Two-stage hepatectomy for colorectal liver metastases: Pathologic response to preoperative chemotherapy is associated with second-stage completion and longer survival
François Quenet, Marie-Hélène Pissas, Hugo Gil, Lise Roca, Sébastien Carrere, Olivia Sgarbura, Philippe Rouanet, Hélène de Forges, Lakhdar Khellaf, Emmanuel Deshayes, et al.

To cite this version:

HAL Id: hal-02448906
https://hal.umontpellier.fr/hal-02448906
Submitted on 22 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License
Two-stage hepatectomy for colorectal liver metastases: pathologic response to preoperative chemotherapy is associated with second-stage completion and longer survival

François Quénet1*, MD, Marie-Hélène Pissas1*, MD, Hugo Gil2, MD, Lise Roca3, MSc, Sébastien Carrère1, MD, Olivia Sgarbura1, MD, Philippe Rouanet1, MD, PhD, Hélène de Forges4, PhD, Lakhdar Khellaf2, MD, Emmanuel Deshayes5, MD, Marc Ychou6, MD, PhD, Frédéric Bibeau2,7, MD, PhD.

1 Surgical oncology Department, Institut régional du Cancer de Montpellier (ICM), Univ. Montpellier, Montpellier, France
2 Anatomopathology Department, Institut régional du Cancer de Montpellier (ICM), Univ. Montpellier, Montpellier, France
3 Biometrics Unit, Institut régional du Cancer de Montpellier (ICM), Univ. Montpellier, Montpellier, France
4 Clinical research Unit, Institut régional du Cancer de Montpellier (ICM), Univ. Montpellier, Montpellier, France
5 Nuclear Medicine Department, Institut régional du Cancer de Montpellier (ICM), Univ. Montpellier, Montpellier, France
6 Medical Oncology Department, Institut régional du Cancer de Montpellier (ICM), Univ. Montpellier, Montpellier, France
7 Anatomopathology Department, Centre Hospitalier Universitaire de Caen, Caen, France

* These two authors are co-first authors

Corresponding author:
Dr François Quénet
Surgical oncology Department,
Institut régional du Cancer de Montpellier (ICM)
208 Avenue des Apothicaires, 34298 Montpellier, France
E-mail: François.quenet@icm.unicancer.fr
Tel: +33 4 67 61 31 03
Fax: +33 4 67 61 37 03

Running head: Pathologic response and longer survival

© 2018 published by Elsevier. This manuscript is made available under the CC BY NC user license https://creativecommons.org/licenses/by-nc/4.0/
ABSTRACT

Background

Two-stage hepatectomy (TSH) of bilobar colorectal liver metastases (CRLM) is widely used and shows encouraging survival results. However, the risk of drop-out after the first-stage remains high and associated with poor survival. The objective of our study was to evaluate the factors associated with long-term survival based on the pathologic response to preoperative systemic chemotherapy in CRLM patients who underwent TSH.

Methods

The pathologic response to preoperative chemotherapy and its effect on second-stage completion and survival were retrospectively evaluated in 67 patients treated between 2003 and 2013.

Results

Fifty-six patients underwent TSH for initially non-resectable CRLM. Chemotherapy was combined with a biotherapy in 32 cases. The Tumor Regression Grade (TRG), modified-TRG and Blazer grade were used to classify patients as responders (TRG and mTRG 1-3, Blazer 0-1) or non-responders (TRG and mTRG 4-5, Blazer 2) after the first stage. Tumor response in the three classifications was associated with second-stage completion (TRG 1-3: OR=4.01 95% CI: 1.12-14.36 p=0.033; mTRG 1-3: OR=3.8 95% CI:1.13-12.6 p=0.03; Blazer 0-1: OR=5.45 95% CI: 1.66-17.85 p=0.005). Triple chemotherapy was also associated with responders. The median overall survival of responders was significantly higher (Blazer 0-1: 42.9 months versus Blazer 2: 20.1 months p=0.018; TRG 1-3: 42.9 months versus TRG 4-5: 25.1 months, p=0.04).

Conclusion

A pathologic response to chemotherapy is associated with second-stage completion and
longer survival. Further studies are needed to try to early identify the patients for whom the benefit of the second surgical stage is less straightforward.

**Keywords:** colorectal cancer, liver metastases, two-stage resection, pathologic response, survival
INTRODUCTION

Liver metastases occur in approximately 50% of patients affected by colorectal cancer and are the most common cause of death in this population\textsuperscript{1,2}. Recent advances in multidisciplinary approaches have changed the prognosis of patients with colorectal liver metastases (CRLM)\textsuperscript{3,4} and technical improvements such as the two-stage hepatectomy (TSH) now allow a higher number of patients with CRLM to be eligible for surgery\textsuperscript{5,6}.

