The prognostic impact of RAS on overall survival following liver resection in early versus late-onset colorectal cancer patients

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BACKGROUND: The impact of molecular aberrations on survival after resection of colorectal liver metastases (CLM) in patients with early-age-onset (EOCRC) versus late-age-onset colorectal cancer (LOCRC) is unknown.

METHODS: Patients who underwent liver resection for CLM with known RAS, BRAF and MSI status were retrospectively studied. The prognostic impact of RAS mutations by age was analysed with age as a categorical variable and a continuous variable.

RESULTS: The study included 573 patients, 192 with EOCRC and 381 with LOCRC. The younger the age of onset of CRC, the greater the negative impact on overall survival of RAS mutations in the LOCRC, EOCRC, and ≤40 years (hazard ratio (HR), 1.64 (95% confidence interval (CI), 1.23–2.20), 2.03 (95% CI, 1.30–3.17), and 2.97 (95% CI, 1.44–6.14), respectively. Age-specific mortality risk and linear regression analysis also demonstrated that RAS mutations had a greater impact on survival in EOCRC than in LOCRC (slope: –4.07, 95% CI –8.10 to 0.04, P = 0.047, R² = 0.08).

CONCLUSION: Among patients undergoing CLM resection, RAS mutations have a greater negative influence on survival in patients with EOCRC, more so in patients ≤40 years, than in patients with LOCRC and should be considered as a prognostic factor in multidisciplinary treatment planning.
METHODS

Study population
Patients who underwent resection of CLM with curative intent at The University of Texas MD Anderson Cancer Center from January 2006 to December 2016 with known RAS, BRAF and MSI status were retrospectively evaluated. Demographic, radiological, surgical, pathological, genetic and medical treatment characteristics were retrieved from electronic medical records. Patients who underwent liver-directed therapy (e.g., radiofrequency ablation, stereotactic radiation therapy) were excluded from the analysis. We followed the Reporting Recommendations for Tumour Marker Prognostic Studies (REMARK) guidelines. This study was approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center.

Multidisciplinary management of CLM
Our institutional approach to surgical management of CLM has been previously detailed. CLM are deemed resectable when a hepatectomy can achieve a negative margin and preserve more than 30% of the standardised total liver volume. Patients with an anticipated insufficient future liver remnant are offered preoperative portal vein embolisation and staged hepatectomy. Patients are typically offered perioperative chemotherapy for a total of 12 cycles. After resection, patients are followed by either medical or surgical oncology with a history, physical examination, CEA and diagnostic imaging (CT chest, pelvis and abdomen with the liver protocol (2.5 mm) or an abdominal MRI) every 4 months for the first 2 years post liver resection and then every 6 months for the next 3 years.

Definitions
EOCRC was defined as CRC in patients <50 years old, and LOCR was defined as CRC in patients ≥50 years old on the date of diagnosis. The primary tumour location was determined based on the surgical pathology report or on colonoscopy. Primary tumours located in the ascending colon or transverse colon were classified as right-sided tumours, and primary tumours located in the splenic flexure, descending colon, or rectum were classified as left-sided tumours. The extrahepatic disease was defined as preoperative radiological findings suggestive of metastatic disease outside the liver or intraoperative findings confirming extrahepatic disease. A positive surgical margin was defined as the presence of tumour cells <1 mm from the transection line. The primary tumour T category and N category were assigned according to the AJCC Cancer Staging Manual, eighth edition.

Somatic gene mutation profiling
RAS and BRAF V600E mutational status were obtained from tissues of the primary tumour or metastatic site, and were assessed using PCR-based DNA sequencing analysis or next-generation sequencing performed on formalin-fixed, paraffin-embedded samples. Standardised testing for RAS mutational status was completed for exons 2 (codons 12 and 13), 3 (codons 59 and 61) and 4 (codons 117 and 146) of the KRAS, NRAS and HRAS genes, respectively. MSI status was determined using immunohistochemical analysis of formalin-fixed, paraffin-embedded tumour tissue.

