HHS Public Access

Author manuscript

Ann Surg. Author manuscript; available in PMC 2024 September 01.

Published in final edited form as:

Ann Surg. 2023 September 01; 278(3): e540–e548. doi:10.1097/SLA.0000000000005771.

Demystifying *BRAF* mutation status in colorectal liver metastases: a multi-institutional, collaborative approach to 6 open clinical questions

Georgios Antonios Margonis, MD, PhD[1,8,*], **Thomas Boerner, MD**[1,*], **Jean-Baptiste Bachet, MD, PhD**[2], **Stefan Buettner, MD, PhD**[3], **Roberto Moretto, MD**[4], **Nikolaos Andreatos, MD**[5], **Andrea Sartore-Bianchi, MD**[6], **Jane Wang, MD**[7], **Carsten Kamphues, MD**[8], **Johan Gagniere, MD, PhD**[9], **Sara Lonardi, MD**[10], **Inger Marie Løes, MD**[11], **Doris Wagner, MD**[12], **Andrea Spallanzani, MD**[13], **Kazunari Sasaki, MD**[5], **Richard Burkhart, MD**[7], **Filippo Pietrantonio, MD**[14], **Emmanouil Pikoulis, MD**[15], **Timothy M. Pawlik, MD, PhD**[16], **Stéphanie Truant, MD, PhD**[17], **Armando Orlandi, MD, PhD**[18], **Anastasia Pikouli, MD**[15], **Nicoletta Pella, MD**[19], **Katharina Beyer, MD**[8], **George Poultsides, MD**[20], **Hendrik Seeliger, MD**[8], **Federico N Aucejo, MD**[5], **Peter Kornprat, MD**[12], **Klaus Kaczirek, MD**[21], **Per Eystein Lønning, MD**[11], **Martin E Kreis, MD**[8], **Christopher L Wolfgang, MD, PhD**[7], **Matthew J Weiss, MD**[7,22], **Chiara Cremolini, MD, PhD**[4], **Stéphane Benoist, MD**[23], **Michael D' Angelica, MD**[1]

[1]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[2]Department of Hepato-Gastroenterology, Hôpital Pitié Salpêtrière, Assistance Publique - Hôpitaux de Paris (AP-HP) and Sorbonne Université, University Pierre and Marie Curie, Paris, France

[3]Department of Surgery, Erasmus MC University Medical Centre, Rotterdam, The Netherlands

[4]Unit of Medical Oncology 2, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy

[5]Department of General Surgery, Digestive Disease Institute, Cleveland Clinic, Cleveland, Ohio

Corresponding Author: Michael I. D'Angelica, MD, Memorial Sloan Kettering Cancer Center, Department of Surgery, 1275 York Ave, New York, NY 10065, Fax: 212-717-3218 dangelim@mskcc.org.

Author contribution: Conception and design, acquisition of data, interpretation of data, drafting and revising the article critically for important intellectual content, and final approval of the version to be published: GAM, TB; Acquisition of data, revising the article critically for important intellectual content, and final approval of the version to be published: JBB, SB, RM, NA, ASB, JW, CK, JG, SL, IML, DW, AS, KS, RB, FP, EP, TMP, AO, AP, NP, KB, GP, HS, FNA, PK, KK, PEL, MEK, CLW, MJW; Conception and design, acquisition of data, revising the article critically for important intellectual content, and final approval of the version to be published: CC, SB, MD

*Contributed equally

Type of study: Multi-institutional, international cohort study

Statistical consultation: The authors would like to thank the biostatistician, Mr. Michail Belias, who is affiliated with Health Evidence, Radboud University Medical Center, Nijmegen, The Netherlands, for the statistical consultation.

Suppl. Figure 1. Flow chart of study cohort

Suppl. Figure 2. Recurrence-free survival after CRLM resection stratified by MSI status

Suppl. Figure 3. Overall survival after CRLM resection stratified by MSI status in patients with V600E mutations

Suppl. Figure 4. Overall survival after CRLM resection of patients with *BRAF* mutated vs co-mutated *KRAS/BRAF* tumors in patients with V600E mutations

Suppl. Figure 5. Overall survival after CRLM resection of patients with upfront resectable vs converted disease in patients with V600E mutations

Suppl. Figure 6. Overall survival after recurrence stratified by *BRAF* codon-specific mutation status

[6]Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda, Milan, Italy; Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, Milan, Italy

[7]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[8]Department of General and Visceral Surgery, Charité Campus Benjamin Franklin, Berlin, Germany

[9]Service de Chirurgie Digestive, CHU Clermont-Ferrand, Inserm, Université Clermont Auvergne, Clermont-Ferrand, France

[10]Oncology Unit 3, Department of Oncology, Veneto Institute of Oncology IOV - IRCCS, Padua, Italy

[11]Department of Clinical Science, University of Bergen, and Department of Oncology, Haukeland University, Hospital, Bergen, Norway

[12]Department of General Surgery, Medical University of Graz, Graz, Austria

[13]Medical Oncology Unit, Azienda Ospedaliero Universitaria di Modena, Modena, Italy

[14]Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.; Oncology and Hemato-oncology Department, University of Milan, Milan, Italy

[15]Third Department of Surgery, School of Medicine, National and Kapodistrian University of Athens, “Attikon” University General Hospital, Athens, Greece

[16]Department of Surgery, Ohio State University, Ohio, USA

[17]Department of Digestive Surgery and Transplantation, CHU Lille, University Lille, Lille, France

[18]Medical Oncology, Comprehensive Cancer Center, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

[19]Department of Oncology, ASUIUD University Hospital of Udine, Udine, Italy

[20]Department of Surgery, Stanford University School of Medicine, Stanford, California, USA

[21]Department of General Surgery, Medical University of Vienna, Vienna, Austria

[22]Department of Surgery, Zucker School of Medicine at Hofstra, Northwell Health Cancer Institute, Lake Success, New York, USA

[23]Department of Digestive Surgery and Surgical Oncology, Bicêtre Hospital, AP-HP, Paris-Sud University, Le Kremlin and Paris-Sud University, Le Kremlin Bicêtre, France

Abstract

Objective: To investigate the clinical implications of *BRAF* mutated (mut*BRAF*) colorectal liver metastases (CRLM).

Summary Background Data: The clinical implications of mut*BRAF* status in CRLM are largely unknown.

