Published in final edited form as:

Ann Surg Oncol. 2013 July ; 20(7): 2166–2171. doi:10.1245/s10434-013-2910-0.

Mutations in specific codons of the *KRAS* oncogene are associated with variable resistance to neoadjuvant chemoradiation therapy in patients with rectal adenocarcinoma

Marjun P. Duldulao, MD¹, Wendy Lee, BA¹, Rebecca A. Nelson, PhD², Wenyan Li, BA¹, Zhenbin Chen, PhD¹, Joseph Kim, MD¹, and Julio Garcia-Aguilar, MD, PhD¹

¹Department of Surgery, City of Hope, Duarte, 91010, CA

²Department of Biostatistics, City of Hope, Duarte, 91010, CA

Abstract

Background—Mutations in *KRAS* and *TP53* are common in colorectal carcinogenesis and are associated with resistance to therapy. Rectal cancers carrying both mutations are less likely to respond to neoadjuvant chemoradiation therapy (CRT) compared to wildtype tumors. Codon-specific *KRAS* mutations are associated with variable resistance to targeted therapies, but their association with rectal cancer response to CRT remains unclear. Our objective was to establish a correlation between specific *KRAS* mutations and rectal cancer response to CRT, and investigate if the correlation was related to a different association between *KRAS* and *TP53* mutations.

Methods—One hundred forty-eight stage II–III rectal cancer patients underwent pre-operative CRT followed by surgery. DNA was extracted from pretreatment tumor biopsies and paired normal surgical tissues and *KRAS* and *TP53* genotyping was performed. Specific *KRAS* mutations were then correlated with tumor response, and with concurrent *TP53* mutation.

Results—Sixty patients had *KRAS* mutation; 12 in codon 13, and 48 in other locations. Eighty patients had *TP53* mutation; 27 had concurrent *KRAS/TP53* mutations. Tumors with any *KRAS* mutation were less likely to have a pCR compared to wildtype *KRAS* ($p=0.006$). Specifically, no tumors with *KRAS* codon 13 mutations had a pCR ($p=0.03$). Tumors with *KRAS* codon 13 mutations also had a higher incidence of concurrent *TP53* mutation compared to tumors with other *KRAS* mutations ($p=0.02$).

Conclusions—Mutations in different *KRAS* codons may have different effects on rectal cancer resistance to CRT. This variable resistance may be related to a different frequency of *TP53* mutations in *KRAS* mutant tumors.

Corresponding Author: Julio Garcia-Aguilar MD, PhD, Department of Surgery, City of Hope, 1500 East Duarte Rd., Duarte, CA 91010; Tel: (626) 471-9309; Fax: (626) 301-8113; jgarcia-aguilar@coh.org.

The authors declare no conflicts of interest associated with this manuscript.

Meeting Presentation: This manuscript was presented at the Society for Surgical Oncology Annual Meeting, March 24th, 2012 in Orlando, FL.

Introduction

Mutation in the *KRAS* oncogene is a common event in colorectal carcinogenesis. [1] *KRAS* mutation causes constitutive activation of the *KRAS* protein and results in dysregulation of downstream processes and uncontrolled cell growth. [2] The presence of *KRAS* mutation has been associated with aggressive tumor behavior such as accelerated tumor growth, early formation of distant metastasis, and resistance to anti-epidermal growth factor receptor (EGFR)-based regimens in metastatic colorectal cancer patients. [3–5]

Most *KRAS* mutations (85%) in colorectal cancers occur in codon 12 or codon 13. [2, 3, 5] Other *KRAS* mutations occur more rarely and are found in only 1% to 3% of tumors. [4] Recent evidence suggests that not all *KRAS* mutations are alike, and that codon-specific *KRAS* mutations may affect different signaling pathways, resulting in variant tumor phenotypes. [6–9] Colorectal cancer patients with *KRAS* codon 13 mutations have different survival after cetuximab compared to other *KRAS* mutations, [10] and studies in colorectal and lung cancers have shown that patients with different codon 12 mutations have disparate prognosis. [11] Specifically, patients with *KRAS* G12V mutations have a worse prognosis than patients with *KRAS* G12D mutations, [2, 3, 11] suggesting that differences in single amino acids may dictate specific oncogenic characteristics of *KRAS* mutations.

