

REVIEW

Targeting the human epidermal growth factor receptor 2 (*HER2*) oncogene in colorectal cancer

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Human epidermal growth factor receptor 2 (*HER2*) is an oncogenic driver, and a well-established therapeutic target in breast and gastric cancers. Using functional and genomic analyses of patient-derived xenografts, we previously showed that a subset (approximately 5%) of metastatic colorectal cancer (CRC) tumors is driven by amplification or mutation of *HER2*. This paper reviews the role of *HER2* amplification as an oncogenic driver, a prognostic and predictive biomarker, and a clinically actionable target in CRC, considering the specifics of *HER2* testing in this tumor type. While the role of *HER2* as a biomarker for prognosis in CRC remains uncertain, its relevance as a therapeutic target has been established. Indeed, independent studies documented substantial clinical benefit in patients treated with biomarker-driven *HER2*-targeted therapies, with an impact on response rates and duration of response that compared favorably with immunotherapy and other examples of precision oncology. *HER2*-targeted therapeutic strategies have the potential to change the treatment paradigm for a clinically relevant subgroup of metastatic CRC patients.

Key words: biomarker, colorectal cancer, *HER2*, targeted therapies

Introduction

Few clinically actionable genetic abnormalities have been identified in primary tumors, metastases, or blood of patients with colorectal cancer (CRC) [1–3]. While the role of human epidermal growth factor receptor 2 (*HER2*) as a biomarker for prognosis in CRC remains uncertain, its role as a therapeutic target is rising [4–8]. *HER2* is also emerging as a negative predictor of response to epidermal growth factor receptor (EGFR)-targeted treatments [9]. Hence, patients with *HER2*-positive CRC might have fewer treatment options and carry an inferior prognosis. In this review, we consider the role of *HER2* in CRC as an oncogenic driver and prognostic and predictive biomarker, present *HER2* testing methods utilized specifically in patients with CRC, and discuss clinical diagnostic and therapeutic data supporting *HER2* as a novel therapeutic target in CRC.

HER2 as an oncogenic driver in CRC

HER2 is the only member of the EGFR family that does not bind ligands; it is activated via heterodimerization with other ligand-bound receptors [10], with the strongest mitogenic signals created by *HER2*–*HER3* heterodimers. *HER2* overexpression, usually caused by gene amplification, allows *HER2* activation even in the absence of ligand bound to the other partners [11]. Overexpression or amplification of *HER2* has been reported in 13%–20% of breast cancers [12], 7%–34% of gastric cancers [13], and 1.9%–14.3% of lung carcinomas [14]. Diverse rates of *HER2* overexpression have been reported in CRC (Table 1 [9, 15–38]), with rates of membranous expression ranging from 2% to 11% [16]. A number of factors may account for these differing rates, including small study populations, different antibodies for immunohistochemistry (IHC), analysis of distinct subgroups of

Table 1. Incidence of HER2 overexpression and association with prognosis in CRC

HER2 testing by IHC/ISH						
References	Patients, N	Stage	Monoclonal antibody	HER2 3+, %	Location	Prognostic role
Ni et al. [15]	4913	–	–	1.4	Colorectal	Yes
Laurent-Puig et al. [9]	1795	III	4B5 Ventana	1.4 (n=21/1457)	Colon	Yes for IHC/FISH only for RFS but not OS ^a
Richman et al. [16]	1914	II–III	A 0485 Dako	1.3	Colorectal	No
	1342	IV	A 0485 Dako	2.2		No
Valtorta et al. [17]	1086	–	4B5 Ventana/ Hercep-Test	4.1	Colorectal	Not assessed
Ingold Heppner et al. [18]	1645	I–IV	Clone SP3	0.5 (1.6 total CISH+)	Colorectal	Trend toward worse survival
Song et al. [19]	106	pT1, pT2, pT3	4B5	7.5 (2/3+)	Colorectal	No
			SP3	3.8 (2/3+)		No
Conradi et al. [20]	264	II/III rectal	PATHWAY (Ventana)	5.9 (n=10/169 pretreatment biopsies)	Rectal	Yes
Kruzszeowski et al. [21]	202	II–IV	A 0485	15.3	Colorectal	No
Kountourakis et al. [22]	106	–	NCL-CB11	2.8 membranous	Colorectal	No
Schuell et al. [23]	77	I–IV	Hercep-Test	3	Colorectal	Trend toward worse survival
Essapen et al. [24]	170	II–III	HM64.13	54.1 (2+, cytoplasmic)	Colorectal	Yes (cytoplasmic, stage III)
				40.0 (2+, membranous)		
McKay et al. [25]	249	I–IV	NCL-CB11	3.2	Colorectal	No
Rossi et al. [26]	156	I–III	–	4 (2/3+)	Colorectal	No
Osako et al. [27]	146	–	Nichirei	4.5 (HER2 amplification)	Colorectal	Yes
Kapitanović et al. [28]	221	–	Ab-3	43 (n=67/155 adenocarcinomas)	Colorectal	Yes

Molecular profiling of HER2						
References	Patients, N	Stage	Method	HER2 amplification, %	Location	Prognostic role
Ross et al. [29]	8874	–	CGS	4.9 (and/or short variant alterations)	Colorectal	Not assessed
Shimada et al. [30]	201	I–IV	CGS	5.0	Colorectal	Not assessed
Gong et al. [31]	138	–	NGS	5.1	Colorectal	Not assessed
Schrock et al. [32]	143	–	ctDNA	4 (≥1 HER2 activating mutation or amplification)	Colorectal	Not assessed
Laurent-Puig et al. [9]	1795	III	NGS	2.9 (5.6 alterations KRAS wild type)	Colorectal	For RFS and OS on anti-EGFR-based first-line therapy
Takegawa et al. [33]	18	–	ctDNA	22 (HER2 gene copy number ratio 1.25)	Colorectal	Not assessed
Edenfield et al. [34]	4110	–	NGS, IHC/ISH	1.8	Colorectal	Not assessed

