J Gastrointest Surg (2017) 21:68–77
DOI 10.1007/s11605-016-3189-9

2016 SSAT PLENARY PRESENTATION

RAS Mutation Is Associated with Decreased Survival in Patients Undergoing Repeat Hepatectomy for Colorectal Liver Metastases

Jason W. Denbo[1] · Suguru Yamashita[1] · Guillaume Passot[1] · Michael Egger[1] · Yun S. Chun[1] · Scott E. Kopetz[2] · Dipen Maru[3] · Kristoffer Watten Brudvik[1] · Steven H. Wei[1] · Claudius Conrad[1] · Jean-Nicolas Vauthey[1] · Thomas A. Aloia[1]

Received: 26 May 2016 / Accepted: 12 June 2016 / Published online: 22 June 2016

Abstract

Background The relationship between *RAS* mutation status and outcome for patients undergoing repeat hepatectomy (RH) for recurrent colorectal liver metastases (CLM) has not been defined.

Objective The objective of this study was to evaluate the relationship between *RAS* mutation status and outcome in patients undergoing RH for CLM.

Methods All patients who underwent RH for CLM with known *RAS* mutation status between January 2005 and November 2014 were identified, and the outcomes of patients with and without *RAS* mutations were compared.

Results Ninety-eight patients underwent RH, of whom 34 (35 %) harbored a *RAS* mutation. Wild-type (WT) and mutant *RAS* groups had similar clinicopathologic characteristics. Median recurrence-free survival (RFS) for patients with WT and mutant *RAS* was 12.2 and 6.1 months, respectively ($p = 0.03$). Median overall survival (OS) for the WT and mutant *RAS* patients were 42.5 and 26.6 months, respectively ($p < 0.01$). On multivariate analysis, *RAS* mutations [hazard ratio (HR) = 1.69, $p = 0.04$] were associated with worse RFS, while multiple tumors (HR = 1.92, $p = 0.045$) and RAS mutations (HR = 2.11, $p = 0.02$) predicted worse OS.

Conclusion Patients with recurrent CLM that harbor *RAS* mutations have worse RFS and OS than patients with WT *RAS*, and *RAS* mutations are independently associated with worse RFS and OS. *RAS* mutation status should be determined prior to RH, as it may impact treatment decisions.

Keywords *RAS* mutation · Colorectal liver metastases · Repeat hepatectomy · Survival

Introduction

The *RAS* family of proteins is involved in cell signaling and regulation of cell division and proliferation.[1,2] *KRAS* mutations occur frequently in colorectal cancer and other types of cancer and have long been implicated in carcinogenesis.[1,2] More recently, there has been a resurgence of interest in *RAS* mutations with the advent of targeted therapies.[3] Van Cutsem et al. published a randomized trial in patients with metastatic epidermal growth factor receptor (EGFR)-positive colorectal cancer in which patients received FOLFIRI alone or in combination with cetuximab.[3] The addition of cetuximab reduced the risk of disease progression, but this risk reduction was limited to patients with wild-type (WT) *KRAS* tumors. Over

✉ Thomas A. Aloia
taaloia@mdanderson.org

[1] Department of Surgical Oncology, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 1484, Houston, TX 77030, USA

[2] Department of Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA

[3] Department of Pathology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA

the next several years, it became apparent that *RAS* mutations were a marker of tumor biology and had potential prognostic value.[4–8] Several series have evaluated patients with colorectal liver metastases (CLM) and have found that *RAS* mutations are associated with worse survival,[4,9] higher rates of lung recurrence,[4,10] poor radiologic and pathologic responses to chemotherapy,[5] and higher rates of margin positive resections.[6] A recent meta-analysis found that *KRAS* mutations were negatively associated with recurrence-free survival (RFS) and overall survival (OS).[11] The preponderance of evidence suggests that *RAS* mutation status in CLM has prognostic significance and may help guide therapy.

Hepatectomy is well accepted as the best treatment for CLM and effectively prolongs survival.[12,13] Despite good long-term survival,[12,13] most patients develop recurrence after hepatectomy for CLM.[14,15] Several studies have demonstrated the safety and efficacy of repeat hepatectomy (RH) in selected patients.[15–18] Despite 5-year survival rates up to 73 % following RH,[16] recurrence rates remain high with median RFS of 7 months.[16] Andreou et al. reported that a positive surgical margin at RH was associated with worse RFS.[16] In summary, although long-term survival can be achieved following RH, recurrence following RH develops early and often. Hepatobiliary surgeons and their multidisciplinary partners are in need of molecular and other biomarkers that will

Table 1 Tissue site used for *RAS* mutational analysis

	Total $n = 98$	WT *RAS* $n = 64$	Mutant *RAS* $n = 34$	p value
Tumor site, *N* (%)				.264
Primary only	27 (28)	21 (33)	6 (18)	
Metastatic only	57 (58)	34 (53)	23 (68)	
Both	14 (14)	9 (14)	5 (15)	

