RESEARCH ARTICLE

Editorial Process: Submission:01/03/2023 Acceptance:04/17/2023

Different Clinicopathological Characteristics in Indonesian Colorectal Patients with *NRAS* Mutations and HER2 Over-Expression

Kiki Lukman[1], Ade Tan Reza[1], Lisa Y. Hasibuan[1], Yunia Sribudiani[2], Birgitta Maria Dewayani[3], Reno Rudiman[1], Etis Primastari[3], Prapanca Nugraha[1]*

Abstract

Objective: This study aims to assess the association of subject characteristics and NRAS mutations with HER2 expression in CRC. **Methods:** This research is a cross-sectional study. The research subjects in this study were colorectal cancer patients in the Digestive Surgery division at Dr. Hasan Sadikin Hospital. There were 58 study subjects. Examination of NRAS mutations was carried out by Polymerase Chain Reaction (PCR) from fresh tumour tissue obtained from surgery or colonoscopy. Meanwhile, HER2 examination used the Immunohistochemistry (IHC) method of paraffin blocks for anatomical pathology examination of the same patients. **Result:** HER2 overexpression was found in 6/58 (10.3%) patients with CRC, and from 8 subjects with NRAS mutations, only 1 subject (1.7%) showed overexpression of HER2. Univariate analysis of HER2 expression showed no significant associations to age, sex, histologic feature, tumor location, and NRAS mutations. A significant association was found between HER2 expression and stage of the CRC with p=0.001. **Conclusion:** There is no association between NRAS mutations and HER2 overexpression in colorectal cancer patients.

Keywords: Colorectal cancer- HER2- IHC- NRAS- PCR

Asian Pac J Cancer Prev, **24 (4)**, 1373-1377

Introduction

Colorectal cancer (CRC) is the third most common cancer in men and the second most common cancer in women. According to GLOBOCAN 2020 data, there were 1.15 million new cases of colon cancer, 0.7 million new cases of rectal cancer, and 50,000 new cases of anal cancer in 2020 globally (Bray et al., 2018). With continued progress, these figures are predicted to increase to 1.92 million, 1.16 million, and 78,000 by 2040. Globally, CRC is one of the cancers with a steadily increasing incidence, accounting for 11% of all cancer diagnoses (Xi and Xu, 2021). Data in Indonesia based on GLOBOCAN in 2020 shows CRC in the fourth position with around 35,000 new cases each year, and an incidence number of 19.1 in men and 15.6 in women per 100,000 population (Bray et al., 2018; Erida et al., 2015).

Colorectal cancer is a complex and genetically heterogeneous disease that drives multiple oncogenic signaling pathways. Pathogenic mechanisms, including microsatellite instability (MSI), CpG island methylation phenotype (CIMP), and chromosomal instability (CIN), represent 80–85% of the causes of all CRC cases (Porru et al., 2018). RAS/RAF/MEK/ERK/MAPK or MAPK/ERK (mitogen-activated protein kinases / extracellular signal-regulated kinases) is the most well-known pathway in the pathogenesis of KKR (Gong et al., 2018). RAS and BRAF are members of the MAPK/ERK pathway that mediates cellular responses to growth signals and are members of the multigene family, of which RAS is composed. of KRAS, NRAS and HRAS, while RAF consists of BRAF, RAF1 (c-Raf), and ARAF (Jafari et al., 2022).

Members of the RAS family are frequently found in mutated, oncogenic forms in human tumors. Because it causes reduced intrinsic GTPase activity and insensitivity to GTPase-activating proteins, the mutated RAS protein is constitutively active, resulting in a constitutively active GTP-bound state and activation of pro-proliferative and tumorigenesis signaling pathways (Irahara et al., 2010; Schirripa et al., 2015). In total, activating mutations in the RAS gene occur in about 20% of all human cancers, mainly at codons 12, 13, or 61. Mutations in KRAS account for about 85% of all RAS mutations in human tumors, NRAS for about 15%, and HRAS for less than

*[1]Department of Surgery, Faculty of Medicine, Universitas Padjadjaran / Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. [2]Department of Basic Medical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. [3]Department of Pathologic Anatomy, Faculty of Medicine, Universitas Padjadjaran / Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. *For Correspondence: rudiman@unpad.ac.id*

1% (Irahara et al., 2010).

