RESEARCH ARTICLE

Editorial Process: Submission:01/03/2024 Acceptance:07/13/2024

The effects of *KRAS* Mutations on the Prognosis of Rectal Cancer Following Neoadjuvant Chemoradiotherapy and Surgery

Ji Sun Park[1], Soon Seong Lee[1], Yoon Sun Choi[2]*

Abstract

Background: Rectal cancers with mutations in the *KRAS* gene have worse prognoses than wild-type malignancies. Variants at codon 12 of *KRAS* have particularly detrimental effects on prognosis. We aimed to analyze whether *KRAS* mutations act as adverse prognostic factors following neoadjuvant concurrent chemoradiotherapy (CRT) and surgery for rectal cancer treatment. **Methods:** We analyzed the effects of *KRAS* mutations on disease-free survival (DFS) and locoregional recurrence-free survival (LRFS) in 125 patients with cT2-4N0-2M0 rectal cancer who underwent surgery following CRT between June 2014 and March 2023 Inje University Busan Paik Hospital. **Results:** The median follow-up period was 39.7 (range, 7.5–98.2) months. There were 25 patients (20.0%) who harbored *KRAS* mutations. Among them, 22 patients (17.6%) had codon 12 variants. Overall, 43 patients (34.4%) showed recurrence, of which 10 (8.0%) had locoregional recurrence and 35 (28.0%) had distant metastases (two occurred simultaneously). DFS was significantly reduced in the patients with *KRAS* mutations ($p = 0.005$). LRFS was also reduced in patients with *KRAS* mutations ($p = 0.039$). DFS and LRFS were also relatively low in the subgroup with *KRAS* mutations at codon 12 ($n = 22$) ($p = 0.003$ and $p = 0.017$, respectively). However, pathologic complete response rate following CRT was not affected by *KRAS* mutations ($p = 0.197$). Overall survival was also not associated with *KRAS* mutations ($p = 0.486$).**Conclusion:** *KRAS* mutation is related to decreased DFS and LRFS, when surgery is performed following neoadjuvant CRT to treat rectal cancer. These effects are particularly pronounced for *KRAS* mutations at codon 12.

Keywords: Rectal cancer- *KRAS* mutation- chemoradiotherapy -clinical oncology

Asian Pac J Cancer Prev, **25 (7)**, 2337-2342

Introduction

Mutations in the *KRAS* gene are common in colorectal cancer (CRC). *KRAS* gene mutations occur in 17–25% of all cancers [1]. In patients with CRC who have *KRAS* gene mutations, the *KRAS* protein continuously stimulates downstream signaling to induce cell proliferation and survival, thereby resulting in a high risk of tumor formation and subsequent deterioration. *KRAS* mutations are associated with relatively fast relapse [2] and poor prognosis [3, 4]. A meta-analysis reported that *KRAS* mutations were not related to the efficacy of neoadjuvant chemoradiotherapy (CRT) [5]. However, according to another recent meta-analysis, *KRAS* mutations are associated with poor disease-free survival (DFS) in CRC [6].

Hotspots for *KRAS* mutations include codons 12, 13, and 61 [7]. Among these, mutations at codon 12 account for more than half of the known *KRAS* mutation subtypes [7]. In particular, in a Japanese multicenter study, patients with metastatic CRC who harbored *KRAS* mutations in codon 12 were found to have poorer treatment outcomes than those without the mutations [8]. Patients with *KRAS* mutations at G12C had the worst progression-free and overall survival (OS) rates [8].

Treatment methods for rectal cancer vary depending on the location, size, and degree of tumor spread. Surgery, radiation therapy, and chemotherapy can all be used, depending on when the tumor is discovered. Early-stage CRC can be treated using endoscopic resection alone. Radiotherapy is administered for locally advanced CRC, most often in combination with chemotherapy prior to surgery. Combined CRT enhances treatment effectiveness and reduces the recurrence rate. Radical resection with pelvic lymph node dissection following neoadjuvant CRT is effective for lowering the recurrence rate in the pelvis and therefore, is currently the standard treatment for locally advanced CRC.

