HER2 as a Predictive Biomarker and Treatment Target in Colorectal Cancer

Astrid De Cuyper, Marc Van Den Eynde, Jean-Pascal Machiels

Abstract

The prognosis of metastatic colorectal cancer (mCRC) is poor. Cetuximab and panitumumab, 2 anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (mAbs), improve the overall survival of patients with *RAS* wild-type mCRC. However, not all patients with *RAS* wild-type mCRC will respond to anti-EGFR mAbs. Several retrospective trials suggest that human epidermal growth factor receptor 2 (*HER2*) amplification could be a predictive biomarker of resistance to anti-EGFR mAbs in patients with metastatic *RAS* and *RAF* wild-type mCRC. Dual HER2 inhibition with trastuzumab plus lapatinib or pertuzumab has shown promising preliminary anti-tumoral efficacy in *RAS* wild-type mCRC. Although these findings need to be confirmed in randomized trials, the data strongly support that *HER2* is an actionable gene in CRC and provide the scientific rationale to test HER2 status on a routine basis in this disease. In this review, we discuss the predictive value of HER2 activation in CRC as well as its potential role as a treatment target.

Clinical Colorectal Cancer, Vol. 19, No. 2, 65-72

Keywords: Drug resistance, Epidermal growth factor receptor, Human epidermal growth factor receptor 2, Gene amplification, Targeted therapy

Introduction

Colorectal cancer (CRC) is the third most frequent cancer in the world. Over the past 2 decades, advances in systemic chemotherapies and targeted therapies, together with surgical resection of metastases, have significantly improved the overall survival (OS) of patients with metastatic colorectal cancer (mCRC). However, the prognosis of mCRC remains poor, with a 5-year survival of 14%.[1]

The activation of the downstream pathways of the epidermal growth factor receptor (EGFR) plays a pivotal role in CRC development and progression. Cetuximab and panitumumab, 2 monoclonal antibodies (mAbs) targeting the EGFR, improve the OS of patients with *RAS* wild-type mCRC. *RAS* mutations (defined as either a mutation in *KRAS* or *NRAS* exon 2, 3, and 4) are well recognized as negative predictive biomarkers of anti-EGFR mAb response and survival benefit.[2] However, not all patients with *RAS* wild-type CRC (for example, right-sided colorectal cancers) will benefit from these agents.[3] Five percent to 10% of CRC also harbor the *V600E BRAF* mutation.[4] These patients have a poor prognosis. The clinical benefit of anti-EGFR mAbs in *V600E BRAF* mCRC is still a matter of debate and is currently being studied.[5] Mutations in *PI3KCA* and *PTEN*, expression of amphiregulin or epiregulin, *EGFR* gene copy number, and miR-31-3p are other investigated predictive biomarkers of anti-EGFR mAb efficacy or resistance.[6-8]

Additionally, human epidermal growth factor receptor 2 (HER2) activation and its downstream signaling pathways have also been implicated in anti-EGFR therapy resistance. Furthermore, besides the EGFR, HER2 could be another treatment target. In this review, we discuss the role of HER2 activation in CRC.

HER2 and CRC

The *HER2* oncogene, located on chromosome 17q21, encodes a transmembrane tyrosine kinase receptor. HER2 is a member of the HER family that includes the EGFR (HER1), HER2, HER3, and HER4 receptors.[9] HER2 has no known ligand but can form heterodimers with EGFR, HER3, and sometimes HER4. Upon dimerization, intracellular tyrosine residues undergo autophosphorylation, triggering a cascade of multiple important signaling pathways including the Ras/Raf/Mek/Erk, the phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR), the Src tyrosine kinase, and the signal transducers and activators of transcription (STAT) pathways. These pathways play an important role in stimulating cellular proliferation, survival, and

Institut Roi Albert II, Service d'Oncologie Médicale, Cliniques Universitaires Saint-Luc and Institut de Recherche Clinique et Expérimentale (POLE MIRO), Université Catholique de Louvain, Brussels, Belgium

Submitted: Jun 26, 2019; Revised: Jan 30, 2020; Accepted: Feb 2, 2020; Epub: Feb 8, 2020

Address for correspondence: Astrid De Cuyper, MD, Institut Roi Albert II, Service d'Oncologie Médicale, Cliniques Universitaires Saint-Luc and Institut de Recherche Clinique et Expérimentale, Université Catholique de Louvain, Ave Hippocrate 10, 1200 Brussels, Belgium
E-mail contact: astrid.decuyper@uclouvain.be

1533-0028/$ - see frontmatter
https://doi.org/10.1016/j.clcc.2020.02.007

angiogenesis. HER2 overexpression may also lead to HER2 dimerization, leading to constitutive activation.[10]

The oncogenic role of HER2 and its therapeutic implications are well-established in breast and gastroesophageal cancers in which amplification or overexpression of HER2 is found in 15% to 30% and 10% to 30% of patients, respectively.[11-14]

HER2 overexpression/amplification is reported in 1.3% to 6.3% of unselected CRC.[15-19] *RAS* and *BRAF* wild-type CRCs are enriched for the presence of *HER2* amplification.[15,17,18,20-22] A higher incidence of HER2 overexpression/amplification is also found in left-sided colon and rectal cancers (versus right-sided colon cancer),[19,22-24] although this is controversial.