HER2 by primary tumor location						
References	Patients, N	Stage	Method	HER2 amplification, % by location	Location	Prognostic role
Weinberg et al. [35]	1602	–	CISH	5.7 left colon (patients ≤45 years) 2.1 left colon (patients ≥65 years)	Colorectal	Not assessed
Marshall et al. [36]	496	–	CISH	5.4 rectum	Colorectal	Not assessed
				3.4 descending colon		
				1.3 right colon		
Siena et al. [37]	33 (all HER2+)	IV	IHC/FISH	64 distal colon	Colorectal	Not assessed
				21 rectal		
				15 proximal colon		
Raghav et al. [38]	97	–	NGS, IHC/ISH	9.3 distal	Colorectal	For PFS on anti-EGFR-based therapy in 2nd/3rd line; not for OS
				5.2 proximal		
Ingold Heppner et al. [18]	1645	I–IV	IHC	2.1 sigmoid colon–rectum 0.9 cecum–descending colon	Colorectal	Trend toward worse survival

^aFor FISH and NGS, RFS and OS analyses performed by pooling HER2 amplifications and mutations.

CGS, comprehensive genomic sequencing; CISH, chromogenic in situ hybridization; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; NGS, next-generation sequencing; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival.

Table 2. Guidelines for HER2 testing in breast, gastric, and CRC

	Negative	Equivocal	Positive
Breast cancer (Wolff et al. [39])	IHC score 0: absence of staining or $\leq 10\%$ weak incomplete membrane staining IHC score 1+: incomplete faint/barely perceptible membrane staining $> 10\%$ ISH < 4 copies or <i>HER2</i> : CEP17 < 2 in $> 10\%$ of cells	IHC score 2+: $> 10\%$ moderate complete or strong or moderate incomplete membrane staining, $\leq 10\%$ strong complete membrane staining ISH mandatory ISH <i>HER2</i> 4–6 copies or <i>HER2</i> : CEP17 < 2 with ≥ 4 and < 6 copies in $> 10\%$ of cells	IHC score 3+: $> 10\%$ strong complete membrane staining ISH not mandatory ISH ≥ 6 copies or <i>HER2</i> : CEP17 ≥ 2 in $> 10\%$ of cells
Gastric cancer surgical specimen (Rüschoff et al. [13])	IHC score 0: no reactivity or membranous reactivity in $< 10\%$ of tumor cells IHC score 1+: faint/barely perceptible membranous reactivity in $\geq 10\%$ of cells; cells reactive only in part of their membrane ISH < 4 copies or <i>HER2</i> : CEP17 < 1.8	IHC score 2+: weak to moderate complete, basolateral, or lateral membranous reactivity in $\geq 10\%$ of tumor cells ISH mandatory ISH <i>HER2</i> 4–6 copies or <i>HER2</i> : CEP17 1.8–2 in $\geq 10\%$ of cells	IHC score 3+: strong complete, basolateral, or lateral membranous reactivity in $\geq 10\%$ of tumor cells ISH not mandatory ISH > 6 copies or <i>HER2</i> : CEP17 > 2 in $\geq 10\%$ of cells
Gastric cancer biopsy (Bartley et al. [40])	IHC score 0: no reactivity or no membranous reactivity in any tumor cell IHC score 1+: tumor cell cluster ^a with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained ISH < 4 copies or <i>HER2</i> : CEP17 < 2	IHC score 2+: tumor cell cluster ^a with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells stained ISH mandatory ISH <i>HER2</i> 4–6 copies or <i>HER2</i> : CEP17 1.8–2.0 in $\geq 10\%$ of cells indicates another 20 cells should be scored in a different target area ^b	IHC score 3+: tumor cell cluster ^a with a strong complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells stained ISH not mandatory ISH <i>HER2</i> > 6 copies or <i>HER2</i> : CEP17 ≥ 2 in $\geq 10\%$ of cells
Colorectal cancer VENTANA (based on Rüschoff et al. [13])	IHC score 0: no staining or staining in $< 10\%$ of cells IHC score 1+: faint, barely perceptible in $> 10\%$ of the cells; segmental or granular ISH < 4 copies or <i>HER2</i> : CEP17 < 1.8	IHC score 2+: weak to moderate staining in $> 10\%$ of cells; circumferential, basolateral, or lateral ISH mandatory ISH <i>HER2</i> 4–6 copies or <i>HER2</i> : CEP17 1.8–2 in $\geq 10\%$ of cells	IHC score 3+: intense in $> 10\%$ of cells; circumferential, basolateral, or lateral ISH mandatory ISH <i>HER2</i> > 6 copies or <i>HER2</i> : CEP17 > 2 in $\geq 10\%$ of cells
Colorectal cancer HERACLES (Valtorta et al. [17])	IHC score 0: no staining IHC score 1+: faint staining (segmental or granular); moderate staining $< 50\%$ of cells (any cellularity); intense staining $\leq 10\%$ cells ^c	IHC score 2+: moderate staining in $\geq 50\%$ of cells ^c ISH mandatory if $> 50\%$ cellularity confirmed following IHC retest ISH <i>HER2</i> : CEP17 ≥ 2 in $\geq 50\%$ of cells	IHC score 3+: intense staining in $\geq 50\%$ of cells ^c ISH not mandatory; intense staining in $> 10\%$ but $< 50\%$ of cells ^c ISH mandatory if $> 10\%$ cellularity confirmed following IHC retest ISH <i>HER2</i> : CEP17 ≥ 2 in $\geq 50\%$ of cells

^aAt least five consecutive cells.
^bIf additional scoring does not allow a definitive result to be rendered, multiple options are feasible: (1) consultation between scorer and pathologist regarding selection of malignant cells or tumor areas for scoring, (2) use of an alternative chromosome 17 probe in a retest to calculate the ratio, (3) selecting a different tumor block, (4) using genomics or an alternative analytic method to evaluate *HER2* amplification.
^cCircumferential, basolateral, or lateral.
CEP, chromosome enumeration probe.

patients with heterogeneous clinico-pathologic CRC characteristics, and application of diverse scoring systems [18]. More recent studies consistently indicate that *HER2* overexpression accounts for approximately 2% of all CRCs [9, 16, 18], increasing up to 5% in stage III [9] or IV *KRAS* exon 2 wild-type tumors [6, 16, 17].