The TSH strategy is widely used since the 2000s\textsuperscript{6} for patients with initially unresectable metastases, becoming resectable after neoadjuvant chemotherapy. This potentially-curative strategy is associated with longer overall survival for patients who completed the two surgical stages. However, the risk of drop-out before the second stage is high, mainly due to disease progression\textsuperscript{7}. Patients who drop-out before the second surgical stage have poorer survival rates, close to those obtained in unresectable patients, and surgery may thus be inappropriate\textsuperscript{7,8}. Studies have suggested that increased exposure to chemotherapy prior to the first hepatectomy may predict failure to complete the second surgical stage\textsuperscript{9} and that the pathologic response to intensive preoperative chemotherapy is a favourable prognostic factor\textsuperscript{10}. However, the prognostic impact of the pathologic response to systemic chemotherapy is probably still underestimated. We thus conducted a retrospective study to demonstrate a possible correlation between the pathologic response to preoperative chemotherapy and survival in CRLM patients who underwent TSH. The objective of our study was to evaluate the factors associated with the completion of the second surgical stage, and, consequently, of progression-free and overall survival, based on the pathologic response to systemic chemotherapy administered preoperatively.

PATIENTS AND METHODS
**Patients**

From January 2003 to January 2013, 899 patients were treated for CRLM in our institution. Among these, patients with multiple, bilobar CRLM initially considered unresectable with a one-stage procedure, and who underwent at least the first stage of a planned TSH, were retrospectively selected for analysis on an intent-to-treat basis. Initial unresectability was defined as the inability to resect all metastases with tumor-free margins while saving a sufficient remnant liver volume to prevent postoperative liver failure. A sufficient remnant liver volume was defined as 30% of the residual liver parenchyma on CT-scan or by estimation of the future remnant liver function with $^{99m}$Tc-mebrofenin 3D SPECT-CT. Patients with a $^{99m}$Tc-mebrofenin clearance rate <2.69%/min/m$^2$ were considered at high risk of postoperative liver failure.

Patients with metachronous metastases diagnosed at least three months after primary tumor diagnosis were also included. Patients with unplanned repeated hepatic resection for recurrence, who underwent a one-stage resection, or who presented with an unresectable extrahepatic abdominal disease were excluded from the study. A limited lung metastatic involvement was not considered a contraindication for hepatic resection. The protocol was approved by the local ethics committee review board. The study was conducted according to the Declaration of Helsinki and European Good Clinical Practice requirements. Patients were informed that their clinical and scientific data could be used for scientific purpose prior to their care in our institution.

**Preoperative chemotherapy and therapeutic strategy**

All patients were treated with intensive chemotherapy before liver resection, with oxaliplatin, irinotecan, 5-fluorouracil (5-FU), leucovorin and capecitabine (5-FU oral form) as doublet or triplet regimens. From 2008, a biotherapy (bevacizumab, cetuximab or panitumumab)
was frequently added, depending on the RAS status. The Response Evaluation Criteria In Solid Tumor (RECIST) evaluation was used to select patients responding to preoperative chemotherapy\textsuperscript{16}.

**Surgery**

During the first operative stage, the future remnant liver was cleared from tumor tissue using wedge resection and/or anatomical resection and/or radiofrequency ablation. Resection of the primary colorectal tumor was performed concomitantly if the surgical liver procedure was not too important and in case the primary tumor was symptomatic. To prevent tumor progression, interval chemotherapy was for most patients administered between the two surgical stages. A portal vein ligation or embolization was performed to prevent postoperative liver failure according to volumetric CT-scan and mebrofenin hepatoscintigraphy data. Resection was mostly a right hepatectomy to achieve a curative resection. Postoperative morbidity at 30 days was graded according to the Clavien-Dindo classification\textsuperscript{17}. Postoperative mortality was defined as death occurring within 90 days following surgery.

**Pathologic response**

All tumor metastases were sampled for pathology examination. Three-\(\mu\)m thick sections of formalin-fixed paraffin-embedded tissues were mounted on SuperFrost Plus glass slides (Menzel, GmbH, Germany) deparaffinized before Hematein-Eosin-Saffron (HES) staining. Three pathologists with hepatobiliary expertise and blinded to the clinical data evaluated the response in all resected metastases, using the Tumor Regression Grade (TRG), the modified TRG and the Blazer classification\textsuperscript{10,18,19}. Challenging evaluation cases were reviewed jointly for final consensus. Patients were classified as pathologic responders (TRG and mTRG 1-3, Blazer 0-1) or non-responders (TRG and mTRG 4-5, Blazer 2) after the first stage. The mean
percentage of residual tumor cells and regression features in the different metastases were used to define the pathologic response. Differentiation, tumour size, number of metastases and resection margins were also reported for each patient.

**Statistical analyses**

Patients’ characteristics were compared using the \( \chi^2 \) or the Fisher’s exact tests for discrete variables. The Student’s t-test and Wilcoxon rank-sum test were used for continuous variables. Factors associated with second-stage completion were analysed using univariate and multivariate logistic regression models. Factors significant at the \( p<0.05 \) level in the univariate analysis and the appropriate pathologic response variable were included in the classical multivariate model, validated using a stepwise automatic method. Survival rates were estimated from the first surgery until the event of interest, death of any cause for OS and recurrence for RFS, using the Kaplan-Meier method, and compared using a log-rank test. Median survival was presented with its 95% confidence interval (95% CI). Patients lost to follow-up were censored at the last documented visit, and those who died without recurrence at the date of death. All statistical analyses were performed using the STATA 11.0 software (StataCorp, College Station, Texas).