Methods: Patients undergoing resection for mut*BRAF* CRLM were identified from prospectively maintained registries of the collaborating institutions. Overall survival (OS) and recurrence-free

survival (RFS) were compared among patients with V600E vs nonV600E mutations, *KRAS/BRAF* co-mutation vs mut*BRAF* alone, MSS vs MSI status, upfront resectable vs converted tumors, extrahepatic vs liver-limited disease, and intrahepatic recurrence treated with repeat hepatectomy (RH) vs non-operative management.

Results: 240 patients harboring *BRAF*-mutated tumors were included. *BRAF* V600E mutation was associated with shorter OS (30.6 vs 144 months, p=0.004), but not RFS compared to nonV600E mutations. *KRAS/BRAF* co-mutation did not affect outcomes. MSS tumors were associated with shorter RFS (9.1 vs 26 months, p<0.001) but not OS (33.5 vs 41 months, p=0.3) compared to MSI-high tumors, while patients with resected converted disease had slightly worse RFS (8 vs 11 months, p=0.01) and similar OS (30 vs 40 months, p=0.4) compared to those with upfront resectable disease. Patients with extrahepatic disease had worse OS compared to those with liver-limited disease (8.8 vs 40 months, p<0.001). RH following intrahepatic recurrence was associated with improved OS compared to non-operative management (41 vs 18.7 months, p=0.004). All results continued to hold true in the multivariable OS analysis.

Conclusions: Although surgery may be futile in patients with *BRAF*-mutated CRLM and concurrent extrahepatic disease, resection of converted disease resulted in encouraging survival in the absence of extrahepatic spread. Importantly, repeat hepatectomy in select patients with recurrence was associated with improved outcomes. Finally, MSI-high status identifies a better prognostic group with regard to RFS while patients with nonV600E mutations have excellent prognosis.

Mini abstract

Although surgery may be futile in patients with *BRAF*-mutated CRLM and concurrent extrahepatic disease, resection of converted disease and repeat hepatectomy in select patients with recurrence may result in reasonable survival. Patients with nonV600E mutations have very favorable prognosis, which renders them a prognostically distinct group.

Keywords

BRAF; CRLM

Introduction

Data on patients with resected *BRAF* mutated (mut*BRAF*) Colorectal Liver Metastases (CRLM) were initially scarce, with only 21 reported cases reported until 2017.[1] In 2018 and 2019, the three largest relevant studies were published: a multi-institutional, international cohort study by Margonis et al which reported on 43 mut*BRAF* patients, a report by Memorial Sloan Kettering Cancer Center (MSKCC) and two European centers which included 35 mut*BRAF* cases, and a French nationwide intergroup study which reported on 66 mut*BRAF* patients.[2–4] These reports demonstrated that median overall survival (OS) after resection is shorter for patients with mut*BRAF* (26–40 months) as compared to wild-type (wt) *BRAF* tumors (60–87 months).

As there are very few nonV600E mutations, previous analyses either did not have these data recorded or were not adequately powered to assess whether patients with resectable

V600E versus nonV600E mutations have differing outcomes. Likewise, we do not know whether microsatellite stability status (MSS vs MSI) or concurrent *KRAS* mutation, a rare phenomenon of biologically unclear significance, influence outcomes in patients with resectable mut*BRAF* CRLMs. More importantly, there is very little data to guide clinical management of patients with resectable mut*BRAF* CRLMs and they are currently treated similarly to those with average risk CRLM. For example, it is not known whether conversion chemotherapy followed by surgery, which is utilized for the majority of patients with unresectable CRLM, is also an effective strategy for patients with mut*BRAF* tumors. It is also unclear whether all patients with resectable mut*BRAF* CRLM have a uniformly good enough prognosis to justify resection or whether specific subgroups have such high risk of systemic spread that resection is oncologically futile, as a group from Johns Hopkins has suggested.[5] It is also unclear whether, in the case of limited intrahepatic recurrence, re-resection is an effective treatment option.

Given the rarity of *BRAF* mutations in resectable patients, addressing these clinically important questions requires an international, multi-institutional collaboration.

Methods

Participating centers, inclusion criteria and recorded variables and outcomes

Prospectively-maintained patient registries from MSKCC, Johns Hopkins Hospital (JHH), the International Genetic Consortium for Colorectal Liver Metastasis (IGCLM) which includes Cleveland Clinic, Stanford University, University of Vienna, University of Graz, Charité - University of Berlin, Haukeland University and the University of Athens, a French nationwide cohort conducted by three cooperative groups [Fédération de Recherche en Chirurgie (FRENCH), Association de Chirurgie Hépato-Bilio-Pancréatique et Transplantation (ACHBT) and Association des Gastro-Enterologues Oncologues (AGEO)] and an Italian multicentric dataset including 7 centers (Azienda Ospedaliero Universitaria Pisana, Veneto Institute of Oncology, Istituto Nazionale dei Tumori, Niguarda Cancer Center, Azienda Ospedaliero Universitaria di Modena, Fondazione Policlinico Universitario A. Gemelli and University Hospital of Udine) were queried for patients who had undergone resection for mut*BRAF* CRLM. The assessed time period differed among institutions, depending on the availability of tumor sections that had undergone molecular analysis (MSKCC, Jan 2000 – Dec 2017; JHH, Jan 2000 – Dec 2017; IGCLM, Jan 2000 – Dec 2017, French cohort, Jan 2012 – Dec 2016, Italian consortium, Jan 2005 – Dec 2017).

Adult patients with mut*BRAF* status (as determined by DNA analysis of either the primary tumor or resected CRLM), were eligible for inclusion and classified as having either *BRAF* V600E or nonV600E mutations. Patients were excluded if they did not undergo a complete gross resection of both the primary tumor and CRLM, or if treatment consisted of ablation only. Patients with resected extrahepatic disease were included; however, the FRENCH/ACHBT/AGEO combined dataset only included patients with resected liver-limited disease. The study was conducted in accordance with the ethical standards of the participating institutions and was approved by the institutional review boards (IRBs). A detailed ethics statement is provided in the Supplementary Appendix.

Clinicopathologic variables included demographic data, T stage, N stage, grade and location (right vs left vs rectal) of the primary tumor, disease-free interval (DFI) between primary tumor resection and diagnosis of liver metastases, carcinoembryonic antigen (CEA) levels at the most recent time before hepatectomy, receipt of pre-hepatectomy chemotherapy, initial resectability status of CRLM, size of the largest CRLM, number of CRLMs, laterality of CRLMs (unilobar vs bilobar), margin status (R0 vs R1, with R1 defined as the presence of tumor cells at the resection margin), performance of concurrent ablation, presence of resected extrahepatic disease, *KRAS* mutational status, MSI status (MSS vs MSI), receipt of post-hepatectomy chemotherapy, and performance of repeat hepatectomy (RH) among patients with intrahepatic recurrence after initial CRLM resection.

