Published in final edited form as:

Cancer. 2014 December 15; 120(24): 3965–3971. doi:10.1002/cncr.28954.

KRAS Mutation Influences Recurrence Patterns in Patients Undergoing Hepatic Resection of Colorectal Metastases

Nancy E. Kemeny, MD^{*}, Joanne F. Chou, MPH^{**}, Marinela Capanu, PhD^{**}, Alexandra N. Gewirtz, BA^{*}, Andrea Cercek, MD^{*}, T. Peter Kingham, MD[†], William R. Jarnagin, MD[†], Y Fong[†], Ronald P. DeMatteo, MD[†], Peter J. Allen[†], Jinru Shia, MD[‡], Celina Ang, MD^{*}, Efsevia Vakiani, MD[‡], and Michael I. D'Angelica, MD[†]

^{*}Department of Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York NY 10065

^{**}Department of Biostatistics, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York NY 10065

[‡]Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York NY 10065

[†]Department of Surgery, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York NY 10065

Abstract

Background—The validity of KRAS mutation as a predictor of recurrence free survival (RFS) or overall survival (OS) is unclear. This study investigated whether KRAS mutation decreases RFS or OS in patients with colorectal cancer who underwent liver resection.

Methods—Patients with resected colorectal liver metastases who were treated with adjuvant hepatic arterial infusion (HAI) plus systemic therapy and in whom KRAS data was available were evaluated. Correlation between KRAS and clinical factors was evaluated using Fisher's exact test. Kaplan-Meier methods were used to estimate median overall RFS and OS.

Results—169 patients were evaluated: 118 KRAS wild type (WT) and 51 mutated (MUT). Three year RFS was 46% for KRAS WT [95%CI: 35-56%], and 30% [95%CI: 16-44%] for MUT patients ($p=0.005$). Three year OS was 95% [87%-98%] and 81% [62%-95%] for KRAS WT and MUT patients, respectively ($p=0.07$). By multivariate analysis, KRAS remained a significant predictor of RFS (HR: 1.9). Cumulative recurrence by 3 years in sites showed: 2% versus 13.4% for bone metastases ($p<0.01$), 2% versus 14.5% for brain ($p=0.05$), 33.2% versus 58% for lung ($p<0.01$), and 30 versus 47% for liver ($p=0.10$) in KRAS WT versus MUT patients, respectively.

Conclusions—In patients with resected colorectal liver metastases treated with adjuvant HAI plus systemic therapy, patients with KRAS mutation had a significantly worse 3 year RFS of 46% versus 30% ($p=0.005$) for KRAS WT vs MUT, respectively. Cumulative incidence of bone, brain,

Dr. Nancy Kemeny Attending Physician, Department of Medicine; Memorial Sloan-Kettering Cancer Center Professor of Medicine, Joan and Sanford I. Weill Medical College of Cornell University 1275 York Avenue New York NY 10065 kemenyn@mskcc.org Tel: 646-888-4180 Fax: 646-888-4541.

Financial disclosures/conflicts of interest/funding sources: none

and lung metastases was significantly higher for KRAS MUT patients as compared to WT patients.

Keywords

colorectal cancer; liver metastases; adjuvant therapy; KRAS mutation; recurrence patterns

Many factors predict overall survival (OS) or recurrence free survival (RFS) for patients with metastatic colorectal cancer such as baseline alkaline phosphatase (alk phos) (1), performance status, white blood cell count (WBC), lactic dehydrogenase (LDH) (2) and carcinoembryonic antigen (CEA) (1, 2). There have been conflicting reports about whether KRAS mutation influences outcome. In one trial of 394 patients, OS was 26.7 months for KRAS wild type (WT) patients and 18 months for KRAS mutated (MUT) patients ($p=0.002$) (3). In their multivariate analysis, KRAS mutation and high LDH were significantly related to worse survival. Others report no significant difference in OS between KRAS WT and MUT patients (4-6). KRAS mutation has also been reported to influence outcomes in patients after liver resection for metastatic disease. Karagkounis showed worse RFS (HR 1.89) and OS (HR 2.13) in those patients with KRAS mutation (7). We performed this study to determine if KRAS status affects outcome in patients treated with adjuvant hepatic arterial infusion (HAI) plus systemic therapy after liver resection.