HER2 protein expression is usually detected by immunohistochemistry (IHC) and *HER2* amplification by fluorescent in situ hybridization (FISH). In contrast to breast and gastric cancers, the diagnostic criteria for HER2 positivity in CRC have not yet been completely standardized and have varied from one study to another.

Valtorta and colleagues proposed and validated a HER2 scoring system in CRC.[25] They defined HER2 positivity as CRC tumors with a HER2 3+ score in more than 50% of tumor cells by IHC or a HER2 2+ score and a *HER2*:CEP17 ratio ≥ 2 in more than 50% of cells by FISH (HERACLES Diagnostic Criteria). These criteria are more stringent than the accepted definition of HER2 positivity in breast and gastric tumors.[26]

HER2 amplification can also be detected by next-generation sequencing (NGS) or comprehensive genomic sequencing. Both tests offer high concordance rates with FISH and/or IHC, thus supporting their use to detect HER2-positive ($HER2^+$) CRC.[27,28]

More recently, an international collaborative project aimed to establish harmonized diagnostic criteria for $HER2^+$ colorectal tumors by considering IHC, FISH, and NGS.[29] Based on an exploratory retrospective cohort of 475 patients with mCRC, and a validation cohort of 16 patients, Fujii et al proposed the following HER2 positivity criteria: IHC 3+ score, or IHC 2+ score and FISH *HER2*:CEP17 ratio ≥ 2.0, in $> 10\%$ of tumor cells for surgically resected specimens. Those criteria were verified by gene copy number (FISH) and copy number variation (NGS) analyses and took into account the heterogeneously expression of HER2 in mCRC cells. Liquid biopsies to detect *HER2* genetic alterations are also attractive as a diagnostic tool, but further investigation is required.[30]

HER2 Activation to Predict Resistance to Anti-EGFR mAbs in CRC

The clinical and biological significance of HER2 signal activation in CRC is an important research topic. *HER2* amplification has been shown to be a mechanism of anti-EGFR treatment resistance in patient-derived CRC xenograft models and cell lines. Bertotti and colleagues collected a large number of patient-derived CRC xenografts to identify predictive biomarkers of response to anti-EGFR inhibitors.[15] *HER2* amplification was observed in 2.2% of unselected mCRC xenografts. However, the prevalence of *HER2* amplification increased to 36% (n = 4/11) when only the CRC xenografts resistant to cetuximab and wild-type for *KRAS/NRAS/BRAF/PIK3CA* were considered. Additionally, *HER2* amplification/overexpression was detected in 13.6% (n = 6/44) of patients with *KRAS* wild-type CRCs progressing on cetuximab or panitumumab, whereas *HER2* amplification was never found in patients with mCRC who responded to anti-EGFR mAbs.[15]

Yonesaka and colleagues demonstrated that HER2 activation, either through *HER2* amplification or heregulin upregulation, results in persistent ERK1/2 signaling and cetuximab resistance.[31] Subsequent inhibition of HER2, or disruption of HER2/HER3 heterodimerization, restored sensitivity to cetuximab in vitro and in vivo. The authors investigated the clinical impact of cetuximab in 233 patients with mCRC. The median progression-free survival (PFS) and OS were longer for patients with *HER2* non-amplified tumors (n = 220) versus those with *HER2* amplified tumors (n = 13) (PFS, 5.0 vs. 3.0 months; OS, 17.1 vs. 10.2 months; $P = .0013$). Jeong and colleagues evaluated the association between *HER2* amplification and cetuximab efficacy in 142 patients with *RAS* and *BRAF* wild-type CRC treated with cetuximab administered as third- or fourth-line systemic treatment. They also found a significantly shorter PFS in patients with *HER2* amplification (n = 7) compared with patients without *HER2* amplification (n = 135): 3.1 versus 5.6 months, respectively ($P = .019$).[32] In the HERACLES studies (diagnostic study and therapeutic studies), 100 of 1485 patients with mCRC were positive for HER2 according to the HERACLES criteria. The outcome of these patients was compared with 116 HER2-negative ($HER2^-$) consecutive patients with mCRC selected as historical controls. When treated with anti-EGFR therapies, $HER2^+$ patients had a lower objective response rate (31.2% vs. 46.9%; $P = .031$) and PFS (5.7 vs. 7.0 months; $P = .087$) than $HER2^-$ patients.[33]

The predictive impact of *HER2* amplification has also been investigated in 2 distinct cohorts of patients with *RAS/BRAF* wild-type mCRC.[34] In cohort 1 (n = 98), *HER2* amplification was tested using in situ hybridization: 13 patients with CRC were *HER2*-amplified (*HER2*:CEP17 ratio ≥ 2.0), and 85 were non-amplified. In cohort 2 (n = 70), molecular screening was carried out using NGS: 16 patients with CRC were *HER2*-amplified (≥ 4 copies), and 54 were *HER2*−non-amplified. In both cohorts, HER2 testing was performed on pre-treatment archival tissue. They compared the efficacy of anti-EGFR therapy in the second- or third-line setting (after prior therapy without anti-EGFR) between *HER2*-amplified and *HER2*−non-amplified groups. The median PFS for patients on anti−EGFR-based regimens was significantly shorter among the *HER2* amplified compared with the *HER2* non-amplified in cohort 1 (2.8 vs. 8.1 months, respectively; hazard ratio, 7.05; 95% confidence interval [CI], 3.4-14.9; $P < .001$). In cohort 2, these findings were validated (2.8 vs. 9.3 months, respectively; hazard ratio, 10.66; 95% CI, 4.5-25.1; $P < .001$). Interestingly, the median PFS for first-line therapy that did not include an anti-EGFR mAb was similar among *HER2*-amplified and *HER2*−non-amplified patients in both cohorts. There was no effect of *HER2* amplification on OS.

