Original Article

Retrospective Review KRAS Mutation Effect on Prognosis in Non-Neoadjuvant Locally Advanced Rectal Cancer

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Background: The KRAS (Kirsten rat sarcoma 2 viral oncogene homolog) mutation is common in colorectal cancer with controversial role in prognosis. The neoadjuvant in management of locally advance rectum by AJCC (American joint committee on cancer) staging were T3 and above, and lymph node involvement possible to affect tissue interpretation. The present study demonstrates prognosis in non-neoadjuvant patients and factors associated with recurrence and metastasis including KRAS mutation.

Objective: Study a KRAS mutation and other factors had effect at 5 years survival, local recurrence and metastasis in non-neoadjuvant rectal cancer.

Materials and Methods: The study was collected from 2006 to 2015 CE, including patients demographic data, pre-operative stage, KRAS status, type of operation, adjuvant chemo-radiation, compliance of adjuvant, recurrence disease with time to recurrence, metastasis with site of metastasis and time to metastasis, and survival data.

Results: Overall there were 277 patients (male 145 and female 132) with a mean age of 60.55±9.06 years. The cancer diagnosis was made at middle rectum 126 and lower rectum in 142 patients. KRAS mutation on codon12 was 6.3%, codon13 was 28.6%, and both were 25.4%. The two years survival was 93.6% and five years survival 74.2%. Local recurrence was 8.3% and distant metastasis 26.0%. The factors associated with local recurrence were at the pre-operative stage, pathological stage III-IV, negative circumferential rectal margin (CRM) and poor compliance with adjuvant chemo-radiation. The factors associated with metastasis were at stage the pre-operative, pathological stage III-IV, received adjuvant treatment, and poor compliance with adjuvant chemo-radiation.

Conclusion: The management of locally advanced rectal cancer in non-neoadjuvant cases, CRM positive is a risk factor. Moreover, poor compliance in adjuvant management is a risk in local and distant metastasis rather than at nodal stages and KRAS mutation status.

Keywords: KRAS, Rectum, Cancer, Non-neoadjuvant

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Today knowledge of gene mutation in colorectal cancer (CRC) is accepted to play a role in prognosis and survival of patients such as with adenoma polyposis coli gene (APC) played a major role in adnoma-carcinoma sequence and in patients where mutation is a risk for developing colorectal cancer; however, it has not generally been included as a factor in clinical prognostic classification and is not generally included in standard CRC-sequencing panels. The development of nextgen sequencing technologies is possible to evaluate extensively a large number of genes and samples to identify its diagnosis, prognosis and potential response to therapy. So, a detailed molecular disease classification and measured molecular parameters correlated with clinical is needed. Endothelial growth factor (EGFR) is one of the pathways that controls by RAS, BRAF, PI3K and PTEN genes and leads to cell proliferation, cell motility and metastasis[1]. The KRAS gene is part of member of RAS gene and may be a gene from which to study mutation in order to predict a response of an anti-epidermal growth factor (EGFR), monoclonal antibodies therapy because it is one of the most common in sporadic colorectal cancer; it is also the site common in mutation codons 12 and 13 on exon 2 and codon 61 on exon 3. Current recommendation suggests studying KRAS mutation before using anti-EGFR monoclonal antibodies therapy in the treatment of patients with metastasis colorectal cancer (mCRC) (due to study compare response in wild and mutated) this is incomprehensible phrasing, needs rewrite-Ed. Only one study in KRAS showed prognosis in colon cancer[2] because in rectum cancer there is a neoadjuvant chemoradiation before surgery to improve local recurrence[3] although radiation is able to cause gene mutation by itself. This study tries to demonstrate the KRAS gene effect and other factors on prognosis in non-radiated rectal cancer.