Methods

The Institutional Review Board allowed a review of patients during the time period of 3/19/03 to 1/19/13 who underwent liver resection followed by adjuvant HAI plus systemic chemotherapy and in whom KRAS data was available. At surgery, all disease was resected or ablated and HAI pump was placed concurrently. One month post surgery, chemotherapy was commenced. Recurrence patterns and survival were evaluated for patients who met the above criteria. Genotyping became standard of care only in 2009 at Memorial Sloan Kettering Cancer Center (MSKCC).

Correlation between KRAS status and clinical factors such as prior chemotherapy, postoperative CEA, clinical risk score (CRS), stage at diagnosis, and whether radiofrequency ablation (RFA) was performed at time of pump placement were evaluated using Fisher's exact test and the Wilcoxon rank sum test. OS and RFS were calculated from time of HAI until the time of any cause of death (for OS) or until the first relapse or death, whichever came first (for RFS). In our cohort, all deaths, except one patient who possibly expired due to myocardial infarction, were related to disease, and hence overall survival was equivalent to disease specific survival. First relapse was confirmed by CT or MRI. Scans were performed every 3 months for the first 2 years, then every 4 months for year 3, every 6 for year 4 and subsequently yearly. OS and RFS were estimated using the Kaplan-Meier method. Log-rank test was used to determine whether survival functions differed by KRAS mutation status and other clinical and pathologic characteristics. Cox proportional hazards model was used to examine association between continuous clinical factors such as baseline CEA and LDH and OS/RFS. Factors significant in the univariate analyses (p value ≤ 0.10) were included in multivariate Cox proportional hazards model examining the effect of

KRAS separately on OS and RFS. Left sided primary tumors were defined as descending colon, rectosigmoid, rectum, and sigmoid colon; all other locations were considered right sided. Positive margin was defined as < 0.1 cm.

Cumulative incidence function was used to estimate the probability of time from start of HAI to recurrence in bone, brain, lung or liver. All p values were based on 2-tailed statistical analysis, and all analyses were performed with SAS version 9.2 (SAS Institute, Cary, North Carolina) and R (2.10.1).

For each tumor, a hematoxylin and eosin-stained (H&E) section was reviewed by a gastrointestinal pathologist (E.V.). DNA was extracted from formalin-fixed paraffin-embedded tumor. Specifically, all H&E slides of every tumor resection were reviewed for confirmation of pathological diagnosis, and to identify the appropriate tissue block for molecular studies. Once a block was selected for KRAS mutation analysis, 10 consecutive sections were then made from the block, one of which was stained with H&E. This H&E slides then served to guide the macro-dissection of the tumor regions from the remaining 9 unstained sections. The quality of whole genome amplified DNA was verified by PCR reactions using 2 control amplicons. Mutations in KRAS (codons 12, 13) were detected using the iPLEX assay (Sequenom, Inc., San Diego, CA), as previously described (8).

In 38 patients, the KRAS was determined from the primary, 119 from liver metastases at the time of resection, and 12 from recurrences after resection. KRAS is considered to be an early event in colorectal carcinogenesis, and its mutation status remains consistent from primary to metastasis as we reported previously (9).

Treatment after resection consisted of HAI infusion of FUDR $0.12 \text{ g/kg} \times 30$ / flow rate mixed with decadron plus heparin administered for a two week infusion monthly. Systemic treatment included fluorouracil infusion and bolus leucovorin plus oxaliplatin (Oxali) if no prior Oxali was administered, and irinotecan based if prior Oxali was used. Treatment details and dose reductions are reported in prior papers (10).

Results

Four hundred and two patients underwent hepatic resection followed by HAI and systemic therapy during the study's time period. Of these, 169 patients had KRAS analysis available; 118 were KRAS WT, and 51 were KRAS MUT (45 G12, 5 G13, 1 K117N). All patients had KRAS G12 evaluation while 162 also had BRAF analysis, of which only 1 patient was mutated (0.6%). Of 91 patients with further evaluations, 3% were NRAS mutated, 10% PI3K mutated, and 0% had MEK 1/2 mutations.

