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Original Article

# Low frequency of HER2 expression in colorectal cancer: A Tunisian single-center study

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**Abstract**

HER2 expression is a potential theranostic and prognostic marker in some cancers, particularly in breast and gastric cancers. However, published data on HER2 expression in colorectal cancer (CRC) remain controversial. This study investigates the immunohistochemical and molecular expression of HER2 in primary CRC and evaluates its clinicopathological and prognostic significance in Tunisian patients. A retrospective analysis was conducted on 144 CRC patients. HER2 status was assessed by immunohistochemistry and tissue microarray analysis, following the diagnostic criteria for gastroesophageal adenocarcinoma. CRC cases with ambiguous results underwent chromogenic in situ hybridization. The mean patient age was 61.9 years (male-to-female ratio: 1.18:1). Tumors were classified as colonic (74.3%) or rectal (25.7%), with 45.8% located in the left colon. Stage III disease was identified in 37.5% of cases, and distant metastases were present in 13.9%. HER2 expression results were as follows: negative (score 0/1+) in 142 cases (98.6%), equivocal (score 2+) in one case (0.7%), and overexpressed (score 3+) in one case (0.7%). No HER2 gene amplification was detected, and none of the metastatic CRC cases showed HER2 immunostaining. These findings suggest that HER2 overexpression and amplification in CRC are rarer than previously reported, highlighting the need for multicenter Tunisian studies to validate these results. The variability in HER2 immunostaining criteria further underscores the importance of a standardized scoring system to ensure consistency in both diagnosis and research.

**Keywords:** Colorectal cancer, HER2, Immunohistochemistry, Chromogenic *in situ* hybridization, Scoring system, Theranostic marker, Tunisian patients.

## 1. Introduction

Colorectal cancer (CRC) represents a significant global public health issue, ranking third in incidence with 1,926,425 new cases and standing as the second leading cause of cancer-related mortality, with 904,019 deaths recorded in 2022 [1]. In Tunisia, a notable increase in CRC incidence has been reported, with rates reaching 14.6 per 100,000 men and 12.2 per 100,000 women [2]. In the era of molecular biology, the objective has shifted beyond the conventional anatomical classification of cancers towards a molecular classification, correlating these profiles with clinicopathological characteristics. CRC exhibits considerable histological heterogeneity and dynamic genetic alterations, with multiple tumor cell clones displaying distinct molecular profiles that respond variably to targeted therapies [3, 4]. This heterogeneity can evolve dynamically during disease progression and under therapeutic pressure, sometimes resulting in tumor progression despite ongoing treatment. Thus, CRC provides a promising framework for the development of personalized oncology and the integration of theranostic biomarkers [5, 6].

Almost 20% of CRC patients are diagnosed with distant metastases, while 50% of those with non-metastatic disease who undergo curative treatment remain at high risk of recurrence [2, 7]. Advanced disease stages and metastatic relapses following curative treatment are the primary contributors to mortality in CRC patients. It is also noteworthy that patients who have exhausted all standard therapeutic options generally have a worse prognosis, even if they remain in relatively good health and continue to pursue additional therapeutic strategies [2, 8-10].

Recent advancements in therapeutic standards have led to an overall survival rate exceeding 30 months in genetically selected patients [8]. For many years, chemotherapy was the conventional treatment for metastatic CRC (mCRC). However, the advent of targeted therapies, notably those utilizing anti-EGFR (epidermal growth factor receptor) agents such as cetuximab and panitumumab,

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which were approved in 2007 and 2008, respectively, have revolutionized the management of mCRC patients with *RAS* wild-type status [2, 11]. These therapies may be used in combination with chemotherapy or after chemotherapy failure. Despite these advancements, the EGFR expression level in CRC does not consistently correlate with therapeutic efficacy, suggesting the existence of primary and secondary resistance mechanisms [12].

Investigating the molecular profile of CRC has revealed mutations, particularly in the *RAS* gene, which contribute to resistance to anti-EGFR therapies [13, 14]. According to scientific societies, *RAS* status (*KRAS* and *NRAS* exons 2, 3, and 4) is the only validated predictive biomarker for response to anti-EGFR therapy [15]. Additional molecular alterations are under investigation to enhance the prediction of therapeutic responses. One such area of research focuses on the human epidermal growth factor receptor 2 (HER2), a member of the tyrosine kinase receptor family [13, 15-17].

HER2 is a member of the epidermal growth factor receptor (EGFR) family, which includes EGFR (ErbB1/HER1), HER3 (ErbB3), and HER4 (ErbB4) [18]. These receptors are expressed in various epithelial, mesenchymal, and neural tissues and play a crucial role in cellular development, proliferation, differentiation, and metabolism. Their functions are mediated through key signaling pathways, including the RAS/RAF/MAPK and PI3K/AKT pathways [19]. Typically, activation of these receptors occurs through homo- or heterodimerization of their tyrosine kinase domains, usually triggered by ligand binding. Notably, HER2 is unique among these receptors as it lacks a known ligand [19].

The *HER2 (ERBB2)* oncogene is located on the long arm of chromosome 17 (17q12; 35.109-35.138 Mb) [20]. The conversion of a proto-oncogene to an oncogene occurs through genetic alterations that affect its structure or regulatory expression. The most prevalent mechanism in human cancers is overexpression, characterized by increased receptor levels in tumor tissues. This overexpression can result from *ERBB2* gene amplification, transcription disruptions leading to uncontrolled protein production, or post-transcriptional modifications [21]. Activating mutations, such as V842I, V777L, and L755S in the kinase domain, as well as the extracellular domain mutation S310F, have been identified in various cancers, including CRC [22].

Over the last decade, HER2 has been extensively studied in various malignancies, notably breast and gastric cancers [23-25]. It has emerged as a potential key marker in CRC management, particularly in metastatic cases [15, 26]. Indeed, HER2 overexpression may represent an alternative mechanism of resistance to anti-EGFR treatments [8, 18, 27]. Furthermore, clinical trials assessing anti-HER2 therapy in mCRC patients have yielded promising results [28-31]. These findings have prompted further exploration of HER2 overexpression in CRC [32, 34]. Despite these promising results, study outcomes have been inconsistent [8]. In this study, we investigated the immunohistochemical and molecular expression of *HER2* in primary CRC at all stages diagnosed in Tunisian patients and explored its clinicopathological, prognostic, and therapeutic implications.

## 2. Materials and methods

### 2.1. Sample collection

We conducted a retrospective and descriptive study on CRC cases referred by the General Surgery Department and diagnosed by the Pathology Department at Sahloul University Hospital, Sousse, Tunisia. Data collection covered two years and six months, from January 1, 2020, to June 30, 2022.

We included primary CRC cases at all stages (I to IV) that underwent surgical resection with lymph node dissection. For rectal cancers, patients were included regardless of whether they had received neoadjuvant therapy. However, we excluded patients with non-carcinomatous colorectal tumors, recurrent CRC, or rectal cancer with a complete response to neoadjuvant therapy. Additionally, patients with synchronous or multiple colorectal tumors were excluded to ensure accuracy in HER2 assessment. Tumors too small for immunohistochemical analysis, such as small residual tumors following neoadjuvant therapy, were also excluded.