These trials all suggest that *HER2* amplification is a biomarker capable of predicting resistance to anti-EGFR mAb in patients with metastatic *RAS and RAF* wild-type mCRC (Table 1). However, the

Table 1 *HER2* Amplification as a Resistance Biomarker for Anti-EGFR mAbs in mCRC

Study Reference	HER2 Detection Method	HER2 Positivity Criteria	Groups	N	PFS, mos	*P* Value	Survival, mos	*P* Value
Yonesaka[31]	FISH	Not specified	*HER2* amplified	13	3	—	10.2	.0013
			HER2 non-amplified	220	5		17.1	
Martin[35]	FISH	*HER2*:CEP17 $\geq$ 2 in the entire tissue section ($\geq$ 90% of the cells)	*HER2*-all-A[a]	6	2.5	.00026	4.2	.0002
			HER2-non-A and *HER2*-minor-A[a]	156	6.7		13	
Jeong[32]	SISH	*HER2*:CEP17 $\geq$ 2.0-2.2	*HER2* amplified	7	3.1	.019	10.1	.488
			HER2 non-amplified	135	5.6		13.5	
Sartore-Bianchi[33]	IHC/FISH	IHC 3+ in $>$ 50% of tumor cells; or IHC 2+ and *HER2*:CEP17 $\geq$ 2 in $>$ 50% of cells	*HER2* positive	100	5.7	.087	44.6	—
			HER2 negative	116	7		43.7	
Raghav[34]	ISH	*HER2*:CEP17 $\geq$ 2.0	Cohort 1					
			HER2 amplified	13	2.8	$<$.001	—	.14
			HER2 non-amplified	85	8.1			
	NGS	*HER2* $\geq$ 4 copies	Cohort 2					
			HER2 amplified	16	2.8	$<$.001	—	.66
			HER2 non-amplified	54	9.3			

Abbreviations: CEP = chromosome enumeration probe; EGFR = epidermal growth factor receptor; FISH = fluorescence in situ hybridization; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry; ISH = in situ hybridization; mAb = monoclonal antibody; mCRC = metastatic colorectal cancer; NGS = next-generation sequencing; PFS = progression-free survival; SISH = silver in situ hybridization.
[a]HER2-all-A was defined as gene amplification (*HER2*:CEP17 $\geq$ 2) in the entire tissue section ($\geq$ 90% of the cells), *HER2*-non-A as the absence of *HER2* gene amplification, and *HER2*-minor-A as *HER2* gene amplification in a minor population (10%-60% of cells).

Table 2 Reported Studies in Patients with HER2$^+$ mCRC

Study	Phase	Treatment	HER2 Positivity Criteria	Patients, n	Line of Treatment	ORR	mPFS	mOS
Ramanathan et al[36]	II	Trastuzumab + irinotecan	IHC 2+/3+	9	1st or 2nd	5/7 (71%)	–	–
Clark et al[37]	II	5-fluorouracil + leucovorin + oxaliplatin + trastuzumab	IHC 2+/3+	21	2nd or 3rd	5/21 (24%)	–	–
Bekaii-Saab et al[38]	I	Paclitaxel + trastuzumab + IL-12	IHC 2+/3+	6	All	0/6 (0%)	–	–
HERACLES-A Sartore-Bianchi et al,[23] Siena et al[39]	II	Trastuzumab + lapatinib	IHC 3+ in > 50% of tumor cells; or IHC 2+ and *HER2*:CEP17 ≥ 2 in > 50% of cells	33	Salvage	10/33 (30.3%)	21 weeks	46 weeks
MyPathway Meric-Bernstam et al[40]	II	Trastuzumab + pertuzumab	FISH/CISH (*HER2*:CEP17 > 2.0 or *HER2* copy number > 6.0), or NGS (copy number gain), or IHC (3+)	57	Salvage	18/57 (32%)	2.9 months	11.5 months

Abbreviations: CEP = chromosome enumeration probe; CISH = chromogenic in situ hybridization; FISH = fluorescence in situ hybridization; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry; mCRC = metastatic colorectal cancer; mOS = median overall survival; mPFS = median progression-free survival; NGS = next generation sequencing; ORR = objective response rate.

currently available data are retrospective and require validation in prospective clinical studies.

HER2 as a Therapeutic Target in CRC

Several drugs that aim to block the HER2 receptor have been developed and are currently undergoing further investigation (Table 2). Trastuzumab is a mAb that targets the extracellular domain of HER2. Pertuzumab is a humanized mAb that binds HER2 and inhibits the heterodimerization of HER2 with other HERs, thereby interfering with downstream HER2 signaling pathways. Trastuzumab emtansine (T-DM1) is a drug conjugate compound that links trastuzumab to a microtubule inhibitor chemotherapy (DM1), to deliver chemotherapy to HER2$^+$ cancer cells. Lapatinib is a tyrosine kinase inhibitor against EGFR1 and HER2. Neratinib is a pan-HER inhibitor able to inhibit all the heterodimers formed by the HER family.