Baseline characteristics did not differ significantly between KRAS WT or KRAS MUT patients (Table 1) except for more mutations in right sided tumors: 26% versus 45% for WT versus MUT, respectively. Clinical Risk Score (CRS) is defined as 1 point for the following variables: node positive primary, disease free interval < 12 months, > 1 tumor, size of metastases > 5 cm, CEA > 200 ng/ml and was not significantly different. CRS above 3 was seen in 43% and 57% of KRAS WT and KRAS MUT patients, ($p=0.10$), respectively. Median follow up was 44.3 months [range 1.41-119 months].

Recurrence Free Survival (RFS)

At the end of the follow-up, we observed 80 events (recurrence or death). The median RFS was 20 months [95% CI: 14-31 months], with a 3 year RFS for the entire group of 41% [95% CI: 33%-50%]. Three year RFS for patients with KRAS WT was 46% [95% CI, 35-56%] and 30% for KRAS MUT [95% CI, 16-44%] (Figure 1, $p=0.005$). In the univariate analysis, factors significantly associated with shorter RFS (Table 2) were prior use of Oxaliplatin (Oxali) (yes/no) median RFS 15 versus 62 months ($p=0.01$), prior use of Bevacizumab (Bev) (yes/no) median RFS 14 versus 26 months ($p=0.03$) and number of lesions (3/2) RFS 15 versus 24 months, respectively ($p<0.01$, Table 2). There was borderline association between RFS and age ($p=0.08$) and bilobar disease ($p=0.07$). Positive tumor margin, baseline CEA and LDH were not significantly associated with RFS ($p=0.33$, $p=0.16$ and $p=0.86$, respectively). In the multivariate analysis (Table 3), the effect of KRAS remained significant after adjusting for prior use of Oxali or Bev, number of lesions, age, bilobar disease and gender (HR: 1.9 [95% CI: 1.16-3.31], $p=0.01$).

Overall Survival (OS)

Median follow up was 48 months. Median OS was not reached and was 91% [95% CI: 84%-95%] at 3 years. OS at 3 years for KRAS WT and KRAS MUT patients was 95% [95% CI: 87%-98%] and 81% [95% CI: 62%-95%] ($p=0.07$), respectively (Figure 2). By univariate analysis, exposure to Oxali prior to resection (prior oxaliplatin vs no prior oxaliplatin: 100% vs 83% at 3 years) ($p<0.01$), and positive resection margin were associated with survival ($p=0.032$). There was borderline association between OS and females: 95% versus 88% for males at 3 years ($p=0.053$) and baseline CEA ($p=0.096$). Baseline LDH was not significantly associated with OS ($p=0.97$). Other patient characteristics did not significantly affect OS. In the multivariate model, the effect of KRAS on OS was not statistically significant after adjusting for prior exposure to Oxali, baseline CEA and positive margin [HR=2.0, 95% CI: 0.87-4.46, $p=0.104$]. Similar results were found when we further adjusted for age and gender.

Cumulative incidence of recurrence to individual sites

At three years, the cumulative incidence of bone metastases was 2% [0-5.7%] versus 13.4% [0.3-26.5%] ($p<0.01$), brain metastases was 2% [0-5.7%] versus 14.5% [0-30%] ($p=0.0533$) and lung metastases in 33.2% [22.7-43.6%] versus 58% [40-76%] ($p<0.01$) in KRAS WT versus MUT, respectively. The cumulative incidence of recurrence in the liver was not statistically significant: 30% [20-41%] and 47.3% [28.7-65.8%] ($p=0.10$) for KRAS WT and MUT, respectively (Table 4).

Discussion

Factors predicting survival in metastatic colorectal cancer have included LDH, WBC, CEA, alk phos (1, 2), performance status, number of metastases and sites involved. Recently, investigators have shown that molecular factors may affect survival (3, 11). A decrease in survival among patients with KRAS MUT tumors has been observed in some studies (3) but others show no difference (5, 6). In the Kirsten Ras In-Colorectal-Cancer Collaborative

