

Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases

CAIRO5

a randomised phase 3 study of the Dutch Colorectal Cancer Group (DCCG)



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Synopsis

Study title	Treatment strategies in colorectal cancer patients with initially unresectable liver-only metastases: CAIRO5, a randomised phase 3 study of the Dutch Colorectal Cancer Group (DCCG)
Study phase	Randomised phase 3
Background	Colorectal cancer patients with initially unresectable liver-only metastases may be cured after downsizing of metastases by induction systemic therapy. However, the optimal induction regimen has not been defined, and no consensus exist on criteria for resectability.
Objectives	To determine the median progression-free survival (PFS) upon induction systemic treatment in colorectal cancer patients with initially unresectable liver-only metastases, stratified by <i>RAS</i> and <i>BRAF</i> tumor mutation status and primary tumor location.
Study design	Colorectal cancer patients with initially unresectable liver-only metastases, as prospectively confirmed by an expert panel according to predefined criteria, and tested for <i>RAS</i> (<i>KRAS</i> exon 2, 3 en 4 and <i>NRAS</i> exon 2 and 3) and <i>BRAF</i> tumor mutation status. Patients with <i>RAS</i> and <i>BRAF</i> wildtype left-sided primary colorectal tumors will be randomised between doublet chemotherapy (FOLFOX or FOLFIRI) plus either bevacizumab or panitumumab, and patients with <i>RAS</i> or <i>BRAF</i> mutant tumors and/or right sided primary colon tumors will be randomised between doublet chemotherapy (FOLFOX or FOLFIRI) plus bevacizumab and triple chemotherapy (FOLFOXIRI) plus bevacizumab. Patient imaging will be reviewed for resectability by a central panel, consisting of at least one radiologist and three surgeons every assessment. Central panel review will be performed prior to randomisation as well as during treatment, as described in the protocol. For study design see also appendix I
Stratification parameters	Patients will be stratified for potential resectability of liver metastases (potentially resectable versus permanently unresectable), serum LDH obtained \leq 4 weeks (normal vs abnormal), treatment centre, <i>BRAF</i> mutation status (wild type versus mutated for <i>RAS</i> wild type patients only) and use of irinotecan- versus oxaliplatin-containing regimen.
Study endpoints	Primary endpoints: median progression-free survival (PFS) Secondary endpoints: R0/1 resection rates, median overall survival, response rate, toxicity, pathological complete response rate (pCR) of resected lesions, postoperative morbidity, and correlation of baseline and follow-up evaluation by the panel with outcome.

Main criteria for inclusion	<ul style="list-style-type: none"> - Histologically proven colorectal cancer - Unresectable metastases confined to the liver according to CT scan, obtained ≤ 3 weeks prior to registration. Unresectability is confirmed by the panel. Patients with small (≤ 1 cm) extrahepatic lesions that are not clearly suspicious of metastases are eligible. - <i>RAS and BRAF</i> mutation status known - Status WHO performance status 0-1 (Karnofsky performance status ≥ 70) - Age ≥ 18 years - No contraindications for liver surgery - In case of primary tumor in situ: tumor should be resectable - In case of resected primary tumor: adequate recovery from surgery - Adequate organ functions, as determined by normal bone marrow function (Hb ≥ 6.0 mmol/L, absolute neutrophil count $\geq 1.5 \times 10^9/L$, platelets $\geq 100 \times 10^9/L$), renal function (serum creatinine ≤ 1.5x ULN and creatinine clearance, Cockcroft formula, ≥ 30 ml/min), liver function (serum bilirubin ≤ 2 x ULN, serum transaminases ≤ 5x ULN) - Life expectancy > 12 weeks - Expected adequacy of follow-up - Written informed consent
Main criteria for exclusion	<ul style="list-style-type: none"> - Extrahepatic metastases (extrahepatic lesions of ≤ 1 cm that are not clearly suspicious for metastases not included) - Unresectable primary tumor - Serious comorbidity or any other condition preventing the safe administration of study treatment (including both systemic treatment and surgery) - Major cardiovascular events (myocardial infarction, severe/unstable angina, congestive heart failure, CVA) within 12 months before registration - Uncontrolled hypertension, or unsatisfactory blood pressure control with ≥ 3 antihypertensive drugs - Previous systemic treatment for metastatic disease; previous adjuvant treatment is allowed if completed ≥ 6 months prior to registration - Previous surgery for metastatic disease - Previous intolerance of study drugs in the adjuvant setting - Pregnant or lactating women <ul style="list-style-type: none"> - Second primary malignancy within the past 5 years with the exception of adequately treated in situ carcinoma of any organ or basal cell carcinoma of the skin, or second primary colorectal cancer

	- Any concomitant experimental treatment.
Treatment	Patients with <i>RAS and BRAF</i> wildtype left-sided primary tumors will be randomised between doublet chemotherapy (FOLFOX or FOLFIRI) plus either bevacizumab or panitumumab. The choice between FOLFOX or FOLFIRI is to the discretion of the local investigator, however, the treatment is restricted to regimens that are specified in the protocol. Patients with <i>RAS</i> or <i>BRAF</i> mutated and/or right-sided primary tumors will be randomised between FOLFOX/ FOLFIRI (investigator choice) plus bevacizumab or 5FU, irinotecan, and oxaliplatin (FOLFOXIRI) plus bevacizumab.
Duration of treatment and follow-up	Patients will be evaluated by CT scan for disease status at baseline (prior to randomisation) and at first evaluation (8-9 weeks of treatment). If deemed necessary by the panel, a panel evaluation will also be performed at second evaluation (16-18 weeks of treatment) and at third evaluation (24-27 weeks of treatment). The assigned systemic treatment should be continued for at least 6 months or earlier in case of resectability, progression of disease, unacceptable toxicity, or patient refusal. If after 6 months the panel concludes that the patient is still not resectable, it is highly unlikely that resectability will be achieved at all. Therefore the chemotherapy regimen may be reconsidered after 6 months of treatment. These patients should continue with the targeted drug in combination with chemotherapy, but the chemotherapy may be altered into a less toxic schedule such as fluoropyrimidine monotherapy. The targeted drug should be continued until progression or unacceptable toxicity. In patients who will become resectable and undergo secondary surgery of liver metastases, the total duration of preoperative and postoperative treatment together should be 6 months, with the chemotherapy schedule being administered according to the assigned treatment arm. However in these patients the targeted drug (bevacizumab or panitumumab) should not be continued after surgery.
Statistics	It is estimated that approximately 160 patients can be entered into the study every year, 48 (30%) are expected to be patients with <i>RAS and BRAF</i> wildtype, left-sided primary colorectal tumors, and 112 (70%) with <i>RAS or BRAF</i> mutated and/or right-sided primary colon tumor. There will be 2 complete separate analyses for patients with wildtype, left-sided primary tumors and patients with mutated tumors and/or right sided primary tumor, hence no adjustment for multiplicity. Patients will be followed until 256 and 257 events in both subgroups are observed for PFS, which is estimated to be 2 years after accrual is completed. This number of events will provide 80% power to detect with a 5% 2-sided logrank test, a true HR of 0.70 in the PFS if it exists. The number of patients randomised will also provide from 71%

	to 91% power to detect a true difference in R0/R1 resection rate from 25% to 40% or to 45% at 5% (2-sided) significance level using the Fisher exact test.
Translational research	Tissue from the primary tumor and, if available, resected liver metastases as well as peripheral blood will be used for translational research on prognostic and predictive factors.

1 Introduction and rationale

Approximately 50% of patients with colorectal cancer (CRC) will develop metastases, and approximately 25% will present with distant metastases at diagnosis. Colorectal cancer disseminates predominantly to the liver. The 5-year overall survival rates in patients with metastatic CRC who participated to clinical trials is currently around 20%. [1] This result has clearly improved during the past 2 decades, and is predominantly due to the increased use of surgical resections of metastases and the increased efficacy of systemic drugs. Fluoropyrimidine-containing chemotherapy plus the anti-VEGF antibody bevacizumab is currently considered to be the standard 1st-line treatment in metastatic CRC. [2-5] In patients with *RAS* and *BRAF* wildtype left-sided primary colorectal tumors, chemotherapy plus cetuximab or panitumumab (anti-EGFR) is a useful alternative. [6-8] Randomised phase 3 studies on a direct comparison between chemotherapy plus either bevacizumab or anti-EGFR antibodies in unselected (i.e. not restricted to liver-only disease) metastatic CRC patients are either ongoing (CALGB 80405) or preliminary results have been presented (FIRE-3 and PEAK trials, to be discussed later).

In patients with resectable liver metastases at diagnosis, a radical resection of these metastases is the first choice of treatment which results in 5-year survival rates in the order of 25-40%. [9] However, only a minority of patients present with resectable metastases. The main reasons for unresectability are liver involvement which is too extensive, or involvement of non-resectable structures. Resection of liver metastases in patients who also have extrahepatic metastases is a matter of debate, and, if done at all, is usually restricted to patients with limited extrahepatic metastases. Evidence for the benefit of induction chemotherapy with the objective to improve resectability rates was already established in 1996, when it was shown that initially unresectable metastases could become resectable (further defined as secondary surgery) after downsizing by chemotherapy. [10] Currently, most phase 2 and 3 studies in metastatic CRC present data on the rate of secondary resections in the subgroup of patients with metastases confined to the liver. However, this almost invariably concerns unplanned retrospective analyses. The rate of secondary surgery in phase 3 studies with unselected metastatic CRC patients (i.e. with metastases not confined to the liver) is usually less than 10%. However, results on this outcome are greatly confounded by the variable use of liver resections among different hospitals and countries. Furthermore, these patients were not specifically screened for extrahepatic disease since the outcome in patients with liver-only metastases usually was not a prospectively defined objective of the study.

1.1 Secondary liver resections after induction systemic treatment

Data from a single institution by Adam et al. [11] have shown that of 1104 patients with metastases confined to the liver, 12.5% of patients became eligible for secondary surgery, and that these patients had a 5-year survival rate of 33%. In another retrospective analysis of 184 patients who underwent radical secondary resection of initially unresectable CRC liver metastases after downsizing by chemotherapy, the 5- and 10-year survival rates were 33% and 27%, respectively. After a follow-up of ≥ 5 years, 16% of 148 patients was considered to

be cured [12]. However, as in most if not all retrospective series, the relative contribution of systemic treatments to survival was not evaluated.

