Predicting treatment outcomes in metastatic colorectal cancer

heterogeneity in patient characteristics,

treatment efficacy and molecular diagnostics

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Predicting treatment outcomes in metastatic colorectal cancer: heterogeneity in patient characteristics, treatment efficacy and molecular diagnostics

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Voorspelling van de behandeluitkomst bij gemetastaseerd colorectaalcarcinoom: heterogeniteit in patiëntkarakteristieken, effectiviteit van de behandeling en moleculaire diagnostiek
(met een samenvatting in het Nederlands)

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“It does not matter how slowly you go, as long as you do not stop.”
- *Confucius*

Aan mijn ouders

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General Introduction

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General Introduction

With 1.4 million new cases and 694,000 deaths in 2012, colorectal cancer (CRC) is one of the most common cancer types and a leading cause of death worldwide[1]. The number of patients diagnosed with CRC has doubled since 1990, which is partly due to the growing and ageing population. Almost half of all CRC patients will develop distant metastases during the course of disease[2–4], which can either present at the time of initial diagnosis (synchronous), or develop during follow-up after initial resection of the primary tumour (metachronous). The liver is the predominant metastatic site in approximately 80% of CRC patients. In 40%-50% of these patients, extrahepatic metastases are also present, which are most commonly situated in the lungs, lymph nodes and peritoneum.

Over the last decades, the availability of new and effective drugs, together with increased possibilities and use of metastasectomy and other local treatment modalities, have markedly improved the prognosis of patients with metastatic colorectal cancer (mCRC)[2,5]. With treatment strategies evolving rapidly, optimal clinical outcomes are being achieved by discussing the treatment approach for each individual patient in a multidisciplinary team (MDT) of experts[6,7]. The first step in this process is to categorise patient subgroups with resectable, potentially resectable or unresectable metastatic disease, as these subgroups of patients have varying prognosis and treatment options.

Resectable metastatic disease

Surgical resection of metastases, either upfront or after downsizing with systemic induction treatment, has a clear survival benefit compared with palliative systemic treatment and offers the best chance for cure[8,9]. However, only a minority of mCRC patients are eligible for metastasectomy with curative intent[8]. When metastases are isolated to the liver, surgical resection of all liver metastases can result in 5-year survival rates between 30%-60%, depending on the study population[10–12]. Surgical possibilities for resecting limited extrahepatic disease have evolved during recent years and are increasingly used[13,14], e.g. metastasectomy in patients with lung metastases, and cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy (HIPEC) in patients with peritoneal metastases[15–17].

Potentially resectable metastatic disease

In mCRC patients with potentially resectable metastatic disease, various local treatment modalities have been introduced in recent years to convert unresectable metastases into resectable metastases. Next to portal embolization and systemic induction treatment, alternative local treatment approaches have been developed. These include radiofrequency ablation (RFA), chemo-embolization (i.e. TACE), radio-embolization (e.g. 90Y-RE), cryosurgery, microwave ablation, stereotactic radiotherapy and isolated liver perfusion[18].

Unresectable metastatic disease

The majority of mCRC patients will present with more advanced, unresectable metastases[8]. In these patients, systemic therapy (with chemotherapy and targeted therapy) is the preferred treatment option, which will be highlighted in the next paragraph. The increased availability and use of effective cytotoxic and targeted agents has resulted in a marked increase in overall survival (OS) in patients with unresectable mCRC during the past decades[2,5].

Systemic treatment for metastatic colorectal cancer

Fluoropyrimidines (5-fluorouracil [5-FU] and capecitabine), oxaliplatin and irinotecan constitute the chemotherapy backbones in the treatment of mCRC[19]. Sequential administration of these agents results in a median OS of 18 to 20 months[20]. The benefit of chemotherapy is further increased by the addition of targeted agents, such as bevacizumab (vascular endothelial growth factor [VEGF] inhibitors), and cetuximab or panitumumab (epidermal growth factor receptor [EGFR] inhibitors) in patients with *RAS* (*KRAS* and *NRAS*) wild-type tumours, resulting in a median OS of ~30 months[20]. The mechanisms of action of anti-VEGF and anti-EGFR inhibitors are depicted in Figure 1. In recent years, other targeted agents, such as the recombinant fusion protein aflibercept and the monoclonal antibody ramucirumab have demonstrated a significant OS benefit over standard care when combined with second-line chemotherapy[21,22]. In patients with refractory mCRC, the multikinase inhibitor regorafenib and trifluridine/tipiracil have demonstrated a significant improvement in OS[23,24]. Immunotherapy represents a promising option for improving survival in a subset of mCRC patients. A recent study showed that pembrolizumab, an antibody to programmed cell death protein 1 (PD-L1), was effective in previously treated mCRC patients with deficient mismatch repair tumours (dMMR), which occurs in 3%-5% of mCRC patients[25]. Another study reported that *HER2* amplification occurs in ~5% of patients with *KRAS* wild-type mCRC, and that dual HER2 inhibition with trastuzumab and lapatinib was effective in refractory patients with *KRAS* exon 2 wild-type, *HER2*-positive mCRC[26]. In the Netherlands, the committee for the evaluation of new oncology drugs (CieBOM) of the Dutch Society for Medical Oncology (NVMO) uses the PASKWIL (Palliative/Adjuvant effectiveness, Specific adverse events, (Kw) quality of life, Impact of treatment and Level of evidence) criteria to assess the value of new oncology drugs. Since aflibercept, ramucirumab and regorafenib do not meet the PASKWIL criteria, these drugs are not advised as standard of care for mCRC in the Netherlands.

In recent years, it has become evident that CRC is a heterogeneous and molecularly complex disease[27], with patients showing varying prognosis and response to treatment. Since few predictive biomarkers are currently available, most systemic treatments are administered with a 'one-size-fits-all' approach, which results in only a limited of number of patients experiencing benefit from often very expensive treatments. Furthermore, many patients are unnecessarily exposed to toxicity, side effects and frequent hospital visits.

Therefore, it is important to clearly discuss the treatment goal and to consciously weigh the pros and cons before initiating systemic therapy. Ultimately, the choice of systemic treatment should be individualised and made in consultation with the patient, depending on tumour- and disease-related characteristics (e.g. biomarkers, symptoms, extent of metastatic disease), patient-related factors (e.g. socioeconomic factors, comorbidity) and treatment-related factors (e.g. estimated survival time, toxicity and side effects)[28].

Prognostic and predictive factors are essential components in the treatment decision-making process, since these factors have the ability to identify patient subgroups with different disease outcomes regardless of treatment (prognostic), and to identify subgroups of patients who are most likely to derive benefit, or not, from a specific treatment (predictive). In particular, predictive markers are urgently needed to optimise patient selection, both for chemotherapeutic and targeted regimens, in order to improve treatment efficacy and patient well-being, and to reduce potential toxicity and high therapy costs[8].

Prognostic factors

Data on the efficacy of new treatment strategies are largely derived from randomised controlled trials. Despite using comparable patient eligibility criteria, phase 3 mCRC trials often show heterogeneity in response and survival outcomes, which is likely explained by differences in prognostic factors. Prognostic factors have the potential to influence the survival of patients to a greater extent than any available treatment regimen. Adequate reporting of patient characteristics in mCRC trials is essential to evaluate whether a trial population is representative of the general population of mCRC patients. Furthermore, stratification will allow for better interpretation of trial results by balancing key prognostic factors between treatment arms[29]. However, there is no consensus on which patient characteristics to report and which stratification factors to use in mCRC trials.

'Classic' clinical prognostic factors are performance status, number of metastatic sites, serum lactate dehydrogenase (LDH), and primary tumour location. Recent studies have identified primary tumour sidedness (proximal versus distal from the splenic flexure) as an important prognostic factor[30,31], although underlying biological mechanisms still have to be clarified. Other potential prognostic factors are primary tumour resection in patients with synchronous metastases, which is currently being investigated in several phase 3 trials[32–34], body mass index (BMI) and muscle loss[35,36].

Established histopathological prognostic factors are tumour extent (T-stage), lymph node status (N-stage), histological subtype, differentiation grade and lymphovascular invasion. Several molecular prognostic factors have been discovered during the last decade. ^{V600E}*BRAF* mutations occur in 5%–10% of mCRC patients and are associated with poor outcome[37,38]. Recent studies have shown that *RAS* mutations, which occur in ~50% of mCRC patients, are also associated with poor prognosis[37,39]. Mismatch repair deficiency, or dMMR, the

underlying cause of microsatellite instability (MSI), has a low prevalence in mCRC (3%–5%) and also indicates a poor prognosis, which is likely driven by its association with ^{V600E}*BRAF* mutations[40,41].

Predictive factors

Although chemotherapy provides the greatest benefit to mCRC patients with unresectable disease, there are no predictive biomarkers to guide treatment response[42]. Similarly, no useful biomarkers have been identified to guide treatment with bevacizumab[43]. Currently, *RAS* mutation status is the strongest predictive biomarker in the management of mCRC. Initial studies showed that patients with *KRAS* exon 2 mutated tumours lacked benefit from anti-EGFR therapy[44]. In recent years, it has become evident that additional *KRAS* and *NRAS* mutations (*RAS* mutations) also have a negative predictive value with regard to anti-EGFR therapy.[45] Therefore, only patients with *RAS* wild-type tumours have an indication for anti-EGFR therapy. Both RAS and BRAF genes encode proteins that are part of the mitogen-activated protein kinase (MAPK) pathway, depicted in Figure 1. *RAS* mutations lead to activation of the MAPK signalling pathway, independent from EGFR activation by binding of the ligand[46]. This results in cellular proliferation and inhibition of apoptosis. As BRAF is downstream of RAS in the MAPK signalling pathway, resistance to anti-EGFR therapy would also be expected for mCRC patients with *BRAF*-mutant tumours. Indeed, two meta-analyses have demonstrated that mCRC patients with ^{V600E}*BRAF*-mutant tumours derive little or no benefit from anti-EGFR therapy[47,48].

Recent studies have also found significant interactions between primary tumour sidedness and anti-EGFR therapy. Survival benefit of anti-EGFR therapy was restricted to patients diagnosed with a distal primary tumour[49,50], which is possibly due to underlying biological differences between proximal and distal carcinomas that still need to be elucidated. Other promising emerging predictive markers for treatment selection in mCRC patients are MSI status for PD-L1 checkpoint inhibition with pembrolizumab[25], and *HER2* amplification for dual HER2-targeted therapy with trastuzumab and lapatinib in patients with *KRAS* exon 2 wild-type tumours[26].

Maintenance treatment

Since mCRC patients have better survival, there has been a shift from continuing systemic treatment until disease progression to that of a 'continuum of care' approach. This approach focuses on optimal exposure of patients to available effective treatment regimens during the course of disease, in order to maximise overall survival, minimise toxicity, and maintain quality of life. The use of systemic treatment is tailored to the clinical setting, including therapy switches prior to disease progression, maintenance treatment, therapy-free intervals, and resection of metastases in selected patients[28,51,52].

Currently, the combination of bevacizumab with fluoropyrimidine-based chemotherapy is a standard option in first-line treatment of mCRC[53–55]. Maintenance treatment is considered the preferred strategy within the continuum of care approach. The phase 3 CAIRO3 and AIO 0207 trials showed that in mCRC patients with stable disease or response after induction treatment with a fluoropyrimidine, oxaliplatin and bevacizumab, maintenance treatment with a fluoropyrimidine and bevacizumab is more effective compared with a treatment break, without compromising quality of life[56–58]. Since not all patients may benefit from maintenance treatment, the next challenge is to discover predictive biomarkers in order to identify subgroups of patients in which a treatment break is safe, and on the other hand those in which maintenance treatment is prerequisite for increased survival.

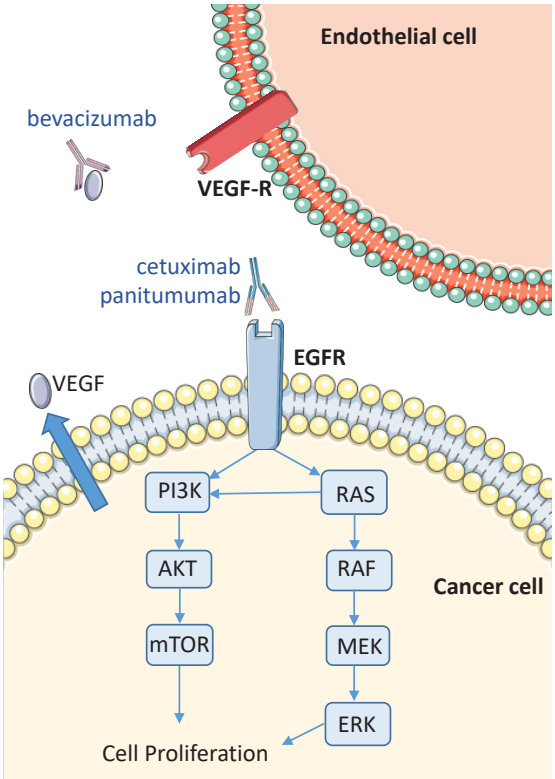

Figure 1. Intracellular signalling in a cancer cell, and the action of VEGF secreted by cancer cells into the extracellular space. VEGF signals to receptors in vascular endothelial cells and can be targeted by the anti-VEGF antibody bevacizumab. At the cancer cell membrane, the EGFR responds to growth signals and subsequently activates the phosphoinositide 3 (PI3)-kinase and MAPK signalling pathways. EGFR can be blocked by the anti-EGFR agents cetuximab and panitumumab. RAS activates multiple parallel pathways, including the PI3-kinase and MAPK pathways. The MAPK pathway consists of signalling from RAF proteins (e.g. BRAF) to mitogen-activated protein kinase (MEK) and extracellular signal-regulated kinase (ERK), which ultimately results enhanced cell proliferation. (own figure; figure legend adapted from Bass[59])

Outline of this Thesis

The research described in this thesis focuses on the search for prognostic and predictive factors in order to optimise treatment outcomes in patients with mCRC.

Several studies have demonstrated that patients with synchronous metastases have unfavourable prognostic characteristics compared to patients with metachronous metastases[60,61]. However, the distribution and prognostic impact of synchronous versus metachronous mCRC is not routinely reported in mCRC studies. **Chapter 2** concerns a systematic review that presents recent trends in inclusion and survival of synchronous versus metachronous mCRC in different types of studies investigating first-line systemic therapy or initial surgical treatment of mCRC patients. Furthermore, we studied the different definitions of synchronous versus metachronous metastatic disease.

Chapter 3 describes the results of a systematic review carried out to provide an overview of the reporting of patient characteristics and stratification factors in phase 3 trials on first-line systemic treatment of mCRC published between 2005 and 2016. We analysed whether the recommendation on standardisation of patient characteristics reporting and stratification factors as proposed by Sorbye et al. in 2007 has been implemented in recent trials[29]. Furthermore, we investigated the reporting of other prognostic factors that may have become relevant in the light of new treatment strategies. Subsequently, we conducted a two-round Delphi survey among international mCRC experts to develop a consensus recommendation on essential patient characteristics and stratification factors in phase 3 trials investigating systemic treatment of mCRC, which is described in **Chapter 4**.

Chapter 5 describes a post-hoc analysis of the CAIRO3 study, a multicentre phase 3 trial conducted by the Dutch Colorectal Cancer Group (DCCG)[56]. With updated follow-up and data regarding sidedness, we defined subgroups according to *RAS* and *BRAF* mutation status and MMR status, and investigated their influence on treatment efficacy of capecitabine plus bevacizumab (CAP-B) maintenance treatment versus observation.

Next generation sequencing (NGS) has been integrated in routine care to guide precision medicine in mCRC, which allows sensitive and quantitative identification of driver gene mutations. The mutant allele fraction (MAF) is defined as the number of mutant reads divided by the total number of reads at a specific genomic position of interest[62]. Assessment of MAFs or MAFs normalised for tumour purity (adjMAFs) in driver genes may have important implications in the therapeutic management of mCRC. **Chapter 6** concerns an exploratory analysis of the CAIRO3 study in which we investigated whether *KRAS* MAFs or adjMAFS are independently associated with prognosis in mCRC patients with *KRAS*-mutant tumours.

Chapter 7 concerns an individual patient data meta-analysis of the phase 3 CAIRO3[56] and AIO 0207[57] trials in order to provide more precise estimates of treatment effects regarding the use of fluoropyrimidine plus bevacizumab maintenance treatment after induction

treatment with combination chemotherapy and bevacizumab, and to identify patient subgroups according to clinical and pathological characteristics that benefit most from fluoropyrimidine and bevacizumab maintenance treatment or observation.

This thesis is completed by a Summary and General Discussion in **Chapter 8**, in which the results and conclusions of the presented studies are summarised and their potential clinical implications will be discussed.

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Chapter 2

Significant increase of synchronous disease in first-line metastatic colorectal cancer trials: results of a systematic review

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Abstract

Background

Although synchronous and metachronous metastases are considered as separate entities of metastatic colorectal cancer (mCRC) with different outcomes, its proportion is reported infrequently. We compared inclusion rates and survival of synchronous versus metachronous mCRC in different types of studies investigating initial systemic therapy or surgical treatment of mCRC.

Methods

We searched PubMed and EMBASE (January 2004 – February 2016) for mCRC studies investigating first-line systemic therapy or surgical treatment of mCRC including information on synchronous versus metachronous metastases. Outcomes were the proportion of synchronous mCRC, and estimated median overall survival (OS) of the total study population. Spearman analysis (r_s) was used to study correlations between outcomes and median year of study enrolment.

Results

We included 46 articles, reporting data from 23 phase 3 randomised controlled trials (RCTs), twenty cohort and three population-based studies (total: 25,941 patients). Seventeen different definitions for synchronous mCRC were identified. In systemic therapy RCTs, we observed an increased proportion of synchronous mCRC during recent years (r_s.77, p<.001). In these trials, estimated median OS slightly improved over time (r_s .48, p=.03). No significant inclusion or survival trends were observed in included cohort and population-based studies.

Conclusions

In recent years, the proportion of patients with synchronous compared with metachronous mCRC enrolled in first-line systemic therapy RCTs increased. Estimated median OS of the total study population in these RCTs slightly increased over time. Many different definitions of synchronous disease were used. Uniform definitions and consistent reporting of the proportion of synchronous versus metachronous metastases could improve cross-study comparisons and interpretation of reported data in all mCRC studies.

Introduction

Approximately half of all colorectal cancer (CRC) patients develop distant metastases during the course of their disease[1–3]. Metastases can either present at the time of initial diagnosis (synchronous) or develop during follow-up (metachronous). Until recently, there has been no international consensus on the definition of synchronous and metachronous metastases. Several studies reported that patients with synchronous metastases have unfavourable clinical and biological characteristics compared with metachronous metastases[4–6]. Survival differences for these groups were found in particular when resection of the primary tumour was taken into account, with best survival outcomes in patients with metachronous metastases and better outcomes in patients with synchronous metastases who underwent a primary tumour resection compared with those with a primary tumour in situ[4,7,8]. Despite these observations, the distribution and prognostic impact of synchronous versus metachronous disease is not routinely reported in metastatic CRC (mCRC) trials[9]. Furthermore, population-based studies reporting on incidence and trends of synchronous and metachronous mCRC are scarce, and their results are conflicting[10–12].

During the last decade, availability of new effective drugs together with increased possibilities and use of metastasectomy have improved the prognosis of mCRC patients[1,13]. As patients with stage I-III disease are being monitored more closely and high quality diagnostic tools to detect metastases have become available, metachronous metastases are probably detected in an earlier stage during follow-up [14,15]. Nowadays, treatment with metastasectomy has a clear survival benefit compared with palliative systemic therapy, which used to be the standard treatment of mCRC[16]. The impact of these progresses on the proportion and survival of mCRC patients with synchronous versus metachronous disease included in randomised controlled trials (RCTs), cohort and population-based studies has not been studied thus far. The aim of this systematic review was to investigate recent trends in inclusion and survival of synchronous versus metachronous mCRC in different types of studies investigating first-line systemic therapy or initial surgical treatment of mCRC.

Methods

Search strategy and selection criteria

We searched PubMed and EMBASE on February 22, 2016 to identify mCRC studies published in English between January 2004 and February 2016. The following keywords were used in our search strategy: ‘metastasis’, ‘colorectal’, ‘cancer’, ‘synchronous’ and ‘metachronous’. We conducted a separate search to identify all phase 3 mCRC studies with synonyms of ‘metastasis’, ‘colorectal’, ‘cancer’ and ‘phase 3 trial’. A detailed literature search strategy is

listed in Supplementary Table 1. Subsequently, reference lists from selected articles were cross-searched for additional relevant studies.

We included full-text articles containing data of randomised phase 3 trials (RCTs), cohort or population-based studies that investigated first-line systemic therapy (i.e. chemotherapy and/or targeted therapy) or initial surgical treatment (i.e. metastasectomy) in adult mCRC patients and included information on synchronous and metachronous metastases. Since stage IV disease is often used interchangeably with synchronous disease, studies categorising mCRC as stage I-III versus stage IV disease were considered eligible. If articles categorised time from initial diagnosis to randomisation into different time intervals, the time interval closest to randomisation was considered as synchronous disease. If articles evaluated the same dataset, only the most recent publication with data on synchronous and metachronous mCRC was included.

Exclusion criteria were: translational studies; studies limited to specific age groups, ethnicity or molecular subgroups (e.g. *KRAS* wild-type); studies focussing on specific metastatic sites (other than liver and lung; e.g. cerebral metastases only); studies investigating beyond first-line systemic therapy or repeat metastasectomy; studies investigating local interventional techniques (e.g. local ablation, embolisation, precision radiotherapy); studies investigating simultaneous surgery of two metastatic organs; studies investigating CRC together with other cancer type(s); and editorials, commentaries, letters, (systematic) reviews or meta-analyses.

Data extraction

All steps in the screening and data extraction process were performed by two independent reviewers (KG, JtL). Disagreements were solved through discussion with a third reviewer (MK or MvO). Study design, patient characteristics and outcomes were extracted from the selected articles. For RCTs, we also obtained inclusion and exclusion criteria, and randomisation technique. Guidelines by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement were followed[17].

Outcomes

The primary outcome was the proportion of patients with synchronous versus metachronous metastases, irrespective of the definition used in the included studies. In addition, when provided, median overall survival (OS) data were collected, as well as the different definitions of 'synchronous' and 'metachronous' metastases. The included studies reported a variety of survival outcomes (e.g. progression-free survival, disease-specific survival or OS), as well as a variety of outcome parameters (e.g. median OS, survival rates or hazard ratios). Since we were particularly interested in the overall prognosis of the different study populations, we have selected median OS as endpoint of interest. When OS was only given for separate

cohorts or treatment arms, median OS of the total study population was estimated by calculating the sample-size weighted mean of the median OS for separate groups (estimated median OS).

Statistical analysis

Three types of mCRC studies were identified: RCTs, cohort and population-based studies. Within these groups, studies were subdivided according to the type of intervention studied: systemic therapy, surgery (i.e. metastasectomy), surgery combined with perioperative systemic therapy, or all interventions (i.e. no focus on specific intervention type). Mean proportions of synchronous disease between different study types and different types of interventions were compared using the Kruskal-Wallis test. To investigate trends in time, we performed correlation analyses between median year of study enrolment and (1) the proportion of synchronous mCRC; and (2) estimated median OS of the total study population. Correlation analyses were performed using the Spearman's rank correlation test (r_s) in case of a minimum sample size of four studies. Data from RCTs, cohort and population-based studies were analysed both combined and separately; as were data from studies investigating systemic and/or surgical treatment. Two-sided *p*-values <.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 21.0 (Armonk, NY: IBM Corp).

Results

Literature search

A flow chart of the literature search is shown in Figure 1. The systematic search identified 6,905 unique publications, of which 6,660 were excluded on the basis of title or abstract. After full-text revision of 245 articles, 46 studies were retained, including 10 eligible studies identified by cross-referencing. In total, 46 studies met the predefined inclusion criteria, reporting data from 23 RCTs[7,18–40], 20 cohort studies[6,41–58], and three population-based studies[59–61].

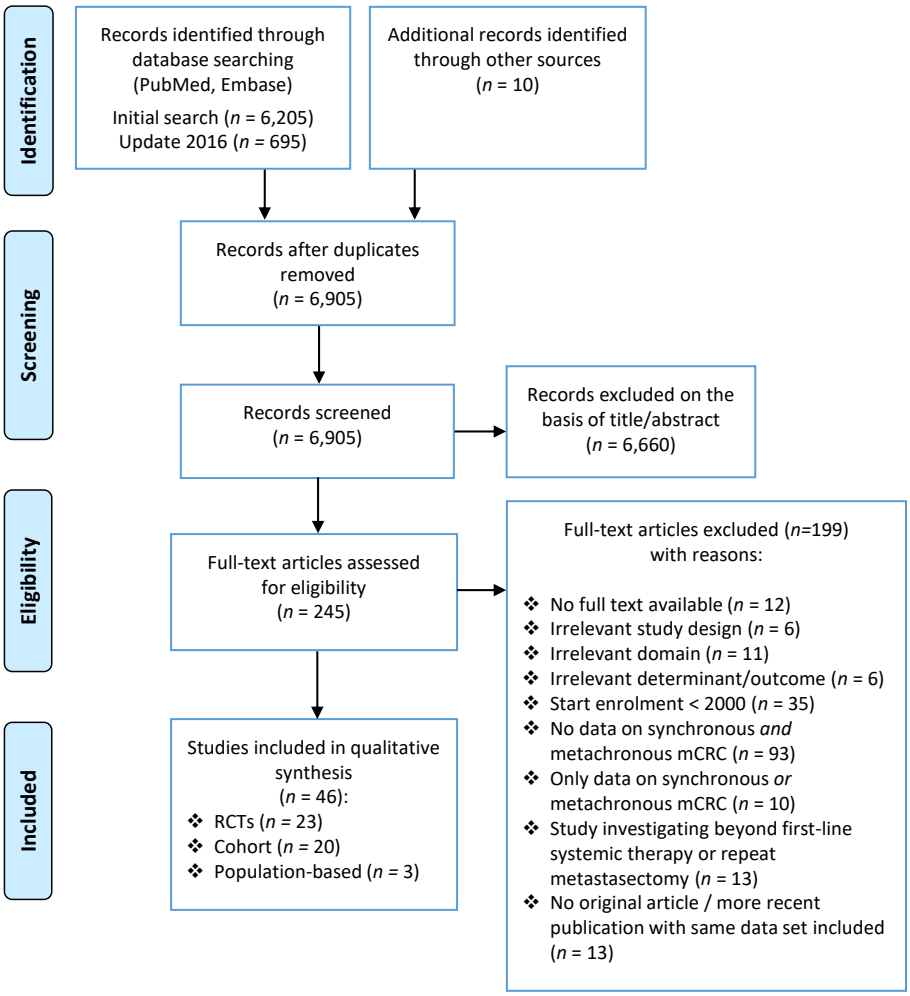

Figure 1. PRISMA flow chart of literature search.