Trastuzumab in combination with chemotherapy improves the survival of patients with *HER2*-amplified breast cancer or gastric cancer.[41,42] Pertuzumab, T-DM1, and lapatinib have demonstrated clinical activity in breast cancer.[43] In contrast, only trastuzumab is currently approved for gastric cancer.

Trastuzumab has been investigated in combination with irinotecan or oxaliplatin-based chemotherapy in 2 phase II trials for patients with mCRC.[36,37] Promising antitumor activity was observed, although the exact contribution of trastuzumab could not be determined owing to the absence of randomization. The trials had to be stopped early owing to poor accrual.

Bertotti and colleagues showed that lapatinib combined either with pertuzumab or cetuximab induced significant tumor regression of cetuximab-resistant *HER2*-amplified mCRC xenografts, whereas HER2 inhibitors (lapatinib or pertuzumab) administered as monotherapy were found to be inactive.[15] These pre-clinical works supported the clinical investigation of dual HER2 inhibition through the combination of 2 HER2 blocking agents. The proof-of-concept HERACLES-A phase II trial was therefore designed to assess the activity of trastuzumab combined with lapatinib in patients refractory to chemotherapy and anti-EGFR therapy with *KRAS* exon 2 wild-type and *HER2*-amplified mCRC.[23] A total of 1299 patients with *KRAS* exon 2 wild-type mCRC were screened. Using the HERACLES diagnostic criteria described above,[25] 69 (5.3%) patients had HER2$^+$ tumors, and 33 were eligible for the trial. The population was heavily pre-treated, with a median of 5 prior regimens administered before inclusion. Patients were treated with oral lapatinib (1000 mg daily) and trastuzumab (4 mg/kg intravenous loading dose, followed by 2 mg/kg weekly) until disease progression. The objective response rate was 30% (95% CI, 17%-47%). Two patients had a complete response, and 8 experienced a partial response. Thirty-nine percent achieved stable disease, giving a disease control rate of 70% (95% CI, 52%-82%). Treatment was well-tolerated with 18% of patients experiencing grade 3 side effects.[39]

Similarly, the MyPathway phase II trial showed promising activity of dual HER2 inhibition with durable responses using a combination of trastuzumab and pertuzumab in HER2$^+$ mCRC.[22,40] Employing a basket trial design, MyPathway continues to evaluate the efficacy of several compounds targeting molecular

Table 3 Ongoing Selected Trials Investigating HER2-based Therapies in CRC (Not Exhaustive)

Study (Reference)	Phase	Treatment	Randomized Controlled Trial	Eligibility
Ongoing trials with HER2 targeted mAbs and TKIs				
TRIUMPH Trial (NCT non-available)	II	Trastuzumab + pertuzumab	No	HER2-positive RAS wt mCRC, refractory to chemotherapy and anti-EGFR mAb
HERACLES-B NCT03225937	II	Pertuzumab + T-DM1	No	HER2 positive, *RAS/BRAF* wt refractory CRC
HERACLES-Rescue NCT03418558	II	T-DM1	No	HER2 positive mCRC who progressed on trastuzumab and lapatinib in the HERACLES-A study
SWOG 1613 NCT03365882	II	Trastuzumab + pertuzumab vs. cetuximab + irinotecan	Yes	*RAS/BRAF* wt mCRC, ≥1 prior therapy without anti-EGFR mAb
NCT03457896	II	Neratinib + trastuzumab or neratinib + cetuximab	No	*KRAS/NRAS/BRAF/PIK3CA* wt mCRC refractory to oxaliplatin and irinotecan with known HER2 status
NCT03065387	I	Neratinib + everolimus or palbociclib or trametinib	No	Advanced or metastatic solid tumors, with *EGFR* mutation/amplification, *HER2* mutation/amplification, *HER3/4* mutation or *KRAS* mutation, refractory to standard therapies
MATCH screening Trial NCT02465060	II	Pertuzumab + trastuzumab cohort	No	*HER2* amplified (≥ 7 CN)
		T-DM1 cohort		*HER2* amplified
		Afatinib cohort		*HER2* activation mutation
Mountaineer NCT03043313	II	Tucatinib + trastuzumab	No	$HER2^+$, *RAS* wt mCRC, refractory to chemotherapy and bevacizumab
Ongoing trials with novel anti-HER2 agents				
NCT03384940	II	DS-8201a	No	Unresectable, recurrent, or metastatic, *RAS/BRAF* wt, $HER2^+$ colorectal adenocarcinoma, after at least 2 prior regimens of standard treatment
NCT02892123	I	ZW25	No	HER2-expressing cancer with evidence of locally advanced (unresectable) and/or metastatic disease, refractory to all standard of care
NCT03821233	I	ZW49	No	HER2-expressing cancer with evidence of locally advanced (unresectable) and/or metastatic disease, refractory to all standard of care
Panther Trial NCT01862003	II	AZD8931 + FOLFIRI	No	*RAS* wt non-resectable, recurrent cancer or mCRC
NCT03602079	I/II	A166	No	Relapsed or refractory cancers expressing HER2 antigen or amplified *HER2*
Ongoing trials with HER2-targeted immunotherapies				
NCT02713984	I/II	Anti-HER2 CAR-modified T cells	No	Relapsed or refractory $HER2^+$ cancer
NCT03740256	I	HER2 chimeric antigen receptor-modified Adenovirus-specific cytotoxic T lymphocytes, in combination with intra-tumor injection of CAdVEC	No	$HER2^+$ solid tumor, after standard first-line therapy, or without available effective treatment options
NCT03319459	I	FAT-NK100 monotherapy cohort	No	Advanced solid tumor malignancies
		FAT-NK100 + trastuzumab cohort		$HER2^+$ advanced breast cancer, $HER2^+$ advanced gastric cancer or other advanced $HER2^+$ solid tumors
		FATNK-100 + cetuximab cohort		Advanced CRC or HNSCC, or other EGFR-positive advanced solid tumors
NCT01376505	I	HER-2 vaccine	No	Metastatic solid tumor after at least 1 line of standard therapy

