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HER2-positive Colorectal Cancer: Molecular Biology, Clinical Significance, and Therapeutic Implications with an Emphasis on Metastatic Disease

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Abstract

Human epidermal growth factor receptor 2 (HER2), the protein product of the *ERBB2* gene, is an emerging therapeutic target in colorectal cancer (CRC). Although HER2 alterations occur in a small proportion of CRCs, advances in molecular characterization and HER2-directed therapies have increased the clinical importance of this biomarker. HER2-positive CRC is biologically heterogeneous, and its clinical significance depends on the type of *ERBB2* alteration (amplification or mutation), the level of HER2 protein expression, coexisting molecular features, and disease setting. *ERBB2* amplification and HER2 overexpression activate oncogenic pathways, including mitogen-activated protein kinase and phosphoinositide 3-kinase-protein kinase B signaling, and may contribute to resistance to anti-epidermal growth factor receptor (EGFR) therapy. HER2-positive CRC is enriched in KRAS/NRAS/rapidly accelerated fibrosarcoma B-type kinase (BRAF) [rat sarcoma GTPase (RAS)/BRAF] wild-type, left-sided, and microsatellite-stable tumors. HER2 overexpression, *ERBB2* amplification, and *ERBB2* mutation are biologically distinct alterations. Accurate biomarker assessment is therefore essential, although HER2 testing criteria in CRC remain less standardized than those in breast and gastric cancers. HER2-directed therapy has become an established strategy for selected patients with previously treated metastatic CRC. Trastuzumab-based dual HER2 blockade, including tucatinib plus trastuzumab, has demonstrated meaningful and durable responses, particularly in HER2-positive, RAS wild-type tumors. Trastuzumab deruxtecan has further expanded treatment options and shown activity in HER2-positive tumors, including RAS-mutant disease, with the greatest benefit observed in immunohistochemistry 3+ tumors. Emerging HER2-targeted antibodies, tyrosine kinase inhibitors, and antibody-drug conjugates may further broaden therapeutic options, although optimal sequencing and mechanisms of resistance remain uncertain.

HER2 has evolved from a biomarker associated with anti-EGFR resistance into an actionable therapeutic target in CRC. Given the rapid expansion of HER2-directed treatment options and testing approaches, this review synthesizes current evidence on HER2 biology, testing, therapeutic strategies, and resistance mechanisms in CRC, with particular emphasis on metastatic disease, to provide an updated, clinically oriented reference for the field.

Keywords: HER2, colorectal cancer, precision oncology

Introduction

Colorectal cancer (CRC) remains a major cause of cancer-related mortality worldwide. For patients with microsatellite-stable (MSS) KRAS/NRAS/rapidly accelerated fibrosarcoma B-type kinase (BRAF) wild-type [hereafter rat sarcoma GTPase (RAS)/BRAF wild-type] disease who progress after standard chemotherapy, anti-vascular endothelial growth factor (VEGF) therapy, and anti-epidermal growth factor receptor (EGFR) therapy, treatment options are often limited. *ERBB2* amplification, with resulting human epidermal growth factor receptor 2 (HER2) overexpression, has emerged as an important treatment target in this setting, similar to its established role in breast and gastric cancers, although it is less common in CRC and is associated with a different molecular profile (1). *ERBB2* amplification has also been implicated in both primary and acquired resistance to EGFR-directed antibodies in RAS/BRAF wild-type metastatic CRC (mCRC) (2,3), making HER2 both a potential treatment target and a clinically relevant mechanism of resistance to anti-EGFR antibodies.

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A central theme of this review is that HER2-positive CRC is not a single, uniform disease. The benefit of HER2-directed therapy depends on several factors, including the level of HER2 expression, RAS/BRAF status, and the type of treatment used, such as an antibody, tyrosine kinase inhibitor (TKI), or antibody-drug conjugate (ADC). A treatment that has demonstrated activity in immunohistochemistry (IHC) 3+, RAS wild-type tumors may not have the same effect in IHC 2+/*in situ* hybridization (ISH)-positive or RAS-mutant tumors. Increasing clinical evidence supports this distinction.

The terminology used in this review: HER2 overexpression, *ERBB2* amplification, and *ERBB2* mutation are biologically distinct and are not used interchangeably. *ERBB2* refers to the gene, and HER2 to the protein. In this review, HER2-positive CRC is defined according to the criteria used in each study. Most antibody- and TKI-based trials included patients with IHC 3+ or IHC 2+/ISH-positive tumors, while trastuzumab deruxtecan (T-DXd) studies also evaluated broader HER2-expression groups. HER2-low is not currently a validated predictive category in CRC. *ERBB2*-mutant tumors without amplification are discussed separately, as current evidence does not support treating them in the same way as *ERBB2*-amplified tumors.

HERACLES-A first demonstrated the activity of dual HER2 blockade (trastuzumab plus lapatinib) in *ERBB2*-amplified, RAS wild-type mCRC (4); MyPathway followed with a chemotherapy-free regimen (5); MOUNTAINEER (tucatinib plus trastuzumab) led to U.S. Food and Drug Administration (FDA) approval (6-8); and DESTINY-CRC01/02 evaluated the ADC T-DXd across a range of HER2 expression levels, with activity concentrated in IHC 3+ tumors, including RAS-mutant disease (9-11). HER2-directed therapy is now written into National Comprehensive Cancer Network (NCCN), European Society for Medical Oncology (ESMO), and Pan-Asian guidance (12-14) as a treatment option for chemorefractory, HER2-positive mCRC.

This narrative review summarizes landmark and recent clinical-trial data on HER2-positive CRC, primarily focusing on metastatic disease, where almost all clinical evidence has been generated. It covers HER2 biology and testing, prognostic and predictive factors, treatment and resistance, sequencing, and emerging therapies. Early-stage and rectal diseases are discussed more briefly, reflecting the limited evidence in these settings. The literature search and selection approach is described in the Literature Search and Review Methodology section, and conference-reported data are clearly distinguished from peer-reviewed publications throughout.

Literature Search and Review Methodology

This article is a narrative review; no formal systematic review or meta-analysis was performed. PubMed/MEDLINE and ClinicalTrials.gov were searched for articles and trial records published or posted on or before 30 June 2026 (date of last search). The main search terms were "HER2" or "*ERBB2*" combined with "colorectal cancer", "colon cancer", or "rectal cancer" terms for testing [IHC, ISH, next-generation sequencing (NGS), circulating tumor DNA], treatment [trastuzumab, pertuzumab, lapatinib, tucatinib, neratinib, pyrotinib, trastuzumab emtansine (T-DM1), T-DXd, zanidatamab, ADC, TKI], and resistance (anti-EGFR resistance). The reference lists of key articles and the current NCCN, ESMO, and Pan-Asian guidelines were also screened.

Priority was given to prospective clinical trials (phase I-III) and their final or updated analyses, followed by biomarker validation, translational, real-world, and guideline publications that specifically addressed HER2 in CRC. Case reports, studies without CRC-specific data, non-English publications, and earlier analyses superseded by updated reports were excluded unless an earlier analysis provided distinct information (for example, the original and long-term HERACLES-A reports).

Abstracts from the American Society of Clinical Oncology (ASCO) Annual Meeting, the ASCO Gastrointestinal Cancers Symposium, the ESMO Congress, and the United States and Canadian Academy of Pathology (USCAP) Annual Meeting (2020-2026) were reviewed to identify studies without corresponding full publications. Conference-reported data were included only when no full publication was available and when the data were considered clinically relevant (for example, randomized trial results, the first clinical data for a new agent, or patient-reported outcomes). These data are identified in the text and tables by the meeting, year, and abstract number; they are interpreted as preliminary and are given less weight than peer-reviewed evidence. When both a congress report and a full publication were available, the peer-reviewed publication was used. Study selection and data extraction were performed by the author; given the narrative design, no formal risk-of-bias assessment was undertaken.