As is the case for primary surgery for resectable liver metastases, the benefit of secondary surgery has not been evaluated in prospective randomised studies. However, given the consistent data from large series, there is little doubt that a radical resection of liver metastases (primary or secondary) prolongs survival. Indeed, in the liver survey database the survival benefits of primary and secondary surgery were shown to be in the same range. [13] The 3, 5, and 10-year survival rates for primary and secondary resections were 63% and 53%, 45% and 33%, and 28% and 19%, respectively. These outcomes are better than have been reported for systemic therapy alone. Again, formal randomised trials on this topic have not been performed, and most likely never will be performed given these results.

Although a radical (R0) resection with tumor-free margins should be attempted in all patients, long-term survival results have been shown for R1 resections as well. [14] Therefore, R1 resections appear to be a relevant surgical outcome as well. An important issue regarding the optimal strategy for this group of patients is that the published series differ in their selection of patients as well as in their definition of resectability. This refers to the maximal size and number of metastases, the use of more extensive surgical procedures such as portal vein embolization and 2-stage resections, and the number of organs involved since some series also include patients with resections of metastases in more than one site. This implies that cross-study comparisons are not valid. Multivariate analyses have identified the size, the number, and the pathological response to induction systemic treatment as independent predictors for survival [12]. A complicating factor is the lack of general consensus on the criteria for resectability, which is nicely illustrated by the recent CELIM study [15]. In this randomised phase 2 study with CRC patients with unresectable liver-only metastases, the CT scans of the liver before and after systemic therapy were retrospectively reviewed in a blinded way by a panel of 7 surgical experts. The panel was asked to vote for one of the following options: initial resection or surgical exploration, initial systemic therapy with the possibility of secondary resection, or permanently unresectable. There was considerable variation in the voting of this panel, with even completely opposing views in 7% of cases. Moreover, one-third of the patients were considered by the reviewers to be resectable at baseline and therefore in retrospect ineligible for the study.

A second important issue is that in the discussions on the optimal strategy that should lead to the downsizing of metastases to allow secondary resections, the assumption is usually made that response rate is a surrogate marker for resection rate. Indeed this hypothesis was supported by a retrospective analysis. [16] However, a downsizing in the number of metastases leading to a technically feasible secondary resection may translate into a worse outcome compared to a downsizing in the size of metastases. This is supported by the observation that more than 80% of liver metastases in complete radiological remission by systemic treatment still contain viable tumor cells. [17] These data strongly suggest that response rate is unlikely to accurately predict the clinical outcome in this patient category, and support the timing of a resection as soon as this appears to be feasible (i.e. not to wait until metastases may have completely regressed). These data also support to resect all sites where lesions in complete remission were located. Lastly, the issue of response rate is further complicated by the observations that the addition of bevacizumab to induction chemotherapy may rather increase the pathological than the objective (RECIST) response of

liver metastases, which may invalidate the use of RECIST response criteria in this setting. [18-20] Morphologic response criteria provide complementary information and have shown to be effective for the prediction of pathological response in patients receiving a bevacizumab-containing regimen. [18, 20]

1.2 Induction treatment with chemotherapy plus either anti-EGFR antibodies or bevacizumab

Given the presumed (but not proven) higher response rates of chemotherapy plus anti-EGFR antibodies (cetuximab, panitumumab) compared to chemotherapy plus bevacizumab in the first-line treatment of metastatic CRC patients (Table 1), the use of cetuximab or panitumumab instead of bevacizumab is advocated by some in patients with potentially resectable metastases. In fact, NICE in the UK has approved the use of cetuximab in this setting for a limited number of cycles. However, these data are all based on cross-study comparisons, and in most studies no independent external review of response rate was performed. In addition, as previously mentioned, the efficacy of bevacizumab plus chemotherapy on liver metastases may not be fully reflected by RECIST response criteria [18-20]. In the randomised phase 2 CELIM study [15] comparing FOLFIRI and cetuximab with FOLFOX and cetuximab in patients with *RAS* wildtype and unresectable liver-only metastases that were either unresectable or ≥ 5 in number, both schedules yielded comparable response rates of 57% and 68%, respectively, and a rate of secondary surgery of 30% and 38%, respectively. In patients with *RAS* and *BRAF* wildtype tumors, the response rate was 72%. The median time of induction treatment in resected patients was 4 months (Table 2).

However, most phase 3 studies which investigated the addition of bevacizumab to chemotherapy showed that bevacizumab also increases the response rate of chemotherapy alone, and these response rates appear not substantially different from response rates of chemotherapy plus anti-EGFR agents (Table 2,3). In two phase 2 studies with chemotherapy plus bevacizumab in patients with unresectable CRC liver-only metastases, response rates of 57% and 73%, and with R0 resection rates of 62% and 93%, respectively, have been reported. [21, 22]. Postoperative morbidity with bevacizumab-containing regimens has been shown to be well within the acceptable range [23], and bevacizumab does not appear to affect the functional recovery of the liver after resection. [24]

In the general population of metastatic CRC patients (i.e. not limited to liver-only metastases) (Table 3), preliminary results have been presented of two randomised studies in which chemotherapy plus bevacizumab was compared with chemotherapy plus cetuximab or panitumumab. In the randomised phase 3 FIRE-3 study in patients with *RAS* wildtype tumors [25], the results of FOLFIRI+cetuximab versus FOLFIRI+bevacizumab were highly similar in response rates (62% and 58%, respectively) and median PFS (10 and 10.3 months, respectively), although there was a difference in median OS in favor of the cetuximab treatment arm. This latter finding may have been caused by post-study treatment, since the OS curves only separated well after the median time to progression. In the randomised phase 2 PEAK study [26] there was a significant benefit for chemotherapy + panitumumab compared to chemotherapy plus bevacizumab in median PFS (10.1 versus 13.1 months,

respectively) and a borderline significant benefit in median OS (28.9 versus 41.3 months, respectively) in patients with *KRAS* wildtype tumors. The small size of this study does not allow definite conclusions. Of note, these studies were not designed to test the optimal sequence of targeted drugs. Moreover, these studies were performed in unselected patients in respect to the number of metastatic sites, and no predefined criteria on secondary resectability and outcome were used. Therefore, based on the currently available data no outright preference is apparent for the addition of either bevacizumab or anti-EGFR antibodies to chemotherapy in this setting. Given the difference in tolerability of these targeted agents, with bevacizumab generally being better tolerated, as well as the fact that the majority of patients will not become resectable and therefore should continue the systemic treatment, the choice of the targeted drug to accompany chemotherapy is highly relevant. In general, an attempt should be made to expose patients to all effective drugs during their course of disease [27], which is possible in the great majority of patients with liver-only metastases [28]. Irinotecan, oxaplatin, bevacizumab and panitumumab are approved drugs and are reimbursed in the 1st and 2nd line treatment of metastatic CRC in The Netherlands.

1.3 Selection of patients for anti-EGFR therapy

Since the initial observation that *KRAS* mutation is a negative predictive factor for anti-EGFR therapy [29], much effort has been made to further optimize patient selection for this therapy. Although the negative predictive value of *KRAS* mutations in codon 13 may be less as initially thought, this finding warrants further validation since this was not been confirmed in other studies. [30-32] More recently, the negative predictive value of *RAS* (*KRAS* exon 2, 3 en 4 and *NRAS* exon 2 and 3) mutations were confirmed [33, 34], with a detrimental effect of panitumumab in patients treated with oxaliplatin-based chemotherapy. [34] *BRAF* mutation was confirmed to be prognostic, but not predictive. [8, 34-37]

However, the benefit, if any, from anti-EGFR treatment for patients with *BRAF* mutated tumors is very limited. [38]. Based on these results, the *BRAF* and *RAS* mutation status (*KRAS* exon 2, 3 en 4 and *NRAS* exon 2 and 3) is currently recommended to select patients with metastatic CRC for anti-EGFR therapy in The Netherlands, with only patients with *RAS* and *BRAF* wildtype tumors being eligible for this treatment. Recent literature demonstrated the relevance of primary tumor localisation in *RAS* or *BRAF* wildtype metastatic colorectal cancer patients. Patients with right-sided primary tumors have no benefit of anti-EGFR therapy as well. [39, 40] Therefore, only patients with *RAS* and *BRAF* wildtype, left-sided primary tumors should be selected for anti-EGFR therapy.

Although it is not expected that the selection by *RAS* mutation status will have different effects for cetuximab and panitumumab, data according to *RAS* mutation status in first-line treatment are currently only available for panitumumab. Therefore, we selected panitumumab as anti-EGFR antibody for this study.

1.4 Choice of chemotherapy regimen in induction treatment

Randomised phase 3 studies have clearly shown that combination chemotherapy with a fluoropyrimidine plus irinotecan or oxaliplatin produces higher response rates compared with fluoropyrimidine monotherapy. Therefore combination chemotherapy is the backbone of systemic treatment when downsizing of metastases is the primary objective. Studies on triple chemotherapy (5FU+oxaliplatin+irinotecan, FOLFOXIRI) have shown high response rate in phase 2 studies, but conflicting results on its survival benefit have been demonstrated in two phase 3 studies [41-43]. However, retrospective analysis of both phase 3 studies showed that the rate of secondary resections was increased, from 12% to 36% and from 7 to 11 patients, respectively. Again, secondary resections were not a prospective or standardized part of the study, and given the inconsistency of the data the use of triple chemotherapy is considered to be promising but not yet standard when downsizing of metastases is the objective. In a phase 2 trial with 57 metastatic CRC patients, FOLFOXIRI plus bevacizumab was shown to be feasible and to result in a high response rate of 77%, with the remaining 23% of patients achieving stabilization of disease as best response. [44] Recently the preliminary results have been presented of a randomised phase 3 study (TRIBE), in which FOLFOXIRI+bevacizumab showed significantly higher response rates (65% versus 53%, respectively), median PFS (12.1 versus 9.7 months, respectively), and median OS (31.0 versus 25.8 months, respectively) when compared to FOLFIRI + bevacizumab [45]. Again, this study also included patients with extrahepatic disease, and did not prospectively investigate the outcome in patients with liver-only metastases according to uniform and predefined criteria. However, these results show that the combination of FOLFOXIRI+bevacizumab is feasible, and encourage further testing in the setting of induction treatment.

With more and more data becoming available on chemotherapy plus targeted agents in metastatic CRC, it becomes also clear that the choice of chemotherapy to accompany the targeted drug may matter. The most convincing argument to date is the observation that the detrimental effect of anti-EGFR antibodies in patients with *RAS* mutated tumors has more often been observed in combination with oxaliplatin- compared with irinotecan-based schedules. [6, 7, 46-48] However, in patients with *RAS* wildtype tumors no outright preference for either irinotecan-based or oxaliplatin-based schedules has been demonstrated, although data from direct comparisons are only available from randomised phase 2 [15] and not yet from phase 3 studies. Although the largest experience with induction chemotherapy in patients with potentially resectable liver metastases has been with oxaliplatin-based schedules, data with irinotecan-based schedules show comparable outcomes [49].