We recently reported that rectal cancers with *KRAS* mutation are less likely to develop a pathologic complete response (pCR) to neoadjuvant chemoradiation (CRT) compared to tumors with wildtype *KRAS*. [12] Interestingly, we also found that none of the tumors with concurrent mutations in the *TP53* tumor suppressor gene and *KRAS* develop a pCR after neoadjuvant CRT. In this study we aimed to establish a potential correlation between specific *KRAS* mutations and rectal cancer response to CRT, and to investigate if that correlation could be related to a different association between the *KRAS* and *TP53* mutations.

Methods

Patients and treatment

This study included 148 patients with Stage II/III rectal cancer enrolled in a prospective multicenter clinical trial investigating the effect of increasing the CRT-to-surgery time interval, and adding chemotherapy during the waiting period ([ClinicalTrials.org](https://clinicaltrials.org/Identifier:NCT00335816) Identifier: NCT00335816). This trial was designed as a series of sequential Phase II trials or study groups (SGs) each with a progressively longer CRT-to-surgery time interval and increasing cycles of pre-operative chemotherapy. This study was approved by an Institutional Review Board (IRB) at each participating institution as well as a central IRB, and informed written consent was obtained from each patient prior to enrollment in the trial. Patients in the present study were pooled from SG1 (n=60), SG2 (n=67) and SG3 (n=21).

Patients in all SGs were treated with CRT; 5-Fluorouracil (FU) 225 mg/m²/day for 7 days in continuous infusion, and a total of 50.4Gy radiation. Patients in SG1 underwent TME an average of 6 weeks after completing CRT (standard of care). Following CRT, patients in SG2 and SG3 with signs of stable disease or disease progression compared with baseline

staging had surgery without further delay. All other patients received 2 and 4 cycles of additional chemotherapy (modified FOLFOX-6), respectively; leucovorin 200 mg/m² or 400 mg/m² plus oxaliplatin 85 mg/m² by 2h infusion, followed by bolus of 5-FU 400 mg/m² and a 46h infusion of 5-FU 2,400 mg/m². Patients in SG2 and SG3 underwent TME an average of 11 and 16 weeks after completing CRT. Further details of this trial are presented elsewhere. [13]

Pathologic staging was performed according to American Joint Committee on Cancer (AJCC) guidelines. [14] Histological sections were stained and examined by two independent blinded pathologists. A pCR was defined as the absence of viable tumor in the entire resected surgical specimen and in the regional lymph nodes.

Sample preparation, DNA extraction, and mutation analysis

Tumor DNA from each patient was obtained from pretreatment biopsy tissue. Tumor cells were isolated using microdissection and genomic DNA was extracted as described previously. [12] Standard polymerase chain reaction (PCR) analysis was performed to detect specific mutations in *KRAS* (exons 2 and 3, which contain the most frequently reported *KRAS* mutations) and *TP53* (exons 4–8) using established primers and PCR conditions. [12] Primer sequences are shown in Supplementary Table 1. *KRAS* and *TP53* mutation status was confirmed by two independently-derived PCR products.

Statistical analysis

Patients were stratified by *KRAS* mutation status, and clinicopathologic characteristics and tumor response (pCR versus non-pCR) were compared between the two groups. Patients were then stratified by tumor response and univariate analysis was performed using the Fisher Exact Test to determine if specific *KRAS* mutations correlated with tumor response. No adjustments were made for multiple comparisons. Correlations were considered significant for two-sided p-values of <0.05. Finally, univariate analysis was performed using the Fisher Exact Test to determine if specific *KRAS* mutations correlated with *TP53* mutations. A p-value of <0.05 was considered statistically significant.

Results

Patient demographics and tumor response

The clinicopathologic characteristics of the 148 patients are shown in Table 1. Eighty-five out of 148 (57%) patients were male and the average age was 57. Almost half of the patients (69 out of 148; 47%) had tumors located in the middle rectum. Initial clinical staging (cT and cN) showed that most patients were cT3 (135 of 148; 91%) and cN1 (106 out of 148; 72%). There was no statistically significant difference in pre-treatment clinical stage between SGs. Evaluation of surgical specimens after CRT revealed that 37 out of 148 (25%) patients had a pCR. There was no statistically significant difference in pCR rate between SGs.