Abbreviations: CN = copy number; EGFR = epidermal growth factor receptor; FOLFIRI = folinic acid, 5-fluorouracil, and irinotecan regimen; HNSCC = head and neck squamous cell carcinoma; mAb = monoclonal antibody; mCRC = metastatic colorectal cancer; TKI = tyrosine kinase inhibitor; wt = wild-type.

alterations in *HER2, EGFR, BRAF*, or hedgehog pathways. Meric-Bernstam and colleagues recently analyzed the efficacy of the pertuzumab/trastuzumab combination in a subset of patients (n = 57) with refractory HER2$^+$ mCRC. HER2 positivity was defined by IHC (3+ staining), FISH/chromogenic in situ hybridization (*HER2*:CEP17 ratio > 2.0, or *HER2* copy number > 6.0), or NGS (copy number increased). In contrast to the HERACLES trial, MyPathway included patients with *KRAS* wild-type (n = 43) and mutated (n = 13) CRC. Eighteen of the 57 patients enrolled achieved an objective response (32%; 95% CI, 20%-45%), including 1 complete response. The median duration of response, PFS, and OS were 5.9, 2.9, and 11.5 months, respectively. When analyzing response rate and survival outcomes according to *KRAS* mutation status, the investigators observed that median PFS and OS were shorter in patients with *KRAS*-mutated tumors than those with *KRAS* wild-type tumors. Similar results were seen regarding response rate: only 1 (8%) of 13 patients with *KRAS*-mutated tumors achieved an objective response versus 17 (40%) of 43 patients with *KRAS* wild-type tumors.

Interestingly, both the HERACLES-A and MyPathway studies investigated the correlation between *HER2* gene copy number and anti−HER2-targeted therapy activity. In MyPathway, the objective response rate was higher in the group of patients with mCRC harboring a *HER-2* copy number higher than the *HER2* median copy number (assessed by NGS on tumor tissue). In HERACLES-A, they also showed a significant association between a high *HER2* gene copy number and better PFS.[30] In an exploratory data analysis of this trial, Siravegna and colleagues proposed a plasmatic copy number of 2.4 and an adjusted plasmatic copy number of 25.82 copies of *HER2* to select patients who should benefit from HER2-targeted therapies.

Liquid biopsy could also be an important diagnosis tool to predict treatment resistance. Indeed, circulating tumor DNA collected before anti-HER2 treatment initiation in HERACLES-A showed alterations in *RAS*/*RAF* in 6 of 7 patients that did not respond to lapatinib and trastuzumab.[44] Interestingly, all patients included in the HERACLES-A study had to have *RAS* wild-type CRC at diagnosis, supporting the role of previous anti-EGFR treatment in the emergence of these *RAS*/*RAF* clones. This highlights the potential role of circulating tumor DNA to characterize iteratively the tumor genomic alterations throughout the disease course.

The question of whether HER2 blockade should be continued after disease progression is unknown. In one report, a patient had a cumulative clinical benefit of 29 months after treatment with several sequential anti−HER-2 therapies: trastuzumab + lapatinib, trastuzumab + pertuzumab, TDM-1, and trastuzumab + capecitabine.[45] This question is being partly evaluated in the HERACLES-RESCUE ongoing phase II trial, which is investigating the activity of T-DM1 in patients with HER2$^+$ mCRC who progressed on lapatinib and trastuzumab in the HERACLES-A trial.

Together, these results suggest that dual HER2 inhibition could have significant efficacy in HER2$^+$ *RAS* wild-type mCRC (Table 2). A high *HER2* gene copy number seems to be predictive of activity. In comparison with the currently available treatments for mCRC refractory to chemotherapy, these aforementioned studies have demonstrated better objective response rates, PFS, and OS.[46,47] Activating *HER2* mutations in mCRC are rare. Theoretically, these patients might also benefit from anti-HER2 therapies, although further investigation is warranted.[40,48,49]

Rationale of Ongoing Trials Targeting HER2 in CRC

To date, only single-arm trials that suggest a role for therapies targeting HER2 in mCRC are available (Table 3). However, to fully assess the potential of this approach, randomized trials need to be conducted. The SWOG 1613 US intergroup phase II clinical trial is a randomized study that is comparing the efficacy of dual anti-HER2 therapy (trastuzumab and pertuzumab) versus anti-EGFR−based therapy (cetuximab and irinotecan) in patients with anti−EGFR-naive, *HER2*-amplified, and *RAS/BRAF* wild-type mCRC with progressive disease after at least 1 prior regimen. A crossover to the HER2 therapy arm after disease progression is scheduled for patients initially included in the control arm. The results of this trial are extremely important and should better define the role of anti-HER2 therapies in mCRC.