HER2 testing in CRC

Although routinely used in breast and gastric cancer, IHC and fluorescent in situ hybridization (ISH; FISH) or silver-enhanced ISH (SISH) techniques have not been modified for assessment of *HER2* overexpression/amplification in CRC. Table 2 [13, 17, 39, 40] and

supplementary Figure S1, available at *Annals of Oncology* online, summarize current IHC/ISH testing guidelines in breast, gastric, and CRC.

Valtorta and colleagues conducted a diagnostic study to define specific IHC/ISH criteria to determine *HER2* positivity in CRC, and to accurately select patients with *HER2*-positive, *KRAS* wild-type metastatic CRC (mCRC) for enrollment in the phase II HERACLES (*HER2* Amplification for Colorectal Cancer Enhanced Stratification) trial of *HER2*-targeted therapy [17].

HER2 protein expression was assessed manually by IHC using the HercepTest antibody (Dako A/S Glostrup, Glostrup, Denmark), and automatically using the VENTANA 4B5 antibody on the BenchMark ULTRA platform (Ventana Medical Systems, Inc. Tucson, AZ, USA). *HER2* amplification was evaluated by FISH using a PathVysion *HER2* DNA Probe Kit (Abbott Laboratories, Abbott Park, IL), and by SISH with a VENTANA 4B5 Inform *HER2* dual-color assay on the BenchMark ULTRA platform [17]. All samples were centrally scored. *HER2* positivity was defined as tumors with *HER2* 3+ score in $\geq 50\%$ of cells by IHC, or *HER2* 2+ score and *HER2*:CEP17 ratio ≥ 2 in $\geq 50\%$ of cells by FISH [17]. Referred to as the HERACLES Diagnostic Criteria, these are more stringent than those adopted for defining *HER2* positivity in breast and gastric tumors.

In an archival cohort of 256 patients tested by an international panel of *HER2* expert pathologists, and a clinical validation cohort of 1277 patients, 5% of patients with *KRAS* wild-type mCRC had *HER2*-positive tumors according to HERACLES Diagnostic Criteria [17, 37]. In recent CRC studies, applying scoring consistent with these criteria, the rate of *HER2* positivity (IHC 2+/3+, or *HER2* ISH amplification) ranged from 1.6% to 6.3% [18, 41], in contrast to the wide-ranging values previously reported (Table 1 [9, 15–38]).

HER2 amplification in CRC has also been explored using molecular techniques such as next-generation sequencing (NGS) and comprehensive genomic sequencing (CGS), with rates ranging from 1.8% to 22.0% (Table 1 [9, 29–34]). Molecular profiling using NGS, IHC, and chromogenic ISH (CISH)/FISH in a large dataset of patients with *HER2*-overexpressing CRC revealed a 1.8% (81/4110 patients) incidence of *HER2* overexpression, with 97% concordance between *HER2* protein expression and gene amplification [34]. Shimada and colleagues retrospectively assessed the *HER2* status of 201 patients with stages I–IV CRC using IHC and FISH compared with using CGS [30]. Ten patients (5%) whose tumors were diagnosed as *HER2* positive by HERACLES Diagnostic Criteria also had *HER2* amplifications according to CGS. *HER2* status and *HER2* amplifications at the primary site were identical in all patients analyzed ($P < 0.001$), indicating the utility of CGS for detecting *HER2*-positive CRC.

The use of liquid biopsies to determine *HER2* status was first explored using blood samples from patients with breast cancer [42, 43] and was recently applied in patients with mCRC [32, 33]. Takegawa and colleagues analyzed circulating tumor DNA (ctDNA) from 18 patients with cetuximab-resistant mCRC, of which four (22%) were classified as *HER2* positive [33]. Concordance of *HER2* amplification between plasma ctDNA and tissue samples was demonstrated by rebiopsy of the metastatic lesion of one of these four patients. In a separate analysis, Schrock and coworkers isolated ctDNA from 143 patients with CRC and identified five patients (4%) with *HER2* activating mutations or amplification [32].

IHC is readily available and successful trials of therapeutic *HER2* blockade have been based on IHC results. However, it is likely that in the near future, molecular screening using NGS may replace IHC. Although NGS is now more expensive, it has the advantage of capturing a wider range of genome abnormalities including *HER2*-activating mutations (see section ‘Are *HER2* mutations actionable therapeutic targets in mCRC?’) and allowing quantitation of gene copy number.

Distribution and prognostic effect of *HER2* in CRC

Clinical and pathologic features of *HER2*-positive CRC

Tumors originating in the right or left side of the colon and rectum differ in their epidemiology, pathology, mutation profile, and presentation, likely due to distinct embryologic origins of the proximal and distal colon [44]. Proximal, or right-sided, tumors are more likely to be hypermethylated or to have microsatellite instability (MSI) than distal tumors [5]. Right-sided tumors are also more common in women and the elderly [45]. Recent meta-analyses showed a consistent and significant worsening in overall survival (OS) in mCRC tumors originating in the right versus the left side of the colon [46–48].