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Materials and Methods

The study was approved by Rajavithi ethical broad committee. The study enrolled patients from January 2006 to January 2015. The inclusion criteria were: 1) Patient age ≥18 years, 2) Adenocarcinoma located at the rectum to anal canal, 3) Clinical staging was advance stage (T3 and above or mesorectal node positive) by American Joint Committee on Cancer staging manual at the time of diagnosis (AJCC), and re-evaluation in staging compared with AJCC manual 8th edition[4] and 4) Patient denied to neoadjuvant therapy. The exclusion was patient lost to follow-up. The KRAS study was done with patients voluntarily and after consent. The specimen for KRAS study was collected at immediate after resection in operative theater. The PCR for KRAS study was 2-step PCR restriction fragmentation length polymorphism and study of codon 12 and 13. The clinical data collected in demographic data included age at diagnosis, sex, co-morbidity, staging of disease in pre- and postoperative, postoperative phase data including surgical complication, hospital stay, adjuvant treatment and its complications, recurrence, metastasis and survival.

Statistical analysis

The descriptive statistics were analysis in number, percent, mean with standard deviation. Kaplan-Meier method was used for survival analysis. Cox-proportion hazard model and Log rank test were used as analysis factors and which had an effect on survival. A *p*-value <0.05 was considered statistically significant (SPSS for Windows version 17.0).

Results

During the study period 2006 to 2015, 300 patients were enrolled , excluding 23 patients lost to follow-up. No patient changed in stage of disease after compared with 8th edition AJCC staging manual. The average follow-up time was 7.23±2.91 year. 63 out of 277 patients were lost for KRAS study. Demographic data of KRAS study group and non-study group are shown in Table 1. All KRAS study found overall mutation rate was 60.3%. Results in mutation shown in Table 2. No difference in demographic data appeared between stages of cancer in KRAS study group, shown in Table 3.

Table 1. Demographic data in all patients non-neoadjuvant locally advance rectal cancer

	Total n = 277	Non test n = 214	K-RAS test sample n = 63
Age (years)	60.55±9.06	60.60±9.09	60.38±9.02
Male	145 (52.3)	113 (52.8)	32 (50.8)
Location cancer			
Low rectum	142 (51.3)	112 (40.4)	30 (47.6)
Middle rectum	126 (45.5)	96 (34.7)	30 (47.6)
Upper rectum	9 (3.2)	6 (2.2)	3 (4.8)
Preoperative CEA	22.71±62.56	20.75±60.54	30.08±69.91
Clinical staging (%)			
II	102 (36.8)	88 (41.1)	14 (22.2)
III	175 (63.2)	126 (58.9)	49 (77.8)
Pathologic staging			
I	8 (2.9)	5 (2.3)	3 (4.8)
II	84 (30.3)	69 (32.2)	15 (23.8)
III	174 (62.8)	134 (62.6)	40 (63.5)
IV	11 (4.0)	6 (2.8)	5 (7.9)
Lateral pelvic node dissection	44 (15.9)	38 (86.4)	6 (13.6)
CRM positive	6 (2.2)	6 (100)	0 (0)
Receive adjuvant chemo radiation	224 (80.1)	171 (76.3)	53 (23.7)
Poor compliance to adjuvant	20 (7.2)	14 (70.0)	6 (30.0)

Value are represented as n (%), mean ± SD

Table 2. K-RAS mutation result

Study K-RAS Mutation	Patients (n)	Percent (%)
K-RAS mutation	38	60.3
K-RAS mutation codon 12 C.35 G>T and K-RAS mutation codon 13 C.38 G>A	16	25.4
K-RAS mutation codon 13 C.38 G>A	18	28.6
K-RAS mutation codon 12 C.35 G>T	4	6.3
Negative	25	39.7

The distribution of KRAS codon mutation by stages was codon 12, stage II 25.0%, stage III 50.0% and stage IV 25.0%. Codon 13 mutation was in stage II 16.7%, stage III 61.1% and stage IV 22.2%. Mutation in both codons in stage II 6.3%, stage III 75.0% and stage IV 18.8%.

The result in prognosis and survival were not different in wild type and mutated typed of KRAS study group by statistics, as shown in Table 4. Comparison of survival, local recurrence and metastasis in KRAS wild and mutated showed in Figure 1. All non-radiated rectal cancer patient had 2 years survival at 93.9%, 5 years survival 74.2% and metastasis 30.0%.

The common sites of metastasis were lung 38.4%, both lung and liver 27.4%, liver 20.5%, carcinomatosis peritoneii 9.6% and other 4.2%. All operable metastasis was 13.9%.