The NRAS proto-oncogene (Neuroblastoma Rat Sarcoma Viral Oncogene Homolog) (locus 1p13.2) is a member of the RAS gene family that encodes proteins involved in signal transmission in cells and participates in the regulation of cell growth. NRAS gene mutations have also been associated with KKR tumors (Jafari et al., 2022; Prior et al., 2020). NRAS is commonly mutated in melanoma and hematopoietic cancers via mapping to chromosome 1. In the tumorigenesis pathway, NRAS mediates both MAPK and PI3K/AKT/MYC signaling. NRAS-induced classical MAPK signaling leads to cyclin D1 expression, cell cycle dysregulation, and promotion of prosurvival pathways. In addition, NRAS effectively prevents Glycogen Synthase Kinase-3 (GSK3)-mediated MYC phosphorylation via PI3K/AKT (phosphoinositide 3-kinase), resulting in increased activity of endogenous MYC protein. NRAS mutations cause RAS GTP to be continuously activated, resulting in malignant proliferation and metastasis (Wang et al., 2014).

Research states that mutations in NRAS, BRAF, and KRAS are mutually exclusive (Meriggi et al., 2014; De Roock et al., 2010). One explanation for the phenotypic differences between KRAS and NRAS is that high expression of KRAS mutants can promote proliferation, while low expression of mutant NRAS can suppress apoptosis (Schirripa et al., 2015; Meriggi et al., 2014; De Roock et al., 2010). NRAS mutations may impair response to anti epidermal growth factor receptor (EGFR) or MoAbs (monoclonal antibodies), and significant differences in median OS (overall survival) were observed in NRAS-WT (wild-type) tumors compared with NRAS mutations (Schirripa et al., 2015). Sullivan et al. (2011) reported that NRAS mutations were associated with a lack of response to cetuximab. In addition, because of the high percentage of resistance to therapy in the same CRC patients, an additional predictive marker for cetuximab-targeted therapy was considered, namely human epidermal growth factor receptor 2 (HER2).

Human epidermal growth factor receptor 2 is a monomeric receptor present on the cell surface. After the ligand binds to its extracellular domain, the HER protein undergoes dimerization and transphosphorylation from its intracellular domain. HER2 lacks a direct activating ligand and may be in a constitutively activated state or become activated after heterodimerization with other HERs, such as HER1 and HER3. Homo- or heterodimerization results in autophosphorylation of tyrosine residues in the cytoplasmic domain of their receptors and induces various signaling pathways, especially MAPK, PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase), and PKC (protein kinase C), triggering cell proliferation, differentiation, angiogenesis, and invasion (Iqbal et al., 2014). Overexpression or amplification of HER2 is an established therapeutic target in breast and gastric cancer. The role of HER2 in CRC is less clear (Muzny et al., 2012).

Several studies have shown that HER2 gene amplification is significantly associated with resistance to cetuximab or panitumumab and is associated with significantly worse progression-free survival (PFS) and overall survival (OS) trends (Bertotti et al., 2011). In CRC, HER2 overexpression and amplification have also been used as potential therapeutic targets. Although several studies have reported the incidence of HER2 overexpression or amplification in CRC, it varies widely, ranging from 0% to 83% (Muzny et al., 2012; Lee et al., 2014). This study aims to assess the relationship between the NRAS mutation and HER2 expression in colorectal cancer patients.

Materials and Methods

Study design and setting

This research is a cross-sectional study. The research subjects in this study were colorectal cancer patients in the Digestive Surgery division at Dr. Hassan Sadikin Hospital. There were 58 study subjects. Examination of NRAS mutations was carried out by polymerase chain reaction (PCR) from fresh tumor tissue obtained from surgery or colonoscopy. Meanwhile, the HER2 examination used the immunohistochemistry (IHC) method of paraffin blocks for anatomical pathology examination. The Dr. Hasan Sadikin Hospital ethics committee approved the study with a waiver of informed consent.

