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Meta-Analysis

Systematic literature review and meta-analysis of HER2 amplification, overexpression, and positivity in colorectal cancer

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Abstract

Background: Colorectal cancer (CRC) is the second most common cause of cancer death globally. Recent clinical trials suggest an emerging role for HER2 as a potential clinically relevant biomarker in CRC. Testing for HER2 in CRC is not standard practice; consequently, the prevalence of HER2 positivity (HER2+) in patients with CRC remains uncertain.

Methods: A systematic literature review and meta-analysis were conducted to generate estimates of proportions of patients with CRC with HER2 overexpression or HER2 amplification and HER2+ (either overexpression or amplification), overall and in patients with rat sarcoma virus (RAS) wild-type cancer. HER2+ was defined as 1) immunohistochemistry with a score of 3+, 2) immunohistochemistry with a score of 2+ and in situ hybridization+, or 3) next-generation sequencing positive.

Results: Of 224 studies identified with information on HER2 in CRC, 52 studies used a US Food and Drug Administration–approved assay and were selected for further analysis. Estimated HER2+ rate was 4.1% (95% confidence interval [CI] = 3.4% to 5.0%) overall (n = 17 589). HER2+ rates were statistically higher in RAS wild-type (6.1%, 95% CI = 5.4% to 6.9%) vs RAS mutant CRC (1.1%, 95% CI = 0.3% to 4.4%; $P < .0001$). Despite limited clinical information, we confirmed enrichment of HER2+ CRC in patients with microsatellite stable and left-sided CRC.

Conclusion: This meta-analysis provides an estimate of HER2+ CRC and confirms enrichment of HER2 in microsatellite stable, left-sided, RAS wild-type CRC tumors. Our work is important given the recently described clinical efficacy of HER2-targeted therapies in HER2+ CRC and informs strategies for incorporation of HER2 testing into standard of care.

Colorectal cancer (CRC) is the third most commonly occurring cancer and the second leading cause of cancer death in the world (1,2). In 2020, an estimated 1 931 590 new cases and 935 173 deaths worldwide were from CRC. The risk of CRC is highest in economically developed countries, which have a higher incidence of major risk factors such as obesity, sedentary lifestyle, and consumption of red meat (3,4). Globally, more than 90% of CRC occurs in individuals older than 50 years (5). However, the incidence of early onset CRC—defined as younger than 50 years at diagnosis—has been increasing in many countries including the United States, Australia, and Canada (5-7). Although survival rates for CRC have statistically improved over the past few decades because of early detection from increased screening and use of effective multimodality therapies in patients with localized disease, the prognosis for patients with advanced and metastatic CRC remains poor, and 5-year overall survival rates are less than 20% (8).

Activating mutations in the mitogen-activated protein kinase pathway are common in CRC, and their presence is predictive and prognostic (9). Mutations in *KRAS*, *NRAS*, and *BRAF* are the most common mitogen-activated protein kinase alterations in CRC and have implications for the selection of targeted treatments as well as predicting resistance to epidermal growth factor receptor–blocking monoclonal antibodies (10). Hence, extended rat sarcoma virus (RAS) and rapidly accelerated fibrosarcoma (RAF) testing is an integral part of clinical care of metastatic CRC. More recently, amplifications in HER2 and erythroblastic oncogene B homolog 2 (ERBB2) have been identified in a small subset of CRC. Current National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology recommend testing for HER2 amplifications for patients with metastatic CRC in tumors without RAS or RAF alterations (11,12). There are no recommendations from the European Society for Medical Oncology regarding the testing of HER2 in CRC (13). HER2 is a known oncogene with therapeutic relevance in breast and gastric cancers (14-19). Data from recent clinical trials including HERACLES-A (HER2 Amplification for Colo-rectaL cancer Enhanced Stratification) (trastuzumab and lapatinib), MyPathway (pertuzumab and

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trastuzumab), DESTINY-CRC01 (trastuzumab deruxtecan), TRIUMPH (pertuzumab and trastuzumab), and MOUNTAINEER (tucatinib and trastuzumab) demonstrate a clinical benefit of HER2-targeted therapy in metastatic CRC with HER2 amplification or overexpression (20-25).

Historically, the standard methods used for detecting HER2 amplifications or overexpression included immunohistochemistry and/or in situ hybridization (ISH) (26). Recently, tissue-based next-generation sequencing has been increasingly adopted in clinical practice and can also identify HER2 amplifications (27-29). However, currently, no US Food and Drug Administration (FDA)–approved criteria or testing assay exist specifically for CRC. Furthermore, because routine testing for HER2 has not historically been performed in CRC, the proportion of patients with HER2-positive (HER2+) CRC who can potentially benefit from HER2-targeted therapy is uncertain, with reported prevalence ranging between 5% and 10% of all CRC patients in published literature (20,25,29). Hence, a comprehensive review of existing literature is needed to better understand HER2 expression in CRC. We performed a systematic literature review and meta-analysis to 1) generate an estimate of the proportion of patients with CRC who have HER2 overexpression, amplification, and/or positivity and 2) explore HER2 overexpression, amplification, and/or positivity in clinically relevant subgroups.

Methods

Systematic literature review methods

The systematic literature review was conducted in accordance with Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (30). The search was implemented in January 2021 and included studies published in the prior 20 years (January 2000 to December 2020) in MEDLINE and Embase databases. Abstracts presented at the following conferences held between 2017 and 2020 were also searched: American Society of Clinical Oncology (ASCO), American Society of Clinical Oncology–Gastrointestinal Cancers Symposium, European Society for Medical Oncology, European Society for Medical Oncology World Gastrointestinal Cancer, American Association for Cancer, and International Society of Gastrointestinal Oncology.

Studies that reported HER2 overexpression, amplification, or HER2+ data for CRC tumor samples were included. Reviews, case reports, case series, letters, and editorials were excluded, as were non-English language studies. Clinical trials of HER2+ patients were included if HER2 expression was tested as part of enrollment criteria and the proportion was reported. Studies that investigated cultured tumor cell lines or nonhuman samples were excluded.