Time of recurrence was defined as the time of the first imaging study that demonstrated definitive or suspicious new tumors. OS and recurrence-free survival (RFS) were calculated from the time of surgery to the time of death and first recurrence, respectively. Initial recurrences were classified as intrahepatic, extrahepatic or both. Extrahepatic recurrence was classified by site, namely recurrence involving the lung parenchyma, peritoneum, primary tumor site, retroperitoneal lymph nodes (including regional portal nodes) or at other sites.

Statistical Analysis

All primary analyses were *pre-specified* to limit bias inherent to exploratory comparisons. We compared long-term outcomes among patients as follows: 1) *BRAF* V600E vs nonV600E mutations, 2) MSS vs MSI mut*BRAF* tumors, 3) co-mutated *KRAS*/*BRAF* vs wt*KRAS*/mut*BRAF* tumors, 4) upfront resectable vs converted disease 5) concurrent extrahepatic vs liver-limited disease, and 6) intrahepatic recurrence treated with repeat hepatectomy (RH) vs non-operative management.

Continuous variables were presented as medians with interquartile ranges (IQR), while categorical variables were reported as counts and percentages. Categorical variables were compared with the chi-square test, whereas continuous variables were compared with the Mann-Whitney U test. The Kaplan-Meier method and the log-rank test were used for univariable survival analysis. Univariable and multivariable Cox regression analyses were performed. The number of factors that were included in the Cox analysis adhered to the requirement of a minimum of 9 outcome events per predictor variable.[6, 7] Since the number of outcome events was limited, we selected factors for Cox analysis based on clinical knowledge and existing literature and not by stepwise selection methods.[8] As noted by Steyerberg et al, stepwise selection in these cases may lead to instable selection, extreme estimated regression coefficients, and overestimation of the performance of the selected model.[8] Lastly, interactions between *BRAF* mutation status and other risk factors were tested. All analyses were conducted using R 5.3.0 (cran.r-project.org).

Results

Baseline characteristics and long-term outcomes of the entire study cohort

A flow chart that demonstrates the cohort selection process is illustrated in Supplemental Figure 1. The baseline characteristics of the 240 patients included in the study cohort are

shown in Table 1. At a median follow-up of 47.5 months (95%CI: 41.4–54.4 months), 125 of 240 patients (52%) had died. Median OS was 35.4 months; the predicted 1, 3 and 5-year OS rate was 86.3%, 49.9% and 33.6%, respectively. During the study period, 178 of 240 patients (74%) developed recurrence. Median RFS was 10.0 months (9.0–12.0); the 1, 3 and 5-year predicted RFS rate was 42.7%, 23.2% and 14.9%, respectively. No interactions between *BRAF* mutational status and other risk factors were found.

V600E vs nonV600E mutations

Data on codon-specific mutations were available for 229 patients. Of those, 182 patients had *BRAF* V600E mutations, while 47 had nonV600E mutations (Table 2 and Supplemental Table 1). Patients with *BRAF* V600E mutations were more likely to be older and have right-sided tumors and less likely to have received pre-hepatectomy chemotherapy and concurrent ablation. Figure 1A demonstrates that patients with V600E mutations had shorter median OS than those with nonV600E mutations (30.6 vs 144 months, p=0.002). Importantly, presence of the V600E mutation remained independently associated with a higher risk of death even after controlling for other factors (Hazard ratio: HR: 3.5: 1.56–7.85; p=0.001) (Table 3). Interestingly, patients with V600E mutations had similar median RFS compared to those with nonV600E mutations (9 vs 11 months, p=0.4). There was no difference in patterns of recurrence between patients with *BRAF* V600E and nonV600E mutations (Supplemental Table 2).

MSS vs MSI tumors

Data on MSI status were available for 194 patients. Of those, 148 had MSS tumors, while 46 had MSI tumors (Supplemental Table 3). Patients with MSS tumors were more likely to be younger and male and more commonly had left-sided primaries with lymph node involvement. The average number of liver metastases was also higher in the MSS group and CRLM distribution was more frequently bilobar. These patients were also more likely to receive post-hepatectomy chemotherapy. Patients with MSS tumors had significantly shorter median RFS (9.1 vs 26 months, p<0.001) (Supplemental Figure 2) compared to those with MSI tumors, a similar numerical trend was noted for median OS (33.5 vs 41, p=0.3), but failed to reach statistical significance (Figure 1B). but there was no significant difference in median OS (33.5 vs 41, p=0.3) (Figure 1B). Importantly, MSI tumors remained independently associated with a lower risk of recurrence even after controlling for other factors (Hazard ratio: HR: 0.47: 0.28–0.78; p=0.005) (Table 4). A subgroup OS analysis restricted to patients with V600E mutations was consistent with the primary OS analysis (Supplemental Figure 3).

wtKRAS/mutBRAF vs co-mutated KRAS/BRAF tumors

Data on *KRAS* status were available for 239 patients. Of those, 222 patients had *BRAF* mutations only, while 17 had co-mutated *KRAS*/*BRAF* tumors. There were no significant differences in baseline characteristics (Supplemental Table 4). Figure 1C demonstrates that patients with wt*KRAS*/mut*BRAF* tumors had similar median OS (35.4 vs 37.1 months, respectively p=0.6) compared to those who harbored co-mutated *KRAS*/*BRAF* tumors. A similar pattern was noted for median RFS (10 vs 10.4 months, wt*KRAS*/mut*BRAF* vs co-mutated *KRAS*/*BRAF* groups, respectively, p=0.8).

Because the *BRAF* nonV600E mutation was associated with favorable outcomes, a comparison between co-mutated *KRAS/BRAF* nonV600E to wt*KRAS*/mut*BRAF* tumors was not pursued. Instead, we plotted the overall survival of patients with co-mutated *KRAS/BRAF* V600E versus *BRAF* V600E alone, which is relevant as it assesses whether the addition of a *KRAS* mutation will worsen the outcomes of patients with V600E mutation alone. Of note, the main finding held true (Supplemental Figure 4).