Group (RASCAL II) trial of 2721 colorectal patients, a mutation of KRAS Codon 12 from glycine to valine significantly increased the risk of recurrence and death in Duke C patients (11). In patients with resected hepatic metastases, the number and size of metastases, synchronous disease, and positive hepatic resection margins are additional prognostic factors (12, 13). In patients who have undergone liver resection, MD Anderson Cancer Center reported a lower 3 year OS of 52% versus 81% ($p=0.002$) and RFS of 13.5% versus 33.5% ($p=0.001$), for KRAS MUT versus WT, respectively. A decrease in 3 year lung RFS in patients with KRAS MUT (34% versus 59%) (14) was also observed. Hagness examined recurrence in patients after liver transplantation and showed an increase in pulmonary metastases (15). A Johns Hopkins review reported a 5 year survival of 49.8% versus 57.4%, and a 3 year RFS of 27.7% versus 34%, for KRAS MUT versus WT, respectively (7).

The present study includes patients who have undergone liver resection followed by adjuvant HAI and systemic therapy. The 3 year RFS was 30% and 46% and 3 year OS 81% and 95%, for KRAS MUT versus WT, respectively. The present trial reported a significant decrease in lung RFS in KRAS MUT patients with 3 year RFS of 42% versus 60.8% for KRAS MUT versus WT. An increase in bone and brain metastases was also seen in KRAS MUT patients. It is unclear whether this is unique to this patient population, since it was not reported in the other studies which may have considered only the first site of metastases or did not report on rarer metastases. Patients with colorectal cancer frequently develop liver metastases with lung as the next most frequent metastatic site. Less common recurrences occur in the central nervous system, skeleton, adrenals, and spleen, accounting for <10% of cases (16). Brain and bone metastases are much more common in breast cancer, and molecular factors may also affect the presence of these metastatic sites, where increased brain metastases (17) and a more rapid development of brain metastases are seen with HER2 positive cells in the primary (18). It has been suggested that CNS migration of some tumor cells might be stimulated by a mechanism mediated by CNS neuregulin (19) of which ErbB2 is an integral member.

A review of recurrence patterns in 1696 patients with resected liver metastases reported 43.2% hepatic recurrence, 21% hepatic and other sites, and 35.8% other sites alone (i.e. 64% recurrence in liver) (20). In the MSKCC trial there was a decrease in liver recurrence in both KRAS WT and MUT (30% and 47%, respectively) compared to the other trials, which likely was related to HAI therapy. A significant decrease in liver recurrence has also been reported with HAI therapy post resection in three randomized trials comparing HAI to systemic therapy(21-23).

In studies analyzing mutations in resected metastases, the prevalence of KRAS mutation was detected more often in lung metastases (62%) and brain metastases (56%) and less often in liver metastases (32%) (24). The same authors studied 859 stage II and III patients and found KRAS mutation in the primary was associated with lung relapse (HR 2.1) ($p=0.007$) but not with relapse in the liver (HR=0.9) (24). Others have also reported more frequent lung metastases in KRAS MUT patients, and less liver metastases in patients with KRAS MUT (25).

Other molecular markers may influence where sites of progression develop in colorectal cancer. PIK3CA mutations were found in a higher number of lung metastases (20%) and brain metastases (23.9%) versus liver metastases (7.7%) (24). Patients with phosphatase and tensin homolog (PTEN) loss had less lung metastases (26). Higher levels of thymidylate synthase gene expression were seen in pulmonary versus liver metastases (27). High levels of dihydropyrimidine dehydrogenase (DPD) have been reported in patients with liver metastases, while lower levels were seen in patients with lung metastases (28). Other studies show poor survival for patients with colon cancer who have KRAS 13 or BRAF mutations, with BRAF patients developing more peritoneal metastases (29).

Do patients with KRAS mutations have tumors with a more aggressive behavior? In paraffin sections of autopsy specimens, aneuploid colorectal carcinomas had significantly higher prevalence of KRAS mutations, 63% vs 37% ($p<0.05$) (30). KRAS mutations also correlated with vascular invasion and hematogenous metastases (31). RAS enhances metastatic ability in several cell types. Varghese H. et al found in a murine model that only micrometastases in RAS-transformed cell lines formed macrometastases with significantly decreased apoptosis in the RAS transformed cells (32). Others also demonstrated RAS causing tumorigenesis and metastatic properties in rodent cells (33). In cholangiocarcinoma, perineural invasion was exclusively associated with KRAS mutations; furthermore, the presence of a KRAS mutation predicted poorer survival (34). In 124 KRAS MUT patients with either resected or sampled colorectal liver metastases, there was a shorter time to the detection of liver metastases with a worse survival in KRAS MUT patients (35).

