DOI: 10.7860/NJLM/2024/61656.2805

Original Article

Pathology Section

Role of HER2/neu in Colorectal Carcinoma: A Cross-sectional Study from a Tertiary Care Centre in West Bengal, India

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ABSTRACT

Introduction: In many tumours, Human Epidermal Growth Factor Receptor 2 (HER2) expression and HER2 amplification are studied as therapeutic and prognostic factors. The incidence of HER2/neu expression and its consequences in patients with colorectal carcinoma has shown conflicting evidence.

Aim: To evaluate the utility of HER2/neu expression in colorectal carcinoma and to find any association between HER2/neu expression, histological grade, and stage of the tumour.

Materials and Methods: This cross-sectional study was conducted on 50 cases of colorectal carcinoma. Patients undergoing surgery for colorectal tumours were selected over an 18-month period (January 2020 to June 2021) in a tertiary care hospital in Kolkata, West Bengal, India. Various clinical and demographic parameters were obtained from the patients, and the specimens were subjected to Immunohistochemical (IHC) staining for HER2/neu. The results were analysed using Statistical Package for the Social Sciences (SPSS) software version 25.0 (IBM, Armonk, New York, USA).

Results: On HER2/neu IHC examination, 30 cases (60%) showed a 3+ score, 4 cases (8%) showed a 2+ score, 7 cases (14%) showed a 1+ score, and the remaining 9 cases (18%) showed a score of 0. HER2/neu expression was found in 30 cases (60%) of colorectal carcinoma. HER2/neu expression showed a significant association with the histological grade of the tumour (p-value=0.020). HER2/neu expression also showed a significant association (p-value <0.001) with histological subtypes of the tumour.

Conclusion: This study revealed a significant association between HER2/neu expression and histological subtypes and grade of the tumour. Moderately differentiated adenocarcinoma showed the highest expression, while mucinous adenocarcinoma and undifferentiated carcinoma showed minimal or absent HER2/neu expression. Thus, HER2/neu can serve as a prognostic and therapeutic marker in colorectal carcinoma. In conclusion, surgery alone is not curative for patients with colorectal cancer, and additional forms of management may be required to improve patient survival. In such cases, HER2/neu expression can guide new adjuvant therapy.

Keywords: Adenocarcinoma, Colorectal, Immunohistochemistry, Prognostic

INTRODUCTION

According to the World Health Organisation (WHO), colorectal cancer is the most common type of gastrointestinal cancer. Colorectal adenocarcinoma is a malignant tumour originating from the epithelial cells in the large bowel, exhibiting glandular or mucinous differentiation. Adenocarcinomas account for 90% of colorectal cancers, with a majority occurring on the left side or in the rectum [1]. Colorectal carcinoma is the third most common cancer in both males and females and ranks second as a leading cause of cancer-related deaths. It is typically diagnosed in individuals in their sixth to seventh decades of life [2]. Colorectal carcinoma is a significant contributor to cancer mortality, with an estimated 1,849,518 new cases recorded worldwide in 2018. According to the WHO, it is the second most common cancer in women and the third most common cancer in men [1].

A study by André T et al., suggests that chemotherapy is an effective adjuvant therapy but does not prevent recurrence [3]. Various studies have reported conflicting data on the expression of HER2/neu in colorectal carcinoma, with a range of 0-83% [4]. HER2/neu is a proto-oncogene located on Chromosome 17q21, encoding a transmembrane protein with tyrosine kinase activity. It plays a role in signal transduction pathways, cell growth, and differentiation. Overexpression of HER2/neu has been associated with increased cell survival and proliferation, as well as reduced apoptotic potential, leading to neoplastic transformation [5]. Amplification and overexpression of HER2/neu genes were initially discovered in breast cancer and were found to be associated with a poor prognosis [6]. HER2/neu expression has also been observed in other malignancies, including gastroesophageal and gastric cancer, colorectal carcinoma, ovarian carcinoma, prostate carcinoma, and lung carcinoma [7]. The grading and staging of colorectal carcinoma were performed according to the WHO classification of tumours of the digestive system, 5th Edition, 2019 [1].

The aim of present study was to understand the clinicopathological spectrum of colorectal cancers in a tertiary care hospital and determine histopathological grading and staging and also to study IHC expression of HER2/neu and assess the association between histological grades and HER2 expression in colorectal cancers.