HER2 Biology in CRC

ERBB2, located on chromosome 17q12, encodes HER2, a receptor tyrosine kinase in the EGFR/HER family. HER2 has no known ligand and primarily signals via dimerization with other HER-family receptors, particularly human epidermal growth factor receptor 3 (HER3). The HER2-HER3 pair is a strong activator of downstream signaling because HER3

directly recruits phosphoinositide 3-kinase (PI3K) (3). *ERBB2* amplification increases HER2 expression on the cell surface and can lead to ligand-independent signaling, often referred to as "HER2 addiction". Amplification, detected by ISH or NGS, is the main alteration associated with HER2 overexpression by IHC and is the basis for patient selection in most clinical trials (2). A smaller subset of CRCs carries activating *ERBB2* mutations without amplification; these tumors represent a distinct subgroup and are discussed separately.

HER2 mainly signals through two pathways (Figure 1). The MAPK pathway (RAS-RAF-mitogen-activated protein kinase kinase-extracellular signal-regulated kinase) promotes tumor cell proliferation, while the PI3K-protein kinase B (AKT)-mechanistic target of rapamycin pathway supports cell survival. Because RAS acts downstream of HER2, KRAS or NRAS mutations can reduce the activity of HER2-targeted therapy and are therefore important factors in treatment selection. Alterations in other components of the PI3K pathway, such as *PIK3CA* mutations or phosphatase and tensin homolog (PTEN) loss, may also contribute to resistance (2). Finally, HER2 expression can be heterogeneous within the same tumor, between primary and metastatic sites, and over time. This has important implications for both HER2 testing and treatment resistance.

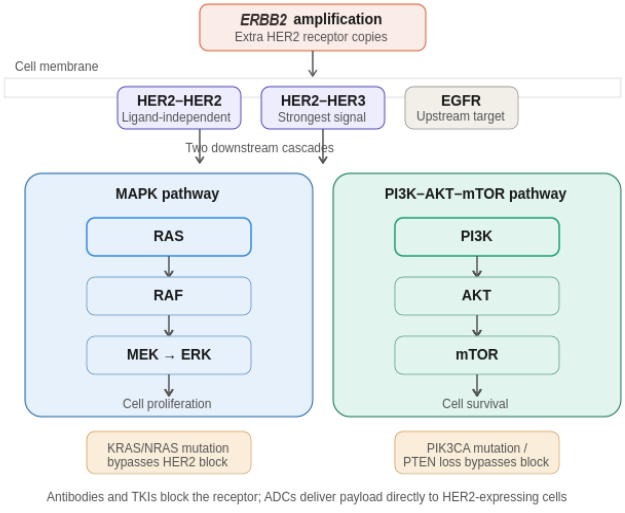


Figure 1. HER2 signaling pathway in colorectal cancer

HER2 receptors, activated by amplification-driven overexpression, dimerize with each other or with HER3 independent of ligand. Signaling proceeds through two cascades: MAPK (HER2→RAS→RAF→MEK→ERK), driving proliferation, and PI3K-AKT-mTOR, driving survival. A KRAS/NRAS mutation bypasses HER2 blockade by activating RAS directly; a *PIK3CA* mutation or PTEN loss can activate the survival arm independently of HER2. Antibodies and TKIs block receptor signaling; antibody-drug conjugates (ADCs) deliver cytotoxic payload directly into HER2-expressing cells (1-3)

ADC: Antibody-drug conjugate, AKT: Protein kinase B, EGFR: Epidermal growth factor receptor, ERK: Extracellular signal-regulated kinase, HER2: Human epidermal growth factor receptor 2, HER3: Human epidermal growth factor receptor 3, MAPK: Mitogen-activated protein kinase, MEK: Mitogen-activated protein kinase kinase, mTOR: Mechanistic target of rapamycin, PI3K: Phosphoinositide 3-kinase, PTEN: Phosphatase and tensin homolog, RAF: Rapidly accelerated fibrosarcoma kinase, RAS: Rat sarcoma GTPase, TKI: Tyrosine kinase inhibitor

Epidemiology and Molecular Characteristics

Frequency and Clinicopathological Associations

ERBB2 amplification or HER2 overexpression is found in 2-3% of unselected mCRC and in 5-14% of RAS/BRAF wild-type tumors (15,16). This group is particularly relevant because most HER2-directed treatments have been studied in RAS wild-type disease. HER2-positive tumors are somewhat more common in the colon than in the rectum and are more often left-sided, which constitute the population that tends to respond to anti-EGFR therapy. HER2 status is generally similar between primary tumors and metastases, but discordance can occur. Therefore, repeat testing should be considered when clinically appropriate.

Co-occurring Alterations and Molecular Subtypes

ERBB2 amplification and activating KRAS or NRAS mutations rarely co-occur, since RAS acts downstream of HER2 in the MAPK pathway (2); most pivotal trials have enrolled predominantly RAS wild-type patients (4-6), with T-DXd a notable exception, having shown activity in RAS-mutant disease as well, mainly in IHC 3+ tumors (see Management of Metastatic HER2-positive CRC). *ERBB2* amplification and *BRAF V600E* mutations rarely occur together. However, this association is less well understood than the relationship between HER2 and KRAS/NRAS alterations. BRAF status should therefore be considered part of the overall molecular profile rather than a universal criterion for HER2-directed therapy.

HER2 positivity is uncommon in microsatellite instability-high/mismatch repair-deficient [microsatellite instability-high (MSI-H)/mismatch repair deficient (dMMR)] tumors, which tend to be hypermutated rather than driven by a single amplified gene; the combination of HER2-positive and MSI-H/dMMR disease is genuinely rare, and the evidence base for HER2-directed therapy in this small subgroup is correspondingly limited (1). In practice, immune checkpoint inhibitors are generally preferred in MSI-H/dMMR metastatic disease, and the place of HER2-directed therapy depends on prior immunotherapy exposure and the individual clinical situation. At the transcriptomic level, *ERBB2* amplification is enriched in the canonical consensus molecular subtype 2 (CMS2) and underrepresented in the immune-active, MSI-enriched CMS1 (1).

ERBB2 Activating Mutations: A Distinct Entity from Amplification

Activating *ERBB2* mutations occur in up to 3% of mCRC cases and can activate the HER2 pathway independently of gene amplification. Unlike *ERBB2* amplification or HER2 overexpression, these mutations are not detected by standard IHC/ISH testing and instead require sequencing. Common variants include S310F, L755S, V777L, V842I, and I655V (16).

Comprehensive genomic profiling studies have found these mutations co-occur more often with alterations in the PI3K, mismatch repair, and Wnt pathways, but show no consistent association with TP53, APC, KRAS, NRAS, or BRAF mutations (16). Some *ERBB2* mutations, particularly L755S and R784G, have been associated with resistance to anti-EGFR therapy, but it is not yet clear whether *ERBB2* mutations predict response to HER2-directed therapy (17). Therefore, patients with an isolated *ERBB2* mutation and no *ERBB2* amplification should be considered a distinct group and not automatically be treated in the same way as patients with *ERBB2*-amplified tumors. Circulating tumor DNA (ctDNA) assays can also detect *ERBB2* alterations and may be useful for non-invasive testing and monitoring over time (18). A summary of HER2 biology and biomarker relationships is provided in Table 1.

HER2 Testing

HER2 testing in CRC is not yet fully standardized. IHC is commonly used as the first-line test, with reflex ISH performed for equivocal IHC 2+ results. NGS can assess *ERBB2* copy number and other key biomarkers, while ctDNA-based NGS may be useful when tissue is unavailable. HER2 results can vary depending on tissue characteristics, the assay and scoring system used, and trial-specific eligibility criteria. These differences should be considered when interpreting the results and comparing data across studies (Table 2, Figure 2).