Statistical analysis
Descriptive statistics were used to summarise demographic and clinical data. Categorical variables were compared by chi-square and Fisher’s exact test. Continuous variables were compared by t test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with the use of the Baptista–Pike method and compared by Fisher’s exact test. Hazard ratios (HRs) and 95% CIs were calculated with the use of the Mantel–Haenszel method. Recurrence-free survival (RFS) was defined as the time in months from the date of the first liver resection to the date of radiological examination or medical evaluation (whichever had occurred first) conclusive of disease recurrence, regardless of the site. OS was defined as the time in months from the date of the first liver resection to the date of death from any cause. The patients who did not have documented recurrence or death or were lost to follow-up were censored on the date of the last contact. RFS and OS were estimated by the Kaplan–Meier method, and the survival curves were compared by the log-rank test. Age-specific mortality risk was estimated by dividing the number of patients in each age-at-diagnosis group who had died by the number of people in the same age group who were exposed to that risk (groups 20–29, 30–39, 40–49, 50–59, 60–69, 70–79 and ≥80 years old).

Prognostic factors were assessed by multivariate analysis using the Cox proportional hazards model, and P values <0.05 were considered statistically significant. The backward stepwise elimination method was used in the variable selection in the regression model. Outliers were detected by using the Robust Regression Followed by the Outlier Identification method. Outliers were also defined as values greater than 1.5 times the interquartile range. Linear regression was used to evaluate the association between continuous variables. The normality of the distribution of variables was examined by the Kolmogorov–Smirnov normality test. Analyses were performed by using SPSS 24.0 software (SPSS, Chicago, IL, USA) and GraphPad Prism software version 8.0.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Study population
A total of 573 patients were eligible and were included in the study. All patients underwent at least one liver resection, 47 underwent two liver resections, 7 underwent three liver resections and 1 underwent four liver resections, for a total of 635 liver resections. The median age of the entire cohort was 54 years (range: 22–81 years). A total of 192 patients (34%) had EOCRC, and 381 patients (66%) had LOCR. The EOCRC group had a higher proportion of women, left-sided tumours and extrahepatic disease compared to the LOCR group (Table 1). Only a small minority of patients were determined to have a BRAF mutation or MSI-H tumour (<3%). The EOCRC and LOCR groups did not differ statistically with respect to the frequency of RAS mutations, BRAF mutation and MSI-H tumours. Most patients received perioperative systemic chemotherapy. Preoperative and postoperative adjuvant chemotherapy were administered to 87 and 69% of the patients, respectively, in both the EOCRC and LOCR cohorts. Perioperative systemic chemotherapy predominantly consisted of oxaliplatin-based regimens. Bevacizumab was used in preoperative chemotherapy in 61% of the patients. Of the 437 patients (76% of the entire cohort) who had pathologic response classification, 224 (51%) had a pathologic major response (<50% viable tumour cells), and 21 (5%) had a pathologic complete response. A positive surgical margin or margins in the liver resection specimen were found in 91 patients (16%).

Association between RAS mutations and other baseline characteristics
An association between RAS mutations and sex was observed in the entire cohort and in the EOCRC and LOCR subgroups (Supplementary Tables 1 and 2). In the entire cohort, RAS mutations were found in 54% (128/238) of women versus 38% (127/335) of men (OR: 1.90, 95% CI 1.36–2.65, P < 0.001). Similarly, an association between RAS mutations and sidedness was observed in the entire cohort and in the EOCRC and LOCR subgroups. In the entire cohort, RAS mutations were found in 60% (87/146) of patients with right-sided tumours versus 39% (168/427) of patients with left-sided tumours (OR: 2.27, 95% CI 1.56–3.30, P < 0.001). An association between sex and sidedness was observed only in the LOCR group, in which women had...
Table 1. Baseline characteristics of the overall population by the timing of onset of colorectal cancer (n = 573).