Studies were screened for inclusion and exclusion criteria by 2 independent reviewers (authors: AK and LB). Disagreements between the 2 reviewers were resolved through discussion, with a third reviewer making the final decision, if needed. Data were extracted by 1 reviewer and validated by an independent reviewer.

Definitions

Classification as HER2+, HER2 overexpression, and HER2 amplification is summarized in Table 1. HER2+ was defined as 1) immunohistochemistry with a score of 3+ plus immunohistochemistry with a score of 2+ and ISH-positive or 2) next-generation sequencing positive. Studies contributing data for HER2+ were required to present both immunohistochemistry and ISH testing (among patients with immunohistochemistry 2+) or next-generation sequencing. Overexpression was defined as immunohistochemistry score of 3+. Amplification was defined as ISH+ or next-generation sequencing positive (ranging from >5 to >7 copy number variations, depending on the platform used) (31,32) (see Table 1).

Table 1. Definitions[a]

Classification	Definition
Amplification	ISH+ Next-generation sequencing +
Overexpression	immunohistochemistry 3+ only
HER2+	immunohistochemistry 3+ plus immunohistochemistry 2+ reflex with ISH+ Next-generation sequencing+

[a] ISH = in situ hybridization.

Subgroup definitions

The overall population was defined as all CRC patients, regardless of RAS status or stage. RAS wild-type (overall) was defined as a subgroup of the overall population limited to RAS wild-type patients. The metastatic CRC population consists of patients with metastatic disease regardless of RAS status. RAS wild-type (metastatic CRC) was defined as a subgroup of the metastatic CRC population limited to RAS wild-type patients. In the analysis of HER2 expression by sidedness, the rectum, rectosigmoid, descending colon, sigmoid colon, and splenic flexure were classified as left side. Cecum, ascending colon, hepatic flexure, and transverse colon were classified as right side.

Meta-analysis methods

Studies were assessed for potential inclusion in a series of meta-analyses to generate point estimates and 95% confidence intervals (CIs) for HER2+, overexpression, and amplification. Assays that used circulating cell–free DNA or circulating tumor DNA only were excluded to ensure comparability to studies that used tissue-based assays. Studies that used assays that are not FDA approved for HER2 (eg, laboratory developed) or did not specify the exact assay used were also excluded. A list of FDA-cleared or -approved assays is presented in Supplementary Table 1 (available online). A sensitivity analysis was conducted that included all studies identified in the literature review that presented data on HER2 status as defined above, regardless of whether the studies reported using an FDA-approved assay. For the RAS wild-type subgroup, studies where patients had previously progressed on an anti–epidermal growth factor receptor regimen were excluded; HER2 expression has previously been correlated with potential resistance to anti–epidermal growth factor receptors, so patients with progression on an anti–epidermal growth factor receptors may not be representative of the broader RAS wild-type population (33-35).

Statistical analysis

Statistical analysis was performed using R Statistical Software (version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria). Heterogeneity was assessed by Higgin I^2 (36,37). If substantial heterogeneity ($I^2 \geq 60\%$) was observed, point estimates were based on a random effects model rather than a fixed effects model.

Results

Cohort identification

Among 903 database references and 137 congress abstracts screened, 224 publications were retained after full-text review.