In conclusion, this study of patients with resected hepatic colorectal metastases followed by adjuvant therapy (HAI and systemic) observed a decrease in RFS with KRAS mutation. This effect persisted after adjusting for factors known to influence recurrence in patients after liver resection. Three year RFS was 48% and 30% for KRAS WT vs MUT patients, respectively ($p=0.005$). Three year OS was 95% vs 81% for KRAS WT vs MUT patients, respectively ($p=0.071$). Others have shown an increase in lung metastases with KRAS mutations, but they have not examined recurrence in other sites. In this study, those with the KRAS mutation had a higher incidence of bone and lung metastases (<0.01) compared to KRAS WT. KRAS MUT patients also appeared to have a higher incidence of brain metastases compared to KRAS WT, although this association was only borderline significant ($p=0.0533$). These trends have to be examined in larger cohorts of patients to see if this is really accurate and whether change in follow-up should be implemented for KRAS MUT patients. Overall survival was not significantly shorter for KRAS MUT patients in this trial, although it was in two other trials examining patients after liver resection with KRAS mutations (7,14). This may be due to the HAI therapy administered post liver resection which decreased hepatic recurrences and thereby may have improved overall survival.

Acknowledgments

This research was supported in part by NIH/NCI Cancer Center Support Grant P30 CA008748.

References

1. Kohne CH, Cunningham D, Di Costanzo F, Glimelius B, Blijham G, Aranda E, et al. Clinical determinants of survival in patients with 5-fluorouracil-based treatment for metastatic colorectal cancer: results of a multivariate analysis of 3825 patients. *Ann Oncol.* 2002; 13(2):308–17. [PubMed: 11886010]
2. Kemeny N, Braun DW. Prognostic Factors in Advanced Colorectal-Carcinoma - Importance of Lactic-Dehydrogenase Level, Performance Status, and White Blood-Cell Count. *Am J Med.* 1983; 74(5):786–94. [PubMed: 6837602]
3. Diaz-Rubio E, Gomez-Espana A, Massuti B, Sastre J, Reboredo M, Manzano JL, et al. Role of Kras Status in Patients with Metastatic Colorectal Cancer Receiving First-Line Chemotherapy plus Bevacizumab: A TTD Group Cooperative Study. *Plos One.* 2012; 7(10)
4. Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, et al. Fluorouracil, Leucovorin, and Oxaliplatin With and Without Cetuximab in the First-Line Treatment of Metastatic Colorectal Cancer. *J Clin Oncol.* 2009; 27(5):663–71. [PubMed: 19114683]
5. Teng HW, Huang YC, Lin JK, Chen WS, Lin TC, Jiang JK, et al. BRAF mutation is a prognostic biomarker for colorectal liver metastasectomy. *J Surg Oncol.* 2012; 106(2):123–9. [PubMed: 22331825]