This study aimed to analyze the impact of *KRAS* mutations on DFS in patients with CRC who underwent

*[1]Department of Nuclear Medicine, Busan Paik Hospital, Inje University College of Medicine, Busan, South Korea. [2]Department of Radiation Oncology, Busan Paik Hospital, Inje University College of Medicine, Busan, South Korea. *For Correspondence: rtyoon@gmail.com*

neoadjuvant chemotherapy and radiation therapy followed by curative surgery.

Materials and Methods

This single-center retrospective study included 125 patients with locally advanced CRC who were treated with neoadjuvant CRT and surgical resection between June 2014 and March 2023 at Inje University Busan Paik Hospital. Radiotherapy was administered on a 45.0–50.4/1.8 Gy schedule. The chemotherapeutic regimen consisted of 5-fluorouracil or Capecitabine. This retrospective study was approved by the institutional review board of Inje University Busan Paik Hospital (No. 2023-09-016).

The primary endpoint was DFS. Secondary outcomes included locoregional recurrence-free survival (LRFS) and pathological complete response rate (pCR). All patients enrolled in this study had CRC, were > 18 years of age, and had undergone neoadjuvant CRT and surgical resection at our hospital. The exclusion criteria were: 1) patients with prior medical histories of other cancers, and 2) patients who were lost to follow-up loss within 3 months following their surgeries.

Deoxyribo nucleic acid (DNA) was obtained from paraffin embedded tumor tissues. *KRAS* codons were amplified by polymerase chain reaction and analyzed.

DFS, LRFS, and OS were measured from the date of diagnosis until death or final follow-up within the study period. SPSS version 25.0 (SPSS Inc., Chicago, IL, USA) and MedCalc (MedCalc Software, Mariakerke, Belgium) were used for all statistical analyses. The chi-squared test was used to examine differences in variables between patients with *KRAS* mutations and those with the wild-type gene. Survival rates were calculated using the Kaplan–Meier method, and prognostic factors were evaluated using univariate analysis with the log-rank test. Independent prognostic factors affecting survival were evaluated using multivariate analysis with the Cox proportional hazards model.

Results

The median follow-up period was 39.7 (range, 7.5–98.2) months

Table 1 shows the basic patient characteristics. The median patient age was 67 years. Men accounted for 67.2% (n = 84), and 24 of the patients (19.2%) had diabetes. Adenocarcinoma was the predominant cancer type (97.6% of the patients), with only three of the patients having mucinous cancer (2.4%). According to clinical stage, cT3 (n = 84, 64.0%) accounted for the largest portion of the cancers in terms of clinical T stage. There were 36 patients (28.8%) with T4 malignancies. Most of the patients (n = 104; cN1 and cN2) had pelvic lymph node metastases when they were diagnosed, but 21 (16.8 %) did not have lymph node metastases. Using a molecular biological analysis, we detected *KRAS* mutations in 25 of the patients (20.0%). Among them, 22 (17.6%) had *KRAS* mutations in codon 12. The most common degree of tumor differentiation was moderate, observed in 85 patients (68.0%). There were 39 patients (31.2%) with well-differentiated tumors, and only one (0.8%) with a poorly differentiated tumor. The most common surgical treatment method used was lower anterior resection, which was performed in 106 patients (84.8%). The remaining 19 underwent abdominoperineal resection. Based on pathology results, 11 of the patients had T0 malignancies, and 85 had N0. Of these, pCR was achieved in eight (6.4%). Neuronal invasion was found in 14 patients (11.2%) and lymphovascular invasion was found in 12 (9.6%). Three of the patients (2.4%) had positive resection margins following their surgeries. Overall, 43 patients (34.4%) showed recurrence during the follow-up period. Among them, 10 (8.0%) had locoregional recurrence, and 35 (28.0%) showed distant metastases. (two occurred simultaneously).