Prevalence of *KRAS* mutation

The results of the *KRAS* mutation analysis for the 148 patients are shown in Table 2. Overall, 60 out of 148 (41%) patients had *KRAS* mutation; 40 of these 60 patients (67%) had mutations in codon 12, 12 (20%) in codon 13, 5 (8%) in codon 61, and 1 (2%) patient each in codons 6, 22 and 64. The most common mutation in *KRAS* codon 13 and the second most common *KRAS* mutation overall was G13D, detected in 11 out of 60 (18%) patients. One other patient carried a *KRAS* codon 13 mutation in G13C. The most common mutation identified was G12D in codon 12; it accounted for over half the *KRAS* mutations identified (31 out of 60; 52%). Other codon 12 mutations detected were: G12V in 7 (12%) patients, G12A in 1 (2%) patient and G12S in 1 (2%) patient. Mutations in the other codons were detected with a much lower prevalence in our patient population. Five out of 60 patients had mutations in codon 61; Q61L and Q61H, each detected in 2 (3%) patients and one patient had a 4 base-pair deletion, which spanned across codons 60 and 61. The remaining *KRAS* mutations were in codon 6 (L6F, one patient), codon 22 (6 base-pair insertion, one patient) and lastly, codon 64 (Y64H, one patient). The distribution of *KRAS* mutations among the three SGs was random, and the differences in the frequency of *KRAS* mutations and codon-specific mutations between SGs were not significant.

KRAS mutation and tumor response

The association of *KRAS* mutation with pCR is shown in Tables 1 and 3. Overall, tumors with any *KRAS* mutation were less likely to have a pCR compared to patients with wildtype *KRAS*; 8 (13%) versus 29 (33%) patients, respectively, $p=0.006$ (Table 1). Specifically, none of the tumors with *KRAS* mutation in codon 13 had a pCR ($p=0.03$) (Table 3).

Correlation of *KRAS* with concurrent *TP53* mutation

Eighty out of 148 (54%) patients had a mutation in *TP53* and 27 patients had concurrent *KRAS/TP53* mutations (Table 4). Patients with any mutation in *KRAS* had a lower prevalence of *TP53* mutation compared to patients with wildtype *KRAS*, although this difference was not statistically significant ($p=0.07$). In patients with *KRAS* mutation, the presence of *KRAS* codon 13 mutation was strongly associated with concurrent *TP53* mutation compared to all other *KRAS* mutations; 9 out of 12 (75%) patients with *KRAS* codon 13 mutations had concurrent *TP53* mutations compared to 18 out of 48 (37%) patients with any other *KRAS* mutation ($p=0.02$).

Discussion

The current study confirms in a larger series our previous observation that rectal cancer patients with tumors carrying any *KRAS* mutation are less likely to have a pCR in response to CRT compared to patients with wildtype *KRAS*. In addition, we show that tumors with *KRAS* codon 13 mutation are particularly resistant to CRT as none of them achieve a pCR. We also found that while tumors with *KRAS* mutation tend to have lower frequency of *TP53* mutation compared to tumors with wildtype *KRAS*, patients with specific *KRAS* codon 13 mutation have a higher frequency of concurrent *TP53* mutation.

Several studies have demonstrated an association between *KRAS* mutation and lower tumor response and poor survival in patients with metastatic colorectal cancer treated with cetuximab-based regimens. [15–18] However, only one study has investigated the association of specific *KRAS* mutation with tumor response in patients with metastatic colorectal cancer treated with best supportive care with or without cetuximab. [10] This study found that patients with the *KRAS* G13D mutation treated with best supportive care (fluoropyrimidine, oxaliplatin or irinotecan) had worse overall survival compared to patients with other *KRAS* mutations or wildtype *KRAS* in univariate analysis but not in multivariate analysis. These data support our findings of less responsiveness to fluoropyrimidine-based CRT plus oxaliplatin chemotherapy in tumors carrying *KRAS* codon 13 mutation. However, the same *KRAS* codon 13 mutation was associated with improved response and survival compared to other *KRAS* mutations in chemotherapy-refractory metastatic colorectal cancer patients treated with cetuximab. [10] A more recent retrospective analysis also indicated that patients with metastatic colorectal cancer carrying the *KRAS* G13D mutation were less likely to respond to first-line chemotherapy and had a shorter progression-free and overall survival compared to patients with wild-type and other *KRAS* mutations. [19]