Upfront resectable vs converted disease

230 patients had data on their initial resectability status. Of those, 48 were deemed to be initially unresectable, but converted following systemic therapy and 182 were upfront resectable. Baseline characteristics of the two groups are summarized in Supplemental Table 5. As expected, patients who received conversion chemotherapy had a higher median number of CRLM (5.5 v 2.6) and more frequent bilobar involvement (60% vs 36%), than those with upfront resectable disease. They also underwent concurrent ablation more frequently (29% vs 15%, respectively). Although median OS was numerically longer in patients with upfront resectable tumors, this was not significant (40 vs 30 months, p=0.4) (Figure 2A). Initially unresectable disease became independently associated with a higher risk of death after controlling for other prognostic factors (HR: 1.95: 1.17–3.26; p=0.01) (Table 3). Patients with upfront resectable tumors had significantly longer median RFS (11 vs 8 months, for patients with upfront resectable vs converted disease, respectively, p=0.01) but resectability status did not remain associated with RFS after controlling for other factors (Table 4). A similar pattern was noted for median RFS (11 vs 8 months, for patients with upfront resectable vs converted disease, respectively, p=0.01). A subgroup OS analysis restricted to patients with V600E mutations was consistent with the primary OS analysis (Supplemental Figure 5).

Liver-limited vs combined intrahepatic/extrahepatic disease

A total of 239 patients had data on the anatomical extent of their disease. Of those, 226 patients had liver-limited disease, while 13 patients also had extrahepatic disease. Figure 2B demonstrates that patients with liver-limited disease had longer median OS (40 vs 8.8 months, p<0.001) compared to those with extrahepatic disease; the latter group fared so poorly that no patient survived beyond 36 months. Concurrent extrahepatic disease remained independently associated with a higher risk of death even after controlling for other prognostic factors (HR: 5.09: 1.68–15.38; p=0.004) (Table 3). A subgroup OS analysis restricted to patients with V600E mutations demonstrated even less favorable outcomes for patients with concurrent extrahepatic disease. Specifically, patients with *BRAF* V600E mutations fared abysmally with a median OS of 6.5 months and an 18-month OS of 0%.

R0 vs R1 resections

Data on surgical margin status was available for 238 patients. Of those, 210 patients underwent R0 resections, while 28 had R1 resections. Supplemental Figure 5 demonstrates that patients who underwent an R0 resection exhibited a trend toward longer median OS (37.1 vs 34.7 months, p=0.09) compared to those who underwent R1 resections. Importantly, an R1 resection was independently associated with a higher risk of death after controlling for other prognostic factors (HR: 1.94; 95% CI: 1.13–3.34; p=0.016) (Table 3). Similarly,

patients who underwent R0 resections exhibited a trend towards longer median RFS (10.55 vs 6.43 months, p=0.06) compared to those who underwent R1 resections.

RH for liver-limited recurrence

A total of 178 patients eventually recurred. Of those, 66 experienced a liver-limited recurrence and were potential candidates for re-resection. Ultimately, 15 patients underwent RH. A total of 178 patients recurred, with 66 experiencing a liver-limited recurrence. Of those, 15 patients underwent RH. Figure 2C demonstrates that patients who underwent RH had longer median OS (41 vs 18.7 months, p=0.004) compared to patients with liver-limited recurrence who were not treated with repeat surgery.

An additional analysis was performed to test whether V600E mutation has prognostic impact on OS after recurrence. The median OS for the V600E subgroup was significantly worse at 17.1 (95%CI 13.7–20.7) months compared to 37.5 (95%CI 23.5-not reached) months for those with a non-V600E mutation (p<0.001) (Supplemental Figure 6). The 5-year OS was also worse at 7.3% vs 39.2%, respectively.

Discussion

Although this is not the first study to report on the prognosis of surgically treated patients with V600E vs nonV600E *BRAF* mutations, prior studies were inconclusive secondary to low power. Specifically, Gagniere et al did not detect a prognostic difference between patients with V600E and nonV600E mutations in a study cohort that included only 25 and 10 such cases, respectively.[3] Bachet et al refrained from performing a comparison given the presence of just 11 patients with nonV600E *BRAF* mutations in their study.[4] Finally, Margonis et al found that the presence of a nonV600E *BRAF* mutation was not a predictor of adverse outcomes, unlike V600E mutations.[2] However, as the authors pointed out, given the very small sample size of nonV600E mutations (n=6), this finding could have been driven by low statistical power (type II error). Prior studies in surgical patients treated *BRAF* mutation status as a binary variable (mut*BRAF* vs wt*BRAF*) and attempts to compare V600E with nonV600E mutations were inconclusive secondary to low power; only 6, 10 and 11 cases with nonV600E mutations were included in the early studies. The present international cohort included 47 patients with nonV600E mutations, far exceeding prior reports and survival analysis in this adequately powered cohort demonstrated the presence of significant differences in outcomes between patients with nonV600E and V600E mutations (median OS: 144 vs 30 months). Interestingly, the median OS of 144 months noted in the nonV600E group even exceeds median OS reported in patients with wt*BRAF* CRLM (60–81 months).[2, 3] This is consistent with prior findings in patients with unresectable mCRC. For example, Cremolini et al demonstrated that patients with nonV600E mutations survived longer than both patients with wt*BRAF* and V600E mut*BRAF* unresectable mCRC (62 vs 35.9 vs 12.6 months, respectively).[9] Similar findings were reported by Jones et al. In their study, patients with nonV600E mutations had a median OS of 60.7 months, compared to 43 and 11.4 months for patients with wt*BRAF* and V600E mut*BRAF* unresectable mCRC, respectively.[10] According to our analysis, patients with nonV600E mutations constitute a

distinct, not uncommon subgroup with a favorable prognosis. that should be considered separately from patients with *BRAF* V600E mutations.