Ethical approval was obtained from the local Human Ethics Committee at Sahloul University Hospital, Sousse, Tunisia, in accordance with the Declaration of Helsinki.

### 2.2. Clinicopathological data

Clinicopathological data, including patient age, gender, tumor site and size, macroscopic appearance, distance from the nearest resection margin, presence of associated macroscopic lesions, and lymph node count after meticulous mesocolon dissection, were collected from pathology reports and clinical records. Additionally, we collected microscopic characteristics, including histological type, histological grade, tumor status (T), lymph node status (N), metastatic status (M), metastatic sites for metastatic cancers, TNM stage (pTNM or ypTNM), and vascular emboli and perineural invasion. Patient follow-up and outcomes were obtained from the Cancer Registry of Central Tunisia.

Histological diagnosis of all tumor cases was independently reviewed by two pathologists (KL and SH) using hematoxylin and eosin-stained sections, according to the 2019 World Health Organization (WHO) classification [34]. Histological grading of adenocarcinomas, not otherwise specified (NOS), followed the College of American Pathologists (CAP) recommendations. TNM staging including pathological TN (pTN) or post-neoadjuvant therapy pathological TN (ypTN), were carried out following the Eighth Edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual [35].

All tissue samples underwent standard fixation in 10% buffered formalin and were subsequently embedded in paraffin. For each case, the most cellular tumor areas were identified using low magnification (x40). Necrotic areas and poorly fixed regions, such as border or crush artifacts, were excluded from the selection.

### 2.3. HER2 expression

HER2 expression was evaluated using immunohistochemistry on tissue microarrays (TMA) derived from archived samples as we previously published [36]. Additionally, whole-slide sections were prepared for 11 mucinous adenocarcinomas, as mucus pools compromised the preparation and interpretation of TMAs. In brief, antigen unmasking was performed on dewaxed and rehydrated sec-

tions using BOND™ Epitope Retrieval ER2 Solution (pH 9) at 100°C for 30 minutes. After blocking endogenous peroxidase activity with the Refine Detection Kit Peroxide Block, the slides were incubated with a pre-diluted rabbit monoclonal anti-c-erbB-2 primary antibody (clone SP3, BOND MAX Leica) for 15 minutes. Immunoreactivity was detected using the BOND-Polymer-Refine Detection Kit on a BOND MAX Leica system, in accordance with the manufacturer's instructions. The immunostaining was visualized with Diaminobenzidine, and the sections were briefly counterstained with hematoxylin and mounted [36].

### 2.4.Evaluation of HER2 expression

HER2 immunostaining was interpreted by two expert pathologists independently using an optical microscope. They evaluated the immunostaining type, extent, and intensity. Each tissue sample was assigned an HER2 expression score based on the diagnostic criteria established by Hofmann et al. [37] and adopted by the College of American Pathologists (CAP), American Society for Clinical Pathology (ASCP), and American Society of Clinical Oncology (ASCO) in the 2016 recommendations for gastroesophageal adenocarcinomas (GEA) [38]. In our study, given the small size of the tissue cylinders, we adhered to the CAP/ASCP/ASCO 2016 recommendations for GEA relevant to biopsies (GEA-b) [38]. For whole tissue sections, we followed the CAP/ASCP/ASCO 2016 guidelines for resection specimens [38]. According to these diagnostic criteria, isolated cytoplasmic/nuclear staining or basal/luminal immunostaining was deemed non-significant and therefore excluded [38].

### *2.5. HER2* gene amplification

HER2 2+ cases underwent additional chromogenic *in situ* hybridization (CISH) testing for gene amplification, as previously described [39]. Briefly, the ZytoDot SPEC ERBB2/CEN probe was applied to 3-µm-thick dewaxed and rehydrated sections following the manufacturer's protocol. Tumor samples were incubated with a cocktail of primary antibodies against digoxigenin and dinitrophenyl, followed by a cocktail of polymerized secondary antibodies: anti-HRP-GOAT and anti-AP-GOAT. Tissue sections were counterstained with hematoxylin, mounted in Faramount (DakoCytomation), and examined under an optical microscope [39].

DIG-labeled hybridization signals appeared as dark green dots (ERBB2 genomic region), while DNP-labeled signals appeared as dark red dots (CEP17). Slides were scanned at low magnification (x100 and x200) to assess heterogeneity. Signal visualization was performed at x400 magnification for clear detection. Interpretation was conducted in the invasive component, excluding necrotic areas, based on at least 200 non-overlapping nuclei. Results were analyzed according to GEA guidelines [38]. Tumors were classified as follows: amplified (≥6 copies per nucleus or HER2/CEP17 ratio ≥2 in 20 to 40 cells); non-amplified (<4 copies per nucleus or HER2/CEP ratio <2); equivocal (4 to 6 copies per nucleus or HER2/CEP17 ratio between 1.8 and 2). For equivocal cases, additional counting of at least 20 more nuclei in other tumor areas or sections was recommended [38].

### 2.6. Statistical analysis

Statistical analysis was performed using SPSS software (Version 25.0, IBM Corp). Descriptive statistics included means for quantitative variables and absolute/relative frequencies (percentages) for qualitative variables.

## 3. Results

### 3.1. Clinicopathological characteristics

A total of 144 patients with CRC were included in the study. The cohort consisted of 78 males (54.2%) and 66 females (45.8%), with a sex ratio of 1.18. Patient ages ranged from 31 to 88 years, with a mean age of 61.9 years. The mean age was 61.4 years in males and 62.5 years in females. Patients younger than 40 years accounted for 6.9% of cases, with the highest frequency reported in the 60–69 year age group (25.7%). Table 1 presents the clinicopathological features of CRC patients.

CRC was diagnosed in 15.3% of patients following an episode of acute intestinal obstruction. Tumor sizes ranged from 1.3 cm to 19 cm, with an average of 5.3 cm. In our study, 107 cases (74.3%) involved colonic cancers, while 37 cases (25.7%) were rectal cancers.

The distribution of colonic tumors showed that descending colon tumors were the most common, accounting for 45.8% of cases (n = 66), followed by ascending colon tumors (22.9%) and transverse colon tumors (5.6%). Among rectal cancers, tumors were most frequently located in the upper rectum (11.8%), followed by the lower rectum (7.6%) and the middle rectum (6.3%).

In our study, the predominant histological type was adenocarcinoma NOS (formerly known as Lieberkühnian adenocarcinoma), accounting for 89.6%, followed by mucinous adenocarcinoma (9.7%) and serrated adenocarcinoma (0.7%). According to the CAP and the Royal College of Pathologists classification, the majority of adenocarcinoma NOS cases were classified as low-grade tumors (93.8%), with the remaining cases being categorized as high-grade tumors (Table 1).

Based on the degree of wall infiltration, tumors were classified as pT3 in 57.6% of cases (n = 83), pT4 in 27.8% of cases (n = 40), and pT2 in 14.6% of cases (n = 21). According to the Eighth Edition of the AJCC classification, colorectal tumors were most frequently diagnosed at stage III (37.5%, n = 54), followed by stage II (34%, n = 49) and stage IV (17.4%, n = 25). Stage I was the least frequent, representing 11.1% of cases.