**RESULTS**

**Patients’ characteristics**

TSH strategy was planned for 67 patients, among whom 11 were excluded because of the lack of pathological sample: 8 because they only had radiofrequency and 3 because the samples were not found (Figure 1 and Table 1). The median age was 59 years (range: 38-77) and there were 25 (44.6%) women. The primary tumor site was colon in 80.4% cases. All CRLM were bilobar at diagnosis, and 90.9% were synchronous. The median time from initial diagnosis
and occurrence of metachronous metastases was 18.3 months (95% CI: 11.8-28.7). RAS was mutated in 30.6% patients and 25.5% had a carcinoembryogenic antigen (CEA) level >200ng/mL.

**Preoperative chemotherapy**

All patients received downstaging chemotherapy. The median number of cycles was 8 (4-25). A doublet regimen, FOLFIRI (5FU+irinotecan) or FOLFOX (5FU+oxaliplatine) was administered to 60.7% patients and FOLFIRINOX (5FU+oxaliplatine+irinotecan) to 33.9% patients. A biotherapy was associated with chemotherapy for 55.3% patients.

**First-stage hepatectomy**

The median hospital stay was 11 days (6-162) (Table 2). A median of 3 (1-5) radiofrequency ablations and 1 (1-6) non-anatomical resection were performed. The median size of the largest metastasis resected was 18mm (3-60). The primary colorectal tumor was resected during this first surgery in 22 patients (39.3%). Postoperative grade ≥3 morbidity at 30 days was reported in 8 (14.3%) patients (Table 2), mainly hepatic abscesses (n=3) and complications linked to the primary colorectal tumor (n=3). There was no postoperative mortality at 90 days.

**Second-stage hepatectomy**

The TSH was completed for 35 (62.5%) patients. Twenty-one patients dropped-out after the first surgical stage. The main reason was disease progression (n=16), including hepatic (n=14) or extra-hepatic (n=1) progression, or both (n=13). Two patients experienced severe complications after the first surgery (one rectal fistula and one abdominal wall complication) which prevented undergoing the second stage; one patient refused the second surgery, and 3 were lost to follow-up.
The median time between the two surgical stages was 6 months (2-24), mainly due to our deliberate policy to administer systemic chemotherapy between the two stages. Interval chemotherapy was administered to 50 (89.3%) patients, with a median of 6 cycles (4-20) (Table 1). Six patients (11%) did not receive interval chemotherapy, mostly patients who had undergone radiofrequency ablations with non-anatomic resections. A portal vein embolization was reported in 17.6% patients. The median hospital stay was 11 days (5-49). A standard right hepatectomy was performed in 31.4% patients, and 23.4% underwent a right lobectomy (Table 2). Postoperative grade ≥3 morbidity was reported in 6 (17.1%) patients mainly hepatic abscesses (n=3) and complications linked to the primary colorectal tumor (n=2). Two patients died of disease progression within 90 days following the second stage. There was no postoperative mortality reported.

**Pathologic response**

A pathologic response (TRG, mTRG 1-3, and Blazer 1-0) was observed in 21 (37.5%), 24 (42.9%), and 30 (53.6%) patients, respectively (Table 3). Patients who completed the two stages were significantly better responders than those who dropped-out, according to the three classifications: 48.6% vs 19.0%, 54.3% vs 23.8% and 68.6% vs 28.6% responders vs non-responders for the TRG, mTRG and Blazer, respectively. The pathologic response was associated with second-stage completion according to the three classifications: TRG 1-3: OR=4.01; 95% CI: 1.12-14.36; p=0.033; mTRG 1-3: OR=3.8; 95% CI: 1.13-12.6; p=0.03; Blazer 0-1: OR=5.45; 95% CI: 1.66-17.85; p=0.005 in univariate analysis. In multivariate analysis, among the three classifications, only the Blazer grade was associated with second stage completion (Blazer 0/1: OR=5.42; 95% CI: 1.53-19.15; p=0.006), together with the initial number of metastases ≤5 (OR=4.06, 95% CI: 1.14-14.47, p=0.009) (Table 4).
The neoadjuvant FOLFIRINOX regimen was associated with a pathologic response according to the three classifications (TRG 1-3: 73.7% vs TRG 4-5: 26.3%; \( p < 0.001; \) mTRG 1-3: 73.7% vs mTRG 4-5: 26.3%; \( p < 0.0001; \) Blazer 0-1: 84.2% vs Blazer 2: 15.8%; \( p = 0.002 \) (Table 5).

