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# Prediction of *KRAS* Mutation in Rectal Cancer Using MRI

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**Abstract.** *Aim: The purpose of the study was to investigate imaging predictors of Kirsten-ras (KRAS) mutations using magnetic resonance imaging (MRI) in patients with rectal cancer. Patients and Methods: A total of 275 patients with rectal cancer were enrolled. They underwent pretreatment rectal MRI, and then KRAS mutation evaluation following surgery. Two reviewers assessed diverse MRI findings associated with rectal cancer. Results: KRAS mutations were detected in 107 (38.9%). KRAS mutations were associated with N stage, gross tumor pattern, axial length of the tumor, and the ratio of the axial to the longitudinal dimensions of the tumor (p=0.0064, p<0.0001, p=0.0003 and p=0.0090). The frequency of KRAS mutations was higher in N2 stage (53.70%), and polypoid tumors (59.09%). Tumors with KRAS mutations exhibited a longer axial length, as well as a larger ratio of the axial to the longitudinal dimensions. Conclusion: KRAS mutations were associated with N stage, a polypoid pattern, axial tumor length, and the ratio of the axial to the longitudinal dimensions of the tumor.*

Rectal cancer is one of the most common malignancies worldwide, and the fourth most common cause of cancer-related deaths (1). In contrast to incidence trends, decreasing rectal cancer mortality rates have been observed in a large number of countries, that can be attributed to improved treatments. Recent developments in molecular biology and associated technologies have initiated a new era of improved chemotherapy regimens, as well as targeted therapeutic agents for rectal cancer (2).

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Rectal cancer develops through multiple steps, with the sequential accumulation of genetic alterations in tumor suppressors and oncogenes (3). Mutations in the Kirsten-ras (*KRAS*) gene occur in approximately 40% of colorectal cancer (CRC), and a number of studies have shown that *KRAS* mutations in CRC are predictive of the response to therapies that use antibodies to target epidermal growth factor receptor (EGFR) (2). Due to the frequency and therapeutic implications of this mutation, *KRAS* testing has been incorporated into routine clinical practice as part of the treatment for metastatic CRC (4).

Magnetic resonance imaging (MRI) is a particularly promising technique for local staging of rectal cancer (5). Although imaging techniques are critical in preoperative workups for determining treatment strategies, associations between pretreatment imaging and genomic expression patterns in patients have not been investigated in depth. If there are associations between MRI features and *KRAS* mutations, supplementary information could be provided *via* genomic analysis to determine the optimal therapeutic strategies for the treatment of rectal cancer, by predicting the tumor response to various treatment modalities. The purpose of this study was to investigate imaging predictors of *KRAS* mutations using rectal MRI.

## Patients and Methods

This work was approved by the Institutional Review Board of our institution (OC16RISI0022). Informed consent was waived due to the retrospective nature of the study. From January 2012 to October 2015, rectal MRI was performed on 367 patients. In total, 275 patients who underwent operations for rectal cancer with or without neoadjuvant chemoradiation therapy, and who underwent histological evaluation for *KRAS* mutation on surgical specimens, were enrolled.

*Initial rectal MRI protocol*. Rectal MRI was performed using a 3.0-T magnetic resonance system (Magnetom Skyra; Siemens, Erlangen, Germany) equipped with 18-channel body-array coils. The imaging protocol included an oblique axial T2-weighted spin-echo sequence [repetition time (TR), 4,890 ms; echo time (TE), 95 ms; slice

