gastrointestinal disorders

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Review

Role of Her-2 in Gastrointestinal Tumours beyond Gastric Cancer: A Tool for Precision Medicine

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Abstract: Gastrointestinal (GI) tumors account for a quarter of all the cancer burden and a third of the global cancer-related mortality. Among them, some cancers retain a dismal prognosis; therefore, newer and innovative therapies are urgently needed in priority disease areas of high-unmet medical need. In this context, HER2 could be a relevant prognostic and predictive biomarker acting as a target for specific drugs. However, if the role of HER2 has been object of investigation for several years in gastric cancer, it is not well established in other GI malignancies. The aim of this narrative review was to portray the current landscape of the potential role of HER2 as a predictive biomarker for GI tumors beyond gastric cancer. In colon cancer, the benefit from anti-HER2 therapies is less clear than in gastric neoplasms for the lack of controlled studies. Pancreatic, biliary tract adenocarcinomas and hepatocarcinoma may derive a less clear clinical benefit by using anti-HER2 agents in HER2 positive tumors. Overall, the results are promising and seem to suggest that the integration of multiple modalities of therapies can optimize the cancer care. However, further prospective trials are needed to validate the use of personalized targeted therapies in this field.

Keywords: biliary cancer; colorectal cancer; cholangiocarcinoma; prognostic factor; predictive; GIST; precision medicine; target therapy; metastatic cancer

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1. Introduction

Tumors arising from the gastrointestinal (GI) tract account for a quarter of all the cancer burden and a third of the global cancer- related mortality [1,2]. In 2018, an estimated 4.8 million new cases and 3.4 million related deaths occurred. The Surveillance, Epidemiology, and End Results population-based (SEER) data estimated that 1- and 3-year cancer specific survival rates for GI tumors are approximately 29% and 6.2% in patients <60 years, and 22.8% and 4.8% in patients ≥60 years old, respectively [3]. However, some GI tumors still retain a dismal prognosis: the 5-year overall survival (OS) rate for advanced gastric cancer is still below 30% and less than 3% for patients with metastatic pancreatic cancer [4,5]. Given the poor survival rates, newer and innovative therapies are urgently needed in priority disease areas of high-unmet medical need.

In the last decade, significant advances have taken place in the diagnosis, treatment, and prognosis of GI tumors, and the progresses in molecular biology have resulted in the introduction of target therapies, positioned as cornerstones of treatment, thus transforming the characterization of tumors and the consideration of therapeutic combinations [6–8].

Gastric and colon cancers spearhead the incidence and mortality of GI cancers, and account for the most data regarding the benefits reached with the incorporation of molecular diagnosis and target therapy in recent years [9,10]. One of the most intensively studied pathways is RAS-RAF- mitogen-activated protein kinase (MAPK), linked to the epidermal growth factor receptor (EGFR) signaling. There are several other transmembrane proteins functioning as receptors that might play a significant role in the GI tumorigenesis, including the human epidermal growth factor receptor 2 (HER2). A growing number of studies consolidated HER2 as a relevant driver of cancerogenesis with a reproducible value as a prognostic and predictive biomarker in upper GI cancers, linking it to a more aggressive biological behavior, actively involved in tumor progression, serosa involvement, local and distant metastases, higher disease stage, and higher frequency of recurrence.

In gastric and esophago-gastric junctional adenocarcinoma (EGJ), HER2 is a known oncogenic driver, with important implications for treatment. Overexpression or amplification, determined by immunohistochemistry (IHC), occurs in a range between 9% and 27% of all the tumors [11]. The relevance of anti-HER2 drugs in GI cancers was established with Trastuzumab in the ToGA trial for patients with advanced and metastatic EGJ and gastric adenocarcinomas. In the HER2 positive tumours, the addition of trastuzumab was associated with a median OS improvement of 2.7 months if compared to chemotherapy alone, with a predictable safety profile. These results established Trastuzumab as the standard of care in the first-line treatment for HER2 positive gastric cancer in combination with platinum-based chemotherapy [12].

Moving from its evidence in gastric cancer treatment, the evaluation of HER2 status is becoming important also in other GI tumor types. Colorectal cancer is one of the most investigated in this field, reporting the strongest evidences existing in the literature beyond gastric cancer regarding the role of HER2 as predictive biomarker [13]. However, in colon cancer the benefit from anti-HER2 therapies is less clear than in gastric neoplasms, for the lack of controlled studies. In fact, although the breakthrough for target therapies came after the discovery of anti-EGFR treatments for RAS-RAF wild type tumors, anti-HER2 therapies have not been found to have the same impact on response or survival in colorectal cancer if compared to the gastric one [14,15].

The use of anti-HER2 in other GI malignancies and non-adenocarcinoma histology variants are recent fields of investigation. Pancreatic, biliary tract adenocarcinomas, and hepatocarcinoma may derive a less clear clinical benefit by using anti-HER2 agents in HER2 positive tumors [8,14,15].

Based on this background, the aim of this narrative review is to portray the current landscape of the potential role of HER2 as a predictive biomarker for GI tumors beyond EGJ and gastric cancer. Therefore, we provided an overview of the HER2 assays and HER2 targeted treatments in this field, alongside with the future perspectives.

2. HER2 pathway and Its Alterations in GI Tumors

The first molecular pathway studied in GI tumors was the EGFR family pathway, which includes EGFR/HER1, HER2/neu, HER3, and HER4 receptors. Each receptor consists of an extracellular ligand-binding domain, an intracellular domain with tyrosine kinases activity, and a short, lipophilic, transmembrane component. The selective binding of ligands to the receptors leads to homo- or hetero-dimerization with other members of the EGFR family, the phosphorylation of intracellular domain, and the activation of downstream pathways including the RAS/RAF/MAPK and phosphatidylinositol-3 kinase/protein kinase-B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathways. Stimulation of these pathways influences many aspects of tumor cell biology, such as proliferation, differentiation, migration, and apoptosis [16].

Among these receptors, HER2 plays a key role in GI tumors. HER2 alterations were historically investigated in breast cancer [17]. However, over the last decades the research has focused also on the role of HER2 in GI tumors. *HER2/neu* gene, located on chromosome 17q21, encodes the HER2 protein; when the oncogene is amplified, it can lead to HER2 receptor overexpression, resulting in a prolongation of trasductional signal with uncontrolled cell growth and tumorigenesis. To date, the specific ligand of this receptor has not been identified yet and it is considered a ligand-independent orphan receptor. Additionally, HER2 mostly acts as the suitable partner for the other EGFR receptors to create heterodimers, especially HER3.