Univariate analysis factors of 5 years survival were Clinical stage III ($p = 0.001$), Pathological stage III-IV ($p<0.001$), poor compliance with adjuvant treatment ($p<0.001$) and lateral pelvic node dissection ($p = 0.021$). Multivariate analysis showed pathologic stage III-IV ($p = 0.006$), poor compliance with adjuvant treatment ($p = 0.006$) and lateral pelvic dissection ($p = 0.049$). Lateral pelvic node dissection was performed in 38 patients and 34 patients had survival of more than 5 years, as shown in Table 5.

Univariate factors of local recurrence were clinical stage III ($p = 0.011$), pathological stage III-IV ($p = 0.017$), circumferential rectal margin (CRM) negative ($p<0.001$) and poor compliance with adjuvant treatment ($p<0.001$). The result numbers of CRM positive were 6 patients (recurrence 5 patients) and CRM negative were 271 patients (recurrence 18 patients) In multivariate analysis factor of local recurrence was poor compliance with adjuvant treatment ($p<0.001$), as shown in Table 6.

For metastasis as shown in Table 7, univariate showed clinical stage III ($p = 0.002$), pathological stage III-IV ($p<0.001$), received adjuvant treatment ($p = 0.010$) and poor compliance with adjuvant treatment ($p = 0.002$). Multivariate analysis showed, significantly poor compliance with adjuvant treatment ($p = 0.023$).

In follow-up period, the overall data of poor compliance in this study were 20 patients (Table 1). The causes were diarrhea 6, neutropenia 4, fatigue 4, non-defined 3 patients, radiation enteritis 2, and postponed by patient 1.

Table 3. Demographic data in K-RAS study group (n = 62)

	Wild, n = 24	Mutated, n = 38	*p*-value
Age (mean ± SD)	59.5±5.69	60.76±10.7	0.548
Male	9 (37.5)	23 (60.5)	0.077
Location			0.389
Low rectum	11 (45.8)	19 (50)	
Middle rectum	13 (54.2)	16 (42.1)	
Upper rectum	0 (0.0)	3 (7.9)	
Clinical staging			0.208
Stage II	7 (29.2)	6 (15.8)	
Stage III	17 (70.8)	32 (84.2)	
Pathological grading			0.193
Stage I	2 (8.3)	1 (2.6)	
Stage II	7 (29.2)	7 (18.4)	
Stage III	15 (62.5)	25 (65.8)	
Stage IV	0 (0.0)	5 (13.2)	

Value are represented as n (%), mean ± SD

Table 4. Prognosis and survival in K-RAS study group

	Wild, n = 24	Mutated, n = 38	*p*-value
2 years survival	23 (95.8)	23 (84.2)	0.232
5 years survival	13 (76.5)	19 (55.9)	0.222
Local recurrence	1 (4.2)	5 (13.2)	0.391
Metastasis	4 (23.5)	17 (47.2)	0.137
Time to metastasis	17.0±13.11	14.52±12.82	0.749
Operable metastasis	0 (0.0)	1 (6.3)	1.000
Poor compliance adjuvant CRT	0 (0.0)	6 (15.8)	0.730