**Survival**

After a median follow-up of 66 months, the median overall survival (OS) of the whole population was 39 months (95% CI: 25.1-42.9), with 3-year and 5-year OS rates of 46.7% (95% CI: 32.6-59.6) and 17.1% (95% CI: 7.1-30.8). In patients who completed TSH, the median OS was significantly higher than that of patients who did not: 50 months (95% CI: 36.2-59.3) versus 18.4 months (95% CI: 12.0-22.2) respectively, with a 3-year OS rate of 71% (95% CI: 51.4-83.8) versus 9.5% (95% CI: 1.6-26.1), hazard ratio (HR)= 0.17 (95% CI: 0.08-0.35, \( p < 0.001 \) (Figure 2). The median relapse-free survival (RFS) of patients who completed the two stages (n=43) was 16.9 months (95% CI: 14.5-21) (Figure 2). The median OS was significantly higher in responders according to the TRG and Blazer classifications: Blazer 0-1: 42.9 months versus Blazer 2: 20.1 months, HR= 2.14 (95% CI: 1.13-4.04), \( p = 0.018, \) and TRG 1-3: 42.9 months versus TRG 4-5: 25.1 months, HR=2.08 (95% CI: 1.03-4.19), \( p = 0.04 \) (Figure 3).

**DISCUSSION**

The present study is, to our knowledge, the first to demonstrate a direct correlation between a favorable pathologic response to chemotherapy and second-stage completion, and thus longer survival. In unresectable CRLM patients, previous studies have shown that a major pathologic response after preoperative treatment was a favorable prognostic factor\textsuperscript{10,18,19}. Yet, its importance is not fully taken into account when taking therapeutic decisions. Among the three classifications, the TRG\textsuperscript{18} considers fibrosis as a characteristic feature of cellular response,
whereas the mTRG\textsuperscript{19} also takes into account the infarct-like necrosis (ILN); the Blazer score\textsuperscript{10} is exclusively based on the percentage of residual tumor cells relative to the total tumor area, whatever the type of regression, and may thus be more efficient to evaluate the impact of intensive induction treatments. Probably because of that reason, the pathologic response was higher assessed with the Blazer score than with both the TRG and mTRG in our series, and it was the only classification associated with second-stage completion in multivariate analysis. Our study also allowed identification of patients treated with FOLFIRINOX as better responders than those treated with the standard regimen. Correlatively, Ychou \textit{et al.} showed that this triplet systemic chemotherapy was associated with a better response, higher resectability and OS rates\textsuperscript{20}.

Very few series have evaluated the impact of the pathologic response in the setting of TSH strategies. Faitot \textit{et al.} showed that the pathologic response was associated with clinical outcome but not with TSH feasibility, contrary to our study\textsuperscript{21}. Another study, Mentha \textit{et al.}, showed in 22 CRLM patients treated with neoadjuvant therapy and TSH that a “dangerous halo” in the pathologic findings was a bad prognosis factor but didn’t correlate the intensity of the pathologic response to survival\textsuperscript{22}. Recently, Pietrantonio \textit{et al.} showed that bevacizumab induced significant better pathological response rates and complete responses compared with cetuximab even if OS was not significantly different\textsuperscript{23}. Moreover, a significant correlation with pathological response was found between the number of resected metastases and bevacizumab allocation.

Numerous studies have suggested that regenerative growth factor levels increased immediately after the first-stage hepatectomy and contributed to tumor recurrence\textsuperscript{24–26}. In this context, administering interval chemotherapy allowed a longer period of natural liver regeneration while preventing tumor progression. It thus was our deliberate policy to administer interval systemic chemotherapy, although its benefits are still controversial.
Tanaka et al.\textsuperscript{27} showed decreased tumour growth and growth factor expression with perioperative chemotherapy, while Muratore et al.\textsuperscript{28} showed no benefit of interval chemotherapy on disease progression between the two surgical stages.

The main drawback of the TSH strategy is that more than 30% patients drop-out after the first-stage procedure (36% patients in our study)\textsuperscript{7,8}. They have a poor prognosis, with survival no longer than that of patients undergoing chemotherapy alone\textsuperscript{7}. The median OS of patients who only underwent the first stage was of 18.4 months (95% CI: 12-22.2) compared to almost 50 months for those who completed the second stage; 3-year OS rates were 9.5%, and 71%, respectively. Completion of the whole procedure allows a similar benefit than that of the single stage reported in patients with initially resectable liver metastases\textsuperscript{29}. It was shown that the main cause of drop-out is disease progression between the two stages\textsuperscript{7}. It is thus essential to identify patients who will not reach the second surgery\textsuperscript{30}. Several studies have identified predictive factors of drop-out\textsuperscript{8,9,30-32}, among which major postoperative complications after the first stage\textsuperscript{31}, older age, CEA level >200ng/ml before portal vein embolization, 3 or more tumours in the future remnant liver\textsuperscript{8}, high number of metastases, and increased exposure to chemotherapy prior to the first hepatectomy\textsuperscript{8}. Mise et al. showed that a RAS mutation should be taken into account to predict response to chemotherapy\textsuperscript{33}. In this study, a pathologic response was more common in patients with wild-type RAS compared with patients with RAS mutations. Passot et al. showed that the RAS mutation independently predicts the oncologic efficacy of TSH\textsuperscript{34}.