The HERACLES scoring system provided the principal historical framework for defining HER2 positivity in pivotal CRC trials: IHC 3+ in >50% of tumor cells, or IHC 2+ with a HER2:chromosome enumeration probe 17 ratio >2 in >50% of cells by fluorescence *in situ* hybridization (FISH) (4,15). This should be understood as a trial-eligibility framework rather than a universal biological definition of HER2 positivity in CRC. It is a higher bar than in breast or gastric cancer, calibrated to the population shown to benefit clinically in early trials; later studies have specifically tested whether this threshold is the right one. Eligibility criteria have varied meaningfully across trials: HERACLES-A, TRIUMPH, and MOUNTAINEER

Table 1. HER2 biology and biomarker relationships in colorectal cancer

Domain	Key feature	Clinical relevance
ERBB2/HER2 (2,3)	Chr. 17q12; no known ligand; preferred partner HER3	Amplification (amp.) defines an actionable subgroup
MAPK pathway (2)	HER2→RAS→RAF→MEK→ERK	RAS mutation confers resistance to HER2 blockade
PI3K-AKT-mTOR (2)	HER2/HER3→PI3K→AKT→mTOR	*PIK3CA/PTEN* alteration implicated in resistance
KRAS/NRAS (4-6)	Largely mutually exclusive with *ERBB2* amp.	Most HER2 regimens validated in RAS WT; T-DXd active in RAS-mutant, mainly IHC 3+
BRAF V600E (1)	Rarely co-occurs with *ERBB2* amp.	Part of broader molecular context, not a strict eligibility rule
MSI-H/dMMR (1)	HER2 positivity infrequent; co-occurrence genuinely rare	Immunotherapy generally preferred; HER2-therapy evidence very limited
MSS (1)	Majority of HER2-positive CRC	HER2 targeting most relevant in MSS disease
CMS2/CMS1 (1)	Enriched/underrepresented for *ERBB2* amp.	May inform future biomarker refinement
ERBB2 point mutations (16)	Up to 3% of mCRC; amp.-independent	Requires sequencing; not a reliable predictor of HER2-therapy response

AKT: Protein kinase B, Amp.: Amplification, Chr.: Chromosome, CMS: Consensus molecular subtype, dMMR: Mismatch repair deficient, ERK: Extracellular signal-regulated kinase, HER2: Human epidermal growth factor receptor 2, HER3: Human epidermal growth factor receptor 3, IHC: Immunohistochemistry, MAPK: Mitogen-activated protein kinase, MEK: Mitogen-activated protein kinase kinase, MSI-H: Microsatellite instability-high, MSS: Microsatellite-stable, mTOR: Mechanistic target of rapamycin, PI3K: Phosphoinositide 3-kinase, PTEN: Phosphatase and tensin homolog, RAF: Rapidly accelerated fibrosarcoma kinase, RAS: Rat sarcoma GTPase, T-DXd: Trastuzumab deruxtecan, WT: Wild-type

Table 2. Factors affecting HER2 testing and classification in colorectal cancer

Category	Specific factors	Practical implication
Pre-analytical	Primary vs. metastatic tissue; tissue age; fixation quality; intratumoral heterogeneity	Same tumor can test differently depending on sample source and timing
Analytical (15)	IHC antibody clone; scoring system used; ISH method (FISH/CISH); NGS copy-number threshold	Results are not automatically interchangeable across platforms/labs
Clinical/trial context (9-14)	Trial-specific eligibility definition (e.g., HERACLES criteria vs. DESTINY-CRC01 broader screening range)	A "positive" result means different things depending on which framework generated it

CISH: Chromogenic *in situ* hybridization, FISH: Fluorescence *in situ* hybridization, HER2: Human epidermal growth factor receptor 2, IHC: Immunohistochemistry, ISH: *In situ* hybridization, NGS: Next-generation sequencing

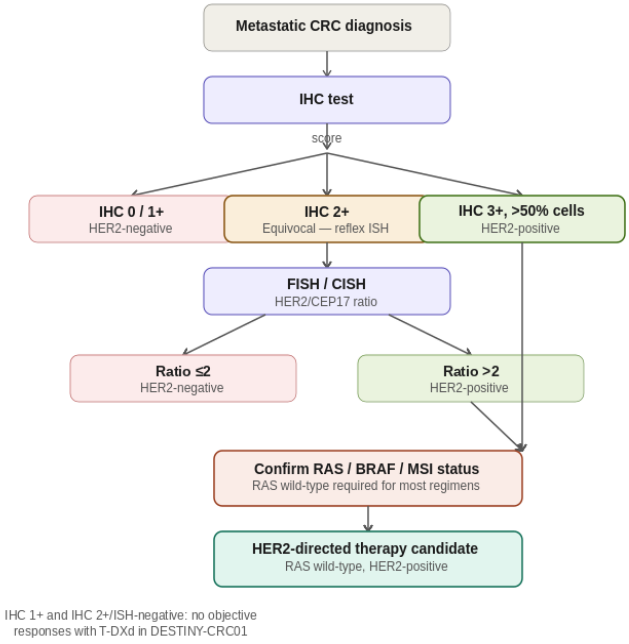


Figure 2. HER2 testing algorithm in colorectal cancer

IHC is generally used as the first-line test, though the overall testing pathway is not fully standardized across institutions (Table 2). IHC 0/1+ is HER2-negative; IHC 3+ in >50% of cells is HER2-positive; IHC 2+ is equivocal and requires reflex FISH/CISH. A HER2:CEP17 ratio >2 on FISH/CISH confirms HER2 positivity under the HERACLES framework. RAS, BRAF, and MSI status are then confirmed, since most HER2-directed regimens are restricted to RAS wild-type disease. No objective responses to T-DXd were observed in IHC 1+ or IHC 2+/ISH-negative tumors in DESTINY-CRC01, and these tumors are managed as HER2-negative under current evidence (10,15)

BRAF: Rapidly accelerated fibrosarcoma B-type kinase, CEP17: Chromosome enumeration probe 17, CISH: Chromogenic in situ hybridization, CRC: Colorectal cancer, FISH: Fluorescence in situ hybridization, HER2: Human epidermal growth factor receptor 2, IHC: Immunohistochemistry, ISH: In situ hybridization, MSI: Microsatellite instability, RAS: Rat sarcoma GTPase, T-DXd: Trastuzumab deruxtecan

required IHC 3+ or IHC 2+/ISH-positive disease, while DESTINY-CRC01 deliberately enrolled a broader range (IHC 1+ through 3+), specifically to test whether T-DXd was active at lower expression levels. The results were informative: in DESTINY-CRC01, the IHC 3+ or IHC 2+/ISH-positive cohort had a 45.3% response rate, while IHC 2+/ISH-negative and IHC 1+ tumors had none (9,10); in DESTINY-CRC02, response was 46.9% in IHC 3+ patients versus just 5.6% in IHC 2+/ISH-positive patients (11). In practical terms, IHC 1+ and IHC 2+/ISH-negative tumors behave as HER2-negative under current evidence, and ESMO/NCCN guidance reflects the HERACLES-aligned threshold (12-14). It is important to note that "HER2-low", a well-established and actionable category in breast cancer, is not yet a validated predictive biomarker in CRC. Therefore, HER2-low results in CRC should be interpreted with caution because current evidence does not support applying the same treatment approach as that used in breast cancer.

HER2 status can differ between the primary tumor and metastases, and can change during treatment: amplification can be lost after HER2-directed therapy (see Mechanisms of Resistance to HER2-targeted Therapy) or, less often, be acquired after anti-EGFR therapy (see HER2 as a Mechanism of Anti-EGFR Resistance). This supports re-biopsy or ctDNA reassessment at key decision points, although, as discussed further under Mechanisms of Resistance to HER2-targeted Therapy, the routine use of ctDNA specifically to select subsequent therapy remains investigational rather than a validated standard.

The Gap Between Evidence and Everyday Practice

These studies consistently report that HER2-directed therapy can be effective, yet many patients with mCRC remain untested for HER2. When one US academic center introduced routine HER2 testing with IHC and ISH for all invasive and mCRC cases, the HER2-positive rate was 4%, consistent with clinical trial data (reported as a USCAP 2022 meeting abstract) (19). However, surveys show that HER2 testing is still not routinely included in mCRC molecular workup, and testing and treatment practices vary across regions and institutions (20). Real-world US data also suggest that many patients with *ERBB2*-amplified mCRC do not receive HER2-directed therapy despite having eligible disease and available guideline-supported treatments (21). Expanding comprehensive genomic profiling was estimated to have only a minimal budget impact, with many missed treatment opportunities related to patients not being tested rather than to the testing method itself (22). Education may also help: an online training program improved both healthcare professionals' confidence and accuracy in identifying HER2 alterations in gastric and CRC (23). Overall, increasing HER2 testing may be as important as developing new HER2-directed treatments.