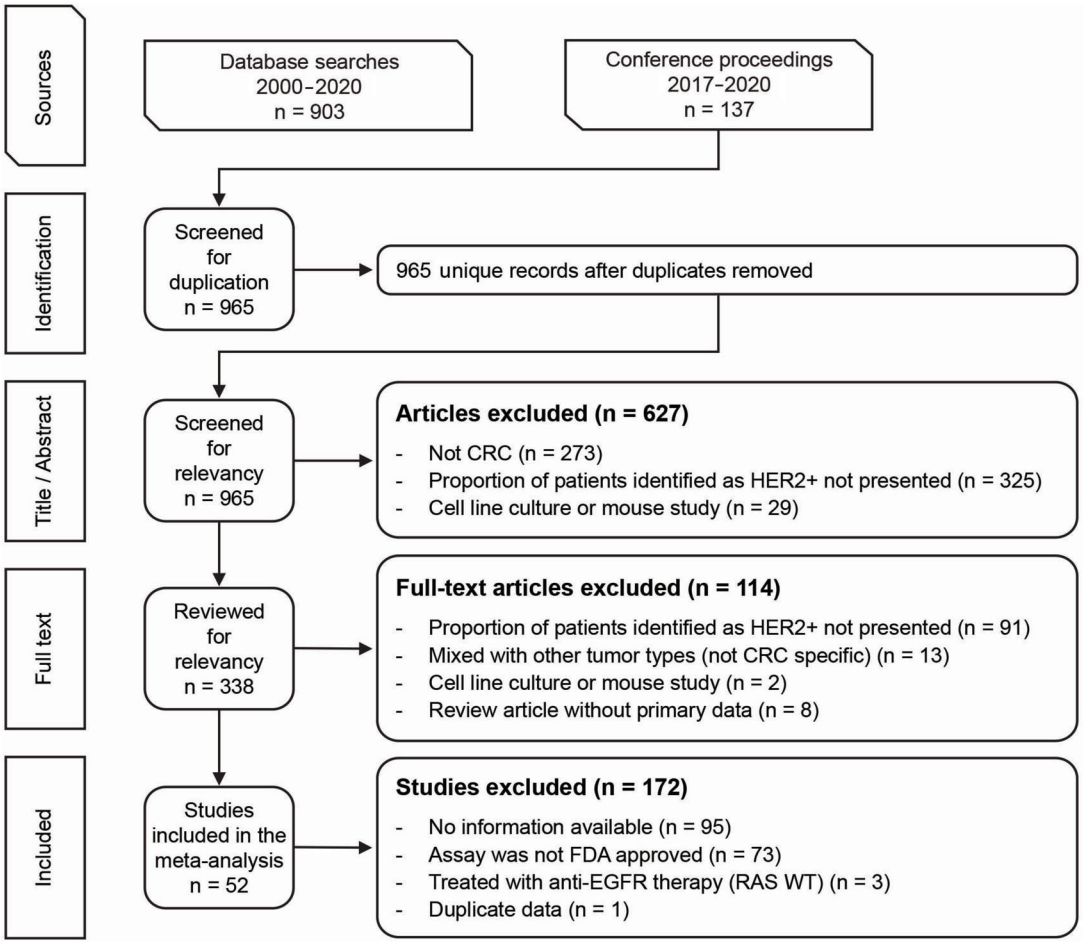

Figure 1. PRISMA diagram. CRC = colorectal cancer; EGFR = epidermal growth factor receptor; RAS = rat sarcoma virus; WT = wild-type.

Of the 224 studies with data pertaining to HER2 status in CRC, 52 studies were confirmed as using an FDA-approved assay and met all criteria for inclusion in the meta-analyses (Figure 1) (Supplementary Tables 2-5, available online). Our primary analysis focused on determining rates of HER2+ (defined as immunohistochemistry with a score of 3+ plus immunohistochemistry with a score of 2+ and ISH-positive or as next-generation sequencing positive; Table 1) in CRC and various CRC disease subsets. However, not all of the 52 studies identified for inclusion in the meta-analyses presented complete information to calculate HER2+—this was particularly the case for studies that used immunohistochemistry and/or ISH only—as both the proportion of patients who scored immunohistochemistry 3+ and immunohistochemistry 2+ and ISH+ were needed to calculate HER2+ rates. Some studies reported only amplification assessed by ISH, whereas other studies reported immunohistochemistry scores only (did not report amplification using ISH) precluding accurate calculation of HER2+ rates. Hence, only select studies of the 52 selected studies using an FDA–approved assay, with all necessary information to calculate HER2+ rates, were included in the primary analysis and will be highlighted in relevant sections. Later in the article, we present a sensitivity analysis to determine HER2+ rate by expanding our analysis to include studies that used non-FDA-approved assays. Last, we derive estimates for rates of HER2 overexpression (ie, immunohistochemistry 3+ only) and HER2 amplification (ie, next-generation sequencing positive or ISH+), when used individually.

Primary analysis

HER2+ in overall study population (both CRC and metastatic CRC)

In the overall population, 26 studies were included in the meta-analysis for HER2+, defined as immunohistochemistry with a score of 3+, or immunohistochemistry with a score of 2+ and ISH-positive, or next-generation sequencing positive (Table 2) (26,31,32,38-59). Among a total of 17 589 patients, HER2+ rates ranged from 2.0% to 12.8% (Table 2; Supplementary Figure 1, available online). Because there was substantial heterogeneity in the rates of HER2+ ($I^2 = 76\%$), a random effects model was used, which revealed a HER2+ point estimate of 4.1% (95% CI = 3.4% to 5.0%) (Figure 2A). Results of this meta-analysis were heavily weighted by 3 studies with sample sizes of at least 1000 patients that reported HER2+ rates between 2.8% and 3.6% (50,52,60). Notably, 1 of the 3 studies reported a HER2+ rate of 2.8% and had a sample size of 8887 (60). Most studies (n = 16) reported HER2+ using immunohistochemistry score 3+ and immunohistochemistry score 2+ reflex with ISH; the remaining 10 studies reported HER2+ using next-generation sequencing (27,31,32,38-48,50-61).

RAS wild-type and RAS mutant subpopulations

Eleven unique studies were limited to RAS wild-type patients (Supplementary Table 3, available online) of which 8 reported data on HER2+ among 4108 patients (Table 3) (20,26,32,45,62-65). Only 2 studies comprising 179 patients contributed HER2+ data in the RAS mutant subgroup (20,46). HER2+ was higher in the

Table 2. HER2 positive and assays used in studies included in the primary meta-analysis of HER2 expression in CRC[a]