The other most frequent alterations of HER2 pathway beyond amplifications are somatic mutations. These are variable according to the cancer type and can lead to pathological uncontrolled signal transduction [18].

An interesting field of research in this regard has focused on the role of HER2 as mechanism of acquired resistance to anti-EGFR agents. In particular, in colorectal cancer the presence of HER2 amplification or overexpression could lead to resistance to those drugs in patients with RAS-RAF wild type tumors [13]. Likewise, the presence of alterations in other EGFRs as well as the loss of HER2 expression after treatment with anti-HER2 drugs could be responsible for the primary and/or acquired resistance to anti HER2 agents, respectively [19–21].

3. HER2 Assessment in GI Tumors

HER2 is a well-known pharmacological target in breast cancer [17], and a variety of anti HER2 agents had deeply changed the treatment practice for both metastatic and early breast cancer, with significant benefit in the outcomes [22]. In the last few years, the assessment of HER2 status (including HER2 overexpression/amplification and HER2 mutations) and the implementation of clinical therapeutic interventions by using anti-HER2 agents, such as Trastuzumab, has become important in non-breast solid cancers, including esophageal, EGJ, gastric, colorectal and possibly small intestinal and pancreato-biliary cancers (Table 1) [23]. Accordingly, it is of paramount importance to develop optimal protocols to assess HER2 status in solid cancers in order to select patients who might derive a benefit by using anti-HER2 treatments.

Table 1. Distribution of HER2 amplifications, overexpression, and mutation in gastrointestinal (GI) tumors [23].

Tumour Types	HER Amplification (%)	HER2 Overexpression (%)	HER2 Mutation (%)
Stomach	11–16	20	3
Biliary Tract	5–15	20	2
Pancreas	2	26	<1
Colorectal	5.8	5	2

Amplification is defined according to the status assessment with molecular diagnostics, like in-situ hybridization. Over-expression is defined based in IHC.

The majority of the evidence available in the literature came from testing HER2 in gastric cancer. In this tumor, in fact, HER2 overexpression is observed in nearly a quarter of the patients, according to the histological tumor type and the primary tumor location [24]. In contrast to breast cancer, HER2 heterogeneity is more common in GI cancers as shown in some studies. In particular, a study by Lee et al. showed that concordant HER2 results between biopsy and surgical specimen were found in 87% of gastric cancer patients; however, 3/7 of discordant results exhibited false negative HER2 status on biopsy assessment and 4/7 specimens showed positive HER2 overexpression on biopsy specimen, whereas surgical specimen had HER2 negative status. This fact could be mostly related to the tumor heterogeneity of gastric neoplasms, so that HER2 variably positive and negative sub clones may co-exist at the same time, with a geographical pattern of positivity. In summary, the HER2 inter-tumoral heterogeneity is substantial [25]. Since HER2 assessment

is often performed in advanced/inoperable or metastatic tumors, small endoscopic samples obtained by esophagogastroduodenoscopy are generally used for histological diagnosis and HER2 evaluation. However, the biopsies used to assess HER2 status are representative only for a limited area of the tumor. On the other hand, small specimens obtained by biopsy might give better antigen preservation and, hence, better HER2 assessment [25]. Therefore, in order to improve the sensibility of HER2 test in gastric cancer and to define a more realistic picture of HER2 status of the tumor (considering the aforementioned intra-tumoral heterogeneity), a minimum of 6–8 endoscopic biopsies from the primitive lesion and surrounding area should be provided for the HER2 assessment [26].

Additionally, the discordance in the HER2 assessment is also an issue between samples taken from primary versus metastatic lesion sites as already shown some years ago by Kim et al. and Perrone et al. [27,28]. In particular, they reported 2.4% and 11% rates in HER2 status in primary versus metastatic sites. According to them, the discordance was mainly related to the positive HER2 conversion from the primary site to the metastatic one [20,21]. Additionally, some studies suggest taking biopsies from both primary and metastatic site in order to overcome the aforementioned discordance in HER2 status. Therefore, in order to address the pre-analytical factors important for HER2 assessment, ASCO/CAP breast cancer guidelines can be followed for the sample collection and preservation to ensure reliable results [29].

Moving from the evidence in gastric cancer, the same criteria for HER2 assessment have been applied also to all GI tumors. Up to date, there are two validated and approved HER2 assessment kits: HERCEP Test Kit (HercepTest™ pharmDx, Agilent, Santa Clara, CA, USA) [30] and Pathway kit (i.e., VENTANA HER2 Dual ISH DNA Probe Cocktail, USA) [29]. In addition, based on ASCO/CAP, in-home built tests can be developed if align with the standard for performance and quality [29].

Combined IHC and molecular protocols, such as in situ hybridation (ISH), can be used to identify HER2 amplified tumors, selecting, in this way, the patients to candidate to anti-HER2 agents. In general, ASCO/CAP designed the protocol for a primary screening of HER2 status with IHC, that is largely available and cost- effective; then, for equivocal cases, confirmation with ISH is required [31]. According to that, three staining variables must be assessed for each sample in order to maximize the concordance rates between IHC and FISH (gold standard test) [31]:

1. *Pattern or Quality of staining (complete, basolateral, or lateral membranous staining versus luminal, cytoplasmic, or nuclear staining)*;
2. *Intensity of staining (weak, moderate, or strong)*;
3. *Fraction of tumor cells that are positive (a cutoff of 10% is used for resection specimens)*.

In colorectal cancers, HER2 gene amplification and subsequent product overexpression have been reported in a range from 0% to 84%. The wide range can be attributed to the differences in scoring system, technical approaches, sample size and heterogeneity of study populations [32]. Then, when using validated scoring systems, membrane and cytoplasmic positivity were observed from 5% to 30%.