Prognostic Significance, Early-stage Disease, and EGFR Resistance

Prognostic versus Predictive Value

The prognostic value of HER2 in early-stage and mCRC remains unclear. Studies have reported inconsistent results, partly because HER2 testing methods have varied and factors such as RAS, BRAF, and MSI status may influence outcomes (24). Some studies suggest that HER2-positive, RAS/BRAF wild-type tumors may have less favorable biological features; however, it remains unclear whether HER2 itself has an independent effect on prognosis. In contrast, the predictive role of HER2 is better established. HER2-positive tumors can benefit from HER2-directed therapy, while HER2 positivity may also identify

patients who are less likely to benefit from anti-EGFR therapy despite being RAS/BRAF wild-type. Overall, the independent prognostic role of HER2 remains uncertain, whereas its value in guiding treatment is increasingly well established.

Early-stage (Stage II/III) and Rectal Disease

No current evidence supports using HER2 status to guide adjuvant treatment in stage II/III CRC; risk stratification still relies on T4 stage, MSI status, lymphovascular invasion, and adequate nodal sampling. HER2 testing is not routinely recommended outside clinical trials for localized disease. HER2-positive rectal cancer shares biological characteristics with HER2-positive colon cancer (RAS/BRAF wild-type, MSS-enriched) has been less studied as an independent entity, and HER2 status does not currently inform selection of neoadjuvant chemoradiotherapy or total neoadjuvant therapy. Early-phase trials of neoadjuvant HER2-directed therapy in resectable HER2-positive colon and rectal cancer are underway (see Emerging Therapies and Ongoing Trials and Table 4)—representing the most advanced attempt to date to bring HER2-directed therapy into a curative setting—but there is not yet evidence to support this outside a trial.

HER2 as a Mechanism of Anti-EGFR Resistance

ERBB2 amplification has been implicated in both primary and acquired resistance to cetuximab or panitumumab in RAS/BRAF wild-type mCRC, although resistance does not occur uniformly in all *ERBB2*-amplified tumors. Studies in patient-derived xenograft models showed that *ERBB2*-amplified, RAS wild-type tumors were resistant to EGFR blockade, while adding HER2 blockade restored sensitivity (2). These findings provided the rationale for the HERACLES trial. A related study showed that activation of *ERBB2* signaling was sufficient to confer cetuximab resistance in preclinical models (3), and serial ctDNA testing has identified *ERBB2*-amplified clones emerging during anti-EGFR therapy after an initial response (25), evidence that the tumor evolves under treatment pressure. Mechanistically, HER2 overexpression sustains MAPK and PI3K-AKT signaling through HER2-HER3 dimers, independent of EGFR. Two practical implications follow: HER2 testing should be considered in RAS/BRAF wild-type patients who fail anti-EGFR therapy, and combined HER2/EGFR-family blockade represents a rational strategy in this resistance setting (see Management of Metastatic HER2-positive CRC and Emerging Therapies and Ongoing Trials).

Management of Metastatic HER2-positive CRC

Most major anti-HER2 trials predominantly enrolled RAS wild-type patients because RAS mutations can activate downstream signaling independent of HER2 and may reduce the effectiveness of HER2-directed therapy. BRAF status has varied across studies. Because BRAF V600E rarely co-occurs with *ERBB2* amplification, BRAF status is better considered part of the overall molecular profile rather than a universal requirement for HER2-directed therapy. Key trials are summarized in Tables 3a and 3b.

HERACLES-A, a single-arm phase II trial, tested trastuzumab plus lapatinib in heavily pretreated, HERACLES-positive (IHC 3+ or IHC 2+ with FISH-confirmed *ERBB2* amplification), RAS wild-type patients with mCRC. In the original 27-patient cohort, the objective response rate (ORR) was 30% and median progression-free survival (PFS) was approximately 5 months (4). A later analysis of an expanded 32-patient cohort, at 6.7 years of follow-up, confirmed durable activity [ORR 28%, median PFS 4.7 months, median overall survival (OS) 10.0 months], with diarrhea (84%), rash (56%), and fatigue (59%) the most common treatment-related adverse events, mostly grade 1-2 (grade 3 fatigue in 16%), and no grade 4/5 events reported (26). This long-term follow-up also identified central nervous system (CNS) progression in 6 of 32 evaluable patients—a hypothesis-generating observation that raises the possibility that HER2-positive mCRC, like HER2-positive breast and gastric cancers, carries some propensity for brain metastases. This finding does not currently justify routine CNS screening in asymptomatic patients, and should be regarded as a signal warranting further study rather than a basis for changing practice (26). MyPathway (trastuzumab plus pertuzumab, IHC 3+ or IHC 2+/ISH-positive) achieved ORR 32%, with median PFS 2.9 months and median OS 11.5 months in 57 evaluable patients, and grade 3-4 treatment-emergent adverse events in 37% (5), later supported by the ASCO-run TAPUR basket trial (ORR 25%, disease control rate 54%) using the same combination (27). The Japanese TRIUMPH trial showed that ctDNA can be used to identify patients with *ERBB2*-amplified mCRC who may benefit from dual HER2 blockade with trastuzumab plus pertuzumab, with response rates of 30% in tissue-positive and 28% in ctDNA-positive patients, compared with 0% in a matched real-world reference population receiving standard salvage therapy (18).

MOUNTAINEER tested tucatinib—a highly HER2-selective oral TKI—plus trastuzumab in chemorefractory, HER2-positive (IHC 3+, IHC 2+/ISH-positive, or NGS-amplified), RAS wild-type mCRC. In the final analysis (median follow-up 32.4 months), the confirmed response rate was 39.3%, median duration of response was 15.2 months, median PFS was 8.1 months, and median OS was 23.9 months (6,7), with manageable toxicity—mainly diarrhea, fatigue, and rash—and tucatinib discontinuation due to adverse events in 5.8% of patients (7). This led to FDA accelerated approval on January 19, 2023—

the first HER2-specific approval in mCRC (8)–and a place in NCCN and ESMO guidance (12,13). A single-agent tucatinib monotherapy arm in the same trial, included to test whether dual blockade was truly necessary, produced a markedly lower ORR of 3% (ESMO Congress 2022, Abstract LBA27) (28), indicating that the combination, rather than tucatinib alone, accounts for the clinical benefit. Patient-reported outcomes from MOUNTAINEER, reported as a congress abstract (ESMO Congress 2022, Abstract 361P), are reassuring on tolerability: health-related quality of life, measured by the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30, remained stable from baseline through the end of treatment in patients receiving tucatinib alone or in combination with trastuzumab (29). The RAS wild-type requirement applies specifically to tucatinib plus trastuzumab and to the other antibody-and TKI-based regimens described in this section, not to HER2-directed therapy as a whole–a distinction that becomes clinically important once T-DXd is considered below. The tucatinib-trastuzumab combination is currently being tested as first-line therapy with modified FOLFOX6 (mFOLFOX6; leucovorin, fluorouracil, and oxaliplatin) in the phase III MOUNTAINEER-03 trial (see Treatment Sequencing in Metastatic Disease).