As to the choice of fluoropyrimidine in the treatment of metastatic CRC patients, there is controversy in the literature about the equivalence of capecitabine and 5-fluorouracil in combination chemotherapy regimens. Published data show increased toxicity and decreased efficacy for capecitabine plus irinotecan (CAPIRI) versus 5-fluorouracil plus irinotecan (FOLFIRI) [50]. Capecitabine plus oxaliplatin (CAPOX) results in lower response rates compared to 5-fluorouracil plus oxaliplatin (FOLFOX), with different toxicity profiles for these regimens [51]. A phase 3 study investigating the added value of cetuximab to chemotherapy

consisting of either FOLFOX or CAPOX [52] showed inferior results for CAPOX compared to FOLFOX as chemotherapy backbone in terms of efficacy and safety. The authors concluded that the use of CAPOX plus cetuximab cannot be recommended. No phase 3 data are available on CAPIRI or with irinotecan+oxaliplatin in combination with targeted therapy, and therefore we restrict the chemotherapy regimens in this study to FOLFOX, FOLFIRI, and FOLFOXIRI.

1.5 Hepatotoxicity of induction treatment

Several studies have shown the feasibility and safety of induction chemotherapy prior to liver resection. The most important message from these studies is that chemotherapy should be of limited duration, since the morbidity of liver resections increases with the prolonged use (i.e. more than 4 months) of chemotherapy [49]. Since the maximal response is usually achieved within this timeframe, there is no clinical need to prolong chemotherapy beyond such a period. [15] Different types of hepatotoxicity have been observed with cytotoxic drugs, with fluoropyrimidines being associated with steatosis, irinotecan with steatohepatitis, and oxaliplatin with sinusoidal obstruction [49]. However, these toxicities have not been shown to result in different safety outcomes, and therefore no regimen of choice can be identified based on the incidence or type of hepatotoxicity.

While the use of anti-EGFR agents prior to liver surgery does not provide reasons for concern, the use of induction bevacizumab may in theory cause excessive bleeding and impaired wound healing and liver regeneration in case of surgery. However, it was shown that bevacizumab can be safely administered up to 5 weeks before liver surgery without affecting wound healing and liver regeneration or causing any excess in morbidity after surgery. [23]

1.6 Conclusions

Secondary resection of liver metastases offers the only chance for cure in patients with initially unresectable liver-only metastases. There are no data from prospective studies with transparent and standardized criteria for staging and resectability in patients with initially unresectable liver-only metastases which may serve as a reference for clinical practice and future studies. The CAIRO5 study is designed to provide clinically relevant data on the optimal strategy that is to be used in these patients. Given the lack of a predictive model that allows the selection of patients in whom secondary resections may be achieved, we propose to include all patients with unresectable liver-only metastases.

The standard induction treatment of patients with initially unresectable liver-only metastases currently consists of combination chemotherapy of a fluoropyrimidine plus either oxaliplatin or irinotecan, with triple chemotherapy (fluoropyrimidine+oxaliplatin+irinotecan) showing promising results. The addition of a targeted drug to chemotherapy has been shown to increase response rates, which provides a clear rationale for use in this setting, but a clear preference for either the anti-VEGF antibody bevacizumab or one of the anti-EGFR antibodies cetuximab or panitumumab has not been demonstrated in this setting.

Given the lack of good prospective data on bevacizumab versus anti-EGFR antibodies in CRC patients with potentially resectable liver metastases, and the clinical relevance of this topic, we propose to randomise patients with *RAS* and *BRAF* wildtype, left-sided primary tumors between these two targeted therapies in combination with a two-drug combination chemotherapy (5-fluorouracil plus either irinotecan, FOLFIRI, or oxaliplatin, FOLFOX, choice of investigator). Given the superior data for panitumumab compared to cetuximab in patients with *RAS* (as opposed to *KRAS*) wildtype tumors in the first-line treatment setting, the former antibody is selected for use in this study. Since anti-EGFR antibodies are not indicated in patients with *RAS* mutated tumors, we propose to randomise patients with *RAS* mutated tumors between FOLFOX/FOLFIRI (choice of investigator) plus bevacizumab and triple chemotherapy (FOLFOXIRI) plus bevacizumab. Given the negative prognostic and predictive value of a *BRAF* mutation (which only occurs in *RAS* wildtype tumors), patients with a *BRAF* mutated tumor will be randomised accordingly to patients with *RAS* mutated tumors [37]. Finally, given the lack of benefit of anti-EGFR therapy in patients with right-sided primary colon tumors, these patients will be randomised accordingly to *RAS* mutated tumors. [39, 40] A further innovative aspect of CAIRO5 is the prospective planning and evaluation of treatment based on mutation status of the tumor and resectability status of metastases.

Table 1. Cross-study comparison of response rates in phase 3 studies with combination chemotherapy plus bevacizumab or anti-EGFR antibody in metastatic CRC patients

chemotherapy + bevacizumab	Response rate		chemotherapy + cetuximab/panitumumab (ref)
Hurwitz (IFL) [2]	45%	57%	CRYSTAL (FOLFIRI) [7]
NO16966 (FOLFOX/CAPOX) [4]	38%	57%	OPUS (FOLFOX) [46]
CAIRO2 (control CAPOX) [48]	50%	59%	COIN (FOLFOX/CAPOX) [52]
PACCE [47]	56%	55%	PRIME (FOLFOX) [6]

Table 2. Studies on induction systemic treatments in patients with initially unresectable liver-only CRC metastases

Author	schedule	N	response rate	R0 resection rate	PFS median	OS median
Folprecht et al. [15]	FOLFIRI + cetuximab FOLFOX + cetuximab	111	57% 68%	30% 38%	N.S.	N.S.
Gruenberger et al. [22]	CAPOX + bevacizumab	56	73%	93%	NS	NS
Bertolini et al. [21]	FOLFOX + bevacizumab	21	57%	62%	12.9 m	22.5 m

NS = not stated

Table 3. Randomised phase 2/3 studies with combination chemotherapy plus targeted therapy in previously untreated patients with metastatic CRC not restricted to the liver

Author	Schedule#	N	Response rate	R0 resection rate	PFS median	OS median
Moosmann et al. [53]	CAPOX + cetuximab	89	45%	N.S.	7.1 m	23.5 m
	CAPIRI + cetuximab		50%		6.2 m	21.1 m
van Cutsem et al. [7]	FOLFIRI + cetuximab	316	57%*	5.1%*	9.9 m*	23.5 m*
	FOLFIRI	350	40%	2.0%	8.4 m	20.0 m
Bokemeyer et al. [46]	FOLFOX+ cetuximab	82	57%*	7.3%	8.3 m*	22.8 m
	FOLFOX	97	34%	3.1%	7.2	18.5 m
Maughan et al. [52]	FOLFOX/CAPOX + cetuximab	362	59%*	N.S.	8.6 m	17.0 m
	FOLFOX/CAPOX	367	50%		8.6 m	17.9 m
Douillard et al. [6]	FOLFOX+ panitumumab	325	55%	8.3%	9.6 (10.1)1 m*	23.9 (25.8)1 m*
	FOLFOX	331	48%	7.0%	8.0 (7.9)1 m	19.7 (20.2)1 m
Hurwitz et al. [2]	IFL + bevacizumab	402	45%*	< 2%	10.6 m*	20.3 m*
	IFL	401	35%		6.2 m	15.6 m
Saltz et al. [4]	FOLFOX/CAPOX + bevacizumab	699	38%	8.4%	9.4 m*	21.3 m
	FOLFOX/CAPOX	701	38%	6.1%	8.0 m	19.9 m
Fuchs et al. [50, 54]	FOLFIRI + bevacizumab	57	58%	N.S.	11.2 m	28.0 m*
	IFL + bevacizumab	60	53%		8.3 m	19.2 m
	FOLFIRI	144	47%		7.6 m	23.1 m
	IFL	141	43%		5.9 m	17.6 m
	CAPIRI	145	39%		5.8 m	18.9 m
Falcone et al. [45]	FOLFOXIRI + bevacizumab	252	65%*	15%	12.1 m*	31.0 m
	FOLFIRI + bevacizumab	256	53%	12%	9.7 m	25.8 m
Heinemann et al. [25]	FOLFIRI + bevacizumab	295	58%	N.S.	10.3 m	25.0 m
	FOLFIRI + cetuximab	297	62%		10.0 m	28.7 m*
Schwartzberg et al. [26]	FOLFOX + bevacizumab	82	49%	N.S.	10.1 m	28.9 m
	FOLFOX + panitumumab	88	56%		13.0 m*	41.3 m
Ye et al. [55]2	FOLFOX/FOLFIRI + cetuximab	70	57%*	26%*	10.2*	30.9*
	FOLFOX/FOLFIRI	68	29%	7%	5.8	21.0

in patients treated with anti-EGFR antibodies: subset of RAS wildtype patients only

1 in patients with RAS wildtype,tumors.

2 in patients with liver-only metastases

N.S. = not stated

* statistically significant difference

2 Objectives

The primary objective of this study in metastatic CRC patients with initially unresectable liver-only metastases is to determine the median progression-free survival (PFS) in each of the 4 study arms upon induction treatment with chemotherapy plus targeted therapy. In case disease progression is documented by the appearance of new metastases in the future liver remnant within 6 weeks after portal vein embolization (Hoekstra et al. Ann Surg 2012) and a complete resection of metastases can still be achieved, than this event is not considered as progression of disease and thus will not be scored as PFS. After complete resection patients will be followed for PFS according to study protocol.

Secondary objectives are to assess the R0/1 secondary resection rate, the median overall survival, response rate, toxicity, pathological complete response rate (pCR) of resected lesions, postoperative morbidity, and correlation of baseline and follow-up evaluation by the panel with outcome. Translational research will be performed on predictive/prognostic biomarkers and imaging methods.