| Characteristic | Early-onset (n = 192) | Late-onset (n = 381) | P value |
|---------------|-----------------------|----------------------|---------|
| Median age at diagnosis (range), y | 42 (22–49) | 59 (50–81) | <0.001 |
| Sex | | | 0.019 |
| Male | 99 (52) | 236 (62) | 0.154 |
| Female | 93 (48) | 145 (38) | |
| RAS status | | | 0.279 |
| Mutated | 77 (40) | 178 (47) | 0.122 |
| Wild-type | 115 (60) | 203 (53) | |
| BRAF status | | | 0.135 |
| Mutated | 5 (3) | 4 (1) | 0.024 |
| Wild-type | 163 (97) | 293 (99) | |
| MSI status | | | |
| MSS | 150 (98) | 204 (97) | 0.739 |
| MSI-H | 3 (2) | 6 (3) | |
| Tumour location | | | |
| Ascending colon | 29 (15) | 93 (25) | 0.012 |
| Transverse colon | 8 (4) | 16 (4) | 0.527 |
| Descending colon | 9 (5) | 32 (8) | 0.122 |
| Rectosigmoid | 146 (76) | 240 (63) | 0.001 |
| Sidedness | | | |
| Right | 37 (19) | 109 (29) | 0.015 |
| Left | 155 (81) | 272 (71) | |
| CEA level > 10 ng/mL | | | |
| Yes | 40 (22) | 101 (27) | 0.213 |
| No | 143 (78) | 271 (73) | |
| Bilobar disease | | | |
| Yes | 39 (21) | 28 (26) | 0.389 |
| No | 149 (79) | 82 (75) | |
| ≥2 liver lesions | | | |
| Yes | 92 (48) | 182 (49) | 1 |
| No | 98 (52) | 193 (51) | |
| Pathologic response | | | |
| <50% viable tumour cells | 73 (46) | 151 (54) | 0.135 |
| ≥50% viable tumour cells | 85 (54) | 128 (46) | |
| Pathologic complete response | | | |
| Yes | 11 (7) | 10 (4) | 0.16 |
| No | 147 (93) | 269 (96) | |
| Margin status | | | |
| Positive | 35 (18) | 56 (15) | 0.279 |
| Negative | 157 (82) | 324 (85) | |
| Extrahepatic disease | | | |
| Yes | 51 (27) | 52 (14) | <0.001 |
| No | 141 (73) | 329 (86) | |
| Preoperative therapy | | | |
| Yes | 167 (87) | 330 (87) | 0.895 |
| No | 24 (13) | 51 (13) | |
| Preoperative bevacizumab | | | |
| Yes | 126 (66) | 222 (58) | 0.102 |
| No | 66 (34) | 159 (42) | |
| Postoperative therapy | | | |
| Yes | 125 (68) | 260 (69) | 1 |
| No | 58 (32) | 119 (31) | |

CEA carcinoembryonic antigen, MSI microsatellite instability, MSI-H high-frequency MSI, MSS microsatellite stable.

Values are presented as No. (%) unless otherwise indicated. p < 0.05 was considered statistically significant.
DISCUSSION

The findings of our study suggest that the prognostic value of RAS mutations in patients who underwent CLM resection differs according to the age of onset of CRC and has a greater influence on survival in patients with EOCRC, especially if diagnosed ≤40 years of age.

Our analysis has several positive aspects. The retrospective design of our single-institution study provides a large and homogeneous population of CRC patients who were uniformly deemed to have surgically resectable CLM disease (100% of the patients had colorectal cancer with liver metastases who underwent surgical resection for curative intent) which allows us to review the role of these biomarkers on the prognosis of both EOCRC and LOCRC. Patients who underwent liver-directed therapy were excluded from this analysis to further support the homogeneity of the patient population. There was the uniformity of the use of neoadjuvant and adjuvant chemotherapy in both groups. Anti-VEGF therapy was the only biologic provided in the neoadjuvant setting. Furthermore, practice patterns at our institution reserved anti-EGFR therapy for the refractory setting especially given the concerning findings of worse PFS when utilised in the neoadjuvant setting.  The long-term median follow-up of 70.1 months also demonstrates adequate follow-up followed by the uniformity of surveillance for all resected patients.

Our study does have its limitations. This is a single-institution, retrospective analysis. The median age of our patient population was younger than the average age of patient in the US impacted by colorectal cancer and is a reflection of the young patient population that is commonly referred to as academic institutions. The primary molecular markers evaluated in this analysis were selected based on published literature and are considered to have clinical relevance. The results of this study support the role of these biomarkers on the prognosis of both EOCRC and LOCRC. However, further studies are needed to confirm these findings in larger, prospective studies.