Table 2 presents a comparison between patients with *KRAS* mutations and those with the wild-type gene. There were no significant differences in terms of clinical disease stages between the two groups. There was no correlation between differentiation (for moderate or poor differentiation) and the presence of *KRAS*

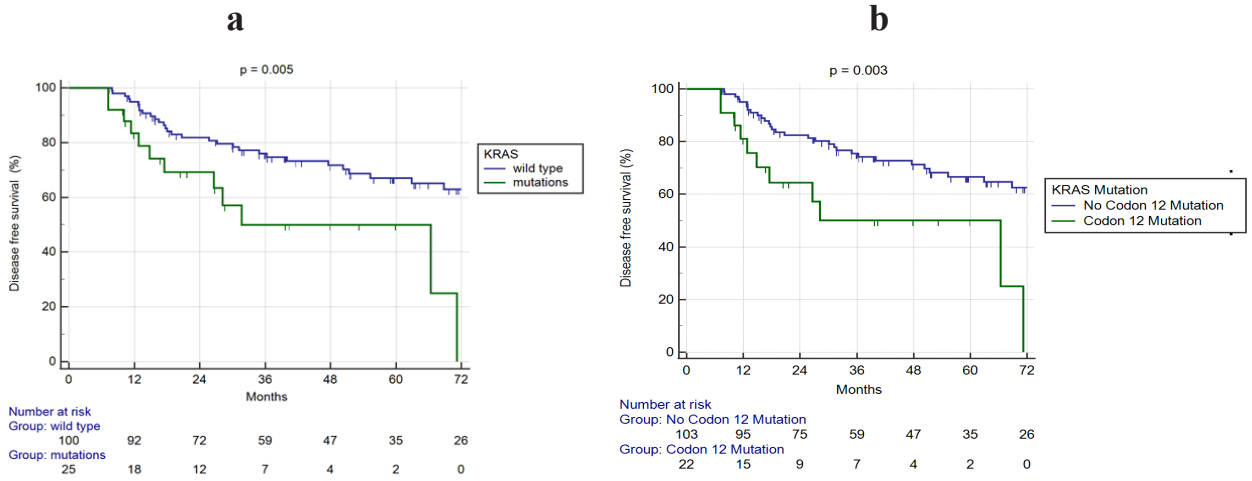

Figure 1. a. Disease free survival (DFS) according to the KRAS mutation status; b. DFS according to the presence of the KRAS mutation in codon 12

Table 1. Patient Characteristics (n = 125)

	No.	(%)
Age		
median (range)	65	(29-83)
≤65	72	(57.6)
>65	53	(42.4)
Sex		
Male	84	(67.2)
Female	41	(32.8)
Diabetes		
Yes	24	(19.2)
No	101	(80.8)
Pathology		
Adenoca	122	(97.6)
Mucinous	3	(2.4)
cT stage		
cT2	9	(7.2)
cT3	80	(64.0)
cT4	36	(28.8)
cN stage		
cN0	21	(16.8)
cN1	50	(40.0)
cN2	54	(43.2)
KRAS mutation		
codon 12	22	(17.6)
codon 13	2	(1.6)
codon 146	1	(0.8)
wild type	100	(80.0)
Differentiation		
WD	39	(31.2)
MD	85	(68.0)
PD	1	(0.8)
Surgery		
LAR		(84.8)
APR		(15.2)
pT stage		
pT0		(8.8)
pT1		(6.4)
pT2		(20.0)
pT3		(60.0)
pT4		(4.8)
pN stage		
pN0		(68.0)
pN1		(24.0)
pN2		(8.0)
Neuronal invasion		
Positive		(11.20)
Negative		(88.80)
Lymphovascular invasion		
Positive		(9.6)
Negative		(90.4)
Resection margin		
Positive		(2.4)
Negative		(97.6)