3 Study design

The study is designed as a randomised phase 3 trial. Tumor tissue from all eligible patients will be tested for *RAS* (*KRAS* exon 2, 3 en 4 and *NRAS* exon 2 and 3) and *BRAF* mutation status prior to randomisation. Patients with *RAS* and *BRAF* wildtype, left-sided tumors (approx. 30%) are being randomised between doublet chemotherapy (5-fluorouracil + irinotecan or oxaliplatin) plus either bevacizumab or panitumumab. Patients with *RAS* or *BRAF* mutated and/or right-sided tumors (approx. 70%) are being randomised between doublet chemotherapy (5-fluorouracil + irinotecan or oxaliplatin) plus bevacizumab or triple chemotherapy (5-fluorouracil + oxaliplatin + irinotecan) plus bevacizumab.

Stratification will be done on the following parameters: resectability of liver metastases (potentially resectable versus permanently unresectable, as decided by the central liver expert panel), serum LDH (normal versus abnormal) and treatment centre. Patients with *RAS* wild type tumours will also be stratified for *BRAF* mutation status (wild type versus mutated, for *RAS* wild type patients only) and use of irinotecan- versus oxaliplatin-containing regimen. For each candidate patient, a liver expert panel of at least 3 surgeons and one radiologist will evaluate the imaging scans for resectability status (potentially resectable versus permanently unresectable) at baseline (prior to randomisation) and, if patient was randomised for trial treatment, at first evaluation (8-9 weeks of treatment). If deemed necessary by the panel, a panel evaluation will also be performed at second evaluation (16-18 weeks of treatment) and at third evaluation (24-27 weeks of treatment).

Patients with resectable metastases at baseline are ineligible for the study. By general consensus among Dutch hepatic surgeons, resectability at baseline for this study is defined as a radical (R0) resection being feasible in a single procedure with surgery alone (see paragraph 11.1). Patients with small (≤ 1 cm) extrahepatic lesions that are not clearly suspicious of metastases are eligible.

4 Study population

4.1 Inclusion criteria

- Histological proof of colorectal cancer
- Initially unresectable metastases confined to the liver according to CT scan, obtained ≤ 3 weeks prior to registration. Unresectability should be confirmed by the liver expert panel. Patients with small (≤ 1 cm) extrahepatic lesions that are not clearly suspicious of metastases are eligible
- Known mutation status of *RAS* and *BRAF*
- WHO performance status 0-1 (Karnofsky performance status ≥ 70)
- Age ≥ 18 years
- No contraindications for liver surgery
- In case of primary tumor in situ: tumor should be resectable
- In case of resected primary tumor: adequate recovery from surgery
- Adequate organ functions, as determined by normal bone marrow function (Hb ≥ 6.0 mmol/L, absolute neutrophil count $\geq 1.5 \times 10^9/L$, platelets $\geq 100 \times 10^9/L$), renal function (serum creatinine $\leq 1.5 \times$ ULN and creatinine clearance, Cockcroft formula, ≥ 30 ml/min), liver function (serum bilirubin $\leq 2 \times$ ULN, serum transaminases $\leq 5 \times$ ULN)
- Life expectancy > 12 weeks
- Expected adequacy of follow-up
- Written informed consent

4.2 Exclusion criteria

- Extrahepatic metastases, with the exception of small (≤ 1 cm) extrahepatic lesions that are not clearly suspicious of metastases
- Unresectable primary tumor
- Serious comorbidity or any other condition preventing the safe administration of study treatment (including both systemic treatment and surgery)
- Major cardiovascular events (myocardial infarction, severe/unstable angina, congestive heart failure, CVA) within 12 months before randomisation
- Uncontrolled hypertension, or unsatisfactory blood pressure control with ≥ 3 antihypertensive drugs
- Previous systemic treatment for metastatic disease; previous adjuvant treatment is allowed if completed ≥ 6 months prior to randomisation
- Previous surgery for metastatic disease
- Previous intolerance of study drugs in the adjuvant setting
- Pregnant or lactating women
- Second primary malignancy within the past 5 years with the exception of adequately treated in situ carcinoma of any organ or basal cell carcinoma of the skin, or second primary colorectal cancer.
- Any concomitant experimental treatment.

5 Evaluations at baseline and follow-up

5.1 Definition of sidedness primary tumor:

Right sided primary tumors: coecum, ascending colon, hepatic flexure, transverse colon.

Left sided primary tumors: splenic flexure, descending colon, sigmoid, rectum.

5.2 Evaluations at baseline

Prior to randomisation, the following test results should be available:

History, physical examination: cardiac/vascular history, other comorbidities, baseline signs and symptoms, concomitant medication, previous history/prior malignancies, WHO performance status, weight, length, blood pressure.

Laboratory test results obtained within 2 weeks before registration: full blood count (Hb, WBC, differential, platelets), serum creatinin, urea, Na, K, Ca, P, Mg, albumin, bilirubin, alkaline phosphatase, ASAT, ALAT, LDH, CEA;. Creatinine clearance Cockcroft Gault calculation.

Imaging test results: CT scan thorax + abdomen prior to randomisation (see below for specifications); a PET scan to confirm the absence of extrahepatic disease is recommended but not mandatory; a MRI scan of liver to assess resectability is recommended but not mandatory.

Study treatment is recommended to start within 4 weeks after baseline CT scan, this period should not exceed 5 weeks.

5.3 Evaluations at follow up

Prior to each cycle until end of treatment or progression (whichever comes first):

Physical examination: evaluation of adverse events (CTCAE criteria), WHO performance status, blood pressure (in bevacizumab-treated patients).

Laboratory test results: full blood count (Hb, WBC, differential, platelets), serum creatinin, Mg (in panitumumb-treated patients), albumin, bilirubin, alkaline phosphatase, ASAT, ALAT, LDH,. Serum CEA should be determined at the time of each radiological evaluation (every 8 weeks).

Every 4 cycles (8 weeks) until progression:

Imaging test results: A CT scan of thorax and abdomen is performed every 8 weeks. Patients who have undergone resection of livermetastases will be followed according to the current national guideline: ultrasound or CT scan of liver every 6 months for 2 years, then every 12 months up to 5 years after surgery; imaging in case of rectal cancer may include a chest X-ray or CT scan of thorax; assessment of serum CEA every 3-6 months for 3 years, then every 6 months up to 5 years after surgery.

Laboratory test results: serum CEA.

Extra bloodsamples (Streck tubes); *optional; only if patient has given informed consent for extra bloodsamples:* Blood will be collected at the following timepoints from

patient who have given informed consent for extra blood sample collecting: baseline, 2 tubes; first evaluation (2 months), 1 tube. In case of surgery: 1 tube <1 week before every surgery + 1 tube 1 day after every surgery + 1 tube every three months until progression for a maximum of three years. In case of no surgery: 1 tube every two months until progression.

6 Assessment of *RAS* and *BRAF* mutation status

RAS and *BRAF* mutation status will be assessed before randomisation. Adequate tumor tissue for this assessment should be available, which may be from either the primary tumor or liver metastases. A high concordance of *RAS* mutation status between the primary tumor and corresponding liver metastases has been shown [55].

Mutation analyses should be carried out according to national pathologist guidelines in a laboratory that is CCKL accredited. An assessment in a large central pathology laboratory is indicated to perform mutation analyses.

In addition, an adequate tissues sample should be made available for post hoc quality control of *RAS* and *BRAF* mutation analysis as well as additional translational research, including TMA construction. Translational research will be addressed in the informed consent of the study. For Pathology flow chart see appendix II.

Patient material will be returned to original hospital after analyses in the central pathology laboratory. Patient material will be returned upmost six months after submitting it, or earlier in case the material is required immediately for diagnostic purposes.

7 Patient registration, randomisation procedure

Patients will be registered by the IKNL clinical research department, tel +31(0) 88 2346500, or email trialbureau@iknl.nl. Only after completion of central panel review and receipt of *RAS/BRAF* mutation status, patients will be randomised. Patients will be stratified for resectability of liver metastases (potentially resectable versus permanently unresectable), serum LDH obtained \leq 4 weeks (normal versus abnormal), use of irinotecan- versus oxaliplatin-containing regimen, *BRAF* mutation status (wild type versus mutated for *RAS* wild type patients only) and reporting institute. The result of randomisation/ treatment assignment will be communicated to the local investigator by email or fax.

For randomisation flow chart see appendix I.

8 Panel procedure and evaluation

8.1 Panel procedure

A central panel is formed of surgeons (recruited from the Dutch Study Group for Liver Surgery in The Netherlands and, if applicable, from participating liver centres outside The Netherlands) and radiologists, who will evaluate the CT scans for resectability status (potentially resectable versus permanently unresectable, see paragraph 11.1). The central

panel will review patient imaging at baseline (prior to randomisation) and at first evaluation (8-9 weeks of treatment). If deemed necessary by the panel, a panel evaluation will also be performed at second evaluation (16-18 weeks of treatment) and at third evaluation (24-27 weeks of treatment). Each evaluation will be done by at least 3 surgeons and 1 radiologist from the panel. Patient images will be uploaded in a program specially designed to share patient imaging on a safe and privacy-respecting manner. For quality and privacy assurance, see chapter 17.

All registered patients will be evaluated by the panel before randomisation.

For panel flow chart see appendix II.

8.2 Radiologist review, including specifications of radiological assessment

A radiologist from the central panel will review all imaging prior to the surgical review. The scans will be reviewed for quality of the imaging and absence of extrahepatic metastases. In case of poor quality or suspicion of extrahepatic metastases, this result will be returned to the local investigator with a recommendation for further analysis, and no surgical panel evaluation will be performed. Patients with small lung lesions < 10 mm without a typical aspect of metastases are eligible for the study. In the absence of extrahepatic metastases the panel radiologist reviews patient imaging according to predefined criteria, among which number, size and segmental location of liver metastases, involvement of major vascular structure and morphological response criteria [18, 20].

8.3 Surgical review, including specifications of surgical assessment

At least 3 surgeons of the panel will evaluate the scans and vote for either of the following 3 categories: 1) resectable liver metastases (in which case the patient is ineligible for the study), 2) potentially resectable liver metastases, or 3) permanently unresectable liver metastases. The chairman of the panel will coordinate the voting and determine the final conclusion

Patients are considered resectable when a R0 resection can be achieved of all lesions with preservation of >25% of total liver volume in one single procedure. The type of resection required is also specified. Patients with marginally resectable liver metastases for whom initial systemic treatment is preferred are to be categorized as potentially resectable liver metastases. These patients are possibly resectable after portal vein embolization, in combination with local ablative techniques such as RFA, or in the setting of a two-stage resection.

If 3 panel surgeons obtain no consensus 2 other panel surgeons will be consulted. Then, the majority vote is accepted as the final vote. In case the vote is 2 vs 2 vs 1, the panel chairman will determine the final conclusion.. The IKNL clinical research department is immediately informed on the result of this vote and type of surgery.