T-DXd is an ADC that pairs an anti-HER2 antibody with a topoisomerase I payload via a cleavable linker (drug-to-antibody ratio ~8:1). Because the released payload can diffuse into neighboring cells, T-DXd can also affect tumor cells regardless of their HER2 status–a "bystander effect" that is clinically relevant given the heterogeneity of HER2 expression in CRC. DESTINY-CRC01 (which screened IHC 1+ through 3+) found responses only in the IHC 3+ or IHC 2+/ISH-positive cohorts (final-analysis ORR 45.3%, median PFS 6.9 months, median OS 15.5 months), with no responses in the lower-expression cohorts. In cohort A (IHC 3+ or IHC 2+/ISH-positive), grade ≥3 treatment-emergent adverse events occurred in 66% and drug-related grade ≥3 events in 55%; adjudicated drug-related interstitial lung disease (ILD)/pneumonitis occurred in 9.3% of patients across all cohorts, with fatal (grade 5) events in 3.5% (9,10). DESTINY-CRC02 compared two doses–5.4 mg/kg (ORR 37.8%) and 6.4 mg/kg (ORR 27.5%)–in a HER2-positive population that notably included RAS-mutant patients. Within the 5.4 mg/kg arm, the response rate was 46.9% in IHC 3+ tumors compared with 5.6% in IHC 2+/ISH-positive tumors, underscoring that HER2 expression intensity, rather than a simple binary positive call, is a strong determinant of benefit. The 5.4 mg/kg dose was selected for its more favorable benefit-risk profile (drug-related grade ≥3 treatment-emergent adverse events 41% vs. 49%; adjudicated drug-related ILD 8% vs. 13%, including one grade 5 event at the higher dose) (11). This is a key practical distinction from the antibody/TKI regimens above: RAS-mutant, HER2-positive disease should not automatically be excluded from consideration for HER2-directed therapy. T-DXd has shown activity in RAS-mutant disease, particularly in patients with IHC 3+ tumors. The RAS-mutant/IHC 3+ subgroup should therefore be distinguished from the broader RAS-mutant/HER2-positive population, and T-DXd may be considered in appropriately selected, previously treated patients, subject to local regulatory approval and guideline recommendations. In April 2024, the FDA granted tumor-agnostic accelerated approval to T-DXd for previously treated unresectable or metastatic HER2-positive (IHC 3+) solid tumors, with DESTINY-CRC02 among the supporting trials, thereby providing T-DXd with a distinct regulatory pathway in CRC alongside tucatinib-trastuzumab's CRC-specific approval. Because ILD/pneumonitis is the safety signal of greatest clinical consequence with T-DXd, baseline pulmonary assessment, patient education on early respiratory symptoms, prompt imaging when symptoms arise, and treatment interruption with grade-appropriate corticosteroid management are standard components of monitoring during therapy.

No randomized trial has directly compared tucatinib-trastuzumab with T-DXd in HER2-positive mCRC, and the two regimens should not be assumed to be interchangeable in the absence of head-to-head data. Treatment choice depends on HER2 expression, RAS status, prior anti-HER2 therapy, and the patient's tolerance of potential toxicities. T-DXd has shown activity in both RAS wild-type and RAS-mutant disease; however, benefit was substantially greater in IHC 3+ tumors, while activity in IHC 2+/ISH-positive tumors was limited in DESTINY-CRC02 (11). T-DXd carries a risk of ILD, whereas tucatinib-trastuzumab is more commonly associated with gastrointestinal and dermatologic toxicity.

Evidence Beyond the Established Regimens

Several other regimens have shown activity in HER2-positive mCRC, but the supporting evidence is smaller, comes from earlier-phase studies, or is less consistent than that for the trials above. They are grouped separately for that reason.

HERACLES-B combined pertuzumab and T-DM1 in 31 patients. It missed its response-rate target (10% ORR) but reached a 68% disease control rate with low toxicity (30). The randomized phase II SWOG-S1613 trial compared pertuzumab plus trastuzumab against cetuximab plus irinotecan in HER2-positive, RAS wild-type mCRC: response rates were similar (34.6% vs. 28.0%) and PFS did not differ significantly, but grade 3 treatment-related adverse events were less frequent with the HER2-directed regimen (23% vs. 46%) (31). Neratinib plus cetuximab showed comparable activity (ORR 33%) in quadruple wild-type disease (32).

Pyrotinib, an irreversible pan-HER TKI, has been combined with trastuzumab in two small Chinese phase II trials. The first reported an ORR of 22.2% (33.3% in RAS wild-type patients) and grade ≥3 diarrhea in 65% (33). A second, later-line trial reported a similar ORR of 23.5%, with median OS of 21.1 months (34). Together, these findings point to modest, consistent activity (ORR approximately 22-24%), with diarrhea as the main toxicity.

Zanidatamab, a bispecific antibody targeting two HER2 epitopes at once, achieved an ORR of 38% in an early-phase trial (35). A separate patient-derived xenograft study across HER2-expressing tumor models identified mesenchymal-epithelial transition factor receptor (MET) amplification as a potential mechanism of acquired resistance, and MET inhibitors enhanced zanidatamab activity in these preclinical models (36).

Two additional agents extend the pipeline; both are reported only in conference proceedings and have not been fully published. HORIZON-CRC01, a randomized phase III trial presented at the ASCO 2026 Annual Meeting (Abstract 3505), tested trastuzumab rezetecan—a HER2 ADC similar to T-DXd—against standard third-line therapy (trifluridine/tipiracil, fruquintinib, or regorafenib) in the same RAS-restricted population as the antibody/TKI regimens above. Among 130 patients, median PFS was 5.5 vs. 2.8 months (hazard ratio 0.33) and ORR was 40.7% vs. 4.5%, with similar rates of grade ≥3 treatment-related adverse events (48.8% vs. 50.0%) (37). An earlier phase I trial of the same drug had reported consistent results (ORR 40.5%, PFS 9.5 months) (38). Zongertinib, an oral HER2-selective TKI already approved for HER2-mutant lung cancer, showed a 42% response rate in a small pooled CRC cohort (n=20) presented as a poster at the ASCO 2026 Annual Meeting (Abstract 3538) (39). Given the limited sample size, this is a preliminary signal rather than established evidence. Neither trastuzumab rezetecan nor zongertinib has changed guidelines to date, but both add to a widening HER2-CRC pipeline that also includes disitamab vedotin, IAH0968, KN026, and several cellular and immune-stimulating therapies now in early testing (see Emerging Therapies and Ongoing Trials). Key trial data are summarized in Table 3a (antibody- and TKI-based regimens) and Table 3b (ADC and bispecific antibodies).

Treatment Sequencing in Metastatic Disease

HER2-directed therapy currently follows standard chemotherapy (fluoropyrimidine, oxaliplatin, and irinotecan), with or without a biologic. First-line treatment remains chemotherapy plus a biologic chosen by RAS/BRAF status, tumor sidedness, and MSI status, though testing for HER2 upfront is increasingly recommended, so the information is available when needed for later lines. MOUNTAINEER-03 is testing whether tucatinib plus trastuzumab should be used as first-line therapy. For RAS wild-type, HER2-positive patients who have already received standard chemotherapy, tucatinib plus trastuzumab and T-DXd are both reasonable options, but they should not be assumed to be interchangeable in the absence of head-to-head data. The choice between them depends on HER2 expression level, RAS status (T-DXd for RAS-mutant disease, particularly with IHC 3+ tumors), prior anti-HER2 exposure, and the toxicity profile that a given patient can tolerate—ILD with T-DXd versus predominantly gastrointestinal toxicity with tucatinib-trastuzumab (Figure 3). Guidance on exactly when HER2-directed therapy becomes available differs slightly by source: NCCN positions it as a second-line-or-later option for HER2-positive mCRC (12), whereas the 2026 ESMO Clinical Practice Guideline and its Living Guideline update continue to place it within third-and-further-line treatment algorithms (13); in practice, the exact line of therapy depends on prior systemic treatment, molecular profile, regulatory approval status, and regimen-specific eligibility rather than a single fixed cut-off.

- **RAS wild-type, HER2-positive, MSS:** Chemotherapy ± anti-EGFR/VEGF (first/second line) → HER2-directed therapy (second or subsequent line, depending on prior systemic therapy, molecular profile, regulatory status, and regimen-specific eligibility), choosing between tucatinib-trastuzumab and T-DXd on the basis above.

- **RAS-mutant, HER2-positive:** Standard RAS-mutant therapy in earlier lines; T-DXd may be considered for previously treated patients, in accordance with guideline recommendations, particularly those with IHC 3+ tumors, in whom its activity has been most evident.

- **BRAF V600E-mutant:** BRAF-directed therapy is the established standard for this subgroup, and its use should be considered according to treatment line and prior therapy. The optimal sequencing of HER2-directed therapy remains unclear, given the rarity of co-occurrence of *BRAF V600E* mutation and *ERBB2* amplification and the absence of prospective data.

- **MSI-H/dMMR:** Immune checkpoint inhibitors are generally preferred, but treatment sequencing should take into account prior immunotherapy exposure and the individual clinical situation. HER2-directed therapy may be considered at progression, although the evidence base for this rare combination is limited.