thickness, 3 mm; flip angle, 150˚; echo train length (ETL), 21; matrix, 256×154; field of view (FOV), 180 mm; bandwidth, 205 Hz/Px], a coronal T2-weighted spin-echo sequence (TR, 4,100 ms; TE, 90 ms; slice thickness, 3 mm; flip angle, 122˚; ETL, 25; matrix, 320×320; FOV, 180 mm; bandwidth, 300 Hz/Px), a sagittal T2-weighted spin-echo sequence (TR, 6,600 ms; TE, 118 ms; slice thickness, 3 mm; flip angle, 132˚; ETL, 35; matrix, 320×320; FOV, 180 mm; bandwidth, 365 Hz/Px), axial diffusion-weighted images (TR, 7,300 ms; TE, 88 ms; slice thickness, 3 mm; flip angle, 90˚; ETL, 45; matrix, 84×59; FOV, 200 mm; bandwidth, 2,205 Hz/Px) with three diffusion weightings (b=0, 500 and 1,000 s/mm$^2$, respectively). Afferent diffusion coefficient (ADC) maps were generated automatically. Gadoteridol (ProHance; Bracco, Konstanz, Germany) was injected intravenously at a dose of 0.2 mmol/kg and a rate of 1.0 ml/s, followed by a 20-ml saline flush. After contrast injection, axial T1-weighted fat-suppressed gradient-echo images (TR, 868 ms; TE, 13 ms; slice thickness, 3 mm; flip angle, 134˚; ETL,3; matrix, 256×141; FOV, 180 mm; bandwidth, 205 Hz/Px) and sagittal T1-weighted fat-suppressed gradient-echo images (TR, 664 ms; TE, 11.0 ms; slice thickness, 3 mm; flip angle, 150˚; ETL, 3; matrix, 320×192; FOV, 180 mm; bandwidth, 200 Hz/Px) were obtained. Patients received bowel preparation, sonography transmission gel as an endorectal contrast agent, and intravenous spasmolytic medication.

*Imaging analysis*. Two radiologists, each with 9 years experience in abdominal imaging, independently reviewed the initial rectal MRI scans, which were obtained prior to any treatment. They evaluated the MRI findings as follows: (a) T stage (1, 2, 3, 4a, 4b); (b) N stage (0, 1, 2); (c) tumor location (below, at, or above peritoneal reflection); (d) tumor gross pattern (polypoid, ulcerative, or circumferential wall thickening); (e) tumor margin (smooth, lobulated, infiltrative); (f) tumor enhancement pattern (homogeneous enhancement, heterogeneous enhancement of less than 50% of the extent of the tumor, heterogeneous enhancement of more than 50% of the extent of the tumor); (g) signal intensity of the T2-weighted image (homogeneous signal intensity, heterogeneous signal intensity of less than 50% of the extent of the tumor, heterogeneous signal intensity of more than 50% of the extent of the tumor); (h) presence of extracellular mucin (which showed a high signal intensity on T2-weighted images); (i) sphincter invasion; (j) perirectal fat infiltration; (k) presence of mesorectal fascia invasion; (l) extramural vascular invasion on MRI; (m) peritoneal invasion; (n) adjacent organ invasion; (o) regional lymph node metastasis; and (p) pelvic side wall lymph node metastasis. One radiologist measured the maximum tumor depth perpendicular to the rectal lumen (*i.e.* the axial length of the tumor) and the maximum extramural tumor depth using axial images. The longitudinal dimensions of the tumor were determined using sagittal or coronal images. The ADCs of the tumors were determined using ADC maps.

*Histological evaluation of KRAS mutation*. Surgical specimens obtained following rectal cancer operations were evaluated histologically. *KRAS* mutation analysis was carried out using DNA extracted from paraffin sections. Mutational analysis for *KRAS* was performed using the CFX96 Real-Time PCR Detection System (Bio-Rad, Philadelphia, PA, USA) with PNA clamp™ KRAS mutation detection kit (Panagene, Inc., Daejeon, Korea).

*Statistical analysis*. The reviewers discussed their evaluations, and reached conclusions by consensus. These consensus results were used in the statistical analyses, except for the interobserver agreement. Associations between the imaging findings upon initial rectal MRI and *KRAS* mutations were assessed using the chi-squared test, Fisher's exact test and the Wilcoxon rank-sum test. The means of continuous variables were used as cut-off values, and the relationships between continuous variables determined from rectal MRI and *KRAS* mutations were assessed using the above-mentioned statistical tests. Statistical significance was considered as $p<0.05$. Interobserver agreement regarding the imaging analyses was assessed using the kappa-value, k. The strength of concordance was interpreted according to k-values as follows: $k\leq0.20$, poor; $0.21\leq k\leq0.40$, fair; $0.41\leq k\leq0.60$, moderate; $0.61\leq k\leq0.80$, good; $0.81\leq k\leq1.00$, very good. Statistical analyses were performed using the SAS software package (ver. 9.3; SAS Institute, Cary, NC, USA), and the DTComPair package for R (ver. 3.1.1; R Foundation for Statistical Computing, Vienna, Austria, www.r-project.org).