Study, year	No. (%)	Country	Metastatic or nonmetastatic	Assay method
Immunohistochemistry 3+ and immunohistochemistry 2+ or ISH+				
Buhmeida et al., 2018 (55)	228 (2.6)	Saudi Arabia	Unclear	Immunohistochemistry (PATHWAY HER2 4B5; Ventana) or BDISH (INFORM HER2 Dual ISH DNA Probe Cocktail)
Conradi et al., 2013 (39)	169 (12.4)	Germany	Mixed	Immunohistochemistry (PATHWAY 4B5; Ventana) or SISH (INFORM HER2 Dual ISH or DNA Probe Cocktail, Ventana)
Dong et al., 2019 (40)	139 (10.1)	China	Unclear	Immunohistochemistry (HercepTest, Dako); FISH (PathVysion HER2 DNA Probe Kit, Abbott)
Fujii et al., 2020 (47)	475 (4.0)	International	Metastatic	Immunohistochemistry (PATHWAY HER2 4B5; Ventana) or FISH (PathVysion HER2 Probe Kit; Abbott)
Kavanagh et al., 2009 (57)	132 (2.3)	Ireland	Mixed	Immunohistochemistry (PATHWAY Ventana 4B5); FISH (INFORM HER2 Probe)
Nathanson et al., 2003 (53)	139 (2.9)	United States	Metastatic	Immunohistochemistry (HercepTest, Dako); FISH (PathVysion)
Park et al., 2018 (58)	139 (2.2)	Korea	Mixed	Immunohistochemistry (PATHWAY HER2 4B5; Ventana) or SISH (Ventana INFORM HER2 DNA dual color)
Ramieri et al., 2010 (59)	50 (2.0)	Italy	Unclear	Immunohistochemistry (PATHWAY 4B5 Ventana) or SISH (INFORM HER2 Ventana)
Sawada et al., 2018 (45)	370 (4.1)	Japan	Metastatic	Immunohistochemistry (PATHWAY HER2 4B5; Ventana); FISH (PathVysion HER2 Probe Kit; Abbott)
Sclafani et al., 2013 (54)	104 (2.9)	Spain, Sweden, UK	Unclear	Immunohistochemistry (PATHWAY HER2 4B5); FISH (INFORM HER2 dual ISH DNA Probe cocktail assay)
Seo et al., 2014 (61)	539 (3.7)	Korea	Mixed	Immunohistochemistry (PATHWAY HER2 4B5; Ventana); SISH (INFORM HER2 DNA)
Song et al., 2014 (48)	106 (3.8)	China	Nonmetastatic	Immunohistochemistry (PATHWAY HER2 4B5; Ventana); FISH (PathVysion); ASCO or CAP guideline used
Wang et al., 2019 (50)	1193 (3.6)	China	Mixed	Immunohistochemistry (PATHWAY HER2 4B5; Ventana); FISH (PathVysion HER2 DNA probe kit)
Yamashiro et al., 2020 (41)	42 (9.5)	Japan	Mixed	Immunohistochemistry (PATHWAY 4B5; Ventana); FISH (Pathvysion HER2 DNA probe kit)
Yun et al., 2018 (51)	331 (3.6)	Korea	Mixed	Immunohistochemistry (HercepTest, Dako) or SISH (INFORM HER2 DNA),
Zhang et al., 2020 (42)	480 (5.8)	China	Nonmetastatic	Immunohistochemistry (PATHWAY anti-HER-2Neu 4B5 rabbit monoclonal primary antibody; Ventana) or FISH (PathVysion HER2 DNA Probe Kit II; Abbott)
Next-generation sequencing				
Battaglin, 2020 (32)	505 (3.2)	United States	Metastatic	Next-generation sequencing (Foundation One)
Dumbrava, 2019 (31)	842 (3.6)	United States	Metastatic	Next-generation sequencing (Oncomine Comprehensive Assay, Ion AmpliSeq Comprehensive Cancer Panel CMS400, FoundationOne or FoundationOne Heme, Guardant360); all were used
Gong, 2017 (43)	138 (5.1)	United States	Metastatic	Next-generation sequencing (FoundationOne)
Hechtman, 2015 (44)	86 (4.7)	United States	Unclear	Next-generation sequencing (MSK-IMPACT)
Hechtman, 2016 (56)	255 (2.4)	United States	Mixed	Next-generation sequencing (MSK-IMPACT)
Lipsyc, 2017 (46)	939 (4.0)	United States	Metastatic	Next-generation sequencing (MSK-IMPACT)
Kato, 2019 (38)	94 (12.8)	United States	Mixed (advanced stage)	Next-generation sequencing (FoundationOne assay)
Raghav, 2019 (52)	1134 (3.1)	United States	Unclear	Next-generation sequencing (MSK-IMPACT, Guardant Health)
Ross, 2018 (60)	8887 (2.8)	United States	Metastatic	Next-generation sequencing (FoundationOne)
Yeh, 2019 (26)	73 (8.2)	Taiwan	Nonmetastatic	Next-generation sequencing (Oncomine Comprehensive Assay)

[a] BDISH = brightfield double in situ hybridization; CISH = chromogenic in situ hybridization; CRC = colorectal cancer; FISH = fluorescence in situ hybridization; ISH = in situ hybridization; mixed = both metastatic and nonmetastatic CRC; SISH = silver in situ hybridization.