Tumour Tissue Collection Blood sampling and PCR Analysis

Tissue samples were collected from a colonoscopy biopsy or surgical resection. Part of the tissue was immediately sent to the Pathology Anatomy Laboratory for Hematoxylin-Eosin (HE) staining, and the remaining tissue was stored in DNA/RNA shield solution (Zymo Research, CA, USA) for further genomic DNA isolation. A maximum of 10 milligrams of fresh colon tissues were dissected into single cells by vortexing them for 40 seconds using the ZR BashingBead Lysis Tube (Zymo Research, CA, USA). The cell suspension was centrifuged at 14000 rpm for 30 seconds. Cell pellets were used for genomic DNA isolation using Quick-DNATM Miniprep (Zymo Research, CA, USA) according to the manufacturer's protocol. The quality of DNA was measured using a NanoDropTM 2000 spectrophotometer (Thermofisher). A polymerase chain reaction of all exons of NRAS was performed. All exons were amplified using the touch-down PCR method with annealing temperatures ranging from 65 ^{o}C to 55 ^{o}C. Sequencing was performed forward, and identified mutations were validated in reverse. The DNA amplification step in pre-sequencing was performed using the Big Dye Terminator V3.3 kit (Applied Biosystem, Foster City, CA, USA) on an ABI 3500 automated sequencer. In Silico, an analysis of identified mutations was performed to predict the functional impact of the mutations. The analysis was performed using three online software programs: Mutation Taster 2021, PolyPhen-2, and SIFT.

HER2 Protein Expression using IHC Analysis

Tissue samples were examined histologically by HE staining for the diagnosis of CRC. The subject's block paraffin was immunohistochemically stained using the monoclonal antibody for HER2 (Brand Cell Marque,

catalogue 237R-24). Two experienced pathologists (B.S.H. and H.Y.) scored independently, following the consensus recommendations for HER2 scoring for CRC, on a 4-point scale (0, 1+, 2+, 3+). As far as HER2 localization is concerned, 2+ and 3+ showed predominantly membrane localization, while 1+ and 0+ showed more staining in the cytoplasm of the tumor cells. Our study focused on the assessment of membranous HER2 expression. +3 HER2 expression was identified as highly positive or over-expression.

Statistical analysis.

The statistical analysis of the variable was performed using the SPSS 26 software (SPSS Inc., Chicago, IL, USA). The comparison of the variables was based on the chi2 test.

Table 1. Characteristics of Research Subjects

Variable		Proportion (%)
Age	Mean	56.83 ± 1.41 year
	Median	56.5 year
Age	< 50	15 (25.9%)
	> 50	43 (74.1%)
Sex	Male	21 (36.2%)
	Female	37 (63.8%)
Histologic	Adenocarcinoma	51 (87.9%)
	Mucinous Adenocarcinoma	5 (8.6%)
	Signet Ring Cell	1 (1.7%)
	Neuroendocrine	1 (1.7%)
Grade	Well Diff	37 (63.8%)
	Moderately Diff	7 (12.1%)
	Poorly Diff	7 (12.1%)
	Specific	7 (12.1%)
Tumor location	Colon	21 (36.2%)
	Rectum	37 (63.8%)
Stage	I	1 (1.7%)
	II	12 (20.7%)
	III	17 (29.3%)
	IV	28 (48.3%)
Metastases	Liver	16 (27.6%)
	Lung	3 (5.2%)
	Bone	1 (1.7%)
	Omentum	1 (1.7%)
	Perforation	1 (1.7%)
	Uterine	1 (1.7%)
	Liver and bone	2 (3.4%)
	Liver and lung	1 (1.7%)
NRAS	Mutation	8 (13.8%)
	Wild type	50 (86.2%)
HER2	0	23 (39.7%)
	+	12 (20.7%)
	++	19 (29.3%)
	+++	6 (10.3%)
HER2	Normal expression	52 (89.7%)
	Over Expression (+++)	6 (10.3%)

Results

Subject characteristics

A total of 58 patients were included in the study. There were 37 female and 21 male patients. The mean age was 56.5 years ± 1.41, and 74.1% of patients were older than 50. Most of the patients showed adenocarcinoma histologically (87.9%), and 63.8% showed well differentiation of the tumor. There were 37 (63.8%) rectal cancer, and 21 (36.2%) colon cancer patients (Table 1). NRAS mutation was shown in 8 (13.8%) patients, and over-expression of HER2 was shown in 6 (10.3%) patients (Table 2).

Univariate analysis of variables and HER2 expression

One variable affecting HER2 expression was statistically significant ($p < 0.05$) in univariate analysis, as shown in Table 3. In stage II, there were 5 (8.6%) subjects with HER2 overexpression, with $p = 0.001$.