- In patients with progression on anti-EGFR therapy, rechecking HER2 status should be considered, since acquired *ERBB2* amplification is a recognized resistance mechanism.

Table 3a. Antibody- and TKI-based anti-HER2 regimens in metastatic CRC

Trial (regimen)	Phase/n	HER2/RAS eligibility	ORR	Median PFS	Median OS	Grade ≥3 AEs (type as reported)/key safety signal	Evidence maturity
HERACLES-A (trastuzumab+lapatinib)-original cohort (4)	II/27	IHC 3+, or IHC 2+ with FISH-confirmed amp. (HERACLES criteria); KRAS ex.2 WT	30%	~5 mo	Not reported	Most common AEs: diarrhea, rash, fatigue	Single-arm phase II (proof-of-concept)
HERACLES-A-long-term follow-up (6.7 y) (26)	II/32	Same criteria, expanded cohort	28%	4.7 mo	10.0 mo	Grade 3 TRAEs: fatigue 16%, diarrhea 3%, rash 3%; no G4/5 (any-grade TRAEs: diarrhea 84%, rash 56%, fatigue 59%)	Single-arm phase II, long-term follow-up
MyPathway (trastuzumab+pertuzumab) (5)	IIa/57	IHC 3+, ISH-positive, or NGS-amp.; RAS mostly WT	32%	2.9 mo	11.5 mo	Grade 3-4 TEAEs 37%; diarrhea, fatigue, nausea most common	Single-arm, multi-basket phase IIa
TAPUR (trastuzumab+pertuzumab) (27)	II/28	*ERBB2* amp./HER2 overexpression	25%	17.2 wk	60.0 wk	Grade 3 AEs/SAEs in 4/38 patients (whole CRC cohort); DCR 54%	Single-arm basket trial, peer-reviewed
SWOG-S1613 (trastuzumab+pertuzumab vs. cetuximab+irinotecan) (31)	II/54	IHC 3+ or IHC 2+/ISH-positive, RAS WT	34.6% vs. 28.0%	4.7 vs. 3.7 mo	NR vs. 24.7 mo	Grade 3 TRAEs 23% vs. 46%; no G4/5	Randomized phase II
MOUNTAINEER (tucatinib+trastuzumab)-final analysis (6-8)	II/84	IHC 3+, IHC 2+/ISH-positive, or NGS-amp.; RAS WT	39.3%	8.1 mo	23.9 mo	Grade ≥3 TEAEs 41%; diarrhea, fatigue; tucatinib discontinued for AEs in 5.8%	Single-arm phase II; basis for FDA approval
TRIUMPH (trastuzumab+pertuzumab, tissue/ctDNA-selected) (18)	II/30	IHC 3+ or FISH-positive (tissue), or ctDNA-amp.	30% (tissue); 28% (ctDNA)	4.0 mo (tissue); 3.1 mo (ctDNA)	10.1 mo (tissue); 8.8 mo (ctDNA)	Any-grade TRAEs 80%; grade 3 TRAEs 10%	Single-arm phase II, peer-reviewed
Neratinib+cetuximab/trastuzumab (32)	Ib/II/~21	Quadruple WT (KRAS/NRAS/BRAF/PIK3CA)	Up to 33%	Not consistently reported	Not consistently reported	Moderate; diarrhea, rash	Early-phase (Ib/II)
Pyrotinib+trastuzumab [Fu et al. (33)]	II/20	HER2-positive; RAS/BRAF WT	22.2% (33.3% RAS-WT)	3.4 mo	Not reported	Diarrhea 65% (G≥3)	Single-arm phase II
Pyrotinib+trastuzumab, later-line [Yang et al. (34)]	II/~34	HER2-positive; RAS/BRAF WT responders	23.5%	6.2 mo	21.1 mo	Diarrhea 30% (G3); DCR 88.2%	Single-arm phase II

Efficacy, safety, and evidence maturity from key trials; RAS wild-type disease unless noted. HERACLES-A rows show the original 27-patient proof-of-concept cohort and the separately reported 32-patient long-term follow-up (6.7 years) as two distinct analyses of an overlapping but expanded population; the two should not be read as a single dataset. Antibody-drug conjugates and bispecific antibodies are summarized separately in Table 3b below. Safety data are shown as reported by each trial, with the event type (TEAE or TRAE) specified; because safety definitions and reporting differ across trials, these rates should not be compared directly. Conference-only results are identified with the meeting, year, and abstract number
AE: Adverse event, amp.: Amplification, ctDNA: Circulating tumor DNA, CRC: Colorectal cancer, DCR: Disease control rate, ex.: Exon, FDA: U.S. Food and Drug Administration, FISH: Fluorescence *in situ* hybridization, G: Grade, HER2: Human epidermal growth factor receptor 2, IHC: Immunohistochemistry, ISH: *In situ* hybridization, mo: Months, NGS: Next-generation sequencing, NR: Not reached, ORR: Objective response rate, OS: Overall survival, PFS: Progression-free survival, SAE: Serious adverse event, TEAE: Treatment-emergent adverse event (all causes), TKI: Tyrosine kinase inhibitor, TRAE: Treatment-related adverse event, wk: Weeks, WT: Wild-type, y: Years

Table 3b. Antibody-drug conjugates and bispecific antibodies in metastatic CRC

Trial (regimen)	Phase/n	HER2/RAS eligibility	ORR	Median PFS	Median OS	Grade ≥3 TRAEs/ key safety signal	Evidence maturity
HERACLES-B (T-DM1+pertuzumab) (30)	II/31	HERACLES criteria, RAS WT	10% (missed endpoint)	4.1 mo	NR	DCR 68%; low rate of grade ≥3 AEs	Single-arm phase II; endpoint not met
DESTINY-CRC01 (T-DXd 6.4 mg/kg, cohort A) (9,10)	II/53	IHC 3+ or IHC 2+/ ISH-positive; RAS WT	45.3%	6.9 mo	15.5 mo	Drug-related grade ≥3 TEAEs 55%; ILD/ pneumonitis 9.3% across all cohorts (grade 5, 3.5%)	Single-arm phase II, peer-reviewed final analysis
DESTINY-CRC02 (T-DXd 5.4 mg/kg) (11)	II/82	IHC 3+ or IHC 2+/ ISH-positive; RAS WT or mutant	37.8% (46.9% in IHC 3+; 5.6% in IHC 2+/ISH-positive)	5.8 mo	13.4 mo	Drug-related grade ≥3 TEAEs 41%; adjudicated ILD 8% (all grade 1-2)	Randomized-dose phase II, peer-reviewed
Zanidatamab (bispecific antibody) (35,36)	I/26	IHC 3+ or IHC 2+/ ISH-positive; KRAS WT	38%	6.8 mo	NR	Generally manageable; MET amp.=resistance mechanism (separate PDX study)	Early-phase, peer-reviewed
HORIZON-CRC01 (trastuzumab rezetecan vs. SOC) (37)	III/130 (2:1)	IHC 3+ or IHC 2+/ ISH-positive; RAS/ BRAF WT	40.7% vs. 4.5%	5.5 vs. 2.8 mo (HR 0.33)	Not yet mature	Grade ≥3 TRAEs 48.8% vs. 50.0%	Randomized phase III; conference-reported (ASCO 2026 Annual Meeting, Abstract 3505), not yet published in full
Trastuzumab rezetecan, phase I (China) (38)	I/37	HER2-expressing, advanced/ unresectable mCRC	40.5%	9.5 mo	22.7 mo	Consistent with T-DXd class safety profile	Early-phase, peer-reviewed
Disitamab vedotin ± immune checkpoint inhibitor combinations (NCT05493683)	Early phase, multiple	HER2-expressing (amplified/ overexpressed or mutated)	Encouraging (early)	Not yet mature	Not yet mature	Under investigation	Early-phase, multiple ongoing trials
Zongertinib pooled analysis (Beamion LUNG-1/ PANTUMOR-1) (39)	Ia/II pooled/20	HER2-altered mCRC	42%	Not yet mature	Not yet mature	DCR 95%; no grade 4/5 events reported	Very small pooled early-phase cohort; conference-reported (ASCO 2026 Annual Meeting, Abstract 3538, poster), not yet published in full