Another limitation of prior studies is the lack of data on MSI status. Our results show that patients with mut*BRAF*/MSI tumors experienced significantly superior RFS than those with mut*BRAF*/MSS tumors (26 vs 9.1 months, respectively; $p<0.001$), but there was no significant difference in median OS. While patients with mut*BRAF*/MSI tumors also had higher median OS, the difference was not statistically significant. Our results are not definitive but suggest that patients with mut*BRAF* tumors should be assessed for MSI status, and those that are MSI-high should be considered a better prognostic group with regard to recurrence-free survival. Furthermore, it should be noted that our cohort's follow-up was completed before pembrolizumab was approved by the FDA and routinely available for mCRC. Thus, it is plausible that patients with MSI-H tumors may have had significantly superior OS compared to those with MSS tumors if they had received immunotherapy. These findings are interesting, not only because the impact of MSI status on patients with mut*BRAF* resected CRLM has not been studied before, but also because the prognostic importance of MSI status in mCRC is controversial and confounded by association with *BRAF* mutation status and other variables.[11, 12]

Concomitant *KRAS* and *BRAF* mutations are exceedingly rare and data on their biologic behavior consist solely of small case series and case reports.[13, 14] The prognostic significance of *KRAS*/*BRAF* co-mutation is therefore a topic of conjecture. The present study included 17 patients with *KRAS*/*BRAF* co-mutations, allowing us to perform a basic survival analysis for the first time. No significant difference in OS or RFS was noted between patients with *KRAS/BRAF* co-mutation and those with *BRAF* mutation alone. This may partially stem from the fact that the rate of *KRAS/BRAF* co-mutation in the nonV600E group was almost double that of the V600E group (11% vs 6%). However, no significant difference in survival was noted also between patients with *KRAS/BRAF* V600E co-mutation and those with *BRAF* V600E mutation alone. In turn, it is possible that the nonV600E/*KRAS* double mutations, which represent one third of the double mutated group, are associated with improved outcomes due to the favorable impact of the nonV600E mutation. Collectively, although limited by the relatively low numbers, there was no trend implying the presence of a substantial underlying difference with near identical survival estimates for both examined subgroups. Thus, on the basis of these data, it seems reasonable to treat these patients similarly to those with *BRAF* mutation alone with respect to prognostication and surgical selection. Thus, on the basis of these data, it seems reasonable to consider these patients as similar to those with *BRAF* mutation alone with respect to prognostication and surgical selection. Nonetheless, when it comes to systemic therapies, patients with *BRAF* mutation alone (i.e., *KRAS* wild-type status) may be treated differently.

Downstaging of initially unresectable CRLM followed by metastasectomy offers a chance for prolonged survival or even cure, an outcome that cannot be accomplished with systemic treatment alone. However, given the overall poor prognosis of patients with mut*BRAF* CRLM, initially unresectable disease has such a high likelihood of occult extrahepatic spread as to render conversion strategies futile. For "all-comers" with CRLM, Adam et

al initially reported a resectability rate of 12.5% after conversion chemotherapy; a recent meta-analysis estimated this rate at 22.5%.[15–17] Although the efficacy of downstaging for mut*BRAF* CRLM cannot be definitively determined without a dedicated prospective study (and the denominator of attempted conversions in patients with mut*BRAF*), while the definitions of 'unresectable disease' vary substantially across centers, the fact that 20% of our cohort (48/230) were patients with converted diseases indirectly suggests that successful downstaging leading to resection is not rare in this patient group. As expected, the median OS of patients who received conversion chemotherapy was lower than those who were resectable upfront due to more extensive baseline disease; similar findings have been reported by Adam et al for "all-comers" with CRLM (5-year OS: 48% vs 33% for upfront resectable vs converted, respectively). Importantly, resectability status was not an independent predictor of OS and patients who underwent conversion chemotherapy still had encouraging prognosis after resection, with a median OS of 30 months and several patients surviving for 5 or more years. These outcomes compare favorably with historical survival rates from medically treated patients with unresectable mut*BRAF* mCRC and suggest that conversion chemotherapy followed by surgery is a viable strategy that should be pursued when feasible.[18, 19]

Identifying subgroups of resectable mut*BRAF* patients who have especially poor post-hepatectomy prognosis is an important challenge because it can spare patients who have technically but not "biologically" resectable disease from a futile operation. Although patients with mut*BRAF* resected liver-limited disease had a very high recurrence rate (85%), they also enjoyed a relatively long median OS of 40 months which compares favorably with historical rates in unresectable mCRC. These results indirectly imply that surgery is justified in these patients, following the same rationale employed in "all-comers" with resectable disease in the absence of clinical trial data. It is far more difficult to make this argument for patients with mut*BRAF* lesions and concurrent extrahepatic disease. While survival in these patients is expected to be low, outcomes proved far worse than anticipated. These patients had a median OS of 9 months (with no patients surviving beyond 36 months) and those with *BRAF* V600E mutations fared abysmally with a median OS of 6.5 months and an 18-month OS of 0%. Even though these estimates are limited by a small sample size, they are so dramatic that surgery may not offer a clinically significant benefit in these extremely high-risk patients. Although methodologically imperfect, the outcomes of patients with resected extrahepatic disease offer at least an indirect glimpse at how likely surgery is to be beneficial in an especially high-risk group. Collectively, while patients with V600E mut*BRAF* tumors and technically resectable, concurrent extrahepatic disease are rare, they form the first well-defined prognostic subgroup in which the benefit of surgery can be questioned.

The surgical margin is the only factor within the surgeon's direct control and thus potentially modifiable. Although surgeons should strive to achieve negative margins, there has been a long-standing debate regarding whether an R0 resection truly confers a survival benefit in CRLM. The present study demonstrated a trend toward improved outcomes among patients with mut*BRAF* tumors who underwent an R0 resection, which was shown to be statistically significant in multivariable analysis; as such, prior reports questioning the prognostic value of margin status do not appear to apply to patients with mut*BRAF*

tumors. Whether this association is reflective of a true benefit from an R0 resection or is secondary to underlying heterogeneity in tumor biology among mut*BRAF* cases is unknown. For example, aggressive tumors may infiltrate diffusely and thus be more likely to have microscopically positive margins. Nonetheless, it is reasonable for surgeons to strive for an R0 resection given the possible survival benefit.

Another important question pertaining to the surgical management of mut*BRAF* patients is whether resection of localized intrahepatic recurrences may benefit these patients. This is a clinically important question because as many as 50–70% of "all-comers" with CRLM will relapse following hepatic resection, with intrahepatic recurrences being especially common.[20] While RH is safe and long-term survival can be achieved, subsequent early recurrence rates are high often negating any benefit from the operation. This renders patient selection highly important. Given that the presence of *KRAS* mutation, another surrogate of tumor biology, has been independently associated with worse OS after RH, it is reasonable to examine whether this is also the case for *BRAF* mutation.[21] While a limited number of patients underwent RH in our cohort, it was associated with a significantly prolonged survival. These results suggest that RH should be considered in selected patients with mut*BRAF* tumors following localized recurrence if technically feasible.