MATERIALS AND METHODS

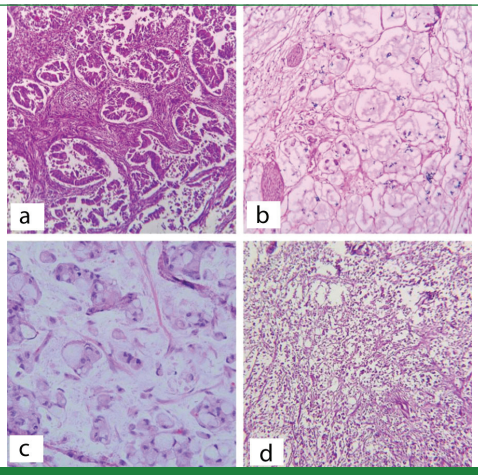
The study was a cross-sectional observational study conducted at the Department of Pathology in collaboration with the Department of Surgery at the Institute of Postgraduate Medical Education and Research in Kolkata, West Bengal, India, after obtaining approval from the Institutional Ethical Committee (IEC No. IPGME&R/IEC/2021/559). A total of 50 patients undergoing surgery for colorectal cancer over an 18-month period (January 2020 to June 2021) were selected.

Inclusion criteria: All surgically resected specimens of colorectal carcinoma and colonoscopic biopsy specimens were included in the study.

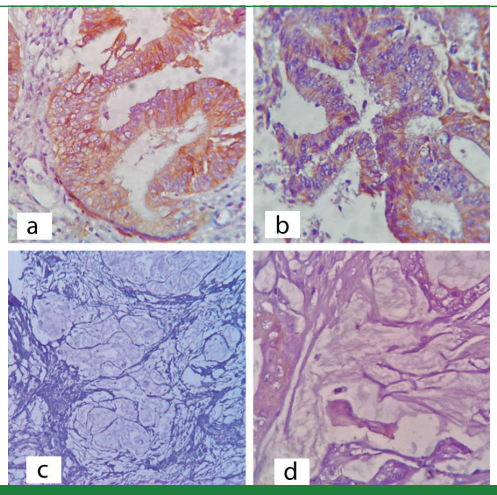
Exclusion criteria: Inflammatory conditions of colorectum, patients unwilling to undergo surgery and critically ill or debilitated patients were excluded from the study.

Procedure

After resection, the specimens were sent to the Pathology Department for Hematoxylin and Eosin (H&E) staining. IHC staining was performed for HER2/neu expression in all cases of colorectal carcinoma. HER2/neu expression was also associated with the subtypes of colorectal carcinoma and other clinicopathological factors such as alcohol consumption, dietary fiber consumption, red meat consumption, smoking, tumour stage, tumour invasion, and lymphovascular invasion [1]. Four-micron sections were prepared from each formalin-fixed and paraffin-embedded tissue sample and stained with an antibody against HER2/neu (polyclonal rabbit anti-human antibody against c-erbB-2 oncoprotein, Dako) [Table/Fig-1,2]. A known HER2/neu-positive case of breast carcinoma was used as a positive control, and a negative control was achieved by omitting the primary antibody.



[Table/Fig-1]: a) Picture showing histology of moderately differentiated adenocarcinoma (X400); b) Picture showing histology of mucinous carcinoma (X400); c) Picture showing histology of signet ring cell carcinoma (X400); d) Picture showing histology of undifferentiated carcinoma (X400).



[Table/Fig-2]: a) Picture showing HER-2/neu IHC 3+in moderately differentiated adenocarcinoma (X400); b) Picture showing HER-2/neu IHC 3+in well differentiated adenocarcinoma (X400); c) Picture showing HER-2/neu IHC in mucinous adenocarcinoma (X400); d) Picture showing HER-2/neu IHC in adenocarcinoma with mucinous component (X400).

Interpretation of HER2/Neu Immunostaining: The interpretation of HER2 immunostaining was done according to the guidelines [Table/Fig-3] [8]. For HER2 immunostaining interpretation, a low-power objective (100X) was used, and immunostaining was observed in a homogeneous membranous population of tumour cells. IHC was used to evaluate the membranous protein expression of HER2/neu in cancer cells. Both the intensity and percentage of staining were assessed using scores ranging from 0 to 3+. A score of 0 or 1+ was considered negative for HER2/neu expression. A score of 2+ was considered equivocal and required confirmation with Fluorescent in situ Hybridisation (FISH) or other available in situ hybridisation techniques.