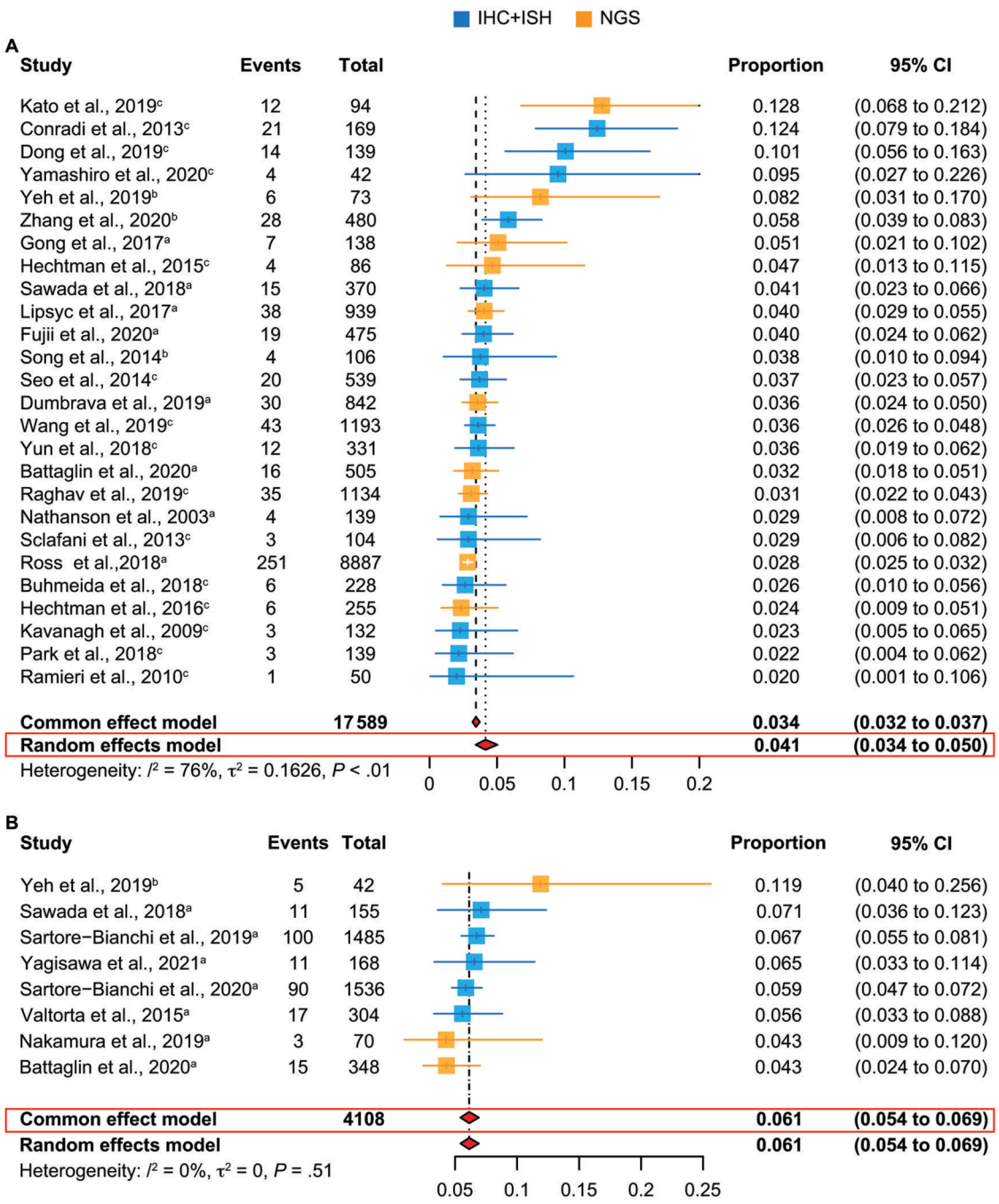

Figure 2. Primary meta-analysis of HER2+ in the overall CRC population (**A**) and the RAS WT CRC population (**B**). The colors **blue** and **orange** indicate studies using IHC+ISH and NGS, respectively, to detect HER2 amplification or overexpression. [a]Metastatic disease; [b]nonmetastatic disease; [c]mixed/unclear. CI = confidence interval; CRC = colorectal cancer; IHC = immunohistochemistry; ISH = in situ hybridization; NGS = next-generation sequencing; RAS = rat sarcoma virus; WT = wild-type.

RAS wild-type population vs the RAS mutant population (6.1% [95% CI = 5.4% to 6.9%] vs 1.1% [95% CI = 0.3% to 4.4%]; $P < .0001$) (Figure 2B).

Metastatic and nonmetastatic populations

In the metastatic population, 8 studies comprising 12 295 patients were included in the meta-analysis for HER2+ (31,32,43,45-47,53,60). HER2+ ranged from 2.8% to 5.1%, with a point estimate of 3.1% (95% CI = 2.8% to 3.4%) (Figure 3A). In the nonmetastatic population, 3 studies comprising 659 patients were included in the meta-analysis for HER2+ (26,42,48). HER2+ ranged from 3.8% to 8.2%, with a point estimate of 5.8% (95% CI = 4.2% to 7.8%). In the metastatic RAS wild-type population (n = 7) comprising 4108 patients, HER2+ ranged from 4.3% to 7.1%, with a point estimate of 6.1% (95% CI = 5.4% to 6.9%) (Figure 3B) (20,32,45,62-65). Only 1 study comprising 42 patients contributed HER2+ data to the nonmetastatic RAS wild-type population (11.9%) (26). Rates of HER2+ were not statistically different between the metastatic and nonmetastatic overall CRC population ($P = .67$) or the metastatic and nonmetastatic RAS wild-type population ($P = .12$).

Table 3. HER2+ and assays used in studies included in the primary meta-analysis of HER2 expression in RAS wild-type CRC[a]

Study, year	No. (%)	Country	Metastatic or nonmetastatic	Assay method
Immunohistochemistry 3+ and immunohistochemistry 2+ or ISH+				
Sartore-Bianchi et al., 2019 (62)	1485 (6.7)	Italy	Metastatic	Immunohistochemistry or FISH
Sartore-Bianchi et al., 2020 (64)	1536 (5.9)	Italy	Metastatic	Immunohistochemistry or FISH
Sawada et al., 2018 (45)	155 (7.1)	Japan	Metastatic	Immunohistochemistry (PATHWAY HER2 4B5 Ventana); FISH (PathVysion HER2 Probe Kit, Abbott)
Valtorta et al., 2015 (20)	304 (5.6)	Italy	Metastatic	Immunohistochemistry (VENTANA 4B5 and HercepTest, Dako) or SISH (Ventana 4B5 Inform HER2 DNAL dual-color assay)
Yagisawa et al., 2021 (63)	168 (6.5)	Japan	Metastatic	Immunohistochemistry (PATHWAY HER2 4B5, Ventana); FISH (PathVysion Abbott)
Next-generation sequencing				
Battaglin et al., 2020 (32)	348 (4.3)	United States	Metastatic	Next-generation sequencing (Foundation One)
Nakamura et al., 2019 (65)	70 (4.3)	Japan	Metastatic	Next-generation sequencing (both Oncomine Comprehensive Assay, Guardant360)
Yeh et al., 2019 (26)	42 (11.9)	Taiwan	Nonmetastatic	Next-generation sequencing (Oncomine Comprehensive Assay)

[a] CRC = colorectal cancer; FISH = fluorescence in situ hybridization; ISH = in situ hybridization; RAS = rat sarcoma virus.

