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 few 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

<table>
<thead>
<tr>
<th>HER2 testing by IHC/ISH</th>
<th>Patients, N</th>
<th>Stage</th>
<th>Monoclonal antibody</th>
<th>HER2 3+, %</th>
<th>Location</th>
<th>Prognostic role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni et al. [15]</td>
<td>4913</td>
<td>–</td>
<td>–</td>
<td>1.4</td>
<td>Colorectal</td>
<td>Yes</td>
</tr>
<tr>
<td>Laurent-Puig et al. [9]</td>
<td>1795</td>
<td>III</td>
<td>4BS Ventana</td>
<td>1.4 (n=21/1457)</td>
<td>Colon</td>
<td>Yes for IHC/FISH only for RFS but not OSa</td>
</tr>
<tr>
<td>Richman et al. [16]</td>
<td>1914</td>
<td>II–III</td>
<td>A 0485 Dako</td>
<td>1.3</td>
<td>Colorectal</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>1342</td>
<td>IV</td>
<td>A 0485 Dako</td>
<td>2.2</td>
<td>Colorectal</td>
<td>No</td>
</tr>
<tr>
<td>Valtorta et al. [17]</td>
<td>1086</td>
<td>–</td>
<td>4BS Ventana/Hercep-Test</td>
<td>4.1</td>
<td>Colorectal</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Ingold Heppner et al. [18]</td>
<td>1645</td>
<td>I–IV</td>
<td>Clone SP3</td>
<td>0.5 (1.6 total CISH+)</td>
<td>Colorectal</td>
<td>Trend toward worse survival</td>
</tr>
<tr>
<td>Song et al. [19]</td>
<td>106</td>
<td>pt1, pt2, pt3</td>
<td>4BS SP3</td>
<td>7.5 (2/3+)</td>
<td>Colorectal</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.8 (2/3+)</td>
<td>Colorectal</td>
<td>No</td>
</tr>
<tr>
<td>Conradi et al. [20]</td>
<td>264</td>
<td>II/III rectal</td>
<td>PATHWAY (Ventana) pretreatment biopsies</td>
<td>5.9 (n=10/169)</td>
<td>Rectal</td>
<td>Yes</td>
</tr>
<tr>
<td>Kruwszewski et al. [21]</td>
<td>202</td>
<td>II–IV</td>
<td>A 0485</td>
<td>15.3</td>
<td>Colorectal</td>
<td>No</td>
</tr>
<tr>
<td>Kountourakis et al. [22]</td>
<td>106</td>
<td>–</td>
<td>NCL-CB11</td>
<td>2.8 membranous</td>
<td>Colorectal</td>
<td>No</td>
</tr>
<tr>
<td>Schuell et al. [23]</td>
<td>77</td>
<td>I–IV</td>
<td>Hercep-Test</td>
<td>3</td>
<td>Colorectal</td>
<td>Trend toward worse survival</td>
</tr>
<tr>
<td>Essapen et al. [24]</td>
<td>170</td>
<td>II–III</td>
<td>HM64.13</td>
<td>54.1 (2+, cytoplasmic) 40.0 (2+, membranous)</td>
<td>Colorectal</td>
<td>Yes (cytoplasmic, stage III)</td>
</tr>
<tr>
<td>McKay et al. [25]</td>
<td>249</td>
<td>I–IV</td>
<td>NCL-CB11</td>
<td>3.2</td>
<td>Colorectal</td>
<td>No</td>
</tr>
<tr>
<td>Rossi et al. [26]</td>
<td>156</td>
<td>I–III</td>
<td>–</td>
<td>4 (2/3+)</td>
<td>Colorectal</td>
<td>No</td>
</tr>
<tr>
<td>Osako et al. [27]</td>
<td>146</td>
<td>–</td>
<td>Nichirei</td>
<td>4.5 (HER2 amplification)</td>
<td>Colorectal</td>
<td>Yes</td>
</tr>
<tr>
<td>Kapitanović et al. [28]</td>
<td>221</td>
<td>–</td>
<td>Ab-3</td>
<td>43 (n=67/155 adenocarcinomas)</td>
<td>Colorectal</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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