The HERACLES study represents the landmark trial in this field [33]. On the base of HER2 amplification data, the authors have defined two order of 2+ and three order of 3+ IHC scores. Indeed, 2+ score with any membrane positivity in <50% of neoplastic cells and 3+ score with intense circumferential, basolateral, and lateral positivity in <10% of neoplastic cells are not considered as positive staining, thus patients are not amenable to anti-HER2 treatments; 2+ score with moderate circumferential, basolateral and lateral positivity in $\geq$50% of neoplastic cells and score 3+ with intense circumferential, basolateral and lateral positivity in $\geq$10% of neoplastic cells but <50% of cells require further IHC confirmation and then ISH documentation of HER amplification before candidating the patients to anti-HER2 treatments. Finally, tumors showing a 3+ score with intense circumferential, basolateral and lateral positivity in $\geq$50% of neoplastic cells, after IHC confirmation, could be candidate to combined therapy without ISH documentation of HER2 amplification, although suggested.

In biliary tract cancers, 10.9% of tumors have HER2 positive state (IHC 3+), whereas HER2 gene amplification is identified in 5–15% of cases. Data from MyPathway (IHC 3+ and/or ISH positive) and SUMMIT (HER2 and/or HER3 alterations identified using next generation sequencing) can be used as reference for assessment of HER2 [34,35]. (Table 2).

Table 2. HER2 assessment criteria used in different Trials in GI cancers' field.

Trial (N of Patients)	Cancer Type/Phase	HER2 Criteria
1-TRIO-013/LOGIC [36] (N = 545)	Gastric/III	IHC 3+ and/or ISH-Positive
2-ToGA [12] (N = 594)	Gastric/III	IHC 3+ and/or ISH-Positive
3-TyTan [37] (N = 261)	Gastric/III	ISH positive
4-MyPathway [34] (N = 13)	Colorectal/II	HER2 Alterations (by NGS)
5-HERACLES [13] (N = 27)	Colorectal/II	IHC 3+ or 2+ and/or ISH positive
6-Ramanathan et al. [38] (N = 9)	Colorectal/II	IHC 3+ or 2+
7-MyPathway [34] (N = 11)	Biliary tract/IIA	IHC 3+ and/or ISH-positive
8- SUMMIT [35] (N = 11)	Biliary tract/II	HER2 and/or HER3 alterations(by NGS)

Abbreviations: NGS: next generation sequencing; IHC: immune-histochemistry; ISH: in-situ hybridization.

Overall, the pathology assessment of HER2 status in GI tumors is still object of debate. Several definitions have been used across the different clinical trials, and the best threshold for the single cancer types predicting the greatest benefit of anti-HER2 medicines is to be identified. In addition, more complexity has emerged with new anti-HER2 molecules, for example, drug-conjugate monoclonal antibodies, which have demonstrated to target cancer cells and provide a benefit across a large range of HER2- positive IHC staining [39]. More interestingly, these compounds have showed activity in HER2-low positive tumors, namely HER2 positive samples at IHC with negative ISH [40]. In such a perspective, the assessment of HER2 staining with IHC alone has been proposed as a screening procedure for multiple innovative compounds that utilize HER2 as a membrane antigen for tailored cytotoxic drug-delivery.

4. A Pharmacological Overview on the Anti-HER2 Biological Agents

The effective pharmacological manipulation of HER2 has resulted in landmark achievements in the treatment of cancer. The identification of pharmacological strategies to target key oncoproteins in human cancers has been declinated through multiple mechanisms, to switch off the pathogenetic drivers by direct binding (monoclonal antibodies or Mab), tackling the intrinsic tyrosine kinase activity (small molecules or TKI), delivering cytotoxic payloads via membrane receptors (antibody-drug conjugate MAb or ADC and radio-conjugate) or enhancing the immune-function of antibodies antibody-dependent cellular cytotoxicity (ADCC).

The first in class anti-HER2 agent implemented in clinical practice has been trastuzumab. Trastuzumab is a MAb IgG1 class, developed as a humanized murine compound able of binding to the extracellular domain IV of HER2 [41]. The binding of trastuzumab to the receptor prevents the dimerization of HER2 that is the key mechanism identified for the oncogenic signal transduction [42]. Such an inhibiting activity results in the dampening of the key downstream transductors and effectors of the cell response to mitogenic signals, namely the MAPK and the PI3K/Akt pathways [43]. Of interest, when PI3K signaling acquires some independency from the HER2 receptor, as observed in the dys-

regulation of the pathway (e.g., for mutational events), the tumor cells appear resistant to trastuzumab—suggesting a key role mediating the therapeutic effects [44]. In addition, trastuzumab seems to trigger the internalization of the HER2 receptor, thus resulting in its degradation [45]. Eventually, trastuzumab retains the functions of immunoglobulins that is the ADCC, recalled as one main mechanism of action of this drug [46]. ADCC mediates humoral and cellular immune responses: the fragment crystallizable region (Fc) of the immunoglobulin of class G1 can be recognized by a specific receptor on some effectors of the immune response, mainly Natural Killer cells, through the receptor FcγRIII [47]. The most relevant toxicity observed with trastuzumab is the cardiac toxicity. Alterations of the heart function have been observed in 1% of the patients; however, the pattern of trastuzumab-related cardiotoxicity is self-limiting in nature, and commonly reversible with a treatment temporary withdrawn [48]. This pattern of toxicity is defined "type 2" cardiotoxicity, as non-cumulative and reversible, possibly due to functional alterations of the HER2 signaling in cardiomyocytes, to distinguish it to the type 1 related to anthracyclines [49].

Even if major successes have been reported with the use of trastuzumab, resistance to this agent ultimately can occur with multiple mechanisms and new compounds have been identified in order to potentiate the frontline therapy or overcome the treatment resistance. HER2 is capable to link to other transmembrane receptors for the activation of the tumorigenic signaling, including with HER3. However, trastuzumab is not able to tackle the hetero-dimerization events, so that HER2/HER3 interaction can occur in the presence of this MAb [50].

Pertuzumab was identified as a MAb capable to bind HER2 in a different domain of trastuzumab, namely the domain II [51]. The current evidence on the pharmacology of trastuzumab and pertuzumab suggests that the exposure of cancer cells to trastuzumab upregulates HER3 (compensatory mechanism) [52]. This fact has provided the rationale to utilize a HER2 double blockade to switch off the HER2 and HER3 mediated signaling in cancer cells, thus providing a synergistic effect [53]. In fact, the entire drug development of pertuzumab has focused on its role as an enhancer of the response of the anti-HER2 treatments, and it is not currently approved for the use as a single agent. Like trastuzumab, the ADCC-inducing capability of pertuzumab seems to be one key mechanism for its antineoplastic activity [54].