In our study, 20 patients with CRC were diagnosed with metastasis. Distant metastases to a single organ were observed in 17 patients (11.8%). Peritoneal metastasis, with or without involvement of additional organs, was reported in three patients (2.1%). The remaining 124 tumors (86.1%) were non-metastatic at diagnosis. Among the metastatic cases, liver metastases were detected in 14 cases (70%), pulmonary metastases in two cases (10%), peritoneal metastases in three cases (15%), and ovarian metastasis in one case (5%).

### 3.2. Detection of HER2 overexpression

According to the GEA-validated diagnostic criteria, membranous HER2 expression was observed in four cases with a score of 1+ (2.8%), one case with a score of 2+ (0.7%), and one case with a score of 3+ (0.7%). The remaining 138 tumors (95.8%) exhibited no HER2 receptor expression (score 0) (Fig. 1). Based on these findings, CRC samples were classified into three categories: HER2-negative tumors (score 0/1+), accounting for 98.6% (n =

**Table 1.** Clinicopathological characteristics of CRC patients.

| Parameters | N | Percentage (%) |
|---|---|---|
| Sex | | |
| Male | 78 | 54.2% |
| Female | 66 | 45.8% |
| Age (Years) | | |
| < 40 | 10 | 6.9% |
| 40-49 | 16 | 11.1% |
| 50-59 | 34 | 23.6% |
| 60–69 | 37 | 25.7% |
| 70-79 | 31 | 21.5% |
| ≥ 80 | 16 | 11.1% |
| Acute intestinal obstruction | | |
| Presence | 22 | 15.3% |
| Absence | 122 | 84.7% |
| Tumor size | | |
| ≤ 4 cm | 57 | 39.6% |
| > 4 cm | 87 | 60.4% |
| Tumor site | | |
| Colonic | 107 | 74.3% |
| Descending colon | 66 | 45.8% |
| Ascending colon | 33 | 22.9% |
| Transverse colon | 8 | 5.6% |
| Rectal | 37 | 25.7% |
| Upper rectum | 17 | 11.8% |
| Lower rectum | 11 | 7.6% |
| Middle rectum | 9 | 6.3% |
| Growth pattern | | |
| Exophytic | 98 | 68.1% |
| Infiltrative | 36 | 25% |
| Ulcerated | 10 | 6.9% |
| Associated lesions | | |
| Absence | 105 | 72.9% |
| Presence | 39 | 27.1% |
| ≤ 100 polyps | 34 | 23.6% |
| > 100 polyps | 2 | 1.4% |
| Mucinous appendix tumor | 2 | 1.4% |
| Colonic diverticula | 1 | 0.7% |
| Surgical margin | | |
| Clear | 140 | 97.2% |
| Involved | 4 | 2.8% |
| Histological type | | |
| Adenocarcinoma NOS | 129 | 89.6% |
| Mucinous adenocarcinoma | 14 | 9.7% |
| Serrated adenocarcinoma | 1 | 0.7% |
| Tumor grade | | |
| Low-grade | 135 | 93.8% |
| High-grade | 9 | 6.2% |
| Vascular emboli | | |
| Presence | 69 | 47.9% |
| Absence | 75 | 52.1% |

| | | |
|---|---|---|
| Perineural invasion | | |
| Presence | 49 | 34% |
| Absence | 95 | 66% |
| Degree of wall infiltration | | |
| pT2 | 21 | 14.6% |
| pT3 | 83 | 57.6% |
| pT4 | 40 | 27.8% |
| Nodular metastasis | | |
| pN0 | 73 | 50.7% |
| pN1 | 45 | 31.3% |
| pN2 | 26 | 18% |
| Distant metastasis | | |
| Presence | 20 | 13.9% |
| Absence | 124 | 86.1% |
| Tumor stage | | |
| Stage I | 16 | 11.1% |
| Stage II | 49 | 34% |
| Stage III | 54 | 37.5% |
| Stage IV | 25 | 17.4% |

142); tumors with equivocal HER2 status (score 2+), representing 0.7% (n = 1); and HER2-overexpressing tumors (score 3+), also comprising 0.7% (n = 1). Additionally, *HER2* gene amplification was not detected in the equivocal HER2 CRC sample.

In our study, none of the metastatic CRC cases exhibited membranous HER2 immunostaining. Furthermore, cytoplasmic HER2 immunostaining without membranous labeling was observed in 16 CRC samples (11.1%) (Fig. 1).

### 3.3. Characteristics of CRC case HER2-overexpressing

The only case of HER2 overexpression (score 3+) was observed in a 37-year-old woman who presented with a mid-rectal tumor following neoadjuvant chemoradiotherapy. Initially, she was diagnosed with a mid-rectal tumor and a solitary hepatic lesion in segment II. The patient received neoadjuvant chemoradiotherapy according to the RAPIDO protocol, which resulted in an estimated 50% radiological response at the primary site and complete resolution of the hepatic lesion (M0).

Macroscopic examination revealed a residual tumor measuring 1.4 cm with a budding morphology. Both longitudinal and circumferential surgical margins were free of tumor. The tumor was classified as a low-grade, well-differentiated adenocarcinoma of the middle rectum. Therapeutic response was graded as TRG2 according to Dowrak's criteria. Perineural invasion was observed, but there were no evidence of vascular emboli. The final pathological stage was ypT3N0 (stage IIA), based on the Eighth Edition of the AJCC Cancer Staging Manual.

HER2 overexpression was further confirmed on the pre-treatment biopsy, which showed an immunohistochemical score of 3+ (Fig. 1), in accordance with the 2016 CAP/ASCO/ASCP guidelines for GEA-b. A HER2-overexpressing invasive breast carcinoma was included as a positive control on the same slide to validate the immunohistochemistry procedure.

Subsequent disease progression was marked by the appearance of secondary pulmonary nodules and recurrence of the hepatic lesion. The patient received three cycles of FOLFOX combined with Avastin, followed by FOLFIRI plus Avastin, but these regimens failed to produce clinical improvement. The tumor continued to progress, with the emergence of an osteosclerotic lesion in the L4 spinous process, pelvic presacral ascites, and a peritoneal carcinomatosis nodule. The patient is considered eligible for anti-