General characteristics

Study characteristics and survival data are presented in Table 1. The included studies comprised data of a total of 25,941 patients (RCTs: *n*=15,461; cohort studies: *n*=5,934; population-based studies: *n*=4,546). Sample sizes ranged from 40 to 2,502 patients. Type of interventions studied were: systemic therapy (*n*=24), surgery (*n*=6), surgery combined with perioperative systemic therapy (*n*=14), or all types of interventions (*n*=2). In studies reporting on gender, age, colon as primary tumour site (as opposed to rectum or rectosigmoid), prior adjuvant chemotherapy or primary tumour resection in their baseline characteristics, distribution of these variables was not significantly different in different study types or intervention types.

Considering all included studies, the proportion of patients with synchronous mCRC varied from 20% to 81% (Figure 2A). Analysed by study type, there was no significant difference in the mean proportion of synchronous mCRC patients included in RCTs (63%), cohort (56%) and population-based studies (69%, p=.37). The mean proportion of synchronous mCRC in studies investigating systemic therapy, surgery or surgery combined with perioperative systemic therapy was 64%, 54% and 57%, respectively (p=.32).

Thirty-five out of 46 studies (76%) reported median OS, of which 29 studies reported median OS for separate treatment arms or cohorts, while six studies only reported median OS of the total study population[43,44,51,54,58,61]. Five studies reported median OS for synchronous and metachronous disease separately[6,41,44,48,59], of which three studies demonstrated a significant OS difference in favour of metachronous mCRC[41,44,59], one study only showed inferior OS in synchronous compared with metachronous mCRC patients in case of no perioperative chemotherapy or unfavourable response to chemotherapy[48] and one study reported no significant OS difference between synchronous versus metachronous mCRC[6].

RCTs

Out of the 23 eligible multicentre RCTs, 20 trials investigated first-line systemic treatment [7,19–27,29–33,36–40] and three trials investigated metastasectomy combined with perioperative systemic therapy [18,34,35]. Eleven trials investigated a targeted therapy containing regimen[7,27,29–33,36–39] (Table 1). Sixteen trials included only patients with unresectable metastases[19–22,24–29,32,33,36], whereas three trials enrolled only patients with resectable or resected metastases[18,34,35]. Four trials did not specify resectability of metastases in their eligibility criteria[23,30,31,40].

The percentage of synchronous mCRC in all RCTs varied from 30% to 85% (Figure 2B). The proportion of patients with synchronous mCRC significantly increased during the last decades (r_s .78, p<.001). This observation remained significant when analysing only systemic therapy RCTs (n=20, r_s .77, p<.001).

Median OS was reported in 22/23 (96%) RCTs (Figure 2C). In RCTs investigating surgery combined with perioperative systemic therapy, estimated median OS varied from 44.8 to 57.8 months (n=2)[18,34]. Estimated OS in systemic therapy RCTs varied from 15.1 to 28.4 months and slightly increased during the last decades (n=20, r_s .48, p=.03)[7,19–27,29–33,36–40].

Cohort studies

Twenty cohort studies were considered eligible, which investigated first-line systemic therapy (n=4)[43,54–56], metastasectomy (n=4)[6,41,53,62], metastasectomy combined with perioperative systemic therapy (n=11)[42,45–52,57,58], and one study considered all types of interventions[44]. The majority of the cohort studies were retrospective (n=17)[6,41–48,50,52–54,56–58,62]; single-centre (n=18)[6,41–45,47–54,56–58,62]; and conducted in a tertiary care facility (n=15)[6,41–43,45,47,48,50,51,53,54,56–58,62] (Table 1).

The proportion of patients with synchronous mCRC in the cohort studies varied from 38% to 76% (Figure 2D). No significant differences in the inclusion of synchronous mCRC patients over time were found; neither when all cohort studies were analysed (r_s .12, *p*=.60), nor when differentiating between studies investigating first-line systemic therapy (r_s -.32, p=.68), metastasectomy (r_s -.80, *p*=.20) or metastasectomy combined with perioperative systemic therapy (r_s .36, *p*=.29).

Median OS was reported in 11/20 (55%) cohort studies (Figure 2E). In cohort studies investigating systemic therapy, metastasectomy or metastasectomy combined with perioperative systemic therapy, estimated median OS ranged from 20.3 to 24.3 months, 22.2 to 44.5 months and 31.5 to 47.8 months, respectively[6,41,43,44,48–51,54,55,58]. There was no significant correlation between estimated median OS and median year of study enrolment when analysing cohort studies investigating metastasectomy combined with perioperative systemic therapy (*n*=5, r_s .10, *p*=.87).

Population-based studies

Out of the three observational population-based studies, two studies mainly focused on surgery of liver metastases[60,61] and one study investigated different intervention types in all kind of CRC metastases [59]. The percentage of synchronous mCRC patients included in these studies varied from 64% to 72% (Figure 2A). Due to the small number of included population-based studies, no correlation analyses were performed for this study type.

Definitions

Definitions and terminology used to describe synchronous versus metachronous metastases varied among the different studies. In total, 17 different definitions to report synchronous versus metachronous metastases were found (Table 2). The terms 'synchronous' versus 'metachronous' metastases were reported in 36 studies. In 18/36 (50%) studies, no specific description of synchronous metastases was given. To distinguish 'synchronous' versus 'metachronous' metastases, some authors used the initial CRC diagnosis as a reference point in time[7,34,37,41,44,53,60], while others incorporated the timing of colorectal surgery in their definition[6,48,57,62]. In these studies, inclusion of patients in the 'synchronous' group varied from 0, 3, 6 to 12 months after the initial diagnosis or colorectal surgery. One study used two different definitions of synchronous disease[41].

Ten publications did not report on the terms 'synchronous' and 'metachronous' metastases. In four of these articles, authors distinguished 'stage I-III' and 'stage IV' disease[8,23,29,38]; three articles reported 'time from initial diagnosis to randomisation' with the first interval ranging until 3 or 6 months after initial diagnosis[24,30,31]; two articles reported 'metastatic disease at first diagnosis'[33,56] and one article reported 'delay in diagnosis of metastasis after primary tumour resection' with the first interval ranging until 12 months after primary tumour resection[35].

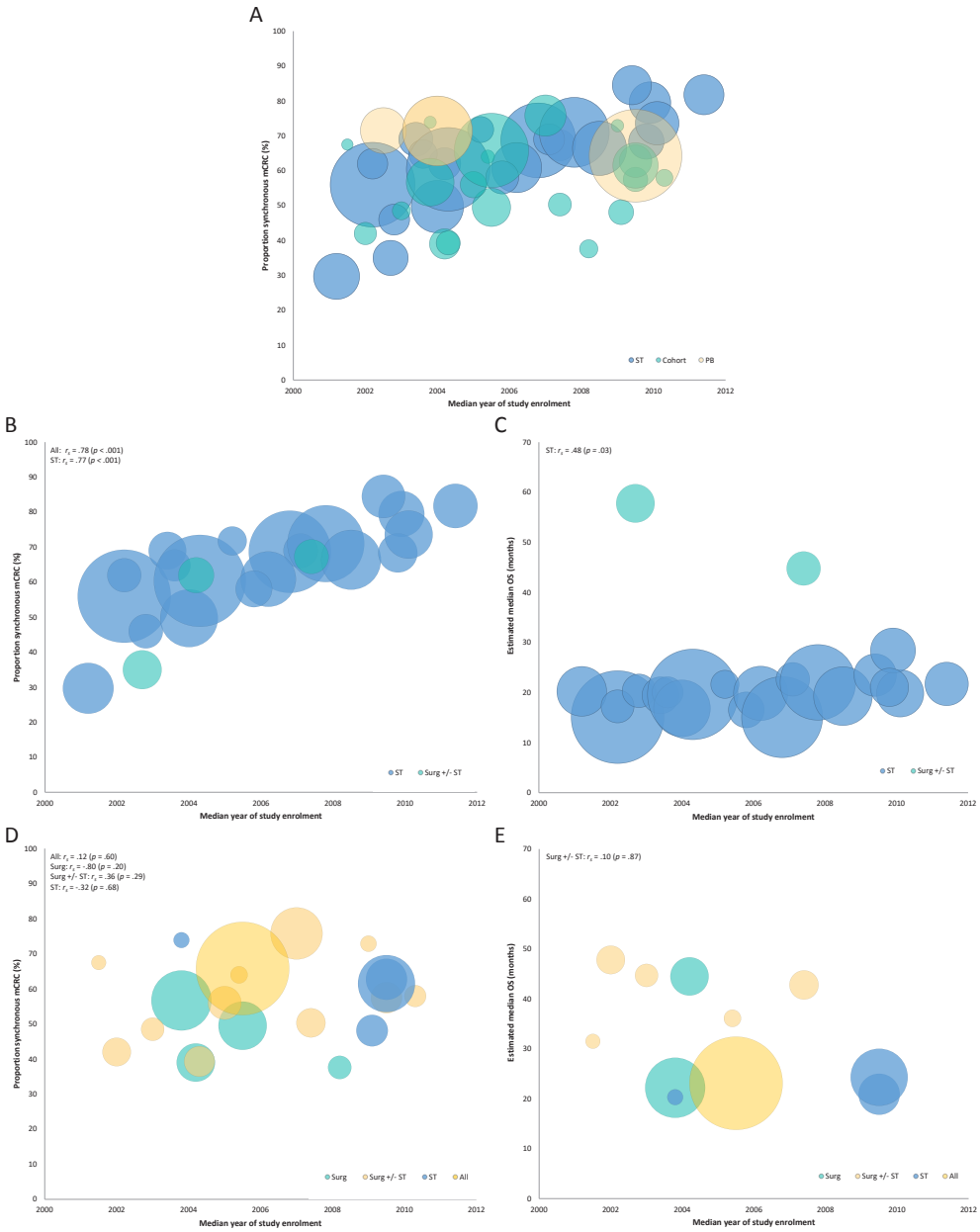

Figure 2. Proportion of synchronous versus metachronous mCRC and estimated median OS of total study population in different study types, plotted against median year of study enrolment. **(A)** All included studies – proportion synchronous versus metachronous mCRC; **(B)** RCTs - proportion synchronous versus metachronous mCRC; **(C)** RCTs - estimated median OS over time; (**D)** cohort studies - proportion synchronous versus metachronous mCRC; (**E**) cohort studies - estimated median OS over time. Dot size represents sample size. All = all interventions; PB = population-based; ST = systemic therapy; Surg = surgical treatment.

Table 1. Study characteristics and outcomes

Author, year [ref]	Enrolment period *Years*	Origin of data	Sample size *N* (% Synchronous mCRC)*	PT resection %	Estimated median OS *Months*	Treatment details
RCTs – First-line systemic therapy						
Adams, 2011[27]	2005-2008	UK, Ireland	1,630 (69)	53	15.1	Continuous vs intermittent FOLFOX / CAPOX
Cassidy, 2008[33]	2003-2005	Multi	2,034 (60)	NR	19.7	CAPOX ± B/placebo vs FOLFOX ± B/placebo
Chibaudel, 2009[26]	2004-2006	France	202 (72)	NR	21.6	mFOLFOX followed by 5FU/LV vs mFOLFOX followed by chemotherapy-free interval
Comella, 2005[22]	2001-2003	Italy	276 (62)	78	17.3	FOLFIRI vs FOLFOX
Comella, 2009[40]	2004-2007	Italy	322 (58)	74	16.6	CAPOX vs FOLFOX
Díaz-Rubio, 2007[23]	2002-2004	Spain	342 (69)	82***	19.5	CAPOX vs FUOX
Hegewisch-Becker, 2015[39]	2009-2013	Germany	472 (82)	74	21.7	FP-B vs B vs observation
Hoff, 2012[31]	2006-2010	Multi	860 (66)	NR	19.4	FOLFOX/CAPOX + cediranib vs FOLFOX/CAPOX + placebo
Koopman, 2007[25]**	2003-2004	Netherlands	803 (50)	82	16.9	Sequential CAP, IRI, CAPOX vs CAPIRI followed by CAPOX
Loupakis, 2014[32]	2008-2011	Italy	508 (80)	NR	28.4	FOLFIRI-B vs FOLFOXIRI-B
Masi, 2011[24]	2001-2005	Italy	244 (65)	NR	20.1	FOLFOXIRI vs FOLFIRI
Passardi, 2015[38]	2007-2012	Italy	370 (24)	76	21.1	FOLFIRI or FOLFOX4 ± B
Pectasides, 2012[29]	2006-2009	Greece	285 (69)	83	22.6	CAPIRI-B vs FOLFIRI-B
Schmoll, 2012[30]	2006-2009	Multi	1,422 (71)	NR	22.1	mFOLFOX6 + cediranib vs mFOLFOX6-B
Seymour, 2007[20]	2000-2003	UK, Cyprus	2,135 (56)	75	15.0	5FU followed by IRI vs 5FU followed by FOLFIRI/FOLFOX vs FOLFIRI/ FOLFOX
Simkens, 2015[7]	2007-2012	Netherlands	557 (74)	59	19.9	CAP-B vs observation
Souglakos, 2006[21]	2000-2004	Greece	283 (46)	NR	20.5	FOLFOXIRI vs FOLFIRI
Tol, 2011[36]**	2005-2006	Netherlands	736 (61)	78	19.9	CAPOX-B vs CAPOX-B + cetuximab
Tournigand, 2006[19]	2000-2002	Multi	620 (30)	NR	20.2	Continuous FOLFOX4 vs FOLFOX7/5FU-LV + intermittent oxaliplatin
Tournigand, 2015[37]	2007-2011	Multi	452 (85)	62	23.5	B vs erlotinib-B
RCTs – Metastasectomy with/without perioperative systemic therapy						
Hebbar, 2014[34]	2004-2010	France	284 (67)	52	44.8	FOLFOX4 vs FOLFOX7 followed by FOLFIRI (after hepatectomy)
Nordlinger, 2013[18]	2000-2004	Multi	364 (35)	NR (PT resected / resectable)	57.8	Hepatectomy ± FOLFOX4
Ychou, 2009[35]	2001-2006	Multi	306 (62)	100	NR	5FU-LV vs FOLFIRI (after hepatectomy)

Cohort studies – First-line systemic therapy						
Kronborg, 2015[54]	2007-2011	Denmark	314 (63)	56	20.9	Intermittent FLIRI-based chemotherapy
Marschner, 2015[55]	2006-2012	Germany	605 (62)	87	24.3	FP chemotherapy with OX or IRI
Ohhara, 2015[56]	2007-2010	Japan	185 (48)	85	NR	FOLFOX4-B vs mFOLFOX6-B vs CAPOX-B
Yoshino, 2007[43]	2002-2004	Japan	46 (74)	70	20.3	mFOLFIRI
Cohort studies – Metastasectomy						
Bredt, 2014[53]	2006-2010	Brazil	101 (38)	100	NR	Hepatectomy
John, 2013[62]	2000-2011	UK	432 (60)	NR	NR	Hepatectomy
Van der Pool, 2010[6]	2000-2008	Netherlands	272 (39)	100	44.5	Hepatectomy
Xu, 2009[41]	2000-2007	China	669 (57)	100	22.2	PT resection ± hepatectomy
Cohort studies – Metastasectomy with/without perioperative systemic therapy						
Barone, 2007[51]	2000-2003	Italy	40 (68)	NR	31.5	Hepatectomy + mFOLFIRI
Boostrom, 2009[50]	2000-2005	USA	99 (48)	100	55.6	Hepatectomy ± FOLFOX/FOLFIRI
Capussotti, 2008[49]	2000-2003	Italy	150 (42)	NR	47.8	Hepatectomy ± chemotherapy
Faron, 2014[42]	2000-2010	France	179 (58)	NR	NR	Hepatectomy ± perioperative FOLFOX
Galizia, 2013[47]	2006-2011	Italy	48 (73)	96	NR	Hepatectomy + chemotherapy
Gur, 2013[58]	2003-2011	USA	157 (50)	NR (PT resection planned)	42.8	Hepatectomy ± perioperative systemic therapy
Mehta, 2008[52]	2003-2005	UK	173 (39)	NR	NR	Hepatectomy vs OX + hepatectomy vs other chemotherapy + hepatectomy
Nakayama, 2015[57]	2007-2013	Japan	88 (58)	NR	NR	Adjuvant OX-based chemotherapy after curative metastasectomy
Ng, 2009[48]	2002-2008	China	55 (64)	100	36.1	Hepatectomy
Nozawa, 2011[45]	2000-2009	Japan	207 (56)	100	NR	Hepatectomy ± 5FU-LV / FOLFOX
Rong, 2014[46]	2002-2012	Multi	501 (76)	100	NR	Hepatectomy + FOLFOX vs hepatectomy + FOLFOX-B
Cohort studies – All interventions						
Dexiang, 2012[44]	2000-2010	China	1,613 (66)	NR	23.1	All interventions
Population-based studies						
Hackl, 2014[60]	2002-2007	Germany	1,426 (71)	NR	NR	Hepatic resection (main focus)
Ksienski, 2010[61]	2002-2004	Canada	618 (72)	NR	15.2	Hepatic resection (main focus)
Kumar, 2014[59]	2006-2012	Australia	2,502 (64)	NR	15.8	All interventions

5FU = 5-fluorouracil; 5FU-LV = 5-fluorouracil + leucovorin; CAP = capecitabine; CAPIRI = capecitabine + irinotecan; CAPOX = capecitabine + oxaliplatin; B = bevacizumab; FLIRI = 5-fluorouracil + leucovorin + irinotecan; FP = fluoropyrimidine; FOLFIRI = 5-fluorouracil + leucovorin + irinotecan; FOLFOX = 5-fluorouracil + leucovorin + oxaliplatin; FOLFOXIRI = 5-fluorouracil + leucovorin + oxaliplatin + irinotecan; FUOX = 5-fluorouracil + oxaliplatin; IRI = irinotecan; Multi = multinational, conducted in several countries; NR = not reported; OX = oxaliplatin; PT = primary tumour; UK = United Kingdom; USA = United States of America.

* Sample size as described in baseline characteristics of included study.

** Data on synchronous and metachronous mCRC and PT resection status were described in the publication of Venderbosch et al.[8]

*** Described as 'previous surgery'; no differentiation between PT resection and metastasectomy.

Table 2. Terminology and definitions of synchronous mCRC

Terminology	Definitions	Studies *N*	References
Synchronous metastases	No further definition given	18	18–22,26,27,32,39,40,43,45,47,49–52,58
	Detected by preoperative imaging or during resection of primary tumour	1	6
	Diagnosed within 3 months of primary diagnosis	1	60
	Diagnosed before, at the same time or within 3 months after colectomy	1	48
	Diagnosed within 3 months, reference point not specified	1	54
	Diagnosed within 6 months of primary diagnosis	3	7,41,53
	Diagnosed within 6 months after resection primary tumour	1	57
	Diagnosed up to 6 months after treatment of primary tumour	1	42
	Based on the existing LiverMetSurvey definition of 6 months	1	46
	Disease-free interval from primary tumour to metastases ≤ 6 months	1	44
	Diagnosed within 12 months of primary diagnosis	2	34,37
	Diagnosed within 12 months after resection primary tumour	1	62
	Stage IV at initial diagnosis	4	41,55,59,61
Other*	Stage IV at initial diagnosis, ‘synchronous’ not reported	4	8,23,29,38
	Stage at first diagnosis: metastatic	2	33,56
	Time from initial diagnosis to random assignment, < 3 months	1	24
	Time from initial diagnosis to random assignment, < 6 months	2	30,31
	Delay in diagnosis of metastasis ≤ 1 year after primary tumour resection	1	35

* Terminology ‘synchronous’ versus ‘metachronous’ metastases not reported.

Discussion

This first systematic review on trends in the proportion and survival of patients with synchronous versus metachronous metastases included in mCRC studies shows a significant increase in the proportion of patients with synchronous compared to metachronous mCRC enrolled in first-line systemic therapy trials, with a slight increase in estimated median OS of the total study population over time. The association between survival and the proportion of synchronous versus metachronous mCRC could not be analysed, since the included RCTs did not differentiate between synchronous and metachronous mCRC in median OS results. No significant trends could be observed in the included cohort or population-based studies.

Prognostic value

Synchronous metastases are considered to be of worse prognostic value compared with metachronous metastases, particularly synchronous disease without a resection of the primary tumour, as is currently being investigated in several RCTs, e.g. the phase 3 CAIRO4 study[7,8,63–65]. Another explanation for the worse prognosis of synchronous disease may be found in biological differences between metastases of synchronous and metachronous origin[5]. Only five studies in our systematic review reported median OS for synchronous and metachronous disease separately and only 26/46 (57%) studies reported the primary tumour resection status. Therefore, no firm conclusions can be drawn on the prognostic value of synchronous versus metachronous mCRC and the resection status of the primary tumour.

In addition, the prognosis of mCRC is highly dependent on whether metastases are resectable. A few studies have reported that an increasing proportion of the mCRC population is eligible for metastasectomy[1,2]. Most of the systemic therapy RCTs included in our systematic review enrolled patients with unresectable metastases only. Our results suggest an increasing amount of patients with synchronous metastases included in first-line systemic therapy RCTs with a possible selection bias of patients with unresectable metastases, both indicating a poor prognosis. This did not seem to have a detrimental effect on estimated median OS of the total study population over time, possibly partly due to the positive influence of advances in systemic therapy, including targeted agents. However, we can only hypothesise on this subject, as the heterogeneity of the included studies with different prognostic groups and the use of different treatments does not justify a clear conclusion.

Cohort and population-based studies

In cohort studies, we found no significant correlations between median year of study enrolment versus proportion of synchronous disease or estimated median OS of the total study population. However, most of the included cohort studies were small, retrospective, single-centre studies conducted in tertiary care facilities. Given these methodological limitations, our results regarding these cohort studies should be interpreted with caution. We hypothesised an increased percentage of patients with synchronous metastases over time in population-based studies, attributable to earlier detection of metastases due to improved quality of imaging techniques, as previously reported in two population-based studies[1,2,13–15]. However, due to the small number of included population-based studies, we could not perform correlation analyses for this study type.

Limitations

Given the extensive literature search with strict adherence to standards of study selection and data extraction, we believe to have selected the most relevant studies in order to answer our research question. The main limitation of this systematic review concerns the infrequent reporting of the proportion of synchronous and metachronous disease in mCRC studies, resulting in only 46 eligible studies in a literature search covering a period of more than a decade. Furthermore, cross-study comparisons were hampered by heterogeneity in study designs, types of intervention and outcomes. In addition, definitions used to describe synchronous versus metachronous metastases varied widely among the included studies. Moreover, most of the studies in which synchronous and metachronous metastases were reported, lacked description of these terms. This supports previous findings that until recently there has been no consensus on what constitutes 'synchronous' and 'metachronous' metastases[4,66–68].

Uniform definitions

Recently, a multidisciplinary expert panel used a modified Delphi method to develop recommendations for managing synchronous CRC liver metastases. The panel recommended that synchronous liver metastases should be defined as metastases detected at or before diagnosis of the primary tumour, and to make a distinction between 'early metachronous' and 'late metachronous' metastases, detected within (early) or after 12 months (late) post-surgery of the primary tumour[67]. We support the use of these uniform definitions to facilitate cross-study comparisons and to gain more insight into the prognostic value of synchronous versus metachronous mCRC.

Conclusion

In first-line systemic therapy RCTs we observed a marked, significant increase of mCRC patients with synchronous compared with metachronous metastases during the last decades. In these RCTs, the estimated median OS of the total study population slightly increased over time. Due to methodological limitations and a restricted number of included studies, our results concerning cohort and population-based studies should be interpreted with caution. Uniform definitions and consistent reporting of the proportion of synchronous and metachronous CRC metastases are essential to gain more insight into differences in clinical outcome and to enable cross-study comparisons in all types of mCRC studies.