A number of studies have investigated the specific association of *KRAS* mutations and rectal cancer response to radiation with different results, but only one study has examined the association of specific *KRAS* mutations and rectal cancer response to CRT. [20] Gaedcke et al. screened 95 patients with rectal cancer treated with 5-fluorouracil and oxaliplatin-based CRT for mutations in codons 12, 13, and 61 of the *KRAS* gene and found no difference in response rate between tumors with *KRAS* mutations, combined or individually, and wildtype tumors. [20] The discrepancy with our results could be explained by the smaller sample size of their series and the use of a five-point tumor regression grade as a measure of response rather than pCR.

Persistent activation of *KRAS* affects numerous downstream pathways that play a role in cancer progression. [21] Several studies suggest that the specific location of *KRAS* mutation within the gene can affect different downstream pathways, which ultimately could affect tumor response to therapy. [8–10] Guerrero et al. examined differences between *KRAS* codon 12 and codon 13 mutations on downstream signaling in transfected NIH3T3 fibroblasts and noted similar *KRAS* activity and upregulation of the MAPK pathway, but differential activation of the AKT, c-Jun N-terminal kinase (JNK), and focal adhesion kinase (FAK) pathways. The functional consequences of differential downstream signaling manifested as altered apoptotic and mitotic rates. [8, 9] Divergent signal transduction between *KRAS* codon 12 and 13 mutations was also demonstrated in *in vitro* studies by De Roock et al. In the presence of cetuximab, SW48 colorectal cancer cells transfected with *KRAS* G12V were still able to signal through to extracellular signal-regulated kinase (ERK), whereas *KRAS* G13D transfected cells did not activate ERK signaling. [10] Other studies have reported an association between the activation or attenuation of several signaling pathways including AKT, JNK, and FAK, and resistance to several chemotherapeutic agents and radiation in multiple cancers. [22–24] Thus, utilization of alternative downstream pathways may provide an avenue of resistance to CRT in rectal cancer patients with different *KRAS* codon mutations.

Our current understanding of colorectal carcinogenesis suggests that colorectal cancer develops as a result of the accumulation of genetic alterations in oncogenes and tumor suppressor genes, and that it is the number rather than the specific mutations that is important for the development of the cancer. The most commonly mutated genes in colorectal cancer are *APC*, *TP53*, and *KRAS*, and while *APC* mutations are common in tumors with *TP53* or *KRAS* mutations, the combination of all three mutations in the same tumor is exceedingly rare, suggesting that the combination of mutations in a specific tumor may not be random. [25] The mutual exclusivity of *KRAS* and *BRAF* mutations in the same tumor seems to be another example of the non-random distribution of mutations in colorectal cancer. [26] Our findings are consistent with these observations and suggest that a non-random combination of mutations may define subtypes of cancer with different biological behavior and even response to therapy.

There are limitations to our study that deserve mention. We had a relatively small sample size, which could account for the low frequency of codon 13 mutations. Additionally, the patient group was not completely homogeneous; there were three treatment arms in our study, and while all patients received pre-operative CRT, some patients also received additional chemotherapy. External validation of our findings will therefore be important to further substantiate our findings.

In conclusion, we demonstrate that rectal cancer patients with *KRAS* codon 13 mutations are resistant to neoadjuvant CRT and do not achieve a pCR. Furthermore, the presence of *KRAS* codon 13 mutation correlates with *TP53* mutation. While it will be important to validate our findings in an independent large patient cohort, screening for these mutations may help to select effective treatments for patients carrying these mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Nicola Solomon, PhD, for assistance in writing and editing the manuscript. This study was supported by the National Institutes of Health (NIH), National Cancer Institute (NCI) R01 Grant CA090559 (JGA). [ClinicalTrials.org](https://clinicaltrials.org) Identifier: NCT00335816.