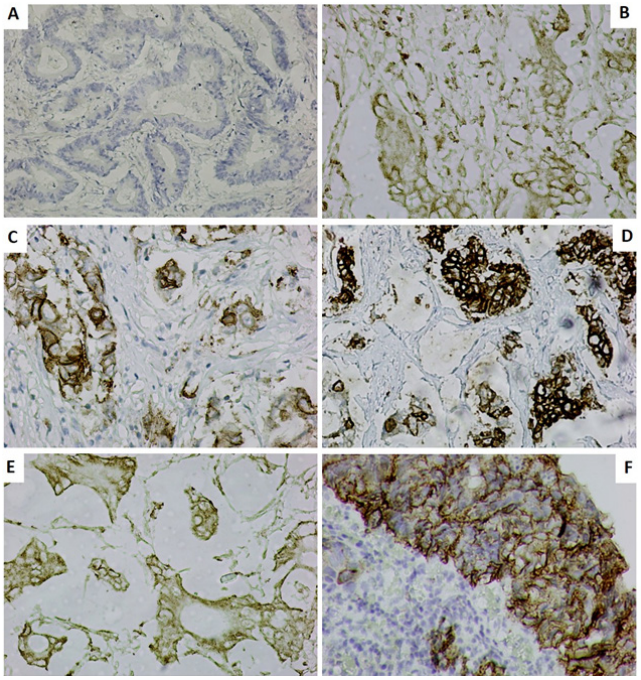


**Fig. 1.** HER2 expression in CRCs by immunohistochemistry. (**A**) Absence of HER2 staining (score 0) (x200). (**B**) HER2 expression (score 1+), showing faint granular basolateral membranous staining (x200). (**C**) HER2 expression (score 2+), showing moderate basolateral membranous staining (x100). (**D**) HER2 overexpression (score 3+), characterized by strong membranous staining (x100). (**E**) Granular cytoplasmic HER2 staining (x100). (**F**) HER2 expression on the pre-treatment biopsy of the HER2-overexpressing CRC case with strong membranous HER2 staining (x200).

EGFR therapy. Pre-treatment molecular testing identified *KRAS* and *NRAS* mutations. Two years after diagnosis, the patient is still alive, but continues to experience recurrent metastatic disease.

## 4. Discussion

In our study, HER2 immunoexpression was absent in most CRC cases, with only 0.7% of tumors exhibiting HER2 overexpression. Additionally, low-level expression was observed in four cases, and one tumor exhibited an equivocal HER2 status without gene amplification. Furthermore, strong membranous HER2 overexpression was identified in one CRC case, consistent consistent with findings from the corresponding pre-treatment biopsy. Cytoplasmic staining without membranous HER2 expression was observed in 11.1% of cases. No HER2 expression was detected in mCRC samples.

Various studies on CRC have evaluated HER2 status using tissue samples from surgical specimens, primary tumor biopsies, or metastatic lesions. Aside from the diagnostic criteria established for GEA [38], no specific guidelines currently exist for assessing HER2 in CRC biopsies. In our study, HER2 expression was assessed using TMA blocks containing 4 mm cores, which are comparable in size to biopsy samples. Therefore, we followed the recommendations for HER2 evaluation in biopsy specimens [38]. Previously, Conradi et al. proposed a minimum threshold of five cohesive tumor cells to define HER2 overexpression in biopsy samples, and recommended assessing HER2 on a representative section of the resected specimen [40]. More recently, Fujii et al. highlighted the challenges of accurately determining HER2 status due to tumor heterogeneity, and advocated for evaluating HER2 on complete, representative sections of the surgical specimen, as biopsy-based assessments may increase the risk of false-negative results [41]. Furthermore, proper tissue fixation is essential for reliable HER2 immunostaining. According to international guidelines, tumor samples from surgical specimens should be fixed in 10% neutral buffered formalin for 24 to 48 hours, starting within one hour of excision, to ensure optimal fixative penetration. Alcohol-based fixatives may cause_false-positive results [42]. Additionally, it is important to maintain the paraffin embedding temperature below 60°C. Once formalin-fixed and paraffin-embedded, tumor samples remain stable for several decades, particularly when stored at room temperature not exceeding 27°C [42].

Assessing HER2 expression using the TMA technique relies on sample miniaturization. Although technically complex and requiring specialized training and expertise, the TMA method allows for cost-effective and high-throughput evaluation of large sample sets, enabling uniform analysis of multiple specimens on a single slide [43]. Nevertheless, this technique may lead to false-negative results, which can be mitigated by analyzing whole-tissue sections. Interestingly, Marx et al. reported that HER2 expression and amplification can be heterogeneous in CRC, recommending the evaluation of multiple regions from the same tumor to ensure diagnostic reliability [44]. Fujii et al. found an intratumoral heterogeneity rate of 36.8% among HER2-positive CRC cases [41]. In contrast, other studies have suggested that HER2 expression in CRC tends to be relatively homogeneous [45, 46]. Ingold Heppner et al. observed heterogeneous HER2 expression and amplification in only four cases [45]. Similarly, in the HERACLES (HER2 Amplification for Colorectal Cancer Enhanced Stratification) clinical trials, only one case of intratumoral heterogeneity was reported [47].

In our study, using the monoclonal HER2 antibody clone SP3, 0.7% of CRCs overexpressed HER2 based on the GEA criteria proposed by Hofmann et al. [37]. Previously reported HER2 overexpression rates in CRC have varied widely, ranging from 1.3% to 82% [48]. A large-scale multicenter British study, which analyzed 3,256 stage II–IV primary CRC samples using the same scoring system as ours, found HER2 overexpression rates of 2.2% in stage IV tumors and 1.3% in stage II–III tumors [46]. Fonotto et al. reported a global membranous HER2 overexpression rate of 5%, significantly lower than the rate observed in breast cancer [8]. The Cancer Genome Atlas identified *HER2* gene amplification in 7% of CRC cases [49]. However, a 2015 meta-analysis involving 2,573 CRC samples estimated an overall HER2 overexpression rate at 16.2% (range: 3.9% to 54.8%), although not all included studies employed immunohistochemistry [50]. Among studies that performed immunohistochemistry, HER2 overexpression or amplification rates ranged from 2% to 18.3% [50]. These discrepancies in HER2 overexpression rates may be attributed to variations in fixation protocols and conditions, tumor volume analyzed, antibody clones used, scoring systems, positivity thresholds, and selection biases, particularly the heterogeneity of the clinicopathological characteristics of CRC samples [8,47,51].

Two standardized immunohistochemical kits commonly used to assess HER2 overexpression are the HercepTest™ kit (DAKO), which employs the polyclonal antibody A485, and the PATHWAY™ kit (VENTANA-Roche), which utilizes the monoclonal antibody 4B5 [28]. The HERACLES clinical trial concluded that the 4B5 clone is superior for detecting HER2 overexpression using a 50% cell positivity cutoff. Specifically, the 4B5 clone exhibited fewer false negatives, ensuring greater sensitivity compared to the A485 polyclonal antibody [47]. Nevertheless, several studies have employed non-standardized HER2 antibodies, including the monoclonal antibody SP3 used in our study, applying either GEA diagnostic criteria [45] or HERACLES criteria [52], with reported HER2 overexpression rates ranging from 1.6% to 3.8%. Interestingly, Song et al. compared HER2 expression using the 4B5 and SP3 clones and found that 4B5 exhibited higher sensitivity and stronger staining intensity [52]. Beyond the pre-analytical factors previously described, several key methodological factors can influence results when using non-standardized immunohistochemistry techniques, including antigen retrieval conditions (buffer type, pH, temperature, and duration), antibody clone and concentration, incubation time, and detection method [42]. Such variations contribute to the substantial discrepancies observed in HER2 overexpression rates across different studies.