HER2/neu score	HER2 pattern of staining in surgical specimen	HER2 pattern of staining in biopsy specimen	Assessment of HER2/neu expression
0	No reactivity or membranous reactivity in <10% of cancer cells	No reactivity or no membranous reactivity in any cancer cell	Negative by IHC
1+	Faint or barely perceptible membranous reactivity in ≥10% of cancer cells; cells are focally membrane positive/reactive	Malignant cell cluster* with a faint and barely perceptible membranous reactivity irrespective of percentage of cancer cells positive	Negative by IHC
2+	Weak to moderate and complete, basolateral, or lateral membranous reactivity in ≥10% of tumour cells	Malignant cell cluster* with a weak to moderate and complete, basolateral, or lateral membranous reactivity irrespective of percentage of cancer cells positive	Equivocal by IHC
3+	Strong and complete, basolateral, or lateral membranous reactivity in ≥10% of cancer cells	Malignant cell cluster* with a strong and complete basolateral, or lateral membranous reactivity irrespective of percentage of cancer cells positive	Positive by IHC

[Table/Fig-3]: Interpretation of HER2/neu staining [8].
*: malignant cell cluster consisting of ≥5 neoplastic cells; IHC: Immunohistochemical

STATISTICAL ANALYSIS

All the data obtained and collected were properly maintained in a Microsoft Excel worksheet. Mean values with standard deviation were calculated for quantitative variables, while qualitative variables were represented by proportions. The Chi-square test was performed to assess the association between clinicopathological parameters. Statistical analyses were conducted using SPSS software version 25.0 (IBM, Armonk, New York, USA). A two-tailed p-value of <0.05 was considered statistically significant.

RESULTS

A total of 50 cases were analysed, with 31 cases (62%) being males and 19 cases (38%) being females. The age of patients ranged from 18 to 80 years, with a mean age of 49.64±14.274 years. The most commonly affected age group was 41-60 years. The ascending colon was the most affected region, observed in 19 out of 50 cases (38%), 13 cases in sigmoid colon (26%), 8 cases in rectum (16%), 5 cases in descending colon (10%), 3 cases in transverse colon (6%) and 2 cases in caecum (4%). The most common histological subtype was Adenocarcinoma NOS, found in 38 out of 50 cases (76%), followed by Mucinous adenocarcinoma with eight cases (16%). Undifferentiated carcinoma and signet ring cell carcinoma were the least common, each accounting for two cases (4%). The majority of tumours were moderately differentiated, with 43 cases out of 50 (86%). Approximately 48% of the tumours (24 out of 50 cases) exhibited lymph node involvement, while 52% (26 out of 50 cases) showed reactive features in the lymph nodes. Invasion upto the muscularis propria was observed in 60% of cases (30 out of 50), followed by invasion up to the subserosa in 34% of cases (17 out of 50) and up to the serosa in 6% of cases (3 out of 50).

HER-2/neu IHC examination revealed that 30 cases (60%) had a 3+ score, four cases (8%) had a 2+ score, seven cases (14%) had a 1+ score, and the remaining nine cases (18%) had a 0 score [Table/Fig-4]. Significant associations were found between HER-2/neu expression and histological subtypes of colorectal carcinoma (p-value <0.001). Adenocarcinoma NOS showed the highest expression. Among the mucinous carcinoma cases, five showed no expression and three showed 1+ expression of HER2/neu out of a total of eight cases. Both cases of signet ring cell carcinoma showed 1+ expression. Both cases of undifferentiated subtype were negative (0) for HER2/neu [Table/Fig-5].

Additionally, a significant association was found between HER2/neu expression and the histological grade of the tumour (p-value=0.020) [Table/Fig-6]. Among the different histological grades of the tumour, moderately differentiated adenocarcinoma showed the highest

Prognostic parameters		HER2/neu Score 0	HER2/neu Score 1	HER2/neu Score 2	HER2/neu Score 3	p-value
Age (years)	1-20	0	1	0	0	0.179
	21-40	3	1	1	7	
	41-60	5	4	2	16	
	61-80	1	1	1	7	
Gender	M	7	3	2	19	0.511
	F	2	4	2	11	
Lymphovascular invasion	Involved	8	4	3	18	0.32
	Not involved	1	3	1	12	
Tumour stage	I	3	4	2	14	0.742
	II	0	0	0	3	
	III	4	3	2	11	
	IV	2	0	0	2	
Tumour invasion	Serosa	1	0	0	2	0.701
	Upto muscularis propria	5	4	4	17	
	Upto subserosa	3	3	0	11	

[Table/Fig-4]: Association between HER2/neu expression and clinicopathological factors.