6. Tol J, Koopman M, Cats A, Rodenburg CJ, Creemers GJM, Schrama JG, et al. Chemotherapy, Bevacizumab, and Cetuximab in Metastatic Colorectal Cancer. *New Engl J Med.* 2009; 360(6):563–72. [PubMed: 19196673]
7. Karagkounis G, Torbenson MS, Daniel HD, Azad NS, Diaz LA, Donehower RC, et al. Incidence and Prognostic Impact of KRAS and BRAF Mutation in Patients Undergoing Liver Surgery for Colorectal Metastases. *Cancer-Am Cancer Soc.* 2013; 119(23):4137–44.
8. Janakiraman M, Vakiani E, Zeng ZS, Pratilas CA, Taylor BS, Chitale D, et al. Genomic and Biological Characterization of Exon 4 KRAS Mutations in Human Cancer. *Cancer Res.* 2010; 70(14):5901–11. [PubMed: 20570890]
9. Vakiani E, Janakiraman M, Shen R, Sinha R, Zeng Z, Shia J, et al. Comparative Genomic Analysis of Primary Versus Metastatic Colorectal Carcinomas. *J Clin Oncol.* 2012; 30(24):2956–62. [PubMed: 22665543]
10. Kemeny NE, Jarnagin WR, Capanu M, Fong YM, Gewirtz AN, DeMatteo RP, et al. Randomized Phase II Trial of Adjuvant Hepatic Arterial Infusion and Systemic Chemotherapy With or Without Bevacizumab in Patients With Resected Hepatic Metastases From Colorectal Cancer. *J Clin Oncol.* 2011; 29(7):884–9. [PubMed: 21189384]
11. Andreyev HJN, Croke DT, Smith DR. Kirsten ras mutations in patients with colorectal cancer: the “RASCAL II” study (vol 85, pg 692, 2001). *Brit J Cancer.* 2001; 85(11):1812.
12. Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer - Analysis of 1001 consecutive cases. *Ann Surg.* 1999; 230(3):309–18. [PubMed: 10493478]
13. Nordlinger B, Guiguet M, Vaillant JC, Balladur P, Boudjema K, Bachellier P, et al. Surgical resection of colorectal carcinoma metastases to the liver - A prognostic scoring system to improve case selection, based on 1568 patients. *Cancer-Am Cancer Soc.* 1996; 77(7):1254–62.
14. Vauthey JN, Zimmitti G, Kopetz SE, Shindoh J, Chen SS, Andreou A, et al. RAS Mutation Status Predicts Survival and Patterns of Recurrence in Patients Undergoing Hepatectomy for Colorectal Liver Metastases. *Ann Surg.* 2013; 258(4):619–27. [PubMed: 24018645]
15. Hagness M, Foss A, Egge TS, Dueland S. Patterns of recurrence after liver transplantation for nonresectable liver metastases from colorectal cancer. *Annals of surgical oncology.* 2014; 21(4): 1323–9. [PubMed: 24370906]
16. Schluter K, Gassmann P, Enns A, Korb T, Hemping-Bovenkerk A, Holzen J, et al. Organ-specific metastatic tumor cell adhesion and extravasation of colon carcinoma cells with different metastatic potential. *Am J Pathol.* 2006; 169(3):1064–73. [PubMed: 16936278]
17. Jung M, Ahn JB, Chang JH, Suh CO, Hong S, Roh JK, et al. Brain metastases from colorectal carcinoma: prognostic factors and outcome. *J Neuro-Oncol.* 2011; 101(1):49–55.