9 Study treatment: systemic therapy

Systemic therapy can be administered at each participating hospital. Until the primary endpoint (PFS) has been reached, systemic treatment should be administered according to protocol, and no other experimental systemic treatment should be administered.

9.1 Doses and schedules

Patients with *RAS* and *BRAF* wildtype, left-sided primary tumors are being randomised between bevacizumab or panitumumab, both in combination with FOLFOX or FOLFIRI (investigator choice). Patients with *RAS* or *BRAF* mutant, and/or right sided primary tumors are being randomised between FOLFOX/FOLFIRI (investigator choice) plus bevacizumab or FOLFOXIRI plus bevacizumab. Other fluoropyrimidines (i.e. capecitabine) are not allowed in these combinations. The choice between FOLFIRI and FOLFOX is to the discretion of the local investigator, and may be selected on a per patient basis (see 9.2).

Based on *RAS* and *BRAF* mutation status or outcome of randomisation, the following schedules:

9.1.1 Systemic treatment schedules

FOLFIRI + bevacizumab

Bevacizumab 5 mg/kg in 15-30 minutes i.v., followed by irinotecan 180 mg/m² i.v. in 60 minutes together with leucovorin 400 mg/m² i.v. in 120 minutes, followed by bolus 5-fluorouracil 400 mg/m² within 4 minutes, all on day 1, followed by continuous infusion of 5-fluorouracil 2400 mg/m² in 46 hours, every 2 weeks

FOLFIRI + panitumumab

Panitumumab 6 mg/kg i.v. (1st dose in 60 minutes, if well tolerated subsequent doses in 30 minutes), followed by irinotecan 180 mg/m² i.v. in 60 minutes together with leucovorin 400 mg/m² i.v. in 120 minutes, followed by bolus 5-fluorouracil 400 mg/m² within 4 minutes, all on day 1, followed by continuous infusion of 5-fluorouracil 2400 mg/m² in 46 hours, every 2 weeks

FOLFOX6 + bevacizumab

Bevacizumab 5 mg/kg in 15-30 minutes i.v., followed by oxaliplatin 85 mg/m² i.v. together with leucovorin 400 mg/m² i.v. in 120 minutes, followed by bolus 5FU 400 mg/m² within 4 minutes, all on day 1, followed by continuous infusion of 5-fluorouracil 2400 mg/m² in 46 hours, every 2 weeks

FOLFOX6 + panitumumab

Panitumumab 6 mg/kg i.v. (1st dose in 60 minutes, if well tolerated subsequent doses in 30 minutes), followed by oxaliplatin 85 mg/m² i.v. together with leucovorin 400 mg/m² i.v.

in 120 minutes, and bolus 5FU 400 mg/m² within 4 minutes, all on day 1, followed by continuous infusion of 5-fluorouracil 2400 mg/m² in 46 hours, every 2 weeks

FOLFOXIRI + bevacizumab

Bevacizumab 5 mg/kg in 15-30 minutes i.v., followed by irinotecan 165 mg/m² i.v. in 60 minutes, followed by oxaliplatin 85 mg/m² i.v. together with leucovorin 400 mg/m² i.v. in 120 minutes, all on day 1, followed by continuous infusion of 5-fluorouracil 3200 mg/m² in 46 hours, every 2 weeks

In patients who are planned for liver surgery, bevacizumab should be discontinued at least 5-6 weeks prior to surgery. During this period patients may receive an additional cycle of chemotherapy without bevacizumab. As to the completion of 6 months of systemic treatment in patients undergoing liver surgery, post-operative (adjuvant) chemotherapy should be initiated within 12 weeks of (final) liver surgery. If the condition of a patient does not permit this, postoperative chemotherapy should be omitted.

For treatment duration, see paragraph 9.2

9.1.2 Change of treatment in case of stable disease at first evaluation

In case of “true” stable disease (which means that there is no decrease in the size of metastases at all) at first evaluation, and the panel concludes that metastases are still potentially resectable, a switch of targeted therapy may be considered in patients with wildtype and left-sided primary tumor in an attempt to downsize liver metastases to achieve resectability. A switch in chemotherapy regimen in patients with RAS/BRAF mutated or right-sided primary tumor is not recommended.

9.1.3 Change of treatment for reasons of toxicity or patient refusal within 6 months

In case unacceptable toxicity despite dose reductions or patient refusal occurs within the first 6 months of treatment that can be attributed to irinotecan or oxaliplatin, the local investigator is free to switch the chemotherapy regimen from FOLFIRI to FOLFOX6 or vice-versa, which is the preferred option in patients that may become resectable, or to 5FU/LV if resectability is not a realistic goal, or when this is considered to be in the interest of the patient. In all these situations the assigned targeted drug should be continued.

In case unacceptable toxicity or patient refusal occurs during treatment with FOLFOXIRI, that can be attributed to a particular chemotherapy drug, the local investigator is free to adapt the schedule accordingly (i.e. to FOLFOX, FOLFIRI, or 5FU/LV according to the abovementioned guidelines), while continuing bevacizumab.

The use of targeted drugs should be continued according to protocol, or should be discontinued in case of unacceptable toxicity or patient refusal. The targeted drug should not be replaced by any other targeted drug during first-line treatment prior to disease progression.

9.1.4 Change of treatment after first progression of disease

Treatment after first progression is not part of the study. However, the following strategies are strongly recommended:

Patients with first progression of disease after 6 months, i.e. who are on maintenance treatment with 5FU/LV + the assigned targeted drug and who have discontinued irinotecan and/or oxaliplatin for other reasons than disease progression (see 10.3), oxaliplatin or irinotecan should be re-introduced upon first progression of disease together with the continued administration of the assigned targeted drug, provided that irinotecan or oxaliplatin were well tolerated. Patients initially treated with FOLFOXIRI may receive FOLFOX or FOLFIRI as re-introduction (to the discretion of the local investigator) with bevacizumab. In patients who did not tolerate the previous administration of irinotecan and/or oxaliplatin, a different second-line regimen should be offered and the assigned targeted drug should be discontinued (see below), although bevacizumab may be continued beyond first progression as this was shown to have a survival benefit [56].

In patients with progression of disease after a systemic therapy-free interval (TFI) due to local therapy of the liver metastases, of more than 3 months, continue initial systemic therapy until second PFS (PFS2) is reached. (For explanation PFS2, see paragraph 15.4)

Patients with first progression of disease in any other situation, i.e. occurring during treatment with FOLFOX, FOLFIRI or FOLFOXIRI plus the assigned targeted drug, or after 6 months of treatment while on maintenance therapy but unable to receive re-introduction of irinotecan and/or oxaliplatin, or in patients in whom a specific drug was discontinued for reasons of toxicity or patient refusal, is to the discretion of the local investigator. **However, an attempt should be made to expose all patients to all available effective drugs.** This is based on the experience with the use of cytotoxic drugs [27], as well as the benefits of second-line treatments including bevacizumab [56, 57] and panitumumab [58] in combination with chemotherapy. The potential value of the use of bevacizumab after progression on first line treatment with chemotherapy plus panitumumab is further supported by a posthoc analysis of the PRIME study [6], which showed superior median OS for patients treated with a bevacizumab-containing regimen (40 versus 26 months, respectively, HR 0.64, descriptive p value p 0.04) [59].

Therefore, the following second-line regimens are **strongly recommended**:

Patients with *RAS* and *BRAF* wildtype tumors:

After FOLFOX + bevacizumab: switch to FOLFIRI + panitumumab*

After FOLFOX + panitumumab: switch to FOLFIRI + bevacizumab

After FOLFIRI + bevacizumab: switch to FOLFOX + panitumumab*

After FOLFIRI + panitumumab: switch to FOLFOX + bevacizumab

* continuation of bevacizumab beyond first progression is also an option [56], in that case panitumumab should be an option in 3rd line in order to expose patients to all available effective drugs.

Patients with *RAS* or *BRAF* mutated and/or right-sided tumors:

After FOLFOX + bevacizumab or FOLFIRI + bevacizumab: switch the chemotherapy to FOLFIRI and FOLFOX, respectively. Given the benefit of continuation of bevacizumab beyond progression [56], bevacizumab may be continued in combination with either schedule in second line.

After FOLFOXIRI + bevacizumab: there is no preferred chemotherapy regimen. Patients progressing during maintenance therapy (5FU/LV+bevacizumab) may be treated with an irinotecan-based regimen in the second line and an oxaliplatin-based regimen in the third line, or vice versa. Given the benefit of continuation of bevacizumab beyond progression [56], bevacizumab may be continued in combination with either schedule in second line. There is no preferred or recommended therapy for patients who experience disease progression during FOLFOXIRI + bevacizumab. Anti-EGFR therapy should not be administered to these patients given the *RAS* or *BRAF* mutation status and location of their tumor.

9.2 Treatment duration

The assigned treatment will be continued for at least 6 months (12 cycles) unless there is earlier progression of disease, unacceptable toxicity, or patient refusal.

9.2.1 Maintenance treatment after 6 months:

If after 6 months the panel concludes that the liver metastases are still not resectable, it is highly unlikely that resectability will be achieved at all. Therefore, the chemotherapy regimen should be reconsidered after 6 months of treatment. These patients should continue with the targeted drug in combination with chemotherapy, but the chemotherapy should be continued as maintenance treatment with 5FU/LV.

Chemotherapy: discontinue irinotecan (FOLFIRI schedule) or oxaliplatin (FOLFOX schedule) or both (FOLFOXIRI schedule) and continue with 5FU/LV + targeted drug according to the following schedule:

5FU/LV + targeted drug:

Bevacizumab/panitumumab, followed by leucovorin 400 mg/m² i.v. in 120 minutes, followed by bolus 5-fluorouracil 400 mg/m² within 4 minutes, all on day 1, followed by continuous infusion of 5-fluorouracil 2400 mg/m² in 46 hours, every 2 weeks. The assigned targeted drug (bevacizumab or panitumumab) should be continued during maintenance treatment with 5FU/LV at the previous dose and schedule.

9.2.2 Continuation of treatment after liver surgery

In patients who become resectable and complete secondary surgery for liver metastases, the total duration of preoperative and postoperative treatment together should be 6 months. As to the completion of 6 months of systemic treatment in patients undergoing liver surgery, post-operative (adjuvant) chemotherapy (without targeted drug) should be initiated within 12 weeks of (final) liver surgery. If the condition of a patient does

not permit this, postoperative chemotherapy should be omitted. The interval between pre- and postoperative systemic treatment should be as short as possible.

