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SYSTEMIC THERAPIES IN COLORECTAL CANCER (RD KIM, SECTION EDITOR)

The Role of HER2 Testing in Advanced Colorectal Cancer

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Abstract

Purpose of Review About 1/3 of all metastatic colorectal cancer (mCRC) patients may harbor a mutation in the *KRAS* or *NRAS* gene suggesting inefficacy of EGFR inhibitors cetuximab and panitumumab. In spite of tailoring treatment in *RAS* wild-type patients to receive EGFR inhibitors, not all show response.

Recent Findings Studies have shown that HER2-neu amplification/alteration in addition to alteration in *BRAF* and *PI3KA* may explain resistance to EGFR inhibitors. Several pre-clinical studies have identified that HER2-neu amplification can result in both de novo and acquired resistance to EGFR inhibitors. Recently, several clinical studies have highlighted the use of single or combination HER2-neu directed therapies in HER2-neu amplified/overexpressed mCRC.

Summary About 5% mCRC patients will demonstrate HER2-neu overexpression and response to HER2-neu-directed therapies can be in the range of 30–38%. Patients not responding to EGFR-inhibitors warrant testing for HER2-neu testing to explain resistance. In the near future, HER2-neu testing is likely to be integrated into our routine clinical practice for management of metastatic colorectal cancer patients.

Keywords Colorectal cancer · HER2-neu · Amplification · ERBB2 · Trastuzumab · Lapatinib · Immunohistochemistry

Introduction

Colorectal cancer (CRC) is the third most common cause of cancer in the USA among both males and females accounting for nearly 8% of all cancer cases. The expected number of new colorectal cancer cases in 2018 is 140,250 and 50,630 deaths [1]. Early stage cancers that are not metastatic have a 5-year survival of over 70%. However, patients with advanced, metastatic CRC have a 5-year survival of less than 15%. There are several biomarkers in CRC that we commonly test for in the advanced, metastatic disease setting to help us guide treatment.

Microsatellite instability high (MSI-h) overall portends good prognosis in advanced CRC as compared to non-MSI-h tumors. Although found in about only 4% of metastatic CRC, recent studies have shown excellent response rates with single agent pembrolizumab (response rate 40%) of patients with MSI-h CRC refractory to standard treatment as compared to non-MSI-h CRC (response rate 0%) [2]. BRAF V600 E mutations are more commonly found in right-sided tumors and overall correlate with poorer outcomes. These are noted in about 8% of all CRC and studies have shown that FOLFOXIRI plus bevacizumab may be better first-line treatment for these patients as compared to FOLFIRI plus bevacizumab [3, 4]. Point mutations in exon 2 and 3 of the *KRAS* gene are found in about 1/3 of all CRC. Additionally, mutations in exon 2, 3, and 4 of *NRAS* gene are found in about 15% of CRC. Patients with advanced CRC, therefore, undergo extended *RAS* testing, as presence of any of these mutations signifies resistance to ant-EGFR inhibitors cetuximab and panitumumab [5].

While these genetic alterations provide important clinical and prognostic information, majority of the patients will lack these alterations or develop resistance to biological agents and, therefore, there is a need to identify newer genetic alterations. Amplification or mutation in *ERBB2* or human epidermal growth factor receptor 2 (HER2) has been observed in several cancers and have oncogenic potential [6, 7]. Almost 15–20% of breast cancer and gastric/gastroesophageal junction adenocarcinomas have *ERBB2* amplification [8, 9].

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Recently, there have been reports of HER2 overexpression in CRC that have been studied to explain lack of response to anti-EGFR inhibitors and also responsive to therapeutic dual anti-HER2 blockade. This review aims to focus on the current knowledge about the biology of *ERBB2* in cancer and colorectal cancer specifically and its clinical applications.