Table 2 Clinicopathologic characteristics of patients undergoing repeat hepatectomy for recurrent CLM with known *RAS* mutation status

Characteristics	Total $n = 98$	WT *RAS* $n = 64$	Mutant *RAS* $n = 34$	p value
Patient demographics, *N* (%)				
Age, median (range)	57 (29–80)	57 (32–76)	57 (29–80)	.40
Male sex	63 (64)	44 (69)	19 (56)	.21
Tumor characteristics, *N* (%)				
Primary tumor				
Rectal	24 (24)	18 (28)	6 (18)	.25
Lymph node metastases	73 (74)	45 (70)	28 (82)	.19
Disease-free interval, months (range)	14.7 (1.6–53)	15.3 (1.6–53)	13.1 (3.4–44)	.09
CLM				
No. of CLM	1 (1-8)	1 (1-8)	1 (1-3)	.65
Solitary	66 (67)	43 (67)	23 (68)	.96
Size of largest CLM (cm)	1.8 (0.6-12)	1.8 (0.6-12)	1.9 (0.6-11)	.93
Treatment variables, *N* (%)				
Chemotherapy	91 (93)	59 (92)	32 (94)	.72
Cycles, >6	32 (33)	22 (34)	10 (29)	.70
Lines, ≥2	4 (4)	3 (5)	1 (3)	.66
Oxaliplatin	41 (45)	23 (39)	18 (56)	.11
Irinotecan	48 (53)	15 (47)	33 (56)	.41
Bevacizumab	47 (52)	30 (51)	17 (73)	.84
Cetuximab	14 (15)	11 (19)	3 (9)	.24
Surgery				
Major resection	22 (22)	16 (25)	6 (18)	.41
Ablation	11 (11)	8 (13)	3 (9)	.58
EBL, ml, median (range)	212 (40-1500)	238 (40-1500)	200 (50-1500)	.64
Pathologic variables, *N* (%)				
Positive margin	12 (12)	7 (11)	5 (15)	.59
Major pathologic response	30 (33)	20 (34)	10 (31)	.18