Discussion

NRAS mutations in this study showed eight subjects

Table 2. Univariate Analysis for Subject Characteristics with NRAS Mutations

	Mutation	Wild Type	Chi-square (p.value)
Age			0.952
Early onset (<50)	2 (3.4%)	13 (22.4%)	
Late onset (>50)	6 (10.3%)	37 (63.8%)	
Sex			0.935
Male	3 (5.2%)	18 (31.0%)	
Female	5 (8.6%)	32 (55.2%)	
Histologic			0.923
Adenocarcinoma	7 (12.1%)	44 (75.9%)	
Mucinous Adenocarcinoma	1 (1.7%)	4 (6.9%)	
Signet Ring Cell	0	1 (1.7%)	
Neuroendocrine	0	1 (1.7%)	
Grade			0.113
Well Diff	3 (5.2%)	34 (58.6%)	
Moderately Diff	1 (1.7%)	6 (10.3%)	
Poorly Diff	3 (5.2%)	4 (6.9%)	
Specific	1 (1.7%)	6 (10.3%)	
Tumor location			0.096
Colon	5 (8.6%)	16 (27.6%)	
Rectum	3 (5.2%)	34 (58.6%)	
Stage			0.435
I	0	1 (1.7%)	
II	1 (1.7%)	11 (19.0%)	
III	1 (1.7%)	16 (27.6%)	
IV	6 (10.3%)	22 (37.9%)	
HER2			0.829
Normal expression	7 (12.1%)	45 (77.6%)	
Over Expression (+++)	1 (1.7%)	5 (8.6%)	

Table 3 Univariate Analysis for Subject Characteristics with HER2 Expression

	Over Expression	Normal	Chi-square (p.value)
Age			0.659
Early onset (<50)	2 (3.4%)	13 (22.4%)	
Late onset (>50)	4 (6.9%)	39 (67.2%)	
Sex			0.293
Male	1 (1.7%)	20 (34.5%)	
Female	5 (8.6%)	32 (55.2%)	
Histologic			0.862
Adenocarcinoma	5 (8.6%)	46 (79.3%)	
Mucinous Adenocarcinoma	1 (1.7%)	4 (6.9%)	
Signet Ring Cell	0	1 (1.7%)	
Neuroendocrine	0	1 (1.7%)	
Grade			0.789
Well Diff	4 (6.9%)	33 (56.9%)	
Moderately Diff	0	7 (12.1%)	
Poorly Diff	1 (1.7%)	6 (10.3%	
Specific	1 (1.7%)	6 (10.3%	
Tumor location			0.291
Colon	1 (1.7%)	20 (34.5%)	
Rectum	5 (8.6%)	32 (55.2%)	
Stage			0.001
I	0	1 (1.7%)	
II	5 (8.6%)	7 (12.1%)	
III	0	17 (29.3%)	
IV	1 (1.7%)	27 (46.6%)	
NRAS			0.829
Wild type	5 (8.6%)	45 (77.6%)	
Mutation	1 (1.7%)	7 (12.1%)	

(13.8%) with positive mutation results. In this study, the proportion of NRAS mutations was higher than in several other studies. A study by Irahara et al., (2010) found NRAS mutations in 2.2% of the 225 CRC patients. A study by Schiripa et al., (2015) found NRAS mutations in 6% of the 785 included CRC patients. A study by Levi et al., (2016) showed NRAS mutations in 6 (5%) of 121 patients with CRC. Ibarra et al., (2020) reported that of 500 CRC patients, only 20 (4%) showed NRAS mutations.

In this study, more NRAS mutations appeared in the colon than the rectum (5 vs. 3), and more NRAS mutations were found in metastatic CRC (mCRC) than early stage CRC (6 vs. 2), but statistically, this difference was not significant. Similar to the studies of Irahara et al., (2011) and Levi et al,.(2016), univariate analysis of NRAS mutations did not show a significant association between NRAS mutations and age, sex, type of anatomic pathology, grade, stage, or location of the tumor. A study by Ibarra et al., (2020) showed that more NRAS mutations appeared in the rectum than the colon (3 vs. 2) and showed better tumor differentiation, but statistically the difference was not significant.