A number of CRC studies have reported differential expression related to the occurrence of *HER2* amplification based on clinical and pathologic features of the tumor, including primary location (Table 1). Analysis of gene expression and DNA copy number data for patients with CRC in the PETACC-3 adjuvant chemotherapy trial revealed that distal carcinomas (splenic flexure, descending colon, rectum) were more likely to be *HER2* or *EGFR* amplified than proximal carcinomas (cecum, ascending, hepatic flexure, transverse colon) [49]. Similar results were reported in advanced CRC, with *HER2* amplifications identified using SISH correlating with a distal location [50]. A retrospective analysis identified higher frequencies of *HER2* overexpression/amplification in rectal cancers compared with descending colon or right colon cancers [36]. A similar trend for more frequent *HER2* overexpression in tumors of the sigmoid colon-rectum than the cecum-descending colon was reported in a large series of primary CRC cases [18]. In the HERACLES-A trial, among 33 patients with *HER2*-positive mCRC, 64% had distal tumors and 21% had rectal tumors [37]. Retrospective data from the phase II EXPERT-C trial, in patients with high-risk, locally advanced rectal cancer receiving neoadjuvant capecitabine and oxaliplatin plus chemoradiotherapy with or without cetuximab, showed a 4.3% prevalence of *HER2* overexpression/amplification using IHC/ISH [51]. These findings are in line with the 5.4% *HER2*-positivity rate for rectal cancers described by Marshall and colleagues [36].

In contrast to studies reporting a correlation between *HER2* amplification and tumor location, other researchers have found no such association. In the PETACC-8 FOLFOX-based adjuvant stage III colon cancer study, no significant differences were seen between patient groups on the basis of tumor location [9]. A retrospective analysis of two independent cohorts of patients with mCRC observed no significant difference in *HER2* expression between right- and left-sided primary tumors [38]. A trend toward a

decreasing frequency of HER2-positive tumors by IHC was noted from colon to rectum in 3/77 (4%) HER2-positive specimens, but this was not statistically significant ($P = 0.251$) [23]. Several studies have considered the relationship between *KRAS* status and *HER2* amplification in CRC. In a meta-analysis of 3256 patients with mCRC, *HER2* amplification using FISH and gene copy number variation was associated with *KRAS/BRAF* wild-type status at all disease stages: 5.2% wild type versus 1.0% mutated tumors ($P < 0.0001$) in stage IV, and 2.1% versus 0.2%, respectively, in stages II–III ($P = 0.01$) [16]. Similarly, in the PETACC-8 study, *HER2* alterations determined using NGS, IHC, and FISH were detected in 42 (5.6%) patients with *KRAS* wild-type tumors compared with 22 (2.4%) patients with *RAS* mutation ($P < 0.001$) [9]. *HER2* amplifications according to SISH were also correlated with *KRAS* wild-type status in 191 patients with mCRC and distant metastases, but only with borderline significance ($P = 0.052$) [50].

In a meta-analysis of 30 studies enrolling 4942 patients with CRC, *HER2* expression assessed by IHC was significantly higher in patients with Duke's stage C/D tumors compared with Duke's stage A/B tumors [odds ratio (OR) 0.335, 95% confidence interval (CI) 0.198–0.568; $P < 0.001$], and in patients with versus without lymph node metastasis (OR 1.987, 95% CI 1.209–3.265; $P = 0.007$) [52]. However, no significant association was found between *HER2* expression and CRC location (rectal versus colon; OR 1.123, 95% CI 0.858–1.468; $P = 0.399$).

Prognostic role of HER2 in CRC

The prognostic role of *HER2* in CRC remains uncertain. A negative prognostic impact of *HER2* overexpression was proposed in earlier studies [27, 28], but more recent trials have found no association between *HER2* amplification and outcome (Table 1 [16, 19, 21, 22, 25]). However, in one of the largest study cohorts examined (1645 patients with stages I–IV CRC), a trend toward worse OS was reported for the 26 (1.6%) patients with *HER2*-positive disease compared with those with *HER2*-negative disease [18]. *HER2* was also identified as a poor prognostic indicator in the PETACC-8 study in patients with stage III colon cancer [9]. *HER2* alterations were present in 66/1689 evaluated patients (3.9%). *HER2* concordant amplification-positive status by both NGS and FISH, and *HER2* mutation status determined by NGS, were associated with shorter time to recurrence [hazard ratio (HR) 1.9, 95% CI 1.1–3.2; $P = 0.03$] and shorter OS (HR 1.7, 95% CI 0.9–3.2; $P = 0.045$). This prognostic value was maintained after adjustment for age, treatment, *RAS* mutation, histologic grade, tumor location, pT and pN status, bowel obstruction or perforation, and vascular or lymphatic invasion. Assessment of the potential prognostic effect of *HER2* amplifications in CRC is hindered by the low incidence of these alterations, potentially explaining the inconsistent results of studies addressing this question. Nevertheless, based on available data, it appears that the negative prognostic effect of *HER2* is not as marked as that of other alterations such as *BRAF* mutation.