CLM colorectal liver metastasis, *EBL* estimated blood loss

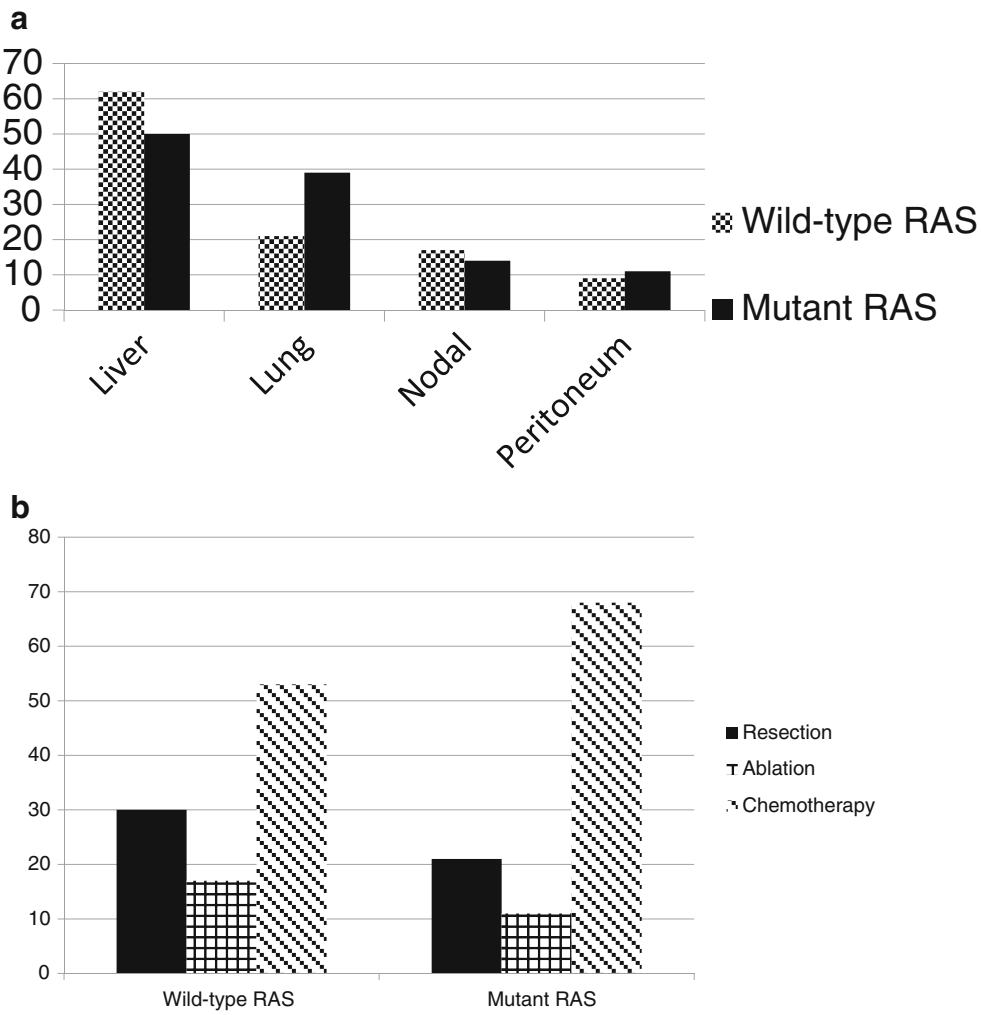

Fig. 1 **a** Recurrence patterns following repeat hepatectomy for recurrent CLM. Percentage of wild-type and mutant *RAS* recurrences in the liver (62 vs. 50 %, $p = 0.32$), lung (21 vs. 39 %, $p = 0.09$), lymph nodes (17 vs. 14 %, $p = 0.76$), and peritoneum (9 vs. 11 %, $p = 0.75$). **b** Treatment of recurrent disease following repeat hepatectomy for recurrent CLM. Percentage of wild-type and mutant *RAS* patients who received an additional resection (30 vs. 21 %, $p = 0.43$), ablation (17 vs. 11 %, $p = 0.46$), or chemotherapy (53 vs. 68 %, $p = 0.21$)

aid in treatment decision making for patients with recurrent metastatic disease.

To date, the relationship between *RAS* mutation status and RH for CLM has not been defined. The present study sought to evaluate the relationship of *RAS* mutation status in patients undergoing RH for recurrent CLM and hypothesized that *RAS* mutation status may help predict which patients would derive the most benefit from RH.

Materials and Methods

Study Population

The Institutional Review Board of the University of Texas MD Anderson Cancer Center approved this study of patients undergoing repeat hepatectomy for CLM. The institutional liver surgery database was used to identify all patients who underwent hepatectomy for CLM with known *RAS* mutation status between January 2005 and November 2014. Next, we identified patients who underwent repeat hepatectomy following recurrence of CLM. Two-stage hepatectomies were considered as one procedure.

Disease Treatment

Computed tomography (CT) of the chest, abdomen, and pelvis with a triphasic liver protocol was used in all patients to assess resectability and extrahepatic disease. After diagnosis of recurrent disease, preoperative oxaliplatin or irinotecan-based chemotherapy was administered to the majority of patients. Parenchymal transection was performed using a two-surgeon technique with the cavitron ultrasonic surgical aspirator (Valleylab, Boulder, CO, USA) and saline-linked cautery (Dissecting Sealer DS 3.0; TissueLink Medical, Inc., Dover, NH, USA) under hepatic inflow and/or outflow occlusion.[19] Following repeat hepatectomy, patients received postoperative chemotherapy and returned every 3–4 months for follow-up, which included surveillance CT scans to assess for recurrence. When recurrence was identified, therapy was at the discretion of the treatment team.

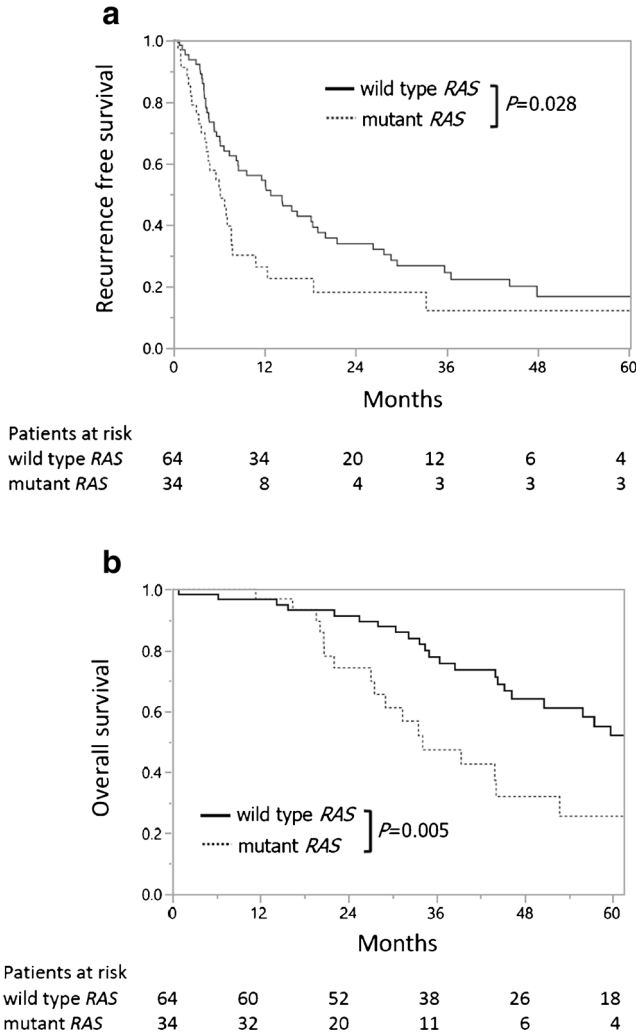

Fig. 2 **a** Recurrence-free survival following repeat hepatectomy for recurrent CLM. Median recurrence-free survival for wild-type and mutant *RAS* patients was 12.2 and 6.1 months, respectively. **b** Overall survival following repeat hepatectomy for recurrent CLM. Median overall survival for wild-type and mutant *RAS* patients was 42.5 and 26.6 months, respectively