HER2 expression in this study showed that there were six subjects (10.3%) with overexpression results. In this study, the proportion of overexpression of HER2 appeared to be higher than in several other studies. In the study of Seo et al., (2014), there were two HER2 assessment cohorts, the first cohort involved 365 patients with CRC, and HER2 overexpression was found in 8 subjects (2.2%), while the second cohort involved 174 patients with stage IV CRC, and HER2 overexpression was present in 5 subjects. (2.9%). Valtorta et al., (2015) conducted a study on 304 patients with CRC. 14 subjects (4.6%) showed HER2 overexpression. In the study by Ross et al., (2018), 148 subjects (1.6%) of 8,887 patients with CRC showed excess HER2 expression. Razzaq et al., (2021) assessed HER2 expression in patients with CRC, out of 17 patients with CRC, there were four subjects (23.52%) with excess HER2 expression. Similar to them, HER2 expression did not show a significant relationship with age, sex, or type of anatomic pathology. In this study, a significant association was only seen at the tumor stage, most of the HER2 overexpression was found at stage II ($p = 0.001$). Meanwhile, in Seo et al., (2014), HER2 overexpression was associated with tumor location and was more frequently found in the rectum than in the colon ($p = 0.013$ in cohort 1, $p = 0.009$ in cohort 2). In study by Razzaq et al., (2021), HER2 overexpression was associated with tumor grade ($p = 0.03$).

A univariate analysis of NRAS mutations with HER2 overexpression showed $p = 0.829$. This indicated that there was no significant relationship between NRAS mutations and HER2 overexpression. A cross-tabulation of HER2 expression and NRAS mutations showed that out of 8 subjects showing NRAS mutations, only one subject had excess HER2 expression. In a similar study conducted by Ross et al., (2018), out of 8,887 patients with CRC, there were 4.3% of subjects with NRAS mutations and 1.6% of subjects with HER2 overexpression. The cross-tabulation of HER2 expression and NRAS mutations showed that 3.3% of NRAS mutation patients showed over-expression of HER2. In this study, the number of patients with mCRC was 28 (48.7%), and six of them (21.4%) had NRAS mutations, and 22 (78.6%) had wild-type NRAS. In contrast, only one (3.6%) of the 28 mCRC patients had HER2 overexpression. In the study of Valentini et al., (2018), out of 29 mCRC patients, two (7%) were found with NRAS mutations, and only one (3.4%) had HER2 overexpression. Our study, similar to those of Ross et al., (2018) and Valentini et al., (2018), showed no significant relationship between NRAS mutations and HER2 overexpression in colorectal cancer patients.

NRAS mutations and HER2 are not the only deterministic carcinogenic factors for CRC, other carcinogenic and clinicopathological factors also contribute, such as patient sex, age, molecular subtype, and tumor stage. This study showed that NRAS mutations and HER2 overexpression were not related to clinicopathological factors, this may be related to the small number of research samples in this study. However, it is important to examine NRAS and HER2 mutations as a consideration for continuing therapy with EGFR inhibitors and anti-HER2 in patients with CRC.

In conclusion , there is no association between NRAS mutations and HER2 overexpression in colorectal cancer patients.

Author Contribution Statement

Ade Tan Reza and Prapanca Nugraha participated in collecting the patient's information. Yunia Sribudiani participated in analyzing the NRAS mutation. Birgitta M. Dewayani and Etis Primaswati participated in the histologic examination of the colorectal diagnosis and the IHC examination of HER2. Kiki Lukman, Lisa Y Hasibuan, and Reno Rudiman analyzed the data, drafted the manuscript, and finalized the manuscript.

Acknowledgements

We thank all the patients who agreed to participate in this study and the trainees and surgical residents who helped carry out this study.

Funding Statement

The preparation of this study was supported by internal funding from Riset Kompetensi Dosen Unpad (RKDU) Faculty of Medicine, Universitas Padjadjaran with register number 2203/UN.6.3.1/PT.00/2022.

Approval

This study was approved as Ade Tan Reza's thesis

Ethical Declaration

The Dr. Hasan Sadikin Hospital ethics committee approved the study with No. Ethical Approval LB.02.01/X.6.5/327/2022.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors have no conflicts of interest to declare.

References

Bray F, Ferlay J, Soerjomataram I, et al (2018). GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, **68**, 394-424.

Bertotti A, Migliardi G, Galimi F, et al (2011). A molecularly annotated platform of patient- derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. *Cancer Discov*, **1**, 508–23.