Histological features	HER2/neu 0	1	2	3	χ^2	p-value
Adenocarcinoma NOS	2	2	4	30	46.867	<0.001
Mucinous adenocarcinoma	5	3	0	0		
Signet ring cell carcinoma	0	2	0	0		
Undifferentiated type	2	0	0	0		
Total	9	7	4	30		

[Table/Fig-5]: Association of HER2/neu with histological subtypes (n=50).
NOS: Not otherwise specified

expression, with 28 out of 50 cases (56%) exhibiting 3+ positivity. Four cases out of 50 (8%) showed equivocal expression (2+), while 11 cases out of 50 (22%) showed negative expression. Two cases of well-differentiated adenocarcinoma showed positive expression (3+). All cases of poorly differentiated adenocarcinoma and both cases of undifferentiated type showed negative expression of HER-2/neu (score 0).

Histological grade	HER2/neu 0	1	2	3	χ^2	p-value
Well differentiated	0	0	0	2	19.663	0.020
Moderately differentiated	6	5	4	28		
Poorly differentiated	1	2	0	0		
Undifferentiated	2	0	0	0		
Total	9	7	4	30		

[Table/Fig-6]: Association of HER2/neu with histological grade of the tumour (n=50).

No significant association was found between HER-2/neu expression and TNM staging, tumour invasion, or Lymphovascular Invasion (LVI) status. Additionally, no statistical associations were found between the histopathological diagnosis of the tumour and alcohol consumption, smoking, excessive red meat consumption, or dietary fiber consumption. No association between the marker and the disease stage was observed.

DISCUSSION

Present study examined HER2/neu expression in 50 cases of colorectal carcinoma. IHC staining was performed, and the results were interpreted based on the intensity, pattern, and percentage of HER2/neu staining, following the ToGA guidelines for scoring [8]. HER2/neu can serve as a predictor of the outcome and response to adjuvant chemotherapy in colon carcinoma. Overexpression of HER2/neu is associated with a poor prognosis, and those who overexpress HER2/neu may respond to Trastuzumab (Herceptin) therapy [9,10]. Ghaffarzadegan K et al., conducted a study on 69 cases of colon carcinoma to assess HER2/neu protein expression. They observed positive HER2/neu staining in 59.4% of cases, cytoplasmic staining in 65.9% of cases, and membranous cytoplasmic staining in 34.1% of cases. They did not find any association between HER2/neu expression and the site or type of the tumour [11]. Half E et al., analysed HER2/neu expression in colorectal carcinoma cells, examining protein expression, HER2/neu amplification, and mRNA levels. They found strong membranous staining in 5% of colorectal carcinomas and cytoplasmic staining in 63.5% of carcinomas. They also found a significant association between cytoplasmic staining and tumour differentiation [12]. The frequency of HER2/neu expression in colorectal cancer has been reported to range from 0-83% in some literature [13,14]. This wide range of HER2/neu overexpression may be attributed to varying staining patterns (cytoplasmic, membranous, or both), differences in tissue fixation intensity, and variations in the antibodies and IHC procedures used.

Block EJ et al., conducted a study and reported that HER2/neu expression can occur both in the membrane and cytoplasm of colorectal carcinoma cells [15]. Studies have also shown that HER2/neu expression in colon carcinoma can serve as a marker for poor prognosis. Additionally, some literature supports the association between cytoplasmic HER2/neu expression in colorectal carcinoma and survival prognosis [15]. In present study, the positive HER2/neu expression in the membrane was 60%. In a larger group of colorectal carcinoma cases, HER2/neu expression was reported in 81.8% of the total number of patients [16]. These variations may be attributed to factors such as sample size, heterogeneity in the study population, racial differences, and technical differences in IHC performance [17,18]. Present study evaluated the results according to the Trastuzumab for GAstric cancer (ToGA) Trial for Scoring HER2 Expression by IHC in Gastric and Gastroesophageal Junction Adenocarcinoma [Table/Fig-3]. Detection of HER2 (ERBB2) gene amplification in colorectal carcinoma was performed according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines, which are similar to those used in breast cancer. HER2 (ERBB2) amplification is defined as a HER2 (ERBB2): CEP17 ratio of ≥2 [19].