Value are represented as n (%), mean ± SD

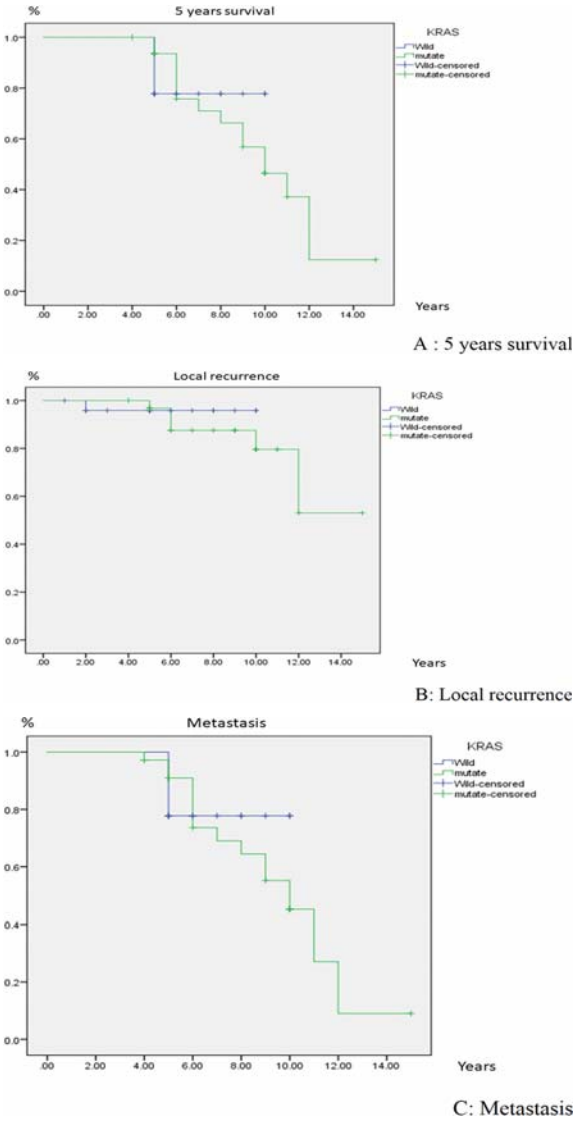

Figure 1. Compare 5 years survival, local recurrence and metastasis in KRAS wild and mutated.

Discussion

The RAS gene family is widely expressed in mammalian cells where encodes four small (21 kDa), cytoplasmic proteins with GTPase activity: H-Ras, K-Ras4a, K-Ras4b, and N-Ras[5]. They function as molecular switches transducing extracellular stimuli such as mitogens and differentiation factors to transcribe factors and cell cycle proteins in the nucleus in order to promote cell growth, differentiation, proliferation and survival[6]. Deregulated RAS signaling results in increased proliferation, angiogenesis, and motility, as well as in decreased apoptosis and in altered cellular metabolism[7]. KRAS gene is one among genetic study to predict a prognosis in colorectal cancer patient because it increases risk to synchronous polyps, high grade dysplasia[8], resistance to chemotherapy and biological target therapy at epidermal growth factor[9]. Most of study done in stage IV disease shows a benefit of study to predict response to anti-EGFR therapy such as progression-free survival in mutated group at 8 months and 18 months in wild type after cetuximab treatment[10] or overall survival 8 months in mutated and 19 months in wild type[11]. Data from previous study indicate that the KRAS mutation is mainly present in codon 12 and 13, 95% of all mutations[12-15]. Other mutations in codons 61, 146, 154 appear rarely, about 5%. In this study there is a high mutation, 67.2%, compared with previous study mutation rates 23 to 52%[10,16]. The possible explanation is all of the data were rectum cancer, different from previous studies and had a combination of results in colon and rectum or colon only. In rectal cancer genetic pathway is related to APC gene mutation. Previous study showed APC/KRAS-mutant embryonic stem cells were significantly enhanced beta-catenin/T-cell factor-mediated transcriptional activation, accompanied by increased beta-catenin nuclear localization leading to tumor multiplicity and malignant behavior[17]. In distribution of codon mutation, this study showed in the same fashion. Codon 13 shows significant high mutation in stage IV disease but the effect still controversial[18]. Recent study shows codon 13 have better response to cetuximab than codon 12 mutation, progression-free survival 4.1 to 4.5 month and 2.3 to 2.8 months, overall survival 9.3 to 10.6 months and 7.4 months, respectively[19,20]. This study did not show significance in survival, recurrence, or metastasis different from previous study in advanced colon cancer which showed significance related to disease progression[21]. To conclude the role of KRAS study is in line with European Society for Medical Oncology (ESMO); the patients with metastatic colorectal cancer must be studied for RAS mutations before initiating anti-EGFR therapy because the ineffectiveness of these treatments in metastatic colorectal cancer, harboring RAS mutation; it also should be avoid because of drug-induced toxicity and unnecessary expenses[22]. In analysis in all non-neoadjuvant rectal cancer, found significant factors in rectal cancer for local recurrence at clinical stage III, CRM positive and poor compliance with adjuvant treatment. The factors related to 5 years survival were pathological stage III, poor compliance with adjuvant treatment and lateral pelvic node dissection. Lastly, factors related to metastasis were clinical stage III, poor compliance with adjuvant treatment and lateral pelvic node dissection. All 3 aspects in survival in this study were in concordance with previous study: reported factors were pathological TNM stage, T substage, CRM status, the number/proportion of involved lymph nodes, extracapsular extension, extranodal deposits, tumor differentiation, lymphovascular invasion (LVI), extramural venous invasion (EMVI) and perineural invasion (PNI)[23-27]. Recently reported improvements in rectal cancer surgery were multidisciplinary team management via improve in R0 resection, 5 years survival in R0 resection, 55.6% and R1 resection, 14.8%[28].