Table 2. Patient Distribution According to the *KRAS* Mutation Status

	KRAS mutation		wild type	
	No.	(%)	No.	(%)
Clinical T4	9	(36.0)	27	(27.0)
Clinical N1-2	10	(40.0)	30	(30.0)
Moderate-poor differentiation	21	(84.0)	65	(65.0)
Perineural invasion	3	(12.0)	11	(11.0)
Lymphovascular invasion	2	(8.0)	10	(10.0)
Pathologic complete response	3	(12.0)	5	(5.0)
Locoregional failure	4	(16.0)	6	(6.0)
Distant failure	9	(36.0)	26	(26.0)
Total	25	(100.0)	100	(100.0)

mutations. Lymphovascular and perineural invasion were not correlated with *KRAS* mutations. No statistical difference between the two groups could be confirmed in terms of the response rate following neoadjuvant treatment and treatment failure after surgical resection. The pCR rate was also unaffected by *KRAS* mutations (p = 0.197). Additionally, *KRAS* mutation was not related to progression during neoadjuvant treatment (n = 11, p = 0.875). Downstaging after neoadjuvant treatment (n = 94, p = 0.680) was also not related to *KRAS* mutation.

The 3-year DFS rate was 71.5%, and the 3-year OS rate was 98.0%. The 3-year LRFS rate was estimated to

Table 3. Univariate and Multivariate Analyses for Disease Free Survival.

	3yr DFS	p-value	HR	(95% CI)	p-value
Diabetes		0.123			
Yes	59.6				
No	74.5				
Clinical T stage		0.08			
T2-3	76				
T4	57.9				
Clinical N stage		0.036	1.486	(0.769-2.873)	0.239
N0	74.3				
N1-2	65.7				
Differentiation		0.557			
WD	73.1				
MD/PD	70.7				
KRAS mutation		0.003	2.852	(1.389-5.853	0.004
Positive	50.1				
Negative	75.5				
Neuronal invasion		0.791			
Positive	77.9				
Negative	70.8				
Lymphovascular invasion		0.028	5.339	(1.213-23.506)	0.027
Positive	58.3				
Negative	73.2				
Resection margin		0.018	2.32	(0.978-5.503)	0.056
Positive	72.7				
Negative	0				