HER2-neu Biology in Colon Cancer

ERBB2 encoding HER2-neu is a member of receptor tyrosine kinases (RTK) that include *ERBB1* (*EGFR*), *ERBB3*, and *ERBB4*. Except *ERBB2*, other members of this family have extracellular ligands that induce formation of active kinase hetero-oilgomers and through downstream activation of *Ras-Raf-MAPK* and *PI3K/Akt/mTOR* induce cell proliferation and survival. *ERBB2* is in a constitutively active configuration by means of which it can bind to other members of the family and activate downstream pathways. *ERBB3*, by itself has weak kinase activity; however upon dimerization with *ERBB2*, forms a strong oncogenic unit resulting in activation of downstream pathways [10–12].

Oncogenic activation of *ERBB2* is possible via (i) amplification of the *ERBB2* gene causing overexpression of HER2-neu, (ii) somatic mutations in tyrosine kinase domain (TKD) or extracellular domain (ECD) of HER2-neu or even large deletions in the ECD resulting in truncated form of *ERBB2*, and (iii) inhibition of the intracellular phosphatases that will dephosphorylate the *ERBB2* receptor and terminate downstream signaling. Amplification of *ERBB2* has been noted in breast cancer, gastric cancer, lung cancer, bladder cancer, ovarian cancer, colorectal cancer, and salivary gland cancer. HER2-neu overexpression has prognostic and therapeutic role in breast cancer. Breast cancers overexpressing HER2-neu are aggressive and have a poorer outcome. While HER2-neu testing in gastric cancer has therapeutic implication, it is not well defined as a prognostic marker. Studies of agents targeting HER2-neu in other cancers are currently ongoing [11]. Mutations in TKD alter the ATP-binding pocket leading to constitutive phosphorylation and activation of *ERBB2*. One of the most common alterations is an in-frame insertion/duplication A775_G776insYVMA within exon 20. Such mutations have been noted in lung, ovarian, breast, gastric, and colorectal cancers. On the other hand, permanent activation of *ERBB2* through ECD mutations is due to reduction-sensitive covalent dimerization. These mutations occur in an area of rich sulfide bonds and result in intermolecular disulfide bone formation, which results in the constitutive dimerization. In the third type of alteration, the ECD lacks substantial parts causing significantly truncated ERBB2 called as p95HER2 or HER2 carboxyl terminal fragments (CTF). These are predominantly found in breast cancer and are implicated in trastuzumab resistance. In very low frequencies, they are also detected in lung cancer [11].

While reports of *ERBB2* amplification causing HER2-neu overexpression in breast and gastric/gastroesophageal junction cancers are well established; recent studies have shown HER2-neu overexpression in CRC cases as well. There are differing rates of HER2-neu overexpression in CRC mostly due to staining pattern and guidelines used to interpret HER2-neu positivity. However, in 2015, Valtorta et al. defined guidelines that were then used for the HERACLES study (described later) [13••]. More recent studies quote HER2-neu overexpression noted in about 2% of all CRC cases [14••]. In stage III or IV *KRAS* exon 2 wild type tumors, the overexpression can be seen in about 5% of the cases [15, 16]. While not perfect, there have been observations about sidedness in HER2-neu overexpressing tumors. A retrospective genomic analysis of the PETACC-3 dataset showed that amplification of *EGFR* and *ERBB2* were more common in distal tumors (splenic flexure descending colon, rectum) as compared to proximal tumors (cecum, ascending colon, hepatic flexure, transverse colon) [17]. Other retrospective studies have also noted a higher proportion of HER2-neu overexpression in tumors of the sigmoid colon-rectum area as compared to cecum-descending colon [18, 19•]. Contrasting to this, there are other studies that have not noted a significant difference in HER2-neu expression pattern based on tumor location [14••, 15]. In a meta-analyses of the QUASAR stage II–III trial and FOCUS and PICCOLO trials (the latter two trials included mCRC patients); it was noted that HER2-neu overexpression was significantly higher in mCRC cases than in stage II–III cases (2.1% versus 0.2%; $p = 0.01$) and that they were significantly more frequent in *KRAS/BRAF* wild-type tumors as compared to mutated tumors (5.2% versus 1.0%; $p < 0.0001$) [16].