The CRM involvement in rectal cancer for oncological outcomes has been well documented as to the

Table 5. Factor associated of 5 years survival rate (n = 182)

Factor	Number (%)	Univariate analysis HR (95% CI)	*p*-value	Multivariate analysis HR (95% CI)	*p*-value
Sex					
Male (ref = Female)	94 (51.6)	1.05 (0.78 to 1.40)	0.762	-	-
Diagnosis					
Upper rectum	6 (3.3)	1	-	-	-
Middle rectum	82 (45.1)	0.80 (0.39 to 2.06)	0.801	-	-
Low rectum	94 (51.6)	0.92 (0.40 to 2.14)	0.838	-	-
Combine resection adjacent organ	27 (14.8)	0.91 (0.61 to 1.37)	0.659	-	-
Pre-operative staging					
Stage II	79 (43.4)	1		1	-
Stage III	103 (56.6)	3.26 (1.65 to 6.42)	0.001*	1.91 (0.90 to 4.08)	0.094
Pathological stage					
Stage I-II	77 (42.3)	1		1	-
Stage III-IV	105 (57.7)	7.44 (2.70 to 20.52)	<0.001*	4.72 (1.55 to 14.40)	0.006*
KRAS mutation	19 (55.9)	0.62 (0.31 to 1.23)	0.170	-	-
Circumferential margin (CRM) negative	182 (100)	0.05 (0.001 to 1.631)	0.091	1.07 (0.39 to 2.89)	0.901
Lateral pelvic node dissection	34 (18.7)	0.64 (0.44 to 0.94)	0.021*	2.55 (1.01 to 7.90)	0.049
Received adjuvant chemo radiation	140 (76.9)	1.02 (0.72 to 1.44)	0.925	-	-
Poor compliance to adjuvant	2 (1.1)	3.90 (2.23 to 6.80)	<0.001*	2.55 (1.31 to 4.94)	0.006*

* = Significant at $p<0.05$

Table 6. Factor of 5 years free recurrence (n = 23)

Factor of free recurrence	Number (%)	Univariate analysis HR (95% CI)	*p*-value	Multivariate analysis HR (95% CI)	*p*-value
Sex					
Male (ref = Female)	13 (56.5)	1.20 (0.52 to 2.78)	0.663	-	-
Diagnosis					
Upper rectum	1 (4.3)	1	-	-	-
Middle rectum	10 (43.5)	0.47 (0.06 to 3.69)	0.470	-	-
Low rectum	12 (52.2)	0.47 (0.06 to 3.72)	0.471	-	-
Combine resection adjacent organ	4 (17.4)	1.33 (0.45 to 3.90)	0.609	-	-
Pre-operative staging					
Stage II	2 (8.7)	1		1	-
Stage III	21 (91.3)	6.523 (1.528 to 27.851)	0.011*	3.702 (0.772 to 17.747)	0.102
Pathological stage					
Stage I-II	1 (4.3)	1		1	-
Stage III-IV	22 (95.7)	11.508 (1.551 to 85.383)	0.017*	3.114 (0.360 to 26.970)	0.302
KRAS mutation	5 (83.3)	1.83 (0.20 to 16.78)	0.592	-	-
Circumferential margin (CRM) negative	18 (78.3)	8.164 (2.944 to 22.643)	<0.001*	1.495 (0.449 to 4.971)	0.512
Lateral pelvic node dissection	2 (8.7)	1.66 (0.39 to 7.12)	0.496	-	-
Received adjuvant chemo radiation	21 (91.3)	-	-	-	-
Poor compliance to adjuvant	12 (52.2)	9.296 (4.076 to 21.202)	<0.001*	6.293 (2.368 to 16.723)	<0.001*

* = Significant at $p<0.05$

Table 7. Factor of metastasis (n = 72)