Several other innovative approaches are also currently under investigation. A phase II trial is studying the efficacy of neratinib plus trastuzumab or neratinib plus cetuximab in patients with "quadruple wild-type" (*RAS/NRAS/BRAF/PIK3CA* wild-type) mCRC based on HER2 status (amplified, wild-type, or mutated). Tucatinib is a novel very potent and highly selective small molecule inhibitor of HER2 that has demonstrated significant activity in preclinical models.[50] MOUNTAINEER is a single-arm phase II study investigating tucatinib in combination with trastuzumab in patients with *HER2*-amplified and *RAS* wild-type pre-treated mCRC.[51] DS-8201a, a drug conjugate mAb, is designed to deliver a novel topoisomerase I inhibitor in a targeted fashion to cells that contain the HER2 antigen, and it is being investigated in a phase II open-label study in subjects with *RAS/BRAF* wild-type mCRC.[52] ZW25 is an engineered bi-specific mAb that targets 2 different epitopes of HER2 with potential immunomodulating and antineoplastic activities. ZW25 is able to stimulate a cytotoxic T-lymphocyte response as well as antibody-dependent cell-mediated cytotoxicity. In addition, ZW25 induces HER2 internalization with inhibition of HER2-mediated tumor growth. A phase I study is ongoing with this compound in a variety of HER2-expressing cancers including CRC.[53] ZW49 is another antibody drug conjugate under investigation that combines a novel auristatin payload (potent anti-cancer agent) with the unique mechanisms of action of the anti-HER2 biparatopic antibody, ZW25. AZD8931 is a tyrosine kinase inhibitor that has been shown to provide equipotent inhibition of EGFR, HER2, and HER3 when investigated in mCRC in combination with FOLFIRI (folinic acid, 5-fluorouracil, and irinotecan) in the phase II part of the PANTHER trial.[54]

mCRC with HER2 genetic alterations can also be included in "basket" trials that investigate various anti-HER2 strategies regardless of tumor type. For example, the MATCH trial is evaluating T-DM1, or pertuzumab combined with trastuzumab, in *HER2*-amplified cancers. Afatinib is also being explored in patients with *HER2*-activating mutated solid tumors. Several immunotherapy approaches targeting HER2 are also under investigation in basket trials. These include (but are not limited to) chimeric antigen receptor T cells (CAR-T) therapy (NCT02713984), HER2 chimeric antigen receptor-modified adenovirus-specific cytotoxic T

lymphocytes (HER2-AdVST) in combination with intra-tumor injection of CAdVEC (an oncolytic adenovirus that is designed to help the immune system) (NCT03740256), FATE-NK 100 (donor-derived natural killer cell product) in monotherapy or in combination with cetuximab or trastuzumab (NCT03319459), and peptide vaccines (NCT01376505).

Discussion and Conclusion

At the time of writing, *RAS* mutations (able to predict resistance to anti-EGFR mAb) and microsatellite instability (MSI) (to select patients for immune checkpoint blockers), are the only clinically validated biomarkers for mCRC. In this context, the identification of new clinically actionable oncogenic drivers is of utmost importance.

HER2 amplification is present in about 1.3% to 6.3% of all CRC. Prospective assessment and validation are crucial to standardize HER2 testing in CRC and to define the appropriate cutoff for positivity in this disease. Importantly, some discrepancies between the molecular profile of the primary tumor and metastases exist (spatial tumor heterogeneity).[55,56] Furthermore, the emergence of *RAS* mutants or $HER2^+$ CRC clones during anti-EGFR therapy has been described (temporal tumor heterogeneity). In this context, liquid biopsies represent an interesting alternative to iterative tumor biopsies and warrant further study.

Early HER2 testing for *RAS/BRAF* wild-type mCRC should be encouraged to help clinicians define the right populations for molecular targeted treatments: *HER2* amplification seems to predict a lack of response to anti-EGFR mAb therapy, and dual HER2 blockade with mAbs (trastuzumab and pertuzumab), or the combination of mAbs with a tyrosine kinase inhibitor (trastuzumab and lapatinib), induces durable tumor responses in about 30% to 40% of patients with $HER2^+$ *RAS* wild-type mCRC refractory to standard systemic therapy. These promising data need to be confirmed in randomized trials, and patients should be enrolled into clinical trials whenever possible.

Acknowledgments

The authors would like to thank Aileen Eiszele, BA (Hons), DipEd, GradDipBus for editorial and writing assistance.

Disclosure

The authors have stated that they have no conflicts of interest.