Multiple scoring systems have been proposed for assessing HER2 expression. Interestingly, Liu et al. compared GEA and HERACLES criteria and concluded that HERACLES system is more suitable for assessing HER2 expression, although both approaches reported similar HER2 amplification (4.1%) and overexpression (3.7%) rates [48]. Furthermore, HER2 overexpression was significantly associated with patient survival and other histoprognostic factors, including left-sided CRC, advanced

lymph node status, and higher pTNM stage [48]. In a Chinese cohort of 664 CRC cases, Sun et al. applied the diagnostic criteria of HERACLES, GEA-s (for surgical specimens), and GEA-b (for applicable to biopsies). In addition, two other scoring systems, immune-reactive scores (IRS), were considered to evaluate the expression of HER2, including the IRS-plus system (IRS-p), and the IRS-multiply system (IRS-m), obtained by adding and multiplying intensity, staining type, and the percentage of positive cells, respectively [53]. These different scoring systems were applied to two 2-mm biopsy punches taken from the center of the tumor and the invasion margin [53]. The IRS-p score had the best specificity and sensitivity and correlated with histoprognostic factors, suggesting that it is the most suitable scoring system for CRC [53]. However, when using the GEA-b criteria, a HER2 overexpression rate of 2.71% was identified, which is higher than the rate reported in our study [53]. More recently, an international collaborative project developed harmonized diagnostic criteria for HER2-positive CRCs using IHC, FISH, and NGS [41]. Inspired by diagnostic criteria for HER2 expression in gastric and breast cancers, Fujii et al. proposed that a 10% threshold of HER2-positive cells is appropriate for diagnosing HER2-positive CRCs, considering the heterogeneous HER2 expression in CRC surgical specimens [41].

Similar to our findings, some previous studies have reported cytoplasmic HER2 expression [54-56]. Although guidelines for determining HER2 overexpression primarily focus on membranous staining, cytoplasmic staining was more frequently observed in CRCs, with reported frequencies ranging from 5% to 63% [8, 57]. Interestingly, Wang et al. reported membranous expression rates between 2.1% and 11%, while cytoplasmic overexpression ranged from 58% to 68.5% [58]. Nevertheless, most previous studies have found no significant correlation with key histoprognostic factors or survival, although some noted correlations with age, tumor size, lymph node status, and the non-mucinous histological type [54, 57, 59]. Furthermore, no significant association was found with *HER2* gene amplification [59]. The presence of cytoplasmic HER2 expression may be attributed to receptor internalization, dimerization, cytoplasmic phosphorylation, intense transcription, or the existence of a truncated HER2 protein [59].

In our study, both the pre-treatment biopsy and the surgical specimen of the CRC case showed the same HER2 status (score of 3+). However, Conradi et al. reported that among rectal cancer cases classified as HER2-negative on biopsy, 21% showed HER2 overexpression in surgical specimens, while 5% of cases that were HER2-positive on biopsy became negative in surgical samples, likely due to the effects of neoadjuvant therapies [40].

The patient exhibiting HER2 overexpression experienced multiple metastatic recurrences involving the lung, liver, bones, and peritoneum. Similarly, a meta-analysis by Pyo et al. demonstrated that HER2 overexpression is significantly associated with lymph node metastases and distant metastases [50]. Furthermore, a recent review highlighted a significant correlation between HER2 overexpression and the number of metastatic sites, with a particularly higher frequency of pulmonary metastases [51].

The prognostic value of HER2 overexpression in mCRCs remains controversial, as studies are limited by the low prevalence of HER2 overexpression [58, 60]. Earlier studies failed to establish HER2 overexpression as an independent prognostic factor [58]. However, more recently, a significant association has been reported with early recurrences and shorter overall survival [58]. Similarly, Sawada et al. reported a poorer prognosis for HER2-positive mCRCs in terms of overall survival in patients with both mutated and wild-type *KRAS*/*BRAF* status [61].

Some limitations were present in our study. Firstly, we utilizes a non-standardized HER2 antibody, the monoclonal antibody SP3. Additionally, the inclusion of samples from post-therapeutic resections may pose a limitation, particularly since the effect of neoadjuvant therapy on HER2 expression in CRCs is poorly documented in existing literature. However, excluding these specimens would have resulted in omitting many distal CRC cases, which are often associated with HER2 overexpression. Moreover, our sample size was too small to extrapolate our findings to the broader Tunisian population, highlighting the need for a multicenter study to further explore our results. Finally, we did not assess intratumoral heterogeneity in our study. Increasing the number of punch biopsies and/or verifying results with whole-section analyses could help strengthen our conclusions.

We observed lower HER2 overexpression and amplification rates in CRC compared to previous reports, highlighting the rarity of this molecular alteration in colorectal oncogenesis. Further multicenter studies in Tunisia are needed to confirm these findings. Additionally, inconsistencies in HER2 immunostaining criteria may cause diagnostic uncertainty; thus, establishing a standardized scoring system is crucial to guide future studies.

## Author contributions

KL and NM worked on the study conception and data acquisition, analysis, and interpretation. WM, AB, MK, ZL, OB, MA and SM contributed to the study conception and data acquisition. SH secured the resources. NM supervised the study and contributed to the manuscript drafting. All authors have read and agreed to the published version of the manuscript.

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## Ethical approval

Approval for the use of archival material for research purposes was obtained from the local Human Ethics Committee at Sahloul University Hospital, Sousse, Tunisia, and the study was conducted in accordance with the Declaration of Helsinki.

## Informed consent

Not applicable.

## Data availability

The supplementary data will be available to the readers when they send the request.

## Conflict of interest

The authors declare no conflict of interest.

## Abbreviations

AJCC: American Joint Committee on Cancer; CISH: chro-

mogenic *in situ* hybridization; CRC: colorectal carcinoma; EGFR: epidermal growth factor receptor; GEA: gastro-esophageal adenocarcinomas; GEA-b: GEA relevant to biopsies; mCRC: metastatic CRC; TMA: tissue microarrays; WHO: World Health Organization.