## Results

In total, 275 patients were included in this study (164 men and 111 women; mean age=65.5 years; age range=32-91 years). *KRAS* mutations were detected in 107 patients (38.9%), whereas 168 patients (61.1%) had no *KRAS* mutation. Table I shows the relationship between *KRAS* mutations and imaging findings for initial rectal MRI. The N stage and tumor gross pattern were associated with *KRAS* mutations ($p=0.0064$ and $p<0.0001$, respectively). The frequency of *KRAS* mutations was higher for N2-stage tumors (53.70%) than for N0-stage tumors (44.93%) and N1-stage tumors (30.92%). The frequency of *KRAS* mutations was higher in polypoid tumors (59.09%) than in ulcerative tumors (24.47%) and circumferential wall thickening tumors (39.13%) (Figure 1). Axial tumor length, and the ratio of the axial to the longitudinal tumor dimensions, were both significantly correlated with *KRAS* mutations. Rectal cancers with *KRAS* mutations exhibited longer axial tumor dimensions than those with no KRAS mutation ($p=0.0003$). The mean of the ratio of the axial to the longitudinal dimensions of the tumor was larger in tumors with *KRAS* mutations than in tumors without ($p=0.0090$). T Stage, tumor location, tumor margin, enhancement pattern, signal intensity on T2WI, presence of extracellular mucin, sphincter invasion, perirectal fat infiltration, mesorectal fascia (MRF) invasion, mrEMVI, peritoneal invasion, adjacent organ invasion, regional or pelvic lymph node metastasis, extramural tumor depth, longitudinal tumor length, and ADC exhibited no statistically significant correlation with the presence of *KRAS* mutation ($p>0.05$).

Table II lists the kappa values of interobserver agreement. The kappa values between the two reviewers were in the range $0.88<k<1.00$ for all imaging findings, which shows very good agreement.

## Discussion

*KRAS*, a commonly mutated oncogene, is part of the mitogen-activated protein kinase signaling pathway associated with CRC (6). Mutations of the *KRAS* gene,