The Cancer Genome Atlas (TCGA) analyses of colorectal patients identified 7% with HER2 alterations in the form of somatic mutations or gene amplifications. The HER2 mutations S310F, L755S, V777L, V842I, and L866M are the similar mutations more commonly seen in breast cancer and non-small cell lung cancer (NSCLC). Early phase studies in breast cancer and NSCLC with these mutations have shown to be sensitivity to tyrosine kinase inhibitors such as neratinib.

Transfection of HER2-neu mutations V842I, V777L, L755S, and S310F into immortalized mouse colon epithelial cells induced ERBB2 signaling pathways with increase in HER2-neu, MAPK, and AKT phosphorylation. These mutations also induced significant growth of colonies in soft agar. These mutations produced resistance to EGFR inhibitors when transfected into cetuximab-sensitive CRC cell lines. PDX studies harboring HER2-neu mutations showed delayed tumor growth when treated with single agent trastuzumab, neratinib, or lapatinib; with growth of tumors post 30 days. However, when these models were treated with dual HER2-

neu targeting therapies such as trastuzumab plus neratinib or trastuzumab plus lapatinib, there was durable tumor response [20]. In a basket trial of 125 patients with HER2-neu mutations including 12 patients with CRC; single agent neratinib had no activity in CRC [21]. This highlights that maybe combination therapies targeting HER2-neu is necessary for treatment of HER2-neu-mutated CRC.

The correlation of microsatellite instability and HER2-neu mutation is not well defined. However, one study found that HER2-neu mutation might be found in about 15% patients with Lynch syndrome or Lynch-like CRC [22].

In a study of comprehensive genomic profiling of 8887 mCRC cases, a total of 569 samples (6.4%) had *ERBB2* (4.8%) or *ERBB3* (1.7%) alterations or both (0.1%). Of the 421 *ERBB2* positive samples, 58.5% were *ERBB2* amplification only, 31.5% were a short variant (SV) sequence alteration and 8.2% has co-occurring SV and amplification alterations of *ERBB2*. None of the *ERBB2* amplified tumors had high tumor mutational burden. MSI-h status correlated with *ERBB2*/*ERBB3* mutations in mCRC with almost 18% of these mutated tumors being MSI-h. None of the *ERBB2* amplified tumors were MSI-h. Alterations in *TP53*, *TOP2A*, and *CDK12* were far more common in *ERBB2* amplified tumors. Alterations in *KRAS*, *BRAF*, and *PI3KA* were less likely in *ERBB2* amplified tumors [7].

Prognostic Role of HER2-neu in Colon Cancer

The prognostic role of *ERBB2* amplification in CRC is not well defined. While some studies have shown a trend towards poor overall survival (OS) and even recurrence-free survival; however, these studies are limited due to small sample sizes of patients with HER2-neu amplification. In the PETACC-8 adjuvant trial of stage III CRC patients, HER2-neu amplification was associated with shorter recurrence-free survival (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; $p = 0.045$) even after adjusting for age, treatment, *RAS* mutation, histological grade, location of the tumor, and pathological T and N stages, bowel obstruction or perforation and vascular or lymphatic invasion [15]. Since the incidence of HER2-neu amplifications is relatively lower than in other cancers or as compared to other alterations in CRC, a definitive conclusion of its prognostic value cannot be yet determined.

Resistance to Anti-EGFR Therapy

EGFR activates downstream signaling pathways of *Ras-BRAF-MEK-ERK* and *PI3K/Akt/mTOR* which control cell proliferation and growth. Alterations in *KRAS*, *NRAS*, or *BRAF* will trigger alternative survival pathways that bypass therapeutic blockade of *EGFR* and thus cause primary resistance to EGFR-inhibitors such as cetuximab and panitumumab [23]. Most common of these alterations occur in exon 2 (41.4%) of *KRAS* at codons 12 and 13. Additionally, mutations can also occur in codons 59, 61, 117, and 146. *KRAS* alterations can also occur in exons 3 and 4. Extended *RAS* testing has now identified alterations in *NRAS* in exon 2, 3, and 4; all of which predict resistance to anti-EGFR antibodies. While these alterations account only 50–60% of mCRC patients that are resistant to anti-EGFR antibodies; discovery of other alterations or biomarkers remains ongoing. Some studies have shown that presence of $BRAF^{V600E}$ mutation is also a predictor of lack of response to EGFR blockade [24].