References

1. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med*. 1988; 319:525–532. [PubMed: 2841597]
2. Andreyev HJN, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: The multicenter “RASCAL” study. *J Natl Cancer Inst*. 1998; 90:675–684. [PubMed: 9586664]
3. Andreyev HJN, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: The ‘RASCAL II’ study. *Br J Cancer*. 2001; 85:692–696. [PubMed: 11531254]
4. Normanno N, Tejpar S, Morgillo F, et al. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. *Nat Rev Clin Oncol*. 2009; 6:519–527. [PubMed: 19636327]
5. Vaughn CP, ZoBell SD, Furtado LV, et al. Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. *Genes Chromosomes Cancer*. 2011; 50:307–312. [PubMed: 21305640]

6. Buhrman G, Wink G, Mattos C. Transformation efficiency of Q61 Ras mutants linked to structural features of the switch regions. *Structure*. 2007; 15:1618–1629. [PubMed: 18073111]
7. Cespedes MV, Sancho FJ, Pavon MA, et al. K-ras Asp12 mutant neither interacts with Raf, nor signals through Erk and is less tumorigenic than K-ras Val12. *Carcinogenesis*. 2006; 27:2190–2200. [PubMed: 16679305]
8. Guerrero S, Casanova I, Farre L, et al. K-ras codon 12 mutation induces higher level of resistance to apoptosis and predisposition to anchorage-independent growth than codon 13 mutation or proto-oncogene overexpression. *Cancer Res*. 2000; 60:6750–6756. [PubMed: 11118062]
9. Guerrero S, Figueras A, Casanova I, et al. Codon 12 and codon 13 mutations at the K-ras gene induce different soft tissue sarcoma types in nude mice. *FASEB J*. 2002; 16:1642–1644. [PubMed: 12207005]
10. De Roock W, Jonker DJ, Di Nicolantonio F, et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. *JAMA*. 2010; 304:1812–1820. [PubMed: 20978259]
11. Keohavong P, DeMichele MA, Melacrinis AC, et al. Detection of K-ras mutations in lung carcinomas: Relationship to prognosis. *Clin Cancer Res*. 1996; 2:411–418. [PubMed: 9816185]
12. Garcia-Aguilar J, Chen Z, Smith DD, et al. Identification of a biomarker profile associated with resistance to neoadjuvant chemoradiation therapy in rectal cancer. *Ann Surg*. 2011; 254:486–493. [PubMed: 21865946]
13. Garcia-Aguilar J, Smith DD, Avila K, Bergsland EK, Chu P, Krieg RM, Timing of Rectal Cancer Response to Chemoradiation Consortium. Optimal timing of surgery after chemoradiation for advanced rectal cancer: preliminary results of a multicenter, nonrandomized phase II prospective trial. *Ann Surg*. 2011; 254(1):97–102. [PubMed: 21494121]
14. Edge, SB., Compton, CC., Fritz, AG., et al. American Joint Committee on Cancer (AJCC) cancer staging manual. 7th. Chicago: Springer, Inc; 2010.
15. De Roock W, Claes B, Bernasconi D, et al. 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*. 2010; 11:753–762. [PubMed: 20619739]
16. De Roock W, Piesseaux H, De Schutter J, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol*. 2008; 19:508–515. [PubMed: 17998284]
17. Lievre A, Bachet J, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol*. 2008; 26:374–379. [PubMed: 18202412]
18. Yen L, Uen Y, Wu D, et al. Activating KRAS mutations and overexpression of epidermal growth factor receptor as independent predictors in metastatic colorectal cancer patients treated with cetuximab. *Ann Surg*. 2010; 251:254–260. [PubMed: 20010090]
19. Tejpar S, Bokemeyer C, Celik I, et al. KRAS mutations and outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. *Ann Oncol*. 2011; 22(Suppl 5):v16–v17.
20. Gaedcke J, Grade M, Jung K, et al. KRAS and BRAF mutations in patients with rectal cancer treated with preoperative chemoradiotherapy. *Radiother Oncol*. 2010; 94:76–81. [PubMed: 19913317]
21. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: Weaving a tumorigenic web. *Nat Rev Cancer*. 2011; 11:761–774. [PubMed: 21993244]
22. Halder J, Landen CN, Lutgendorf SK, et al. Focal adhesion kinase silencing augments docetaxel-mediated apoptosis in ovarian cancer cells. *Clin Cancer Res*. 2005; 11:8829–8836. [PubMed: 16361572]
23. Liu LZ, Zhou XD, Qian G, et al. AKT1 amplification regulates cisplatin resistance in human lung cancer cells through the mammalian target of rapamycin/p70S6K1 pathway. *Cancer Res*. 2007; 67:6325–6332. [PubMed: 17616691]
24. Tournier C, Hess P, Yang DD, et al. Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science*. 2000; 288:870–874. [PubMed: 10797012]