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Supplementary Material

Supplementary Table 1. Search strategy in PubMed and EMBASE
Date of search: February 22, 2016

PubMed	
Search for all mCRC studies reporting on synchronous versus metachronous disease	
1	Synchronous [tiab] OR synchronic [tiab] OR synchronicity [tiab] OR synchronously [tiab] OR Stage IV [tiab] OR stage 4 [tiab] OR stage four [tiab]
2	Metachronous [tiab] OR metachronic [tiab] OR metachronicity [tiab]
3	#1 OR #2
4	Neoplasm Metastasis [MESH] OR neoplasm metastasis [tiab] OR Metastatic [tiab] OR metastases [tiab] OR metastasis [tiab] OR metastasize [tiab] OR metastasized [tiab] OR metatasised [tiab] OR Disease Progression [MESH] OR disease progression [tiab] OR advanced [tiab]
5	Intestine, large [MESH] OR Colorectal [tiab] OR Colon [tiab] OR colonic [tiab] OR Rectal [tiab] OR rectum [tiab]
6	Colorectal Neoplasms [MESH] OR Cancer [tiab] OR cancers [tiab] OR Neoplasms [MESH] OR neoplasm [tiab] OR neoplasia [tiab] OR neoplasms [tiab] OR Tumour [tiab] OR tumours [tiab] OR tumor [tiab] OR tumors [tiab] OR Malignant [tiab] OR malignancy [tiab] OR malignancies [tiab] OR Carcinoma [MESH] OR carcinoma [tiab] OR carcinomas [tiab]
7	#3 AND #4 AND #5 AND #6
8	Filters: NOT (“animals”[MeSH Terms] NOT “humans”[MeSH Terms]) AND English[lang] AND (“2004/01/01”[PDAT] : “2016/02/22”[PDAT])
Additional search for phase 3 trials on mCRC	
9	Phase 3 [tiab] OR phase III [tiab] OR phase three [tiab] OR Randomized Controlled Trial [tiab] OR Randomized Controlled Trials as topic [MESH] OR Clinical Trials, phase III as topic [MESH]
10	#4 AND #5 AND #6 AND #9
11	Filters: NOT (“animals”[MeSH Terms] NOT “humans”[MeSH Terms]) AND English[lang] AND (“2004/01/01”[PDAT] : “2016/02/22”[PDAT])

EMBASE	
Search for all mCRC studies reporting on synchronous versus metachronous disease	
1	Synchronous:ab,ti OR synchronic:ab,ti OR synchronicitiy:ab,ti OR synchronously:ab,ti OR 'stage IV':ab,ti OR 'stage 4':ab,ti OR 'stage four':ab,ti
2	Metachronous:ab,ti OR metachronic:ab,ti OR metachronicity:ab,ti OR metachronously:ab,ti
4	#1 OR #2
5	'metastasis'/exp OR 'neoplasm metastasis':ab,ti OR metastatic:ab,ti OR metastasis:ab,ti OR metastases:ab,ti OR metastasize:ab,ti OR metastasized:ab,ti OR metastasised:ab,ti OR advanced:ab,ti OR 'disease progression':ab,ti
6	'large intestine'/exp OR colorectal:ab,ti OR colon:ab,ti OR colonic:ab,ti OR rectal:ab,ti OR rectum:ab,ti OR rectum:ab,ti
7	'large intestine tumor'/exp OR 'malignant neoplastic disease'/exp/mj OR cancer:ab,ti OR cancers:ab,ti OR neoplasm:ab,ti OR neoplasms:ab,ti OR neoplasia:ab,ti OR tumor:ab,ti OR tumors:ab,ti OR tumour:ab,ti OR tumours:ab,ti OR malignant:ab,ti OR malignancy:ab,ti OR malignancies:ab,ti OR carcinoma:ab,ti OR carcinomas:ab,ti
8	#4 AND #5 AND #6 AND #7
9	Quick limits: Humans; Publication types: Article; Source: Embase; Date limits: Records added to Embase from 01-01-2014 to 22-02-2016; Language: English
Additional search for phase 3 trials on mCRC	
10	'phase 3 clinical trial'/exp OR 'randomized controlled trial'/exp OR 'phase 3':ab,ti OR 'phase iii':ab,ti OR 'phase three':ab,ti OR 'randomized controlled trial':ab,ti
11	#5 AND #6 AND #7 AND #10
12	Quick limits: Humans; Publication types: Article; Source: Embase; Date limits: Records added to Embase from 01-01-2014 to 22-02-2016; Language: English

Chapter 3

Reporting of patient characteristics and stratification factors in phase 3 trials investigating first-line systemic treatment of metastatic colorectal cancer: a systematic review

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Submitted

Abstract

Background

Patient characteristics and stratification factors are important factors influencing trial outcomes. Uniform reporting on these parameters would facilitate cross-study comparisons and extrapolation of trial results to clinical practice. In 2007, standardization on patient characteristics reporting and stratification in metastatic colorectal cancer (mCRC) trials was proposed. We investigated the reporting of prognostic factors and implementation of this proposal in mCRC trials published from 2005-2016.

Methods

We searched PubMed and Embase (January 2005–June 2016) for first-line phase 3 mCRC trials. Patient characteristics reporting and use of stratification factors were extracted and analyzed for adherence to the proposal from 2007.

Results

Sixty-seven trials (35,315 patients) were identified, reporting 48 different patient characteristics (median: 9 [range: 5-18] per study). Age, gender, performance status, primary tumor site and adjuvant chemotherapy were frequently reported (87-100%), in contrast to laboratory values as alkaline phosphatase, lactate dehydrogenase and white blood cell count (10-25%). We identified 29 different stratification factors (median: 3 [range: 1-9] per study). The most common strata were performance status and treatment center (>60%). A median of 8/12 (range: 4-11) of the proposed parameters was reported. Although the percentage of studies reporting each factor slightly increased over time, there was no significant correlation between publication year and adherence to the proposal from 2007.

Conclusions

We observed persistent heterogeneity in the reporting of patient characteristics and use of stratification factors in first-line mCRC trials. The proposal from 2007 has not led to increased uniformity of patient characteristics reporting and use of stratification over time. There is an urgent need to address this issue to improve interpretation of trial results.

Introduction

Randomized controlled trials are considered the gold standard for evaluating the efficacy of new treatment strategies. Patient characteristics are probably the most important factors determining trial outcomes, since many characteristics are of prognostic value. Randomization of a sufficient number of patients increases the odds of balanced distribution of potential prognostic factors. In addition, stratification can be used to balance several key prognostic factors between treatment arms, which also reduces the risk of bias in pre-planned subgroup analysis. For statistical efficiency, the number of strata are usually kept to a minimum[1,2], which requires the challenging task of identifying a minimal set of clinically relevant variables to use as stratification factors. In many cases, prognostic factors have a stronger impact on survival than any available treatment regimen. Therefore, uniform trial reporting of patient characteristics and use of stratification factors is essential to enable a valid comparison of treatment arms, to facilitate cross-study comparisons, and to evaluate whether study populations are representative of the general patient population.

Sorbye et al.[3] observed considerable heterogeneity in the reporting of patient characteristics and use of stratification factors in mCRC trials published between 2001 and 2005, indicating a lack of consensus on the importance and use of prognostic factors. The authors found that only gender, age, performance status (PS), prior adjuvant therapy, site and location of metastases were frequently reported in the trials. Other prognostic factors were often missing, particularly laboratory values. The authors proposed a standardization of patient characteristics reporting and stratification factors (Table 1). The adoption of these recommendations in mCRC trials published in more recent years has not been evaluated.

The aims of this systematic review are (1) to provide an overview of the reporting of patient characteristics and stratification factors in phase 3 mCRC trials of first-line systemic treatment published between 2005 and 2016; (2) to analyze whether standardization of reporting of patient characteristics and stratification factors as proposed by Sorbye et al.[3] has been used in trials published since 2009; and (3) to investigate the reporting of other prognostic factors that may have become relevant in the light of new treatment strategies.

Methods

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement[4].

Search strategy

We performed a systematic literature search in PubMed and Embase on June 6, 2016 to identify mCRC studies published in English between January 2005 and June 2016. The search strategy included 'metastasis', 'colorectal', 'cancer' and 'phase 3 trial' as keywords (Supplementary Table 1). After selection of eligible studies, references from selected articles were cross-searched for additional relevant studies.

Study selection

We included full-text publications of phase 3 trials investigating first-line systemic treatment (i.e. chemotherapy, targeted therapy) in adult mCRC patients. When several articles evaluated the same data set, the original trial publication was selected. Studies were excluded if they were: restricted to systemic therapy beyond first-line, prematurely closed, primarily investigating patient reported outcome measures or cost-effectiveness, investigating nutrition supplements or alternative/traditional medicine; including tumor types other than mCRC; and commentaries, editorials, letters, (systematic) reviews or meta-analyses.

Data extraction

Two independent reviewers (KG, RM) performed all steps in the screening and data extraction process. In case of disagreement, consensus was reached through discussion with a third reviewer (MK or MvO). Study characteristics, relevant eligibility criteria, reported baseline characteristics and stratification factors were extracted from the selected articles. After screening the eligibility criteria of the selected articles, eligibility criteria concerning the following items were extracted from all studies: location of metastases, measurable disease, primary tumor resection status, previous therapy for metastatic disease and molecular biomarkers.

Sorbye Adherence Score

Sorbye et al.[3] suggested a minimum set of 12 baseline characteristics to be reported in studies concerning systemic treatment of mCRC (Table 1). We established a 'Sorbye Adherence Score' by allocating one point for each factor reported, creating a variable with a range of 0-12 per included study. Assessment of PS according to the Karnofsky scale instead of ECOG/WHO was allowed.

Table 1. Suggested patient characteristics and stratification factors in studies of medical treatment of mCRC[3].

Patient characteristics	
Age	Median
Gender	
Performance status (PS)	ECOG or WHO. PS 0, 1 and 2
Site of primary tumor	Colon vs rectum
Surgery of primary tumor	
Prior adjuvant chemotherapy	
Prior radiotherapy	
Metastatic sites	1 vs >1
Location of metastases	Liver vs other
Lactate dehydrogenase (LDH)	>UNL or 1.5 UNL
Alkaline phosphatase (ALP)	>UNL
White blood cell (WBC) count	>10 x 10^9/l
Stratification factors	
Center	
Performance status	
Laboratory value	ALP or LDH
Number of metastatic sites	1 vs >1
For later line trials	
Prior chemotherapy or targeted therapy	
Feasibility of metastasectomy after systemic treatment	If applicable

Statistical analysis

Frequencies of all reported baseline characteristics, stratification factors and the Sorbye Adherence Score were extracted from the selected articles. Descriptive statistics were reported as numbers (%) and mean (SD) or median (range), where appropriate. Proportions of reported baseline characteristics and stratification factors were adjusted for relevant eligibility criteria and treatment strategies. For example, studies enrolling only patients who underwent resection of the primary tumor were excluded when determining the reported frequency of 'surgery of primary tumor'. In addition, only studies that incorporated an induction treatment period were included when determining reported frequencies of variables concerning induction treatment. We were unable to adjust the reported frequency of 'surgery of metastases' for relevant eligibility criteria, as most studies did not specify whether previous surgery of metastases was allowed. RECIST criteria recommend that trials with objective tumor response as primary endpoint enrol only patients with measurable disease[5]. As measurable disease is not considered a prognostic factor, the parameter of measurability was excluded from the analyses of baseline characteristics. We used Spearman's rank correlation coefficient (r_s) to assess the correlation between Sorbye Adherence Score and year of publication. In addition, we compared the reporting of the proposed parameters[3] in studies published until 2009 and thereafter. A two-sided *P*-value <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics, version 21.0 (Armonk, NY: IBM Corp).

Results

Literature search

Our search identified 4,273 publications, of which 254 were excluded as duplicates. Of the 4,019 remaining articles, 3,919 were excluded on the basis of title or abstract. After full-text revision of 100 articles, 67 studies met the predefined inclusion criteria, and therefore we report data from these 67 trials. Cross-referencing revealed no additional relevant studies. Results of the literature search are shown in Figure 1.

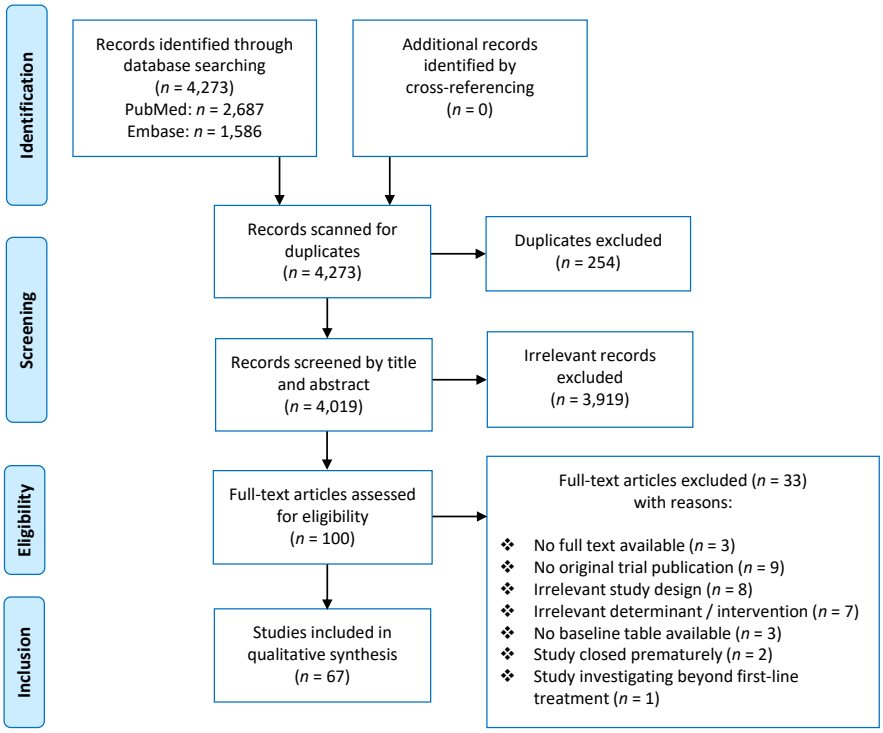

Figure 1. PRISMA flow chart of literature search.

Study and patient characteristics

Study characteristics are presented in Supplementary Table 2. Sixty-seven phase 3 trials were identified, including a total of 35,315 patients. A median of 410 (range: 120 - 2,135) patients was enrolled per study. Thirty-three studies investigated a regimen containing targeted therapy[6–37]. One study exclusively enrolled patients with *KRAS* wild-type tumors[13].

A total of 49 different baseline characteristics was identified with a median of 9 (range: 5-18) characteristics per study (Supplementary Table 3). Seven patient characteristics were reported in >50% and 18 characteristics were reported in ≥10% of the included studies

(Figure 2). Age, gender, PS, primary tumor site and prior adjuvant chemotherapy were most frequently reported (87-100%). In contrast, laboratory values ALP, LDH and WBC count were reported in less than 30% of the studies. Patient characteristics that were reported in >10% of the included studies but that were not proposed by Sorbye et al. [3] included: synchronous or metachronous metastases (*n*=23; 34%), tumor differentiation (*n*=12; 18%), ethnicity (*n*=10; 15%), carcinoembryonic antigen (CEA) (*n*=10; 15%), stage at first diagnosis (*n*=8; 12%) and time from first diagnosis to randomization (*n*=8; 12%). For 'surgery of primary tumor' we excluded one study enrolling only patients who underwent a primary tumor resection[20]. Patient characteristics reported in ≥10% of the 43 studies published since 2009 are depicted in Supplementary Figure 1.

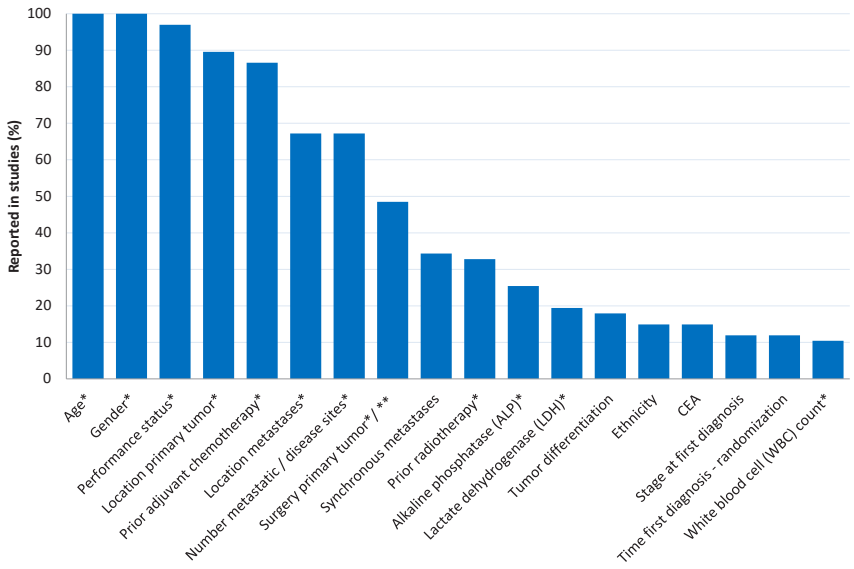

Figure 2. Patient characteristics reported in 67 phase 3 trials on first-line systemic treatment of mCRC published in 2005-2016. CEA = carcinoembryonic antigen. * Characteristics suggested by Sorbye et al.[3]. ** One study excluded enrolling only patients who underwent a primary tumor resection[20].

Sorbye Adherence Score

The reporting of patient characteristics as suggested by Sorbye et al. in the original publication[3] and in studies published from 2005-2008 compared to 2009-2016 is presented in Table 2; the percentage of studies reporting each factor slightly increased over time. Overall, the median Sorbye Adherence Score was 8 out of 12 (range: 4-11). Thirty-one (46%) of the 67 studies reported 7 or fewer of the 12 patient characteristics proposed by Sorbye et al.[36,7,11,12,14,16,17,20,23–25,27–29,35,37–52]. None of the studies reported on all 12 characteristics. There was no significant correlation between the year of publication and the Sorbye Adherence

Score, neither when all studies were included in the analysis (r_s 0.13; *P*=0.31; Supplementary Figure 3), nor when only studies published from 2009 were analyzed (r_s -0.05; *P*=0.75).

Table 2. Comparison of patient characteristics reporting proposed by Sorbye et al.[3] in trials investigating systemic treatment of mCRC published between 2001-2005, 2005-2008 and 2009-2016.

	Reported in studies		
Must be reported [3]	**2001-2005 *N*(%)***	**2005-2008 *N*(%)****	**2009-2016 *N*(%)*****
Age	140 (98)	24 (100)	43 (100)
Gender	142 (99)	24 (100)	43 (100)
Performance status	131 (92)	23 (96)	42 (98)
Site of primary tumor	108 (76)	21 (88)	39 (91)
Surgery of primary tumor	37 (26)	8 (33)	24 (57)#
Prior adjuvant chemotherapy	90 (63)	20 (83)	38 (89)
Prior radiotherapy	55 (38)	7 (29)	15 (35)
Metastatic sites	81/127 (64)§	14 (58)	31 (72)
Location of metastases	106/127 (83)§	15 (63)	30 (70)
Lactate dehydrogenase (LDH)	13 (9)	2 (8)	11 (26)
Alkaline phosphatase (ALP)	12 (8)	6 (25)	11 (26)
White blood cell (WBC) count	7 (5)	2 (8)	5 (12)

* Reported in 143 phase 2 or 3 trials concerning first-line or second-line systemic treatment of mCRC published between 2001-2005[3]. ** Reported in 24 phase 3 trials concerning first-line systemic treatment of mCRC published between 2005 and 2008. *** Reported in 43 phase 3 trials concerning first-line systemic treatment of mCRC published between 2009 and 2016. # Corrected for one study exclusively enrolling patients who underwent primary tumor resection[20]. § Studies with patients with only liver or peritoneal metastases excluded[3].

Other patient characteristics

Patient characteristics reported in <10% of the studies included: surgery of metastases, Köhne prognostic score, number of metastases, *KRAS* and *BRAF* mutation status, weight loss and laboratory values hemoglobin, albumin, platelet count and serum CA 19-9 (Supplementary Table 3). Four studies reported on *KRAS* mutation status[14,25,32,33]; one study reported on *BRAF* mutation status[25]. Measurable disease was used in eligibility criteria of 56/67 studies (84%); 8 of the remaining 11 studies reported measurable disease status in their baseline characteristics[7,24,33,42,51,53–55]. Concerning 15 studies that investigated an induction treatment regimen, the response, type and duration of induction treatment were reported in 20%-33% of the studies[9,19,22,23,25,30–32,34,37,48,54,56–58].

Stratification factors

Stratification was performed in 55/67 studies (82%). A median of 3 (range: 1-9) stratification factors was used per study. Stratification factors reported in >10% of included studies are shown in Figure 3. Strata used in >10% of the studies published from 2009 are depicted in Supplementary Figure 2. Twenty-nine different parameters were used as stratification

factors, of which PS (*n*=40; 73%) and treatment center *(n*=34; 62%) were the most common (Supplementary Table 4). Other stratification factors suggested by Sorbye et al.[3] were infrequently used, including number of metastatic or disease sites (*n*=14; 26%), ALP *(n*=11; 20%) and LDH *(n*=6; 11%). Stratification factors used in >10% of included studies that were not suggested by Sorbye et al. [3] included prior adjuvant chemotherapy (*n*=27; 49%), age (*n*=9; 16%), and location of metastases (*n*=8; 15%). More than 10% of the 13 studies that used stratification and investigated a treatment strategy containing an induction phase reported strata concerning response to induction therapy and whether oxaliplatin was given during induction treatment[19,22,25,30–32,34,37,48,54,56–58].

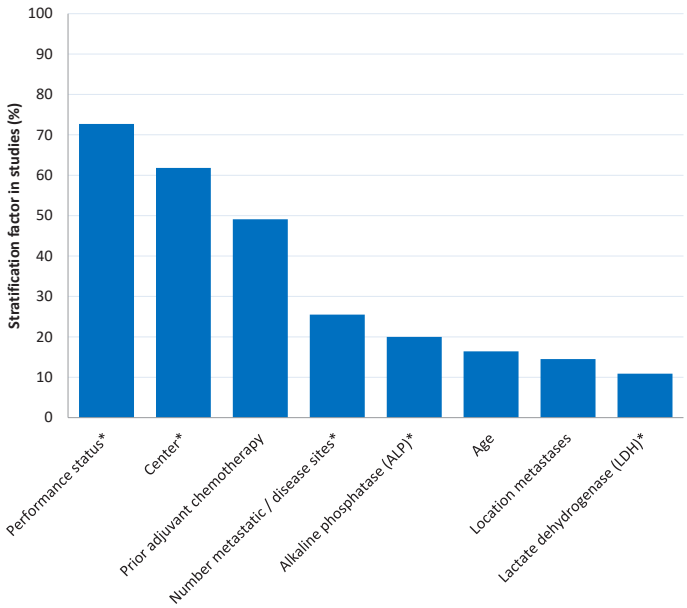

Figure 3. Stratification factors used in 55 phase 3 trials on first-line systemic treatment of mCRC published in 2005-2016. * Stratification factors suggested by Sorbye et al.[3].

Discussion

In this systematic review of 67 phase 3 trials concerning first-line treatment of mCRC published between 2005 and 2016, we observed significant heterogeneity with respect to reported patient characteristics and stratification factors. In studies published from 2005-2008 compared to 2009-2016, there was only a slight improvement in the reporting of patient characteristics as suggested by Sorbye et al. in 2007[3]. Therefore, we must conclude that the proposed standardization of these items has not been widely implemented. In addition,

novel prognostic factors that have become important due to availability of targeted drugs (e.g. *BRAF* mutation status) were not frequently reported.

We performed an extensive literature search and have selected relevant phase 3 mCRC trials published in the past ten years. We established a Sorbye Adherence Score and found that almost half of the included studies reported 7 or fewer of the 12 proposed characteristics[3]. Only age, gender, PS, primary tumor site and prior adjuvant chemotherapy were frequently reported. Laboratory values were infrequently reported, including important prognostic factors such as serum LDH, ALP and WBC count[59–61]. Since we included trials published between 2005 and 2016, some studies were initiated and/or published before the publication by Sorbye et al.[3] became available in 2007. However, if their proposal had been widely implemented, an increase in the reporting of proposed patient characteristics would have been expected in studies published after 2009. Even in these studies, there was only a slight improvement in uniformity of patient characteristics reporting as proposed in the original publication[3] compared with studies published in earlier years. In addition, we observed no correlation between the Sorbye Adherence Score and year of publication, neither when all studies were included in the analysis, nor when only studies published from 2009 were analyzed. The Sorbye Adherence Score varied considerably among different publications within a single journal (e.g. Journal of Clinical Oncology: range 4-9; Annals of Oncology: range 4-9; Lancet Oncology: range 6-10; Supplementary Table 2). Variation in reporting of patient characteristics was even observed in two reports on different arms of a single trial[56,62]. Our findings suggest that there is still no consensus on which patient characteristics should be reported in first-line mCRC trials, and that a substantial part of the observed variation could be a result of decisions made by journal editors during the publication process. The persistent heterogeneity and missing information in the reporting of patient characteristics limits cross-study comparisons, and in particular the possibility to evaluate if the trial population is a reflection of the general population, more often a worse prognostic group with inferior median OS, or a selection of a good prognostic group with better median OS[63]. The CONSORT (Consolidated Standards of Reporting Trials) statement represents an evolving guideline to improve the reporting quality of randomized controlled trials[64]. In the light of the present study, we believe that CONSORT 2010 item number 15 ('A table showing baseline demographic and clinical characteristics for each group') should be expanded to provide better guidance to both authors and journal editors on how to improve reporting of trial results.

We also observed great variation in the use of stratification factors. Of the strata suggested by Sorbye et al.[3], only treatment center and PS were frequently used. As the need for stratification and number of strata depends on the study design and, in particular, the number of included patients[2], it is not possible to define an optimal set of stratification factors. However, given the observed heterogeneity, it is unlikely that the reported

stratification factors were selected with any consistency. We recommend to define a set of minimum and evidence-based characteristics that should be considered as stratification factors.

We did not identify frequent use of novel prognostic factors, such as *(K)RAS* and *BRAF* mutation status. The prognostic role of these molecular markers in mCRC patients has only been recently established[65,66]. In particular, *BRAF* mutations are important as they are strong prognostic factors, and since patients with *BRAF*-mutant tumors are probably underrepresented in mCRC trials[67]. Admittedly, the majority of trials included in this review were designed and conducted when these biomarkers were yet unknown. With personalized treatment becoming increasingly realistic, the use of molecular biomarkers leading to a 'molecular risk profile' consisting of several biomarkers will gain more importance[68]. However, in addition to defining molecular subgroups, the importance of reporting routine clinical and pathological parameters should not be neglected, as not all patients with tumors expressing a specific molecular marker will respond to a certain targeted therapy. The implication of these routine parameters may also evolve over time. Sorbye et al. [3] initially proposed to report primary tumor site by distinguishing colon versus rectum. However, there is growing evidence that tumors arising from different sides of the colon (left versus right) have different prognosis and therapy response in mCRC patients[69,70].