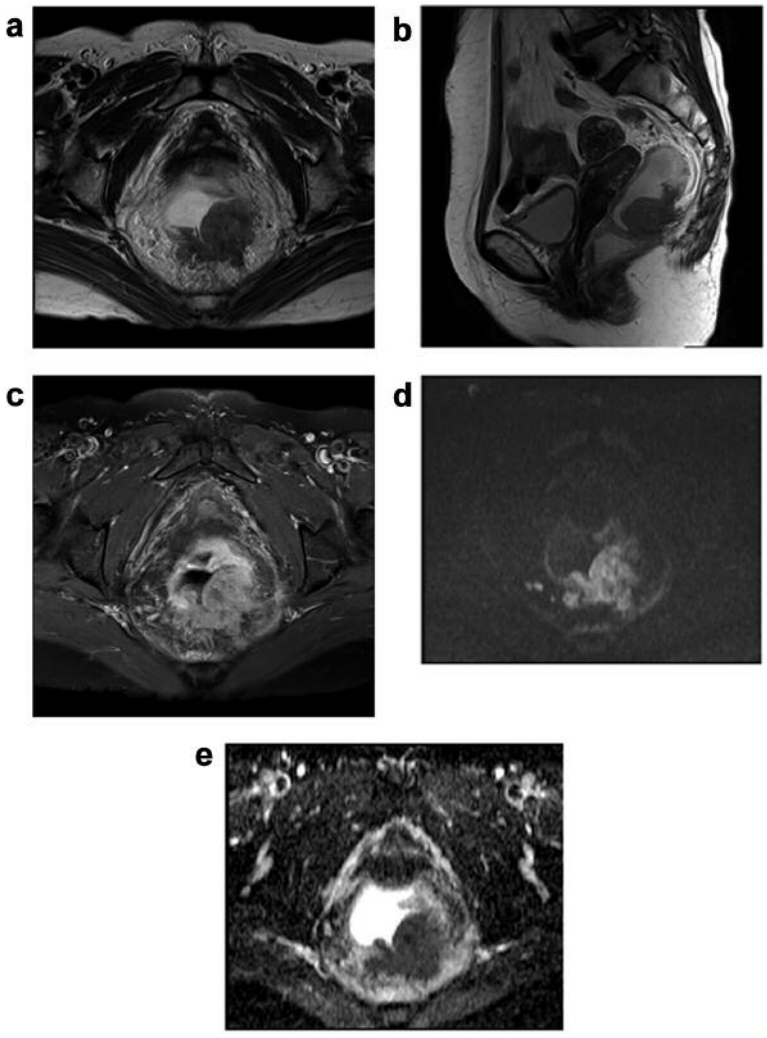


Figure 1. *A 64-year-old female patient with rectal cancer with Kirsten-ras (KRAS mutation. T2-Weighted turbo-spin-echo oblique axial (a) andT2-weighted sagittal (b) magnetic resonance images, both showing rectal mass with a polypoid pattern, a relatively long axial tumor length (2.8 cm), and a relatively large ratio of the axial to the longitudinal dimensions of the tumor (0.7). Several enlarged lymph nodes were observed in the mesorectum along the superior rectal vessel, suggesting N2 stage. c: An axial contrast-enhanced T1-weighted magnetic resonance image, showing a rectal tumor with penetration through the rectal wall into the mesorectal fat and fascia. d: The rectal mass exhibited a high signal intensity on the diffusion-weighted image (b=1,000 s/mm$^2$). e: An apparent diffusion coefficient map, showing diffusion restriction.*

especially at codon 12, have been assumed to be independent risk factors for reduced overall survival in patients with CRC (3). The mutation status of the *KRAS* gene has also been reported to be associated with treatment response during chemotherapy (2, 3). Therefore, the clinical relevance of mutations of the *KRAS* gene to the prognoses of patients with CRC has increased in recent years, and detecting *KRAS* mutation status can enable more accurate risk stratification and improved identification of appropriate therapeutic strategies (3). Recently, several studies have shown the potential of MRI examination as a non-invasive method to assess tumor biology (6, 7). However, MRI has not been widely used in the investigation of genomic expression. Here, we investigated the association between observable MRI features and *KRAS* mutations, and used the resulting data to assess the potential of MRI as an indicator of the genetic mutation status of patients with rectal cancer.

We found that *KRAS* mutations were significantly associated with the gross tumor pattern, the axial tumor length, and the ratio of the axial to the longitudinal dimensions of the tumor. *KRAS* mutations are thought to be related to the conversion of low-grade to high-grade adenoma status during the early stages of colorectal tumorigenesis, and are significant in protrusive growth

Table I. *The relationship between imaging findings on initial rectal magnetic resonance imaging and the presence of Kirsten-ras (KRAS) mutation. Data are the number of patients (%) unless stated otherwise.*