25. Samowitz WS, Slattery ML, C S, et al. APC mutations and other genetic and epigenetic changes in colon cancer. *Mol Cancer Res.* 2007; 5:165–170. [PubMed: 17293392]
26. Vasovcak P, Pavlikova K, Sedlacek Z, et al. Molecular genetic analysis of 103 sporadic colorectal tumours in Czech patients. *PLoS One.* 2011; 6:e24114. [PubMed: 21901162]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Synopsis

Rectal cancer response to neoadjuvant chemoradiation therapy may be affected by specific *KRAS* mutations. We show that rectal cancers harboring *KRAS* codon 13 mutations are resistant to neoadjuvant chemoradiation therapy and do not develop a pathologic complete response.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Patient demographics

Factors	Total n=148	<i>KRAS</i> ^{wt1} n=88 (59%)	<i>KRAS</i> ^{mut2} n=60 (41%)	p-value
Age, years, median (range)	57 (25–87)	57 (32–85)	57 (25–87)	1.00
Gender				0.13
Male	85 (57%)	46 (52%)	39 (65%)	
Female	63 (43%)	42 (48%)	21 (35%)	
Pre-treatment T stage				
cT2	11 (7%)	7 (8%)	4 (7%)	0.75
cT3	135 (91%)	79 (90%)	56 (93%)	0.47
cT4	2 (2)	2 (2%)	0 (0%)	0.32
Pre-treatment N stage				0.37
cN0	34 (23%)	18 (20%)	16 (27%)	
cN+	114 (77%)	70 (80%)	44 (73%)	
Tumor location ³				
Lower	46 (31%)	25 (28%)	21 (35%)	0.38
Middle	69 (47%)	42 (48%)	27 (45%)	0.70
Upper	13 (9%)	9 (10%)	4 (7%)	0.47
Tumor response				0.006
pCR ⁴	37 (25%)	29 (33%)	8 (13%)	
Non-pCR	111 (75%)	59 (67%)	52 (87%)	

¹ wt: wildtype

² mut: mutant.

³ The tumor location for 20 patients was not recorded and was therefore not included in the analysis for tumor location.

⁴ pCR: pathologic complete response.

Table 2Prevalence of specific *KRAS* mutations

<i>KRAS</i> mutation	n=60
Codon 12	40 (67%)
G12D	31 (52%)
G12V	7 (12%)
G12A	1(2%)
G12S	1 (2%)
Codon 13	12 (20%)
G13D	11 (18%)
G13C	1 (2%)
Codon 61	5 (8%)
Q61L	2 (3%)
Q61H	2 (3%)
4 base-pair deletion	1 (2%)
Codon 6	1 (2%)
L6F	
Codon 22	1 (2%)
6 base-pair insertion	
Codon 64	1(2%)
Y64H	

Table 3*KRAS* mutations and tumor response

<i>KRAS</i> Status	n=148	pCR ^I	Non-pCR	p-value
Wildtype	88	29 (33%)	59 (67%)	
Codon 13 mutation	12	0 (0%)	12 (100%)	0.03 [*]
Other <i>KRAS</i> mutations ²	48	8 (17%)	40 (83%)	

^{*} *KRAS* codon 13 mutation correlates significantly with resistance to CRT compared to all other *KRAS* mutations.

^I pCR: pathologic complete response.

² Other *KRAS* mutations: Mutations in codons 12, 61, 6, 22, and 64 as listed in Table 2.

Table 4Correlation of *KRAS* mutations with concurrent *TP53* gene mutation

<i>KRAS</i> Status	Total n=148	<i>TP53</i> ^{wt1} n=68	<i>TP53</i> ^{mut2} n=80	p-value
Wildtype	88	35 (40%)	53 (60%)	
Codon 13 mutation	12	3 (25%)	9 (75%)	0.02 *
Other <i>KRAS</i> mutations ³	48	30 (63%)	18 (37%)	

* *KRAS* codon 13 mutation correlates significantly *TP53* mutation compared to other *KRAS* mutations.

¹ wt: wildtype

² mut: mutant

³ Other *KRAS* mutations: Mutations in codons 12, 61, 6, 22, and 64 as listed in Table 2.