References

1. Siegel RL, Miller KD, Fedewa SA, et al. Colorectal cancer statistics, 2017. *CA Cancer J Clin* 2017; 67:177-93.
2. Sorich MJ, Wiese MD, Rowland A, Kichenadasse G, McKinnon RA, Karapetis CS. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. *Ann Oncol* 2015; 26:13-21.
3. Arnold D, Lueza B, Douillard JY, et al. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. *Ann Oncol* 2017; 28:1713-29.
4. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008; 26:5705-12.
5. Sanz-Garcia E, Argiles G, Elez E, Tabernero J. BRAF mutant colorectal cancer: prognosis, treatment, and new perspectives. *Ann Oncol* 2017; 28:2648-57.
6. De Roock W, De Vriendt V, Normanno N, Ciardiello F, Tejpar S. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. *Lancet Oncol* 2011; 12:594-603.
7. Martins M, Mansinho A, Cruz-Duarte R, Martins SL, Costa L. Anti-EGFR therapy to treat metastatic colorectal cancer: not for all. *Adv Exp Med Biol* 2018; 1110:113-31.
8. Laurent-Puig P, Grisoni ML, Heinemann V, et al. Validation of miR-31-3p expression to predict cetuximab efficacy when used as first-line treatment in RAS wild-type metastatic colorectal cancer. *Clin Cancer Res* 2019; 25:134-41.
9. Sergina NV, Moasser MM. The HER family and cancer: emerging molecular mechanisms and therapeutic targets. *Trends Mol Med* 2007; 13:527-34.
10. Moasser MM. The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. *Oncogene* 2007; 26:6469-87.
11. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989; 244:707-12.
12. Cronin KA, Harlan LC, Dodd KW, Abrams JS, Ballard-Barbash R. Population-based estimate of the prevalence of HER-2 positive breast cancer tumors for early stage patients in the US. *Cancer Invest* 2010; 28:963-8.
13. Rakha EA, Pinder SE, Bartlett JM, et al. National Coordinating Committee for Breast Pathology. Updated UK Recommendations for HER2 assessment in breast cancer. *J Clin Pathol* 2015; 68:93-9.
14. Ruschoff J, Hanna W, Bilous M, et al. HER2 testing in gastric cancer: a practical approach. *Mod Pathol* 2012; 25:637-50.
15. Bertotti A, Migliardi G, Galimi F, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. *Cancer Discov* 2011; 1:508-23.
16. Ingold Heppner B, Behrens HM, Balschun K, et al. HER2/neu testing in primary colorectal carcinoma. *Br J Cancer* 2014; 111:1977-84.
17. Richman SD, Southward K, Chambers P, et al. HER2 overexpression and amplification as a potential therapeutic target in colorectal cancer: analysis of 3256 patients enrolled in the QUASAR, FOCUS and PICCOLO colorectal cancer trials. *J Pathol* 2016; 238:562-70.
18. Sawada K, Nakamura Y, Yamanaka T, et al. Prognostic and predictive value of HER2 amplification in patients with metastatic colorectal cancer. *Clin Colorectal Cancer* 2018; 17:198-205.
19. Seo AN, Kwak Y, Kim DW, et al. HER2 status in colorectal cancer: its clinical significance and the relationship between HER2 gene amplification and expression. *PLoS One* 2014; 9:e98528.
20. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; 487:330-7.
21. Nam SK, Yun S, Koh J, et al. BRAF, PIK3CA, and HER2 oncogenic alterations according to KRAS mutation status in advanced colorectal cancers with distant metastasis. *PLoS One* 2016; 11:e0151865.
22. Hainsworth JD, Meric-Bernstam F, Swanton C, et al. Targeted therapy for advanced solid tumors on the basis of molecular profiles: results from MyPathway, an open-label, phase IIa multiple basket study. *J Clin Oncol* 2018; 36:536-42.
23. Sartore-Bianchi A, Trusolino L, Martino C, et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2016; 17:738-46.
24. Missiaglia E, Jacobs B, D'Ario G, et al. Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features. *Ann Oncol* 2014; 25:1995-2001.
25. Valtorta E, Martino C, Sartore-Bianchi A, et al. Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. *Mod Pathol* 2015; 28:1481-91.
26. Siena S, Sartore-Bianchi A, Marsoni S, et al. Targeting the human epidermal growth factor receptor 2 (HER2) oncogene in colorectal cancer. *Ann Oncol* 2018; 29:1108-19.
27. Edenfield WJ, Chung KY, Gatalica Z, Basu GD. Molecular profiling of HER2-positive colorectal cancer for identification of multiple potential drug targets. *J Clin Oncol* 2014; 32(15 Suppl):e14508.
28. Shimada Y, Yagi R, Kameyama H, et al. Utility of comprehensive genomic sequencing for detecting HER2-positive colorectal cancer. *Hum Pathol* 2017; 66:1-9.
29. Fujii S, Magliocco AM, Kim J, et al. International harmonization of provisional diagnostic criteria for ERBB2-amplified metastatic colorectal cancer allowing for screening by next-generation sequencing panel. *JCO Precis Oncol* 2020; 4:6-19.
30. Siravegna G, Sartore-Bianchi A, Nagy RJ, et al. Plasma HER2 (ERBB2) copy number predicts response to HER2-targeted therapy in metastatic colorectal cancer. *Clin Cancer Res* 2019; 25:3046-53.
31. Yonesaka K, Zejnullahu K, Okamoto I, et al. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. *Sci Transl Med* 2011; 3:99ra86.
32. Jeong JH, Kim J, Hong YS, et al. HER2 Amplification and cetuximab efficacy in patients with metastatic colorectal cancer harboring wild-type RAS and BRAF. *Clin Colorectal Cancer* 2017; 16:e147-52.
33. Sartore-Bianchi A, Amatu A, Porcu L, et al. HER2 positivity predicts unresponsiveness to EGFR-targeted treatment in metastatic colorectal cancer. *Oncologist* 2019; 24:1395-402.
34. Raghav K, Loree JM, Morris JS, et al. Validation of HER2 amplification as a predictive biomarker for anti–epidermal growth factor receptor antibody therapy in metastatic colorectal cancer. *JCO Precis Oncol* 2019.
35. Martin V, Landi L, Molinari F, et al. HER2 gene copy number status may influence clinical efficacy to anti-EGFR monoclonal antibodies in metastatic colorectal cancer patients. *Br J Cancer* 2013; 108:668-75.