## References


1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A (2024) Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 74(3):229-263. https://doi.org/10.3322/caac.21834.
2. Makhlouf NA, Abdel-Gawad M, Mahros AM, Lashen SA, Zaghloul M, Eliwa A, Elshemy EE, Ali-Eldin Z, Abdeltawab D, El-Raey F, Omran D, Khalaf M, Fanous N, Abdelmohsen AS, Abu-Elfatth A, Abdelghani M, Farouk M, Abdelaziz M, Alboraie M (2021) Colorectal cancer in Arab world: A systematic review. World J Gastrointest Oncol 13(11):1791-1798. https://doi.org/10.4251/wjgo.v13.i11.1791.
3. Benson AB, Venook AP, Al-Hawary MM, Arain MA, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Farkas L, Garrido-Laguna I, Grem JL, Gunn A, Hecht JR, Hoffe S, Hubbard J, Hunt S, Johung KL, Kirilcuk N, Krishnamurthi S, Messersmith WA, Meyerhardt J, Miller ED, Mulcahy MF, Nurkin S, Overman MJ, Parikh A, Patel H, Pedersen K, Saltz L, Schneider C, Shibata D, Skibber JM, Sofocleous CT, Stoffel EM, Stotsky-Himelfarb E, Willett CG, Gregory KM, Gurski LA (2021) Colon Cancer, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 19(3):329-359. https://doi.org/10.6004/jnccn.2021.0012
4. Ding ZY, Piao Y, Jiang T, Chen J, Wang YN, Yu HY, Zheng ZD (2024) Effects of postoperative treatment with chemotherapy and cellular immunotherapy on patients with colorectal cancer. World J Gastrointest Surg 16(10):3202-3210. https://doi.org/10.4240/wjgs.v16.i10.3202
5. Takeda M, Yoshida S, Inoue T, Sekido Y, Hata T, Hamabe A, Ogino T, Miyoshi N, Uemura M, Yamamoto H, Doki Y, Eguchi H (2025) The role of KRAS mutations in colorectal cancer: Biological insights, clinical implications, and future therapeutic perspectives. Cancers (Basel) 17(3):428. https://doi.org/10.3390/cancers17030428.
6. Meyiah A, Khan FI, Alfaki DA, Murshed K, Raza A, Elkord E (2025) The colorectal cancer microenvironment: Preclinical progress in identifying targets for cancer therapy. Transl Oncol 53:102307. https://doi.org/10.1016/j.tranon.2025.102307.
7. Puzzo M, De Santo M, Morelli C, Leggio A, Catalano S, Pasqua L (2025) Colorectal cancer: Current and future therapeutic approaches and related technologies addressing multidrug strategies against multiple level resistance mechanisms. Int J Mol Sci 26(3):1313. https://doi.org/10.3390/ijms26031313.
8. Fanotto V, Ongaro E, Rihawi K, Avallone A, Silvestris N, Fornaro L, Vasile E, Antonuzzo L, Leone F, Rosati G, Giuliani F, Bordonaro R, Scartozzi M, De Maglio G, Negri FV, Fasola G, Aprile G (2016) HER-2 inhibition in gastric and colorectal cancers: tangible achievements, novel acquisitions and future perspectives. Oncotarget 7(42):69060-76904. https://doi.org/10.18632/oncotarget.11264.
9. Tatsuta K, Sakata M, Kojima T, Booka E, Kurachi K, Takeuchi H (2025) Updated insights into the impact of adjuvant chemotherapy on recurrence and survival after curative resection of liver or lung metastases in colorectal cancer: a rapid review and meta-analysis. World J Surg Oncol 23(1):56. https://doi.org/10.1186/s12957-025-03714-4.
10. Matsuda T, Fujimoto A, Igarashi Y (2025) Colorectal cancer: epidemiology, risk factors, and public health strategies. Digestion 106(2):91-99. https://doi.org/10.1159/000543921.
11. Arnold D, Seufferlein T (2010) Targeted treatments in colorectal cancer: state of the art and future perspectives. Gut 59(6):838-858. https://doi.org/10.1136/gut.2009.196006
12. Hecht JR, Mitchell E, Neubauer MA, Burris HA 3rd, Swanson P, Lopez T, Buchanan G, Reiner M, Gansert J, Berlin J (2010) Lack of correlation between epidermal growth factor receptor status and response to Panitumumab monotherapy in metastatic colorectal cancer. Clin Cancer Res 16(7):2205-2213. https://doi.org/10.1158/1078-0432.CCR-09-2017.
13. Greally M, Kelly CM, Cercek A (2018) HER2: An emerging target in colorectal cancer. Curr Probl Cancer 42(6):560-571. https://doi.org/10.1016/j.currproblcancer.2018.07.001.
14. Valentini AM, Cavalcanti E, Di Maggio M, Caruso ML (2018) RAS-expanded mutations and HER2 expression in metastatic colorectal cancer: A new step of precision medicine. Appl Immunohistochem Mol Morphol 26(8):539-544. https://doi.org/10.1097/PAI.0000000000000475.
15. Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM, Funkhouser WK, Kopetz SE, Lieu C, Lindor NM, Minsky BD, Monzon FA, Sargent DJ, Singh VM, Willis J, Clark J, Colasacco C, Rumble RB, Temple-Smolkin R, Ventura CB, Nowak JA (2017) Molecular biomarkers for the evaluation of colorectal cancer. Am J Clin Pathol 147(3):221-260. https://doi.org/10.1093/ajcp/aqw209.
16. Siena S, Sartore-Bianchi A, Marsoni S, Hurwitz HI, McCall SJ, Penault-Llorca F, Srock S, Bardelli A, Trusolino L (2018) Targeting the human epidermal growth factor receptor 2 (HER2) oncogene in colorectal cancer. Ann Oncol 29(5):1108-1119. https://doi.org/10.1093/annonc/mdy100.
17. Sartore-Bianchi A, Amatu A, Porcu L, Ghezzi S, Lonardi S, Leone F, Bergamo F, Fenocchio E, Martinelli E, Borelli B, Tosi F, Racca P, Valtorta E, Bonoldi E, Martino C, Vaghi C, Marrapese G, Ciardiello F, Zagonel V, Bardelli A, Trusolino L, Torri V, Marsoni S, Siena S (2019) HER2 positivity predicts unresponsiveness to EGFR-targeted treatment in metastatic colorectal cancer. Oncologist 24(10):1395-1402. https://doi.org/10.1634/theoncologist.2018-0785.
18. Wieduwilt MJ, Moasser MM (2008) The epidermal growth factor receptor family: biology driving targeted therapeutics. Cell Mol Life Sci 65(10):1566-1584. https://doi.org/10.1007/s00018-008-7440-8.
19. Talukdar S, Emdad L, Das SK, Fisher PB (2020) EGFR: An essential receptor tyrosine kinase-regulator of cancer stem cells. Adv Cancer Res 147:161-188. https://doi.org/10.1016/bs.acr.2020.04.003.
20. Popescu NC, King CR, Kraus MH (1989) Localization of the human erbB-2 gene on normal and rearranged chromosomes 17 to bands q12-21.32. Genomics 4(3):362-366.
21. Hassan G, Seno M (2022) ERBB Signaling Pathway in Cancer Stem Cells. Adv Exp Med Biol 1393:65-81. https://doi.org/10.1007/978-3-031-12974-2_3.
22. Kavuri SM, Jain N, Galimi F, Cottino F, Leto SM, Migliardi G, Searleman AC, Shen W, Monsey J, Trusolino L, Jacobs SA, Bertotti A, Bose R (2015) HER2 activating mutations are targets for colorectal cancer treatment. Cancer Discov 5(8):832-841. https://doi.org/10.1158/2159-8290.CD-14-1211.
23. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, Bilous M, Ellis IO, Fitzgibbons P, Hanna W, Jenkins RB, Press MF, Spears PA, Vance GH, Viale G, McShane LM, Dowsett M (2018) Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/

College of American Pathologists Clinical Practice Guideline Focused Update. Arch Pathol Lab Med 142(11):1364-1382. https://doi.org/10.5858/arpa.2018-0902-SA.