A pathologic response was shown to be a strong prognostic factor after preoperative chemotherapy and surgical resection of CRLM\textsuperscript{35,36} and, in another field, of colorectal distant metastasis such as peritoneal carcinomatosis\textsuperscript{37}. However, its use for treatment decision remains limited. Our study shows some limitations as it was retrospective and monocentric,
and with a relatively limited number of patients. However, it is the first to show a significant correlation between a pathologic response to chemotherapy and second stage completion and consecutively, a better survival and, considering the design limitations, proofs of these correlations seem quite robust. We hope it to be a first step towards further investigations correlating histologic and radiologic findings. Among these, identifying non-responders patients before the first surgical step, which is unfortunately not yet possible, would allow sparing some patients from a not necessarily-needed surgery.

**CONCLUSION**

A pathologic response to preoperative chemotherapy after the first-stage hepatectomy is associated with completion of the second surgical stage and of longer survival. The accurate assessment of the pathologic response to induction treatments represents an important contribution from pathologists for treatment decision for these patients, and may allow avoiding resections for patients for whom the TSH strategy benefit seems less straightforward.

**Acknowledgments**

The authors thank the patients and their families. They also thank the Drs. Emmanuelle Samalin, Fabienne Portales, and Thibault Mazard for their participation to the study, and Pr. Bernard Saint-Aubert for providing expertise and interesting discussions all along the project. This research was supported by the SIRIC Montpellier Cancer Grant INCa_Inserm_DGOS_12553.

**Authors’ contributions**

Study design: FQ, FB.
Collection of data: FQ, MHP, HG, LK, SC, OS, PR, ED, MY, FB.
Analysis and interpretation of data: LR, FQ, FB, MHP.
Drafting of the manuscript: FQ, MHP, HF, FB.
Revising critically the manuscript: FQ, MHP, FB, HF, OS, PR, SC, LR
Final validation of the manuscript: FQ, MHP, HG, LR, SC, OS, PR, HF, LK, ED, MY, FB.

**Disclosure:** All the other authors have no conflict of interest to disclose in relation with the present study.
References


Figure legends

**Figure 1**
Flowchart of the study

**Figure 2**
A: Overall survival in patients who completed or did not complete the two-stage resection;
B: Relapse-free survival of patients who completed the second stage.

**Figure 3**
Overall survival depending on the pathological response, according to the TRG (A), mTRG (B) and Blazer (C) classifications. A: TRG classification: responders (TRG 1,2,3) *versus* non-responders; B: mTRG classification: responders (mTRG 1,2,3) *versus* non-responders (4,5); C: Blazer classification: responders (Blazer 0,1) *versus* non-responders (Blazer 2).

**Tables**

*Table 1:* Patients’ characteristics

*Table 2:* Surgical characteristics of the first and second stages

*Table 3:* Patients’ and tumor characteristics, and histologic response in the “first-stage only” and the “two-stages completed” groups

*Table 4:* Factors associated with second-stage completion (uni- and multivariate analyses)

*Table 5:* Correlation between the neoadjuvant FOLFIRINOX regimen and the pathologic response
### Table 1: Patients’ characteristics

<table>
<thead>
<tr>
<th>Patients’ characteristics</th>
<th>n=56</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>med (range) 59 (38-77)</td>
</tr>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td>n (%)</td>
</tr>
<tr>
<td>Male</td>
<td>31 (55.4)</td>
</tr>
<tr>
<td>Female</td>
<td>25 (44.6)</td>
</tr>
<tr>
<td><strong>Site of primary tumor</strong></td>
<td>n (%)</td>
</tr>
<tr>
<td>Colon</td>
<td>45 (80.4)</td>
</tr>
<tr>
<td>Rectal</td>
<td>11 (19.6)</td>
</tr>
<tr>
<td><strong>N+ stage</strong></td>
<td>n (%) 42 (75.0)</td>
</tr>
<tr>
<td><strong>Metastases,</strong></td>
<td>n (%)</td>
</tr>
<tr>
<td>Synchronous</td>
<td>50 (90.9)</td>
</tr>
<tr>
<td>Metachronous</td>
<td>5 (9.1)</td>
</tr>
<tr>
<td><strong>Initial number of metastases</strong></td>
<td>n (%)</td>
</tr>
<tr>
<td>0-5</td>
<td>28 (50.9)</td>
</tr>
<tr>
<td>5-10</td>
<td>7 (12.7)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>20 (36.4)</td>
</tr>
<tr>
<td><strong>Neoadjuvant chemotherapy regimens</strong></td>
<td>n (%)</td>
</tr>
<tr>
<td>Doublet therapy (Folfox or Folfiri)</td>
<td>34 (60.7)</td>
</tr>
<tr>
<td>Triplet therapy (Folfirinox)</td>
<td>19 (33.9)</td>
</tr>
<tr>
<td>Biotherapy</td>
<td>31 (55.3)</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>14 (25)</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>16 (28.6)</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td><strong>Missing</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Neo-adjuvant chemotherapy regimens</strong></td>
<td>n (%)</td>
</tr>
<tr>
<td>Doublet therapy (Folfox or Folfiri)</td>
<td>42 (75)</td>
</tr>
<tr>
<td>Triplet therapy (Folfirinox)</td>
<td>6 (10.7)</td>
</tr>
<tr>
<td>Biotherapy</td>
<td>37 (66.1)</td>
</tr>
<tr>
<td><strong>Number of cycles,</strong></td>
<td>med (range) 8 (4-25)</td>
</tr>
<tr>
<td><strong>Missing</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>Interval chemotherapy regimens</strong></td>
<td>n (%)</td>
</tr>
<tr>
<td>Doublet therapy (Folfox or folfiri)</td>
<td>50 (89.3)</td>
</tr>
<tr>
<td>Triplet therapy (Folfirinox)</td>
<td>6 (10.7)</td>
</tr>
<tr>
<td><strong>Number of cycles,</strong></td>
<td>med (range) 6 (4-20)</td>
</tr>
<tr>
<td><strong>Preoperative CEA plasma level (ng/ml)</strong></td>
<td>n (%)</td>
</tr>
<tr>
<td>&gt; 200</td>
<td>13 (25.5)</td>
</tr>
<tr>
<td><strong>Missing</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>RAS status</strong></td>
<td>n (%)</td>
</tr>
<tr>
<td>Mutated</td>
<td>15 (30.6)</td>
</tr>
<tr>
<td><strong>Missing</strong></td>
<td>7</td>
</tr>
</tbody>
</table>