Strengths of this systematic review include the comprehensive literature search and strict compliance to criteria for study selection and data extraction. Differences between our analysis and the one performed by Sorbye et al.[3] are that we only included phase 3 trials on first-line systemic treatment of mCRC, whereas Sorbye et al. included both phase 2 and 3 trials investigating first-line as well as second-line treatment of mCRC. We restricted our analysis to studies in first-line mCRC, as it is unlikely that our topic of interest will greatly differ between studies in first- and second-line treatment. In addition, the definition of second-line treatment has become complicated due to the various first-line treatment strategies (stop-and-go, maintenance treatment, reintroduction of initial treatment, etc.). We further limited our analysis to phase 3 trials since these are most relevant for guidance of treatment in general practice.

Conclusion

In conclusion, our results show that the proposal for standardization of patient characteristics and stratification factors made in 2007[3] has not led to an increase in uniformity of reporting patient characteristics and use of stratification factors. We observed large heterogeneity in the reporting of these items in first-line mCRC trials published between 2005 and 2016. There is an urgent need to reach international consensus on a standardized set of patient characteristics and, if possible, stratification factors in order to improve trial reporting, interpretation of trial results, and cross-study comparisons. In order to reach consensus, we have performed a modified Delphi survey among medical oncologists with widespread experience in conducting phase 3 mCRC trials. In a follow-up paper, we present a consensus recommendation on a minimum set of essential patient characteristics and stratification factors to include in phase 3 trials investigating systemic treatment of mCRC.

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Supplementary Material

Supplementary Table 1. Search strategy in PubMed and EMBASE
Date of search: June 6, 2016

PubMed	Syntax
#1	Disseminat* [tiab] OR Metasta* [tiab] OR Advanced [tiab] OR Disease progression [MeSH Terms] OR Neoplasm metastasis[MeSH Terms]
#2	Colorectal[tiab] OR rectal[tiab] OR rectum[tiab] OR colon[tiab] OR colonic[tiab] OR intestine, large[MeSH Terms]
#3	Neoplas*[tiab] OR cancer[tiab] OR cancers[tiab] OR tumor[tiab] OR tumors[tiab] OR tumour[tiab] OR tumours[tiab] OR carcinom*[tiab] OR malignan*[tiab] OR neoplasms[MeSH Terms]
#4	Phase III[tiab] OR phase three[tiab] OR Phase 3[tiab] OR RCT[tiab] OR Randomized controlled trial[tiab] OR clinical trial[tiab] OR Randomized controlled trial [PT] OR clinical trial[MeSH Terms]
#5	("animals"[MeSH Terms] NOT "humans"[MeSH Terms])
	#1 AND #2 AND #3 AND#4 NOT #5
FILTERS	Publication date from 2005/01/01 to 2016/06/06. Language: English.

EMBASE	Syntax
#1	disseminat*:ab,ti OR metasta*:ab,ti OR advanced:ab,ti OR 'metastasis'/exp OR 'neoplasm metastasis':ab,ti OR 'disease progression':ab,ti
#2	colorectal:ab,ti OR rectal:ab,ti OR rectum:ab,ti OR colon:ab,ti OR colonic:ab,ti OR 'large intestine':ab,ti OR 'large intestine'/exp
#3	neoplas*:ab,ti OR cancer:ab,ti OR cancers:ab,ti OR tumor:ab,ti OR tumors:ab,ti OR tumour:ab,ti OR tumours:ab,ti OR arcinoma*:ab,ti OR malignan*:ab,ti OR 'malignant neoplastic disease'/mj OR 'large intestine tumor'/exp
#4	'phase iii':ab,ti OR 'phase three':ab,ti OR 'phase 3':ab,ti OR 'rct':ab,ti OR 'randomized controlled trial':ab,ti OR 'clinical trial':ab,ti OR 'randomized controlled trial'/exp OR 'phase 3 clinical trial'/exp
#5	[embase]/lim NOT [medline]/lim
#6	[humans]/lim AND [english]/lim AND [1-1-2005]/sd NOT [6-6-2016]/sd
#7	#1 AND #2 AND #3 AND #4 AND #5 AND #6

Supplementary Table 2. Study characteristics of 67 RCTs of first-line systemic treatment of mCRC published in 2005 - 2016

Author, year	Journal	Enrolment period *Years*	Origin of data	Sample size *N**	Single-/ multi-center	Treatment strategy	SAS
Adams, 2011 [56]	Lancet Oncol	2005-2008	UK, Ireland	1,630	M	Continuous vs intermittent FOLFOX / CAPOX	8
Aparicio, 2016 [71]	Ann Oncol	2003-2010	France	282	M	LV5FU2 vs sLV5FU2 vs LV5FU2 + Iri vs FOLFIRI	9
Aranda, 2009[72]	Ann Oncol	2001-2005	Spain	346	M	FOLFIRI vs FUIRI	9
Carrato, 2013[6]	JCO	2007-2009	International	768	M	FOLFIRI + sunitinib vs placebo	6
Cassidy, 2008[38]	JCO	2003-2004	International	634	M	CAPOX vs FOLFOX4	7
Chibaudel, 2009[54]	JCO	2004-2006	France	202	M	mFOLFOX followed by 5-FU/LV vs mFOLFOX followed by CT-free interval	9
Chong, 2006[7]	Ann Oncol	1998-2001	International	422	M	5-FU/LV + 3H1 anti-idiotype monoclonal antibody vs 5-FU/ LV + placebo	4
Colucci, 2005[73]	JCO	1999-2002	Italy	360	M	FOLFIRI vs FOLFOX4	8
Comella, 2005[74]	Ann Oncol	2001-2003	Italy	276	M	Ox + HD-FA + 5-FU vs Iri + HD-FA + 5-FU	8
Comella, 2009[75]	J Cancer Res Clin Oncol	2004-2007	Italy	322	M	FOLFOX vs CAPOX	9
Correale, 2014[39]	J Immunother	2005-2010	Italy	120	M	GOLFIG vs FOLFOX4	6
Cunningham, 2009[76]	Ann Oncol	NR	International	725	M	5-FU ± LV followed by 5-FU CIV or LV5FU2 vs oxaliplatin + 5-FU ± LV followed by Ox + 5-FU CIV or FOLFOX4	9
Cunningham, 2013[8]	Lancet Oncol	2007-2010	International	280	M	CAP vs CAP-B	8
Díaz-Rubio, 2012[9]	Oncologist	2006-2008	Spain	480	M	CAPOX-B induction followed by B alone vs CAPOX	8
Díaz-Rubio, 2007[77]	JCO	2002-2004	Spain	342	M	CAPOX vs FUOX	8
Douillard, 2010[10]	JCO	2006-2008	International	1,183	M	FOLFOX4 vs FOLFOX4 + Pan	8
Ducreux, 2006[50]	Oncology	1997-2001	France	294	M	LV5FU2 vs LD-LV5FU2 vs HD-5-FU vs raltitrexed	7
Ducreux, 2011[49]	Int J Cancer	2003-2004	France	306	M	CAPOX vs FOLFOX6	5
Ducreux, 2011[78]	Lancet Oncol	2002-2006	France	410	M	Sequential (LV5FU-FOLFOX6-FOLFIRI) vs combination (FOLFOX6-FOLFIRI) therapy	9
Falcone, 2007[79]	JCO	2001-2005	Italy	244	M	FOLFOXIRI vs FOLFIRI	8
Fischer von Weikersthal, 2011[80]	EJC	2000-2004	Germany	479	M	mIROX vs FUFIRI	10
Fuchs, 2007[52]	JCO	2003-2004	International	430	M	(FOLFIRI vs mIFL vs CAPIRI) with celvoxib vs placebo	4
Gamelin, 2008[40]	JCO	NR	France	208	M	Standard 5-FU/LV vs pharmacokinetic dose-adjusted 5-FU/ LV	6
Giacchetti, 2006[41]	JCO	1998-2002	International	564	M	chronoFLO4 vs FOLFOX2	7
Glimelius, 2008[81]	Ann Oncol	2001-2004	International	567	M	Iri + Nordic bolus 5-FU/LV vs Iri + LV5FU2	9
Goldberg, 2006[42]	JCO	2001-2002	USA, Canada	305	M	rIFL vs FOLFOX4	4
Guan, 2011[11]	Chin J Cancer	2007-2008	China	203	M	mIFL + B vs mIFL	7
Hagman, 2015[30]	Ann Oncol	2010-2012	Sweden, Denmark	231	M	CAPOX/FOLFOX or CAPIRI/FOLFIRI + B induction (18 weeks), followed by B vs B + erlotinib (*KRAS* WT) or B vs metronomic CAP (*KRAS* MT)	8

Author, year	Journal	Enrolment period *Years*	Origin of data	Sample size *N**	Single-/ multi-center	Treatment strategy	SAS
Hecht, 2009[28]	JCO	2005-2006	USA	1,053	M	Ox-based CT and Iri-based CT with B / Pan regimens	6
Hecht, 2011[12]	JCO	2003-2004	International	1,168	M	FOLFOX4 + PTK/ZK vs FOLFOX4 + placebo	5
Heinemann, 2014[13]	Lancet Oncol	2007-2012	Germany, Austria	592	M	FOLFIRI + cetuximab vs FOLFIRI + B	10
Hegewisch-Becker, 2015[31]	Lancet Oncol	2009-2013	Germany	472	M	FP + Ox + B induction (24 weeks), followed by FP + B vs B vs observation	8
Hochster, 2008[29]	JCO	2002-2004	USA	373	M	mFOLFOX6 vs bFOL vs CAPOX vs all arms + B	6
Hoff, 2012[35]	JCO	2006-2010	International	860	M	FOLFOX/CAPOX + cediranib vs FOLFOX/CAPOX + placebo	6
Hong, 2012[51]	Lancet Oncol	2008-2009	South Korea	168	M	CAPOX vs S-1 + Ox	7
Hospers, 2006[43]	Ann Oncol	1999-2002	NL	302	M	5-FU/LV vs Ox + LV + 5-FU	7
Hurwitz, 2005[44]	JCO	NR	USA	313	M	IFL + placebo vs IFL + B vs 5-FU + LV + B	4
Johnsson, 2013[34]	Ann Oncol	2007-2009	Denmark, Sweden	249	M	Doublet CT + B followed by maintenance B vs maintenance B + erlotinib	8
Koeberle, 2015[37]	Ann Oncol	2007-2012	Switzerland	262	M	B continuation or not after B-based CT	5
Köhne, 2005[53]	JCO	1999-2001	International	430	M	AIO HD-FU/FA vs HD-FU/FA + Iri	8
Koopman, 2007[45]	Lancet	2003-2004	NL	820	M	Sequential CAP-Iri-CAPOX vs combination CAPIRI-CAPOX	7
Labianca, 2011[48]	Ann Oncol	2001-2005	Italy	293	M	Continuous vs intermittent FOLFIRI	6
Loupakis, 2014[25]	NEJM	2006-2012	Italy	508	M	FOLFIRI + B vs FOLFOXIRI + B induction followed by 5-FU + B maintenance	6
Luo, 2016[58]	Ann Oncol	2010-2013	China	274	M	CAPOX or FOLFOX induction (18 vs 24 weeks), followed by CAP vs observation	8
Maughan, 2011[62]	Lancet	2005-2008	UK, Ireland	1,630	M	Ox + 5-FU vs Ox + 5-FU + cetuximab vs intermittent CT	10
Nogué, 2005[82]	EJC	1997-2000	Spain	237	M	FT/LV vs 5-FU/LV	9
Passardi, 2015[14]	Ann Oncol	2007-2012	Italy	376	M	FOLFIRI / FOLFOX4 ± B	7
Pectasides, 2012[15]	BMC Cancer	2006-2008	Greece	285	M	CAPIRI + B vs FOLFIRI + B	8
Porschen, 2007[83]	JCO	2002-2004	Germany, Austria	474	M	CAPOX vs FUFOX	8
Saltz, 2012[16]	Clin Color Cancer	NR	USA	247	M	mFOLFOX6 + B vs FOLF-CB	7
Saltz, 2008[17]	JCO	2004-2005	International	1,401	M	CAPOX + placebo vs FOLFOX4 + placebo vs CAPOX-B vs FOLFOX4 + B	7
Schmoll, 2012[18]	JCO	2006-2009	International	1,614	M	Cediranib + B-matched placebo vs B + cediranib matched placebo; each combined with FOLFOX6	8
Seymour, 2007[55]	Lancet	2000-2003	UK, Cyprus	2,135	M	5-FU/LV, followed by irinotecan vs 5-FU/LV followed by combination CT vs combination CT	9
Seymour, 2011[47]	Lancet	2004-2006	UK	459	M	5-FU/LV vs Ox/5-FU vs CAP vs CAPOX	6
Simkens, 2015[19]	Lancet	2007-2012	NL	558	M	6x CAPOX-B induction (18 weeks) followed by CAP-B vs observation	8

Author, year	Journal	Enrolment period *Years*	Origin of data	Sample size *N**	Single-/ multi-center	Treatment strategy	SAS
Souglakos, 2006[84]	Br J Cancer	2000-2004	Greece	283	M	FOLFIRI vs FOLFOXIRI	8
Stathopoulos, 2010[20]	Oncology	2004-2008	Greece	222	S	FOLFIRI vs FOLFIRI + B	4
Tebbutt, 2010[21]	JCO	2005-2007	International	471	M	CAP vs CAP-B vs CAP-B + mytomycin	8
Tol, 2009[27]	NEJM	2005-2006	NL	755	M	CAPOX-B vs CAPOX-B + cetuximab	7
Tournigand, 2006[57]	JCO	2000-2002	International	620	M	FOLFOX4 until PD vs 6x FOLFOX7, maintenance without Ox, reintroduction FOLFOX7	8
Tournigand, 2015[32]	Lancet Oncol	2007-2011	International	700	M	Pre-amendment: mFOLFOX7 + B vs mCAPOX-B induction (3 months), followed by B vs B + erlotinib. Post-amendment: mFOLFOX7 + B vs mCAPOX-B induction (3 months) followed by FP + B maintenance (3 months) vs FOLFIRI + B (6 months); followed by B vs B + erlotinib	10
Tsavaris, 2007[46]	Chemotherapy	2000-2003	Greece	120	M	5-FU/LV followed by Iri vs Iri followed by 5-FU/LV	5
Tveit, 2012[22]	JCO	2005-2007	International	566	M	FLOX vs FLOX-cetuximab vs continuous cetuximab+ intermittent FLOX	9
Van Cutsem, 2009[26]	NEJM	2004-2005	International	1,198	M	FOLFIRI vs FOLFIRI-cetuximab	11
Yalcin, 2013[23]	Oncology	2008-2009	Turkey	123	M	6x CAPOX-B induction (18 weeks) followed by CAPOX-B or CAP-B	6
Yamada, 2013[24]	Lancet Oncol	2009-2011	Japan	511	M	mFOLFOX6 + B vs S-1 + Ox + B	6
Yamazaki, 2016[33]	Ann Oncol	2008-2012	Japan	395	M	FOLFIRI + B vs mFOLFOX6 + B	9

5-FU = 5-fluorouracil; 5FU/LV = 5-fluorouracil + leucovorin; B = bevacizumab; CAP = capecitabine; CAP-B = capecitabine + bevacizumab; CAPIRI = capecitabine + irinotecan; CAPOX = capecitabine + oxaliplatin; CAPOX-B = capecitabine + oxaliplatin + bevacizumab; chronoFLO4 = chronomodulated infusion of 5-fluorouracil + leucovorin + oxaliplatin; CIV = continuous intravenous infusion; CT = chemotherapy; FA = folinic acid; FLOX = 5-bolus fluorouracil + leucovorin + oxaliplatin; FOL = bolus and infusion 5-fluorouracil + leucovorin + oxaliplatin; FOLF-CB = 5-fluorouracil + leucovorin + cetuximab + bevacizumab; FOLFIRI = 5-fluorouracil + leucovorin + irinotecan; FOLFOX = 5-fluorouracil + leucovorin + oxaliplatin; FOLFOXIRI = 5-fluorouracil + leucovorin + oxaliplatin + irinotecan; FP = fluoropyrimidine; FT = oral tegafur; FUFOX = 5-fluorouracil + folinic acid + oxaliplatin; FUIRI = 5-fluorouracil + irinotecan; FUOX = 5-fluorouracil + oxaliplatin; GOLFIG = gemcitabine + oxaliplatin + 5-FU/LV + granulocyte-macrophage colony-stimulating factor + interleukin-2; HD = high-dose; IFL = irinotecan + bolus 5-fluorouracil + leucovorin; Iri = irinotecan; LD = low-dose; LV = leucovorin; LV5FU2 = leucovorin + 5-fluorouracil; M = multi-centre; mIROX = irinotecan + oxaliplatin; NA = not applicable; NL = the Netherlands; NR = not reported; Ox = oxaliplatin; Pan = panitumumab; PD = progressive disease; PTK/ZK = valatanib; S = single-centre; SAS = Sorbye Adherence Score; UK = United Kingdom; USA = United States of America. * Sample size as described in baseline characteristics of included study.

Supplementary Table 3. All reported patient characteristics in 67 phase 3 studies of first-line systemic treatment of metastatic colorectal cancer published in 2005-2016

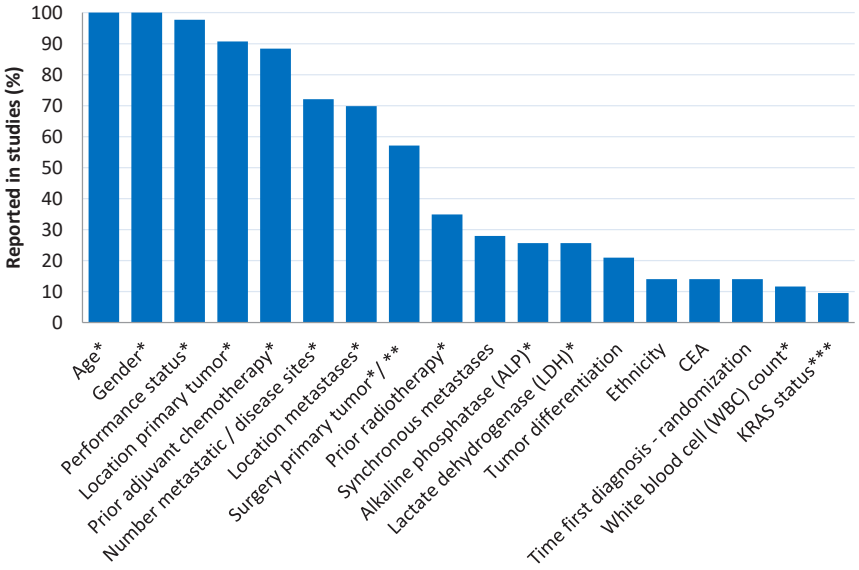
	Reported in studies	%	Usually reported
Age*	67	100	Median (range)
Gender*	67	100	
Performance status*	65	97	ECOG / WHO
Location primary tumor*	60	90	Colon vs rectum
Prior adjuvant chemotherapy*	58	87	
Location metastases*	45	67	Liver, lung
Number metastatic / disease sites*	45	67	1 vs >1 or 1 vs 2 or ≥ 3
Surgery primary tumor*/ **	32/66	49	
Synchronous metastases	23	34	
Prior radiotherapy*	22	33	
Alkaline phosphatase (ALP)*	17	25	>UNL or >300
Lactate dehydrogenase (LDH)*	13	19	>UNL
Tumor differentiation	12	18	Well / moderate / poor / undifferentiated
Ethnicity	10	15	
CEA	10	15	>UNL or >5 or >10 or >100
Stage at first diagnosis	8	12	Stage I-IV or local regional vs metastatic
Time first diagnosis - randomization	8	12	
White blood cell (WBC) count*	7	10	>10 x 10^9/l or >8,000 cells/ml
Primary tumor local recurrence	6	9	
Surgery metastases	5	8	
Köhne prognostic score	5	8	Low / intermediate / high risk
KRAS mutation status	4	6	
Center location	3	5	
Symptoms disease	3	5	
CA 19.9	2	3	
Hemoglobin	2	3	
Number metastases	2	3	
Liver involvement	2	3	
Time diagnosis mCRC - randomization	2	3	
Time adjuvant therapy - randomization	2	3	
History disease comorbidity	2	3	
Weight loss	2	3	>5%
Weight	1	2	
BRAF mutation status	1	2	
Platelet count	1	2	
Neutrophil count	1	2	
Albumin	1	2	
Liver covariate	1	2	
Previous dose modifications	1	2	
Time primary tumor - metastases	1	2	
Concomitant medication	1	2	
Frailty index	1	2	
Reason trial participation	1	2	
Number of assessable patients	1	2	
Stratification groups	1	2	
Response to induction treatment ***	5/15	33	CR/PR vs SD
Regimen induction treatment ***	4/15	27	
Duration induction treatment ***	3/15	20	

CEA = carcinoembryonic antigen; mCRC = metastatic colorectal cancer; ULN = upper normal limit. *Characteristics suggested by Sorbye et al.[3] ** One study excluded enrolling only patients who underwent a primary tumor resection[20]. *** Only 15 studies included investigating a regimen containing induction treatment [9,19,22,23,25,30–32,34,37,48,54,56–58].

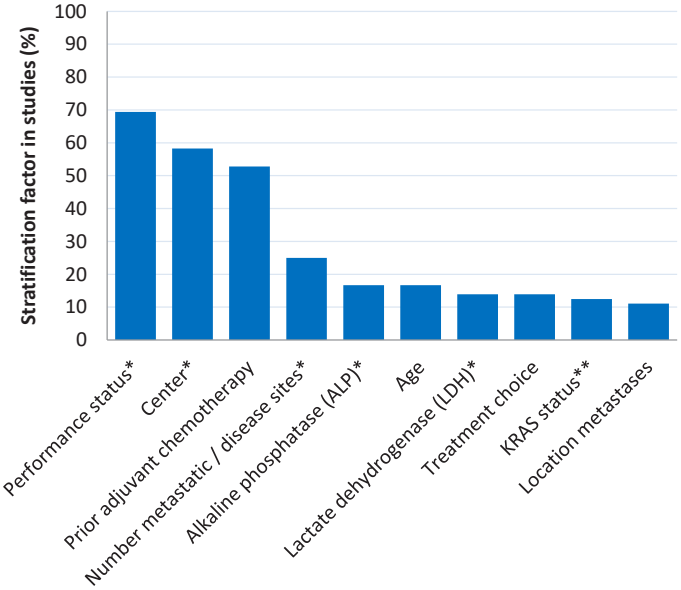
Supplementary Table 4. All stratification factors used in 67 phase 3 studies of first-line systemic treatment of metastatic colorectal cancer published in 2005-2016

	Reported in studies	%
Performance status*	40	73
Center location*	34	62
Prior adjuvant chemotherapy	27	49
Number metastatic/disease sites*	14	26
Alkaline phosphatase (ALP)*	11	20
Age	9	16
Location metastases	8	15
Lactate dehydrogenase (LDH)*	6	11
Treatment choice	5	9
Location primary tumor	4	7
KRAS status**	3	6
Clinician	2	4
White blood cell (WBC) count	2	4
Surgery primary tumor	2	4
Chemotherapy dose	2	4
Prognostic score	2	4
Gender	1	2
Prior immunotherapy	1	2
Prior oxaliplatin	1	2
Extent liver involvement	1	2
Tumor size	1	2
Liver covariate	1	2
Albumin	1	2
Concomitant medication	1	2
Metastases present	1	2
Measurable lesion	1	2
Response to induction treatment ***	7/13	54
Oxaliplatin during induction treatment ***	2/13	15
Duration induction treatment ***	1/13	8

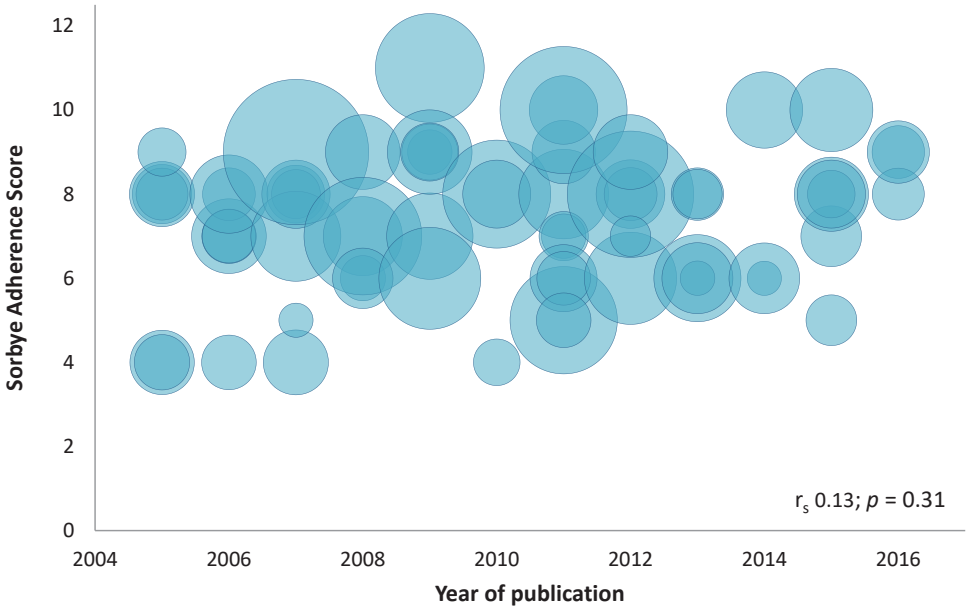
* Characteristics suggested by Sorbye et al.[3] ** One study excluded enrolling only patients who underwent a primary tumor resection[20]. ** One study excluded enrolling only patients with *KRAS* wild-type tumors [13]. **** Only 13 studies included that investigated a regimen containing induction treatment and used stratification [19,22,25,30–32,34,37,48,54,56–58].



Supplementary Figure 1. Patient characteristics reported in 43 phase 3 studies of first-line systemic treatment of metastatic colorectal cancer published in 2009-2016. CEA = carcinoembryonic antigen. * Characteristics suggested by Sorbye et al. [3]** One study excluded enrolling only patients who underwent a primary tumor resection[20]. *** One study excluded enrolling only patients with *KRAS* wild-type status[13].