HER2 as a negative biomarker for EGFR-targeted treatments in CRC

CRCs are a molecularly heterogeneous group of tumors that often harbor mutations in *KRAS*, *BRAF*, or *PIK3CA*, as well as *HER2*

amplifications. These genetic alterations confer resistance to EGFR-targeted therapies in patients with CRC [1, 53, 54], although currently only selection based on *RAS* status is recommended for excluding patients from anti-EGFR treatment [55–57]. Using data generated from patient-derived mCRC xenografts, Bertotti and coworkers identified *HER2* amplification as a negative predictor of response to cetuximab [1, 4]. Two retrospective clinical series supported that activation of *HER2* signaling causes resistance to cetuximab [58, 59]. Yonesaka and colleagues evaluated the clinical impact of *de novo HER2* amplification in 233 cetuximab-treated patients [58]. Median progression-free survival (PFS) and OS were almost halved in patients with *HER2*-amplified ($n = 13$) versus nonamplified tumors ($n = 220$): PFS 89 versus 149 days, respectively; OS 307 versus 515 days, respectively; $P = 0.0013$, log-rank test [58]. Survival outcomes were negatively influenced by *HER2* gene copy number in a second series of 170 patients with *KRAS* wild-type mCRC treated with cetuximab or panitumumab alone or in combination with chemotherapy [59]. Raghav and coworkers analyzed the impact of *HER2* amplification on the efficacy of anti-EGFR monoclonal antibody therapy in *RAS* and *BRAF* wild-type mCRC [38]. They tested *HER2* amplification in a first cohort of 97 patients using IHC and dual ISH (*HER2*: CEP17 ≥ 2.2), and validated their findings in a second cohort of 99 patients, which comprised 37 cases of *HER2* amplification identified by NGS (*HER2* gene copy number ≥ 4) and 62 *HER2* nonamplified control patients pretreated with anti-EGFR antibodies. Median PFS on anti-EGFR therapy was significantly shorter in patients with *HER2* amplification versus non-*HER2*-amplification (2.9 months versus 8.1 months, HR 5.0, $P < 0.0001$). These findings were confirmed in the validation cohort, in which 69 patients received anti-EGFR treatment after first-line therapy; median PFS was significantly shorter for patients harboring *HER2*-amplified versus nonamplified tumors (2.8 months versus 9.3 months, HR 6.6, $P < 0.0001$). Notably, these subgroups had a similar OS ($P = 0.86$) and PFS while on first-line therapy ($P = 0.62$) [38]. Finally, in the HERACLES-A study, conducted exclusively in *HER2*-positive mCRC, Sartore-Bianchi and colleagues reported that patients who had previously received panitumumab or cetuximab, evaluable according to rigorous clinical criteria, were resistant to such therapy [6].

As summarized in Table 3 [4, 6, 37, 38, 58, 59], the results from these experimental and clinical studies concur that *HER2* activation substitutes for EGFR dependence in a fraction of patients with CRC. From a clinical perspective, this notion could potentially impact on optimal therapeutic sequence; however, it should be interpreted with caution since the data are retrospective and need to be validated in prospective clinical studies of patients treated with cetuximab or panitumumab.

HER2 as a novel therapeutic target in CRC

HER2 has been investigated as a therapeutic target in mCRC in several small studies during the last decade, but with differing outcomes (Table 4 [7, 37, 60, 61]). A phase II study assessed the combination of trastuzumab and FOLFOX therapy as second- or third-line treatment of patients with mCRC [61]. Notably, patients with IHC *HER2* 2+ tumors were eligible for enrollment and no ISH testing was planned. Overall, 26 of 653 (4%) of

Table 3. Studies addressing the predictive role of HER2 to EGFR-targeted therapies in mCRC

Reference	Patients, N	HER2 amplification, % of patients	Method	Prediction of resistance to cetuximab or panitumumab
Bertotti et al. [4]	85	13.6 (KRAS WT, cetuximab- or panitumumab-resistant)	IHC/FISH (LSI HER2/CEP17 PathVysion probe)	HER2 amplification or overexpression in 6/44 (13.6%) patients with KRAS WT tumors without objective response to cetuximab or panitumumab versus 0/45 (0%) patients with objective response ($P < 0.05$) ^a
Yonesaka et al. [58]	233 (182 KRAS WT)	5.6 (6.0 KRAS WT)	FISH (LSI HER2 SO/CEP17 PathVysion probe)	Median OS longer in HER2 nonamplified versus HER2-amplified (515 versus 307 days, $P = 0.0013$) ^b
Martin et al. [59]	162 (KRAS WT)	20 (HER2: CEP17 ≥ 2 in $\geq 10\%$ of tumor cells) 6 [HER2: CEP17 ≥ 2 in $\geq 90\%$ of tumor cells (HER2-all-A profile)]	FISH (LSI HER2-neu/CEP17 probe)	Median PFS longer in HER2 FISH+ versus HER2 FISH- (7.4 versus 3.9 months, HR 2.00, 95% CI 1.42–2.83, $P < 0.0001$) ^c
Sartore-Bianchi et al. [6] and Siena et al. [37]	33 [36]	100	IHC/FISH	No objective response to cetuximab or panitumumab in 15/27 patients [6] with HER2-positive tumors assessed for sensitivity to cetuximab or panitumumab according to rigorous criteria
Raghav et al. [38]	97 (cohort 1); 99 (cohort 2)	14% cohort 1; RAS/BRAF-WT cohort 2; not reported	IHC/ISH cohort 1 ^d ; NGS cohort 2 ^e	Median PFS on anti-EGFR therapy shorter in patients with HER2-amplified versus HER2 nonamplified tumors (2.9 versus 8.1 months, HR 5.0, $P < 0.0001$) [cohort 1]; (2.8 versus 9.3 months, HR 6.6, $P < 0.0001$) [cohort 2]

^aData supported by a molecularly annotated platform of patient-derived xenografts.

^bAcquired HER2 amplification in two cases of secondary resistance to cetuximab.

^cHER2 FISH+: HER2 gene copy number gain (presence of ≥ 4 copies of the HER2 gene in $\geq 40\%$ of cells) and HER2-amplified (HER2 gene amplification defined as HER2: CEP17 ≥ 2 in $\geq 10\%$ of cells).

^dHER2 amplification defined as HER2: CEP17 ≥ 2.2 .

^eHER2 amplification defined as HER2 ≥ 4 copies.