Histological Evaluation and *RAS* Mutation Analysis

All surgical specimens underwent histologic evaluation by the pathology department, and the presence of CLM was confirmed, the surgical margins were assessed, and the percentage of viable tumor cells was recorded. A positive margin was defined as viable tumor cells <1 mm from the margin.[20] A major pathologic response to preoperative chemotherapy was defined as ≤49 % viable tumor cells.[21] DNA from the primary tumor or CLM was used to determine *RAS* mutation status as previously described.[4,6] We screened for mutations in *KRAS* codons 12 and 13 in all patients and for mutations in *KRAS* codons 61 and 146 and *NRAS* codons 12, 13, and 61 in the majority of patients treated since 2012.[6,22] The lower limit of detection of the assay was approximately one mutant allele in the background of nine wild-type alleles.[6] Mutations in *KRAS* and *NRAS* codons were collectively reported as *RAS* mutations.

Statistics

Clinicopathologic variables for patients undergoing first repeat hepatectomy for CLM with WT and mutant *RAS* tumors were compared. Quantitative and qualitative variables were expressed as frequency and median (range). Comparisons between groups were analyzed with chi-square or Fischer's exact test for categorical data and the Mann-Whitney test for continuous data. RFS and OS were calculated using the Kaplan-Meier method from the date of repeat hepatectomy until the date or recurrence and last follow-up, respectively, and compared using the log-rank test. Univariate analysis was performed to evaluate potential variables associated with RFS and OS. Potential variables included gender, age, primary tumor location (colon vs. rectum), primary tumor lymph node status, number of CLM, size of largest CLM, carcinoembryonic antigen (CEA) level, preoperative chemotherapy (cycles, lines, and agents), types of hepatectomy [major (≥3 Couinaud's segments) vs. minor], estimated blood loss (EBL), radiofrequency ablation (RFA), margin status of repeat hepatectomy (R0 vs. R1), and RAS mutational status (mutant vs. wild-type). All variables associated with RFS or OS on univariate analysis with p value <0.10 were included in a multivariate cox proportional hazard model. p Value <0.05 was considered statistically significant. All statistical analysis was performed using SPSS version 17.2 (SPSS Inc., Chicago, IL, USA).

Results

Patient Characteristics

During the study period, 922 patients with known *RAS* mutation status underwent curative-intent hepatectomy. Ninety-eight of these were first repeat hepatectomies—64 patients had wild-type *RAS* tumors and 34 (35 %) had tumors with a mutant *RAS*. For the majority of patients, *RAS* mutational analysis was performed on a metastatic tumor (Table 1). Of note, 14 patients had *RAS* mutational analysis performed on a primary tumor and a metastatic site. The mutational status from the primary and metastatic site was concordant in all 14 of these cases. *KRAS* mutations ($n = 32$) predominated and two patients were found to have *NRAS* mutations. *KRAS* mutations were identified in exon 12 ($n = 22$), exon 13 ($n = 8$), and exon 61 ($n = 2$).

Clinicopathologic variables were similar between WT and mutant *RAS* groups (Table 2). Two thirds of patients had a solitary CLM prior to repeat hepatectomy. One third of

Table 3 Univariate and multivariate analysis of recurrence-free survival (RFS)

	RFS (%)[a]					
	N	1 year	3 years	Univariate P[b]	Hazard ratio	Multivariate P[c]
All patients	98	45	20	–	–	–
Background characteristics						
Sex						
Male	63	48	21	0.341	–	–
Female	35	40	18			
Age at hepatectomy, years						
≥60	38	44	25	0.160	–	–
<60	60	46	18			
Primary tumor						
Primary tumor location						
Rectum	24	46	25	0.899	–	–
Colon	74	45	18			
Lymph node metastasis						
Yes	73	46	19	0.277	–	–
No	25	44	25			
Liver metastasis						
Preoperative chemotherapy						
Yes	91	60	25	0.359	–	–
No	7	83	21			
Preoperative chemotherapy (cycles)						
≤6	73	44	19	0.942	–	–
>6	25	47	22			
Fluorouracil-based chemotherapy regimen						
Oxaliplatin						
Yes	41	41	15	0.706	–	–
No	57	47	23			
Irinotecan						
Yes	48	43	22	0.912	–	–
No	50	47	17			
Use of bevacizumab						
Yes	47	46	23	0.489	–	–
No	51	45	18			
Use of anti-EGFR						
Yes	14	50	29	0.739	–	–
No	84	44	18			
CEA (ng/mL)						
≥5	43	42	23	0.675	–	–
<5	55	48	19			
Number of tumors[d]						
Multiple	32	35	11	*0.042*	1.47 (0.90–2.37)	0.124
Solitary	66	50	24			
Maximum size of tumor, cm[d]						
≥3	23	26	7	*0.015*	1.65 (0.93–2.82)	0.085
<3	75	50	24			
Residual tumor						
R1	12	22	0	0.191	–	–
R0	86	48	22			
Estimated blood loss, ml[d]						