Supplementary Figure 2. Stratification factors used in 36 phase 3 studies of first-line systemic treatment of metastatic colorectal cancer published in 2009-2016. * Stratification factors suggested by Sorbye et al.[3]. ** One study excluded enrolling only patients with *KRAS* wild-type status[13].



Supplementary Figure 3. Sorbye Adherence Score plotted against year of study publication in 67 phase 3 studies of first-line systemic treatment of metastatic colorectal cancer published in 2005-2016. Dot size represents sample size.

Chapter 4

Consensus statement on essential patient characteristics in systemic treatment trials for metastatic colorectal cancer: supported by the ARCAD Group

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Submitted

Abstract

Background

Patient characteristics and stratification factors are key features influencing trial outcomes. However, there is substantial heterogeneity in reporting of patient characteristics and use of stratification factors in phase 3 trials investigating systemic treatment of metastatic colorectal cancer (mCRC). We aimed to develop a minimum set of essential baseline characteristics and stratification factors to include in such trials.

Methods

We performed a modified, two-round Delphi survey among international experts with wide experience in the conduct and methodology of phase 3 trials of systemic treatment of mCRC.

Results

Thirty mCRC experts from 15 different countries completed both consensus rounds. A total of 14 patient characteristics were included in the recommended set: age, performance status, primary tumor location, primary tumor resection, prior chemotherapy, number of metastatic sites, liver-only disease, liver involvement, surgical resection of metastases, synchronous versus metachronous metastases, *(K)RAS* and *BRAF* mutation status, MSI/MMR status, and number of prior treatment lines. A total of 5 patient characteristics were considered the most relevant stratification factors: *RAS*/*BRAF* mutation status, performance status, primary tumor sidedness, and liver-only disease.

Conclusions

This survey provides a minimum set of essential baseline patient characteristics and stratification factors to include in phase 3 trials of systemic treatment of mCRC. Inclusion of these patient characteristics and strata in study protocols and final study reports will improve interpretation of trial results and facilitate cross-study comparisons.

Introduction

Metastatic colorectal cancer (mCRC) is a heterogeneous disease, with patients experiencing varying prognosis and treatment response. Trials investigating systemic treatment of mCRC often demonstrate heterogeneity in response and survival outcomes, which could partly be explained by differences in prognostic factors. However, there is no consensus on which patient characteristics should be reported as baseline characteristics, and what stratification factors should be used to balance key prognostic factors between treatment arms. This complicates cross-study comparisons and extrapolation of trial results to the general patient population. We have previously addressed a comparable situation in early-stage colon cancer, which has resulted in clear recommendations on endpoint definitions for phase 3 adjuvant trials[1].

Following a proposal made in 2007 on standardization of patient characteristic reporting and stratification in trials investigating systemic treatment of mCRC[2], we performed a systematic review to investigate the implementation of this proposal and reporting of prognostic factors in phase 3 trials of first-line treatment of mCRC published between 2005 and 2016 (Chapter 3). In this systematic review, including more than 35,000 mCRC patients from 67 phase 3 trials, we observed persistent heterogeneity in the reporting of patient characteristics and use of stratification factors. Apparently, the proposal made in 2007 has not resulted in uniform reporting of patient characteristics and use of stratification factors over time. In addition, novel prognostic factors that have become relevant in the light of new targeted agents were infrequently reported.

There is an urgent need for an international consensus on reporting of patient characteristics and stratification in mCRC trials. Although standardization of stratification factors in mCRC trials is difficult to establish due to different study designs, reaching consensus on a standardized set of baseline characteristics would greatly improve trial reporting, interpretation of results, and future meta-analyses. The Delphi method is the preferred technique to systematically obtain expert opinions for this purpose[3,4]. In a Delphi survey, experts are asked for their opinion on a specific issue, and repeatedly polled with controlled feedback regarding the polled opinions in order to encourage consensus between the experts[5].

With the use of a two-round Delphi survey, we aimed to (1) reach consensus on a minimum set of essential patient characteristics to include in the study protocol and to report as baseline characteristics in final reports of phase 3 trials investigating systemic treatment of mCRC, and (2) to present a minimum set of prognostic factors that are currently considered the most important stratification factors in these trials.

Methods

Participants

We performed a two-round Delphi survey among international experts with experience in the conduct and methodology of phase 3 trials of systemic treatment of mCRC. Eligible experts were identified from the member list of the Aide et Recherche en Cancérologie Digestive (ARCAD) working group[6], and received an electronic invitation to participate in the survey.

Selection of patient characteristics

To determine a preliminary list of patient characteristics, we retrieved all baseline characteristics reported in 67 phase 3 mCRC trials published between January 2005 and June 2016 that were included in a systematic review, of which results are presented in a companion paper (Chapter 3). Reported baseline characteristics were grouped by members of the study team (KG, MK). Overlapping baseline characteristics and variables that were deemed to be too rare or specific were excluded. In addition, novel prognostic factors that have potentially become relevant during recent years were added to the list.

Consensus rounds

The consensus procedure consisted of a modified Delphi survey of two rounds (Figure 1), resulting in a recommended and suggested set of baseline characteristics. The survey was done online on a secure survey website. Non-responders received up to three reminders.

In round 1, a preliminary list of patient characteristics was presented to the experts. Experts were asked to rate the importance (i.e. not/moderately/very important) of reporting each variable as a baseline characteristic in final reports of phase 3 trials of systemic treatment of mCRC. They could vote for as many patient characteristics as desired. Experts were asked to give their preferred definition of 'primary tumor location' and 'synchronous versus metachronous metastases' if they considered these variables 'very important'. Furthermore, they could suggest baseline characteristics that were not already mentioned in the list. Finally, experts were asked to provide a maximum of four prognostic factors that they considered the most relevant stratification factors in phase 3 trials of systemic treatment of mCRC.

Following round 1, variables rated as 'very important' by ≥67% of the experts were included in the recommended set. Variables rated as 'not important' by ≥50% of the experts were excluded. Remaining variables were presented in round 2. Additional patient characteristics mentioned during round 1 were evaluated by the study team, grouped if possible, and presented in round 2. Preferred definitions of 'primary tumor location' and 'synchronous versus metachronous mCRC' were evaluated. Definitions with most votes plus

additional definitions suggested by experts were entered in round 2. Prognostic factors that were reported as most relevant stratification factors were summarized and presented.

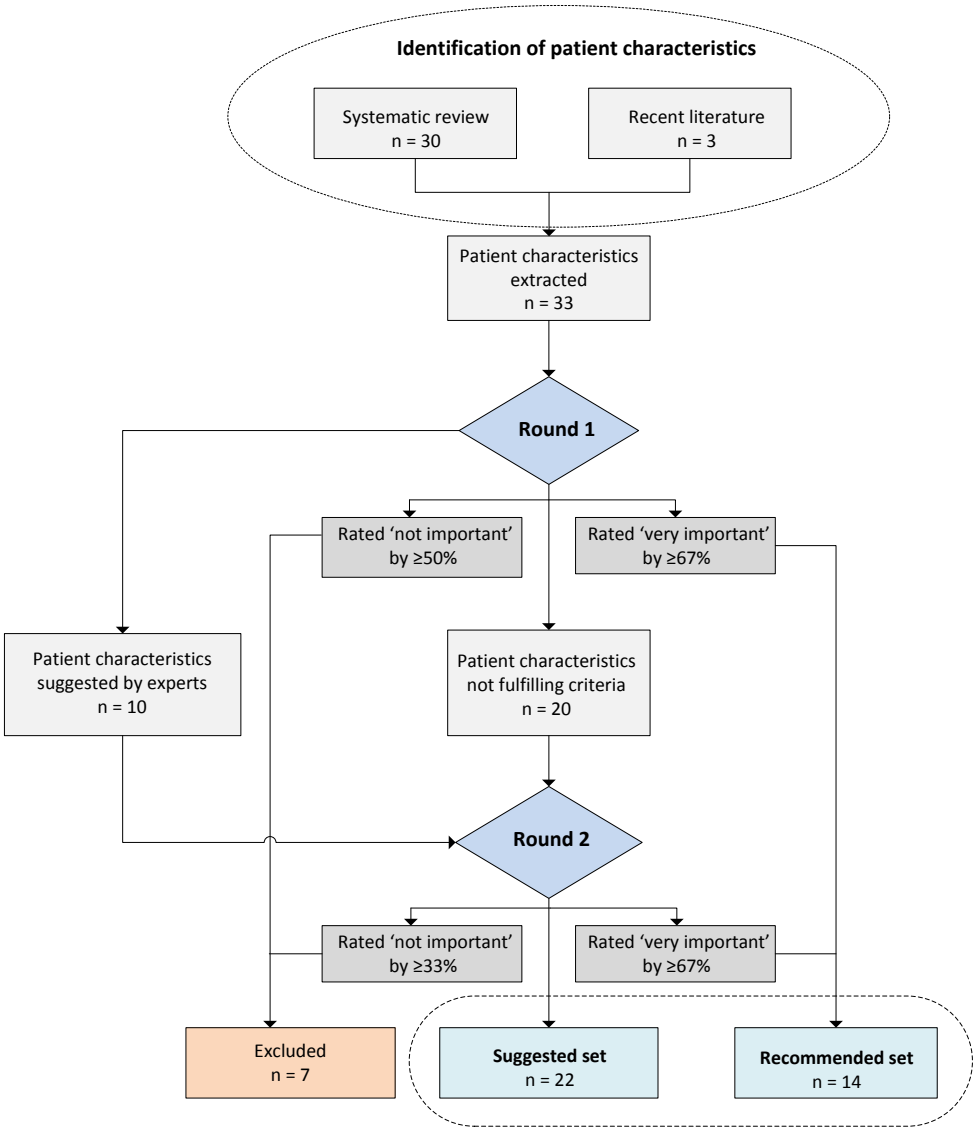

Figure 1. Flow chart of consensus procedure

Second round forms were sent to all responders of the first round, accompanied by feedback on results of round 1. The second round consisted of the same list of baseline characteristics as round 1, except those rated as 'very important' by ≥67% or 'not important'

by ≥50% of the experts, plus additional characteristics suggested in round 1. Procedures in round 1 and 2 were comparable. In round 2, all experts were asked for their preferred subdivision of 'primary tumor location' and 'synchronous versus metachronous metastases'. Prognostic factors that were voted the most relevant stratification factors during round 1 were presented. In addition to the three prognostic factors that received the highest number of votes in round 1, experts were asked to choose a maximum of three prognostic factors that they considered relevant to include as stratification factors in phase 3 trials of systemic treatment of mCRC.

After round 2, variables rated as 'very important' by ≥67% of the experts were included in the recommended set. Remaining variables were incorporated in the suggested set, unless a variable was rated as 'not important' by ≥33% of the experts. Variables not fulfilling the criteria to be included in either the recommended or suggested set were excluded. Preferred definitions of 'primary tumor location' and 'synchronous versus metachronous metastases' were compared with results from round 1. Prognostic factors that were reported as most relevant stratification factors were summarized and compared with results from round 1.

Definition of recommended and suggested set

Patient characteristics rated as 'very important' by ≥67% of the experts in round 1 or 2 were incorporated in the recommended set. The suggested set was assembled after round 2, and included all patient characteristics that were not incorporated in the recommended set, unless a variable was rated as 'not important' by ≥33% of the experts.

Stratification factors

Following both consensus rounds, we assembled an overview of prognostic factors that are currently considered the most important stratification factors in phase 3 trials of systemic treatment of mCRC. Based on these results, we provided a minimum set of stratification factors to include in systemic treatment trials for mCRC.

Statistical analysis

Data were analyzed using Excel and SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as absolute numbers (%).

Results

Participants

Sixty-two experts were contacted, of whom 29 medical oncologists and 1 statistician from 15 different countries participated in both consensus rounds. All participants had known expertise in the field of mCRC based upon experience in designing and conducting mCRC trials, publications, and national/international committee leadership.

Selection of patient characteristics

In round 1, 33 patient characteristics were presented to the experts, subdivided into different categories: demographics; disease characteristics (primary tumor and metastasis); prior treatment; laboratory testing and biomarkers; and disease symptoms. In round 2, 29 patient characteristics were presented to the experts, subdivided into the same categories as in round 1, plus an additional category concerning specific baseline characteristics for later line trials. In both rounds, patient characteristics were formulated with examples in parentheses.

Consensus rounds

During round 1, 13 characteristics were rated as 'very important' by ≥67% of the experts and were directly included in the recommended set (Table 1; Figure 2). One characteristic was rated as 'not important' by ≥50% of the experts and was therefore excluded. The remaining characteristics plus eleven additional characteristics suggested by the experts were entered in round 2. After round 2, one additional characteristic was rated as 'very important' by ≥67% of the experts and was included in the recommended set (Table 1; Figure 3). Six characteristics were excluded from further analysis, as they were rated 'not important' by ≥33% of the experts. The remaining 22 characteristics were added to the suggested set (Table 2).

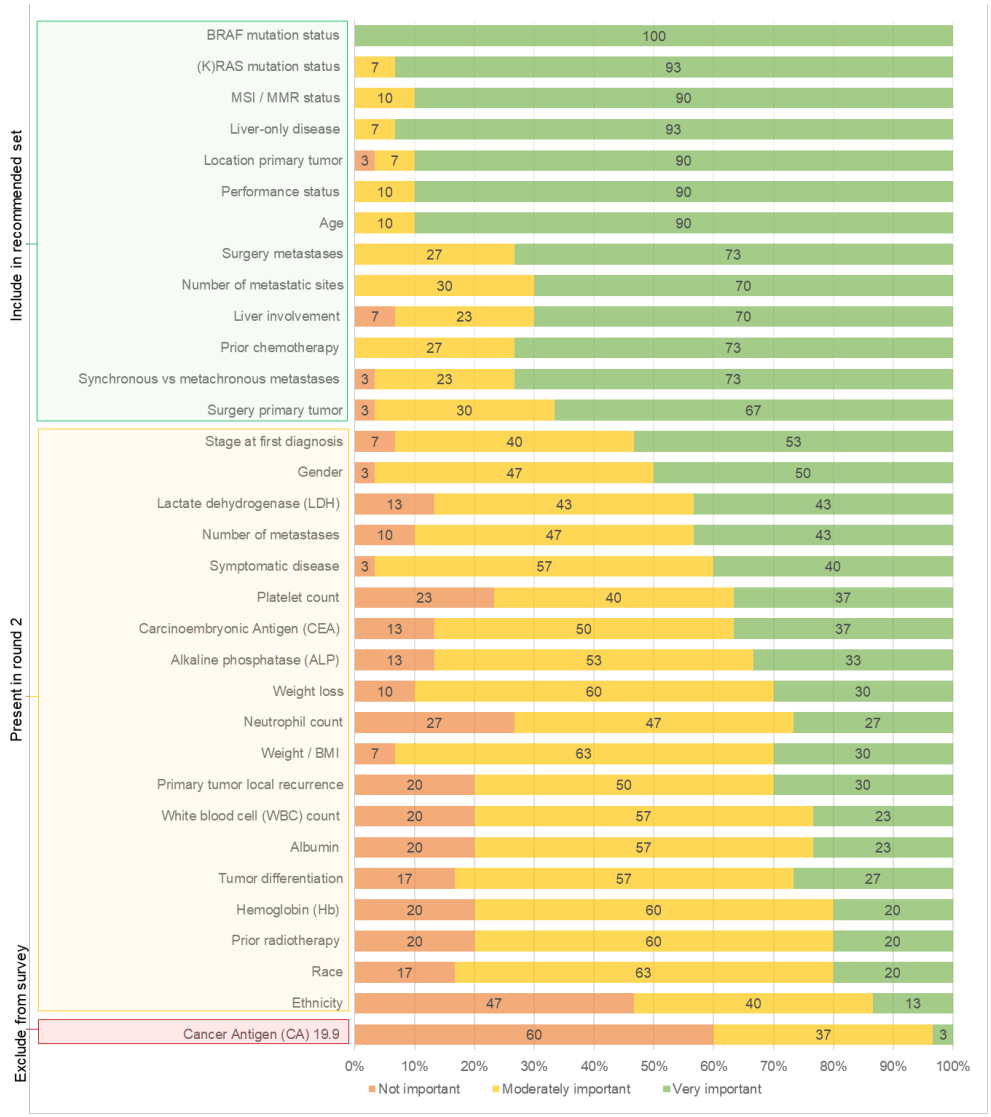

Figure 2. Baseline characteristics – Consensus round 1.

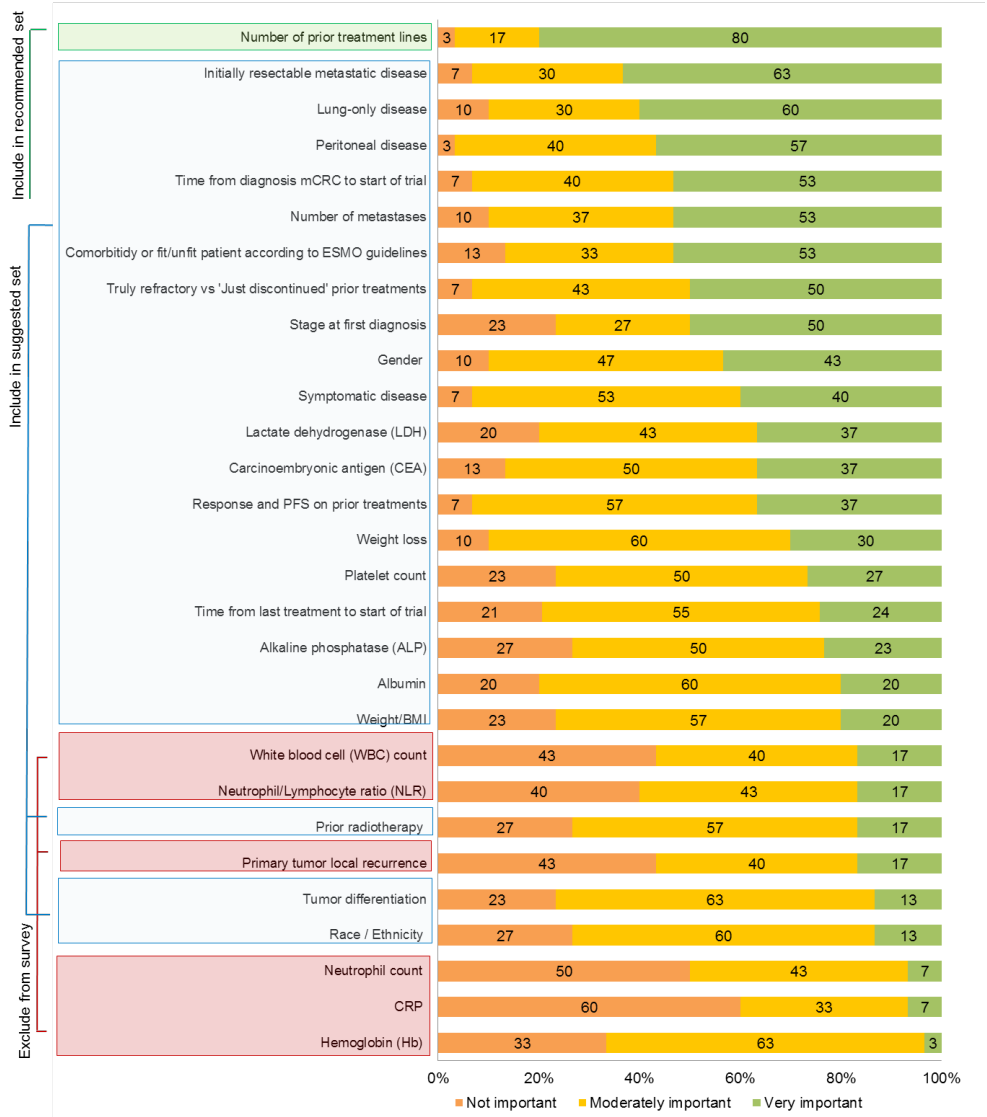

Figure 3. Baseline characteristics – Consensus round 2.

Final consensus statement

A total of 14 patient characteristics were included in the essential, recommended set of baseline characteristics to include in the study protocols and final reports of phase 3 trials investigating systemic treatment of mCRC (Table 1). Twenty-two patient characteristics were incorporated in the suggested set, as their possible clinical value was not ruled out by the experts (Table 2). For both sets, recommendations were made on how to report a specific item.

Preferred definitions

In round 1, 29 (97%) experts gave their preferred definition of 'primary tumor location'. All experts gave their preferred subdivision of primary tumor location in round 2. Following both rounds, the definition with the highest number of votes was: right colon (cecum up to and including transverse colon) versus left colon (splenic flexure up to and including sigmoid) versus rectum (rectosigmoid and rectum)(Supplementary Figure 1).

Table 1. Recommended set of baseline characteristics to report in phase 3 trials on systemic treatment of mCRC

Recommended set	
Age	Median (range); <70 vs ≥70 years
Performance status	ECOG / WHO, 0 vs 1-2
Location primary tumor	Right colon vs Left colon vs Rectum*
Surgery primary tumor	Yes or No
Prior chemotherapy	Yes or No
Number of metastatic sites	1 vs >1 (primary tumor excluded)
Liver-only disease	Yes or No
Liver involvement	Yes or No
Surgery metastases	Yes or No
Synchronous versus metachronous metastases	**
(K)RAS mutation status	Wild-type or Mutant
BRAF mutation status	Wild-type or Mutant
MSI / MMR status	MSI or MSS; dMMR or pMMR
For later line trials	
Number of prior treatment lines	1, 2, >2

The recommended set should be regarded as a minimum set of essential characteristics to include in the study protocol and baseline table of final reports of phase 3 mCRC trials. * Preferred definitions of primary tumor location: right colon (cecum up to and including transverse colon), left colon (splenic flexure up to and including sigmoid), rectum (rectosigmoid to rectum). ** Preferred definitions of synchronous vs metachronous metastases are depicted in Supplementary Figure 2. MSI = microsatellite instability. MSS = microsatellite stable. MMR = mismatch repair. dMMR = deficient MMR. pMMR = proficient MMR

Table 2. Suggested set of baseline characteristics to report in phase 3 trials on systemic treatment of mCRC

Suggested set	
Gender	Male or Female
Race / Ethnicity	Race: e.g. White, Black, Asian, Other; Ethnicity: e.g. Hispanic, Not Hispanic
Prior radiotherapy	Yes or No
Stage at first diagnosis	I-III vs IV
Tumor differentiation	Well vs Moderate vs Poor vs Undifferentiated
Lactate dehydrogenase (LDH)	Normal vs > UNL
Alkaline phosphatase (ALP)	Normal vs > UNL
Carcinoembryonic antigen (CEA)	Normal vs > UNL
Albumin	< LLN vs Normal
Platelet count	<400 vs ≥ 400 x 10^9/L
Initially resectable metastatic disease	Yes or No
Lung-only disease	Yes or No
Peritoneal disease	Yes or No
Number of metastases	1 vs >1
Comorbidity or Fit vs Unfit patient	According to ESMO guidelines
Weight / BMI	Underweight (BMI<18·5 kg/m^2); Normal (18·5-24·9 kg/m^2), Overweight (25-29·9 kg/m2), Obese (≥30 kg/m2)
Weight loss	>5% or >10% during last 3 or 6 months
Symptomatic disease	Yes or No
For later line trials	
Truly refractory vs 'Just discontinued' prior treatments	
Time from diagnosis mCRC to start of treatment	< or ≥18 months
Response and PFS on prior treatments	best response to prior treatment: CR/PR, SD, PD; PFS: median, in months
Time from last treatment to start of trial	in months

CR/PR = complete or partial response. LLN = lower limit of normal. PD = progressive disease. PFS = progression-free survival. UNL = upper normal limit.

In round 1, twenty-two (73%) experts gave their preferred definition of 'synchronous versus metachronous metastases'. All experts gave their preferred subdivision of 'synchronous versus metachronous metastases' in round 2. Definitions with the highest number of votes were: synchronous (diagnosed ≤6 months following CRC diagnosis) versus metachronous (diagnosed >6 months following CRC diagnosis); and synchronous (diagnosed before or at time of CRC diagnosis) versus early metachronous (≤0-12 months following CRC diagnosis) versus late metachronous (>12 months following CRC diagnosis)(Supplementary Figure 2).

Stratification factors

In round 1, prognostic factors that were considered the most important stratification factors in phase 3 trials on systemic treatment of mCRC were: *RAS/BRAF* mutation status, performance status and primary tumor sidedness. Liver-only disease received the highest number of votes in round 2 (Figure 4).

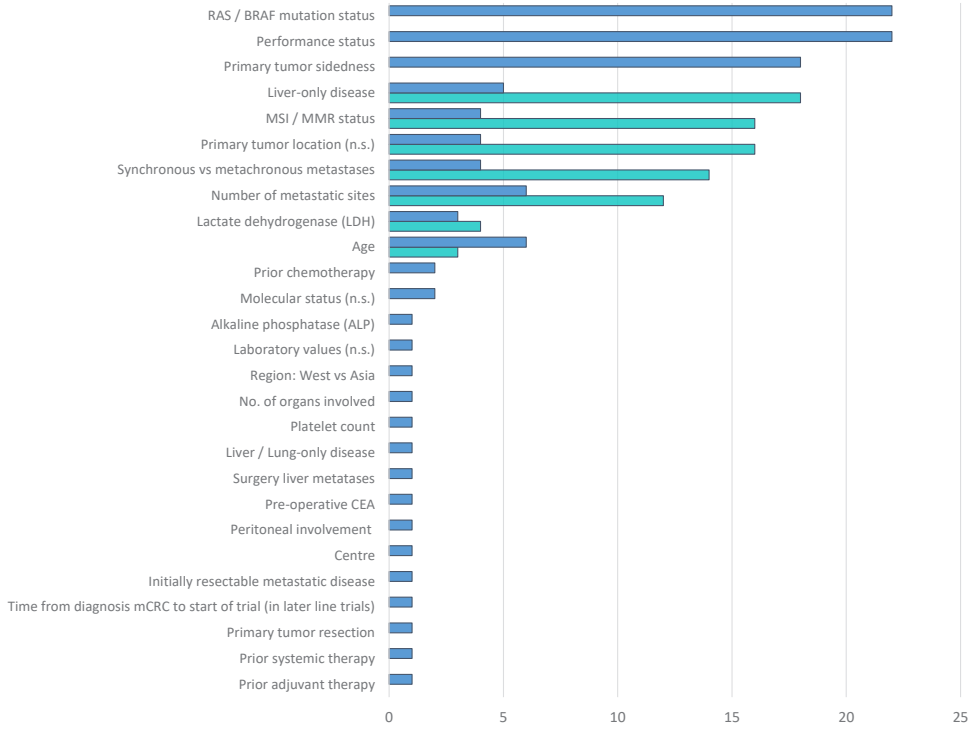

Figure 4. Stratification factors – Consensus round 1 (blue) and 2 (green).