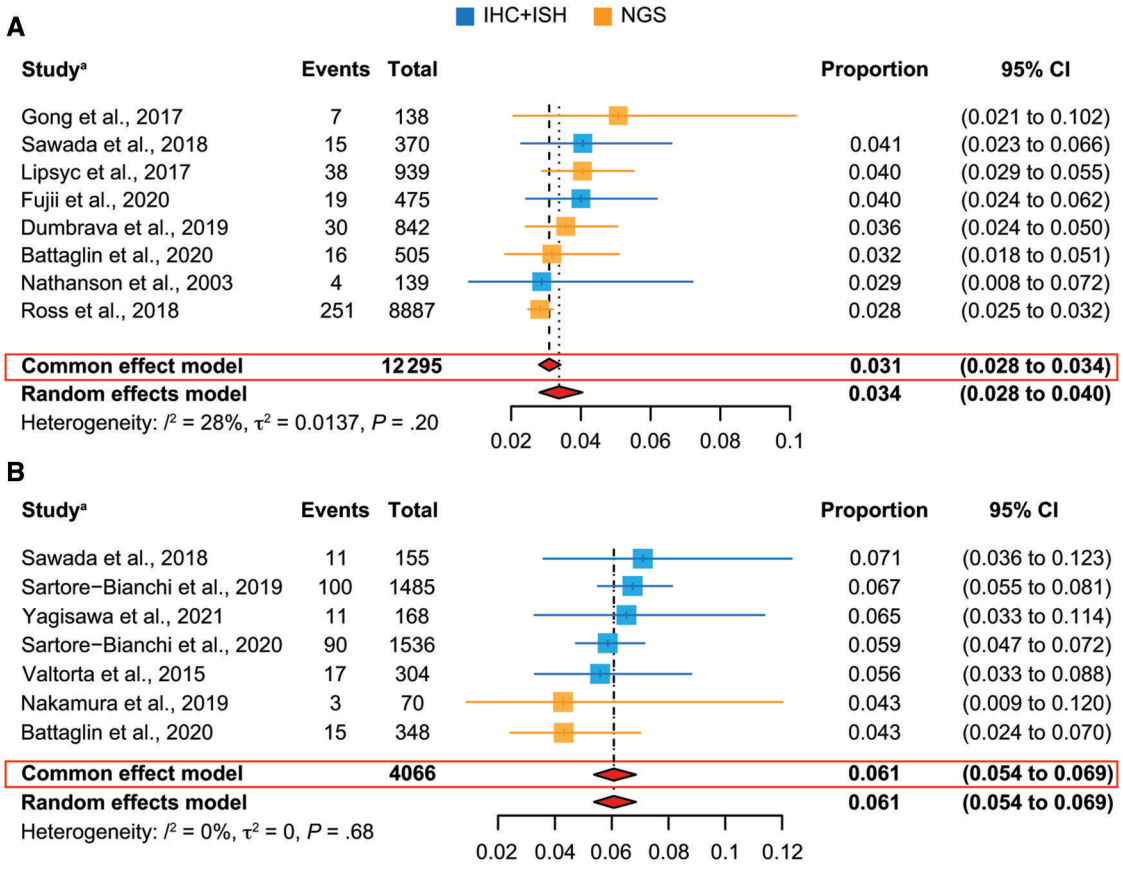

Figure 3. Primary meta-analysis of HER2+ in the metastatic CRC population (**A**) and RAS WT and metastatic CRC population (**B**). The colors **blue** and **orange** indicate studies using IHC+ISH and NGS, respectively, to detect HER2 amplification or overexpression. CI = confidence interval; CRC = colorectal cancer; IHC = immunohistochemistry; ISH = in situ hybridization; NGS = next-generation sequencing; RAS = rat sarcoma virus; WT = wild-type. [a]Metastatic disease.

Sidedness

For each study that provided HER2+ information by tumor location, data were extracted and pooled into left and right side, as defined in Subgroup Definitions. Three studies comprising 1018 patients (330 right sided and 688 left sided) reported HER2+ by right- and left-side primary tumor location. Rates of HER2+ were statistically higher in left-sided CRC patients compared with right-sided CRC patients (5.8% [95% CI = 4.3% to 7.8%] vs 2.7% [95% CI = 1.4% to 5.2%]; P = .03). For rectum only, 6 studies comprising 2020 patients reported HER2+ (26,39,42,54,58,60). HER2+ ranged from 2.2% to 12.4%, with a point estimate of 4.7% (95% CI = 2.8% to 8.0%). Rates of HER2+ for rectum only were numerically higher but not statistically significantly different compared with right-sided CRC patients (P = .23). Because our analysis of

Table 4. Summary of HER2+ point estimates from primary analysis restricted to studies using FDA-approved assays

Disease setting	No. of studies[a]	No. of patients	HER2+ point estimates (95% CI)	P[e]
Overall population, CRC and metastatic CRC	26	17 589	4.1% (3.4% to 5.0%)	—
RAS wild-type, CRC and metastatic CRC	8	4108	6.1% (5.4% to 6.9%)	<.0001
RAS mutant, CRC and metastatic CRC	2	179	1.1% (0.3% to 4.4%)	
Metastatic CRC	8	12 295	3.1% (2.8% to 3.4%)	.6672
Nonmetastatic CRC	3	659	5.8% (4.2% to 7.8%)	
Left-sided CRC[b]	3	688	5.8% (4.3% to 7.8%)	.02926
Right-sided CRC[b]	3	330	2.7% (1.4% to 5.2%)	
Rectum only[c]	6	2020	4.7% (2.8% to 8.0%)	.23014[d]

[a] Only studies using FDA-approved assays for HER2 detection and reporting complete information to allow estimate HER2+ rates are included. CI = confidence interval; CRC = colorectal cancer; FDA = Food and Drug Administration; RAS = rat sarcoma virus. Em dash indicates not assessed.
[b] Left side was defined as rectum, rectosigmoid, descending colon, sigmoid colon, and splenic flexure. Right side was defined as cecum, ascending colon, hepatic flexure, and transverse colon.
[c] Includes rectum only and studies that investigated rectal patients only.
[d] Right-sided CRC vs rectum only.
[e] Test of proportions (z score) was used to calculate the P value.

left-sided tumors included rectal primaries, we could not compare rates of HER2+ between rectum and left-sided (nonrectum) patients.

Microsatellite stability and microsatellite instability

A total of 11 studies reported microsatellite stability status of which 2 studies also reported HER2+. Among these 2 studies with 186 patients, HER2+ was enriched in microsatellite stability CRC (98.8%-100%) and scarce in microsatellite instable-high CRC (0%-1.2%) (32,60). Two other studies reported HER2 amplification (using ISH) and microsatellite stability status, with similar trends observed (Supplementary Tables 4 and 5, available online) (42,66).