table

| Characteristic          | Categories | Univariate analysis | Multivariate analysis |
|-------------------------|------------|---------------------|-----------------------|
|                         |            | Median OS (95% CI), mo | HR (95% CI) | P value | HR (95% CI) | P value |
| Age at diagnosis        | <50 y (n = 192) | 70.8 (55.5–86.1) | 0.94 (0.73–1.23) | 0.693 | 1.17 (0.77–1.77) | 0.460 |
|                         | ≥50 y (n = 381) | 68.6 (59.3–77.8) |                       |          |               |          |
| Sex                     | Male (n = 335) | 75.5 (65.7–85.3 m) | 0.75 (0.59–0.95) | 0.021 | 0.88 (0.66–1.17) | 0.393 |
|                         | Female (n = 238) | 58.9 (47.4–70.3) |                       |          |               |          |
| RAS status              | Mutated (n = 255) | 52.5 (45.2–59.7) | 1.74 (1.36–2.22) | <0.001 | 1.58 (1.23–2.03) | <0.001 |
|                         | Wild-type (n = 318) | 78.4 (66.0–90.8) |                       |          |               |          |
| BRAF status             | Mutated (n = 9) | 47.2 (37.9–90.7) | 1.32 (0.54–3.21) | 0.536 | 2.01 (0.60–6.66) | 0.252 |
|                         | Wild-type (n = 456) | 72.2 (63.4–81.0) |                       |          |               |          |
| MSI status              | MSI-H (n = 9) | Not reached | 0.32 (0.04–2.30) | 0.259 | 0.28 (0.03–2.09) | 0.218 |
|                         | MSS (n = 354) | 81.4 (67.8–95.0) |                       |          |               |          |
| Sidedness               | Right (n = 146) | 54.2 (47.7–60.7) | 1.63 (1.26–2.12) | <0.001 | 1.57 (1.20–2.05) | 0.001 |
|                         | Left (n = 427) | 76.3 (65.3–87.2) |                       |          |               |          |
| CEA level > 10 ng/mL    | Yes (n = 141) | 64.5 (54.1–75.0) | 1.19 (0.90–1.56) | 0.211 | 1.15 (0.72–1.83) | 0.535 |
|                         | No (n = 414) | 72.0 (62.9–81.2) |                       |          |               |          |
| ≥2 liver lesions        | Yes (n = 263) | 61.1 (50.3–71.8) | 1.26 (0.99–1.60) | 0.057 | 0.88 (0.58–1.33) | 0.565 |
|                         | No (n = 294) | 77.9 (69.2–86.6) |                       |          |               |          |
| pCR                     | Yes (n = 21) | 90.2 (40.4–139.9) | 0.59 (0.28–1.27) | 0.182 | 0.63 (0.28–1.43) | 0.278 |
|                         | No (n = 416) | 67.3 (57.5–77.2) |                       |          |               |          |
| Margin status           | Positive (n = 91) | 66.6 (51.3–82.0) | 0.89 (0.73–1.43) | 0.894 | 1.16 (0.65–2.04) | 0.607 |
|                         | Negative (n = 481) | 70.6 (61.4–79.8) |                       |          |               |          |
| Extrahepatic disease    | Yes (n = 103) | 46.0 (32.5–59.5) | 1.62 (1.21–2.17) | 0.001 | 1.54 (1.15–2.07) | 0.004 |
|                         | No (n = 470) | 72.2 (63.7–80.7) |                       |          |               |          |
| Postoperative therapy   | Yes (n = 385) | 77.8 (67.6–88.0) | 0.66 (0.51–0.85) | 0.001 | 0.66 (0.51–0.85) | 0.001 |
|                         | No (n = 177) | 57.1 (48.3–65.8) |                       |          |               |          |

Table 2. Results of univariate and multivariate analysis of predictors for overall survival (OS) in the overall population (n = 573).

CEA carcinoembryonic antigen, HR hazard ratio, MSI microsatellite instability, MSI-H high-frequency MSI, MSS microsatellite stable, pCR pathologic complete response.

p < 0.05 was considered statistically significant.

of patients with EOCRC and wild-type RAS, the subgroup with EOCRC and RAS mutations (n = 77) had higher proportions of women, right-sided tumours and extrahepatic disease (Supplementary Table 1). A multivariate analysis of the LOCRC patients showed that RAS mutations, right-sided tumours, extrahepatic disease and lack of postoperative chemotherapy were associated with worse OS (Table 4). Since patients with age near 50 years were excluded from this analysis to further support the homogeneity of the patient population. There was the uniformity of the use of neoadjuvant and adjuvant chemotherapy in both groups. Anti-VEGF therapy was the only biologic provided in the neoadjuvant setting. Furthermore, practice patterns at our institution reserved anti-EGFR therapy for the refractory setting especially given the concerning findings of worse PFS when utilised in the neoadjuvant setting. The long-term median follow-up of 70.1 months also demonstrates adequate follow-up followed by the uniformity of surveillance for all resected patients.
Table 3. Results of univariate and multivariate analysis of predictors for overall survival (OS) in patients with EOCRC (n = 192).