De Roock W, Claes B, Bernasconi D, et al (2010). Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: A retrospective consortium analysis. *Lancet Oncol*, **11**, 753–62.

Erida Y, Aminah H, Yulianti H, Hernowo BS (2015). Vitamin D Receptor (VDR) and Phosphatidylinositol 3-Kinase (PI3K) Independently Affected Colorectal Adenocarcinoma Differentiation. *Indones J Clin Pharm*, **4**, 264–74.

Gong S, Xu D, Zhu J, et al (2018). Efficacy of the MEK Inhibitor Cobimetinib and its Potential Application to Colorectal Cancer Cells. *Cell Physiol Biochem*, **47**, 680–93.

Jafari M, Laraqui A, Baba W, et al (2022). Prevalence and patterns of mutations in RAS/RAF/MEK/ERK/MAPK signaling pathway in colorectal cancer in North Africa. *BMC Cancer*, **22**, 1-4.

Ibarra HE, Jiang X, Gallegos-Gonzalez EY, et al (2020). KRAS, NRAS, and BRAF mutation prevalence, clinicopathological association, and their application in a predictive model in Mexican patients with metastatic colorectal cancer: A retrospective cohort study. *PLoS One*, **15**.

Irahara N, Baba Y, Nosho K, et al (2010). NRAS mutations are rare in colorectal cancer. Diagnostic Mol Pathol.19(3):157–63.

Iqbal N (2014). Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. *Mol Biol Int*, **2014**, 1–9.

Lee WS, Park YH., Lee JN, et al (2014). Comparison of HER2 expression between primary colorectal cancer and their corresponding metastases. *Cancer Med*, **3**, 674–80.

Levi M, Prayogi G, Sastranagara F, et al (2018). Clinicopathological Associations of K-RAS and N-RAS Mutations in Indonesian Colorectal Cancer Cohort. *J Gastrointest Cancer*, **49**, 124–31.

Meriggi F, Vermi W, Bertocchi P, et al (2014). The Emerging Role of NRAS Mutations in Colorectal Cancer Patients Selected for Anti-EGFR Therapies. *Rev Recent Clin Trials*, **9**, 8–12.

Muzny DM, Bainbridge MN, Chang K, et al (2012). Comprehensive molecular characterization of human colon and rectal cancer. *Nature*, **487**, 330–7.

Porru M, Pompili L, Caruso C, et al (2018). Targeting kras in metastatic colorectal cancer: Current strategies and emerging opportunities. *J Exp Clin Cancer Res*, **37**, 1–10.

Prior IA, Hood FE, Hartley JL (2020). The frequency of ras mutations in cancer. *Cancer Res*, **80**, 2669–974.

Razzaq EA, Venkatachalam T, Bajbouj K, et al (2021). HER2 overexpression is a putative diagnostic and prognostic biomarker for late-stage colorectal cancer in North African patients. *Libyan J Med*, **16**.

Ross JS, Fakih M, Ali SM, et al (2018). Targeting HER2 in colorectal cancer: The landscape of amplification and short variant mutations in ERBB2 and ERBB3. *Cancer*, **124**, 1358–73.

Schirripa M, Cremolini C, Loupakis F, et al (2015). Role of NRAS mutations as prognostic and predictive markers in metastatic colorectal cancer. *Int J Cancer*, **136**, 83–90.

Seo AN, Kwak Y, Kim DW, et al (2014). HER2 status in colorectal cancer: Its clinical significance and the relationship between HER2 gene amplification and expression. *PLoS One*, **9**.

Sullivan KM, Kozuch PS (2011). Impact of KRAS Mutations on Management of Colorectal Carcinoma. *Patholog Res Int*, **2011**, 1–11

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**, 539–44.

Valtorta E, Martino C, Sartore-Bianchi A, et al (2015). Assessment of a HER2 scoring system for colorectal cancer: Results from a validation study. *Mod Pathol*, **28**, 1481–91.

Wang Y, Velho S, Vakiani E, et al (2013). Mutant N-RAS Protects Colorectal Cancer Cells from Stress-Induced Apoptosis and Contributes to Cancer Development and ProgressionN-Ras in Colorectal Cancer. *Cancer Discovery*, **3**, 294-307.

Xi Y, Xu P (2021). Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol*, **14**, 101174.