CEA: Carcinoembryonic antigen
Table 2: Surgical characteristics of the first and second stages

<table>
<thead>
<tr>
<th>First-stage perioperative characteristics</th>
<th>n=56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of stay (days)</td>
<td>med (range) 11 [6-162]</td>
</tr>
<tr>
<td>Associated resection of primary tumor</td>
<td>n (%) 22 (39.3)</td>
</tr>
<tr>
<td>Side of hepatic clearance resection</td>
<td>n (%)</td>
</tr>
<tr>
<td>Right</td>
<td>6 (10.7)</td>
</tr>
<tr>
<td>Left</td>
<td>40 (71.4)</td>
</tr>
<tr>
<td>Right + Left</td>
<td>10 (17.9)</td>
</tr>
<tr>
<td>Anatomic minor liver resection (segmentectomy)</td>
<td>n (%) 15 (22.3)</td>
</tr>
<tr>
<td>Median number per patient Radiofrequency</td>
<td>n (%) 46 (82.1)</td>
</tr>
<tr>
<td>Median number per patient Wedge resection</td>
<td>n (%) 1 [1-6]</td>
</tr>
<tr>
<td>Median number per patient</td>
<td>med (range) 25 (44.6)</td>
</tr>
<tr>
<td>Median number per patient</td>
<td>med (range) 3 [1-5]</td>
</tr>
<tr>
<td>Largest diameter of the resected metastases (mm),</td>
<td>med (range) 18 [3-60]</td>
</tr>
<tr>
<td>Missing</td>
<td>2</td>
</tr>
<tr>
<td>Resection margins (mm), Contact margins with tumor,</td>
<td>med (range) 2 [1-25]</td>
</tr>
<tr>
<td>Missing</td>
<td>n (%) 10 (17.8)</td>
</tr>
<tr>
<td>Morbidity grade ≥3</td>
<td>n (%) 8 (14.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Second-stage perioperative characteristics</th>
<th>n=35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of stay (days)</td>
<td>med (range) 11 [5-49]</td>
</tr>
<tr>
<td>Delay between the 2 stages (months)</td>
<td>med (range) 6.7 [2.1-24.5]</td>
</tr>
<tr>
<td>Portal embolization</td>
<td>n (%) 6 (17.6)</td>
</tr>
<tr>
<td>Right hepatectomy¹</td>
<td>n (%) 11 (31.4)</td>
</tr>
<tr>
<td>Right lobectomy²</td>
<td>n (%) 8 (22.8)</td>
</tr>
<tr>
<td>Left hepatectomy³</td>
<td>n (%) 2 (5.7)</td>
</tr>
<tr>
<td>Segmental resections (≥2)*</td>
<td>n (%) 6 (17.1)*</td>
</tr>
<tr>
<td>Morbidity grade ≥3</td>
<td>n (%) 6 (17.1)</td>
</tr>
</tbody>
</table>

¹ Segments 5 to 8; ² Segments 4 to 8; ³ Segments 2 to 4

* Two patients had a single segmentectomy, but also had a right hepatectomy extended to segment 4 during the first surgical stage.
Table 3: Patients’ and tumor characteristics, and histologic response in the “first-stage only” and the “two-stages completed” groups