In case of incomplete secondary surgery for liver metastases and 1st line systemic treatment was interrupted for more than 3 months and PFS is reached on first postoperative imaging, then initial systemic treatment should be resumed until PFS2. In case disease progression is documented by the appearance of new metastases in the future liver remnant within 6 weeks after portal vein embolization (Hoekstra et al. Ann Surg 2012) and a complete resection of metastases can still be achieved, than this event is not considered as progression of disease and thus will not be scored as PFS. After complete resection patients will be followed for PFS according to study protocol. In case of a liver-first approach, with the primary tumor still in situ, the chemotherapy should be resumed right after last liver surgery and primary tumor resection should be postponed until completion of 6 months peri-operative systemic treatment. However, given the lack of benefit of adding a targeted drug to chemotherapy in the adjuvant setting of stage III colon cancer (C-08, AVANT, and NO147 trials) as well as of resected liver metastases (EPOC trial), the targeted drug should not be continued after surgery. For postoperative treatment, the same recommendations for continuation of treatment in case of toxicity or patient refusal are applicable as mentioned before.

9.3 Dose modifications for toxicity, reporting of SAE

Toxicity will be scored according to Common Terminology Criteria for Adverse Events 4.0. In particular grade 3 and 4 will be tabulated (both related- and all reported toxicities) and aggregated as worst toxicity in particular relevant time intervals with respect to the intervention (neo-adjuvant, post-surgery, follow-up). Toxicities will be compared between the different randomised groups with either Chi-square tests or Fishers' exact tests whenever appropriate. Dose modifications and dose delays should be administered and applied according to standard practice.

9.4 Evaluation of treatment

Tumor response will be evaluated according to RECIST 1.1 criteria as well as morphological criteria [18].

A central panel (see chapter 8) of at least 3 surgeons and one radiologist will evaluate CT scans of thorax and abdomen of all patients at baseline prior to randomisation, at first evaluation after 8 weeks (usually after four 2-weekly cycles of FOLFOX/FOLFIRI/FOLFOXIRI) and at second evaluation at 16 weeks (usually after eight 2-weekly cycles of FOLFOX/FOLFIRI/FOLFOXIRI). At second evaluation the panel will decide whether it is useful (in terms of resectability) or not to have a third panel evaluation after 24 weeks (usually after twelve 2-weekly cycles). The objective of the evaluation at baseline is to exclude patients with initially resectable metastases, to assess potentially resectable metastases versus permanently unresectable metastases, and at subsequent evaluations to assess resectability. Criteria for resectability are the possibility of achieving R0 resection of all metastases with preservation of >25% of total liver volume. If 3 panel surgeons obtain no

consensus, 2 other panel surgeons will be consulted, then the majority vote is accepted as the final vote. The chairman of the panel will coordinate the voting and determine the final conclusion. In case the vote is 2 vs 2 vs 1, the panel chairman will determine the final conclusion. In case of a non-unanimous decision on (potential) resectability, the vote of the majority of the surgeons in the panel will be final. Of note: the criteria for resectability during treatment are different from baseline!

When there is doubt whether the future remnant liver is sufficient (i.e. >25%), the panel will recommend to perform CT-volumetry of total liver and future liver remnant.

In case the liver metastases of any patient will become resectable according to the panel based on CT scans, a FDG-PET scan is recommended but not mandatory to exclude extrahepatic metastases or liver metastases that were not demonstrated by CT scan [60], and a MRI of liver to exclude liver metastases not visible on CT scan [61]. The final decision to perform resection will be made based on the outcome of the available imaging. Resection may be planned after portal vein embolization, in combination with local ablative techniques such as RFA, or as a two-stage resection. When lesions have disappeared under treatment, resection should include all original sites if possible.

Patients will be evaluated at their own site for tumor response according to RECIST 1.1 criteria. The panel radiologists will perform the measurements for the RECIST 1.1 for as long as a patient is evaluated by the panel. Progression-free survival is calculated from the date of randomisation to first progression, and overall survival from the date of randomisation to death. Patients will be evaluated at the start of each treatment cycle for toxicity according to CTCAE version 4.0.

10 Study treatment: surgery

Liver surgery should only be performed in designated centers that meet the national criteria by the NVvH (Netherlands Association for Surgery). These criteria can be found on the NVvH website (www.heelkunde.nl, normering). Until the primary endpoint (PFS) has been reached, no experimental local treatment of liver metastases should be administered.

10.1 Criteria for resectability, surgical procedures

Patients with resectable metastases at baseline are ineligible for the study. Although there is no formal international consensus on resectability of liver metastases, the panel will adhere to the following guidelines:

Resectability at baseline:

- based on preoperative imaging, all lesions are resectable with a tumor-free margin of at least 3 mm, leaving a minimum remnant liver volume of 25-30% in normal livers, and 35-40% in compromised livers (fibrosis/cirrhosis, steatosis), in a single procedure employing resection(s) only.

Resectability during study:

- based on preoperative imaging, all lesions appear resectable with a tumor-free margin of at least 3 mm, leaving a minimum remnant liver volume of 25-30% in normal livers, and 35-40% in compromised livers. Assessment of remnant liver volume may be required using CT-volumetry.
 - in case a tumor-free margin of < 3 mm can not be achieved for all lesions with preservation of sufficient remnant liver volume,, liver resection may be combined with a local ablative technique such as radiofrequency ablation (RFA).
 - in case a minimum remnant liver volume of 25-30% in normal livers, and 35-40% in compromised livers (fibrosis/cirrhosis, steatosis) is not feasible, preoperative portal vein embolization (PVE) should be performed [62] in designated centers. Increase in remnant liver volume is assessed 3 weeks after PVE using CT-volumetry. According to currently available evidence, chemotherapy will be continued following PVE to prevent tumor progression during liver hypertrophy [63]. Alternatively, a two-stage resection may be performed. During the first stage usually involving the lesser resection, concomitant embolization or ligation is performed of the portal vein branch to the liver lobe that will be resected in the second stage.
 - in case of complete radiological response, an attempt should be made to resect all original sites of the liver in which the lesions were previously detected. When this is not feasible, it is acceptable to leave disappeared metastases *in situ* with the intention of subsequent approaches for recurrent metastases [64] .
 - during all surgical procedures, fresh frozen tumor tissue should be collected.
- In case the primary tumor is still *in situ*, this tumor should be resected at a time which is considered to be medically appropriate.

10.2 Definitions of R0 and R1 resection

R0 resection indicates a microscopically margin-negative resection, in which no gross or microscopic tumor remains in the tumor bed. R1 resection indicates the removal of all macroscopic disease, but microscopic margins are positive for tumor.

10.3 Use of repeat hepatectomy, resection of new extrahepatic metastases

The use of repeat-hepatectomies, local ablative techniques and resection of new extrahepatic metastases should be performed according to standard or local practice.

10.4 Local treatment (surgery, radiotherapy) for primary tumor in patients with synchronous metastases

Patients with a resectable primary tumor *in situ* are eligible for the study, provided that this tumor does not require immediate surgery for symptoms such as obstruction, bleeding, etc. If immediate surgery is required, patients may be eligible for study participation after successful recovery from surgery, and if all eligibility criteria are met. Patients with minor or no symptoms of their primary tumor may be included in the study, and should receive

subsequent surgical treatment for their primary tumor in case of the development of local symptoms, or in case liver metastases become eligible for radical surgery. Patients with synchronous metastases of rectal cancer are generally treated with local radiotherapy 5x5 Gy [59].

10.5 Complications after liver surgery

Surgical complications will be collected separately. Complications will be registered using the Clavien-Dindo grading system for the classification of surgical complications. [65] Complications will be compared between the different randomised groups with either Chi-square tests or Fishers' exact tests whenever appropriate

11 Follow-up and disease evaluation during and after protocol treatment

During systemic treatment, patients will be evaluated until disease progression by CT scan of thorax and abdomen every 8 weeks. In case of R0/1 resection of the liver metastases, CT scan evaluation should be performed every 6 months until disease progression. After disease progression, patients will be followed for overall survival. The trial will be considered completed when all patients have reached the primary endpoint of the study.

Toxicity of systemic treatment will be assessed prior to each treatment cycle. After resection of liver metastases, patients will be evaluated for surgical morbidity during 2 months.

12 Study flow charts

See table 4 for flow chart of the CAIRO5.

Table 4: Flow chart CAIRO5

	Registration	Baseline/ randomisation	First 6 months				In case of surgery **	After 6 months		After end of treatment
			Prior to each cycle	During cycle 1 and 2	After every cycle	Every 8 weeks		Prior to each cycle	Every 8 weeks	
Informed consent	X									
In/ exclusion criteria	X	Request for confirmation								
Tissue for pathology	X						X			
Upload imaging for review ¹	X					X				
Imaging and measurement	X*					X			X	
History and Physical examination, incl WHO PS	X		X					X		
Concomitant medication		X	X				X	X		
Clinical Chemistry and hematology ²	X		X			X		X		
AE/ toxicity ³		X			X		X			
Follow up										X

CTCAE 4.0 and RECIST 1.1 are used

* **Study treatment is recommended to start within 4 weeks after baseline CT scan, this period should not exceed 5 weeks**

** In patients who become resectable and undergo secondary surgery for livermetastases, the total duration of preoperative and postoperative treatment together should be 6 months, with the chemotherapy schedule being continued postoperatively according to the preoperative schedule.

1 upload of imaging (e.g. CT-scan of abdomen and thorax) for panel review until panel agrees that panel review for a patient can be terminated

2 see paragraph 5 for detailed instructions

3 SAEs to be reported from moment of registration until 30 days after protocol treatment

13 Informed consent procedure

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which they will be exposed, and the mechanism of treatment allocation. They will be informed about the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. Patients will also be asked to agree to the use of patient tissues for trial purposes.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever she/ he wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered in the study. The written informed consent form should be signed and personally dated by both the patient and the physician.

14 Reporting of SAE

14.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

14.2 AEs, SAEs and SUSARs

14.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to protocol treatment.

All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. AEs will be scored according to CTCAE version 4.0. CTCAE version 4.0 can be found on the EORTC website:

<http://www.eortc.be/services/doc/ctc/>

14.2.2 Serious adverse events (SAEs)

An adverse event (AE) is any symptom, sign, illness or experience, which develops or worsens in severity from informed consent to up to 30 days following the last administration of any of the study drugs or up to PFS following surgery (liver or primary tumor). Intercurrent illnesses or injuries should be regarded as adverse events. Progression of the malignancy is not considered a SAE unless the outcome is fatal within the safety reporting period.