In the attempt to enforce the ADCC capability of the MAb, the bioengineering technology has provided a strategic manipulation of the Fc domain, by increasing the FcγRIII binding capacity to the immune effectors [55]. Margetuximab was designed as an anti-HER2 chimeric immunoglobulin G, capable to bind the same HER2 epitope of trastuzumab with an increased affinity to the FcγRIII and lower binding capacity to CD32B, an FcγR inhibitory receptor [56]. When used in trastuzumab- resistant models, margetuximab demonstrated an improved antitumor activity, prompting its implementation in clinical trials [57].

Some fusion molecules have been introduced with the aim to use a single antibody with multiple functions, and/or enhance the immune- activity. In general, bi-functional antibodies are engineered MAb capable to bind two different epitopes, aiming to bind to multiple antigens on the cancer cells (e.g., HER2 and HER3) or recruit different cell types (e.g., immune-cells). Zenocutuzumab, ZW25, PRS-343, and GBR1302 are bi-functional monoclonal antibodies under clinical development for the treatment of HER2- positive tumors. Zenocutuzumab and ZW25 are bi-functional MAb, designed to bind epitopes on the transmembrane receptors on cancer cells [56]. Zenocutuzumab is able to recognize HER2 and HER3 with enhanced ADCC, while ZW25 binds to the domain II and IV of HER2, recapitulating the activity of trastuzumab and pertuzumab. On the other hand, PRS-343 and GBR1302 are MAb designed to bind immune- and cancer cells. PRS-343 recruits the co-stimulatory immune-checkpoint protein CD137 (4-1BB), an immune- activating member of the tumor necrosis factor receptor superfamily and HER2; GBR1302 binds to the T-cell receptor and HER2 [57]. The use of single molecules with double activities is a promising strategy for the treatment of cancer, intending to provide a contemporary physical linkage

of multiple epitopes or produce a favorable milieu of immune-activity by linking effector cells to cancer cells [58].

MAbs have been also invested with a novel role that is the delivery of potent cytotoxic chemotherapeutics specifically into cancer cells [40]. An ADC is composed of a MAb plus a cytotoxic agent linked by a molecular linker [59]. The first approved ADC for the treatment of breast cancer was trastuzumab emtansine (T-DM1). T-DM1 is an ADC combining trastuzumab to mertansine, an inhibitor of the microtubular assembly [40]. The second ADC approved for breast cancer is trastuzumab-deruxtecan (T-DXd, DS-8201), composed of trastuzumab conjugated to a topoisomerase I inhibitor [60]. When binding to the cancer cells via HER2, T-DXd is internalized by the cells and cleaved by lysosomal cysteine proteases, thus releasing the payload. The payload can exert intra-cellular direct cytotoxic activities in the targeted cells and can induce bystander effects in the tumoral milieu, thus enhancing the overall antineoplastic activity e.g., on HER2 heterogeneous tumors with variable expression of the membrane oncoprotein [61]. Though developed for the precise delivery of chemotherapy agents, some spillover of the conjugated compounds can be observed, resulting in some classic toxicities of chemotherapy. For example, the use of T-DXd is associated with nausea in three-quarter of the patients, fatigue and alopecia in a half; in addition, moderate to severe hematological toxicities has been reported in one-fifth of patients, including anemia and neutropenia. Of relevance, the accumulation of the cytotoxic compounds in the lungs has been associated with interstitial lung disease, the most concerning toxicity from ADCs, prompting the treatment discontinuation in the largest proportion of the patients and being lethal in around 2% of the cases [62]. Several other ADCs anti-HER2 are on the pipeline for the treatment of solid tumors, including Trastuzumab duocarmazine (SYD985), Disitamab Vedotin (RC48-ADC) and the bi-specific ADC MEDI4276. Envisioning the use of MAb as "carriers" of pharmaco-therapeutics, some have suggested using trastuzumab conjugates to deliver radioisotopes (radio-immunotherapy) [63]. The first in class anti-HER2 compound implemented in clinical trials is 212Pb-TCMC-trastuzumab, a radio-immunoconjugate of trastuzumab with an alpha emitting isotope [64]. More recently, the conjugates of 227Th have emerged as favored compounds, alpha- emitting and with a better stability both in serum and vivo conditions [65]. The advantage to use alpha vs. beta-emitters is related to short half-lives, short rage of actions to deliver localized higher burden of radiations [66]. So far, none of the anti-HER2 radio-immunoconjugates is approved up to date.

Another strategy to tackle the pathogenetic HER2 signaling in tumors is to target the tyrosine kinase activity with TKI. The use of TKI against HER2 has been the second strategy implemented in the clinical practice for patients with HER2- positive breast tumors, with the advent of lapatinib. HER2 TKIs block the ATP binding pocket, thus preventing auto-phosphorylation of the receptor and resulting in the downregulation of the transduction signal. While the kinome of the small molecules can be quite promiscuous, these TKIs are mainly distinguished by their ability to block reversibly or irreversibly HER2 and their capacity to cross the blood- brain barrier. All the TKI used in the treatment of HER2-positive cancers are administered as oral agents. As HER2 blockers, a special precaution for the cardiac effects should be provided for patients taking these molecules, as for trastuzumab. Additionally, according to their capability to bind to other protein of the EGFR family, cutaneous and GI effects are also reported [67]. Neratinib and Pyrotinib are irreversible HER2 inhibitors targeting HER1/EGFR, HER2, and HER4; Poziotinib is an irreversible pan-HER blocker with activity on key pathogenetic mutations of HER1 [68]. Lapatinib and tucatinib are reversible HER2 blockers; however, while lapatinib exerts pharmacological activity on HER2 and on HER1/EGFR, tucatinib is HER2 very selective resulting in a better safety profile and less skin or intestinal side effects [69]. In addition, tucatinib has a better bioavailability in the central nervous system, thus widely suggested for the control of HER2- positive brain (secondary) tumors [70].