Discussion

This study provides a recommended and suggested set of patient characteristics to include in the study protocol and baseline table of final reports of phase 3 trials investigating systemic treatment of mCRC. Following a systematic review including more than 35,000 mCRC patients from 67 phase 3 trials (Chapter 3), we performed a two-round Delphi survey to develop a consensus recommendation based on the opinions of 30 international experts in the field of mCRC. Furthermore, we present a minimum set of prognostic factors that are currently considered the most important stratification factors in phase 3 trials on systemic treatment of mCRC.

Patient characteristics

The recommended set includes 14 patient characteristics (Table 1) that should be regarded as a minimum set of essential characteristics to include in the study protocol and baseline table of final reports of phase 3 mCRC trials. The suggested set consists of 22 patient characteristics, of which a selection may be considered for inclusion in the baseline table (Table 2). For both sets, recommendations were made on how to report these characteristics. Clearly, the final set of baseline characteristics will depend on the study objectives, eligibility criteria, treatment line and drugs evaluated.

Based on a literature review, Sorbye et al. made a proposal in 2007 on standardization of patient characteristic reporting and stratification in systemic treatment trials for mCRC[2]. Overall, there was high concordance between patient characteristics included in our recommended set and their proposal[2]. An important difference was that none of the laboratory values suggested by Sorbye et al. fulfilled the criteria to be included in our recommended set. Laboratory values were only included in the suggested set (e.g. LDH, ALP), or even excluded from both sets (e.g. WBC count, Hb). Laboratory values were also infrequently reported in mCRC trials studied in our systematic review (Chapter 3). Although several studies have reported the importance of abnormal laboratory values as prognostic factors in mCRC[7,8], our findings confirm that general acceptance of their prognostic value has not been reached. Furthermore, gender was not included in our recommended set, though all trials included in our systematic review (Chapter 3) reported this item in the baseline characteristics. A possible explanation could be that although gender differences influence CRC incidence and gene expression patterns[9], its independent prognostic significance in mCRC is unclear and most likely reflects biological differences which are now increasingly understood as noted below.

Molecular and genetic testing has become increasingly important to define different subtypes of mCRC[10]. Since the prognostic value of *RAS* and *BRAF* mutation status and MSI/MMR status has only been established in recent years[11–14], it seems logical that these prognostic factors were not yet incorporated in the proposal made in 2007[2], but will now be included. It is likely that in upcoming years, other molecular or genetic markers will be identified to complement the established prognostic factors. Nonetheless, 'classic' clinical and pathological characteristics currently cannot be disregarded, since biomarkers or gene expression profiles with high predictive specificity are not yet available.

Preferred definitions

There is increasing evidence that in mCRC, tumors arising from different sides of the colon (left versus right) have different prognosis and response to anti-EGFR therapy[15,16]. Almost all experts acknowledged the importance of using primary tumor location or sidedness as a baseline characteristic and/or stratification factor in mCRC trials. The preferred subdivision

of primary tumor location was: right colon (cecum up to and including transverse colon) versus left colon (splenic flexure up to and including sigmoid) versus rectum (rectosigmoid and rectum)(Supplementary Figure 1). We recommend to use this definition in the baseline table of future mCRC trials to improve cross-study comparisons. Furthermore, it has been hypothesized that differences between right-sided and left-sided tumors arise from a non-random distribution of molecular characteristics that change gradually along the length of the colorectum[17]. Until these underlying mechanisms have been clarified, we advise to specify the exact primary tumor location (i.e. anatomical segment of the colorectum) in the Case Report Forms (CRFs) of mCRC trials, which will facilitate future meta-analyses.

Although most experts acknowledged the importance of reporting synchronous versus metachronous metastases as baseline characteristics, there was no consensus on the preferred definition. This is in line with a recent systematic review which showed that many different definitions of synchronous disease were used in mCRC studies[18]. Following our survey, two definitions received the highest number of votes (Supplementary Figure 2). We recommend to use one of these definitions in future mCRC trials to gain more insight into differences in clinical outcome in patients with synchronous and metachronous mCRC. Until consensus is reached, collecting the root elements (i.e. date of initial diagnosis CRC, date of first distant metastasis) in the CRFs of mCRC trials will be helpful in deriving synchronous versus metachronous disease with different definitions.

Stratification factors

Following our survey, prognostic factors that are currently considered the most relevant strata in mCRC trials are WHO performance status, *RAS/BRAF* mutation status, primary tumor sidedness, and liver-only disease. Performance status was the only prognostic factor that was also suggested in 2007[2]; the prognostic value of *RAS/BRAF* mutation status and primary tumor sidedness has only recently been established[11–13,15,16], and more local ablative treatment options have become available for patients with liver-only disease[19]. We recommend this minimum set of stratification factors in future mCRC trials investigating systemic treatment. Since the number and type of strata is dependent on multiple factors, including treatment line and drugs evaluated, this set can be adjusted and/or supplemented with one or more trial-specific strata.

Limitations and strengths

This study has some limitations. The systematic review used to compile a list of patient characteristics to present in round 1 included first-line mCRC trials published between 2005 and 2016 (Chapter 3). Therefore, recently identified prognostic factors could have been missed. However, novel prognostic factors were added to the list by members of the study team, and experts' suggestions were presented in round 2. Furthermore, our

Delphi survey consisted of two rounds. It is possible that characteristics almost fulfilling the criteria to be included in the recommended set after two rounds would have made it after a third round. However, there are no guidelines for Delphi surveys regarding the number of rounds to be performed. Likewise, consensus criteria for the recommended and suggested set were not based on validated guidelines, since these are non-existent, but on considerations of the study team to create manageable sets of baseline characteristics. Due to the large heterogeneity in study reporting in current mCRC trials, we were not able to assess the level of evidence of the prognostic value of all recommended characteristics. Our recommendation may facilitate the standardization of data collection and reporting of mCRC trials across all lines of treatment. This will provide better evidence as to how the actual prognostication works out in each treatment setting. Therefore, implementation of our recommendation in future mCRC trials will enable evaluation of whether this minimum set of recommended patient characteristics fulfils its intended purpose.

A strength of our consensus procedure is that it is based on both literature evidence and expert opinions. Thirty international experts with experience in conducting phase 3 mCRC trials participated in this survey. All were members of the ARCAD Group, a worldwide collaboration of clinicians, statisticians and scientists specializing in gastrointestinal cancer, whose ultimate goal is to develop more efficient clinical trials[6]. Another strength is that each expert voted independently, which encourages an honest opinion based on their clinical expertise in conducting mCRC trials.

Conclusion

This is the first consensus recommendation among international mCRC experts on essential patient characteristics and stratification factors in phase 3 trials investigating systemic treatment of mCRC. In future mCRC trials, inclusion of this minimum set of essential baseline characteristics and strata in study protocols and final study reports will greatly improve trial reporting, interpretation, and cross-study comparisons.

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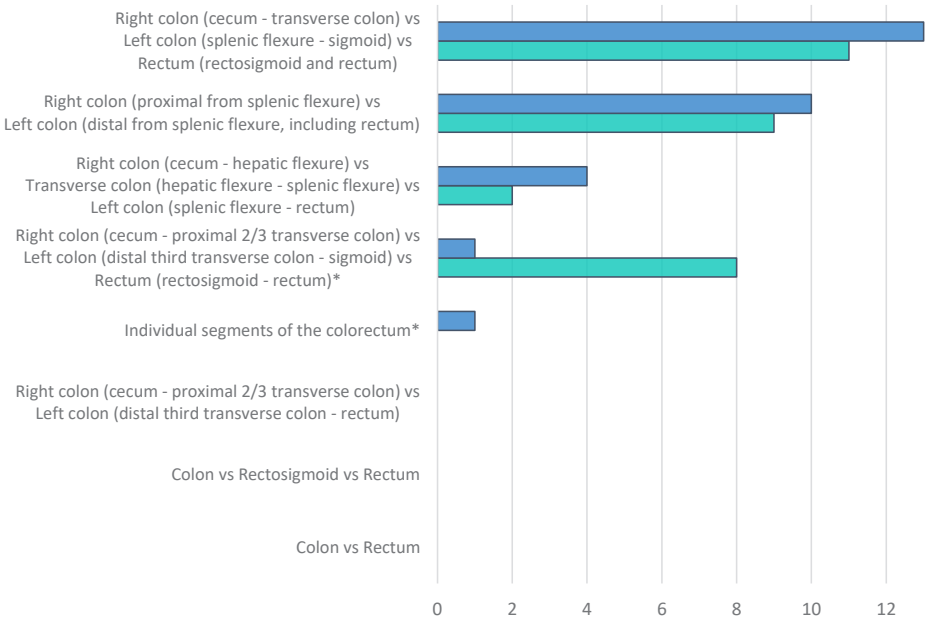
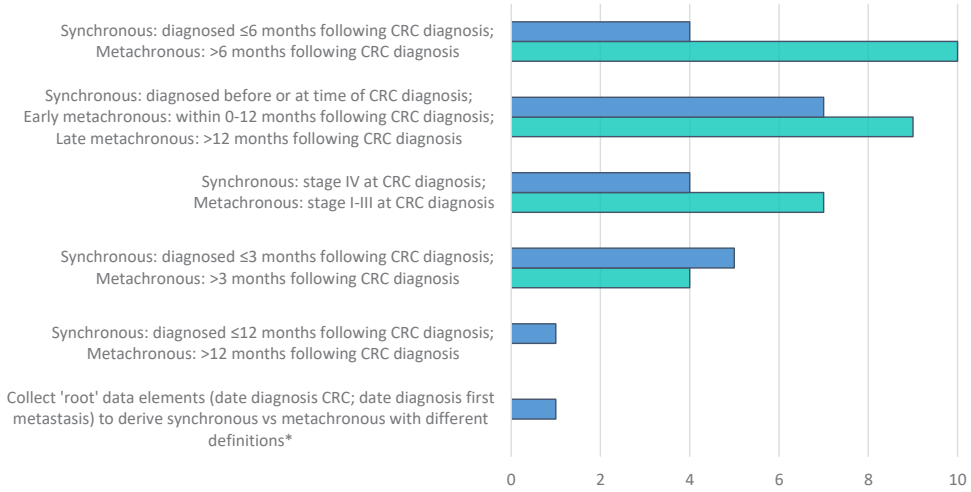
Supplementary Material



Supplementary Figure 1. Preferred definition of primary tumor location – Consensus round 1 (blue) and 2 (green). * Definition suggested by experts during round 1.



Supplementary Figure 2. Preferred definition of synchronous versus metachronous metastases – Consensus round 1 (blue) and 2 (green). * Definition suggested by experts during round 1.

Chapter 5

Maintenance treatment with capecitabine and bevacizumab versus observation in metastatic colorectal cancer: updated results and molecular subgroup analyses of the phase 3 CAIRO3 study

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Abstract

Background

The phase 3 CAIRO3 study showed that capecitabine plus bevacizumab (CAP-B) maintenance treatment after six cycles capecitabine, oxaliplatin and bevacizumab (CAPOX-B) in metastatic colorectal cancer (mCRC) patients is effective, without compromising quality of life. In this post hoc analysis with updated follow-up and data regarding sidedness, we defined subgroups according to *RAS/BRAF* mutation status and mismatch repair (MMR) status, and investigated their influence on treatment efficacy.

Methods

A total of 558 patients with previously untreated mCRC and stable disease or better after six cycles CAPOX-B induction treatment were randomised to either CAP-B maintenance treatment (*n*=279) or observation (*n*=279). Upon first progression, patients were to receive CAPOX-B reintroduction until second progression (PFS2, primary end point). We centrally assessed *RAS/BRAF* mutation status and MMR status, or used local results if central assessment was not possible. Intention-to-treat stratified Cox models adjusted for baseline covariables were used to examine whether treatment efficacy was modified by *RAS/BRAF* mutation status.

Results

RAS, BRAF mutations and MMR deficiency were detected in 240/420 (58%), 36/381 (9%) and 4/279 (1%) patients, respectively. At a median follow-up of 87 months (IQR 69-97), all mutational subgroups showed significant improvement from maintenance treatment for the primary end point PFS2 (*RAS/BRAF* wild-type: HR 0.57 [95%CI 0.39-0.84]; *RAS*-mutant: HR 0.74 [0.55-0.98]; ^{V600E}*BRAF*-mutant: HR 0.28 [0.12-0.64]) and secondary end points, except for the *RAS*-mutant subgroup regarding overall survival. Adjustment for sidedness instead of primary tumour location yielded comparable results. Although right-sided tumours were associated with inferior prognosis, both patients with right-sided and left-sided tumours showed significant benefit from maintenance treatment.

Conclusions

CAP-B maintenance treatment after six cycles CAPOX-B is effective in first-line treatment of mCRC across all mutational subgroups. The benefit of maintenance treatment was most pronounced in patients with *RAS/BRAF* wild-type and ^{V600E}*BRAF*-mutant tumours.

Introduction

Integrating targeted therapies into the management of metastatic colorectal cancer (mCRC) has significantly improved outcome of mCRC patients during recent years. Combining bevacizumab with fluoropyrimidine-containing chemotherapy is considered a standard option in first-line treatment of mCRC.[1-2] Since not all mCRC patients benefit from systemic therapy, predictive biomarkers are needed to optimize patient selection. Up to now, there is no validated biomarker for the efficacy of bevacizumab-based chemotherapy.

Only a few CRC biomarkers are being used in clinical practice, e.g. *RAS*, *BRAF* mutation status, and mismatch repair (MMR) status. Furthermore, there is growing evidence that primary tumour sidedness influences prognosis and therapy response in mCRC patients.[3] *RAS (KRAS* and *NRAS*) mutations occur in ~50% of mCRC patients and are negative predictors of outcome to anti-EGFR therapy.[4] Recently, it has been found that *RAS* mutations are associated with poor prognosis.[5-6] ^{V600E}*BRAF* mutations occur in ~5%-10% of mCRC patients and are also associated with poor outcome.[5,7] Moreover, studies suggest that mCRC patients with ^{V600E}*BRAF*-mutant tumours derive little or no benefit from anti-EGFR antibodies.[8] Deficient MMR (dMMR), the underlying cause of microsatellite instability, has a low prevalence in mCRC (3%-5%) and indicates a poor prognosis, which is likely driven by its association with ^{V600E}*BRAF* mutations.[9,10]

The phase 3 CAIRO3 study showed that in mCRC patients with stable disease (SD) or better after six cycles induction treatment with capecitabine, oxaliplatin and bevazicumab (CAPOX-B), maintenance treatment with capecitabine and bevacizumab (CAP-B) is more effective compared with observation, without compromising quality of life.[11] However, maintenance treatment may not be considered as cost-effective, and better patient selection would improve clinical decision-making and reduce therapy costs.[12]

In this post hoc analysis with updated follow-up and data regarding primary tumour sidedness, we aimed to define patient subgroups according to *RAS/BRAF* mutation status and MMR status, and investigate their impact on efficacy of CAP-B maintenance treatment versus observation.

Methods

Study design and participants

CAIRO3 was an open-label, multicentre phase 3 trial conducted by the Dutch Colorectal Cancer Group. Study design, eligibility criteria, ethical approvals, treatment regimens and outcomes have been reported elsewhere.[11] Previously untreated mCRC patients with stable disease (SD), partial response (PR), or complete response (CR) according to Response

Evaluation Criteria in Solid Tumours (RECIST, version 1.1) after six cycles CAPOX-B were randomised (1:1) to observation or CAP-B maintenance treatment. Upon first progression, patients in both arms were to receive CAPOX-B reintroduction. If CAPOX-B reintroduction was not possible after all due to persisting sensory neuropathy (grade ≥2) or any other reason, treatment choice was left to the local investigator's discretion. All patients provided written informed consent. Separate informed consent was asked for tissue collection.

Molecular assessment

From patients with informed consent for tissue collection, formalin-fixed, paraffin-embedded (FFPE) tissue of the primary tumour or metastases was retrieved from pathology archives for central study testing. Furthermore, pathology reports concerning primary tumour and metastases were obtained from all participants to collect results from prior local assessment of mutation status and MMR/MSI status. These results were used to supplement results obtained by central study testing.

FFPE tissue sections were prepared of the primary tumour (*n*=346) or metastasis (*n*=19). H&E stained sections were reviewed by experienced pathologists (ML, SMW) to determine the tumour cell percentage (≥10% required for next generation sequencing) and to encircle tumour areas for macro-dissection. Next generation sequencing of 50 genes' hotspot regions (including *KRAS* exons 2-4, *NRAS* exons 2-4, and *BRAF* exons 11, 15) included in the Ion AmpliSeq™ Cancer Hotspot Panel v2 (Life Technologies) was carried out using the Ion Torrent PGM System™ (Life Technologies), as previously described.[13]

In patients with available primary tumour resection material, MMR protein expression was determined by immunohistochemistry on tissue microarrays (TMAs). Of each FFPE block, 1.5mm punches for assembling TMAs were accomplished as previously reported.[14] Four 4µm sections of every TMA were stained in an automated immunostainer (Ventana BenchMark Ultra, Roche) with antibodies against MLH1 (clone G168-15; BD Pharmingen), PMS2 (clone EP51; Dako), MSH2 (clone FE11; Calbiochem), and MSH6 (clone ERP3945; Abcam). Two independent observers (KG, ML) carried out the scoring. In case of discordance, a third observer's opinion (GJO) was final. MMR protein staining patterns were evaluated as previously described.[9] Tumours were considered dMMR if they showed loss of expression in ≥1 MMR proteins, and proficient MMR (pMMR) if no loss of expression was observed.

Outcomes

The primary end point was second progression-free survival (PFS2), defined as the interval between randomisation until second progression while under CAPOX-B reintroduction, or first progression while under maintenance or observation for patients in whom CAPOX-B was not reintroduced, or until death, discontinuation or end of trial for patients without a second progression. Secondary end points included: interval between randomisation until

first progression (PFS1), interval between randomisation until second progression on any treatment (TT2PD), and overall survival (OS). TT2PD was considered equal to OS if no further treatment was registered beyond PFS1. Patients without recurrence or alive at time of the present analysis were included as censored data. Data cut-off of the initial analysis was 6 January 2014. In this updated analysis, we used follow-up data received before 21 March 2017.

Statistical analysis

First, we assessed overall treatment effect in the total study population. Patients with available *KRAS*, *NRAS*, *BRAF* and MMR status were included in the subgroup analyses. The Kappa statistic was carried out to determine consistency between mutation status and MMR status acquired through central study testing versus local assessment. In case of discordance, central study testing results were used.

We estimated survival curves of each treatment group and molecular subgroup with the Kaplan-Meier method. Furthermore, we assessed the impact of primary tumour sidedness (right colon: cecum-transverse colon; left colon: splenic flexure-rectum) on outcome in the total study population and mutational subgroups.

We investigated the influence of mutation status on treatment efficacy in three subgroups: patients with *RAS* plus *BRAF* wild-type status, *RAS*-mutant tumours (patients with concomitant *BRAF* mutations excluded), and ^{V600E}*BRAF*-mutant tumours (patients with concomitant *RAS* mutations excluded). We used intention-to-treat Cox proportional hazard models to estimate hazard ratios (HRs), including interaction terms between *RAS* and ^{V600E}*BRAF* mutation status and treatment allocation. Analyses were stratified according to previous adjuvant chemotherapy, response to induction treatment, WHO PS, and serum LDH. Additional adjustments were made for age, sex, stage, primary tumour location, primary tumour resection, number of metastatic sites, synchronous versus metachronous metastases, dose reduction during induction treatment, and interval between CRC diagnosis and randomisation.

To assess the influence of sidedness on mutational analyses, we carried additional analyses adjusted for sidedness (right versus left colon) instead of primary tumour location (colon versus rectosigmoid versus rectum). Furthermore, we aimed to investigate the influence of sidedness on treatment efficacy, and whether this was dependent on *RAS* plus *BRAF* mutation status. Patients with synchronous left-sided and right-sided tumours were excluded from these analyses, as were patients of which sidedness could not be determined. We report nominal, two-sided *P*-values (significance level set to 0.05), without adjustment for multiple testing. Analyses were carried out using IBM SPSS Statistics 21 and R version 3.0.3.

Results

Between May 2007 and October 2012, 558 patients were randomised to observation or maintenance treatment (supplementary Figure 1). One patient withdrew informed consent before treatment initiation. *RAS, BRAF*, and MMR status were available in 420 (75%), 381 (68%), and 279 (50%) patients, respectively, acquired through central or local assessment. *KRAS, NRAS, BRAF* and MMR status were available through both central and local assessment in 193, 11, 48 and 0 patients, respectively. For these patients, there was high agreement between central and local assessment (Supplementary Table 1).
RAS-mutant, *BRAF*-mutant and dMMR tumours were detected in 242 (58%), 36 (9%) and four (1%) patients, respectively. The prevalence of mutations was comparable between treatment arms (Supplementary Table 2). Of 371 *RAS*/*BRAF* assessable patients, 140 patients had *RAS* plus *BRAF* wild-type tumours. Of 242 patients with a *RAS*-mutant tumour, 224 were *KRAS*-mutant, 19 were *NRAS*-mutant, 1 had both a *KRAS* and *NRAS* mutation, and 2 had a concomitant *BRAF* mutation (1 ^{V600E}*BRAF* mutation; 1 $^{non\text{-}V600}$*BRAF* mutation). Of 36 patients with a *BRAF*-mutant tumour, 31 were ^{V600E}*BRAF*-mutant, and 5 were $^{non\text{-}V600}$*BRAF*-mutant. One out of the four patients with dMMR had a ^{V600E}*BRAF*-mutant tumour. Mutation variants are shown in Supplementary Table 3. Compared with the total study population, the *RAS/BRAF* wild-type subgroup contained more males with left-sided tumours, while ^{V600E}*BRAF* mutations were more prevalent in females, patients with WHO PS 0, right-sided tumours, synchronous metastases, and elevated platelet count (Table 1). Compared with ^{V600E}*BRAF* mutations, $^{non\text{-}V600}$*BRAF* mutations occurred more frequently in patients with left-sided tumours and metachronous metastases (Supplementary Table 4).

The median duration of follow-up was 87 months (IQR 69-97), compared with 48 months (IQR 36-57) at time of the primary analysis. By 21 March 2017, 531 (95%) patients had died, and 14 (3%) patients had not progressed. The outcome of maintenance treatment versus observation was improved for all end points. This benefit was statistically significant, except for OS (Table 2).

Table 1. Patient characteristics

	Total study population *n*=557	*RAS/BRAF* WT *n*=140	*RAS* MT *n*=240[a]	^{V600E}*BRAF* MT *n*=30[b]
Age				
Median (range)	64 (26-81)	65 (26-80)	64 (39-81)	64 (47-78)
Sex				
Male	361 (65%)	106 (76%)	151 (63%)	15 (50%)
Female	196 (35%)	34 (24%)	89 (37%)	15 (50%)
WHO performance status				
0	345 (62%)	91 (65%)	147 (61%)	22 (73%)
1	212 (38%)	49 (35%)	93 (39%)	8 (27%)
Serum lactate dehydrogenase				
Normal	245 (44%)	66 (47%)	104 (43%)	12 (40%)
Above normal	312 (56%)	74 (53%)	136 (57%)	18 (60%)
Prior adjuvant chemotherapy				
Yes	188 (34%)	42 (30%)	85 (35%)	8 (27%)
No	369 (66%)	98 (70%)	155 (65%)	22 (73%)
Best response to induction treatment				
Stable disease	191 (34%)	39 (28%)	88 (37%)	9 (30%)
Partial or complete response	366 (66%)	101 (72%)	152 (63%)	21 (70%)
Site of primary tumour				
Right colon[c]	122 (22%)	16 (11%)	62 (26%)	20 (67%)
Left colon[d]	406 (73%)	117 (84%)	171 (71%)	8 (27%)
Colon n.o.s.	19 (3%)	3 (2%)	5 (2%)	1 (3%)
Multiple sites	10 (2%)	4 (3%)	2 (1%)	1 (3%)
Number of metastatic sites				
1	229 (41%)	61 (44%)	97 (40%)	10 (33%)
>1	302 (54%)	70 (50%)	135 (56%)	18 (60%)
Unknown	26 (5%)	9 (6%)	8 (3%)	2 (7%)
Interval of metastases and primary tumour resection status				
Synchronous[e], resection	180 (32%)	54 (39%)	83 (35%)	12 (40%)
Synchronous, no resection	230 (41%)	49 (35%)	89 (37%)	14 (47%)
Metachronous	147 (26%)	37 (26%)	68 (28%)	4 (13%)
Platelet count at start induction treatment				
< 400 x 10^9/L	346 (62%)	92 (66%)	150 (63%)	13 (43%)
≥ 400 x 10^9/L	163 (29%)	39 (28%)	68 (28%)	13 (43%)
Unknown	48 (9%)	9 (6%)	22 (9%)	4 (13%)
Treatment arm				
Observation	279 (50%)	61 (44%)	128 (54%)	15 (50%)
Maintenance	278 (50%)	79 (56%)	112 (46%)	15 (50%)

Data are *n* (%) unless otherwise specified. Due to rounding, not all percentages total 100. MT= mutant; WT= wild-type. [a] Two patients with concomitant *BRAF* MT tumour excluded. [b] One patient with concomitant *RAS* MT tumour excluded. [c] Right colon: cecum to transverse colon. [d] Left colon: splenic flexure to rectum. [e] Synchronous metastases: distant metastases discovered ≤ 6 months after diagnosis of the primary tumour.