Hospitalization due to signs and symptoms of disease progression should always be reported as an SAE. Adverse events are classified as either serious or non-serious.

A serious adverse event (SAE) is any event that is:

- fatal
- life-threatening
- requires or prolongs hospitalisation
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event.

Important medical events are those which may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the patient, and may require intervention to prevent one of the other serious outcomes. Any SAE, which occurs after the study period and is considered to be possibly related to study treatment or study participation should be recorded and reported immediately.

14.2.3 Recording of adverse events (AE)

Information on all AEs should be recorded at each contact on the AE-module of the CRF. Grading will be done according to the NCI-CTCAEAE version 4.0 or if not applicable, the event will be graded as 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening.

14.2.4 Reporting of serious adverse events (SAE)

The local investigators are responsible for reporting SAEs. All SAEs, whether or not considered to be related to the study treatment, must be reported by fax or e-mail to the central data management (IKNL Clinical research department; e-mail: trialbureau@iknl.nl) within 24 hours, using the completed SAE report form.

The DCCG as the initiator (“verrichter” in the terminology of the Dutch law) is responsible for SAE assessment and reporting to the authorities in accordance with all requirements of the Dutch law. The DCCG has delegated these responsibilities to the principal investigator of this study.

All SAEs will be reported by the IKNL clinical research department to the medical ethical committee. Half yearly a line listing of all reported SAEs will be send to the METC via ToetsingOnline. In case of fatal cases or cases compromising patient safety, the SAE will be reported via ToetsingOnline. within 7 days after the case was reported to IKNL.

14.2.5 Suspected Unexpected Serious Adverse Reaction (SUSARs)

Unexpected adverse reactions are SUSARs if the following three conditions are met:

- the event must be serious (see chapter 9.2.2);

- there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
- the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in: Summary of Product Characteristics (SPC) for an authorised medicinal product; Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal ToetsingOnline to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered. Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. IB for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorized medicinal product).

The IKNL clinical research department is responsible for the expedited reporting of the SUSARs through the web portal Toetsing Online to the METC:

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

14.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

14.4 Data Safety Monitoring Board

In the CAIRO5 a DSMB is established to perform ongoing safety surveillance and to perform interim analyses on the safety data. This committee is an independent committee. The composition of the DSMB will be described in the DSMB charter and it should be clear that each member has no conflict of interest with the sponsor of the study. The tasks and responsibility of the DSMB are described in the DSMB charter.

The advice(s) of the DSMB will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

15 Study statistics, sample size, planned analyses

15.1 Sample size considerations - primary endpoint

The study is designed as a randomised phase 3 trial with progression free survival (PFS) as primary endpoint. Two hypotheses will be tested simultaneously:

- In patients with (K or N)RAS and BRAF wildtype (WT) with a left-sided primary colorectal tumor it is hypothesized that FOLFOX or FOLFIRI + panitumumab will improve PFS as compared to FOLFOX or FOLFIRI + bevacizumab.
- In patients with (K or N)RAS or BRAF mutant and/or right-sided primary colon tumor it is hypothesized that FOLFOXIRI + bevacizumab will improve PFS as compared to FOLFOX or FOLFIRI + bevacizumab.

Given recent literature, it is expected that about 30% of the patients will have RAS (KRAS exon 2, 3 en 4 and NRAS exon 2 and 3) and BRAF wildtype, left-sided primary colorectal tumors. While 70% will have RAS or BRAF mutated and/or right-sided primary colon tumors [66]. Patients with RAS and BRAF wildtype left-sided primary colorectal tumors will be randomised between doublet chemotherapy (FOLFOX or FOLFIRI) plus bevacizumab or panitumumab and patients with RAS or BRAF mutated and/or right sided colon tumors will

be randomised between doublet chemotherapy (FOLFOX or FOLFIRI) + bevacizumab or triple chemotherapy (FOLFOXIRI) + bevacizumab.

(K or N)RAS and BRAF wildtype

The median PFS in RAS and BRAF wildtype patients is 12.8 months, for RAS mutant patients it is 10.9 months and for BRAF mutant patients it is 7 months. The median PFS in RAS wildtype patients (BRAF mutation included) with a right-sided primary tumor is 7.6 - 9 months. [67] The median PFS of RAS and BRAF wildtype patients with a left-sided primary colorectal tumor is 11.6 months.

The treatment is assumed to reduce the hazard rate for PFS by 30%. To detect such an improvement in PFS (hazard ratio of 0.7, e.g. to a median of approximately 14.3 months), with 80% power and a two-sided logrank test at 5%, 256 events need to be observed. Assuming an accrual period of 4 years and 3 months and an analysis at 2 years follow-up after inclusion of the last patient (total study duration 75 months) a total of 290 RAS and BRAF WT patients with left-sided primary tumors (15 in each arm) should be enrolled including a planned interim analysis after 128 events. (see chapter 15.2 interim analysis).

15.2 Interim analysis

For the primary endpoint of PFS one interim analysis and a final analysis will be performed, equally spaced based on the number of events (approximately at 50%) of the way through the trial. At the interim analysis both futility and efficacy will be considered. The trial may be discontinued in either subgroup (RAS/BRAF wildtype, RAS/BRAF mutated and left or right-sided primary tumor patients) when the treatment is very efficacious, but the trial may also be discontinued early in either subgroup if the new treatment is unlikely to show superiority to control based on the interim analysis.

(K or N)RAS and BRAF wildtype and left-sided primary tumor

Statistical details:

Analysis	Value	Futility	Efficacy
IA 1: 50%	Z-value	0.41	2.75
N: 226	HR	0.93	0.61
Events: 128	p (1-sided)	0.3397	0.003
39.9 months	P{Cross} if HR=1	0.6603	0.003
	P{Cross} if HR=0.7	0.0538	0.2335
Final analysis	Z-value	1.98	1.98
N: 290	HR	0.78	0.78
Events: 256	p (1-sided)	0.0238	0.0238
75 months	P{Cross} if HR=1	0.9761	0.0239
	P{Cross} if HR=0.7	0.2	0.8

As a consequence of the interim analysis, if the trial continues as planned, a total of 256 events need to be observed and 290 patients need to be enrolled.

It is estimated that 160 patients per year could be entered into the study; 48 patients (30%) will have RAS and BRAF wildtype tumors and a left-sided primary colorectal cancer, 290 in 6 years.

(K or N)RAS or BRAF mutation and/or right-sided primary tumor

It is expected that about 70% will have RAS or BRAF mutation and/or a right-sided primary colon cancer. For this group the following parameters are assumed, a median PFS of about 8,7 months and a HR of about 0.7. The inclusion of patients with a RAS or BRAF mutation and/or a right-sided primary colon tumor will be closed after 274 patients have been randomised to guarantee at least 80% power.

Analysis	Value	Futility	Efficacy
IA 1: 50\%	Z-value	0.41	2.75
N: 204	HR	0.93	0.62
Events: 129	p (1-sided)	0.3397	0.003
37.8 months	P{Cross} if HR=1	0.6603	0.003
	P{Cross} if HR=0.7	0.0538	0.2335
Final analysis	Z-value	1.98	1.98
N: 274	HR	0.78	0.78
Events: 257	p (1-sided)	0.0238	0.0238
75 months	P{Cross} if HR=1	0.9761	0.0239
	P{Cross} if HR=0.7	0.2	0.8

It is estimated that 160 patients per year could be entered into the study; 112 patients (70%) will have RAS or BRAF mutated and/or a a right-sided primary colon cancer; 274 in 2 years and 6 months.

Statistical analysis – survival endpoints

Analysis of the primary endpoint will be based on the ‘intention-to-treat’ population. PFS curves by treatment arm will be calculated and depicted by means of the Kaplan Meier technique and will be compared using the (stratified) logrank test. Hazard ratios and 95% confidence intervals will be calculated with a (stratified) cox-proportional hazard analysis.

15.3 Subgroup analysis

Planned subset analyses will be performed in the following subgroups:

- patients with unresectable but potentially resectable versus permanently unresectable metastases (panel decision),
- *RAS* and *BRAF* mutation status,
- R0 versus R1 resected patients,
- outcome of FOLFIRI versus FOLFOX-treated patients,
- prognostic role of *RAS* and *BRAF* mutation status.

Subgroups analysis will be presented by means of forest plots with 99% confidence intervals for the comparison of treatment versus control within the subcategories. Tests for interaction of treatment by subgroups will be performed using cox-proportional hazard model.

15.4 Secondary endpoints

R0/1 resection rate will be a secondary endpoint. The number of patients randomised will provide from 71% to 91% power for the comparison of the secondary endpoint, the resection rate, to detect a true difference from 25% to 40% or to 45% at 5% (2-sided) significance level using the Fisher exact test, in favour of chemotherapy plus panitumumab in RAS and BRAF wildtype patients and in favor of FOLFOXIRI plus bevacizumab in RAS or BRAF mutated patients.

Secondary PFS (PFS2) will be a secondary endpoint. In case PFS is reached after interruption of 1st line systemic treatment of more than 3 months because of planned local therapies of liver metastases/primary tumor, and the initial systemic treatment is resumed after PFS, then PFS2 is analyzed. PFS2 is calculated from the date of randomization to progression upon resumption of 1st line systemic treatment, i.e. discarding progression during interruption of this treatment. In case a systemic treatment different from 1st line treatment is initiated after interruption of 1st line treatment, PFS2 is not applicable.

Secondary study endpoints also include the median overall survival, 3- and 5-year overall survival rates, tumor response rate, toxicity, pathological complete response rate (pCR) of resected lesions, postoperative morbidity, and correlation of baseline and follow-up evaluation by the panel with outcome.

16 Translational research

16.1 Central pathology review

Central review of the pathology will be performed at the dept. of Pathology, VUmc Amsterdam and will include tumor typing, grading and assessment of histological prognostic factors, including proliferation and apoptosis. Participating pathologists will be requested to submit when possible, tumor and normal tissue for studies related to the research questions of the trial. Preferably also fresh tissue will be collected when possible. All studies will be performed on tissue that has already been obtained from patients for diagnostic purposes. No tissue will be collected with the sole purpose of research. Written informed consent will be obtained from patients prior to tissue and peripheral blood collection.

16.2 General aim

The general aim of translational research in the CAIRO5 study is to improve the clinical outcome of CRC patients. We aim to validate molecular biomarkers to improve the clinical management of metastatic CRC patients through translational research coupled to the DCCG CAIRO5 clinical trial, thereby specifically addressing the following unmet clinical needs:

1. Identify the subgroup of metastatic CRC patients with unresectable liver-only metastases that will achieve sufficient downstaging to benefit from secondary liver

resection; i.e. to match tumor biology with responsiveness to systemic therapy (*therapy prediction*).