Table 3 (continued)

	RFS (%)[a]					
	N	1 year	3 years	Univariate P[b]	Hazard ratio	Multivariate P[c]
>750	9	22	0	0.052	1.43 (0.63–2.91)	0.371
≤750	89	47	23			
Surgical procedure						
Major resection	22	62	16	0.818	–	–
Minor resection	76	40	22			
Concomitant RFA						
Yes	11	64	21	0.315	–	–
No	87	43	20			
RAS mutation status[d]						
Mutant	34	26	12	*0.028*	1.69 (1.03–2.72)	*0.037*
Wild-type	64	54	24			
Extrahepatic disease						
Yes	9	44	11	0.444	–	–
No	89	45	21			

Values in parentheses represent 95 % confidence intervals.

EGFR epidermal growth factor receptor, *CEA* carcinoembryonic antigen, *CLM* colorectal liver metastases, *RFA* radiofrequency ablation

[a] Kaplan-Meier analysis

[b] Log rank test

[c] Cox regression model

[d] Variables entered into the Cox regression model

patients received >6 cycles of chemotherapy prior to repeat hepatectomy. A major hepatectomy was performed in 22 % of patients, and 11 % of patients had an RFA. On pathologic evaluation, 12 patients were found to have a positive margin. There were no major differences between the WT and mutant *RAS* groups with regards to demographics, tumor characteristics, treatments, or pathologic features.

Recurrence

At a median follow-up of 35 months, 75 (77 %) patients had developed a recurrence. The two most common sites of disease recurrence were the liver and lungs. Liver recurrence was seen in 62 and 50 % of WT and mutant *RAS* patients, respectively, while lung recurrence was seen in 21 and 39 %, respectively (Fig. 1a). Recurrent disease was treated with local therapy in 31 (41 %) patients—47 % of WT and 32 % of mutant *RAS* patients (Fig. 1b). The differences in recurrence patterns and treatment of recurrent disease were not significantly different between the two groups.

Survival

The median RFS and OS for the entire cohort were 7.8 and 34.6 months, respectively. Patients with mutant *RAS* had a median RFS of 6.1 months, compared to 12.2 months for those with WT *RAS* ($p = 0.03$) (Fig. 2a). The median overall survival was 26.6 versus 42.5 months for mutant and wild-type RAS, respectively ($p = 0.005$) (Fig. 2b).

Univariate and Multivariate Analyses

Patient, tumor, treatment, and pathologic variables were incorporated into a multivariate analysis to determine which factors impacted RFS and OS. On univariate analysis, multiple tumors, tumor size >3 cm, EBL >750 ml, and RAS mutation status were associated with RFS, but only *RAS* mutation status [HR = 1.69 (1.03–2.72), $p = 0.04$] was associated with RFS on multivariate analysis (Table 3). Gender, multiple tumors, major hepatectomy, and RAS mutation status were associated with OS on univariate analysis, but only multiple tumors [HR = 1.92 (1.02–3.54), $p = 0.045$] and *RAS* mutation status [HR = 2.11 (1.11–3.98), $p = 0.02$] were associated with OS (Table 4).

Discussion

The present study is the first to investigate the relationship of *RAS* mutation status and outcomes following repeat

Table 4 Univariate and multivariate analysis of overall survival (OS)

	OS (%)[a]					
	Number	3 years	5 years	Univariate P[b]	Hazard ratio	Multivariate P[c]
All patients	98	69	44	–	–	–
Background characteristics						
Sex[d]						
Female	35	60	31	0.091	1.35 (0.72–2.51)	0.351
Male	63	74	52			
Age at hepatectomy, years						
≥60	38	68	50	0.523	–	–
<60	60	69	39			
Primary tumor						
Primary tumor location						
Rectum	24	76	40	0.380	–	–
Colon	74	66	45			
Lymph node metastasis						
Yes	73	67	44	0.898	–	–
No	25	72	44			
Liver metastasis						
Preoperative chemotherapy						
Yes	91	67	41	0.181	–	–
No	7	83	67			
Preoperative chemotherapy (cycles)						
≤6	73	66	48	0.685	–	–
>6	25	76	35			
Fluorouracil-based chemotherapy regimen						
Oxaliplatin						
Yes	41	64	38	0.723	–	–
No	57	71	46			
Irinotecan						
Yes	48	68	42	0.702	–	–
No	50	69	46			
Use of Bevacizumab						
Yes	47	68	44	0.728	–	–
No	51	70	44			
Use of anti-EGFR						
Yes	14	71	46	0.774	–	–
No	84	69	44			
CEA (ng/mL)						
≥5	43	72	44	0.321	–	–
<5	55	66	44			
Number of tumors[d]						
Multiple	32	59	33	0.073	1.92 (1.02–3.54)	*0.045*
Solitary	66	73	50			
Maximum size of tumor, cm						
≥3	23	49	33	0.347	–	–
<3	75	74	47			
Residual tumor						
R1	12	28	28	0.286	–	–
R0	86	74	46			
Estimated blood loss, ml						