In a patient-derived xenograft (PDX) study, *ERBB2* amplification was noted in models that were *KRAS/NRAS/BRAF/PIK3CA* wild-type and insensitive to cetuximab treatment. This was supported by enrichment of HER2-neu amplification in *KRAS* wild-type patients that were nonresponsive to anti-EGFR antibodies [25]. In cell line studies, 3 of the 7 cetuximab-resistant CRC clones demonstrated *ERBB2* amplification. Combination treatment of cetuximab with trastuzumab resulted in growth inhibition. Treatment with lapatinib was able to restore sensitivity to cetuximab in these cell lines. There was also significantly higher level of circulating heregulin in patient samples that developed resistance to cetuximab. Thus, aberrant *ERBB2* signaling causing either *ERBB2* amplification or increased levels of heregulin have been implicated in either de novo or acquired resistance of CRC to anti-EGFR antibodies [26•].

In 233 patients treated with cetuximab, the progression-free survival (PFS) in HER2-neu-amplified tumors as compared to HER2-neu-nonamplified tumors was 2.9 months versus 4.9 months and the OS was 10.1 months versus 17 months respectively ($p = 0.0013$) [26•]. In another study of *KRAS* wild-type mCRC patients treated with EGFR inhibitors, *ERBB2* gene copy number negatively correlated with survival [27]. In a study published by Raghav et al. which tested for HER2-neu amplification using immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and next generation sequencing (NGS) in two cohorts of patients with *RAS* and *BRAF* wild-type mCRC. The first cohort comprising 97 patients on treatment with EGFR inhibitors showed a significantly worse PFS in HER2-neu amplified tumors as compared to HER2-neu nonamplified tumors (2.9 months versus 8.1 months, HR 5.0; $p < 0.0001$). These findings were confirmed in the second cohort (validation cohort) of patients already treated with EGFR inhibitors, which also showed that median, PFS was significantly shorter in patients with HER2-neu amplified tumors (2.8 months) as compared to HER2-neu nonamplified tumors (9.3 months) (HR 6.6; $p < 0.0001$) [28]. While HER-neu amplification is noted upon retrospective review of cases resistant to EGFR inhibitors, it is not well established if there is indeed emergence of HER2-neu amplification as patients are treated with EGFR inhibitors.

Preclinical studies of cetuximab-resistant and HER2-neu amplified PDX models treated with single agent HER2 tyrosine kinase inhibitors or anti-HER2 antibodies have not shown great efficacy. However, the combination of trastuzumab or pertuzumab with lapatinib demonstrated tumor shrinkage [25, 29]. These data have prompted further clinical studies as outlined below.

HER2-neu Testing in Colorectal Cancer

While there are validated IHC scoring systems for breast and gastric HER2-neu expression, IHC scoring for other solid tumors including CRC is not so robust. The scoring system for breast and gastric cancer also differs since gastric cancers are more heterogeneous than breast cancers. Unlike breast and gastric cancer, reports of HER2-neu overexpression vary widely (0 to 83%) based on different studies and more important staining pattern on immunohistochemistry (IHC) [30, 31]. HER2-neu expression between primary and metastatic site in CRC can be discordant in about 15% cases and can also change over time [30].