Regardless of treatment arm, OS was significantly different across the *RAS/BRAF* wild-type [24.1 months (95%CI 21.3-26.9)], *RAS*-mutant [19.5 months (17.7-21.2)] and ^{V600E}*BRAF*-mutant subgroups [13.6 months (8.5-18.8)] (*P*=0.012; Supplementary Table 5). Patients in the $^{non\text{-}V600E}$*BRAF*-mutant subgroup showed a non-statistically significant increase in median OS compared with the ^{V600E}*BRAF*-mutant subgroup. Patients with dMMR versus pMMR tumours showed inferior outcome, but differences were not statistically significant. The prevalence of $^{non\text{-}V600E}$*BRAF* mutations and dMMR was too low to investigate their influence on treatment efficacy. Patients with right-sided (*n*=122) versus left-sided tumours (*n*=406) had a significantly worse median OS [15.7 months (95%CI 13.1-18.2) *vs* 21.8 months (20.2-23.5), *P*=0.010; Supplementary Table 6]. Within mutational subgroups, patients with right-sided tumours also showed inferior OS, though differences were not statistically significant.

In the adjusted analyses regarding treatment efficacy, maintenance treatment significantly improved PFS1 in all mutational subgroups (Table 2; Figure 1A). Likewise, all mutational subgroups showed significant benefit from maintenance treatment for the primary end point PFS2: *RAS/BRAF* wild-type: HR 0.57 (95%CI 0.39-0.84); *RAS*-mutant: HR 0.74 (0.55-0.98); ^{V600E}*BRAF*-mutant: HR 0.28 (0.12-0.64)(Table 2; Figure 1B). Maintenance treatment also significantly improved TT2PD across all mutational subgroups (Table 2; Figure 1C). Regarding OS, the *RAS*/*BRAF* wild-type and ^{V600E}*BRAF*-mutant subgroups showed significant benefit from maintenance treatment, in contrast to the *RAS*-mutant subgroup (Table 2; Figure 1D). Interaction tests between treatment arm and mutation status were statistically significant for TT2PD ($P_{interaction}$=0.021) and OS ($P_{interaction}$=0.028; Table 2). When mutational subgroup analyses were adjusted for sidedness instead of primary tumour location, comparable efficacy results were observed (data not shown).

Both patients with right- and left-sided tumours showed significant benefit from maintenance treatment for all end points, except for patients with left-sided tumours regarding OS (Supplementary Table 7). No significant interactions were found between treatment arm and sidedness. As *RAS* plus *BRAF* mutation status was not available for all patients, sample sizes were too small to investigate whether treatment efficacy according to sidedness was influenced by mutation status.

In the total study population, the proportion of patients that received subsequent treatment of mCRC was comparable between treatment arms (Supplementary Table 8). The proportion of patients that did not receive subsequent treatment was highest in the ^{V600E}*BRAF*-mutant subgroup. Eighteen patients with *RAS*-mutant tumours received anti-EGFR antibodies before *(K)RAS* mutation status was widely implemented in daily practice as a predictive marker: 13 patients with *KRAS* mutations outside exon 2 underwent anti-EGFR therapy before extended *RAS* testing was a routine procedure, and five patients received anti-EGFR therapy despite the presence of a *KRAS* exon 2 mutation.

Table 2. Efficacy results in *RAS* and *BRAF* mutational subgroups

	Total study population		***RAS/BRAF* WT**		***RAS* MT**		**^{V600E}*BRAF* MT**	
	***n*=557**		***n*=140**		***n*=240[a]**		***n*=30[b]**	
	Obs (*n*=279)	**Maint (*n*=278)**	**Obs (*n*=61)**	**Maint (*n*=79)**	**Obs (*n*=128)**	**Maint (*n*=112)**	**Obs (*n*=15)**	**Maint (*n*=15)**
PFS1								
Events	275	268	61	76	127	110	14	12
Median (months)	4.1	8.5	5.2	8.8	4.1	8.4	2.0	9.5
95% CI	3.9-4.2	6.6-10.3	3.8-6.6	5.9-11.6	3.9-4.2	6.2-10.6	0.2-3.9	3.9-15.0
HR	0.38		0.36		0.40		0.19	
95% CI	0.31-0.46		0.25-0.54		0.30-0.54		0.08-0.44	
P-value	<0.0001		<0.0001		<0.0001		<0.0001	
PFS2								
Events	274	266	60	75	127	109	14	12
Median (months)	8.6	11.6	9.0	13.3	8.9	11.2	5.7	13.0
95% CI	7.0-10.1	10.0-13.3	6.6-11.4	10.0-16.7	6.7-11.2	9.6-12.9	2.2-9.2	7.1-18.8
HR	0.64		0.57		0.74		0.28	
95% CI	0.53-0.77		0.39-0.84		0.55-0.98		0.12-0.64	
P-value	<0.0001		0.004		0.038		0.002	
TT2PD								
Events	272	264	60	74	126	109	14	12
Median (months)	11.4	13.9	12.4	15.4	11.6	15.4	7.4	13.0
95% CI	10.2-12.7	12.1-15.6	10.4-14.3	11.4-19.4	9.8-13.5	12.0-18.7	3.5-11.3	7.1-18.8
HR	0.63		0.60		0.71		0.20	
95% CI	0.52-0.76		0.41-0.87		0.53-0.94		0.08-0.46	
P-value	<0.0001		0.008		0.017		0.001	
OS								
Events	268	263	59	73	124	109	14	12
Median (months)	18.2	21.6	19.0	25.7	18.7	20.9	13.6	15.8
95% CI	16.1-20.3	19.5-23.7	13.9-24.1	22.3-29.1	16.6-20.8	18.1-23.7	10.1-17.2	7.8-23.8
HR	0.86		0.68		0.98		0.32	
95% CI	0.71-1.03		0.46-1.00		0.73-1.30		0.14-0.73	
P-value	0.100		0.047		0.867		0.007	

CI= confidence interval. HR= hazard ratio for maintenance treatment versus observation. Maint= maintenance. MT= mutant. Obs= observation. WT= wild-type. [a] Two patients with concomitant *BRAF* mutations excluded. [b] One patient with concomitant *RAS* mutation excluded. Likelihood ratio-based test for interaction between treatment and mutation status: PFS1: *P*=0.239; PFS2: *P*=0.079; TT2PD: *P*=0.021; OS: *P*=0.028.

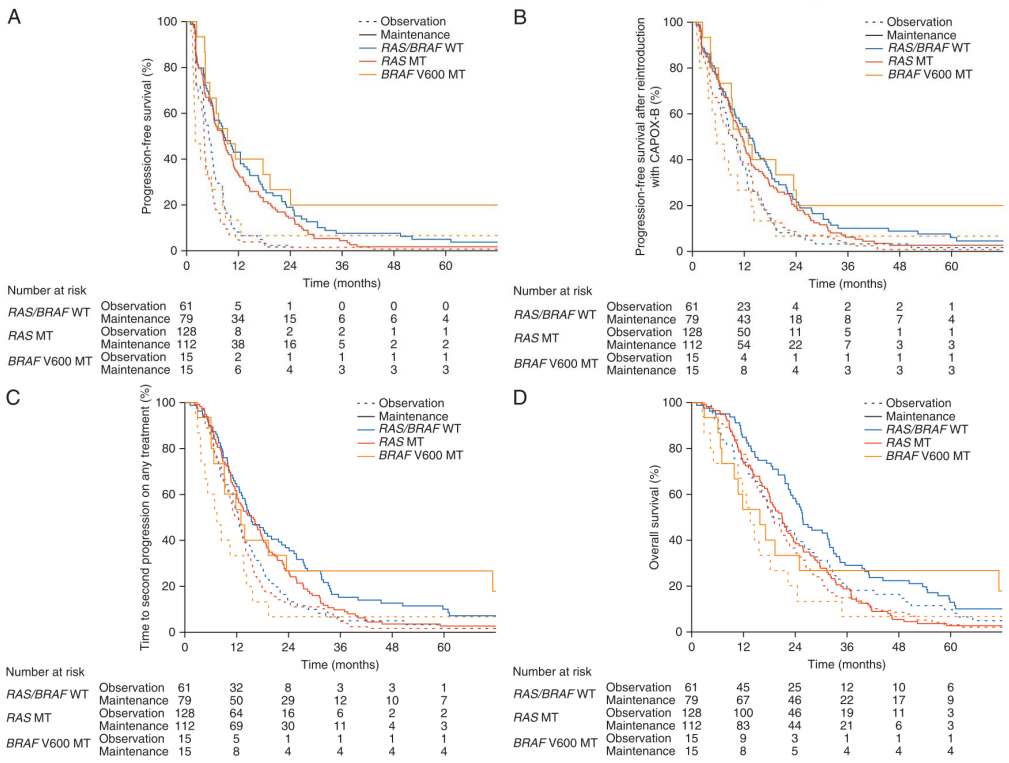

Figure 1. Kaplan-Meier curves for progression-free and overall survival according to *RAS* and *BRAF* mutation status. (A) Progression-free survival. (B) Progression-free survival after CAPOX-B reintroduction. (C) Time to second progression on any treatment. (D) Overall survival. MT, mutant; WT, wild-type.

Discussion

This post hoc analysis with updated follow-up confirms the benefit of CAP-B maintenance treatment versus observation in first-line treatment of mCRC, with significant results for PFS1, PFS2 (primary end point) and TT2PD. With an improvement of 3.4 months, the OS benefit remained clinically meaningful, though not statistically significant. Patients with *RAS/BRAF* wild-type tumours had favourable prognosis compared with patients with *RAS*-mutant or ^{V600E}*BRAF*-mutant tumours, and right-sided tumours were associated with inferior outcome compared with left-sided tumours. Maintenance treatment was more effective compared with observation across all mutational subgroups, except for the *RAS*-mutant subgroup regarding OS. When mutational subgroup analyses were adjusted for sidedness instead of primary tumour location, comparable efficacy results were observed. Both patients with right- and left-sided tumours showed significant benefit from maintenance treatment.

The CAIRO3 study consisted of a selected subgroup of patients, since only patients with SD or better after six cycles CAPOX-B were included. Nevertheless, the prevalence of *KRAS* (47%)*, NRAS* (5%)*, RAS (*58%) and *BRAF* (9%) mutations was comparable with results from other first-line mCRC trials.[5,15] However, the prevalence of dMMR (1%) was lower than expected.[10] Individual patient data of the CAIRO2 study (CAPOX-B ± cetuximab; eligibility criteria comparable to CAIRO3), showed a high prevalence of dMMR (10/65; 15%) and ^{V600E}*BRAF* mutations (10/59; 17%) in patients with progressive disease or toxicity within the first six cycles of CAPOX-B.[7] We therefore cannot exclude that a considerable number of patients with dMMR and ^{V600E}*BRAF*-mutant tumours was not eligible for CAIRO3 due to disease progression or toxicity during induction treatment.

Consistent with other first-line trials, patients with *RAS/BRAF* wild-type tumours had a favourable prognosis compared with patients with *RAS*-mutant or ^{V600E}*BRAF*-mutant tumours.[5,6,15] The ^{V600E}*BRAF*-mutant subgroup showed inferior OS compared with *RAS/BRAF* wild-type and *RAS*-mutant subgroups, corresponding with the negative prognostic value of ^{V600E}*BRAF* mutations.[5,6] Patients with dMMR compared with pMMR showed a numerically inferior OS, in line with the poor prognosis of dMMR in mCRC.[10] Interestingly, patients with $^{non\text{-}V600}$*BRAF*-mutant tumours showed a numerically superior OS compared with the ^{V600E}*BRAF*-mutant subgroup. Despite the small sample size, our findings correspond with a recent report describing that $^{non\text{-}V600}$*BRAF* mutations represent a distinct molecular subtype of mCRC with good prognosis.[16] In line with other studies, patients with right-sided compared with left-sided tumours showed inferior OS.[3] Within mutational subgroups, patients with right-sided tumours were also associated with inferior OS, though differences were not statistically significant.

Subgroup analyses in the primary analysis showed significant interactions with OS and maintenance treatment for CR/PR as best response to induction treatment, and synchronous disease with a resected primary tumour.[11] The present analysis shows a significant interaction between treatment arm and mutation status regarding TT2PD and OS. Our subgroup analyses were exploratory in nature. Therefore, possible explanations for a statistically significant benefit from maintenance treatment or a lack thereof remain speculative, and do not allow definite conclusions. Furthermore, as the CAIRO3 study population concerns a selected group of patients, our findings may not be used to assess the biology of mutational subgroups within mCRC in general. Nonetheless, every mutational subgroup showed significant benefit from maintenance treatment for all end points, except for the *RAS*-mutant subgroup regarding OS. In patients with *RAS/BRAF* wild-type tumours, the marked increase in median OS of 6.7 months (19.0 versus 25.7 months) suggests a clinically relevant benefit from maintenance treatment. Moreover, despite the negative prognostic value of ^{V600E}*BRAF* mutations, these patients also showed good response to maintenance treatment. Although the *RAS*-mutant subgroup showed significant benefit from maintenance treatment for PFS1,

PFS2, and TT2PD, effect sizes were less pronounced compared with the *RAS/BRAF* wild-type and ^{V600E}*BRAF*-mutant subgroups. Furthermore, although maintenance treatment resulted in a 2.2-month increase in median OS in the *RAS*-mutant subgroup, this did not translate into a statistically significant OS benefit from maintenance treatment. However, it must be emphasised that the CAIRO3 study was not designed or powered to detect a difference in OS. This end point can be highly influenced by subsequent treatment lines. Regarding subsequent treatments, we found no clear imbalances between treatment arms that could have influenced our OS results. Altogether, our findings show that maintenance treatment is effective across all mutational subgroups.

Several mCRC trials have examined observation versus maintenance treatment with bevacizumab-based chemotherapy[15,17-18], but mutational data are only available of the AIO 0207 study (observation versus fluoropyrimidine + bevacizumab versus bevacizumab). Consistent with our findings, the authors showed that both patients with all wild-type status (*RAS* plus ^{V600E}*BRAF* wild-type) or any mutation (*RAS*- or ^{V600E}*BRAF*-mutant) experienced greater benefit from doublet maintenance treatment versus observation in PFS1.[15] Different from our analysis, their mutational analyses did not show significant results for time to failure of strategy and OS, which could be explained by differences in study design, induction treatment duration, and exclusion criteria.

Our subgroup analyses may have been subject to bias as *RAS/BRAF* mutation status and MMR status were not available for all patients, comparable to other first-line mCRC trials.[5,15] However, baseline characteristics were comparable between the total study population and mutational subgroups, and potential confounders were adjusted for in multivariable analyses. Although sidedness data was not available for all patients, mutational analyses adjusted for sidedness instead of primary tumour location yielded comparable results. Both patients with right-sided and left-sided tumours showed significant improvement from maintenance treatment for all end points, except for patients with left-sided tumours regarding OS.

In conclusion, this updated analysis of the CAIRO3 study confirms the effectiveness of CAP-B maintenance treatment after six cycles of CAPOX-B in first-line treatment of mCRC. Our findings suggest that all mutational subgroups derive a significant benefit from maintenance treatment, which was most pronounced in patients with *RAS/BRAF* wild-type or ^{V600E}*BRAF*-mutant tumours.

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Supplementary Material

Supplementary Table 1. Mutation status and MMR status acquired through central study testing and local assessment in daily clinical practice

	Central study testing	Local assessment	Total (*n* =)	Overlapping results[b] (*n* =)	Agreement[c] (%)	Kappa[c] (95% CI, *p*-value)
KRAS	363 (77%)	109 (23%)	472	193	93%	0.87 (0.79-0.94, *p*<0.001)
NRAS	365 (98%)	7 (2%)	372	11	91%	0.62 (-0.04-1.28, *p*=0.026)
RAS	363 (86%)	57 (14%)	420[a]	NA	NA	NA
BRAF	360 (94%)	21 (6%)	381	48	96%	0.73 (0.37-1.08, *p*<0.001)
MMR	237 (85%)	42 (15%)	279	0	NA	NA

Data are *n* (%) unless otherwise specified. MMR = mismatch repair. NA = not applicable. [a] Including two patients with *NRAS* status acquired through central study testing, and *KRAS* status acquired through local assessment. [b] Number of patients of which *KRAS, NRAS, BRAF* and MMR status was available through both central study testing and local assessment. [c] Agreement between results acquired through both central study testing and local assessment.

Supplementary Table 2. Prevalence of mutations and dMMR

	Observation	Maintenance	Total
Total study population	279	278	557
***KRAS* mutant**	120 / 234 (51%)	104 / 238 (44%)	224 / 472 (47%)
***NRAS* mutant**	9 / 185 (5%)	10 / 187 (5%)	19 / 372 (5%)
***RAS* mutant**	128[a] / 209 (61%)	114 / 211 (54%)	242[b] / 420[c] (58%)
***BRAF* mutant**	17 / 188 (9%)	19 / 193 (10%)	36[d] / 381 (9%)
***RAS* or *BRAF* mutant**	145 / 206 (70%)	131[e] / 210 (62%)	276 / 416 (66%)
dMMR	3 / 137 (2%)	1 / 142 (1%)	4 / 279 (1%)

Data are *n / N* (%) unless otherwise specified. dMMR = deficient mismatch repair. [a] One patient had a tumour with a concomitant *KRAS* and *NRAS* mutation. [b] Two patients had a tumour with a concomitant *BRAF* mutation. [c] The number of patients with available *RAS* mutation status also included patients with *KRAS*-mutant tumours of which *NRAS* mutation status could not be determined. [d] Two patients had a tumour with a concomitant *RAS* mutation. [e] Two patients had a tumour with a concomitant *RAS* and *BRAF* mutation.

Supplementary Table 3. *KRAS*, *NRAS* and *BRAF* mutation variants

	Number of variants
***KRAS* exon 1 (*n* = 1)**	
Unknown	1
***KRAS* exon 2 (*n* = 207)**	
G12D	59[a,b]
G12V	58[a]
G12S	12
G12C	15[a]
G12A	11[c]
G12F	1
G12L	1
G12R	1
G13D	37[a]
G13C	2
AG11-12AV	1
Unknown	5
Unknown, codon 12	1
Unknown, codon 13	3
***KRAS* exon 3 (*n* = 4)**	
Q61H	3
Q61L	1
***KRAS* exon 4 (*n* = 13)**	
A146T	8
A146V	4
A146QQ	1
***NRAS* exon 2 (*n* = 4)**	
G12D	3
G13D	1
***NRAS* exon 3 (*n* = 14)**	
Q61H	2
Q61K	9[d,e]
Q61R	1
Q61L	2
***NRAS* exon 4 (*n* = 1)**	
R102*	1[b]
***BRAF* exon 11 (*n* = *2*)**	
G460V	1[c]
G469A	1
***BRAF* exon 15 (*n* = 34)**	
V600E	31[e]
D594A	1
D594G	2

Frequencies based on analysed populations. [a] Three patients had a tumour with a double *KRAS* mutation (G12D+G13D, G12D+G12C, and G12V+G13D). [b] One patient had a tumour with a *KRAS* G12D and *NRAS* R102* mutation. [c] One patient had a tumour with a *KRAS* G12A and *BRAF* G460V mutation. [d] One patient had a tumour with a double *NRAS* mutation (Q61K+ E132V). [e] One patient had a tumour with an *NRAS* Q61K and ^{V600E}*BRAF* mutation.

Supplementary Table 4. Patient characteristics according to *BRAF* mutation status

	^{V600E}***BRAF*** **MT** ***n*** **= 31**	$^{non\text{-}V600}$***BRAF*** **MT** ***n*** **= 5**
Age		
Median (range)	64 (47-78)	62 (52-73)
Sex		
Male	16 (52%)	2 (40%)
Female	15 (48%)	3 (60%)
WHO performance status		
0	23 (74%)	2 (40%)
1	8 (26%)	3 (60%)
Serum lactate dehydrogenase		
Normal	12 (39%)	1 (20%)
Above normal	19 (61%)	4 (80%)
Prior adjuvant chemotherapy		
Yes	8 (26%)	1 (20%)
No	23 (74%)	4 (80%)
Best response to induction treatment		
Stable disease	10 (33%)	1 (20%)
Partial or complete response	21 (68%)	4 (80%)
Site of primary tumour		
Right colon[a]	20 (65%)	1 (20%)
Left colon[b]	9 (29%)	4 (80%)
Colon n.o.s.	1 (3%)	0 (0%)
Multiple sites	1 (3%)	0 (0%)
Number of metastatic sites		
1	11 (35%)	2 (40%)
>1	18 (58%)	3 (60%)
Unknown	2 (6%)	0 (0%)
Interval of metastases and primary tumour resection status		
Synchronous[c], resection	12 (39%)	1 (20%)
Synchronous, no resection	15 (48%)	1 (20%)
Metachronous	4 (13%)	3 (60%)
Platelet count at start induction treatment		
< 400 x 10^9/L	14 (45%)	3 (60%)
≥ 400 x 10^9/L	13 (42%)	2 (40%)
Unknown	4 (13%)	0 (0%)
Treatment arm		
Observation	15 (48%)	2 (40%)
Maintenance	16 (52%)	3 (60%)

Data are *n* (%) unless otherwise specified. Due to rounding, not all percentages total 100. MT = mutant. N.o.s. = not otherwise specified. [a] Right colon: cecum – hepatic flexure. [b] Left colon: splenic flexure – sigmoid. [c] Synchronous metastases: metastases discovered ≤ 6 months after diagnosis of the primary tumour.

Supplementary Table 5. Kaplan-Meier estimates of clinical outcome according to molecular subgroups

	***RAS/BRAF* WT** ***n* = 140**	***RAS* MT** ***n* = 240**[a]	**^{V600E}*BRAF* MT** ***n* = 30**[b]	**$^{non\text{-}V600}$*BRAF* MT** ***n* = 4**[b]	**pMMR** ***n* = 275**	**dMMR** ***n* = 4**
PFS1						
Events	137	237	26	4	270	4
Median (months)	6.2	4.7	5.4	2.1	5.7	2.1
95% CI	5.5 - 6.9	3.6 - 5.7	1.9 - 8.8	0.0 - 13.5	4.9 - 6.6	0.0 - 11.6
P-value		0.045[c]		0.804[d]	0.748[e]	
PFS2						
Events	135	236	26	4	267	4
Median (months)	11.1	10.4	9.0	11.4	10.7	8.0
95% CI	8.9 - 13.2	8.8 - 12.0	6.3 - 11.8	0.1 - 22.7	9.4 - 12.0	0.0 - 17.4
P-value		0.350[c]		0.761[d]	0.268[e]	
TT2PD						
Events	134	235	26	4	266	4
Median (months)	13.5	13.0	9.2	11.4	13.4	8.0
95% CI	11.5 - 15.4	11.5 - 14.4	4.7 - 13.7	0.0 - 27.8	12.2 - 14.6	1.7 - 14.2
P-value		0.210[c]		0.945[d]	0.236[e]	
OS						
Events	132	233	26	4	263	4
Median (months)	24.1	19.5	13.6	30.4	21.4	13.6
95% CI	21.3 - 26.9	17.7 - 21.2	8.5 - 18.8	0.0 - 64.1	19.1 - 23.7	9.5 - 17.7
P-value		0.012[c]		0.640[d]	0.040[e]	

CI = confidence interval. dMMR = deficient mismatch repair. MT = mutant. pMMR = proficient mismatch repair. WT = wild-type. [a] Two patients with concomitant *BRAF* MT tumour excluded. [b] One patient with concomitant *RAS* MT tumour excluded. [c] *P*-value for overall comparison between *RAS/BRAF* WT, *RAS* MT and ^{V600E}*BRAF* MT subgroups. [d] *P*-value for overall comparison between ^{V600E}*BRAF* MT and $^{non\text{-}V600E}$*BRAF* MT subgroups. [e] *P*-value for overall comparison between pMMR and dMMR subgroups.

Supplementary Table 6. Kaplan-Meier estimates of clinical outcome according to mutation status and primary tumour sidedness

	Total study population (*n* = 528)		***RAS/BRAF* WT (*n* = 133)**		***RAS* MT[a] (*n* = 233)**		**^{V600E}*BRAF* MT[b] (*n* = 28)**	
	Right *n* = 122	**Left *n* = 406**	**Right *n* = 16**	**Left *n* = 117**	**Right *n* = 62**	**Left *n* = 171**	**Right *n* = 20**	**Left *n* = 8**
PFS1								
Events	118	397	16	114	61	170	18	6
Median (months)	4.2	5.9	5.7	6.3	4.1	5.2	4.3	5.7
95% CI	3.6-4.9	5.4-6.4	0.0-13.2	4.9-7.7	3.8-4.4	4.1-6.4	2.6-6.1	0.0-12.8
P-value[c]	0.381		0.749		0.465		0.487	
PFS2								
Events	117	396	16	113	60	170	18	6
Median (months)	8.4	10.7	11.1	11.1	8.3	10.7	8.4	5.7
95% CI	6.4-10.5	9.6-11.7	4.1-18.1	8.9-13.2	5.2-11.3	9.3-12.0	4.9-11.9	0.0-16.3
P-value[c]	0.403		0.870		0.711		0.469	
TT2PD								
Events	117	392	16	112	60	169	18	6
Median (months)	10.5	13.2	13.4	13.5	11.1	13.3	8.4	13.0
95% CI	8.2-12.8	12.3-14.1	6.9-19.8	11.4-15.5	7.5-14.6	11.7-14.8	4.9-11.9	0.0-27.3
P-value[c]	0.163		0.617		0.476		0.383	
OS								
Events	117	388	16	111	60	167	18	6
Median (months)	15.7	21.8	21.7	24.3	16.5	21.6	11.8	15.7
95% CI	13.1-18.2	20.2-23.5	16.8-26.6	21.5-27.1	13.2-19.8	19.7-23.5	7.6-16.0	8.3-23.1
P-value[c]	0.010		0.574		0.127		0.314	

CI = confidence interval. MT = mutant. WT = wild-type. Patients with primary tumour location 'colon n.o.s.' or 'multiple sites' were excluded from the analysis. [a] Two patients with concomitant *BRAF* MT tumour excluded. [b] One patient with concomitant *RAS* MT tumour excluded. [c] *P*-value for overall comparison between patients with right-sided and left-sided tumours.