Efficacy, safety, and evidence maturity from key trials. To allow like-for-like presentation, grade ≥3 treatment-related (drug-related) adverse events are shown where reported (HERACLES-B reported only a low rate of grade ≥3 AEs); cross-trial comparisons should nevertheless be made with caution. Conference-only results are identified with the meeting, year, and abstract number

AE: Adverse event, amp.: Amplification, ASCO: American Society of Clinical Oncology, DCR: Disease control rate, HER2: Human epidermal growth factor receptor 2, HR: Hazard ratio, IHC: Immunohistochemistry, ILD: Interstitial lung disease, ISH: *In situ* hybridization, mCRC: Metastatic colorectal cancer, MET: Mesenchymal-epithelial transition factor, mo: Months, NCT: ClinicalTrials.gov identifier, NR: Not reached, ORR: Objective response rate, OS: Overall survival, PDX: Patient-derived xenograft, PFS: Progression-free survival, SOC: Standard of care, T-DM1: Trastuzumab emtansine, T-DXd: Trastuzumab deruxtecan, TEAE: Treatment-emergent adverse event, TRAE: Treatment-related adverse event, WT: Wild-type

Mechanisms of Resistance to HER2-targeted Therapy

Some HER2-positive, RAS wild-type patients do not respond despite appropriate selection, suggesting that a binary positive/negative test does not fully capture the degree to which a given tumor depends on HER2 signaling. Possible explanations include HER2 heterogeneity, low-level pathway alterations that standard testing misses, and a weaker degree of HER2 addiction than the biomarker alone suggests. Among patients who respond, resistance eventually emerges through several routes (Figure 4):

- **HER2 Loss or Heterogeneity:** Selection of HER2-negative or HER2-low subclones, detectable by re-biopsy or serial ctDNA (25).
- **New *ERBB2* Mutations:** Kinase-domain changes that alter drug binding or downstream signaling (16,17).
- **RAS Activation:** Acquired KRAS/NRAS mutations that reactivate MAPK signaling.
- **PI3K Pathway Activation:** Acquired *PIK3CA* mutation or PTEN loss (16).

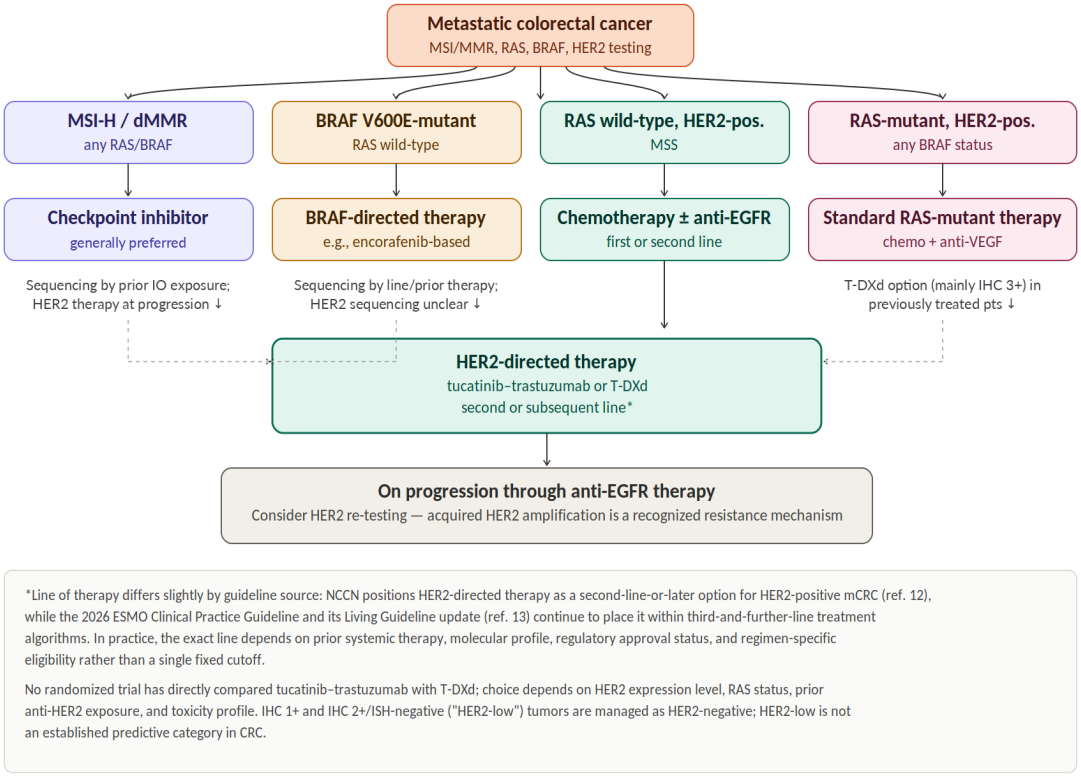


Figure 3. Treatment algorithm for metastatic HER2-positive colorectal cancer

First-line treatment is chosen by RAS, BRAF, and MSI status. For MSI-H/dMMR tumors, immune checkpoint inhibitors are generally preferred, with sequencing guided by prior immunotherapy exposure and the individual clinical situation; the role of HER2-directed therapy at progression is less well defined given how rare this combination is. RAS wild-type, MSS tumors receive chemotherapy ± anti-EGFR therapy in the first or second line, with HER2-directed therapy available from the second or subsequent line depending on prior systemic therapy, molecular profile, regulatory status, and regimen-specific eligibility; NCCN positions HER2-directed therapy as a second-line-or-later option, while the 2026 ESMO guideline places it within third-and-further-line algorithms (12,13); tucatinib-trastuzumab and T-DXd should not be assumed to be interchangeable in the absence of head-to-head data. RAS-mutant tumors receive standard RAS-mutant therapy, with T-DXd a HER2-directed option in previously treated patients, particularly those with IHC 3+ tumors. For BRAF V600E-mutant tumors, BRAF-directed therapy is considered according to treatment line and prior therapy; the optimal sequencing with HER2-directed therapy remains unclear. HER2 status should be rechecked on progression through anti-EGFR therapy, since acquired *ERBB2* amplification is a recognized resistance mechanism (12,13)

BRAF: Rapidly accelerated fibrosarcoma B-type kinase, dMMR: Mismatch repair deficient, EGFR: Epidermal growth factor receptor, ESMO: European Society for Medical Oncology, HER2: Human epidermal growth factor receptor 2, IHC: Immunohistochemistry, ISH: In situ hybridization, MMR: Mismatch repair, MSI-H: Microsatellite instability-high, MSS: Microsatellite-stable, NCCN: National Comprehensive Cancer Network, RAS: Rat sarcoma GTPase, T-DXd: Trastuzumab deruxtecan, VEGF: Vascular endothelial growth factor

- **MET Amplification:** An alternative receptor tyrosine kinase taking over signaling (36).
- **EGFR Reactivation:** The tumor reverting to HER-family signaling through EGFR (2,3).

Serial ctDNA assessment can identify emerging resistance alterations of the kind listed above and is increasingly incorporated into investigational adaptive treatment strategies (18,25) (see Emerging Therapies and Ongoing Trials); its routine use to select subsequent therapy, however, has not yet been validated for standard practice.