<table>
<thead>
<tr>
<th>n (%)</th>
<th>First stage only n=21</th>
<th>2 stages completed n=35</th>
<th>Total n=56</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60</td>
<td>8 (38.1)</td>
<td>21 (60)</td>
<td>29 (51.8)</td>
<td>ns</td>
</tr>
<tr>
<td>≥ 60</td>
<td>13 (61.9)</td>
<td>14 (40)</td>
<td>27 (48.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>Male</td>
<td>13 (61.9)</td>
<td>18 (51.4)</td>
<td>31 (55.4)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8 (38.1)</td>
<td>17 (48.6)</td>
<td>25 (44.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Primary tumor localization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>17 (81)</td>
<td>28 (80)</td>
<td>45 (80.4)</td>
<td>ns</td>
</tr>
<tr>
<td>Rectum</td>
<td>4 (19)</td>
<td>7 (20)</td>
<td>11 (19.6)</td>
<td></td>
</tr>
<tr>
<td><strong>T stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>1 (4.8)</td>
<td>5 (14.3)</td>
<td>6 (10.7)</td>
<td>ns</td>
</tr>
<tr>
<td>III/IV</td>
<td>16 (76.2)</td>
<td>28 (80)</td>
<td>44 (78.6)</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>4 (19)</td>
<td>2 (5.7)</td>
<td>6 (10.7)</td>
<td></td>
</tr>
<tr>
<td><strong>N stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>2 (9.5)</td>
<td>9 (25.7)</td>
<td>11 (19.6)</td>
<td>p=0.03</td>
</tr>
<tr>
<td>N+</td>
<td>16 (76.2)</td>
<td>26 (74.3)</td>
<td>42 (75)</td>
<td></td>
</tr>
<tr>
<td>Nx</td>
<td>3 (14.3)</td>
<td>0</td>
<td>3 (5.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Metastases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number ≤ 5</td>
<td>6 (28.6)</td>
<td>22 (64.7)</td>
<td>28 (50.9)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Number &gt; 5</td>
<td>15 (71.4)</td>
<td>12 (35.3)</td>
<td>27 (49.1)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synchronous</td>
<td>19 (90.5)</td>
<td>31 (91.2)</td>
<td>50 (90.9)</td>
<td>ns</td>
</tr>
<tr>
<td>Metachronous</td>
<td>2 (9.5)</td>
<td>3 (8.8)</td>
<td>5 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>CEA level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200 ng/dl</td>
<td>13 (76.5)</td>
<td>25 (73.5)</td>
<td>38 (74.5)</td>
<td>ns</td>
</tr>
<tr>
<td>&gt;200 ng/dl</td>
<td>4 (23.5)</td>
<td>9 (26.5)</td>
<td>13 (25.5)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><strong>RAS status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutated</td>
<td>5 (29.4)</td>
<td>10 (31.3)</td>
<td>15 (30.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Non-mutated</td>
<td>12 (70.6)</td>
<td>22 (68.8)</td>
<td>34 (69.4)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td><strong>Chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoadjuvant</td>
<td>21 (100)</td>
<td>32 (91.4)</td>
<td>53 (94.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Intervalt</td>
<td>19 (90.5)</td>
<td>31 (88.6)</td>
<td>50 (89.3)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>All-grade morbidity after the first stage (Clavien-Dindo)</strong></td>
<td>6 (28.6)</td>
<td>9 (25.7)</td>
<td>15 (26.8)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Histologic response</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders (1/2/3)</td>
<td>4 (19.0)</td>
<td>17 (48.6)</td>
<td>21 (37.5)</td>
<td>p=0.045</td>
</tr>
<tr>
<td>Non-responders (4/5)</td>
<td>17 (81.0)</td>
<td>18 (51.4)</td>
<td>35 (62.5)</td>
<td></td>
</tr>
<tr>
<td>mTRG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders (1/2/3)</td>
<td>5 (23.8)</td>
<td>19 (54.3)</td>
<td>24 (42.9)</td>
<td>p=0.026</td>
</tr>
<tr>
<td>Non-responders (4/5)</td>
<td>16 (76.2)</td>
<td>16 (45.7)</td>
<td>32 (57.1)</td>
<td></td>
</tr>
<tr>
<td>Blazer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders (0/1)</td>
<td>6 (28.6)</td>
<td>24 (68.6)</td>
<td>30 (53.6)</td>
<td>p=0.004</td>
</tr>
<tr>
<td>Non-responders (2)</td>
<td>15 (71.4)</td>
<td>11 (31.4)</td>
<td>26 (46.4)</td>
<td></td>
</tr>
</tbody>
</table>

CEA: Carcinoembryonic antigen; TRG: Tumor Regression Grade; mTRG: modified TRG
Table 4: Factors associated with second-stage completion (uni- and multivariate analyses)