The complex treatment landscape of development of anti-HER2 agents includes some innovative approaches to cancer treatment. The presence of HER2 on some tumor

cells and the wide utilization of it as a cancer- specific antigen (though not exclusive of tumors) has suggested its role for the development of preventive or therapeutic anticancer vaccines. Peptide- based vaccines (e.g., HER2 369–377) have been engineered and used in preliminary studies for cancer patients, with promising results [71]. Immune-stimulating effects have been variously reported, with an acceptable safety profile and no major myocardial toxicities [72]. Additionally, dendritic cell-based vaccines have been developed, using autologous cells pulsed with HER2 peptides [73]. Eventually, cell-based strategies have also been proposed, using adoptive T-cell therapies. These strategies generate T cells with chimeric antigen receptor, to enhance the recognition of HER2 epitopes and tailor precisely the cancer cells expressing the cancer-associated antigen [74]. Variously combined or as single agents, these molecules represent appealing new strategies to treat solid tumors, including GI tumors positive to HER2; however, definitive results with prompt clinical applications beyond gastric cancers are still await.

5. Role of HER2 across Gastrointestinal Cancer Types

5.1. Colorectal Cancer

Colorectal cancer (CRC) has benefited from anti-HER2 targeted therapy [23,75]. HER2 overexpression in CRC is an emerging biomarker and accounts for around 5% of all cases [76,77]. Patients with HER2-positive CRC have been enrolled in several early phase I-II trials in order to investigate various combinations encompassing HER2 targeted drugs. Previously, a phase II trial (NCT00003995) tested the addition of trastuzumab to irinotecan in the first and second lines for advanced CRC [38]. HER2 status was assessed by IHC and verified by fluorescent in situ hybridization (FISH). Despite the discordant findings between these techniques, patients harboring HER2 amplification had partial response to this combination. Unfortunately, the trial was closed earlier because of poor accrual [38]. Similarly, another phase II trial (NCT00006015) that combined trastuzumab to FOLFOX regimen as a second line treatment in the metastatic disease was also terminated because of the lack of sufficient accrual [78]. Trastuzumab was also combined to a farnesyl protein transferase inhibitor (tipifarnib) in a phase I trial in molecularly unselected patients; it proved to have an acceptable safety profile (NCT00005842). However, this new agent failed to show improved survival for this indication in a phase III trial [79]. Since then, most clinical studies showed some signs of early efficacy but without further development [80,81]. Later, the combination of pertuzumab, the EGFR inhibitor cetuximab, and irinotecan has been evaluated in the second line by a phase I trial (NCT00551421) in a molecularly unselected group of patients, refractory to first-line chemotherapy plus cetuximab [82]. The trial terminated early due to the safety issues of the combination. The reported objective response rate was 14% [82]. Recently, the phase II HERACLES-A trial evaluated the objective response rate of dual trastuzumab and lapatinib in metastatic CRC patients with HER2 overexpression and amplification and *KRAS* exon 2 wildtype [13,83]. At a median follow-up of 94 weeks, the combination resulted in 8/27 objective responses (30%, 95% confidence interval (CI): 14–50%) with one complete response (3%). The reported median progression-free survival (PFS) was 4.7 months (95% CI: 3.7–6.1), and the median OS was 10.0 months (95% CI: 7.9–15.8). Interestingly, intracranial progression was reported in 19% of the patients [83]. Grade 3 adverse events were reported in 22% of the patients. The second cohort (NCT03225937) of this trial evaluated the combination of pertuzumab and T-DM1, reporting an objective response of 10% (95% CI: 0–28); the proportion of patients with stable disease was 70% (95% CI: 50–85) [33]. However, this study did not meet its primary endpoint.

The efficacy of trastuzumab combined with tucatinib was explored in CRC patients with HER2 overexpression or amplification in the phase II open-label MOUNTAINEER trial in the second line setting [84]. A CLIA-certified HER2 IHC or next generation sequencing (NGS) test or an FDA-approved FISH test confirmed molecular status. Trastuzumab and tucatinib produced an overall response rate of 55% (12/22). After a median follow-up of 10.6 months, median PFS was 6.2 months (95% CI, 3.5-not reached (NR)), and me-

dian OS was 17.3 months (95% CI: 12.3-NR). The planned primary completion date of the trial will be in August 2021 [84]. Additionally, the phase II MyPathway basket trial has assessed the combination of trastuzumab and pertuzumab in 57 patients with HER2-amplified metastatic CRC [34]. Objective responses were observed in 32% of the patients (95% CI: 20–45); one patient had a complete response (2%). More recently, the TAPUR phase II study that enrolled 28 HER2-amplified CRC patients and treated with the pertuzumab/trastuzumab combination showed objective responses in 14% of the patients (14% (95% CI: 4–33) [85].

The ADC of trastuzumab was investigated in the phase II DESTINY-CRC01 trial that evaluated the efficacy of trastuzumab deruxtecan, focusing only on HER2 expressing CRC [86]. The confirmed objective response rate was 45.3% (95% CI: 19.8–70.1%), with a disease control rate of 83.0% and a median PFS of 6.9 months (95% CI: 4.1 months-NR). The authors emphasized recognizing and managing treatment-related interstitial lung disease, which occurred in 6.4% of the patients [86].

In conclusion, targeting HER2 amplified/overexpressed CRC is still under investigation and the current data are not mature yet to provide actionable findings for this group of tumors. There are currently more than 40 recorded clinical studies ongoing trials in this field [87]. This may potentially provide promising findings in the future for improving outcomes.

5.2. Small Bowel Cancers

Small bowel cancer is a rare subset of GI tumor (3% of all), whose genome profile is still under debate. Up to date, in fact, it is not very clear if these tumors might be more similar to the stomach, duodenum, or colon ones and, therefore, the systemic treatments in case of metastatic disease is mainly based on the chemotherapy schedules generally used in other GI malignancies (e.g., FOLFOX schedule) [2]. Therefore, there is a lack of target treatment for tumors, which arise from the small bowel. However, the small bowel cancers show deeply differences from CRC, with a poor prognosis (poorer than CRC), mostly due to a late diagnosis. Additionally, the knowledge available today seems to report a genetic profile more similar to the stomach cancer, but with some peculiarities. Schrock et al. analyzed 7559 samples from patients affected by gastric (*n*: 889), CRC (*n*: 6353) and small bowel cancer (*n*: 317) with the aim to compare the genomic profile of those tumors [88]. The genomic alterations in small bowel tumors were different from either CRC or gastric ones, confirming that those tumors represent a distinct entity. Additionally, they revealed to have HER2 mutations in 8.2% (versus 9.5% of gastric cancer and 5.1% of CRC) of samples, HER2 amplifications in 2.2% and both co-occurring alterations in three cases. The most important activating mutations were S310F/Y, V777L, V842I, D769Y and L755S. These alterations could represent possible targets for personalized treatment also in this group of tumors. However, further investigations are needed in order to evaluate the role of HER2 in small bowel carcinomas and to eventually design prospective randomized trials with target therapies in this field.