| Characteristic       | Categories     | Univariate analysis | Multivariate analysis |
|----------------------|----------------|---------------------|-----------------------|
|                      |                | Median OS (95% CI), mo | HR (95% CI) | P value | HR (95% CI) | P value |
| Sex                  | Male (n = 99) | 70.8 (48.8–92.8) | 0.82 (0.53–1.27) | 0.384 | 1.08 (0.60–1.94) | 0.782 |
|                      | Female (n = 93) | 71.2 (48.8–92.8) |                      |       |           | |
| RAS status           | Mutated (n = 77) | 53.3 (44.5–62.1) | 2.03 (1.30–3.17) | 0.002 | 1.90 (1.20–3.02) | 0.006 |
|                      | Wild-type (n = 115) | 82.9 (52.3–113.4) |                      |       |           | |
| BRAF status          | Mutated (n = 5)  | 111.6 (0.0–∞)   | 1.22 (0.38–3.93) | 0.730 | 1.18 (0.34–4.04) | 0.784 |
|                      | Wild-type (n = 163) | 68.4 (33.7–83.2) |                      |       |           | |
| MSI status           | MSI-H (n = 3)   | Not reached | 0.04 (0.0–82.8) | 0.424 | 0.0 (0.0–∞) | 0.969 |
|                      | MSS (n = 150)   | 72.0 (33.3–90.8) |                      |       |           | |
| Sidedness            | Right (n = 37)  | 47.8 (24.8–76.8) | 1.84 (1.11–3.02) | 0.016 | 1.71 (0.99–2.94) | 0.050 |
|                      | Left (n = 155)  | 81.7 (58.5–105.0) |                      |       |           | |
| CEA level > 10 ng/mL | Yes (n = 40)    | 71.2 (41.9–100.5) | 1.10 (0.65–1.87) | 0.698 | 1.23 (0.62–2.45) | 0.544 |
|                      | No (n = 143)    | 68.4 (53.0–83.8) |                      |       |           | |
| ≥2 liver lesions     | Yes (n = 83)    | 56.4 (39.4–73.4) | 1.48 (0.95–2.30) | 0.077 | 1.60 (1.02–2.49) | 0.038 |
|                      | No (n = 102)    | 72.2 (52.5–91.9) |                      |       |           | |
| pCR                  | Yes (n = 11)    | 90.2 (0.0–∞) | 0.63 (0.19–2.01) | 0.438 | 0.57 (0.13–2.41) | 0.446 |
|                      | No (n = 147)    | 70.8 (54.8–86.8) |                      |       |           | |
| Margin status        | Positive (n = 35) | 72.2 (0.0–∞)  | 0.85 (0.48–1.52) | 0.597 | 1.06 (0.53–2.12) | 0.856 |
|                      | Negative (n = 157) | 68.4 (51.8–85.1) |                      |       |           | |
| Extrahepatic disease | Yes (n = 51)    | 53.3 (33.1–73.5) | 1.76 (1.11–2.80) | 0.015 | 1.64 (1.00–2.66) | 0.046 |
|                      | No (n = 141)    | 72.2 (60.2–84.2) |                      |       |           | |
| Postoperative therapy | Yes (n = 125)  | 72.2 (44.0–100.4) | 0.71 (0.44–1.12) | 0.144 | 0.64 (0.40–1.02) | 0.064 |
|                      | No (n = 58)     | 59.5 (43.8–75.1) |                      |       |           | |

*CEA* carcinoembryonic antigen, HR hazard ratio, MSI microsatellite instability, MSI-H high-frequency MSI, MSS microsatellite stable, pCR pathologic complete response. p < 0.05 was considered statistically significant.