<table>
<thead>
<tr>
<th>Univariate analysis</th>
<th>Odd ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRG 1/2/3</td>
<td>4.01</td>
<td>1.12-14.36</td>
<td>0.033</td>
</tr>
<tr>
<td>mTRG 1/2/3</td>
<td>3.8</td>
<td>1.13-12.67</td>
<td>0.030</td>
</tr>
<tr>
<td>Blazer 0/1</td>
<td>5.45</td>
<td>1.66-17.85</td>
<td>0.005</td>
</tr>
<tr>
<td>Initial number of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metastases ≤5</td>
<td>4.6</td>
<td>1.4-14.9</td>
<td>0.011</td>
</tr>
</tbody>
</table>

| Multivariate analysis | | | |
|-----------------------| | | |
| Blazer 0/1            | 5.42 | 1.53-19.15 | 0.006 |
| Initial number of     |       |           |       |
| metastases ≤5        | 4.06  | 1.14-14.47 | 0.009 |

TRG: Tumor Regression Grade; mTRG: modified TRG; 95% CI: 95% Confidence interval
Table 5: Correlation between the neoadjuvant FOLFIRINOX regimen and the pathologic response

<table>
<thead>
<tr>
<th></th>
<th>No FOLFIRINOX n=43</th>
<th>FOLFIRINOX n=24</th>
<th>Total n=67</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TRG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders (1/2/3)</td>
<td>7 (18.9)</td>
<td>14 (73.7)</td>
<td>21 (37.5)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Non-responders (4/5)</td>
<td>30 (81.1)</td>
<td>5 (26.3)</td>
<td>35 (62.5)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>6</td>
<td>5</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><strong>mTRG</strong></td>
<td></td>
<td></td>
<td></td>
<td>p=0.001</td>
</tr>
<tr>
<td>Responders (1/2/3)</td>
<td>10 (27.0)</td>
<td>14 (73.7)</td>
<td>24 (42.9)</td>
<td></td>
</tr>
<tr>
<td>Non-responders (4/5)</td>
<td>27 (73.0)</td>
<td>5 (26.3)</td>
<td>32 (57.1)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>6</td>
<td>5</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><strong>Blazer</strong></td>
<td></td>
<td></td>
<td></td>
<td>p=0.001</td>
</tr>
<tr>
<td>Responders (0/1)</td>
<td>14 (37.8)</td>
<td>16 (84.2)</td>
<td>30 (53.6)</td>
<td></td>
</tr>
<tr>
<td>Non-responders (2)</td>
<td>23 (62.2)</td>
<td>3 (15.8)</td>
<td>26 (46.4)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>6</td>
<td>5</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

TRG: Tumor Regression Grade; mTRG: modified TRG
Figure 1: Flowchart of the study

899 patients treated for CRLM

67 patients with planned TSH

56 patients selected for analysis

35 patients completed TSH

832 patients not eligible for TSH

11 patients excluded
- 3 patients with missing anapathological samples
- 8 patients who underwent radiofrequency ablation only

21 patients dropped-out before the 2nd stage
- 16 disease progression (14 hepatic, 1 extra-hepatic, 13 both)
- 2 severe complications after 1st-stage
- 3 lost to follow-up
Figure 2

A. Overall survival (OS) in patients who completed or did not complete the two-stage resection

HR=0.18; 95%CI [0.09-0.36]; \( p<0.001 \)

* OS was defined as the time between the date of first surgery to death. The Kaplan-Meier estimates of probabilities of OS were estimated in all patients who completed or not the two-stage resection

B. Relapse-free survival (RFS) of patients who completed the second stage

* RFS was defined as the time between the date of first surgery to the relapse event. The Kaplan-Meier estimates of probabilities of RFS were estimated in all patients who completed the two-stage resection
Figure 3: Overall survival depending on the pathological response, according to the TRG (A), mTRG (B) and Blazer (C) classifications.

A. TRG classification: responders (TRG 1,2,3) versus non-responders (4,5)

![Graph A with Kaplan-Meier estimates](image)

HR=2.08; 95%CI [1.03-4.19], \( p=0.040 \)

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>Resp</th>
<th>No rep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>21</td>
<td>35</td>
</tr>
<tr>
<td>Non reps</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>

* OS was defined as the time between the date of first surgery to death. The Kaplan-Meier estimates of probabilities of OS depending on the TRG pathological response were estimated for responders (TRG 1,2,3) versus non-responders (TRG 4,5)

B. mTRG classification: responders (mTRG 1,2,3) versus non-responders (4,5)

![Graph B with Kaplan-Meier estimates](image)

HR=21.88; 95%CI [0.96-3.69]; \( p=0.063 \)

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>Resp</th>
<th>No rep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>24</td>
<td>32</td>
</tr>
<tr>
<td>Non reps</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

* OS was defined as the time between the date of first surgery to death. The Kaplan-Meier estimates of probabilities of OS depending on the mTRG pathological response were estimated for responders (TRG 1,2,3) versus non-responders (TRG 4,5)
C. Blazer classification: responders (Blazer 0,1) versus non-responders (Blazer 2)

* OS was defined as the time between the date of first surgery to death. The Kaplan-Meier estimates of probabilities of OS depending on the Blazer pathological response were estimated for responders (Blazer 0,1) versus non-responders (Blazer 2)