5.3. Gastrointestinal Stromal Tumors (GIST)

Several small series in Europe and USA found no GIST patient to be positive for HER2. Yan et al. examined 37,992 patients with diverse cancer types, including 143 GIST. Nevertheless, no GIST expressed a positive HER2 status [89]. Likewise, another trial including 94 GIST failed to show gene amplification for HER2 [90]. However, a small Egyptian study including 32 patients showed a 43.7% positivity in HER2 status [91]. Additionally, it reported that high-risk grade, tumor size, mitotic count, and increased risk of relapse were significantly correlated with HER2 positivity. Interestingly, a large study from China [92] involving 453 GIST patients showed a positive HER2 status in 52.3% of patients, even if the author used a non-conventional scoring system for the HER2 assessment.

Therefore, the role of HER2 in GIST is not still defined. However, further investigations also exploring the heterogeneity of HER2 expression according to ethnicity could be of interest.

5.4. Biliary Tract Cancer and Pancreatic Cancer

5.4.1. Biliary Tract Tumors

Cancers of the biliary tract includes different kind of tumors, which are intrahepatic and extrahepatic cholangiocarcinoma and gallbladder carcinoma. The highest incidence rates are reported in India, Pakistan, Korea, Japan, and some South American countries [93]. Surgery is considered the only curative approach; however, only few patients have an early stage amenable to surgical resection at diagnosis. Therefore, the majority of patients are diagnosed with a locally advanced disease; those patients show high recurrence rates even after adjuvant chemotherapy [94].

Regarding the molecular profile, the knowledge about carcinogenesis and genetic alterations in the biliary tract cancers is still incomplete. However, some reports in the literature have showed an HER2 overexpression in 10–82% biliary tumors [95]. Additionally, a metanalysis showed a higher HER2 overexpression in extrahepatic than intrahepatic cholangiocarcinoma (19% versus 4.8%). Among patients with HER2 overexpression, almost 60% of patients has HER2 amplification too [96]. Another case series showed an HER2 positivity in 16.6% of gallbladder cancer [97].

The role of HER2 as predictive or prognostic biomarker is still unclear in the field of biliary tract cancers. In this regard, Vivaldi et al. did one of the largest retrospective mono-institutional analysis of 100 patients with biliary tract tumors after curative surgery [98]. HER2 positivity was found in 11% of patients and was associated with a poorer disease-free survival (DFS): 10.6 versus 20.9 months in HER2 positive and HER2 negative patients, respectively. However, the HER2 status alone did not affect the OS. Another Japanese analysis evaluated the HER2 status in samples from 454 patients with biliary tract cancers who had undergone to curative surgery (intrahepatic cholangiocarcinoma: 110 patients, perihilar extrahepatic cholangiocarcinoma: 67, distal extrahepatic cholangiocarcinoma: 19, gallbladder carcinomas: 80, ampullary carcinomas: 79) [99]. HER2 positivity was seen in 14.5% of tumors (intrahepatic cholangiocarcinoma: 3.7%, perihilar extrahepatic cholangiocarcinoma: 3%, distal extrahepatic cholangiocarcinoma: 18.5%, gallbladder carcinomas: 31.3%, ampullary carcinomas: 16.4%) [99]. Unlike the work by Vivaldi et al. [98], in this analysis HER2 positive tumors were mainly differentiated with low rate of invasion. Therefore, the prognostic role of HER2 is still unclear.

Regarding the therapeutic application, up to date there are no randomized controlled trials in the field of biliary tract cancers showing efficacy of anti-HER2 agents. In fact, all the evidence about their activity in those tumors are retrospective or at an early stage of development, such as preclinical (in vitro and in vivo models) or from phase I trials [100]. In particular, in a retrospective series from a single institution, 14 patients with metastatic HER2 positive biliary tract cancers (9 gallbladder cancer patients and 5 cholangiocarcinoma patients) had received trastuzumab, lapatinib, and pertuzumab, alone or in association with chemotherapy [101]. In this experience, the authors recorded a complete response in one patient with gallbladder cancer, whereas 4 patients had a partial response by RECIST. The median duration of response was 40 weeks. No response was seen in patients affected by HER2 positive cholangiocarcinoma, even if they had higher proportion of HER2 overexpression or mutation. However, these results should be considered with caution due to the limitations of the study, mainly related to the heterogeneity of treatment and the retrospective design. Another case report describes a good response by using trastuzumab in combination with Paclitaxel in the second line treatment of a patients affected by HER2 positive metastatic gallbladder cancer. Then, lapatinib plus FOLFOX was evaluated in a phase I trial, regardless of HER2 status, showing partial responses in 2/34 patients [102].

Among phase II trials, the California consortium and the MyPathway trial are the best match for anti-HER2 treatments in this field [103,104]. In the first one, 19 patients with advanced biliary tract tumors were treated with lapatinib. However, no objective responses were seen in those patients and only five patients had a stable disease as best response at the radiological assessment [103]. The MyPathway trial is an open label basket trial aiming to find the genomic peculiarities of each kind of tumors in order to develop a personalized

target therapy for each alteration. It included 11 patients with HER2 positive biliary tract tumors (eight patients had HER2 amplifications and three had somatic mutations), showing good responses after treatment with the target anti-HER2 agents trastuzumab and pertuzumab (four patients had a partial response and five patients a stable disease as best response) [104]. Then, another basket trial (SUMMIT) evaluated the use of neratinib in patients with HER2 positive solid tumors, including nine patients with HER2 positive biliary tract tumors, who had a 22% overall response rate (ORR) [105].

In conclusion, the evidence regarding a potential role of HER2 in the field of biliary tract cancers is immature at the time of writing. Therefore, further phase III trials are needed in order to consider HER2 as a potential target for effective target treatments in this field.