Table 4. Results of univariate and multivariate analysis of predictors for overall survival (OS) in patients with LOCRC (n = 381).

| Characteristic       | Categories     | Univariate analysis | Multivariate analysis |
|----------------------|----------------|---------------------|-----------------------|
|                      |                | Median OS (95% CI), mo | HR (95% CI) | P value | HR (95% CI) | P value |
| Sex                  | Male (n = 236) | 75.5 (64.2–86.8) | 0.70 (0.52–0.94) | 0.019 | 0.90 (0.63–1.30) | 0.591 |
|                      | Female (n = 145) | 56.8 (43.2–70.4) |                      |       |           | |
| RAS status           | Mutated (n = 178) | 52.2 (39.0–65.4) | 1.64 (1.23–2.20) | 0.001 | 1.53 (1.13–2.05) | 0.005 |
|                      | Wild-type (n = 203) | 78.3 (62.8–93.9) |                      |       |           | |
| BRAF status          | Mutated (n = 4)  | 47.2 (4.6–89.8) | 1.44 (0.35–5.85) | 0.605 | 1.98 (0.47–8.24) | 0.345 |
|                      | Wild-type (n = 293) | 78.2 (68.3–88.1) |                      |       |           | |
| MSI status           | MSI-H (n = 6)   | Not reached | 0.59 (0.08–4.26) | 0.603 | 1.12 (0.12–9.74) | 0.916 |
|                      | MSS (n = 204)   | 83.2 (69.2–97.3) |                      |       |           | |
| Sidedness            | Right (n = 109) | 54.3 (47.1–61.6) | 1.56 (1.15–2.12) | 0.004 | 1.53 (1.12–2.09) | 0.007 |
|                      | Left (n = 272)  | 76.3 (64.5–88.1) |                      |       |           | |
| CEA level > 10 ng/mL | Yes (n = 101)  | 62.6 (52.7–72.5) | 1.22 (0.88–1.69) | 0.215 | 1.19 (0.86–1.66) | 0.286 |
|                      | No (n = 271)    | 74.6 (64.2–84.9) |                      |       |           | |
| ≥2 liver lesions     | Yes (n = 180)   | 63.5 (49.8–77.1) | 1.18 (0.88–1.57) | 0.256 | 1.01 (0.71–1.44) | 0.937 |
|                      | No (n = 192)    | 78.2 (66.6–89.9) |                      |       |           | |
| pCR                  | Yes (n = 10)    | 93.6 (0.0–∞) | 0.58 (0.21–1.58) | 0.291 | 0.80 (0.25–2.58) | 0.713 |
|                      | No (n = 269)    | 64.9 (51.7–78.1) |                      |       |           | |
| Margin               | Positive (n = 56) | 62.6 (47.7–77.5) | 1.15 (0.75–1.74) | 0.509 | 1.02 (0.56–1.86) | 0.944 |
|                      | Negative (n = 324) | 72.1 (61.9–82.3) |                      |       |           | |
| Extrahepatic disease | Yes (n = 52)    | 45.1 (33.3–56.9) | 1.57 (1.07–2.32) | 0.021 | 1.52 (1.03–2.25) | 0.033 |
|                      | No (n = 329)    | 72.1 (62.3–82.0) |                      |       |           | |
| Postoperative therapy | Yes (n = 260)  | 78.2 (66.7–89.7) | 0.64 (0.47–0.86) | 0.004 | 0.66 (0.48–0.89) | 0.008 |
|                      | No (n = 119)    | 56.8 (46.1–67.5) |                      |       |           | |

*CEA* carcinoembryonic antigen, HR hazard ratio, MSI microsatellite instability, MSI-H high-frequency MSI, MSS microsatellite stable, pCR pathologic complete response. p < 0.05 was considered statistically significant.
and (RAS mutation status in patients with surgical resection and had reduced OS relative to Fig. 1 Overall survival by RAS mutation status. Overall survival by RAS mutation status in patients with (a) early-age-onset (n = 192) and (b) late-age-onset (n = 381) colorectal cancer.

with surgical resection and had reduced OS relative to KRAS MT patients.

Our findings may be influenced by a subgroup of patients with RAS mutations who had a higher risk of death than patients with RAS mutations in the remaining population. These outliers were identified by two concordant methods. These patients accounted for 10% of the patients in the entire cohort and had increased rates of poor prognostic factors in addition to RAS mutations. This raises the hypothesis that there is a subgroup of patients with RAS mutations who have an extremely high risk of death after CLM resection. The absence of statistically significant differences in the rates of poor prognostic factors between the outliers with RAS mutations and the patients with RAS mutations in the remaining population was probably due to the small sample size of outliers. The lower frequency of patients in the extremely young and old age groups might explain the presence of the outliers. However, a definitive explanation would only be possible with an analysis of a much larger population of patients in those age groups (<35 years and >70 years).