Overall HER2+ point estimates from our primary analysis are summarized in Table 4.

Sensitivity analysis

A total of 125 unique studies were included in the sensitivity analysis of all studies regardless of whether an FDA-approved assay was used. A total of 80 HER2+ studies were analyzed in the sensitivity analysis of which 10 reported HER2+ rates higher than the highest HER2+ rate (12.8%) identified in the primary analysis. Of the 10 studies that reported HER2+ rates higher than 12.8%, 3 reported substantially higher HER2+ rates of 35.1%, 47.3%, and 53.6%. Despite inclusion of these outliers, results of the sensitivity analysis were similar to the primary analysis, with 4.6% (95% CI = 3.7% to 5.7%) HER2+ in the overall CRC population (vs 4.1% [95% CI = 3.4% to 5.0%], in the primary analysis), as the sensitivity analysis was heavily weighted by 13 studies with sample sizes of at least 1000 patients that reported HER2+ rates between 1.6% and 5.0% (Supplementary Figure 2, available online). In the sensitivity analysis of the 12 studies that presented data for the RAS wild-type population, HER2+ was estimated at 7.9% (95% CI = 4.9% to 12.6%), which was slightly higher than the estimate generated in the primary analysis (6.1%, 95% CI = 5.4% to 6.9%), most likely attributed to a study that appears to be an outlier, reporting an HER2+ rate of 57.1% in the RAS wild-type population (Supplementary Figure 3, available online) (67).

Re-analysis of HER2 point estimates using only HER2 overexpression or HER2 amplification

Finally, we wanted to understand whether these estimates would be different if HER2 overexpression (using immunohistochemistry alone) or amplification (using ISH or next-generation sequencing) were used as inclusion metrics individually. In the overall population, 28 studies comprising 7333 patients were included in the meta-analysis for HER2 overexpression. Overexpression ranged from 0% to 32.3%, with a point estimate of 3.5% (95% CI = 2.5% to 4.9%). Notably, the 2 studies that reported the highest proportion of patients with HER2 overexpression (immunohistochemistry with a score of 3+) were conducted in the Middle East and Pakistan (Supplementary Table 2, available online) (68,69). A total of 66 HER2 overexpression studies were included in the sensitivity analysis of inclusion regardless of whether an FDA-approved assay was used. Results of the sensitivity analysis were similar to the primary analysis, with 4.5% (95% CI = 3.4% to 6.0%) HER2 overexpression.

In the overall population, 32 studies comprising 18 750 patients were included in the meta-analysis for HER2 amplification. HER2 amplification ranged from 0.9% to 20.2%, with a point estimate of 4.6% (95% CI = 3.6% to 5.8%). A total of 79 HER2 amplification studies comprising 55 839 patients were included in the sensitivity analysis of inclusion regardless of whether an FDA-approved assay was used. Results of the sensitivity analysis were similar to the primary analysis, with 4.2% (95% CI = 3.3% to 5.2%) HER2 amplification in the overall population. Studies eligible for inclusion in the sensitivity analysis generally reported higher rates of HER2 expression. For instance, Qiu et al. (67) and Herreros-Villanueva et al. (70) conducted studies that did not use an FDA-approved assay and reported RAS wild-type HER2 amplification rates of 57.1% and 26.3%, respectively. Among FDA-approved studies only, the highest rate of RAS wild-type HER2 amplification reported was 11.9% (19).

Hence, the point estimates from our meta-analysis, considering all included studies, suggest that HER2 overexpression and amplification rates are similar. Most individual studies that reported both HER2 overexpression (immunohistochemistry with a score of 3+) and amplification (ISH-positive) found discordance between 0.8% and 3.5% but as high as 14.0% (range = 0%-14.0%) (26,39,40,42,45,47,48,50,53-55,57-59,61).

Discussion

Recent studies suggest that HER2 is a clinically relevant biomarker in CRC. To the best of our knowledge, this is the first meta-analysis to consolidate and generate point estimates for HER2+, HER2 overexpression, and HER2 amplification in CRC. Rigorous inclusion and exclusion criteria, such as only including FDA-approved assays, were applied to provide the most reliable data for this meta-analysis. As routine testing for HER2 has not

historically been performed in CRC, the point estimate generated in this meta-analysis may have important implications for better identifying patients who could benefit from receiving HER2-targeted therapy.

This literature review identified few studies that reported relevant HER2 information for RAS wild-type patients. The estimated RAS wild-type HER2+ rate from our study of 6.1% is consistent with 5%-7% range reported in recent studies (20,35,60,71-75). Results of our sensitivity analysis where the requirement for an FDA-approved assay was relaxed produced a slightly higher estimate at 7.9%. Our point estimates may underestimate the true HER2+ in the RAS wild-type population because of strict inclusion and exclusion criteria. In addition, we excluded studies where the study population was treated with anti–epidermal growth factor receptor therapy, which may be enriched for HER2+ (33-35).

Our results support the clinical observation that HER2 is enriched in left-sided CRC (62,76,77). The higher HER2 expression in left-sided cancers may be driven in part by rectal cancer, which has reported HER2+ rates of 2.2%-12.4% but occasionally as high as 26.7% in resected specimens (39,67). In addition, HER2+ is enriched in microsatellite stable CRC and scarce in microsatellite instable CRC, as has been noted elsewhere (78). Interestingly, a recent study reported high rates of microsatellite instable–high tumors in patients with HER2 mutations but none in patients with HER2 amplification, which may indicate an inverse relationship between microsatellite instable status and the 2 HER2 alterations in CRC (67). We also found that rates of HER2 in metastatic CRC were slightly lower than the estimates generated for CRC overall. This could be related to underlying risk of distant disease in HER2+ CRC; however, our findings related to HER2+ in various CRC clinical subsets need to be interpreted with caution given the limited number of studies that contributed to the data.