5.4.2. Pancreatic Cancer

The prevalence and diagnostic criteria for HER2 status in pancreatic cancer remain unclear as well as its role as predictive or prognostic biomarker. In general, few experiences have reported an HER2 positivity in 24–26% of pancreatic adenocarcinomas [106]. Additionally, the data available in the literature showed no correlation between the HER2 status and the outcome in pancreatic cancer patients [107].

Regarding the therapeutic implications of HER2, preclinical studies have evaluated the role of trastuzumab in human pancreatic cancer cell lines both in vitro and in vivo [108]. Cells with high level of HER2 positivity had the best response to treatment, whereas there were no significant responses in those with low HER2 expression. A similar anti-tumor effect of trastuzumab was seen in another in vitro study in combination with gemcitabine, causally related to HER2 level expression [109]. Moving from the preclinical to the early drug development field, a phase II study evaluated the efficacy of using trastuzumab in combination with capecitabine in 17 HER2 positive pancreatic cancer [106]. The trial showed a median OS and PFS of 23.55 and 6.9 months, respectively, without improvement in the outcomes if compared with the "historical" data of chemotherapy alone [106].

Another single arm study by Safran et al. evaluated the response rates by using Trastuzumab and gemcitabine in 34 patients with metastatic HER2 positive pancreatic adenocarcinoma [110]. Thirteen patients had >50% reduction of CA 19–9 levels; median OS was 7 months, and 1-year OS rate was 19%. Nevertheless, trastuzumab appeared not to give a benefit if compared with chemotherapy (gemcitabine) alone.

In conclusion, the evidence regarding a potential role of HER2 in the field of pancreatic cancers is immature and not conclusive at the time of writing. Therefore, further phase III trials are needed in order to consider HER2 as a potential target for effective target treatments in this field.

6. Future Directions and New Strategies for HER2-Positive GI Tumors

The progress in molecular tumor biology is growing, and the research focusing on targeted therapies in GI cancers is incessant. Currently, more than one-third of the ongoing clinical trials with anti-HER2 agents evaluates the combination of chemotherapy and monoclonal antibodies or TKI. More commonly, these agents are conceived to target HER2- amplified tumors; however, with the advent of conjugated MAb, some studies are ongoing against tumor types positive to HER2 at IHC without an ISH-confirmed tumor amplification (HER2-positive, non-HER2 oncogene driven tumors).

A third of the enrolling phase I or II trials assess bispecific antibodies (zanidatamab, zenocutuzumab, SBT6050) and antibody-drug conjugates (trastuzumab emtansine, trastuzumab deruxtecan, disitamab vedotin, ZW49). A smaller segment of the ongoing clinical trials evaluates the efficacy of the combination of trastuzumab and pertuzumab or of MAb with TKIs.

Table 3. Ongoing trials using anti-HER2 agents in gastrointestinal tumors.

Name of Drug, Intervention	Tumor Type	Phase	Planned Primary Completion Date (PCD)	Study Name, Trial ID
Monoclonal antibodies and their combinations				
trastuzumab + chemotherapy	HER2 positive colorectal, biliary tract and liver carcinomas	II	July 2021	NCT03185988
trastuzumab + gemcitabine-cisplatin chemotherapy	HER2 amplified biliary tract cancer (first-line)	II	NA	CTRI/2019/11/021955 [111]
trastuzumab + gemcitabine-cisplatin chemotherapy	HER2 positive biliary cancer	II	September 2020	BILHER, NCT03613168
trastuzumab + pertuzumab	HER2 amplified (RASwt) CRC	II	March 2023	TRIUMPH, UMIN000030505 and UMIN000027887 [112,113]
trastuzumab + pertuzumab	HER2 amplified solid tumors (basket trial)	II	NA	JUPITER, jRCT2031180150 [114]
trastuzumab + pertuzumab versus a combination of cetuximab and irinotecan (CETIRI)	HER2 amplified CRC (KRAS/NRAS/BRAF wild type)	II	April 2021	SWOG S1613, NCT03365882 [115]
trastuzumab or pertuzumab combined with the PD-L1 inhibitor atezolizumab	HER2 overexpressed or amplified solid tumors	II	April 2024	NCT04551521
Bispecific antibodies. Dual HER-2 signal blockade				
Zanidatamab	refractory colorectal cancer	II	NA	NA [116]
Zanidatamab	HER2 amplified biliary tract cancer	IIb	July 2022	NCT04466891
Zanidatamab	HER2 amplified biliary tract cancer	II	September 2025	ChiCTR2000036161
zanidatamab with or without selected chemotherapies	HER2 expressing colorectal, hepatocellular, and pancreatic neoplasms	I	January 2022	NCT02892123
zenocutuzumab	solid tumors with neuregulin-1 gene (NRG1) fusions	I/II	September 2021	NCT02912949
SBT6050 (TLR8 agonist conjugated to a HER2 directed monoclonal antibody)	HER2 expressing solid tumors	I/Ib	August 2023	NCT04460456

Table 3. *Cont.*

Name of Drug, Intervention	Tumor Type	Phase	Planned Primary Completion Date (PCD)	Study Name, Trial ID
Antibody-drug conjugates (ADCs)				
trastuzumab emtansine	HER2-amplified colorectal cancer (after the use of trastuzumab and lapatinib)	II	NA	HERACLES-RESCUE, NCT03418558 [117]
trastuzumab emtansine	HER2-amplified or mutant solid tumors, including colorectal and hepatocellular cancer (basket trial)	II	NA	NCT02675829 [118]
trastuzumab deruxtecan	HER2 positive solid tumors, including biliary tract cancer, pancreatic cancer and colorectal cancer	II	March 2023	DESTINY-PanTumor02, NCT04482309
trastuzumab deruxtecan	HER2 positive biliary tract cancer (second-line)	II	NA	HERB, JMA-IIA00423 [119]
trastuzumab deruxtecan	HER2 amplified cancers, including colorectal, biliary tract and pancreatic cancer	II	July 2022	HERALD, JapicCTI-194758 [120]
trastuzumab deruxtecan + avelumab	HER2 expressing or mutant, solid tumors	I	NA	NA
disitamab vedotin	HER2 overexpressed metastatic biliary tract cancer (second-line)	II	August 2022	NCT04329429
ZW49 (anti-HER2 bispecific comprised of a yet undisclosed cytotoxic drug)	HER2 expressing solid tumors	I	May 2021	NCT03821233
Tyrosine kinase inhibitors (TKIs)				
lapatinib or trastuzumab and GEMOX chemotherapy	HER2 pathway altered extrahepatic cholangiocarcinoma and gallbladder carcinoma (first line)	II	December 2019	NCT02836847