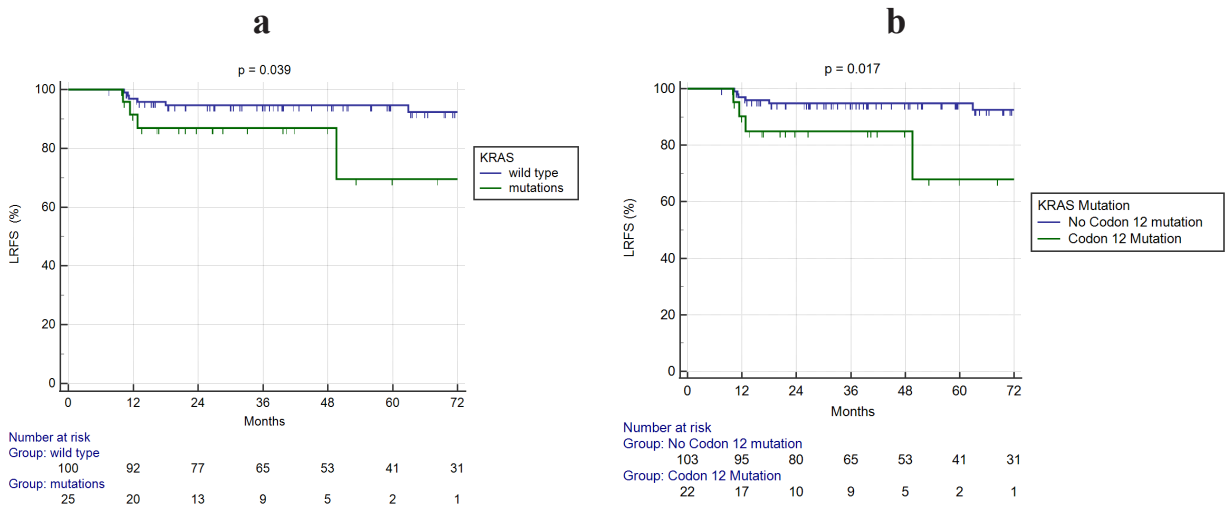

Figure 2. a. Locoregional recurrence-free survival (LRFS) according to the *KRAS* mutation status; b. LRFS according to the presence of the *KRAS* mutation in codon 12

be 93.1%. Table 3 summarizes the prognostic factors for DFS. Our univariate analysis of DFS showed that clinical N stage (p = 0.036), the presence of mutated *KRAS* (p = 0.003), lymphovascular invasion (p = 0.028), and resection margin status (p = 0.018) all had statistically significant effects on DFS. Clinical T stage also tended to be associated with DFS (p = 0.080). A multivariate analysis confirmed that mutated *KRAS* (p = 0.004) and lymphovascular invasion (p = 0.027) were independent prognostic factors for DFS.

Figure 1A shows that DFS was reduced in patients with *KRAS* mutations (p = 0.005). Figure 1B shows that DFS was also significantly reduced in the subgroup of patients with the mutation at codon 12 (p = 0.003). However, OS was not associated with mutated *KRAS* (p = 0.486). Figure 2A shows that LRFS was significantly reduced in patients with *KRAS* mutations (p = 0.039). It was also significantly reduced in patients with mutation at codon 12 (p = 0.017, Figure 2B).

Discussion

This study showed that *KRAS* mutations are associated with poor DFS in patients with CRC. Additionally, the presence of *KRAS* mutations was found to represent a poor prognostic factor for LRFS. This study also confirmed that the presence of *KRAS* mutations had a negative impact on prognosis in CRC, even after neoadjuvant CRT and surgery had been used to treat it.

We also found certain aspects that differed from the characteristics of *KRAS* mutations that have been reported in previous studies. We found no correlation between pCR following neoadjuvant CRT and the presence of *KRAS* mutations, which contradicts the findings of Chow et al. [9]. This implies that *KRAS* mutation is not closely related to the direct response of the disease to chemoradiation, which is similar to what was reported by Zhou et al. [10]. In contrast to what was reported in a study by Shin et al. [11], we did not find an association between tumor N stage and mutated *KRAS*. In contrast to the findings of Siderlis' study [12], distant recurrence rate did not differ based on *KRAS* mutation status.

In this study, *KRAS* mutations were located in codon 12 for most of the patients. According to a previous study [8], *KRAS* mutations at codon 12 are associated with poor prognoses. In this study, the *KRAS* mutation frequency was lower (20.0%) than that reported previously in other studies. However, most of the *KRAS* mutations in our patient cohort were in codon 12. There seemed to be a higher frequency of *KRAS* mutations at codon 12 in our patients than that reported previously [13]. Therefore, it is necessary to analyze whether the occurrence of *KRAS* mutations differs depending on ethnicity and region.

Patients with CRC who have *KRAS* mutations require comparatively precise and personalized treatment. In the past, the *KRAS* mutation was considered 'undruggable' [14]. However, *KRAS* inhibitors have since been tested both alone and as components of combination therapies [14-16]. Agents targeting G12C have also been developed and are currently undergoing clinical trials [17, 18, 7]. Sotorasib and Adagrasib have been used to treat refractory CRC in clinical trial settings [19, 20]. The effectiveness of *KRAS* G12C inhibitors is lower in CRC than in lung cancer, owing to resistance [19]. However, when Adagrasib, a *KRAS* G12C inhibitor, was used in combination with Cetuximab in a recent trial, promising antitumor effects were observed in patients with metastatic CRC [21]. Moreover, in patients with *KRAS* mutations, dose-escalated radiotherapy can improve poor treatment outcomes. As a result of these new approaches, we believe that the general prognosis of CRC is expected to improve in the near future, even for patients with *KRAS* mutations.