HR, hazard ratio; mCRC, metastatic colorectal cancer; WT, wild type.

screened tumor blocks scored HER2 $\geq 2+$. Among 21 evaluable patients, 5 (24%) achieved a partial response. However, the low rate of HER2 positivity precluded completion of the trial. Results of a subsequent phase II study of first- or second-line trastuzumab plus irinotecan in nine patients with HER2-overexpressing advanced CRC were also reported [60]. HER2 overexpression by IHC was evident in 11 of 138 (8.0%) screened tumors (HER2 2+ in 5 patients and HER2 3+ in 6 patients). Partial responses were observed in five of seven evaluable patients (71%), with responses maintained for ≥ 6 weeks in four patients. The median survival time was 14 months. The study was closed prematurely due to lack of accrual.

A case report was also published of two patients with mCRC and liver metastases who responded to capecitabine, oxaliplatin, and lapatinib while on a clinical trial, but HER2 status was not determined in these patients [62].

The inconclusive efficacy findings and poor accruals observed in these studies most likely relate to the absence of a number of important prerequisites in study design, including a mechanistically based HER2-targeted preclinical strategy, patient selection using a validated HER2 scoring system, and a preplanned sample size based on the actual estimated incidence of HER2 amplification. Moreover, due to concomitant chemotherapy in some studies, it is difficult to establish the specific contribution of HER2 blockade on therapeutic outcome.

Using xenografts derived from patients with mCRC in preclinical therapeutic trials, Bertotti and colleagues identified amplified HER2 as an effective therapeutic target in cetuximab-resistant CRC [1, 4]. Patient-derived mCRC xenografts with HER2 amplification were sensitive to HER2-blockade with trastuzumab in combination with lapatinib, but not to either agent alone. These preclinical data formed a strong rationale for

Table 4. Clinical studies exploiting HER2 as a target for mCRC

Reference	Phase	Patients, N	HER2 overexpression, %	Treatment	Line of treatment	Objective response rate, %
<i>Published studies</i>						
Ramanathan et al. [60]	II	9 ^a	3.6 (2+) 4.3 (3+)	Trastuzumab and irinotecan	1st/2nd	71
Clark et al. [61]	II	21 ^b	4.0 (2+/3+)	5-FU, leucovorin, oxaliplatin and trastuzumab	2nd/3rd	24
HERACLES-A; Siena et al. [37]	II	33	21 (2+) 79 (3+)	Trastuzumab and lapatinib	Salvage	30.3
MyPathway; Hainsworth et al. [8]	II	37	100 ^c	Trastuzumab and pertuzumab	Salvage	38
<i>Ongoing studies</i>						
HERACLES-B; Siena et al. [37]	II	30	100 (17, 2+; 83, 3+)	Pertuzumab and T-DM1	2nd/3rd	Not reported
HERACLES-RESCUE; Siena et al. [37]	II	9	100	T-DM1	Salvage	Not reported
S1613 (NCT03365882)	II	Not available	Not reported	Trastuzumab and pertuzumab or cetuximab and irinotecan	2nd or later	Not reported
MODUL (NCT02291289)	II	Not available	Not reported	Capecitabine, trastuzumab, and pertuzumab	Biomarker-driven maintenance therapy after first-line FOLFOX + bevacizumab induction	Not reported
NCT03384940	II	Not available	Not reported	DS-8201 (investigational trastuzumab conjugated with deruxtecan)	3rd	Not reported

^aThe study was prematurely closed due to low accrual.

^bThe low rate of HER2 positivity precluded completion of the trial.

^cPatients with HER2 mutations were also eligible; see text for details.

DS-8201, trastuzumab deruxtecan; 5-FU, 5-fluorouracil; T-DM1, trastuzumab emtansine.

clinical trials targeting HER2 genetic alterations in patients with mCRC [1, 4, 63], paving the way for the HERACLES studies. Recently, results of the HERACLES-A phase II trial of dual HER2-targeted therapy (trastuzumab plus lapatinib) in CRC were presented [6, 37]. This proof-of-concept trial was conducted in patients with *KRAS* wild-type, HER2-positive mCRC who were refractory to standard-of-care treatments, including cetuximab or panitumumab. The HER2 status of the patients was determined using the CRC-specific HERACLES Diagnostic Criteria [17]. At the data cutoff of 28 February 2017, 33 patients had been enrolled and were evaluable for response. Complete responses were observed in two patients (6.1%) and partial responses in eight patients (24.2%), giving an overall response rate (ORR) of 30.3% (Table 4) [37]. Patients with *HER2* gene copy number ≥ 9.6 had significantly longer time to progression (median 26.6 weeks versus 13.4 weeks; $P = 0.0001$) and longer OS (median 53.1 weeks versus 34.0 weeks; $P = 0.0058$) than those with *HER2* gene copy number < 9.6 [37]. The combination regimen was well tolerated in this heavily pretreated population (median of five prior regimens), with no grade 4/5 adverse events and no withdrawals due to patient

request [37]. Novel methods of generating additional evidence to make this regimen widely available in clinical practice are needed.

Based on preliminary data from the phase II MyPathway trial, investigating agents targeting the HER2, EGFR, BRAF, or Hedgehog pathways in tumors for which these therapies are not currently indicated, the CRC cohort was expanded to enroll 37 patients with HER2-amplified/overexpressed mCRC who had exhausted standard treatments [8]. Patients received a combination of trastuzumab and pertuzumab. The ORR was 38% (95% CI 22.2–56.4) (Table 4). Four (11%) patients had stable disease for > 4 months [8]. Median PFS was 4.6 months (95% CI 1.6–9.8) and median OS was 10.3 months (95% CI 7.2–22.1) [7]. ORR was higher in patients with wild-type versus mutated *KRAS* (52.0% versus 0%, respectively), and in patients with left-sided colon cancer (42.9%) or rectal cancer (45.5%) versus right-sided colon cancer (12.5%) [7].