The study has some limitations. The study population was heterogeneous but, given the rarity of *BRAF* mutations in surgical cohorts it would not have been possible to conduct the study without multi-institutional, international cooperation. For example, although a uniform definition of resectability of CRLM has been adopted after 2000 (i.e., complete resection with preservation of at least two disease-free liver segments with viable vascular inflow, outflow, biliary drainage, and adequate FLR volume), there may still be differences across centers or within the same center over time as this multi-institutional study spanned 18 years and 7 countries.[22] Nonetheless, the heterogeneity of the database adds to the generalizability of the findings. Even though this study included an unprecedented number of patients, the sample size in a number of patient subgroups (e.g., double *KRAS/BRAF* mutations and patients with extrahepatic disease) was still limited, possibly reducing the accuracy of survival estimates. However, this should not preclude from these analyses because, as Miguel Hernan has recently stated, the solution to observational analyses with imprecise effect estimates is not avoiding observational analyses with imprecise estimates, but rather encouraging the conduct of many observational analyses which will allow subsequent meta-analyses to provide a more precise pooled effect estimate.[23] The limited sample size also reduced the number of variables of interest that could be included in the univariable and multivariable analysis. However, the dataset of this study can be potentially used in future patient level meta-analyses to allow for more extensive survival analyses. Of note, given the study cohort included only patients with *BRAF* mutations, we were unable to include data from patients with wt*KRAS*/wt*BRAF* tumors. This is a limitation of the study and future studies should include this patient group to evaluate the impact of double *KRAS/BRAF* co-mutation more precisely. Lastly, as this was a retrospective observational study, selection bias and confounding by indication were certainly present.

In summary, this multi-institutional collaboration allowed us to assemble the largest cohort of patients with mut*BRAF* CRLM reported to date. We addressed a number of *pre-specified,*

clinically and biologically important questions with far more statistical power than prior reports. We found that patients with nonV600E mutations have very favorable prognosis and should be considered separately from patients with V600E mutations. We found that patients with nonV600E mutations have very favorable prognosis, which renders them a prognostically distinct group. Moreover, our findings suggest that MSI-high status identifies a better prognostic group with regard to RFS, while the co-existence of *KRAS* and *BRAF* mutations likely does not result in different prognosis than *BRAF* mutation alone. The results of the study also have specific implications for the practicing surgeon, which are on the whole positive. While the co-existence of extrahepatic spread and V600E mutations was associated with extremely poor prognosis and should prompt re-evaluation of the role of metastasectomy, repeat hepatectomy in select patients with recurrence was associated with improved outcomes. Importantly, conversion chemotherapy followed by resection also resulted in encouraging survival. While the BEACON trial recently demonstrated the evolving potential of innovative targeted therapy among patients with *BRAF* V600E mutations, time-honed surgical techniques continue to have an important role in the management of these high-risk patients.[24]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement:

The authors would like to thank Dr. Nefeli Bampatsikou for the excellent technical support.

Funding:

Partially funded by Italian Ministry of Health, Ricerca Finalizzata 2019, grant code J99C21000260001. The funding organization had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

This work was funded by National Cancer Institute award P30-CA008748. The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

References

1. Tosi F, Magni E, Amatu A, et al. Effect of KRAS and BRAF Mutations on Survival of Metastatic Colorectal Cancer After Liver Resection: A Systematic Review and Meta-Analysis. Clin Colorectal Cancer. 2017;16:e153–e163. [PubMed: 28216246]
2. Margonis GA, Buettner S, Andreatos N, et al. Association of BRAF Mutations With Survival and Recurrence in Surgically Treated Patients With Metastatic Colorectal Liver Cancer. JAMA Surg 2018;153:e180996. [PubMed: 29799910]
3. Gagniere J, Dupre A, Gholami SS, et al. Is Hepatectomy Justified for BRAF Mutant Colorectal Liver Metastases?: A Multi-institutional Analysis of 1497 Patients. Ann Surg 2020;271:147–154. [PubMed: 29995686]
4. Bachet JB, Moreno-Lopez N, Vigano L, et al. BRAF mutation is not associated with an increased risk of recurrence in patients undergoing resection of colorectal liver metastases. Br J Surg 2019;106:1237–1247. [PubMed: 31183866]

5. Karagkounis G, Torbenson MS, Daniel HD, et al. Incidence and prognostic impact of KRAS and BRAF mutation in patients undergoing liver surgery for colorectal metastases. Cancer. 2013;119:4137–4144. [PubMed: 24104864]
6. Concato J, Peduzzi P, Holford TR, et al. Importance of events per independent variable in proportional hazards analysis. I. Background, goals, and general strategy. J Clin Epidemiol 1995;48:1495–1501. [PubMed: 8543963]
7. Peduzzi P, Concato J, Feinstein AR, et al. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. J Clin Epidemiol 1995;48:1503–1510. [PubMed: 8543964]
8. Steyerberg EW, Vergouwe Y. Towards better clinical prediction models: seven steps for development and an ABCD for validation. Eur Heart J 2014;35:1925–1931. [PubMed: 24898551]
9. Cremolini C, Di Bartolomeo M, Amatu A, et al. BRAF codons 594 and 596 mutations identify a new molecular subtype of metastatic colorectal cancer at favorable prognosis. Ann Oncol 2015;26:2092–2097. [PubMed: 26153495]
10. Jones JC, Renfro LA, Al-Shamsi HO, et al. (Non-V600) BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. J Clin Oncol 2017;35:2624–2630. [PubMed: 28486044]
11. Sinicrope FA, Shi Q, Smyrk TC, et al. Molecular markers identify subtypes of stage III colon cancer associated with patient outcomes. Gastroenterology. 2015;148:88–99. [PubMed: 25305506]
12. Tran B, Kopetz S, Tie J, et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer. 2011;117:4623–4632. [PubMed: 21456008]
13. Sahin IH, Kazmi SM, Yorio JT, et al. Rare Though Not Mutually Exclusive: A Report of Three Cases of Concomitant KRAS and BRAF Mutation and a Review of the Literature. J Cancer. 2013;4:320–322. [PubMed: 23569465]
14. Larki P, Gharib E, Yaghoob Taleghani M, et al. Coexistence of KRAS and BRAF Mutations in Colorectal Cancer: A Case Report Supporting The Concept of Tumoral Heterogeneity. Cell J 2017;19:113–117. [PubMed: 28580315]
15. Lam VW, Spiro C, Laurence JM, et al. A systematic review of clinical response and survival outcomes of downsizing systemic chemotherapy and rescue liver surgery in patients with initially unresectable colorectal liver metastases. Ann Surg Oncol 2012;19:1292–1301. [PubMed: 21922338]
16. Adam R, Delvart V, Pascal G, et al. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. Ann Surg 2004;240:644–657; discussion 657–648. [PubMed: 15383792]
17. Imai K, Adam R, Baba H. How to increase the resectability of initially unresectable colorectal liver metastases: A surgical perspective. Ann Gastroenterol Surg 2019;3:476–486. [PubMed: 31549007]
18. Olthof PB, Huiskens J, Wicherts DA, et al. Survival after associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) for advanced colorectal liver metastases: A case-matched comparison with palliative systemic therapy. Surgery. 2017;161:909–919. [PubMed: 28038862]
19. Simkens LH, van Tinteren H, May A, et al. Maintenance treatment with capecitabine and bevacizumab in metastatic colorectal cancer (CAIRO3): a phase 3 randomised controlled trial of the Dutch Colorectal Cancer Group. Lancet 2015;385:1843–1852. [PubMed: 25862517]
20. Wicherts DA, de Haas RJ, Salloum C, et al. Repeat hepatectomy for recurrent colorectal metastases. Br J Surg 2013;100:808–818. [PubMed: 23494765]
21. Denbo JW, Yamashita S, Passot G, et al. RAS Mutation Is Associated with Decreased Survival in Patients Undergoing Repeat Hepatectomy for Colorectal Liver Metastases. J Gastrointest Surg 2017;21:68–77. [PubMed: 27334313]
22. Adam R, De Gramont A, Figueras J, et al. The oncosurgery approach to managing liver metastases from colorectal cancer: a multidisciplinary international consensus. Oncologist 2012;17:1225–1239. [PubMed: 22962059]
23. Hernan MA. Causal analyses of existing databases: no power calculations required. J Clin Epidemiol 2022;144:203–205. [PubMed: 34461211]