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

For IHC 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

<table>
<thead>
<tr>
<th></th>
<th>Negative</th>
<th>Equivocal</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breast cancer</strong></td>
<td>IHC score 0; absence of staining or ≤10% weak incomplete membrane staining</td>
<td>IHC score 2+: &gt;10% moderate complete or strong or moderate incomplete membrane staining, ≤10% strong complete membrane staining</td>
<td>IHC score 3+: &gt;10% strong complete membrane staining</td>
</tr>
<tr>
<td>(Wolff et al. [39])</td>
<td>IHC score 1+: incomplete faint/barely perceptible membrane staining &gt;10%</td>
<td>ISH mandatory</td>
<td>ISH not mandatory</td>
</tr>
<tr>
<td></td>
<td>ISH &lt;4 copies or HER2: CEP17 &lt;2 in &gt;10% of cells</td>
<td>ISH HER2 4–6 copies or HER2: CEP17 &lt;2 with ≥4 and &lt;6 copies in &gt;10% of cells</td>
<td>ISH ≥6 copies or HER2: CEP17 ≥2 in &gt;10% of cells</td>
</tr>
</tbody>
</table>

| **Gastric cancer surgical specimen** | IHC score 0: no reactivity or membranous reactivity in <10% of tumor cells | IHC score 2+: weak to moderate complete, basolateral, or lateral membranous reactivity in ≥10% of tumor cells | IHC score 3+: strong complete, basolateral, or lateral membranous reactivity in ≥10% of tumor cells |
| (Rüschhoff et al. [13]) | IHC score 1+: faint/barely perceptible membranous reactivity in ≥10% of cells; cells reactive only in part of their membrane | ISH mandatory | ISH not mandatory |
|                     | ISH <4 copies or HER2: CEP17 <1.8 | ISH HER2 4–6 copies or HER2: CEP17 1.8–2 in ≥10% of cells | ISH >6 copies or HER2: CEP17 ≥2 in >10% of cells |

| **Gastric cancer biopsy** | IHC score 0: no reactivity or no membranous reactivity in any tumor cell | IHC score 2+: tumor cell clustera with a weak to moderate complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells stained | IHC score 3+: tumor cell clustera with a strong complete, basolateral, or lateral membranous reactivity irrespective of percentage of tumor cells stained |
| (Bartley et al. [40]) | IHC score 1+: tumor cell clustera with a faint/barely perceptible membranous reactivity irrespective of percentage of tumor cells stained | ISH mandatory | ISH not mandatory |
|                     | ISH <4 copies or HER2: CEP17 <2 | ISH HER2 4–6 copies or HER2: CEP17 1.8–2 in ≥10% of cells | ISH HER2 >6 copies or HER2: CEP17 ≥2 in ≥10% of cells |

| **Colorectal cancer VENTANA** | IHC score 0: no staining or staining in <10% of cells | IHC score 2+: weak to moderate staining in >10% of cells; circumferential, basolateral, or lateral | IHC score 3+: intense in >10% of cells; circumferential, basolateral, or lateral |
| (based on Ruschhoff et al. [13]) | IHC score 1+: faint, barely perceptible in >10% of the cells; segmental or granular | ISH mandatory | ISH not mandatory |
|                     | ISH <4 copies or HER2: CEP17 <1.8 | ISH HER2 4–6 copies or HER2: CEP17 1.8–2 in ≥10% of cells | ISH HER2 >6 copies or HER2: CEP17 ≥2 in >10% of cells |
|                     | IHC score 0: no staining | IHC score 2+: moderate staining in 50% of cells | IHC score 4+: intense staining in >50% of cells |
|                     | IHC score 1+: faint staining (segmental or granular); moderate staining <50% of cells (any cellularity); intense staining ≤10% cellsa | ISH mandatory if >50% cellularity confirmed following IHC retest | ISH not mandatory; intense staining in >10% but <50% of cellsa |
|                     | ISH <4 copies or HER2: CEP17 <2 | ISH HER2: CEP17 ≥2 in >50% of cells | ISH HER2: CEP17 ≥2 in ≥50% of cells |