The main strength of this study is that it confirmed that *KRAS* mutations have a negative impact on DFS, even in patients who have received neoadjuvant CRT and surgery for treatment of locally advanced CRC. One positive aspect of this study was that subtype analysis was performed at the codon level, and its impact on prognosis was confirmed.

This study has some limitations. First, it was a retrospective study; hence, it may suffer from a selection bias. Second, the follow-up period was relatively short.

Finally, we did not confirm whether poor OS was related to *KRAS* mutations. We expect differences in OS to become apparent in future studies with relatively long follow-up periods. In summary, *KRAS* mutations reduce DFS and LRFS, even when surgery is performed following neoadjuvant concurrent CRT, in patients with CRC. These detrimental effects are particularly pronounced for *KRAS* mutations in codon 12. It is necessary to analyze whether other types of cancer with *KRAS* mutations have a negative prognostic effect on recurrence after treatment.

Author Contribution Statement

Conceptualization: YSC, SSL. Formal Analysis: YSC, JSP, SSL. Investigation: YSC, JSP, SSL. Methodology: JSP, YSC. Writing – Original Draft: YSC, JSP. Writing – Review & Editing: All authors.

Acknowledgements

Funding statement

The research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HR21C1003).

Patient consent

The study was conducted without informed consent from the patient, since it was a retrospective study (IRB of Inje University Busan Paik Hospital No. 2023-09-016).

Any conflict of interest

The researchers claimed no conflict of interest.