24. Kopetz S, Grothey A, Yaeger R, et al. Encorafenib, Binimetinib, and Cetuximab in BRAF V600E-Mutated Colorectal Cancer. N Engl J Med 2019;381:1632–1643. [PubMed: 31566309]

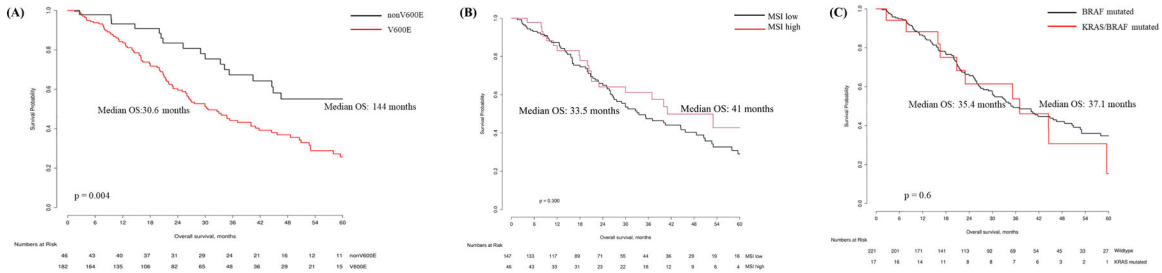

Figure 1.

Overall survival after CRLM resection (A) stratified by *BRAF* codon-specific mutation status, (B) stratified by MSI status, (C) of patients with *BRAF* mutated vs co-mutated *KRAS/BRAF* tumors

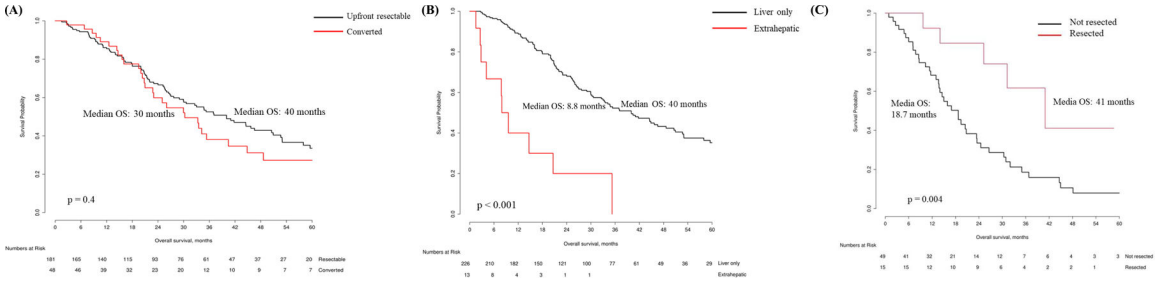

Figure 2.

Overall survival after CRLM resection of patients (A) with upfront resectable vs converted disease, (B) with liver-limited vs combined intrahepatic/extrahepatic disease, (C) who underwent repeat hepatectomy for liver-limited recurrence vs those who did not

Table 1.

Clinicopathological and treatment characteristics of patients with *BRAF* mutated tumors

Characteristics		*BRAF* Mutant (n=240)
Median Age (IQR)		63.1 (56.0, 71.0)
Sex (%)	Male	125 (52)
	Female	115 (48)
Primary Tumor Site (%)	Right Colon	132 (55)
	Left Colon	71 (30)
	Rectum	35 (15)
T Stage (%)	0–2	29 (12.2)
	3–4	209 (87.8)
Primary Tumor Nodal Status (%)	Negative	67 (28)
	Positive	171 (72)
Primary Tumor Grade (%)	0–1	72 (50.7)
	2–3	70 (49.3)
Median CEA Level, μg/L (IQR)		6.1 (2.8, 20.4)
Median DFI, months (IQR)		0.0 (0.0, 9.2)
Synchronous disease		79 (32.9)
Pre-hepatectomy Chemotherapy (%)	No	98 (41)
	Yes	142 (59)
Conversion (%)	Upfront resectable	182 (79)
	Converted	48 (21)
Median Diameter of Largest CRLM, cm (IQR)		2.3 (1.5, 4.1)
Median Number of CRLM ([IQR)		2.0 (1.0, 4.0)
Bilobar Distribution (%)	No	143 (60)
	Yes	97 (40)
Surgical Margin Status (%)	R0	210 (88)
	R1	28 (12)
Ablation Associated with Hepatectomy (%)	No	197 (82)
	Yes	43 (18)
Extrahepatic Disease (%)	No	226 (95)
	Yes	13 (5)
***KRAS* (%)**	Wild type	222 (93)
	Mutated	17 (7)
MSI Status (%)	MSS	148 (76)
	MSI	46 (24)
Post-hepatectomy Chemotherapy (%)	No	61 (26)
	Yes	178 (74)
Type of chemotherapy		
5-FU/Capecitabine		14 (9.8)
Oxaliplatin-based		74 (52.1)
Irinotecan-based		23 (16.2)

Characteristics	*BRAF* Mutant (n=240)
Oxaliplatin + Irinotecan based	17 (12)
HAI	14 (9.8)

Table 2.