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

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 CRGs [9, 16, 18], increasing up to 5% in stage III [9] or IV KRAS exon 2 wild-type tumors [6, 16, 17].
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 ≥50% of cells by IHC, or HER2 2+ score and HER2 CEP17 ratio ≥2 in ≥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 capcitabine 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 FOLFIRI-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 KRAS 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, KRAS mutation, histologic grade, tumor location, pT and pN status, bowel obstruction or perforation, or 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 315 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
screened tumor blocks scored HER2 ≥ 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 3. Studies addressing the predictive role of HER2 to EGFR-targeted therapies in mCRC

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patients, N</th>
<th>HER2 amplification, % of patients</th>
<th>Method</th>
<th>Prediction of response to cetuximab or panitumumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bertotti et al. [4]</td>
<td>85</td>
<td>13.6 (KRAS WT, cetuximab- or panitumumab-resistant)</td>
<td>IHC/FISH (LSI HER2/C EP17 PathVysion probe)</td>
<td>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&lt;0.05)²</td>
</tr>
<tr>
<td>Yonesaka et al. [58]</td>
<td>233 (182 KRAS WT)</td>
<td>5.6 (6.0 KRAS WT)</td>
<td>FISH (LSI HER2 SO/C EP17 PathVysion probe)</td>
<td>Median OS longer in HER2 nonamplified versus HER2-amplified (S15 versus 307 days, P=0.0013)⁹</td>
</tr>
<tr>
<td>Martin et al. [59]</td>
<td>162 (KRAS WT)</td>
<td>20 (HER2: CEP17 ≥ 2 in ≥10% tumor cells) 6 (HER2: CEP17 ≥ 2 in ≥90% of tumor cells (HER2-all-A profile))</td>
<td>FISH (LSI HER2-neu/C EP17 probe)</td>
<td>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&lt;0.0001)¹²</td>
</tr>
<tr>
<td>Sartore-Bianchi et al. [6] and Siena et al. [37]</td>
<td>33 [36]</td>
<td>100</td>
<td>IHC/FISH</td>
<td>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</td>
</tr>
<tr>
<td>Raghav et al. [38]</td>
<td>97 (cohort 1); 99 (cohort 2)</td>
<td>14% cohort 1; RAS/BRCAF-WT cohort 2; not reported</td>
<td>IHC/ISH cohort 1³, NGS cohort 2⁶</td>
<td>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&lt;0.0001) [cohort 1]; (2.8 versus 9.3 months, HR 6.6, P&lt;0.0001) [cohort 2]</td>
</tr>
</tbody>
</table>

*Data supported by a molecularly annotated platform of patient-derived xenografts.
²Acquired HER2 amplification in two cases of secondary resistance to cetuximab.
³HER2 FISH+: HER2 gene copy number gain (presence of ≥4 copies of the HER2 gene in ≥40% of cells) and HER2-amplified (HER2 gene amplification defined as HER2: CEP17 ≥ 2 in ≥10% of cells).
⁴HER2 amplification defined as HER2: CEP17 ≥ 2.2.
⁵HER2 amplification defined as HER2 ≥ 4 copies.
⁶HR, hazard ratio; mCRC, metastatic colorectal cancer; WT, wild type.
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/C21 > 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].

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

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

\[a\] The study was prematurely closed due to low accrual.

\[b\] The low rate of HER2 positivity precluded completion of the trial.

\[c\] Patients with HER2 mutations were also eligible; see text for details.

DS-8201, trastuzumab deruxtecan; 5-FU, 5-fluorouracil; T-DM1, trastuzumab emtansine.
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 or L866M, treatment with trastuzumab, neratinib, or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in trastuzumab plus either neratinib or lapatinib produced durable tumor regression, similar to that observed in these HER2-directed therapies. Therefore, 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 >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

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 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 >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...
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 straightforward 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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is currently an employee of Roche. SJM owns stock in LabCorp.
FP-L has received honoraria from Roche, acted in a consulting or
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accommodation, or expenses from Roche. SS is an employee of
Roche, owns stock in Roche, and has received travel, accommodation,
or expenses from Roche. AB has received honoraria from
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