Table 3. *Cont.*

Name of Drug, Intervention	Tumor Type	Phase	Planned Primary Completion Date (PCD)	Study Name, Trial ID
lapatinib and trastuzumab combined with chemotherapy for HER2 mutant or HER3 mutant/amplified, trastuzumab combined with gemcitabine-cisplatin or gemcitabine-oxaliplatin chemotherapy for HER2 amplified	HER2/3 mutant or amplified biliary tract cancers	II	NA	CTRI/2020/05/025147
neratinib	solid tumors with activating mutations in EGFR or HER2 including colorectal and biliary cancers	II	NA	SUMMIT, NCT01953926 [105]
neratinib + cetuximab (NC) or neratinib + trastuzumab (NT)	quadruple wild-type (KRAS/NRAS/BRAF/PIK3CA wild-type) colorectal cancer, HER2-mutant patients receive NT, HER2 amplified or HER2- "wild-type" (non-mutated and non-amplified) patients receive NC	II	December 2020	NCT03457896
poziotinib	solid tumors with activating mutations in EGFR or HER2 including colorectal cancer	II	June 2023	NCT04172597
tucatinib + trastuzumab + FOLFOX chemotherapy	colorectal, hepatocellular and gallbladder cancers, and cholangiocarcinoma (molecularly unselected, safety and pharmacokinetics only)	I	May 2021	NCT04430738
pyrotinib with or without trastuzumab	HER2 amplified colorectal cancer	II	December 2020	NCT04380012
pyrotinib with or without trastuzumab	HER2 amplified colorectal cancer	II	July 2022	NCT03843749

Table 3. *Cont.*

Name of Drug, Intervention	Tumor Type	Phase	Planned Primary Completion Date (PCD)	Study Name, Trial ID
pyrotinib + trastuzumab + FOLFOX6 chemotherapy in the preoperative setting	HER2 positive colorectal cancer	II	September 2025	ChiCTR2000037827
afatinib + capecitabine chemotherapy	solid tumors, including biliary tract and pancreatic cancers	I/Ib	January 2022	NCT02451553
afatinib + GEMOX chemotherapy after radical surger	HER2 pathway altered gallbladder carcinoma	II	May 2021	NCT04183712
afatinib + nivolumab	HER2 or HER3 mutant metastatic extrahepatic cholangiocarcinoma or gallbladder carcinoma	II	December 2021	ChiCTR1800018149
TAS0728 (HER2 inhibitor)	solid tumors with HER2 or HER3 abnormalities, including biliary tract cancer with HER2 or HER3 mutation, colorectal cancer with HER2 mutation or amplification	I/II	January 2021	NCT03410927
		Vaccines		
chimeric HER-2 B-cell peptide vaccines	solid tumors including colorectal cancer	I	December 2021	NCT01376505
NANT pancreatic cancer vaccine combined with several agents (Aldoxorubicin, ALT-803, ETBX-011, ETBX-021, ETBX-051, ETBX-061, GI-4000, GI-6207, GI-6301, haNK for infusion, bevacizumab, capecitabine, cyclophosphamide, fluorouracil, leucovorin, nab-paclitaxel, oxaliplatin, avelumab)	pancreatic adenocarcinoma	Ib/II	December 2019	NCT03586869
FATE-NK100 (allogeneic CD3- CD19- CD57+ NKG2C+ NK cells)	HER2 positive solid tumors including colorectal cancer, hepatocellular and pancreatic cancer	I	October 2021	NCT03319459
ACE1702 (anti-HER2 oNK cells)	HER2 expressing solid tumors	I	December 2021	NCT04319757

Interesting results are awaited from HER2-directed vaccine trials (chimeric HER-2 B-cell peptide, NANT pancreatic cancer vaccine, FATE-NK100, ACE1702), an innovative approach in the immune-stimulation against tumors. In fact, HER2 has been commonly referred as a tumor- association antigen, with the potentiality to be recognized by the immune system, thus targeting specifically the cancer cells. In addition, for HER2 mutant tumors, TKIs (neratinib, poziotinib, and TAS0728) are under evaluation in clinical trials, as single agents or in combination with immune- agents (i.e., afatinib plus nivolumab). Then, Atezolizumab with trastuzumab or pertuzumab (NCT04551521), avelumab with trastuzumab deruxtecan, nivolumab combined with afatinib (ChiCTR1800018149) represent all possible future immunotherapy combinations and are under clinical investigation (see Table 3 for additional details).

Finally, while the current standard for the evaluation of the treatment response is based on radiological assessment criteria, multiple trials will provide valuable information about the utility of blood-based initial detection of HER2 amplification and the role of circulating tumor DNA (NCT04183712), resulting in dynamic and non-invasive modalities to monitor treatment responses and anticipate resistance to therapies.

7. Conclusions

The landscape of anti-HER2 therapeutics for non-gastric GI tumors is rapidly evolving, and innovative paradigms of cancer treatment are emerging. While nonspecifically considered an agnostic marker, the amplification of HER2 has been recognized as a driver mechanism of tumorigenesis in selected tumor types beyond breast cancer—and the benefits of anti-HER2 therapies confirmed in multiple disease areas. In addition, the demonstration of a membrane stain at IHC of HER2 is emerging as a possible "agnostic-like" marker for cancer treatment, using treatment delivery modalities that target the cancer cells via membrane antigen- recognition. This is the case of ADCs, therapeutic vaccines and Chimeric antigen receptor T cells—which utilize HER2 as a "non-self" recognition signal, delivering cytotoxic agents or immune-mediated cytotoxic responses, respectively—thus not primarily aiming to switch-off the HER2 pathological signaling, as for TKIs or classic MAb. Which strategy is the most promising and in what setting is expected will be determined by clinical trials. So far, the results are promising and seem to suggest that the integration of multiple modalities of therapies can optimize the cancer care, including the combination of multiple innovative therapies.

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