References

1. Arrington AK, Heinrich EL, Lee W, Duldulao M, Patel S, Sanchez J, et al. Prognostic and predictive roles of kras mutation in colorectal cancer. Int J Mol Sci. 2012;13(10):12153-68. https://doi.org/10.3390/ijms131012153.
2. Chatila WK, Kim JK, Walch H, Marco MR, Chen CT, Wu F, et al. Genomic and transcriptomic determinants of response to neoadjuvant therapy in rectal cancer. Nat Med. 2022;28(8):1646-55. https://doi.org/10.1038/s41591-022-01930-z.
3. Dai D, Wang Y, Zhu L, Jin H, Wang X. Prognostic value of kras mutation status in colorectal cancer patients: A population-based competing risk analysis. Peer J. 2020;8:e9149. https://doi.org/10.7717/peerj.9149.
4. Diez-Alonso M, Mendoza-Moreno F, Gomez-Sanz R, Matias-Garcia B, Ovejero-Merino E, Molina R, et al. Prognostic value of kras gene mutation on survival of patients with peritoneal metastases of colorectal adenocarcinoma. Int J Surg Oncol. 2021;2021:3946875. https://doi.org/10.1155/2021/3946875.
5. Clancy C, Burke JP, Coffey JC. Kras mutation does not predict the efficacy of neo-adjuvant chemoradiotherapy in rectal cancer: A systematic review and meta-analysis. Surg Oncol. 2013;22(2):105-11. https://doi.org/10.1016/j.suronc.2013.02.001.
6. Peng J, Lv J, Peng J. Kras mutation is predictive for poor prognosis in rectal cancer patients with neoadjuvant chemoradiotherapy: A systemic review and meta-analysis. Int J Colorectal Dis. 2021;36(8):1781-90. https://doi.org/10.1007/s00384-021-03911-z.
7. Zhu G, Pei L, Xia H, Tang Q, Bi F. Role of oncogenic kras in the prognosis, diagnosis and treatment of colorectal cancer. Mol Cancer. 2021;20(1):143. https://doi.org/10.1186/s12943-021-01441-4.
8. Chida K, Kotani D, Masuishi T, Kawakami T, Kawamoto Y, Kato K, et al. The prognostic impact of kras g12c mutation in patients with metastatic colorectal cancer: A multicenter retrospective observational study. Oncologist. 2021;26(10):845-53. https://doi.org/10.1002/onco.13870.
9. Chow OS, Kuk D, Keskin M, Smith JJ, Camacho N, Pelossof R, et al. Kras and combined kras/tp53 mutations in locally advanced rectal cancer are independently associated with decreased response to neoadjuvant therapy. Ann Surg Oncol. 2016;23(8):2548-55. https://doi.org/10.1245/s10434-016-5205-4.
10. Zhou P, Goffredo P, Ginader T, Thompson D, Hrabe J, Gribovskaja-Rupp I, et al. Impact of kras status on tumor response and survival after neoadjuvant treatment of locally advanced rectal cancer. J Surg Oncol. 2021;123(1):278-85. https://doi.org/10.1002/jso.26244.
11. Shin YR, Kim KA, Im S, Hwang SS, Kim K. Prediction of kras mutation in rectal cancer using mri. Anticancer Res. 2016;36(9):4799-804. https://doi.org/10.21873/anticanres.11039.
12. Sideris M, Moorhead J, Diaz-Cano S, Haji A, Papagrigoriadis S. Kras mutant status may be associated with distant recurrence in early-stage rectal cancer. Anticancer Res. 2017;37(3):1349-57. https://doi.org/10.21873/anticanres.11454.
13. Meng M, Zhong K, Jiang T, Liu Z, Kwan HY, Su T. The current understanding on the impact of kras on colorectal cancer. Biomed Pharmacother. 2021;140:111717. https://doi.org/10.1016/j.biopha.2021.111717.
14. Porru M, Pompili L, Caruso C, Biroccio A, Leonetti C. Targeting kras in metastatic colorectal cancer: Current strategies and emerging opportunities. J Exp Clin Cancer Res. 2018;37(1):57. https://doi.org/10.1186/s13046-018-0719-1.
15. Li ZN, Zhao L, Yu LF, Wei MJ. Braf and kras mutations in metastatic colorectal cancer: Future perspectives for personalized therapy. Gastroenterol Rep (Oxf). 2020;8(3):192-205. https://doi.org/10.1093/gastro/goaa022.
16. van de Haar J, Ma X, Ooft SN, van der Helm PW, Hoes LR, Mainardi S, et al. Codon-specific kras mutations predict survival benefit of trifluridine/tipiracil in metastatic colorectal cancer. Nat Med. 2023;29(3):605-14. https://doi.org/10.1038/s41591-023-02240-8.
17. Cefalì M, Epistolio S, Palmarocchi MC, Frattini M, De Dosso S. Research progress on kras mutations in colorectal cancer. J Cancer Metastatis Treat. 2021. https://doi.org/10.20517/2394-4722.2021.61.
18. Ryan MB, Coker O, Sorokin A, Fella K, Barnes H, Wong E, et al. Kras(g12c)-independent feedback activation of wild-type ras constrains kras(g12c) inhibitor efficacy. Cell Rep. 2022;39(12):110993. https://doi.org/10.1016/j.celrep.2022.110993.
19. Ciardiello D, Maiorano BA, Martinelli E. Targeting kras(g12c) in colorectal cancer: The beginning of a new era. ESMO Open. 2023;8(1):100745. https://doi.org/10.1016/j.esmoop.2022.100745.
20. Ji J, Wang C, Fakih M. Targeting kras (g12c)-mutated advanced colorectal cancer: Research and clinical developments. Onco Targets Ther. 2022;15:747-56. https://

doi.org/10.2147/OTT.S340392.
21. Yaeger R, Weiss J, Pelster MS, Spira AI, Barve M, Ou SI, et al. Adagrasib with or without cetuximab in colorectal cancer with mutated kras g12c. N Engl J Med. 2023;388(1):44-54. https://doi.org/10.1056/NEJMoa2212419.