| Imaging factor | | | *KRAS* mutation | | |
|---|---|---|---|---|---|
| | | Total (N=275) | No (n=168) | Yes (n=107) | *p*-Value |
| T Stage | 1 | 10 (3.64) | 4 (40.00) | 6 (60.00) | 0.4731 |
| | 2 | 50 (18.18) | 29 (58.00) | 21 (42.00) | |
| | 3 | 134 (48.73) | 84 (62.69) | 50 (37.31) | |
| | 4a | 67 (24.36) | 44 (65.67) | 23 (34.33) | |
| | 4b | 14 (5.09) | 7 (50.00) | 7 (50.00) | |
| N Stage | 0 | 69 (25.09) | 38 (55.07) | 31 (44.93) | 0.0064* |
| | 1 | 152 (55.27) | 105 (69.08) | 47 (30.92) | |
| | 2 | 54 (19.64) | 25 (46.30) | 29 (53.70) | |
| Tumor location | Below PR | 99 (36.00) | 52 (52.53) | 47 (47.47) | 0.0891 |
| | At PR | 134 (48.73) | 89 (66.42) | 45 (33.58) | |
| | Above PR | 42 (15.27) | 27 (64.29) | 15 (35.71) | |
| Tumor gross pattern | Polypoid | 66 (24.00) | 27 (40.91) | 39 (59.09) | <0.0001* |
| | Ulcerative | 94 (34.18) | 71 (75.53) | 23 (24.47) | |
| | Circumferential wall thickening | 115 (41.82) | 70 (60.87) | 45 (39.13) | |
| Tumor margin | Smooth | 45 (16.36) | 25 (55.56) | 20 (44.44) | 0.5099 |
| | Lobulated | 111 (40.36) | 72 (64.86) | 39 (35.14) | |
| | Infiltrating | 119 (43.27) | 71 (59.66) | 48 (40.34) | |
| Enhancement pattern | Homogeneous | 128 (46.55) | 75 (58.59) | 53 (41.41) | 0.3545 |
| | Heterogeneous <50% | 97 (35.27) | 58 (59.79) | 39 (40.21) | |
| | Heterogeneous ≥50% | 50 (18.18) | 35 (70.00) | 15 (30.00) | |
| Signal intensity on T2WI | Homogeneous | 182 (66.18) | 113 (62.09) | 69 (37.91) | 0.4437 |
| | Heterogeneous <50% | 57 (20.73) | 31 (54.39) | 26 (45.61) | |
| | Heterogeneous ≥50% | 36 (13.09) | 24 (66.67) | 12 (33.33) | |
| Presence of extracellular mucin | No | 258 (93.82) | 159 (61.63) | 99 (38.37) | 0.4767 |
| | Yes | 17 (6.18) | 9 (52.94) | 8 (47.06) | |
| Sphincter invasion | No | 258 (93.82) | 158 (61.24) | 100 (38.76) | 0.8431 |
| | Yes | 17 (6.18) | 10 (58.82) | 7 (41.18) | |
| Perirectal fat invasion | No | 60 (21.82) | 33 (55.00) | 27 (45.00) | 0.2738 |
| | Yes | 215 (78.18) | 135 (62.79) | 80 (37.21) | |
| MRF invasion† | No | 131 (55.98) | 77 (58.78) | 54 (41.22) | 0.5010 |
| | Yes | 103 (44.02) | 65 (63.11) | 38 (36.89) | |
| mrEMVI | No | 217 (78.91) | 130 (59.91) | 87 (40.09) | 0.4364 |
| | Yes | 58 (21.09) | 38 (65.52) | 20 (34.48) | |
| Peritoneal invasion | No | 198 (72.00) | 117 (59.09) | 81 (40.91) | 0.2753 |
| | Yes | 77 (28.00) | 51 (66.23) | 26 (33.77) | |
| Adjacent organ invasion | No | 263 (95.64) | 162 (61.60) | 101 (38.40) | 0.5467 |
| | Yes | 12 (4.36) | 6 (50.00) | 6 (50.00) | |
| Regional lymph node metastasis | No | 72 (26.18) | 40 (55.56) | 32 (44.44) | 0.2622 |
| | Yes | 203 (73.82) | 128 (63.05) | 75 (36.95) | |
| Pelvic lymph node metastasis | No | 227 (82.55) | 138 (60.79) | 89 (39.21) | 0.8256 |
| | Yes | 48 (17.45) | 30 (62.50) | 18 (37.50) | |
| Mean extramural tumor depth (cm) (SD) | | 0.41 (5.03) | 0.37 (4.54) | 0.47 (5.66) | 0.3056 |
| Mean ATL (cm) (SD), | | 1.41 (0.92) | 1.30 (0.87) | 1.58 (0.99) | 0.0003* |
| Mean LTL (cm) (SD), | | 3.84 (1.56) | 3.80 (1.44) | 3.91 (1.73) | 0.7914 |
| Mean ATL/LTL ratio (SD) | | 0.40 (0.24) | 0.36 (0.20) | 0.46 (0.29) | 0.0090* |
| Mean ADC ($10^{-6}$ $mm^2/s$) (SD)† | | 964.81 (216.90) | 966.78 (207.20) | 961.69 (232.45) | 0.6230 |

ATL: Axial tumor length; LTL: longitudinal tumor length; PR, peritoneal reflection; T2WI, T2-weighted image; SD, standard deviation; MRF, mesorectal fascia; mrEMVI, extramural vascular invasion on MRI; ADC, apparent diffusion coefficient. †Factor with missing values (missing values are deleted listwise). *Statistically significant.