Factor	Number (%)	Univariate analysis HR (95% CI)	*p*-value	Multivariate analysis HR (95% CI)	*p*-value
Sex					
Male (ref = female)	39 (54.2)	1.12 (0.70 to 1.79)	0.683	-	-
Diagnosis					
Upper rectum	2 (2.8)	1	-	-	-
Middle rectum	32 (44.4)	0.95 (0.23 to 3.94)	0.938	-	-
Low rectum	38 (52.8)	0.97 (0.23 to 4.05)	0.962	-	-
Combine resection adjacent organ	13 (18.1)	0.71 (0.39 to 1.3)	0.262	-	-
Pre-operative staging					
Stage II	15 (20.8)	1		1	-
Stage III	57 (79.2)	2.457 (1.390 to 4.342)	0.002*	1.55 (0.82 to 2.93)	0.181
Pathological stage					
Stage I-II	9 (12.5)	1		1	-
Stage III-IV	63 (87.5)	3.634 (1.807 to 7.306)	<0.001*	2.11 (0.94 to 4.74)	0.071
KRAS mutation	17 (81.0)	0.73 (0.24 to 2.28)	0.586	-	-
Circumferential margin (CRM) negative	67 (93.1)	2.198 (0.879-5.497)	0.092	-	-
Lateral pelvic node dissection	5 (6.9)	2.044 (0.821 to 5.089)	0.125	-	-
Received adjuvant chemoradiation	65 (90.3)	0.357 (0.164 to 0.781)	0.010*	0.54 (0.24 to 1.21)	0.136
Poor compliance to adjuvant	15 (20.8)	2.428 (1.369 to 4.306)	0.002*	1.98 (1.10 to 3.58)	0.023*

* = Significant at $p<0.05$

increased risk of both local recurrence and distant metastases[29-31], but the management to accomplish CRM is controversial. Recent guideline by American society of colon and rectal surgeons suggest routine neoadjuvant to reduce local recurrence[32]. But the another guideline by Japanese society for cancer of the colon and rectum has not adopted neoadjuvant in routine practice[33] and lastly from ESMO group support a selective neoadjuvant base on prediction of CRM involvement by MRI study[34]. In this study, CRM is related to local recurrence in the same as previous study from univariate analysis but not significant in multivariate analysis. In Thailand, neoadjuvant is not routinely adopted in practice due to patient selection after consent and information. Although our early study reported better outcomes in 2 years and 5 years disease-free survival in neoadjuvant group and adjuvant group, 81.8% vs. 73.5% and 70.4% vs. 46.1%, respectively[35].

In lateral pelvic dissection (LPND): This factor relates to 5 years survival. The result was different from JCOG 0212 that reported significantly better results in local recurrence[36] because the author performed lateral pelvic node dissection in positive extra-mesorectal pelvic lymph node involvement by pre-operative imaging or intra-operative positive extra-mesorectal pelvic lymph node. By the way, previous study showed the result of LPND is comparable to neoadjuvant in 5 years local recurrence 9.3% to 10.9%[37-39] and 6% to 8.7%[40-42], respectively.

About the compliance, this study had 20 patients (7.2%) in poor compliance with adjuvant treatment due to adjuvant related complications. The author did not collect all complications from adjuvant treatment. However, overall serious complications from adjuvant chemoradiation were 3 to 10%[43] and in grade 3, 4 toxicities that preclude patients for adjuvant treatment, 11 to 12%[44]. The present study result is comparable. In case of poor compliance, it can occur because postoperative complications[45] and adjuvant treatment. Recent study has shown shortened adjuvant FOLFOX regimen did not compromise 5 years survival in colon cancer[46] but in rectum, it is not reported. Meticulous technique in radiation and good operative results are helpful to improve compliance to adjuvant treatment.

What is already known on this topic?

1) KRAS mutation testing is required before anti-EGFR treatment, 2) KRAS gene is one of RAS gene family but others have effect on prognosis too, such as NRAS.

What this study adds?

1) First study in KRAS mutation in rectal cancer, 2) study provides data in the benefit of lateral pelvic node dissection, and 3) confirms significant CRM in rectal cancer surgery.

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Potential conflicts of interest

The authors declare no conflicts of interest.

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