2. Identify a) the subgroup of metastatic CRC patients who underwent secondary liver-resection at high risk to develop recurrence; and b) by retrospective analysis the subgroup of stage III CRC patients who are at risk of developing liver metastases, in order to anticipate surveillance and intervention (*disease prognosis*).
3. Develop and validate minimal invasive diagnostics (e.g. blood sampling) for reading out tumor biology, to monitor treatment response and disease recurrence (*disease monitoring*).

16.3 Materials and Methods

This translational research project concerns a detailed molecular analysis of CAIRO5 tumor tissue and blood for validation of known (and identification of novel) predictive, prognostic, disease and drug monitoring biomarkers. Formalin-fixed paraffin-embedded (FFPE) tissues of primary tumors will be collected from all participating mCRC patients, as well as the liver metastases of ~200 patients who became eligible for secondary resection upon tumor downstaging. Blood samples will be collected at various timepoints before, during, and after surgery and systemic treatment, as described in detail in the Streck tubes lab manual. We will generate tissue microarrays (TMAs) from primary tumors and CRC liver metastases to perform immunohistochemical stainings and determine protein biomarker expression levels. Moreover, DNA will be isolated from FFPE material to determine chromosome copy number alterations by whole genome shallow sequencing; and to determine somatic mutations in cancer genes as well as germline variation in drug metabolizing genes by whole exome sequencing.

Microvesicles/exosomes will be isolated from blood and analyzed for cancer-specific protein- and miRNA disease monitoring biomarkers.

All data will be stored in a sustainable and queryable manner, in line with the CTMM TraIT Dutch national translational research IT project. In particular, clinical information will be stored using Open Clinica, while clinical and molecular profiling data will be integrated using tranSMART.

16.4 Biomarker validation

The CAIRO5 clinical trial offers access to a unique unselected liver-only mCRC patient cohort, unlike most studies that were previously analyzed for identification of candidate biomarkers. Hence, biomarker validation will be achieved by combining CAIRO5 clinical information with molecular profiling data.

- *Predictive biomarkers*: DNA methylation status of DCR1 will be examined in primary tumors and liver metastases of all patients that receive a treatment regimen that includes irinotecan (FOLFOXIRI and FOLFIRI; n=~435) and associated with progression free survival (PFS) and OS. Likewise, gain of 6q will be examined in the same population for responsiveness to 5FU-based and irinotecan combination

therapy. Loss of 5q12.1-12.3 will be associated with PFS and OS upon 5FU-based treatment (all patients), while loss of 5q34 will be associated with PFS and OS of patients treated with bevacizumab in combination with FOLFOXIRI or FOLFOX (n~350).

- *Prognostic biomarkers*: Protein expression of VEGFA, EGFR, PTGS2 (COX2), AURKA, SERPINB5, KCNQ1 and other potentially prognostic markers of interest will be examined in primary tumors and metastases of mCRC patients who receive secondary resection (n = ~200), and will be associated with recurrence and OS. Protein expression of these markers will also be examined in a separate cohort of stage III CRC patients who did not develop recurrence and liver metastases.
- *Disease monitoring biomarkers*: Microvesicles/exosomes will be isolated from blood taken before, during, and after surgery and drug treatment. Microvesicle-associated abundance of approximately 40 proteins, among which MCM5, SERPINB5, AGRN, and IPO4, will be measured by targeted mass spectrometry and correlated to disease status. Meanwhile, CRC- and metastases-associated miRNA candidate biomarkers will be identified and validated in the same population by miRNA sequencing analysis.
- Circulating tumor DNA (ctDNA) and other potentially prognostic, predictive and disease monitoring markers of interest will be isolated from all taken blood samples.
- Data analyses: For all comparisons described, multivariate analyses will be performed that take various known prognostic clinical parameters as well as the spectrum of most common somatic mutations in cancer genes into account. In addition, we will analyze novel parameters such as recurrent structural genomic variations and effects of germline variation.

17 Ethical and legal aspects

This study will be conducted in accordance to the standards of Good Clinical Practice, in agreement with the Declaration of Helsinki (latest amendment) and with Dutch law in general and with the W.M.O. (Wet Medisch-wetenschappelijk Onderzoek met mensen) in particular.

Before they agree to participation in this trial, all patients will be provided with written information in the form of a Patient Information Sheet.

The formal written consent of a patient must be obtained before initiation of any study-specific procedure.

17.1 Independent physician

In accordance with Dutch law and the WMO, an independent physician has been assigned to this study. Dr. M.J. Kersten, department of Hematology, AMC Amsterdam, tel. 020-5665955, who is not otherwise involved in this study, has agreed to act as the independent physician.

17.2 Insurance

In accordance with Dutch law and the W.M.O., an insurance policy, covering all participating patients, has been effected with AON Risk Solutions.

18 Quality

18.1 Monitoring

This study will be monitored based on the recommendations as described in the brochure “Kwaliteitsborging mensgebonden onderzoek 2.0” published October 2012 by the Dutch Federation of University Medical Centres (NFU). The monitor plan is based on the judgment of the principle investigators that the study treatment takes negligible risk for the participating patients.

The trial will be monitored by independent qualified monitors, local and central oncology data managers of IKNL clinical research department. A comprehensive description of the aspects and frequency of monitoring can be found in a separate monitoring plan filed in the Trial Master File and the Investigator Site Files.

Surgical reports and pathology reports will be collected and stored anonymised.

18.2 Quality assurance

Quality is assured in this trial by the follow aspects:

- Only panel members and study coordinators have access to the program used by the panel. The statisticians and employees from the central IKNL clinical research departement will not have access to the program used by the panel.
- All information stored in the study database will be anonymised according to laws and regulations.
- Accounts for the panel program or the study database will be handed out according to role-description as noted on the signature logs.
- A plan about the panel processes, including privacy statements, is part of the Trial Master File
- A data management plan, including validation plan, is part of the Trial Master File
- All staff working on this trial is competent, able and qualified, as shown by their curriculum vitae, which will be stored in the Trial Master File.

19 Public disclosure and publication policy

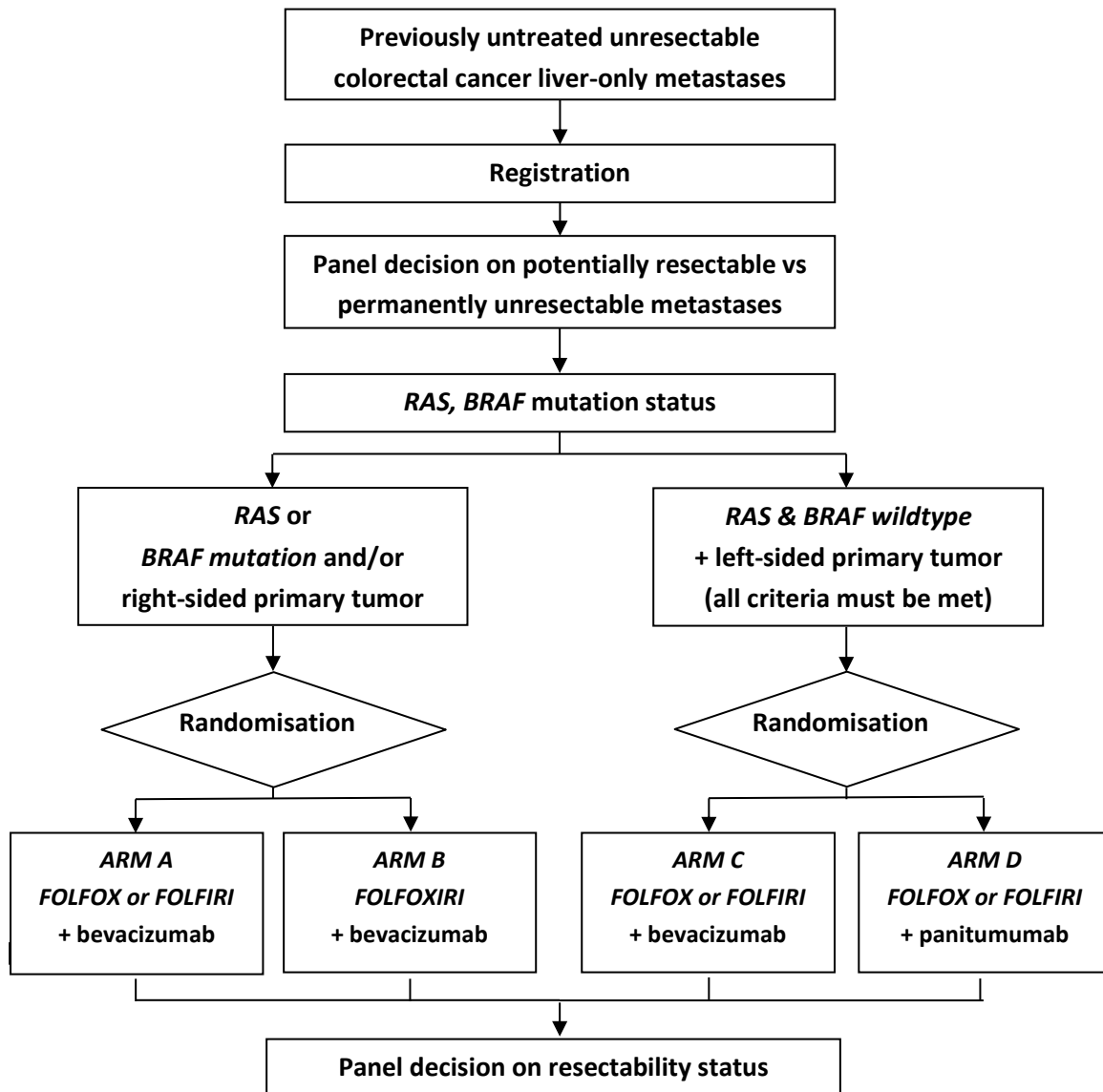
Authorship will include at least the principal investigators, all members of the liver panel, the statistician, the principal datamanager, the trial coordinator, the top ten local investigators (one medical oncologist and liver surgeon per site) regarding to inclusion numbers, and any other person that made an significant contribution to CAIRO5 which latter is decided by the principal investigators.

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21 Appendix I: CAIRO5 study design



22 Appendix II: Flow chart for registration, randomisation, pathology and central panel review

Flow chart for registration, randomisation, pathology, radiology, central panel