The National Comprehensive Cancer Network (NCCN) panel recommends using FISH only for cases with HER2/neu 2+ expression. The NCCN guidelines also suggest adding trastuzumab with chemotherapy only for patients with IHC 3+ expression or IHC 2+ expression with evidence of HER2/neu amplification by in situ hybridisation (HER2 (ERBB2): CEP17 ratio ≥2). Trastuzumab is not recommended if the HER2/neu score is 0 or 1+ [20]. Two other phase II trials, TRIUMPH and TAPUR, confirmed the activity of trastuzumab plus pertuzumab. The TAPUR trial aimed to describe the efficacy and

safety of available targeted anticancer drugs in patients with advanced cancer. In the cohort of HER2-amplified mCRC, which included 28 patients, the Overall Response Rate (ORR) was 14%, and the disease control rate for at least 16 weeks was 50% [21]. Another ongoing phase II study, DESTINY-CRC02 (study of trastuzumab deruxtecan), aims to determine the safety and efficacy of two doses (5.4 mg/kg and 6.4 mg/kg) in patients with HER2/neu overexpression, RAS wild-type variant, or mutant metastatic colorectal carcinoma [22]. All these studies shed light on the importance of focusing on the pattern and intensity of HER2/neu staining in colorectal carcinomas. The recent approval of lapatinib, an intracellular kinase inhibitor, for trastuzumab resistance in breast cancer patients suggests that it may also have potential in the treatment of colorectal carcinoma [23]. This is especially relevant considering the role of cytoplasmic HER2/neu expression in the pathogenesis of colorectal carcinoma, similar to that in breast cancer. Conradi LC et al., conducted a study on 225 resection specimens of advanced rectal carcinomas after neoadjuvant radiochemotherapy and found that HER2/neu-positive tumours had better survival (CSS; $p<0.03$) after five years compared to negative cases. They concluded that HER2/neu-positive tumours may have a better 5-year Cancer Specific Survival (CSS) than HER2/neu-negative tumours [24].

Limitation(s)

However, there are some limitations. Firstly, since it was conducted in a single institute and the total number of cases was limited, it may not be possible to generalise the findings to the entire population. This study can be considered as a part of a larger study to reach a definitive conclusion. Secondly, due to the short study period, follow-up and survival analysis were not possible. It would be recommended to gather data on follow-up to evaluate the prognostic utility of these markers and their ability to determine tumour behaviour. Lastly, authors were unable to evaluate any markers in the study using gene amplification techniques due to financial constraints.

CONCLUSION(S)

This study revealed a significant association between HER2/neu expression and the histological subtypes and histological grade of the tumour. Adenocarcinoma NOS showed strong HER2/neu expression, while mucinous adenocarcinoma and undifferentiated carcinoma had minimal or absent HER2/neu expression. These findings highlight the importance of routine evaluation of HER2/neu expression in colorectal carcinoma, along with other prognostic parameters. It is important to note that surgery alone is not curative in patients with advanced colon cancer, and additional forms of therapy may be required to improve patient survival. HER2 overexpression could serve as a guideline for targeted trastuzumab therapy in these patients, similar to its use in breast carcinoma.

