOR		
hsp90 inhibitors	Tanespimycin	hsp90	Up-regulation of alternative pathways	NF- $\kappa$ B, <i>MAPK</i>
	Retaspimycin	hsp90		
	AUY922	hsp90		

of precision medicine, there are increasing evidences that digital image analysis tools are able to capture the whole spectrum of *erbB2* IHC expression patterns and therefore represent useful tools for determining *HER2* status and its heterogeneity in DSC<sup>[12,98,99]</sup>. Both the membranous and nuclear features of the cells should be identified and scored, while the settings for cell count and differentiation between stroma and neoplasia should take into account the morphologic features of the cells (*e.g.*, curvature, color intensity, size, roundness, compactness, elongation) as well as each histologic pattern (*e.g.*, glandular, solid). Digital image analysis technologies grant a rapid and quantitative record of the percentage of stained tumor cells and their membrane staining distribution, allowing a precise and reproducible patients' stratification also capturing intra-tumor heterogeneity<sup>[12]</sup>. To verify equivocal IHC results, FISH, silver *in situ* hybridization (SISH), chromogenic *in situ* hybridization (CISH) assays are widely performed<sup>[8,12,57,93,96]</sup>. In particular, FISH identifies the number of *HER2* gene copies in conjunction with the number of chromosome 17 centromere (CEP17) copies<sup>[94]</sup>. This scoring is considered more objective and quantitative than IHC, however FISH reproducibility is strictly dependent on technical issues (*e.g.*, thickness of tissue sections)<sup>[8,100-103]</sup>. On the other hand, CISH is re-emerging as a more cost-effective assay, using conventional enzymatic reactions and being applicable to standard formalin-fixed paraffin embedded (FFPE) tissues<sup>[104]</sup>. This method shows high levels of quality and reproducibility, particularly in GC<sup>[105]</sup>. In a way akin to CISH, SISH is a rapid automated assay that can be interpreted using conventional microscopies, allowing pathologists to evaluate *HER2* status within the context of tissue morphology<sup>[103,106]</sup>.

### Overcoming resistance to *erbB2*-targeted therapies

Resistance to trastuzumab and other anti-*erbB2* therapies is an event that may occur during the course of therapy or *de novo* (Table 2)<sup>[107]</sup>. Drug resistance has been widely studied in breast cancer but not in the DSC, with subsequent lack of a detailed molecular characterization of this phenomenon. Intra-tumor and tumor-to-metastasis heterogeneity are among the most important characteristics that determine resistance to anti-*erbB2* therapy in DSC and should always be taken into account when selecting patients eligible for these treatments and clinical trials<sup>[12,19,52,96,108]</sup>. Indeed, there are several molecular evidences that genetic heterogeneity is not restricted to passenger genes but that also *bona fide* driver genetic alterations such as *HER2* gene amplification can be heterogeneously distributed within a given tumor<sup>[109]</sup>. The therapeutic implications of this concept are yet to be ascertained, although it is intuitive that only the *HER2*-positive neoplastic population would be sensitive to anti-*erbB2* drugs<sup>[110]</sup>. Furthermore, it is not clear whether *HER2* amplification is an early event and subsequently lost in the *HER2*-negative components, or whether *HER2* amplification might be subclonally acquired at a relatively late stage of tumorigenesis<sup>[12,27]</sup>. In addition, somatic mutations in *HER2* have been described in a small subset of DSC and there are functional evidences that at least a subset of mutations targeting *HER2* might be responsible for the development of resistance to trastuzumab therapy, in a way akin to breast cancer<sup>[8,109]</sup>. For example, alterations leading to increased heterodimerization of *HER2* with *EGFR* or *HER3* are thought to induce resistance to trastuzumab therapy<sup>[107]</sup>. Furthermore, cleavage of the full-length *erbB2* protein produces a truncated membrane-

associated fragment called p95HER2 with increased kinase activity in GC cell lines<sup>[111,112]</sup>. Up to 30% of breast cancers may harbor this alteration, showing poor prognosis and lower rates of response to trastuzumab therapy compared to patients with full-length *HER2*<sup>[111]</sup>. Furthermore, 11% of HCC but none of BTC have been found to harbor *HER2* (H878Y) somatic mutation occurring in the tyrosine kinase domain, resulting in c.2632C>T<sup>[81,113,114]</sup>. This specific mutation has been proposed as a predictor of response to *HER2*- and/or *EGFR*-targeted therapy in HCC. Interestingly, a phase II trial observed that the dual *EGFR/HER2* tyrosine kinase inhibitor lapatinib was active in HCC but not in BTC, suggesting that mutations in the tyrosine kinase domain of *HER2* in HCC may underlie responsiveness to agents that target *HER2* and/or *EGFR*<sup>[115]</sup>. Generally, erbB2-directed therapy appears to be beneficial in erbB2-positive gallbladder cancers; however, tumors harboring *HER2* mutation (V777L), in the kinase domain, followed into the non-responders category<sup>[91]</sup>. In addition to the alterations in erbB2 receptors, mutations in genes involved in the signaling pathways activated by these receptors are also correlated with failure of therapeutic response to erbB2 inhibitors<sup>[116]</sup>. A preclinical trial is testing the hypothesis that *HER2* amplification might be used, under certain conditions, as a “molecular bait” for trastuzumab-entansine precision chemotherapy to overcome anti-erbB2 resistance in *HER2*-positive metastatic CRC<sup>[117]</sup>. Moreover, *PIK3CA* mutations and *PTEN* inactivation could affect the effectiveness of erbB2-targeting therapy. Thus, it might be advantageous to clarify not only *HER2* alterations but also the PI3K-Akt pathway status to optimize *HER2*-targeting therapy<sup>[118]</sup>. In this regard, massively parallel sequencing and bioinformatic analyses are likely to represent the next frontier in the identification of complex mechanisms of trastuzumab resistance in this broad group of tumors<sup>[8,118-120]</sup>. A better knowledge of the biology underpinning *HER2* status in the digestive system should be regarded as a priority for the development of effective strategies to overcome resistance.

## CONCLUSIONS: BUILDING UP INDIVIDUAL THERAPEUTIC SCHEMES

Substantial progress has been made in the management of patients with advanced-stage DSC in recent years, with the realization of tangible improvements in terms of outcome and life quality. In particular, trastuzumab has greatly improved the therapeutic approach to patients with advanced GC but not yet in other DSC. However, no validated *HER2* testing strategies are available for non-gastric DSC and subsequently tailored treatments are yet to be implemented in this broad group of malignancies. Several drugs targeting erbB2 or its downstream signals are under development in CRC, GBC, and EC, including

ongoing phase 3 clinical trials in CRC. Nowadays, the focus on *HER2* expression/amplification status alone is not able to capture the underlying mechanisms of disease progression and resistance. In this setting, *PIK3CA* mutation or *PTEN* loss has been evaluated as a possible predictive biomarker and has also been used as one of the inclusion criteria. Understanding the interplay between *HER2* and the PI3K-Akt pathway alterations would be pivotal in the development of new therapeutic strategies. Further studies focused on the epistasis between molecular alterations and associations between molecular alterations and tumor microenvironment are warranted to accurately and robustly predict anti-erbB2 treatment outcome in DSC. Thus, a comprehensive clinical and pathogenomic approach is fundamental in appropriately characterizing *HER2* status in DSC at an individualized level for both precision therapy and accurate prognostication.

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