Clinicopathological and treatment characteristics of patients with V600E vs nonV600E *BRAF* mutated tumors

Characteristics		nonV600E	V600E	P
n		47	182	
Median Age (IQR)		61.0 (53.3, 66.0)	64.8 (57.0, 72.6)	0.03
Sex (%)	Male	28 (60)	92 (51)	0.269
	Female	19 (40)	90 (49)	
Primary Tumor Site (%)	Right Colon	15 (32)	110 (61)	0.001
	Left Colon	20 (43)	51 (28)	
	Rectum	12 (26)	19 (11)	
T Stage (%)	0	1 (2)	0 (0)	0.182
	1	1 (2)	4 (2)	
	2	6 (13)	16 (9)	
	3	29 (62)	101 (56)	
	4	10 (21)	59 (33)	
Primary Tumor Nodal Status (%)	Negative	17 (36)	48 (27)	0.199
	Positive	30 (64)	132 (73)	
Primary Tumor Grade (%)	0	0 (0)	5 (5)	0.003
	1	19 (66)	46 (45)	
	2	6 (21)	49 (48)	
	3	4 (14)	2 (2)	
Median CEA Level, μg/L (IQR)		7.0 (2.8, 41.7)	6.4 (2.7, 20.0)	0.549
Median DFI, months (IQR)		0.0 (0.0, 12.8)	0.0 (0.0, 9.0)	0.111
Pre-hepatectomy Chemotherapy (%)	No	10 (21)	85 (47)	0.002
	Yes	37 (79)	97 (53)	
Conversion (%)	Upfront	30 (65)	144 (82)	0.015
	Converted	16 (35)	32 (18)	
Median Diameter of Largest CRLM, cm (IQR)		2.5 (1.5, 5.5)	2.2 (1.5, 4.0)	0.282
Median Number of CRLM (IQR)		2.0 (1.0, 4.5)	2.0 (1.0, 4.0)	0.578
Bilobar Distribution (%)	No	25 (53)	112 (62)	0.298
	Yes	22 (47)	70 (38)	
Surgical Margin Status (%)	R0	41 (89)	159 (88)	0.81
	R1	5 (11)	22 (12)	
Concurrent Ablation (%)	No	34 (72)	155 (85)	0.039
	Yes	13 (28)	27 (15)	
***KRAS* (%)**	Wild type	42 (89)	171 (94)	0.208
	Mutated	5 (11)	10 (6)	
Post-hepatectomy Chemotherapy (%)	No	11 (23)	48 (26)	0.678
	Yes	36 (77)	134 (74)	

Table 3.

Overall Survival Univariable and Multivariable Analyses

	UV	pUV	MV	pMV
CEA level	1.001 [1.000–1.001]	0.005	1.000 [1.000–1.001]	0.404
Tumor Size	1.028 [0.962–1.098]	0.418	1.009 [0.889–1.144]	0.89
Tumor Number	1.001 [0.995–1.008]	0.707	1.009 [0.997–1.022]	0.138
Primary Tumor Nodal Status	1.799 [1.182–2.739]	0.006	1.850 [1.014–3.376]	0.045
Synchronous	0.786 [0.544–1.136]	0.2	0.798 [0.454–1.403]	0.433
R1 Resection	1.530 [0.937–2.499]	0.089	1.829 [0.855–3.914]	0.12
Ablation Associated with Hepatectomy	0.697 [0.427–1.138]	0.149	0.556 [0.254–1.216]	0.141
V600E mutation	2.043 [1.246–3.348]	0.005	3.503 [1.563–7.853]	0.002
Co-mutated KRAS/BRAF	1.183 [0.636–2.198]	0.595	1.360 [0.538–3.436]	0.516
MSI	0.760 [0.460–1.257]	0.286	1.060 [0.565–1.989]	0.856
Extrahepatic Disease	5.669 [2.923–10.996]	<0.001	5.089 [1.684–15.382]	0.004
Pre-hepatectomy Chemotherapy	0.774 [0.544–1.102]	0.156	1.130 [0.654–1.953]	0.661
Post-hepatectomy Chemotherapy	0.557 [0.375–0.827]	0.004	0.440 [0.239–0.813]	0.009
Converted CRLM	1.214 [0.799–1.845]	0.364	1.650 [0.859–3.170]	0.133

Table 4.

Recurrence-free Survival Univariable and Multivariable Analyses

			Multiple imputations	
	UV	**pUV**	**MV**	**pMV**
CEA level	1.001 [1.000–1.001]	0.005	1.000 [1.000–1.001]	0.153
Tumor Size	1.047 [0.985–1.114]	0.14	1.064 [0.981–1.155]	0.132
Tumor Number	1.002 [0.997–1.007]	0.381	1.036 [0.967–1.109]	0.311
Primary Tumor Nodal Status	1.759 [1.237–2.501]	0.002	1.867 [1.232–2.830]	0.004
Synchronous disease	1.002 [0.997–1.007]	0.381	1.001 [0.993–1.008]	0.889
R1 Resection	1.532 [0.979–2.399]	0.062	1.331 [0.795–2.230]	0.273
Ablation Associated with Hepatectomy	1.189 [0.819–1.725]	0.363	0.980 [0.593–1.620]	0.937
V600E mutation	1.164 [0.805–1.684]	0.42	1.406 [0.913–2.165]	0.121
Co-mutated KRAS/BRAF	1.080 [0.625–1.867]	0.782	0.819 [0.407–1.648]	0.573
MSI	0.444 [0.284–0.696]	<0.001	0.469 [0.280–0.785]	0.005
Extrahepatic Disease	2.197 [1.118–4.317]	0.022	1.844 [0.760–4.472]	0.174
Pre-hepatectomy Chemotherapy	0.884 [0.653–1.197]	0.425	0.893 [0.604–1.321]	0.567
Post-hepatectomy Chemotherapy	1.038 [0.714–1.507]	0.847	0.777 [0.504–1.196]	0.249
Converted CRLM	1.598 [1.118–2.283]	0.01	1.463 [0.895–2.392]	0.127