In support of the available preclinical and clinical data, anecdotal case reports of patients with HER2-positive mCRC who have achieved substantial clinical benefit with targeted anti-HER2 therapy have recently been published [64–66].

Challenges and open issues toward clinical application

Are HER2 mutations actionable therapeutic targets in mCRC?

A number of *HER2* activating mutations, sometimes in concomitance with *HER2* amplification, have been identified in CRC. These mutations are present in approximately 7% of CRCs, based on TCGA data, and may co-exist with *HER2* gene amplification in around 20% of cases [63]. They are similar to those seen in breast cancer and include kinase domain single nucleotide variations such as V842I, V777L, and L755S, and extracellular domain mutations such as S310F [1, 5, 63]. Introduction of these four mutations into immortalized mouse colon epithelial cells triggered *HER2* signaling pathways, with increases in *HER2*, MAPK, and AKT phosphorylation noted relative to *HER2* wild-type transduced cells [63]. These *HER2* mutations also dramatically increased the number of colonies formed in soft agar, demonstrating enhanced anchorage-independent growth. Furthermore, these *HER2*-activating mutations produced resistance to the EGFR monoclonal antibodies, cetuximab or panitumumab, when transfected into two cetuximab-sensitive CRC cell lines [63]. In patient-derived CRC xenografts containing the *HER2*-activating mutations S310Y or L866M, treatment with trastuzumab, neratinib, or lapatinib alone delayed tumor growth, but after 30 days, the mice developed large tumors. However, dual *HER2*-targeted therapy with trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in *HER2*-amplified mCRC [63]. Lack of effect of neratinib monotherapy in *HER2*-mutant CRC was recently confirmed in a basket trial of 125 patients with different tumors harboring *HER2* mutations, including 12 patients with CRC [67]. In the latter cohort, there were no objective responses and median PFS was just 1.8 months. In the MyPathway trial, eight patients with mCRC had tumors that harbored *HER2* mutations and one of these (12.5%) achieved an investigator-assessed objective response with the combination of pertuzumab and trastuzumab (H. Hurwitz, personal communication). Overall, these data indicate that monotherapy with *HER2* small molecule inhibitors is ineffective. It remains to be determined whether *HER2*-directed combination therapy with monoclonal antibodies or monoclonal antibodies plus small molecule inhibitors can be effective in *HER2*-mutated tumors.

What are the determinants of resistance to HER2-directed therapies?

Understanding the mechanisms of primary and secondary resistance to *HER2* blockade is a priority to develop more effective and additional lines of therapy, because 40%–50% of patients treated within the HERACLES-A and MyPathway trials did not achieve partial response or prolonged stable disease despite *HER2* amplification [6, 8]. Moreover, even in patients displaying disease control, secondary resistance occurs in almost all cases. Preclinical models of *HER2* therapeutic blockade were carried out in quadruple negative mCRC (i.e. *KRAS*, *NRAS*, *PIK3CA*, and *BRAF* wild-type) since aberrations in one or more of these effectors could impact on and compensate for the inhibition exerted by *HER2*-directed therapy [4, 6, 7]. Based on these findings, the

HERACLES-A trial was performed in *KRAS* exon 2 wild-type patients, thus positive clinical results can be applied to this subset of patients only. Anecdotally, we treated two patients harboring *KRAS* exon 2 mutations off-study, and neither obtained a partial response (S. Siena and A. Sartore-Bianchi, personal communication). The MyPathway study included patients with *KRAS*-mutated CRC, but none of these patients responded to *HER2*-directed therapy [7], confirming the biologic rationale underlying resistance.

Extensive studies utilizing pre- and post-treatment tissue samples are ongoing to define the molecular basis of primary and secondary resistance to trastuzumab and lapatinib in *HER2*-positive mCRC. Preliminary data obtained by molecular profiling of plasma samples collected during the HERACLES-A trial suggest that alterations affecting *HER2* parallel pathways controlled by receptor tyrosine kinases, and/or downstream effectors such as RAS and PI3KA, are implicated in primary and secondary resistance to *HER2* blockade (G. Siravegna, unpublished data). Once completed, these analyses may have important implications for the selection of patients with mCRC most likely to benefit from anti-*HER2* therapies, as well as providing the basis for designing new treatments in patients who experience disease progression.

When to test for HER2 and how an HER2-directed therapy may integrate in the treatment algorithm of mCRC

Based on the consistent therapeutic actionability of dual *HER2* inhibition in refractory mCRC [6, 8], it is feasible that a similar effect may be obtained by targeting *HER2* in earlier lines of treatment and testing for *HER2* alongside other biomarkers being assessed at the time of diagnosis of metastatic disease [68]. *HER2* amplification in mCRC can be considered an orphan molecular entity. Difficulties in developing a targeted treatment of this cancer, up to the level of regulatory approval, are being addressed with tools developed in the context of international initiatives [69]. While speculative in the absence of randomized data, hypotheses can be made regarding clinical trials with *HER2*-directed therapies aimed at defining optimal positioning and combination partners in the treatment algorithm of mCRC (Figure 1). National and international collaborations and translational studies designed to understand mechanisms of resistance to *HER2* inhibition will be of paramount importance to the successful development of these trials. Based on available results [6, 8], these studies should be restricted to *KRAS* exon 2 wild-type tumors. Although data regarding the potential impact on sensitivity exerted by other *RAS* mutations outside *KRAS* exon 2 are lacking, application of expanded *RAS* wild-type criteria might be more realistic. Finally, since *HER2* amplification might act as a negative predictor of response to EGFR-targeted treatments, *HER2*-directed therapy before cetuximab or panitumumab in the continuum of care of these patients should be considered a reasonable option.