Table 4 (continued)

	OS (%)[a]					
	Number	3 years	5 years	Univariate P[b]	Hazard ratio	Multivariate P[c]
>750	9	50	17	0.411	–	–
≤750	89	71	48			
Surgical procedure[d]						
Major resection	76	65	35	0.050	1.87 (0.87–4.65)	0.113
Minor resection	22	80	74			
Concomitant RFA						
Yes	11	67	53	0.554	–	–
No	87	69	43			
RAS mutation status[d]						
Mutant	34	47	26	*0.005*	2.11 (1.11–3.98)	*0.024*
Wild-type	64	78	52			
Extrahepatic disease						
Yes	9	60	45	0.691	–	–
No	89	70	44			

Values in parentheses represent 95 % confidence intervals

EGFR epidermal growth factor receptor, *CEA* carcinoembryonic antigen, *CLM* colorectal liver metastases, *RFA* radiofrequency ablation

[a] Kaplan-Meier analysis

[b] Log rank test

[c] Cox regression model

[d] Variables entered into the Cox regression model

hepatectomy for recurrent CLM. Thirty-four (35 %) of 98 patients undergoing RH were found to have mutant *RAS* tumors—32 *KRAS* mutations and 2 *NRAS* mutations. Brudvik et al. reported a *RAS* mutation rate of 36 % in patients undergoing hepatectomy for CLM.[6] Another recent study reported a *KRAS* mutation rate of 39.5 % in CLM.[23] In CLM, the most commonly identified *RAS* mutation is *KRAS*, which compromises about 80 % of identified mutations with the remaining being mutations in *NRAS*.[4,5] In this cohort, *KRAS* mutations were most frequently identified exons 12 and 13 (69 and 25 %, respectively). The incidence and specific type of *RAS* mutations found in the present RH study is similar to the larger population of patients who undergo hepatectomy for CLM.

It is well established that recurrence after hepatectomy for CLM remains high; up to 73 % of patients will develop recurrence.[14,15] In the current study, at a median follow-up of 35 months following RH, 75 (77 %) patients had developed recurrence. Andreou et al. reported a recurrence rate of 70 % after a median follow-up of 33 months after RH for CLM.[16] In the current study, recurrence in the liver and lung were the most common at 57 and 28 %, respectively. Of note, lung recurrence was seen in 21 % of patients with WT tumors compared to 39 % in those with mutant *RAS* tumors ($p = 0.09$). Previous studies demonstrate that the lung recurrence following hepatectomy for CLM is more commonly seen in patient with *RAS* mutations.[4,10] Although the current study did not find a statistically significant difference, this is likely due to sample size, and this may represent a clinically significant difference in concordance with other studies.

In the current study, about 40 % of the patients who developed recurrence following RH were able to undergo local therapy (resection 27 % and ablation 15 %). It would stand to reason that patients with mutant *RAS* tumors would be less likely to undergo local therapy, if they have the same rate of liver recurrence and a higher rate of lung recurrence following RH, but this was not seen in the current study; local therapy was utilized in 47 and 32 % of WT and mutant *RAS* patients, respectively. Recurrence following RH for CLM is common; however, patients with *RAS* mutant tumors may have a higher risk of lung recurrence, and when they do recur, they appear less likely to be candidates for aggressive therapy.

Recurrence-free survival following RH for CLM remains poor. Andreou et al. previously reported a median progression-free survival of 7 months in patients undergoing repeat hepatectomy for CLM, after excluding patients treated with ablation.[16] Herein, we report a median RFS of 7.8 for the entire cohort of patients following RH for CLM. Fortunately, as discussed above, many of these early recurrences have treatment options. Andreou et al. reported a positive surgical margin at RH as predictor of worse PFS.[16] In the current study, a positive surgical margin occurred in 12 % of the patients undergoing RH, but was not associated with RFS. *RAS*

mutation status (HR = 1.69, $p = 0.037$) was the only predictor of RFS, and the median RFS for WT and mutant RAS patients were 12.2 and 6.1 months, respectively ($p = 0.028$). These data indicate that *RAS* mutations are a predictor of poor RFS.

Despite high recurrence rates, overall survivals following RH for CLM have remained comparable to first resection patients.[16,18] This undoubtedly reflects appropriate selection of patients with favorable tumor biology for RH.[24] Luo et al. conducted a systematic review and meta-analysis to determine predictors of survival following RH for CLM.[24] They found six predictors of survival following RH: disease-free interval >1 year, solitary CLM, unilobar CLM, size ≤5 cm, lack of extrahepatic disease, and R0 resection.[24] These reported predictors of survival are similar to the numerous scoring systems and predictive models that have been previously published for first hepatectomies.[25–27] A comparable set of factors was evaluated in the current study following RH, but only multiple tumors (HR = 1.92, $p = 0.045$) and mutant *RAS* (HR = 2.11, $p = 0.005$) were found to be associated with worse OS. Furthermore, the OS for patients with wild-type and mutant *RAS* was 42.5 and 26.6 months, respectively ($p = 0.005$). These data strongly suggest that *RAS* mutation status needs to be incorporated into historical prognostic models as it is a powerful predictor of OS.