36. Ramanathan RK, Hwang JJ, Zamboni WC, et al. Low overexpression of HER-2/neu in advanced colorectal cancer limits the usefulness of trastuzumab (Herceptin) and irinotecan as therapy. A phase II trial. *Cancer Invest* 2004; 22: 858-65.
37. Clark JW, Niedzwiecki D, Hollis D, Mayer R. Phase II trial of 5-fluororuacil (5-FU), leucovorin (LV), oxaliplatin (Ox), and trastuzamab (T) for patients with metastatic colorectal cancer (CRC) refractory to initial therapy. *Onkologie* 2003; 26:13-46.
38. Bekaii-Saab TS, Roda JM, Guenterberg KD, et al. A phase I trial of paclitaxel and trastuzumab in combination with interleukin-12 in patients with HER2/neu-expressing malignancies. *Mol Cancer Ther* 2009; 8:2983-91.
39. Siena S, Sartore-Bianchi A, Trusolino L, et al. Final results of the HERACLES trial in HER2-amplified colorectal cancer. *Cancer Res* 2017; 77:CT005.
40. Meric-Bernstam F, Hurwitz H, Raghav KPS, et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. *Lancet Oncol* 2019; 20:518-30.
41. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; 344:783-92.
42. Bang YJ, Van Cutsem E, Feyereislova A, et al, ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; 376:687-97.
43. Harbeck N, Gnant M. Breast cancer. *Lancet* 2017; 389:1134-50.
44. Siravegna G, Lazzari L, Crisafulli G, et al. Radiologic and genomic evolution of individual metastases during HER2 blockade in colorectal cancer. *Cancer Cell* 2018; 34:148-62.e7.
45. Martinelli E, Troiani T, Sforza V, et al. Sequential HER2 blockade as effective therapy in chemorefractory, HER2 gene-amplified, RAS wild-type, metastatic colorectal cancer: learning from a clinical case. *ESMO Open* 2018; 3:e000299.
46. Li J, Qin S, Xu R, et al. CONCUR Investigators. Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2015; 16:619-29.
47. Mayer RJ, Van Cutsem E, Falcone A, et al. RECOURSE Study Group. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. *N Engl J Med* 2015; 372:1909-19.
48. Gharib E, Salmanipour R, Nazemalhosseini Mojarad E, et al. HER2(+) mCRC patients with exon 20 R784G substitution mutation do not respond to the cetuximab therapy. *J Cell Physiol* 2019; 234:13137-44.
49. Kavuri SM, Jain N, Galimi F, et al. HER2 activating mutations are targets for colorectal cancer treatment. *Cancer Discov* 2015; 5:832-41.
50. Peterson S, de Vires P, Piasecki J, Rosler R. Tucatinib, a HER2 selective kinase inhibitor, is active in patient derived xenograft (PDX) models of HER2-amplified colorectal, esophageal and gastric cancers. *Ann Oncol* 2017; 28:v573-94.
51. Strickler JH, Niedzwiecki D, Zemla T, et al. A phase II, open label study of tucatinib (ONT-380) combined with trastuzumab in patients with HER2+ metastatic colorectal cancer (mCRC)(MOUNTAINEER). *J Clin Oncol* 2017; 35(15 Suppl):TPS3624.
52. Doi T, Shitara K, Naito Y, et al. Safety, pharmacokinetics, and antitumour activity of trastuzumab deruxtecan (DS-8201), a HER2-targeting antibody-drug conjugate, in patients with advanced breast and gastric or gastro-oesophageal tumours: a phase 1 dose-escalation study. *Lancet Oncol* 2017; 18:1512-22.
53. Hausman DF, Hamilton EP, Beeram M, Thimmarayappa J, Ng G, Meric-Bernstam F. Phase 1 study of ZW25, a bispecific anti-HER2 antibody, in patients with advanced HER2-expressing cancers. *J Clin Oncol* 2017; 35(4 Suppl):TPS215.
54. Hickinson DM, Klinowska T, Speake G, et al. AZD8931, an equipotent, reversible inhibitor of signaling by epidermal growth factor receptor, ERBB2 (HER2), and ERBB3: a unique agent for simultaneous ERBB receptor blockade in cancer. *Clin Cancer Res* 2010; 16:1159-69.
55. Lee WS, Park YH, Lee JN, Baek JH, Lee TH, Ha SY. Comparison of HER2 expression between primary colorectal cancer and their corresponding metastases. *Cancer Med* 2014; 3:674-80.
56. Shan L, Lv Y, Bai B, Huang X, Zhu H. Variability in HER2 expression between primary colorectal cancer and corresponding metastases. *J Cancer Res Clin Oncol* 2018; 144:2275-81.