The phase II HERACLES (HER2 Amplification for ColorectaL Cancer Enhanced Stratification) trial used the HercepTest antibody (Dako A/S Glostrup, Glostrup, Denmark), and HER2-neu expression was assessed as follows: (a) manually by IHC using Hercep Test antibody (Dako A/S Glostrup, Glostrup, Denmark); (b) automated IHC using VENTANA 4B5 antibody on BenchMark ULTRA platform (Ventana Medical Systems, Inc. Tucson, AZ, USA); and (c) using fluorescence in situ hybridization (FISH) with PathVysion HER2 DNA Probe Kit (Abbott Laboratories, Abbott Park, IL). HER2-neu positivity was defined as HER2 3+ (intense staining) in ≥ 50% of cells or HER2 2+ and HER2:CEP17 ≥ 2 in ≥ 50% of cells. IHC staining pattern can be circumferential, basolateral or lateral [13••]. Using these HERACLES diagnostic criteria, 5% of KRAS wild-type metastatic CRC (mCRC) were HER2-neu positive. Similarly, other recent studies using these criteria, have now reported HER2-neu positivity ranging from 1.6 to 6.3% [14••]. Molecular testing such as next-generation sequencing (NGS) or comprehensive genomic sequencing (CGS) detect HER2-neu amplification in 1.8 to 22.0% of CRC cases. In a study using NGS, IHC and FISH methods for detection of HER2-neu overexpression, 1.8% of CRC cases were found to be HER2-neu overexpressing. There was a 97% concordance between HER2-neu protein expression and gene amplification. CGS reliably assesses HER2-neu amplification with excellent concordance to IHC scoring using HERACLES criteria. CGS also has good concordance among primary and metastatic sites for HER-neu amplification. Recently, circulating tumor DNA (ctDNA) also has excellent and reliable results for HER2-neu amplification when compared to HER2-neu expression in tissue biopsy [14••]. While at this time, molecular techniques may not be cost-effective for assessment of HER2-neu expression; in the future a comprehensive NGS panel for CRC can be developed.

Clinical Studies

In a phase II study of trastuzumab and FOLFOX post first-line treatment for mCRC, 4% patients (26 of 653) were screened to have tumors HER2-neu ≥ 2+ and among 21 evaluable tumors, 24% achieved a partial response. The trial halted due to poor accrual [14••]. In another phase II study of irinotecan plus trastuzumab as first- or second-line treatment of advanced CRC, 8% (11 of 138) patients had HER2-neu overexpressing (HER2-neu 2+ or 3+) tumors by IHC and 71% of 11 evaluable patients had partial responses [32]. In a case report, 2 patients with mCRC with liver metastases treated with cepecitabine, oxaliplatin, and lapatinib on a clinical trial showed good response to the combination. HER2-neu statuses of these patients were, however, not reported [33]. In a more recent case report of a patient with *KRAS*, *NRAS*, *BRAF*, *PIK3CA* wild-type mCRC, but *ERBB2* amplified confirmed on IHC, FISH, and NGS that had failed four prior lines of standard treatment, experienced significant clinical benefit with sequential treatment with trastuzumab plus lapatinib, pertuzumab plus trastuzumab, trastuzumab emtansine, trastuzumab plus capecitabine [34].

The HERACLES trial is an open-label multicenter trial conducted in Italy in HER2-neu positive, *KRAS* exon 2 (codons 12 and 13) wild-type, mCRC after progression on standard therapies. HER2-neu positivity was interpreted per the HERACLES criteria as described above. There are two cohorts in the study. Cohort A (HERACLES A) is with combination of trastuzumab and lapatinib. Treatment involved trastuzumab 4 mg/kg loading dose and then 2 mg/kg weekly. Lapatinib was dosed at 1000 mg per day. Each cycle length was 21 days with trastuzumab given weekly and lapatinib daily. In case of adverse events, lapatinib dose was lowered to 750 mg daily. Primary endpoint of the study was objective response rate including complete or partial responses. Secondary endpoints were progression free survival (PFS) and safety. One thousand two hundred ninety-nine patients with *KRAS* exon 2 (codons 12 and 13) wild type mCRC were screened, of which 69 (5.3%) were tested positive for HER2-neu amplification. Thirty-three patients were evaluable for response. Paired primary and metastatic site tissue for HER2-neu testing was available for 3 patients and the paired samples showed 100% concordance for the HER2-neu expression score. Seventy-five percent of patients had received at least four prior therapies. None of the 15 patients treated previously with anti-EGFR inhibitors had an objective response to the drugs. Sixty percent patients had tumors of the