The retrospective design of this study is a potential limitation. Only patients with known *RAS* mutation status were included in the study, and the decision of whether or not to perform a *RAS* mutational analysis was up to the discretion of the treatment team. This having been said, the denominator cohort of patients with genetic evaluation was large, and the outcomes are well tracked with a long median follow-up interval. Another area of challenge in the study of *RAS* mutations is the tissue source, be it primary tumor, previous metastatic tissue, or the current metastatic tissue under treatment. Importantly, on the 14 patients with both primary and metastasis tissue assessed, there was *RAS* mutation concordance in 100 %. Lastly, *RAS* mutation testing has rapidly evolved and will continue to progress. Over the first many years of this study, only *KRAS* exons 12 and 13 were analyzed, so it is possible that some patients were classified as WT *RAS* and actually possessed a mutation in an exon that was undetectable at that time. However, correction of any potential misclassifications of this type would be likely to identify even larger difference in outcomes between WT and mutant *RAS* patients.

Conclusion

In general, patients with CLM that harbor *RAS* mutations have worse RFS and OS than patients with WT RAS. This study demonstrates that in the specific population of patients with recurrent liver CLM, RAS mutational status has prognostic value, with *RAS* mutations independently associated with worse RFS and OS. Thus, *RAS* mutation status should be determined prior to RH, as it may impact treatment decisions.

Acknowledgments The University of Texas MD Anderson Cancer Center is supported by the National Institutes of Health through Cancer Center Support Grant P30CA016672.

References

1. Forrester K, Almoguera C, Han K, Grizzle WE, Perucho M: Detection of high incidence of K-ras oncogenes during human colon tumorigenesis. *Nature* 1987, **327**(6120):298-303.
2. Khosravi-Far R, Campbell S, Rossman KL, Der CJ: Increasing complexity of Ras signal transduction: involvement of Rho family proteins. *Advances in cancer research* 1998, **72**:57-107.
3. Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pinter T, Lim R, Bodoky G *et al*: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *The New England journal of medicine* 2009, 360(14):1408-1417.
4. Vauthey JN, Zimmitti G, Kopetz SE, Shindoh J, Chen SS, Andreou A, Curley SA, Aloia TA, Maru DM: RAS mutation status predicts survival and patterns of recurrence in patients undergoing hepatectomy for colorectal liver metastases. *Annals of surgery* 2013, 258(4):619-626; discussion 626-617.
5. Mise Y, Zimmitti G, Shindoh J, Kopetz S, Loyer EM, Andreou A, Cooper AB, Kaur H, Aloia TA, Maru DM *et al*: RAS mutations predict radiologic and pathologic response in patients treated with chemotherapy before resection of colorectal liver metastases. *Annals of surgical oncology* 2015, 22(3):834-842.
6. Brudvik KW, Mise Y, Chung MH, Chun YS, Kopetz SE, Passot G, Conrad C, Maru DM, Aloia TA, Vauthey JN: RAS Mutation Predicts Positive Resection Margins and Narrower Resection Margins in Patients Undergoing Resection of Colorectal Liver Metastases. *Annals of surgical oncology* 2016.
7. Karagkounis G, Torbenson MS, Daniel HD, Azad NS, Diaz LA, Jr., Donehower RC, Hirose K, Ahuja N, Pawlik TM, Choti MA: Incidence and prognostic impact of KRAS and BRAF mutation in patients undergoing liver surgery for colorectal metastases. *Cancer* 2013, 119(23):4137-4144.
8. Nash GM, Gimbel M, Shia J, Nathanson DR, Ndubuisi MI, Zeng ZS, Kemeny N, Paty PB: KRAS mutation correlates with accelerated metastatic progression in patients with colorectal liver metastases. *Annals of surgical oncology* 2010, **17**(2):572-578.
9. Schirripa M, Bergamo F, Cremolini C, Casagrande M, Lonardi S, Aprile G, Yang D, Marmorino F, Pasquini G, Sensi E *et al*: BRAF and RAS mutations as prognostic factors in metastatic colorectal cancer patients undergoing liver resection. *British journal of cancer* 2015, 112(12):1921-1928.
10. Pereira AA, Rego JF, Morris V, Overman MJ, Eng C, Garrett CR, Boutin AT, Ferrarotto R, Lee M, Jiang ZQ *et al*: Association between KRAS mutation and lung metastasis in advanced colorectal cancer. *British journal of cancer* 2015, 112(3):424-428.
11. Brudvik KW, Kopetz SE, Li L, Conrad C, Aloia TA, Vauthey JN: Meta-analysis of KRAS mutations and survival after resection of colorectal liver metastases. *The British journal of surgery* 2015, 102(10):1175-1183.
12. Abdalla EK, Vauthey JN, Ellis LM, Ellis V, Pollock R, Broglio KR, Hess K, Curley SA: Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases. *Annals of surgery* 2004, 239(6):818-825; discussion 825-817.