18. Duchnowska R, Szczylik C. Central nervous system metastases in breast cancer patients administered trastuzumab. *Cancer Treat Rev.* 2005; 31(4):312–8. [PubMed: 15979804]
19. Ritch PA, Carroll SL, Sontheimer H. Neuregulin-1 enhances motility and migration of human astrocytic glioma cells. *J Biol Chem.* 2003; 278(23):20971–8. [PubMed: 12600989]
20. de Jong MC, Pulitano C, Ribero D, Strub J, Mentha G, Schulick RD, et al. Rates and patterns of recurrence following curative intent surgery for colorectal liver metastasis: an international multi-institutional analysis of 1669 patients. *Ann Surg.* 2009; 250(3):440–8. [PubMed: 19730175]
21. Kemeny MM, Adak S, Gray B, Macdonald JS, Smith T, Lipsitz S, et al. Combined-modality treatment for resectable metastatic colorectal carcinoma to the liver: Surgical resection of hepatic metastases in combination with continuous infusion of chemotherapy - An intergroup study. *J Clin Oncol.* 2002; 20(6):1499–505. [PubMed: 11896097]
22. Kemeny N, Huang Y, Cohen AM, Shi WJ, Conti JA, Brennan MF, et al. Hepatic arterial infusion of chemotherapy after resection of hepatic metastases from colorectal cancer. *New Engl J Med.* 1999; 341(27):2039–48. [PubMed: 10615075]
23. Lygidakis NJ, Sgourakis G, Vlachos L, Raptis S, Safioleas M, Boura P, et al. Metastatic liver disease of colorectal origin: The value of locoregional immunochemotherapy combined with systemic chemotherapy following liver resection. Results of a prospective randomized study. *Hepato-Gastroenterol.* 2001; 48(42):1685–91.
24. Tie J, Lipton L, Desai J, Gibbs P, Jorissen RN, Christie M, et al. KRAS Mutation Is Associated with Lung Metastasis in Patients with Curatively Resected Colorectal Cancer. *Clin Cancer Res.* 2011; 17(5):1122–30. [PubMed: 21239505]
25. Kim MJ, Lee HS, Kim JH, Kim YJ, Kwon JH, Lee JO, et al. Different metastatic pattern according to the KRAS mutational status and site-specific discordance of KRAS status in patients with colorectal cancer. *Bmc Cancer.* 2012; 12
26. Price TJ, Hardingham J, Lee C, et al. Prognostic impact and the relevance of PTEN copy number alterations in patients with advanced colorectal cancer (CRC) receiving bevacizumab. *Cancer Medicine.* 2013; 2:277–285. [PubMed: 23930204]
27. Gorlick R, Metzger R, Danenberg KD, Salonga D, Miles JS, Longo GSA, et al. Higher levels of thymidylate synthase gene expression are observed in pulmonary as compared with hepatic metastases of colorectal adenocarcinoma. *J Clin Oncol.* 1998; 16(4):1465–9. [PubMed: 9552053]
28. Akagi Y, Kinugasa T, Mizobe T, Kawahara A, Kage M, Shirouzu K. Expression of Dihydropyrimidine Dehydrogenase, Orotate Phosphoribosyl Transferase and Thymidylate Synthase in Patients with Primary Colorectal Cancer, and Associations with Site of First Metastasis. *Anticancer Res.* 2012; 32(6):2277–82. [PubMed: 22641663]
29. Tran B, Kopetz S, Tie J, Gibbs P, Jiang ZQ, Lieu CH, et al. Impact of BRAF Mutation and Microsatellite Instability on the Pattern of Metastatic Spread and Prognosis in Metastatic Colorectal Cancer. *Cancer-Am Cancer Soc.* 2011; 117(20):4623–32.
30. Suchy B, Zietz C, Rabes HM. K-Ras Point Mutations in Human Colorectal Carcinomas - Relation to Aneuploidy and Metastasis. *Int J Cancer.* 1992; 52(1):30–3. [PubMed: 1500224]
31. Tanaka M, Omura K, Watanabe Y, Oda Y, Nakanishi I. Prognostic Factors of Colorectal-Cancer - K-Ras Mutation, Overexpression of the P53-Protein, and Cell Proliferative Activity. *J Surg Oncol.* 1994; 57(1):57–64. [PubMed: 7914949]
32. Varghese HJ, Davidson MTM, MacDonald IC, Wilson SM, Nadkarni KV, Groom AC, et al. Activated Ras regulates the proliferation/apoptosis balance and early survival of developing micrometastases. *Cancer Res.* 2002; 62(3):887–91. [PubMed: 11830548]
33. Webb CP, Van Aelst L, Wigler MH, Vande Woude GF. Signaling pathways in Ras-mediated tumorigenicity and metastasis. *P Natl Acad Sci USA.* 1998; 95(15):8773–8.
34. Chen TC, Jan YY, Yeh TS. K-ras Mutation is Strongly Associated with Perineural Invasion and Represents an Independent Prognostic Factor of Intrahepatic Cholangiocarcinoma after Hepatectomy. *Annals of surgical oncology.* 2012; 19:S675–S81. [PubMed: 22805857]
35. Nash GM, Gimbel M, Shia J, Nathanson DR, Ndubuisi MI, Zeng ZS, et al. KRAS Mutation Correlates With Accelerated Metastatic Progression in Patients With Colorectal Liver Metastases. *Annals of surgical oncology.* 2010; 17(2):572–8. [PubMed: 19727962]