distal colon. Two patients had complete responses and 8 patients had partial response. Thus, the overall objective response rate was 30.3%. The median duration of response was 38 weeks. Thirteen of the 33 patients had stable disease and, therefore, the disease control rate was 70%. Median PFS was 21 weeks and median OS was 46 weeks. Most common adverse events (AEs) were diarrhea, rash, fatigue, paronychia, and conjunctivitis. No grade 4 or 5 AEs were noted. Only 18% patients had grade 3 AEs (4- fatigue; 1- skin rash; 1- increased bilirubin). Through exploratory analyses, *HER2* gene copy number 9.45 or above as determined by quantitative PCR was predictive of response as well as prognostic. Median PFS was longer (29 weeks versus 16 weeks) in patients with tumors expressing HER2-neu above this threshold versus lower than 9.45 [35•, 36].

There is a cohort B in the study (HERACLES B) with combination of pertuzumab and antibody drug conjugate trastuzumab-emtansine. Patients will receive pertuzumab 840 mg intravenous load, flowed by 420 mg intravenously every 3 weeks. Trastuzumab-emtansine was administered as 3.6 mg/kg intravenously on day 1 of each subsequent 3-week cycle. Twelve patients have been enrolled to this cohort. Of the eight evaluable for responses, 7 patients had clinical benefit with two partial responses.

HERACLES RESCUE is a phase II trial testing the efficacy of sequential trastuzumab-emtansine in patients with HER2-neu amplified mCRC that have progressed on trastuzumab and lapatinib combination within the HERACLES trial. Treatment is administered at a dose of 3.6 mg/kg intravenously every 21 days [37]. Final results from this trial are yet pending.

In a phase IIa basket trial of all solid tumors refractory to standard treatment options and that harbored alterations in HER2-neu, *EGFR*, *BRAF*, and Hedgehog signaling pathway received specific treatments. One hundred fifty-one patients had HER2-neu alterations out of total of 230 patients. Thirty-seven CRC patients had HER2-neu amplification or overexpression. These patients treated with pertuzumab and trastuzumab showed a response rate of 38% (14 partial responses). The median duration of response was 11 months. The disease control rate was 48.6% [38•]. Median PFS was 4.6 months and median OS 10.3 months. Response rates were higher in *KRAS* wild-type tumors as compared to mutant (52% versus 0%). Response rates were highest in rectal tumors (45.5%) followed by left-sided tumors (42.9%) and then right-sided tumors (12.5%) [14••].

Results from another basket trial of *ado*-trastuzumab emtansine in HER2-neu altered tumors were recently reported. This included HER2-neu mutant lung cancers, HER2-neu overexpressing lung cancers, bladder, and urinary tract cancers and other cancers including endometrial, salivary gland, and CRC. Sixty-two patients were enrolled, 7 of which were mCRC. ORR in the cohort of salivary gland tumors was 100%, 43% in lung cancers and 25% in endometrial cancers. The ORR in the mCRC was 0%. Of these 7 patients, 5 were *RAS* wild type, 1 was *KRAS* mutant and the other was *NRAS* mutant [39]. In a case reported by Parikh et al., a patient with *KRAS*, *NRAS*, *BRAF* wild-type, MSS mCRC, and HER2-neu amplified showed durable response with single agent *ado*-trastuzumab emtansine [31].