REFERENCES

[1] Digestive System Tumours. WHO Classification of Tumours. Tumours of Colon and Rectum. 2019; 5th Edition Chapter 6/.
[2] Rosai & Ackerman. Surgical pathology. First south Asia edition. Gastrointestinal and hepatobiliary tract. Chapter 17, Pp 677.
[3] André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med. 2004;350(23):2343-51.
[4] Schuell B, Gruenberger T, Scheithauer W, Zielinski Ch, Wrba F. HER 2/neu protein expression in colorectal cancer. BMC Cancer. 2006;6:123.
[5] Ung L, Chua TC, Merrett ND. Targeting HER2 amplifications in gastric cancer. Gastrointestinal Cancer Targets Ther. 2014;4:11-22.
[6] Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A. Human breast cancer: Association of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987;235:177-82.
[7] Hansford S, Kaurah P, Li-Chang H, Woo M, Senz J, Pinheiro H, et al. Hereditary diffuse gastric cancer syndrome CDH1 mutations and beyond. JAMA Oncol. 2015;1(1):23-32.
[8] Rüschoff J, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca F, et al. HER2 testing in gastric cancer: A practical approach. Mod Pathol. 2012;25:637-50. Doi: 10.1038/modpathol.2011.198.
[9] Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER-2 for metastatic breast cancer that overexpresses HER-2. N Engl J Med. 2001;344(11):783 92.
[10] Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, et al. Efficacy and safety of trastuzumab as a single agent in the first line treatment of HER-2 over expressing metastatic breast cancer. J Clin Oncol. 2002;20(3):719-26.
[11] Ghaffarzadegan K, Sharifi N, Vosooghynia H, Shakeri T, Lari S, Nassiri G, et al. Her-2/neu expression in colon adenocarcinoma and its association with clinicopathologic variables. IJBMS. 2006;9(1):64-69.
[12] Half E, Broaddus R, Danenberg KD, Danenberg PV, Ayers GD, Sinicrope FA, et al. Her-2/neu receptor expression, localization and activation in colorectal cancer cell lines and human tumours. Int J Cancer. 2003;108(4):540-48.
[13] Kapitanović S, Radosević S, Kapitanović M, Andelinović S, Ferencić Z, Tavassoli M, et al. The expression of p185 (HER-2/neu) correlates with the stage of disease and survival in colorectal cancer. Gastroenterology. 1997;112(4):1103-13.
[14] Osako T, Miyahara M, Uchino S, Inomata M, Kitano S, Kobayashi M, et al. Immunohistochemical study of the c-erbB-2 protein in colorectal cancer and its association with patient survival. Oncology. 1998;55(6):548-55.
[15] Blok EJ, Kuppen PJ, van Leeuwen JE, Sier CF. Cytoplasmic overexpression of HeR2: A key factor in colorectal cancer. Clin Med Insights Oncol. 2013;7:41-51.
[16] McKay JA, Loane JF, Ross VG, Ameyaw MM, Murray GI, Cassidy J, et al. C-erbB-2 is not a major factor in the development of colorectal cancer. Br J Cancer. 2002;86(4):568-73. Doi: 10.1038/sj.bjc.6600127. [PubMed: 11870539].
[17] Li S, Buchbinder E, Wu L, Bjorge JD, Fujita DJ, Zhu S, et al. EGFR and HER2 levels are frequently elevated in colon cancer cells. Discov Rep. 2014;1(1):e1.
[18] Seo AN, Kwak Y, Kim DW, Kang SB, Choe G, Kim WH, et al. HER2 status in colorectal cancer: Its clinical significance and the relationship between HER2 gene amplification and expression. PLoS One. 2014;9(5):e98528. Doi: 10.1371/journal.pone.0098528. [PubMed: 24879338].
[19] Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for humanepidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. Clin Oncol. 2013;31(31):3997-4013.
[20] Ajani JA, Bentrem DJ, Besh S, D'Amico TA, Das P, Denlinger C, et al. Gastric cancer, version 2.2013: Featured updates to the NCCN Guidelines.J Natl Compr Canc Netw. 2013;11(5):531-46.
[21] Gupta R, Garrett-Mayer E, Halabi S, Mangat PK, D'Andre SD, Meiri E, et al. Pertuzumab plus trastuzumab (P1T) in patients (Pts) with colorectal cancer (CRC) with ERBB2 amplification or overexpression: Results from the TAPUR Study. J Clin Oncol. 2020;38:4s (suppl; abstr 132).
[22] Raghav KPS, Yoshino T, Guimbaud R, Chau I, Eynde MVD, Maurel J, et al. Trastuzumab deruxtecan in patients with HER2-overexpressing locally advanced, unresectable, or metastatic colorectal cancer (mCRC): A randomized, multicenter, phase 2 study (DESTINY-CRC02). J Clin Oncol. 2021;39:15s (suppl; abstr TPS3620).
[23] Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med. 2006;355(26):2733-43.
[24] Conradi LC, Styczen H, Sprenger T, Wolff HA, Rodel C, Nietert M, et al. Frequency of HER-2 positivity in rectal cancer and prognosis. Am J Surg Pathol. 2013;37(4):522-31.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 24, 2022
- Manual Googling: May 29, 2023
- iThenticate Software: May 31, 2023 (14%)

ETYMOLOGY: Author Origin

EMENDATIONS: 10

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Nov 23, 2022**
Date of Peer Review: **Jan 11, 2023**
Date of Acceptance: **Jun 01, 2023**
Date of Publishing: **Jan 01, 2024**