Figure 1. shows the 3 year RFS for KRAS WT versus KRAS MUT truncated at 60 months.

Figure 2. shows the 3 year OS for KRAS WT versus KRAS MUT truncated at 60 months.

Table 1

Baseline Characteristics of KRAS WT versus KRAS MUT

	KRAS WT (N=118)	KRAS MUT (N=51)	P Value
Baseline CEA Median [range]	2.6 [0.8 – 49.4]	2.4 [0.9 – 125.9]	0.52
Baseline LDH Median [range]^a	156 [99-320]	164 [90-227]	0.27
Age, median [range]	54 [27-83]	56 [36-79]	0.45
Gender			0.86
Female	48 (41%)	22 (43%)	
Male	70 (59%)	29 (47%)	
Dukes Stage			0.08
B	56 (47%)	17 (33%)	
C	62 (53%)	34 (67%)	
Prior Chemotherapy			0.94
No	19 (16%)	8 (16%)	
Yes	99 (84%)	43 (84%)	
Clinical Risk Score			0.10
(0,1,2)	67 (57%)	22 (43%)	
(3,4,5)	51 (43%)	29 (57%)	
Lesions			0.64
1	32 (27%)	11 (22%)	
2	24 (20%)	13 (25%)	
3+	53 (48%)	23 (48%)	
Margin			0.13
Positive	7 (6%)	7 (14%)	
Negative	109 (94%)	44 (86%)	
Prior Oxaliplatin			0.15
No	46 (39%)	14 (27%)	
Yes	72 (61%)	37 (73%)	
Primary tumor (Right or Left)			0.016
Right	31 (26%)	23 (45%)	
Left	87 (74%)	28 (55%)	

^a Note: 3 patients (2 WT and 1 MUT) were missing baseline LDH

Table 2Recurrence Free Survival Univariate Analysis^a

Characteristics	Number	1 YEAR RFS %	3 YEAR RFS %	MEDIAN RFS	P Value
Prior Chemotherapy					0.69
No	27	81	44	20.0	
Yes	138	65	41	19.4	
Prior Oxaliplatin					0.01
No	60	78	51	62.0	
Yes	105	61	34	15.4	
Prior Bevacizumab					0.03
No	120	71	47	26.4	
Yes	45	60	29	14.0	
Sex					0.53
F	69	62	46	26.1	
M	96	72	38	19.3	
Synchronous Tumor					0.16
N	52	76	49	31.4	
Y	113	64	37	19.3	
Bilobar					0.07
N	83	74	46	24.0	
Y	82	62	37	15.4	
CRS score					0.40
0,1,2	88	70	44	23.2	
3,4,5	77	66	38	19.3	
Lesions					<0.01
1	41	73	65	NR ^b	
2	37	78	48	24.0	
3+	74	65	28	15.4	
Largest size of tumor					0.78
<3cm	81	0-64	0-40	19.3	
>=3cm	84	0-72	0-43	24.0	
Margins					0.33
Positive	14	55	31	13.4	
Negative	149	69	42	20.0	
Pump dose					0.24
<42%	70	60	38	15.6	
>=42%	80	74	44	26.1	
Systemic Dose					0.96
<83%	66	0-68	0-44	20.7	

Characteristics	Number	1 YEAR RFS %	3 YEAR RFS %	MEDIAN RFS	P Value
>=83%	76	0.71	0.40	19.3	
Age					0.08
<55	82	0.61	0.34	16.5	
>=55	83	0.75	0.49	28.9	
Primary Tumor (Right or Left)					0.18
Right	53	68	33	17.8	
Left	112	68	45	26.1	

^a 4 patients excluded who recurred prior to pump treatment

^b NR=not reached

Table 3

Multivariate RFS model

Characteristics	95%CI	P Value
KRAS (MUT vs WT)	1.9 (1.16-3.31)	0.0102
Prior Oxaliplatin (Yes vs No)	1.4 (0.8-2.4)	0.2027
Prior Bevacizumab (Yes vs No)	1.03 (0.58-1.81)	0.9222
Number of Lesions		
3+ vs 1	2.7 (1.3-5.5)	0.0117
2 vs 1	1.4 (0.6-3.1)	0.4153
Age		
>=55 vs <55	0.7 (0.44-1.16)	0.179
Bilobar (No vs Yes)	1.2 (0.7-2.09)	0.407
Gender (F vs M)	0.9 (0.5-1.4)	0.858

Table 4

Cumulative Incidence by 3 Years in 169 Patients

Site of Recurrence	KRAS WT (N=118)	KRAS MUT (N=51)
Bone	2% (0%-5.7%)	13.4% (0.3%-26.5%)
Brain	2% (0%-5.7%)	14.5% (0%-30%)
Lung	33.2% (22.7%-43.6%)	58% (40%-76%)
Liver	30% (20%-41%)	47.3% (28.7%-65.8%)