Table II. *Kappa statistics of interobserver agreement for imaging findings of initial rectal magnetic resonance imaging.*

| Imaging factor | Kappa (95% confidence interval) |
|---|---|
| T Stage | 0.93 (0.90-0.97) |
| N Stage | 0.95 (0.92-0.98) |
| Tumor location | 0.98 (0.96-1.00) |
| Tumor gross pattern | 0.96 (0.92-0.99) |
| Tumor margin | 0.94 (0.90-0.97) |
| Enhancement pattern | 0.97 (0.94-0.99) |
| Signal intensity on T2WI | 0.94 (0.89-0.98) |
| Presence of extracellular mucin | 0.88 (0.77-1.00) |
| Sphincter invasion | 1.00 (1.00-1.00) |
| Perirectal fat invasion | 0.98 (0.95-1.00) |
| MRF invasion | 0.93 (0.88-0.98) |
| mrEMVI | 0.90 (0.84-0.97) |
| Peritoneal invasion | 0.96 (0.92-0.99) |
| Adjacent organ invasion | 0.88 (0.75-1.00) |
| Regional lymph node metastasis | 0.98 (0.95-1.00) |
| Pelvic lymph node metastasis | 0.97 (0.94-1.00) |

T2WI, T2-weighted image; MRF, mesorectal fascia; mrEMVI, extramural vascular invasion on magnetic resonance imaging.

(8-10). Many studies of the pathology of CRCs have reported that a low incidence of *KRAS* mutations is associated with a flat appearance during the early stage of carcinogenesis; furthermore, non-polypoid early carcinomas exhibit a low incidence of *KRAS* mutations, whereas polypoid cancer has a high incidence of *KRAS* mutation (8, 11-14). The results of this study revealed that tumors with a polypoid pattern (as determined from MRI images) exhibited more frequent *KRAS* mutations than those with a flat morphology of ulcerative tumors, and tumors with circumferential wall-thickening patterns. This result is concordant with previous pathology studies using radiological imaging. A larger ratio of the axial to the longitudinal dimensions of the tumor, in combination with a longer axial tumor length, was found to be positively correlated with an increased frequency of *KRAS* mutations. This indicates that a polypoid tumor morphology is a quantitative indicator of *KRAS* mutation, which can be measured using MRI.

*KRAS* mutations are widely assumed to be associated with advanced CRC, as well as lymph node metastasis and higher tumor stage (15-17). Here, we found that *KRAS* mutations were associated with the N stage, as determined using rectal MRI. This is consistent with the results of previous studies (15-17), although this is limited by the relatively low sensitivity for nodal staging using rectal MRI (18). *KRAS* mutations may affect lymph node metastasis, and the existence of *KRAS* mutations may facilitate lymph node metastasis, increasing the likelihood of a higher N stage (16, 19).

To date, the clinical significance of *KRAS* mutations in patients with CRC has been the subject of debate; however, an association has been reported between the lack of a response to targeted anti-EGFR drugs and *KRAS* mutations in patients with advanced rectal cancer (2). Stratification of patients according to the presence of *KRAS* mutations may be helpful in order to select effective treatments and to predict prognoses in patients with rectal cancer. Rectal MRI is widely used as the primary examination in patients with rectal cancer for diagnosis, tumor staging and evaluation of the therapeutic response following treatment. These associations of pretreatment tumor staging and tumor responses with rectal MRI data can be used to predict the prognoses of rectal cancer. Additional interpretation of imaging features associated with *KRAS* mutations identified using rectal MRI may increase the efficacy of stratification of patients with rectal cancer, as well as the selection of treatment protocols. The heterogeneity of the *KRAS* mutation status within a primary CRC tumor has been established (20). Mutational testing of some parts of tumors acquired from biopsy may fail to detect *KRAS* mutations in specimens from the outer portion of the tumor (*i.e.* may give false-negative result). MRI may disclose features associated with *KRAS* mutations, thereby providing an opportunity to correct misdiagnoses by suggesting possible false-negatives from histological assessments.

In conclusion, *KRAS* genetic mutations were associated with the following MRI features: higher N stage, polypoid pattern, greater axial tumor length, and greater ratio of the axial to the longitudinal dimensions of the tumor. This study shows that rectal MRI has potential as a non-invasive imaging modality to predict *KRAS* mutation status, and help determine appropriate therapeutic strategies for patients with rectal cancer.

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