Emerging Therapies and Ongoing Trials

Next-generation HER2 ADCs (new payloads and linkers) and HER2/HER3 dual-targeting bispecific antibodies are in early development, aiming to block the dominant HER2-HER3 axis more completely than a HER2-only agent can. HER2-directed chimeric antigen receptor T-cell and T-cell receptor-engineered cell therapies are being explored, though CRC brings its own challenges: low-level HER2 expression in normal tissue raises the risk of on-target, off-tumor toxicity, and the MSS tumor microenvironment tends to suppress immune activity. Combining HER2-directed therapy, particularly ADCs (which can trigger immunogenic cell death), with checkpoint inhibitors is being investigated in early-phase studies, with interest in whether this could bring immunotherapy benefit to MSS HER2-positive disease. ctDNA-guided adaptive trial designs–adjusting treatment as resistance markers appear–represent an important

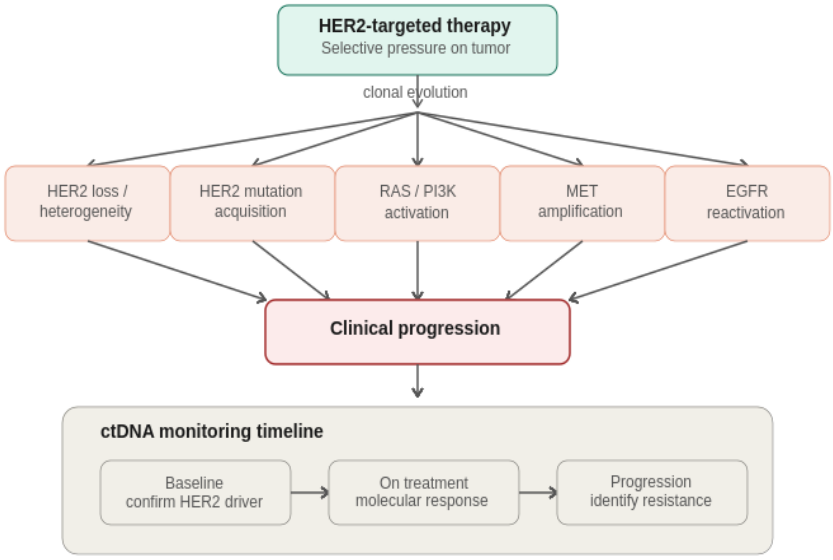


Figure 4. Mechanisms of resistance to HER2-targeted therapy

Under the selective pressure of HER2-directed therapy, clonal evolution can produce clinical progression through several convergent routes: loss of HER2 expression or emergence of HER2-low subclones, acquisition of new *ERBB2* kinase-domain mutations, activation of RAS or the PI3K pathway downstream of HER2, MET amplification providing an alternative signaling route, or reactivation of EGFR signaling. ctDNA monitoring at baseline, on treatment, and at progression is an investigational strategy for identifying which of these mechanisms is driving relapse

ctDNA: Circulating tumor DNA, EGFR: Epidermal growth factor receptor, HER2: Human epidermal growth factor receptor 2, MET: Mesenchymal-epithelial transition factor receptor, PI3K: Phosphoinositide 3-kinase, RAS: Rat sarcoma GTPase

Table 4. Selected ongoing HER2-directed trials in colorectal cancer

Trial	Phase	Regimen	Population	Endpoint	Status
MOUNTAINEER-03 (NCT05253651)	III	Tucatinib+trastuzumab+mFOLFOX6, first-line	HER2+, RAS WT mCRC	PFS	Ongoing
Zanidatamab before surgery (NCT07405476)	II	Neoadjuvant zanidatamab ± surgical resection	HER2+, RAS WT, resectable colon/rectal cancer	pCR, safety	Ongoing
Zongertinib in HER2-altered CRC (NCT06581432, NCT06324357)	Early phase	Monotherapy and combinations (incl. T-DXd)	HER2-altered mCRC	ORR, safety	Ongoing

HER2: Human epidermal growth factor receptor 2, mCRC: Metastatic colorectal cancer, mFOLFOX6: Modified leucovorin, fluorouracil, and oxaliplatin, NCT: ClinicalTrials.gov identifier, ORR: Objective response rate, pCR: Pathological complete response, PFS: Progression-free survival, RAS: Rat sarcoma GTPase, T-DXd: Trastuzumab deruxtecan, WT: Wild-type

direction for how these trials are being designed, though, as noted above, this remains an investigational rather than a standard-of-care application of ctDNA. The common thread across these approaches is a movement from a binary HER2-positive/negative call toward a fuller characterization of HER2 dependency: amplification level, expression intensity, co-occurring mutations, transcriptomic signals, and longitudinal ctDNA dynamics. HER2-directed therapy is also being tested earlier in the disease course, including first-line tucatinib-trastuzumab combined with mFOLFOX6 (MOUNTAINEER-03) and neoadjuvant zanidatamab for resectable disease. Selected ongoing trials are summarized in Table 4.

Discussion

The development of HER2-directed therapy in CRC illustrates the increasing role of molecular stratification in the management of metastatic disease. HERACLES and MyPathway established proof of concept; MOUNTAINEER produced the first HER2-specific regulatory approval for this disease; T-DXd extended the reach of HER2-directed therapy through its bystander effect and activity in RAS-mutant disease, particularly in IHC 3+ tumors; 2026 conference data (HORIZON-CRC01, zongertinib) indicate that the pipeline continues to widen, although these specific findings await full publication.

Most of the underlying evidence consists of phase II, basket-trial, or single-arm studies, with small patient numbers, limited randomized comparisons, and HER2 definitions that have shifted across studies. The OS benefit and the head-to-head comparison of the different regimens remain incompletely defined–a gap that MOUNTAINEER-03 and other phase III trials are intended to close. Testing is not

fully standardized, and whether IHC 2+/ISH-negative disease represents its own actionable category, as "HER2-low" has in breast cancer, remains unresolved in CRC and should not be assumed by analogy.

Several questions will likely shape the field over the next several years: whether HER2-directed therapy should be moved earlier, into first-line metastatic or neoadjuvant settings for rectal disease; which patients stand to benefit most, given that amplification level, expression intensity, RAS/BRAF status, and CMS subtype likely all contribute to a fuller picture of HER2 dependency than any single threshold; and how the growing number of active regimens–tucatinib-trastuzumab, T-DXd, trastuzumab-pertuzumab, and newer agents such as trastuzumab rezetecan and zongertinib–should be sequenced in the absence of head-to-head data.

Conclusion

HER2 is a clinically actionable but heterogeneous biomarker in CRC. Clinical benefit depends on the interaction among *ERBB2* amplification, expression intensity, RAS/BRAF status, and treatment platform, rather than on a single positive/negative determination.

Three points follow for clinical practice. First, the distinction between IHC 3+ or IHC 2+/ISH-positive disease and IHC 1+ or IHC 2+/ISH-negative disease is a clear, definitive boundary supported by consistent trial data, and not a mere technicality. Second, tucatinib-trastuzumab and T-DXd should not be assumed to be interchangeable in the absence of head-to-head data; the choice between them should be guided by HER2 expression level, RAS status, and toxicity considerations. Third, HER2 reassessment at progression–by tissue re-biopsy or ctDNA–is a reasonable strategy for understanding resistance, though its routine use for treatment selection remains investigational rather than the standard of care.

The principal unmet needs are: earlier-line integration; validation of ctDNA-based monitoring; next-generation platforms addressing HER2 heterogeneity; and closing the testing gap described under The Gap Between Evidence and Everyday Practice, which may affect outcomes for more patients than any single new agent. The central question in this field is shifting from whether a colorectal tumor is HER2-positive to which molecular subgroup it belongs to and how that subgroup should be treated.

Footnotes

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Evaluation Questions

Based on the manuscript: "HER2-positive Colorectal Cancer: Molecular Biology, Clinical Significance, and Therapeutic Implications with an Emphasis on Metastatic Disease"

1. Which alteration may confer resistance to anti-EGFR therapy in RAS wild-type mCRC?

A. BRCA1 mutation

B. ERBB2 amplification

C. NTRK fusion

D. POLE mutation

E. ATM deletion

2. Which HER2 result requires reflex ISH testing in CRC?

A. IHC 0

B. IHC 1+

C. IHC 2+

D. IHC 3+

E. HER2-negative

3. Which HER2-directed regimen has demonstrated activity in RAS-mutant mCRC?

A. Trastuzumab–lapatinib

B. Tucatinib–trastuzumab

C. Trastuzumab–pertuzumab

D. Trastuzumab deruxtecan

E. Tucatinib monotherapy

4. Which toxicity is particularly important with trastuzumab deruxtecan?

A. Hemorrhagic cystitis

B. Interstitial lung disease

C. Peripheral neuropathy

D. Nephrotoxicity

E. Hemolytic anemia

5. Which mechanism may cause acquired resistance to HER2-targeted therapy?

A. MET amplification

B. BRCA2 loss

C. ALK fusion

D. IDH1 mutation

E. RET fusion

Correct Answers: 1B, 2C, 3D, 4B, 5A