Table 1 List of ongoing trials in HER2-neu overexpressing/amplified mCRC

Trial ID	Phase	Treatment	Target number of patients	Primary endpoint
Colorectal cancer specific				
NCT03384940	II	DS-8201a intravenously every 3 weeks	90 (HER2-neu expressing CRC)	ORR
NCT03457896	II	Neratinib plus trastuzumab or neratinib plus cetuximab	35 (mCRC KRAS, NRAS, BRAF, PIK3CA wild-type)	PFS
NCT03043313	II	Tucatinib plus trastuzumab	25 (RAS wild-type advanced CRC, HER2-neu amplified/overexpressed)	ORR
NCT03365882 (S1613)	II	Trastuzumab and pertuzumab versus cetuximab and irinotecan	130 (HER2-neu amplified advanced CRC)	PFS
All solid tumors with CRC included				
NCT03410927	I/II	TAS0728, an oral covalent binding inhibitor of HER2	204 (all solid tumors with a cohort for HER2 mutated or amplified CRC)	Safety, tolerability, ORR
NCT03602079	I/II	A166	82 (includes HER2-neu expressing CRC)	Safety, ORR
NCT03319459	I	FATE-NK100, a donor derived NK cell product in combination with cetuximab	100 (cohort for advanced CRC)	Safety
NCT01376505	I	HER2-neu peptide vaccine comprising measles virus epitope MVF-HER-2 (266–296) and MVF-HER-2 (597–628)	36 (all solid tumors including CRC)	Type and duration of immune response; clinical benefit

CRC colorectal cancer, *ORR* overall response rate, *PFS* progression-free survival

There are several new HER2-neu targeting agents that are now being tested in clinical trials. A list of the ongoing trials is detailed in Table 1. Trastuzumab deruxtecan (DS-8201a) is a novel HER2-neu targeting antibody drug conjugate that has been recently studied in a phase I trial comprising HER2-neu positive breast cancer, HER2-neu low breast cancer, HER2-neu positive gastric cancer and other HER2-neu solid tumors. The cohort of other HER2-neu positive solid tumors comprised of CRC. Of the 31 evaluable patients in this cohort, the overall response rate (ORR) was 38.7% and disease control rate of 83.9%. The median PFS was 12.1 months. In the entire study population, grade 3 or more adverse events were noted in 50.2% patients and 4.1% (10 patients) died due to treatment-related adverse events. Most common AEs were hematological in nature. Five patients died of interstitial lung disease or pneumonitis as result of treatment [40]. A trial is currently ongoing studying the efficacy of DS-8201a in HER2-neu expressing CRC.

In another phase I trial, ZW25, a bispecific HER2-neu targeted antibody (binds to both ECD4 and ECD2 which are trastuzumab binding and pertuzumab binding domains respectively) was assessed in multiple HER2-neu expressing cancers including mCRC. Five mCRC patients were enrolled, of which 3 were evaluable for treatment response. Each of these patients had partial response, stable disease, and progressive disease respectively [41].

These studies highlight that response rates with HER2-neu targeting drugs in HER2-neu expressing mCRC can range from 0 to 52%. This means that there are pathways that explain resistance to HER2-neu blockade. It has been hypothesized and studied that some of these pathways could be functioning parallel to HER2-neu or even downstream such as *RAS* or *PI3KA* [14].

Conclusions

Various biomarkers have been used to guide treatment decisions in mCRC. While it is well established that *RAS* wild-type tumors respond to EGFR inhibitors cetuximab or panitumumab, response rates are small and therefore there is concern for alternative pathways that may be causing this resistance. HER2-neu amplification or overexpression has been discovered as one of the mechanisms by which *KRAS*, *NRAS*, *BRAF*, and even *PIK3CA* wild-type mCRC tumors can develop either de novo or acquired resistance to EGFR inhibitors. Although, about 5% of mCRC tumors are found to have HER2-neu amplification or overexpression, multiple studies have now shown that HER2-neu-targeted therapies in this group of patients are efficacious. While single agents may have low activity, combination treatments have yielded response rates of 30–38%. In light of these findings, it is highly encouraged to test for HER2-neu expression as part of standard biomarker testing at the time of mCRC diagnosis to help guide treatment decisions.

Compliance with Ethical Standards

Conflict of Interest Rutika Mehta received reimbursement for travel expenses from Bristol-Myers Squibb and served as the site PI for a clinical trial.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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