A sensitivity analysis was conducted to explore the impact of our strict inclusion criteria limiting studies to those that used FDA-approved assays, and no statistically significant differences were found in HER2+ rates. With less stringent criteria, the sensitivity analysis included 3 studies that reported substantially higher HER2+ rates of 35.1%-53.6% than the highest HER2+ rate of 12.8% identified in the primary analysis, contributing to the generation of a slightly higher point estimate than that of the primary analysis. However, results of the primary analysis were robust to inclusion of these outliers, as results were heavily weighted by 13 studies with large sample sizes that reported HER2+ rates between 1.6% and 5.0%. For the RAS wild-type subgroup, the sensitivity analysis yielded slightly higher results (difference of 1.8%-2.5%) than the meta-analysis of FDA-approved studies only. This difference is most likely attributable to 1 study that appears to be an outlier, reporting an HER2+ rate of 57.1% in the RAS wild-type population.

We used strict definitions of HER2+, overexpression, and amplification to enhance the precision of our estimates by reducing heterogeneity because of differences in assays, unvalidated laboratory-developed assays, and differences in scoring criteria. As such, only a fraction of studies identified in the systematic literature review could ultimately be included in the quantitative analysis.

Our study has several limitations. First, studies that used circulating cell–free DNA or circulating tumor DNA–based assays for identification of HER2+ CRC were excluded from this meta-analysis to ensure comparability between studies. It is important to note the increasingly vital role of liquid biopsies in cancer care, which have shown great potential to change clinical practice. In metastatic CRC, both gene-specific and panel-based ctDNA assays show a high degree of concordance with tissue-based methods for identification of single nucleotide variations and small indels in key molecular drivers (79,80). However, more data are needed to confirm concordance between the 2 approaches in identifying copy number alterations such as amplifications in HER2. This is in part because of the varied algorithms used for identification of copy number changes making cross-comparison of presence and level of amplifications identified inherently challenging between assays. Second, although this study sought to consolidate information on HER2 status in CRC, it did not examine the impact of detecting HER2 mutations in CRC. Preclinical studies suggest that HER2 mutations are oncogenic and predict resistance to epidermal growth factor receptor–directed therapy in CRC but are amenable to targeted therapy with irreversible HER2 inhibitors such as neratinib or afatinib (81). Interestingly, HER2 mutations lead to increased intracellular-to-cell membrane cycling of the HER2 protein allowing effective internalization and, hence, efficacy of HER2–antibody drug conjugates (82). This approach has shown success in lung cancer where HER2 mutant cases showed impressive clinical benefit with HER2–antibody drug conjugates, trastuzumab deruxtecan (83). These exciting data support continued work in further clinical characterization and therapeutic targeting of HER2 mutations in CRC. Lastly, there were residual differences between assays used to determine HER2 status in different studies that could not be controlled for despite restricting the criteria to only include FDA-approved assays. For example, studies used different HER2 scoring criteria (eg, HERACLES, ASCO–College of American Pathologists [CAP] breast, ASCO–CAP gastric), which can influence how patients are classified. The gastric criteria define immunohistochemistry score of 2+ as weak to moderate complete, basolateral or lateral membranous reactivity in at least 10% of cancer cells, whereas the HERACLES diagnostic criteria define immunohistochemistry score of 2+ as moderate in at least 50% of tumor cells (21,84). Similarly, the 2007 ASCO–CAP recommendation considered a patient HER2 amplified if the HER2 to chromosome enumeration probe 17 (CEP17) ratio was more than 2.2, whereas the HERACLES diagnostic criteria defined HER2 amplification as a HER2/CEP17 ratio of at least 2.0. Differences in determining amplification between next-generation sequencing and fluorescence ISH also exist. Additionally, some studies that defined overexpression differently than how this meta-analysis defined it could not be included if counts for immunohistochemistry score of 3+ were not presented. Hence, consensus on standardized HER2 scoring criteria and testing is paramount to better select patients who may benefit from HER2-directed therapies.

Across the majority of included studies, 2%-6% of CRC patients were HER2+, with a point estimate of 4.1% (95% CI = 3.4% to 4.8%). HER2+ was found to be higher in RAS wild-type and left-sided primary tumors and rare in those with microsatellite instable-high CRC. Results of this meta-analysis help elucidate the proportion of patients with HER2 who may potentially benefit from emerging HER2-targeted therapies in the CRC landscape. Our work highlights the importance of testing HER2 status in patients with advanced or metastatic CRC especially in those with RAS wild-type and microsatellite stable disease.

Data availability

The data underlying this article are available in the article and its online supplementary material.

Author Contributions

Harshabad Singh, MBBS, MD (Conceptualization; Data curation; Writing—review & editing), Ashley Kang, MPH (Formal analysis; Writing—original draft; Writing—review & editing), Lisa Bloudek, PharmD, MS (Formal analysis; Writing—original draft; Writing—review & editing), Ling-I Hsu, PhD (Conceptualization; Data curation; Writing—review & editing), Maria Corinna Palanca-Wessels, MD, PhD (Conceptualization; Data curation; Writing—review & editing), Michael Stecher, MD (Conceptualization; Data curation; Writing—review & editing), Muriel Siadak, MD (Conceptualization; Data curation; Writing—review & editing), and Kimmie Ng, MD, MPH (Conceptualization; Data curation; Writing—review & editing).

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Conflicts of interest

AK and LB are employees of Curta, Inc, which received funding from Seagen Inc in connection with this research. LH, MPW, MSt, and MSi are employees of Seagen Inc and hold stock/stock options in Seagen Inc. HS received research funding from AstraZeneca; HS received consulting fees from Dewpoint Therapeutics, Merck. KN received institutional research funding from Pharmavite, Evergrande Group, Janssen, and Revolution Medicines; KN participated on advisory boards or received consulting fees from GlaxoSmithKline, Bicara Therapeutics, CytomX, Pfizer, Seagen, and Bayer.

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