Since the use of postoperative therapy was not mandatory and it was a significant predictor for OS on the overall population on multivariate analysis, imbalances in the rates of postoperative therapy among subgroups could influence our findings. However, there was no difference in the use of postoperative therapy by age and by RAS status (Supplementary Tables 1 and 2).

Our findings regarding the prognostic value of RAS mutations in patients with EOCRC are concordant with previous reports of lower tumour mutational burden in this patient group. Tumour mutational burden increases significantly with age and maybe up to 2.4 times as high in someone diagnosed with cancer at 90 years of age as it is in someone diagnosed with cancer at 10 years of age. We were able to find no similar studies examining other RAS-driven malignancies, such as pancreatic cancer and non-small cell lung cancer (NSCLC), possibly because few young patients experience these cancers. However, a study in a cohort of NSCLC patients demonstrated that patients younger than 50 years had a higher probability of harbouring a targetable genotype. This suggests that age might be an underappreciated marker for specific mutational profiles. Interestingly, we found no such association of age with BRAF mutation or MSI-H. This is most likely because our cohort had very low rates of these two molecular abnormalities.

The development of tailored therapies for RAS-driven malignancies has been elusive. A recently presented Phase 1 trial showed encouraging results of targeted therapies for the specific KRASG12C mutation. The study evaluated the efficacy of a RASG12C inhibitor (AMG 510) in patients who had locally advanced or metastatic solid tumours and harbouring RASG12C mutation. Of ten patients with NSCLC, five patients showed a partial response, and four had stable disease. These findings had a minimal benefit in the colorectal cohort. Our study suggests that RAS mutations have a significantly greater impact on survival after CLM resection in patients with EOCRC than in patients with LOCRC. In addition, in the presence of extrahepatic disease, consideration of liver resection in an EOCRC patient should be considered with caution. With next-generation sequencing (NGS) now as a standard of care, it is likely additional molecular markers will be identified in the EOCRC versus LOCRC patient population that may be prognostic and possibly predictive for OS following CLM resections. Inclusion of adjunct tools, such as immunoscore, a robust and validated test of the host immune reaction measuring CD3+, CD8+, and T-cell densities within the tumour and/or detection of ctDNA may be considered for determination of the risk of recurrence. To our knowledge, this is the first study determining the influence of RAS mutations on overall survival from the perspective of the age of onset in RAS-driven colorectal cancer liver resections.

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AUTHOR CONTRIBUTIONS

A.A.J. worked in conception, data collection, data analysis, statistical analysis, paper writing and paper review. T.V. in data collection, data analysis, statistical analysis, paper writing and paper review. B.J. in data analysis, paper writing and paper review. Y.K. in data collection, data analysis and paper review. S.H.W. in data collection, data analysis and paper review. J.N.V. in conception, data collection, data analysis, paper writing and paper review. C.E. in conception, data analysis, statistical analysis, paper writing and paper review.
Fig. 2  Age-specific mortality risk by RAS status. Age-specific mortality risk was estimated by dividing the number of patients in each age-at-diagnosis group who had died by the number of people in the same age group who were exposed to that risk (groups 20–29, 30–39, 40–49, 50–59, 60–69, 70–79 and ≥80 years of age). The area between the curves is the sum of the hazard ratios of death; the larger the area, the stronger the age-specific prognostic value of RAS mutations. RAS mutations had a stronger prognostic value in early-onset CRC. The fact that the curves are closer around 50 years suggests that the risk is similar in patients diagnosed with CRC in middle age.

ADDITIONAL INFORMATION
Ethics approval and consent to participate This study was approved by the Institutional Review Board (IRB) of The University of Texas MD Anderson Cancer Center. The informed consent was waived by the IRB. The study was performed in accordance with the Declaration of Helsinki.

Consent to publish Not applicable.

Data availability The datasets of the study contain personal health information and are confidential. These are available to readers upon reasonable request.

Competing interests The authors declare no competing interest.

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