24. Zhang J, Yuan C, Ma X (2025) Efficacy and safety of different drugs in patients with HER2-positive gastric cancer: network meta-analysis. Syst Rev 14(1):40. https://doi.org/10.1186/s13643-025-02777-4.
25. Aoki Y, Nakayama I, Shitara K (2025) Human Epidermal Growth Factor Receptor 2 positive advanced gastric or esophagogastric adenocarcinoma: Reflecting on the past to gain a new insights. Curr Oncol Rep 27(1):15-29. https://doi.org/10.1007/s11912-024-01626-2.
26. Yagisawa M, Sawada K, Nakamura Y, Fujii S, Yuki S, Komatsu Y, Yoshino T, Sakamoto N, Taniguchi H (2021) Prognostic value and molecular landscape of HER2 low-expressing metastatic colorectal cancer. Clin Colorectal Cancer 20(2):113-20.e1. https://doi.org/10.1016/j.clcc.2020.11.002.
27. Chang J, Xu M, Wang C, Huang D, Zhang Z, Chen Z, Zhu X, Li W (2022) Dual HER2 targeted therapy with pyrotinib and trastuzumab in refractory her2 positive metastatic colorectal cancer: A result from HER2-FUSCC-G Study. Clin Colorectal Cancer 21(4):347-353. https://doi.org/10.1016/j.clcc.2022.07.003.
28. Meric-Bernstam F, Hurwitz H, Raghav KPS, McWilliams RR, Fakih M, VanderWalde A, Swanton C, Kurzrock R, Burris H, Sweeney C, Bose R, Spigel DR, Beattie MS, Blotner S, Stone A, Schulze K, Cuchelkar V, Hainsworth J (2019) Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. Lancet Oncol 20(4):518-530. https://doi.org/10.1016/S1470-2045(18)30904-5.
29. Sartore-Bianchi A, Lonardi S, Martino C, Fenocchio E, Tosi F, Ghezzi S, Leone F, Bergamo F, Zagonel V, Ciardiello F, Ardizzoni A, Amatu A, Bencardino K, Valtorta E, Grassi E, Torri V, Bonoldi E, Sapino A, Vanzulli A, Regge D, Cappello G, Bardelli A, Trusolino L, Marsoni S, Siena S (2020) Pertuzumab and trastuzumab emtansine in patients with HER2-amplified metastatic colorectal cancer: the phase II HERACLES-B trial. ESMO Open 5(5):e000911. https://doi.org/10.1136/esmoopen-2020-000911.
30. Siena S, Di Bartolomeo M, Raghav K, Masuishi T, Loupakis F, Kawakami H, Yamaguchi K, Nishina T, Fakih M, Elez E, Rodriguez J, Ciardiello F, Komatsu Y, Esaki T, Chung K, Wainberg Z, Sartore-Bianchi A, Saxena K, Yamamoto E, Bako E, Okuda Y, Shahidi J, Grothey A, Yoshino T; DESTINY-CRC01 investigators (2021) Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): a multicentre, open-label, phase 2 trial. Lancet Oncol 22(6):779-789. https://doi.org/10.1016/S1470-2045(21)00086-3.
31. Strickler JH, Cercek A, Siena S, André T, Ng K, Van Cutsem E, Wu C, Paulson AS, Hubbard JM, Coveler AL, Fountzilas C, Kardosh A, Kasi PM, Lenz HJ, Ciombor KK, Elez E, Bajor DL, Cremolini C, Sanchez F, Stecher M, Feng W, Bekaii-Saab TS; MOUNTAINEER investigators (2023) Tucatinib plus trastuzumab for chemotherapy-refractory, HER2-positive, RAS wild-type unresectable or metastatic colorectal cancer (MOUNTAINEER): a multicentre, open-label, phase 2 study. Lancet Oncol 24(5):496-508. https://doi.org/10.1016/S1470-2045(23)00150-X.
32. Sartore-Bianchi A, Trusolino L, Martino C, Bencardino K, Lonardi S, Bergamo F, Zagonel V, Leone F, Depetris I, Martinelli E, Troiani T, Ciardiello F, Racca P, Bertotti A, Siravegna G, Torri V, Amatu A, Ghezzi S, Marrapese G, Palmeri L, Valtorta E, Cassingena A, Lauricella C, Vanzulli A, Regge D, Veronese S, Comoglio PM, Bardelli A, Marsoni S, Siena S (2016) Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. Lancet Oncol 17(6):738-746. https://doi.org/10.1016/S1470-2045(16)00150-9.
33. Roy-Chowdhuri S, Davies KD, Ritterhouse LL, Snow AN (2022) ERBB2 (HER2) alterations in colorectal cancer. J Mol Diagn 24(10):1064-1066. https://doi.org/10.1016/j.jmoldx.2022.07.001.
34. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA (2020) WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. Histopathology 76(2):182-188. https://doi.org/10.1111/his.13975.
35. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR, Winchester DP (2017) The Eighth edition AJCC cancer staging manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin 67(2):93-99. https://doi.org/10.3322/caac.21388.
36. Missaoui N, Landolsi H, Mestiri S, Essakly A, Abdessayed N, Hmissa S, Mokni M, Yacoubi MT (2019) Immunohistochemical analysis of c-erbB-2, Bcl-2, p53, p21WAF1/Cip1, p63 and Ki-67 expression in hydatidiform moles. Pathol Res Pract 215(3):446-452. https://doi.org/10.1016/j.prp.2018.12.015.
37. Hofmann M, Stoss O, Shi D, Büttner R, van de Vijver M, Kim W, Ochiai A, Rüschoff J, Henkel T (2008) Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology 52(7):797-805. https://doi.org/10.1111/j.1365-2559.2008.03028.x.
38. Bartley AN, Washington MK, Ventura CB, Ismaila N, Colasacco C, Benson AB 3rd, Carrato A, Gulley ML, Jain D, Kakar S, Mackay HJ, Streutker C, Tang L, Troxell M, Ajani JA (2016) HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: Guideline from the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology. Arch Pathol Lab Med 140:1345-1363. https://doi.org/10.5858/arpa.2016-0331-CP.
39. Hanna WM, Kwok K (2006) Chromogenic in-situ hybridization: a viable alternative to fluorescence in-situ hybridization in the HER2 testing algorithm. Mod Pathol 19(4):481-487. https://doi.org/10.1038/modpathol.3800555.
40. Conradi LC, Styczen H, Sprenger T, Wolff HA, Rödel C, Nietert M, Homayounfar K, Gaedcke J, Kitz J, Talaulicar R, Becker H, Ghadimi M, Middel P, Beissbarth T, Rüschoff J, Liersch T (2013) Frequency of HER-2 positivity in rectal cancer and prognosis. Am J Surg Pathol 37(4):522-531. https://doi.org/10.1097/PAS.0b013e318272ff4d.
41. Fujii S, Magliocco AM, Kim J, Okamoto W, Kim JE, Sawada K, Nakamura Y, Kopetz S, Park WY, Tsuchihara K, Kim TW, Raghav K, Yoshino T (2020) International harmonization of provisional diagnostic criteria for ERBB2-amplified metastatic colorectal cancer allowing for screening by next-generation sequencing panel. JCO Precis Oncol 4:6-19. https://doi.org/10.1200/PO.19.00154.
42. Penault-Llorca F, Chenard MP, Bouché O, Emile JF, Bibeau F, Metges JP, André T, Monges G. HER2 et cancer gastrique. Recommandations pour la pratique clinique en 2011 (2011) [HER2 and gastric cancer. Recommendations for clinical practice in 2011]. Ann Pathol 31(2):78-87. https://doi.org/10.1016/j.annpat.2011.03.001.
43. Kononen J, Bubendorf L, Kallioniemi A, Bärlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP (1998) Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 4(7):844-847. https://doi.org/10.1038/nm0798-844.
44. Marx AH, Burandt EC, Choschzick M, Simon R, Yekebas E, Kaifi JT, Mirlacher M, Atanackovic D, Bokemeyer C, Fiedler W,