Available data suggest a $\geq 30\%$ ORR with dual *HER2* inhibition, which compares favorably with results of second-line standard treatment options in patients with *RAS* wild-type CRC previously treated with a first-line regimen including an anti-EGFR component. In patients who do not receive an anti-EGFR in first-line, second-line chemotherapy plus anti-EGFR

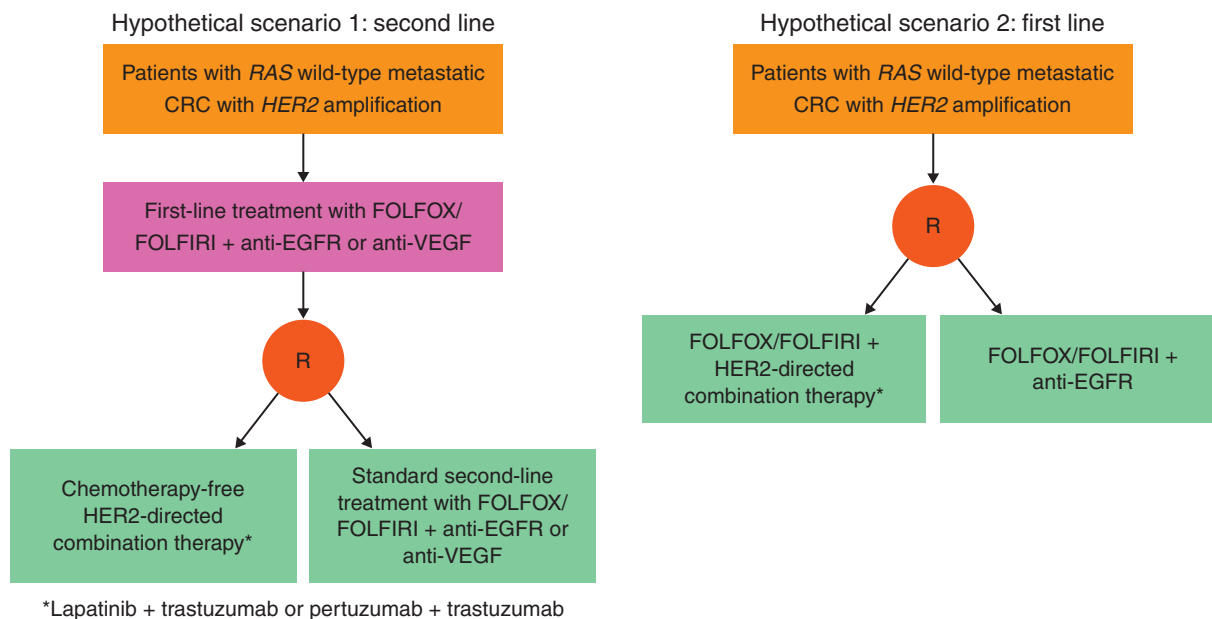


Figure 1. Scenarios for positioning of clinical trials with HER2-targeted treatments in mCRC.

(especially in *RAS* and *BRAF* selected cases [70]) might produce good responses, but given the potential negative predictive role of *HER2* amplification, this is unlikely to be the case in this patient population. In light of these considerations, and of the expected lower toxicity of a chemotherapy-free regimen, a dual HER2-targeted combination might be an optimal choice for a trial in this setting, either after FOLFOX/FOLFIRI with an anti-EGFR or a vascular endothelial growth factor (VEGF)-targeted component, and compared with standard second-line options.

A hypothetical alternative might be starting with an HER2-targeted component during first-line treatment. However, it is uncertain whether a chemotherapy-free HER2-targeted regimen would perform better than standard FOLFOX/FOLFIRI or FOLFOXIRI+/- EGFR- or VEGF-targeted options, which produce ORRs of 59%–65% in this setting [71, 72]. Therefore, a combination of HER2-directed treatment with chemotherapy should be considered, taking into account safety issues for the combination and the fact that HER2 might act as a negative predictive biomarker of response to EGFR-targeted treatment. For the latter reason, comparison between a FOLFOX/FOLFIRI backbone with an HER2-directed agent versus the same chemotherapy with an anti-EGFR agent might offer a straight-forward answer on the optimal sequencing in *RAS* wild-type tumors, and whether or not to offer an anti-EGFR drug upfront to these patients.

Conclusion

HER2 amplification is a clinically relevant genetic alteration in mCRC as documented by the HERACLES [6, 37] and MyPathway [7, 8] studies. This biomarker can be screened for with established diagnostic tools [16, 17], occurs in a sizable 5% of patients with *KRAS* wild-type mCRC, and can potentially act as a predictor of lack of benefit to anti-EGFR monoclonal antibodies [4].

HER2-targeted therapy compares favorably with emerging therapeutic strategies for mCRC such as *BRAF*-directed therapy and immunotherapy with checkpoint inhibitors. *HER2* amplification displays an incidence similar to that of MSI-high (MSI-H) tumors (5%) [73] and lower than that of *BRAF* mutations (10%); however, compared with *BRAF*-directed combinations (ORR 16%–21%; median PFS 4.2 months [74]), responses achieved so far in clinical studies with HER2-directed therapies are higher (ORR 30%–38% [6, 8]) and more durable (median PFS 5.2 months [6]), resembling results obtained with checkpoint inhibitors in MSI-H tumors [75]. The toxicity of HER2-targeted combinations is also less than *BRAF*- or MSI-H-directed therapeutics [74, 75]. Thus, HER2-directed therapies appear to reconcile the merits of precision medicine (rapid and deep induction of tumor shrinkage) with those of immunotherapy (durable responses and better tolerability).

Although evidence from phase III trials with HER2-targeted agents is lacking, randomized studies will take a long time to achieve results in such a selected population [69]. The strong underlying biologic rationale [4], consistent actionability at the therapeutic level [6, 8], and favorable comparison with other precision medicine approaches support consideration for conditional approval of HER2-targeted agents for clinical use by regulatory agencies.

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