13. Fernandez FG, Drebin JA, Linehan DC, Dehdashti F, Siegel BA, Strasberg SM: Five-year survival after resection of hepatic metastases from colorectal cancer in patients screened by positron emission tomography with F-18 fluorodeoxyglucose (FDG-PET). *Annals of surgery* 2004, 240(3):438-447; discussion 447-450.
14. de Jong MC, Pulitano C, Ribero D, Strub J, Mentha G, Schulick RD, Choti MA, Aldrighetti L, Capussotti L, Pawlik TM: Rates and patterns of recurrence following curative intent surgery for colorectal liver metastasis: an international multi-institutional analysis of 1669 patients. *Annals of surgery* 2009, 250(3):440-448.
15. Saiura A, Yamamoto J, Koga R, Takahashi Y, Takahashi M, Inoue Y, Ono Y, Kokudo N: Favorable outcome after repeat resection for colorectal liver metastases. *Annals of surgical oncology* 2014, 21(13):4293-4299.
16. Andreou A, Brouquet A, Abdalla EK, Aloia TA, Curley SA, Vauthey JN: Repeat hepatectomy for recurrent colorectal liver metastases is associated with a high survival rate. *HPB : the official journal of the International Hepato Pancreato Biliary Association* 2011, 13(11):774-782.
17. Petrowsky H, Gonen M, Jarnagin W, Lorenz M, DeMatteo R, Heinrich S, Encke A, Blumgart L, Fong Y: Second liver resections are safe and effective treatment for recurrent hepatic metastases from colorectal cancer: a bi-institutional analysis *Annals of surgery* 2002, 235(6):863-871.
18. Battula N, Tsapralis D, Mayer D, Isaac J, Muiesan P, Sutcliffe RP, Bramhall S, Mirza D, Marudanayagam R: Repeat liver resection for recurrent colorectal metastases: a single-centre, 13-year experience. *HPB : the official journal of the International Hepato Pancreato Biliary Association* 2014, 16(2):157-163.
19. Aloia TA, Zorzi D, Abdalla EK, Vauthey JN: Two-surgeon technique for hepatic parenchymal transection of the noncirrhotic liver using saline-linked cautery and ultrasonic dissection. *Annals of surgery* 2005, 242(2):172-177.
20. Pawlik TM, Scoggins CR, Zorzi D, Abdalla EK, Andres A, Eng C, Curley SA, Loyer EM, Muratore A, Mentha G *et al*: Effect of surgical margin status on survival and site of recurrence after hepatic resection for colorectal metastases. *Annals of surgery* 2005, 241(5):715-722, discussion 722-714.
21. Blazer DG, 3rd, Kishi Y, Maru DM, Kopetz S, Chun YS, Overman MJ, Fogelman D, Eng C, Chang DZ, Wang H *et al*: Pathologic response to preoperative chemotherapy: a new outcome end point after resection of hepatic colorectal metastases. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2008, 26(33): 5344-5351.
22. Passot G, Chun YS, Kopetz SE, Zorzi D, Brudvik KW, Kim BJ, Conrad C, Aloia TA, Vauthey JN: Predictors of Safety and Efficacy of 2-Stage Hepatectomy for Bilateral Colorectal Liver Metastases. *Journal of the American College of Surgeons* 2016.
23. Kawamata H, Yamashita K, Kojo K, Ushiku H, Ooki A, Watanabe M: Discrepancies between the K-ras mutational status of primary colorectal cancers and corresponding liver metastases are found in codon 13. *Genomics* 2015, 106(2):71-75.
24. Luo LX, Yu ZY, Huang JW, Wu H: Selecting patients for a second hepatectomy for colorectal metastases: an systemic review and meta-analysis. *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology* 2014, 40(9):1036-1048.
25. 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. *Annals of surgery* 1999, 230(3):309-318; discussion 318-321.
26. Nordlinger B, Guiguet M, Vaillant JC, Balladur P, Boudjema K, Bachellier P, Jaeck D: Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Francaise de Chirurgie. *Cancer* 1996, 77(7):1254-1262.
27. Hill CR, Chagpar RB, Callender GG, Brown RE, Gilbert JE, Martin RC, 2nd, McMasters KM, Scoggins CR: recurrence following hepatectomy for metastatic colorectal cancer: development of a model that predicts patterns of recurrence and survival. *Annals of surgical oncology* 2012, 19(1):139-144.

All authors/coauthors contributed significantly to the manuscript and fulfilled the requirements for authorship as per the guidelines of the International Committee of Medical Journal Editors (ICMJE).

This manuscript was presented in the Presidential Plenary session at the 2016 Annual Meeting of the Society for Surgery of the Alimentary Tract (SSAT) on 5/22/2106 in San Diego, CA.