Terracciano L, Sauter G, Izbicki JR (2010) Heterogenous high-level HER-2 amplification in a small subset of colorectal cancers. Hum Pathol 41(11):1577-1585. https://doi.org/10.1016/j.humpath.2010.02.018.

45. Ingold Heppner B, Behrens HM, Balschun K, Haag J, Krüger S, Becker T, Röcken C (2014) HER2/neu testing in primary colorectal carcinoma. Br J Cancer 111(10):1977-1984. https://doi.org/10.1038/bjc.2014.483.
46. Richman SD, Southward K, Chambers P, Cross D, Barrett J, Hemmings G, Taylor M, Wood H, Hutchins G, Foster JM, Oumie A, Spink KG, Brown SR, Jones M, Kerr D, Handley K, Gray R, Seymour M, Quirke P (2016) HER2 overexpression and amplification as a potential therapeutic target in colorectal cancer: analysis of 3256 patients enrolled in the QUASAR, FOCUS and PICCOLO colorectal cancer trials. J Pathol 238(4):562-570. https://doi.org/10.1002/path.4679.
47. Valtorta E, Martino C, Sartore-Bianchi A, Penaullt-Llorca F, Viale G, Risio M, Rugge M, Grigioni W, Bencardino K, Lonardi S, Zagonel V, Leone F, Noe J, Ciardiello F, Pinto C, Labianca R, Mosconi S, Graiff C, Aprile G, Frau B, Garufi C, Loupakis F, Racca P, Tonini G, Lauricella C, Veronese S, Truini M, Siena S, Marsoni S, Gambacorta M (2015) Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. Mod Pathol 28(11):1481-1491. https://doi.org/10.1038/modpathol.2015.98.
48. Liu F, Ren C, Jin Y, Xi S, He C, Wang F, Wang Z, Xu RH, Wang F (2020) Assessment of two different HER2 scoring systems and clinical relevance for colorectal cancer. Virchows Arch 476(3):391-398. https://doi.org/10.1007/s00428-019-02668-9.
49. The Cancer Genome Atlas Network (2012) Comprehensive molecular characterization of human colon and rectal cancer. Nature 487:330-337.https://doi.org/10.1038/nature11252.
50. Pyo JS, Kang G, Park K (2016) Clinicopathological significance and diagnostic accuracy of HER2 immunohistochemistry in colorectal cancer: a meta-analysis. Int J Biol Markers 31(4):e389-e394. https://doi.org/10.5301/jbm.5000208.
51. Ahcene Djaballah S, Daniel F, Milani A, Ricagno G, Lonardi S (2022) HER2 in colorectal cancer: The long and winding road from negative predictive factor to positive actionable target. Am Soc Clin Oncol Educ Book 42:1-14. https://doi.org/10.1200/EDBK_351354.
52. Song Z, Deng Y, Zhuang K, Li A, Liu S (2014) Immunohistochemical results of HER2/neu protein expression assessed by rabbit monoclonal antibodies SP3 and 4B5 in colorectal carcinomas. Int J Clin Exp Pathol 7(7):4454-4460.
53. Sun Q, Li Q, Gao F, Wu H, Fu Y, Yang J, Fan X, Cui X, Pu X (2023) HER2 overexpression/amplification status in colorectal cancer: a comparison between immunohistochemistry and fluorescence in situ hybridization using five different immunohistochemical scoring criteria. J Cancer Res Clin Oncol 149(2):579-592. https://doi.org/10.1007/s00432-022-04230-8.
54. Shabbir A, Mirza T, Khalid AB, Qureshi MA, Asim SA (2016) Frequency of Her2/neu expression in colorectal adenocarcinoma: a study from developing South Asian Country. BMC Cancer 16(1):855. https://doi.org/10.1186/s12885-016-2912-y.
55. Baiocchi G, Lopes A, Coudry RA, Rossi BM, Soares FA, Aguiar S, Guimarães GC, Ferreira FO, Nakagawa WT (2009) ErbB family immunohistochemical expression in colorectal cancer patients with higher risk of recurrence after radical surgery. Int J Colorectal Dis 24(9):1059-1068. https://doi.org/10.1007/s00384-009-0702-6.
56. Blok EJ, Kuppen PJ, van Leeuwen JE, Sier CF (2013) Cytoplasmic overexpression of HER2: a key factor in colorectal cancer. Clin Med Insights Oncol 7:41-51. https://doi.org/10.4137/CMO.S10811.
57. Buhmeida A, Assidi M, Al-Maghrabi J, Dallol A, Sibiany A, Al-Ahwal M, Chaudhary A, Abuzenadah A, Al-Qahtani M (2018) Membranous or cytoplasmic HER2 expression in colorectal carcinoma: Evaluation of prognostic value using both IHC & BDISH. Cancer Invest 36(2):129-140. https://doi.org/10.1080/07357907.2018.1439054.
58. Wang G, He Y, Sun Y, Wang W, Qian X, Yu X, Pan Y (2020) Prevalence, prognosis and predictive status of HER2 amplification in anti-EGFR-resistant metastatic colorectal cancer. Clin Transl Oncol 22(6):813-822. https://doi.org/10.1007/s12094-019-02213-9.
59. Kountourakis P, Pavlakis K, Psyrri A, Rontogianni D, Xiros N, Patsouris E, Pectasides D, Economopoulos T (2006) Clinicopathologic significance of EGFR and Her-2/neu in colorectal adenocarcinomas. Cancer J 12(3):229-236. https://doi.org/10.1097/00130404-200605000-00012.
60. Kavanagh DO, Chambers G, O'Grady L, Barry KM, Waldron RP, Bennani F, Eustace PW, Tobbia I (2009) Is overexpression of HER-2 a predictor of prognosis in colorectal cancer? BMC Cancer 9:1. https://doi.org/10.1186/1471-2407-9-1.
61. Sawada K, Nakamura Y, Yamanaka T, Kuboki Y, Yamaguchi D, Yuki S, Yoshino T, Komatsu Y, Sakamoto N, Okamoto W, Fujii S (2018) Prognostic and predictive value of HER2 amplification in patients with metastatic colorectal cancer. Clin Colorectal Cancer 17(3):198-205. https://doi.org/10.1016/j.clcc.2018.05.006.