Supplementary Table 7. Efficacy results in the total study population according to primary tumour sidedness

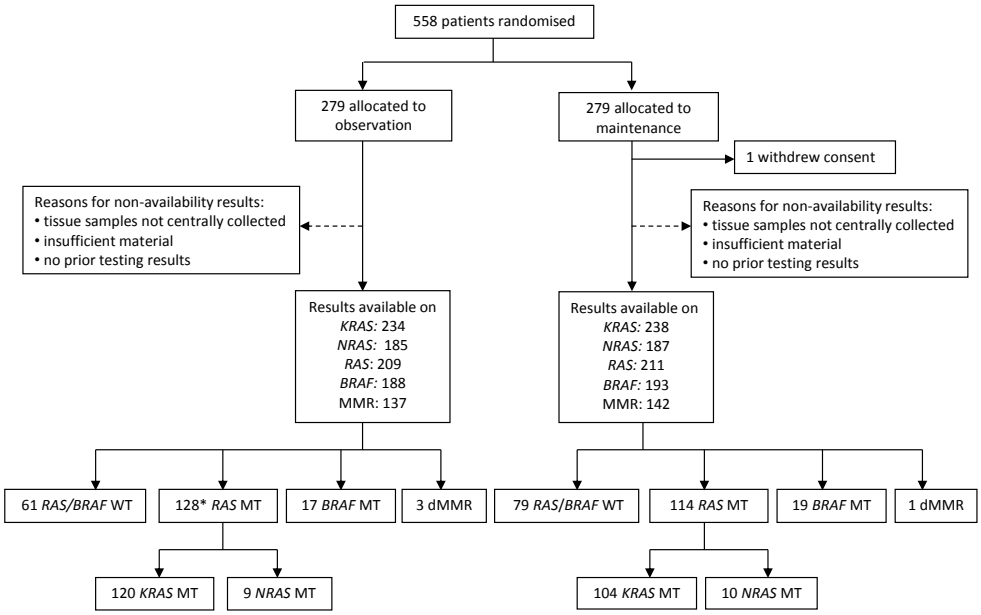
	Total study population (*n* = 557)			
	Right		Left	
	Observation	Maintenance	Observation	Maintenance
	n = 62	*n* = 60	*n* = 203	*n* = 203
PFS1				
Events	61	57	201	196
Median (months)	3.5	7.4	4.1	8.8
95% CI	2.3-4.6	3.1-11.6	3.9-4.4	6.7-10.8
HR	0.36		0.38	
95% CI	0.24-0.53		0.30-0.47	
P-value	<0.001		<0.001	
PFS2				
Events	61	56	200	196
Median (months)	6.6	9.9	10.3	12.0
95% CI	5.1-8.1	6.1-13.7	8.8-11.7	10.1-13.9
HR	0.58		0.65	
95% CI	0.39-0.86		0.53-0.81	
P-value	0.006		<0.001	
TT2PD				
Events	61	56	198	194
Median (months)	8.5	13.4	12.6	14.0
95% CI	6.6-10.4	10.0-16.7	11.4-13.8	12.2-15.8
HR	0.50		0.68	
95% CI	0.34-0.74		0.55-0.84	
P-value	0.001		<0.001	
OS				
Events	61	56	194	194
Median (months)	14.2	17.7	21.3	22.5
95% CI	12.7-15.8	14.4-20.9	18.8-23.7	20.5-24.4
HR	0.64		0.95	
95% CI	0.43-0.94		0.77-1.17	
P-value	0.024		0.619	

CI = confidence interval. HR= hazard ratio for maintenance treatment *vs* observation. Patients with primary tumour location 'colon n.o.s.' or 'multiple sites' were excluded from the analysis. Likelihood ratio-based test for interaction between treatment and primary tumour sidedness: PFS1: p=0.802; PFS2: p=0.575; TT2PD: p=0.178; OS: p=0.074.

Supplementary Table 8. Therapy received during the course of metastatic disease

	Total study population *n* = 557			*RAS/BRAF* WT *n* = 140		*RAS* MT *n* = 240[a]		^{V600E}*BRAF* MT *n* = 30[b]	
	Obs	**Maint**	**Total**	**Obs**	**Maint**	**Obs**	**Maint**	**Obs**	**Maint**
	n = 279	*n* = 278	*n* = 557	*n* = 61	*n* = 79	*n* = 128	*n* = 112	*n* = 15	*n* = 15
Capecitabine (CAP)	279 (100%)	278 (100%)	557 (100%)	61 (100%)	79 (100%)	128 (100%)	112 (100%)	15 (100%)	15 (100%)
Oxaliplatin (Ox)	279 (100%)	278 (100%)	557 (100%)	61 (100%)	79 (100%)	128 (100%)	112 (100%)	15 (100%)	15 (100%)
Bevacizumab (Bev)	279 (100%)	278 (100%)	557 (100%)	61 (100%)	79 (100%)	128 (100%)	112 (100%)	15 (100%)	15 (100%)
Anti-EGFR therapy	48 (17%)	54 (19%)	102 (18%)	18 (30%)	25 (32%)	10 (8%)	8 (7%)	3 (20%)	3 (20%)
Irinotecan	165 (59%)	162 (58%)	327 (59%)	32 (52%)	52 (66%)	86 (67%)	64 (57%)	6 (40%)	3 (20%)
Mitomycin	1 (0.4%)	1 (0.4%)	2 (0.4%)	-	1 (1%)	-	-	-	-
Regorafenib	2 (0.8%)	1 (0.4%)	3 (0.5%)	-	1 (1%)	2 (2%)	-	-	-
Aflibercept/placebo	8 (3%)	4 (1.4%)	12 (2%)	-	2 (3%)	4 (3%)	-	1 (7%)	-
Ramucirumab/placebo	2 (0.7%)	2 (0.7%)	4 (0.7%)	-	2 (3%)	2 (2%)	-	-	-
Tegafur-uracil	3 (1%)	5 (2%)	8 (1%)	1 (2%)	3 (4%)	2 (2%)	1 (0.9%)	-	-
Cisplatin	1 (0.4%)	-	1 (0.2%)	-	-	-	-	-	-
Paclitaxel	-	1 (0.4%)	1 (0.2%)	-	-	-	1 (0.9%)	-	-
Experimental study drug	9 (3%)	4 (1.4%)	13 (2%)	3 (5%)	3 (4%)	4 (3%)	-	-	-
Dendritic cell vaccinations	1 (0.4%)	-	1 (0.2%)	1 (2%)	-	-	-	-	-
No agent other than CAP, Ox, Bev	104 (37%)	103 (37%)	207 (37%)	25 (41%)	22 (28%)	38 (30%)	46 (41%)	9 (60%)	11 (73%)
Metastasectomy	8 (3%)	12 (4%)	20 (4%)	2 (3%)	5 (6%)	4 (3%)	4 (4%)	-	-
Radioembolisation	2(0.7%)	1 (0.4%)	3 (0.5%)	1 (2%)	1 (1%)	-	-	-	-
Radiofrequency ablation	3 (1%)	1 (0.4%)	4 (0.7%)	1 (2%)	-	2 (2%)	1 (0.9%)	-	-
Transarterial chemoembolization	-	1 (0.4%)	1 (0.2%)	-	1 (1%)	-	-	-	-
No agent other than CAP, Ox, Bev / No other therapy (incl. surgical / radiological interventions)	100 (36%)	99 (36%)	199 (36%)	24 (39%)	20 (25%)	36 (28%)	45 (40%)	9 (60%)	11 (73%)

Data are *n* (%) unless otherwise specified. Maint = maintenance treatment. MT = mutant. Obs = Observation. WT = wild-type. [a] Two patients with concomitant *BRAF* mutations excluded. [b] One patient with concomitant *RAS* mutation excluded.



Supplementary Figure 1. Trial profile.
dMMR = deficient mismatch repair. MMR = mismatch repair. MT = mutant. WT = wild-type. *One patient had a concomitant *KRAS* and *NRAS* mutation.

Chapter 6

Association between *KRAS* mutant allele fraction and overall survival in metastatic colorectal cancer patients treated in the phase 3 CAIRO3 study

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Abstract

Background

The poor prognostic value of *KRAS* mutations in metastatic colorectal cancer (mCRC) has recently been established. Mutant allele fractions (MAFs) or MAFs normalized for tumor purity (adjMAFs) of CRC driver genes may have important implications in the therapeutic management of mCRC. To investigate whether *KRAS* MAFs or adjMAFS are independent prognostic factors in mCRC, we analyzed the distribution within the phase 3 CAIRO3 study population, and their impact on overall survival in patients with *KRAS*-mutant tumors.

Methods

FFPE samples from the CAIRO3 study of capecitabine plus bevacizumab (CAP-B) maintenance treatment versus observation in previously untreated mCRC patients with stable disease or better after six cycles capecitabine, oxaliplatin plus bevacizumab (CAPOX-B) were analyzed by next-generation sequencing. In patients with *KRAS*-mutant tumors, we analyzed the association between *KRAS* MAFs or adjMAFs and overall survival, both linearly and by restricted cubic splines (RCS) using Cox models, adjusted for potential confounders.

Results

We analyzed a total of 170 patients with *KRAS*-mutant tumors, in which we observed marked heterogeneity in *KRAS* MAFs (median 29.9%; IQR 22.4%-40.1%) and adjMAFs (median 0.96; IQR 0.69-1.32). Median OS varied among *KRAS* MAF and adjMAF tertiles; differences were not statistically significant. In multivariable Cox regression analysis with and without RCS, we observed no significant (non-)linear associations between either *KRAS* MAFs or adjMAFs and OS.

Conclusions

In this exploratory analysis, *KRAS* MAFs or adjMAFs were not independently associated with OS in mCRC patients with *KRAS*-mutant tumors treated with CAP-B maintenance treatment versus observation after six cycles CAPOX-B.

Introduction

During recent years, next-generation sequencing (NGS) has been integrated in routine care to guide precision treatment in patients with metastatic colorectal cancer (mCRC). NGS allows for sensitive detection of mutations in putative driver genes and digital quantification of the mutational burden. The mutant allele fraction (MAF) is defined as the number of mutant reads divided by the total number of reads at a specific genomic position of interest[1]. Assessment of MAFs in driver genes may have important implications in the therapeutic management of mCRC. To date, however, the clinical implementation of mutational analyses has largely been of a dichotomous nature. The majority of studies report genomic mutations only as mutant or wild-type, at a MAF threshold of 5%.

Approximately 50% of mCRC patients carry tumors with *RAS* (*KRAS* and *NRAS*) mutations, which are negative predictors of outcome to anti-EGFR therapy[2]. Two studies have reported that mCRC patients with *KRAS* MAFs of ≥1% show resistance to anti-EGFR therapy[3,4]. The poor prognostic value of *RAS* mutations in mCRC patients has recently been established[5,6]. Two studies have investigated the prognostic value of *KRAS* MAF[1,7] with different methods and conflicting outcomes. In mCRC patients with *KRAS*-mutant tumors receiving first-line treatment including bevacizumab, a *KRAS* MAF >40% (median value) was significantly associated with decreased PFS and OS[7]. Since MAFs in tissue samples can be largely influenced by tumor purity (fraction of neoplastic cells in the sample) and ploidy (copy number gains or losses of wild-type/mutant alleles), other investigators partially adjusted the MAFs of driver gene mutations by normalizing it to the neoplastic cell content of the sample, i.e. the 'adjusted MAF' (adjMAF)[1]. Patients with *RAS*-mutant tumors showed a worse prognosis compared with patients with all wild-type tumors, though an association between *KRAS* adjMAF levels and overall survival(OS) was not observed[1]. Following these studies' conflicting results, it remains unclear whether *KRAS* MAFs or adjMAFs are independently associated with prognosis in mCRC patients with *KRAS*-mutant tumors. Furthermore, the potential prognostic implications of *KRAS* MAFs or adjMAFS have never been studied within a clinical trial.

A recent post hoc analysis of the phase 3 CAIRO3 study showed that maintenance treatment with capecitabine and bevacizumab (CAP-B) after six cycles capecitabine, oxaliplatin and bevacizumab (CAPOX-B) is effective in first-line treatment of mCRC across all mutational subgroups[8]. Regardless of treatment arm, patients with *RAS*-mutant tumors had a worse median OS compared to patients with *RAS/BRAF* wild-type tumors[8]. In order to investigate whether *KRAS* MAFs or adjMAFs are independent prognostic factors in mCRC, we analyzed (1) the distribution within the CAIRO3 study population, and (2) their clinical impact on overall survival in patients with *KRAS*-mutant tumors.

Methods

Study design and participants

CAIRO3 was an open-label, multicenter phase 3 trial carried out by the Dutch Colorectal Cancer Group (DCCG). Study design, eligibility criteria, ethical approvals, treatment regimens and outcomes have been reported previously[9]. mCRC patients with stable disease (SD), partial response (PR), or complete response (CR) according to Response Evaluation Criteria in Solid Tumours (RECIST, version 1.1) after initial treatment with six cycles of CAPOX-B were randomized (1:1) to observation or CAP-B maintenance treatment. Patients in both arms were to receive CAPOX-B reintroduction upon first progression. If CAPOX-B reintroduction was not feasible, the choice of treatment was left to the local investigator's discretion. All patients provided written informed consent. Separate informed consent was asked for tissue collection.

Molecular assessment

A detailed description of the molecular assessment with NGS has been reported elsewhere[8]. In short, formalin-fixed, paraffin-embedded (FFPE) tissue of the primary tumor or metastases was retrieved from pathology archives for central study testing. FFPE tissue sections were prepared of the primary tumor (*n*=346) or metastasis (*n*=19). Tumor purity, or tumor cell percentage, was defined as the percentage of tumor cells, compared with the total number of cells (including surrounding stromal and immune/inflammatory cells). The tumor purity was assessed by one of two experienced pathologists (ML, SMW). A minimum tumor purity of 10% was required for NGS. Following DNA extraction, tumor samples were analyzed with the Ion AmpliSeq™ Cancer Hotspot Panel v2 (Life Technologies) using NGS on the Ion Torrent PGM System™ (Life Technologies) with an average coverage depth of 1000x, as previously described[10]. Annotated and filtered variants were manually checked using IGV (Integrative Genomics Viewer)[11] by an experienced technician (ES) and investigator (KG), and by an experienced molecular biologist (WL) in case of discussion. Variants were checked for reads being >500x, mutant reads >30x, and whether the variant was not a homopolymer stretch[10]. All *KRAS* variants with a MAF of ≥5% were annotated as genomic mutations. Low frequency *KRAS* variants (MAF 1%-5%) were discussed and considered as genomic mutations in case of sufficient coverage depth and tumor purity.

Outcomes

The endpoint in this exploratory analysis was overall survival, defined as the interval between randomization until death, discontinuation or end of trial for patients who were still alive. Patients that were alive at time of this analysis (data cut-off: 21 March 2017) were included as censored data.

Statistical analysis

Out of patients with available NGS data, only patients with a *KRAS*-mutant tumor were included in the statistical analysis. Since patients with (*K*)*RAS* wild-type status have an indication for anti-EGFR therapy, which contributes to a better prognosis, we did not include patients with *KRAS* wild-type tumors as a reference group. Patients with samples carrying multiple *RAS* mutations were excluded from the analysis, as the impact of multiple *RAS* mutations on prognosis of mCRC patients is unknown.

KRAS mutant allele fraction

First, we assessed the distribution of *KRAS* MAFs among patients with *KRAS*-mutant tumors, and estimated survival curves according to *KRAS* MAF tertiles with the Kaplan-Meier method. Second, we used univariable and multivariable Cox proportional hazard models to investigate the (log)linear association between *KRAS* MAF and OS. Multivariable analyses were adjusted for the following potential confounders: treatment arm, age, sex, tumor purity, prior adjuvant chemotherapy, best response to induction treatment, WHO performance status, serum LDH at randomization, primary tumor location, primary tumor resection, stage, synchronous versus metachronous metastases, number of metastatic sites, dose reduction during induction treatment, and interval between CRC diagnosis and randomization.

Next, we assessed the potential non-linear relationship between *KRAS* MAF and OS with univariable and multivariable Cox models with restricted cubic splines (RCS), adjusted for the same potential confounders as mentioned above. RCS are a smoothly joined sum of polynomial functions that can flexibly examine a relationship between covariates and outcome without assuming any relationship a priori[12]. Additionally, RCS permits objective identification of a threshold by visualization of the model output. The spline was defined using four knots at the 5th, 35th, 65th, and 95th percentiles. Furthermore, we tested whether there was an interaction between *KRAS* MAF and tumor purity in relation to OS. Lastly, we analyzed whether a cut-off based on a median value of *KRAS* MAF was associated with OS differences, as previously investigated[7].

KRAS adjusted mutant allele fraction

In tissue samples, MAFs can be largely influenced by tumor purity and ploidy[1]. Therefore, using a different approach, we calculated *KRAS* adjusted MAFs (adjMAFs) by normalizing the MAFs to the neoplastic cell content of the tissue section used for NGS (*KRAS* MAF / tumor purity). Subsequently, we performed the same statistical analyses as described for *KRAS* MAF in order to investigate the potential linear and non-linear association between *KRAS* adjMAF and OS. Since *KRAS* adjMAF was already normalized for tumor purity, we did not include tumor purity as a potential confounder in the multivariable Cox models.

We report nominal (i.e. without multiple testing adjustment), two-sided *p*-values (significance level set to 0.05). Statistical analyses were carried out using IBM SPSS Statistics 23 and R version 3.0.3.

Results

Study design and participants

Between May 2007 and October 2012, 558 mCRC patients were randomized to observation or maintenance treatment. One patient withdrew informed consent before treatment initiation. NGS data were available in 365/558 (65%) patients. Out of these patients, *KRAS* mutation status was available in 363 patients. *KRAS* mutations were detected in 172/363 (47%) patients. Two patients with a double *RAS* mutation (one with a double *KRAS* mutation [G12V and G13D]; one with a concomitant *KRAS* [G12D] and *NRAS* [R102*] mutation) were excluded from the analysis, since the prognostic impact of multiple *RAS* mutations is unknown.

Thus, a total of 170 patients with *KRAS*-mutant tumors (primary tumor (*n*=163) or metastases (*n*=7)) were included in the analysis (Supplementary Figure 1). Median tumor purity was 30% (range: 10%-80%). Median OS in patients with *KRAS*-mutant tumors was 18.2 months (95%CI 15.8-20.6). Specific *KRAS* mutation variants are shown in Supplementary Table 1. One patient had a concomitant $^{\text{non-V600}}$*BRAF* mutation (G460V). Mismatch repair status had been tested in 108/170 patients, of which two had mismatch repair deficiency (dMMR).

Association between KRAS MAF and OS

KRAS MAF distribution and patient characteristics

We observed a marked heterogeneity in the distribution of *KRAS* MAFs among patients with *KRAS*-mutant tumors (Figure 1A), with a median of 30% (IQR 22.4%-40.1%). Patient characteristics according to *KRAS* MAF tertiles are shown in Table 1. The percentage of patients with synchronous mCRC increased with increasing tertile of *KRAS* MAF, as well as the percentage of patients with stable disease as best response to induction treatment, and right-sided colon tumors. Subsequent therapies received during the course of metastatic disease were comparable between *KRAS* MAF tertiles (Supplementary Table 2).

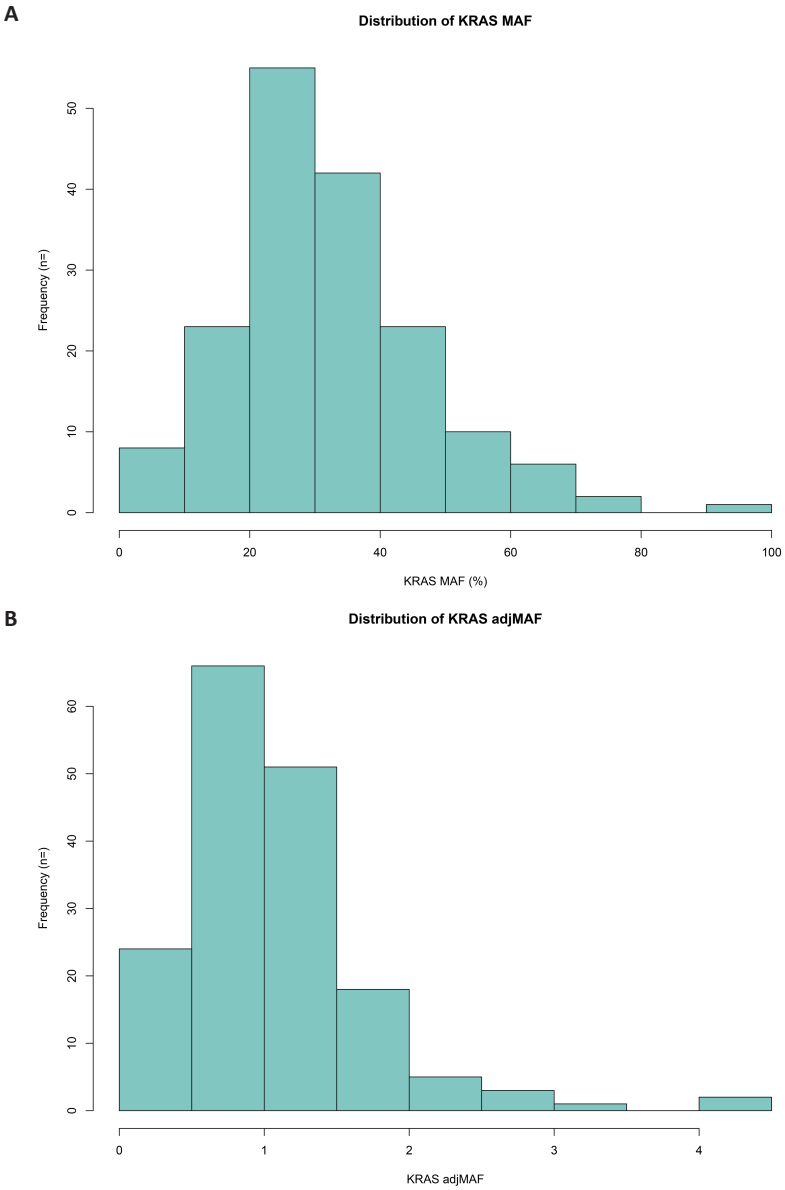

Figure 1. Distribution of (A) *KRAS* MAFs and (B) *KRAS* adjMAFs among 170 mCRC patients with *KRAS*-mutant tumors.

Table 1. Patient characteristics according to *KRAS* MAFs and *KRAS* adjMAFs

		KRAS MAF (n=170)			*KRAS* adjMAF (n=170)		
	Total study population	**First tertile (lowest – 23.9%)**	**Second tertile (>23.9 – 35.6%)**	**Last tertile (>35.6% – highest)**	**First tertile (lowest – 0.77)**	**Second tertile (>0.77 – 1.16)**	**Last tertile (>1.16 – highest)**
	***n*=557**	***n*=55**	***n*=57**	***n*=58**	***n*=56**	***n*=57**	***n*=57**
Age							
Median (range)	64 (26-81)	65 (41-81)	64 (43-78)	64 (44-81)	63 (43-79)	64 (46-81)	66 (41-78)
Sex							
Male	361 (65%)	35 (64%)	37 (65%)	36 (62%)	35 (63%)	37 (65%)	36 (63%)
WHO performance status							
0	345 (62%)	34 (62%)	33 (58%)	33 (57%)	35 (63%)	31 (54%)	34 (60%)
1	212 (38%)	21 (38%)	24 (42%)	25 (43%)	21 (38%)	26 (46%)	23 (40%)
Serum lactate dehydrogenase							
Above normal	312 (56%)	32 (58%)	27 (47%)	34 (59%)	32 (57%)	39 (68%)	22 (39%)
Prior adjuvant chemotherapy							
Yes	188 (34%)	22 (40%)	20 (35%)	21 (36%)	16 (29%)	23 (40%)	24 (42%)
No	369 (66%)	33 (60%)	37 (65%)	37 (64%)	40 (71%)	34 (60%)	33 (58%)
Best response to induction treatment							
Stable disease	191 (34%)	19 (35%)	23 (40%)	25 (43%)	24 (43%)	23 (40%)	20 (35%)
Partial or complete response	366 (66%)	36 (66%)	34 (60%)	33 (57%)	32 (57%)	34 (60%)	37 (65%)
Site of primary tumor							
Right colon[a]	122 (22%)	11 (20%)	13 (23%)	21 (36%)	12 (21%)	13 (23%)	20 (35%)
Left colon[b]	406 (73%)	42 (76%)	44 (77%)	35 (60%)	42 (75%)	44 (77%)	35 (61%)
Colon n.o.s.	19 (3%)	1 (2%)	0 (0%)	2 (3%)	1 (2%)	0 (0%)	2 (4%)
Multiple sites	10 (2%)	1 (2%)	9 (0%)	0 (0%)	1 (2%)	9 (0%)	0 (0%)
Number of metastatic sites							
1	229 (41%)	23 (42%)	23 (40%)	23 (40%)	26 (47%)	27 (47%)	16 (%)
>1	302 (54%)	30 (55%)	34 (60%)	33 (57%)	29 (53%)	30 (53%)	38 (%)
Unknown	26 (5%)	2 (4%)	0 (0%)	2 (3%)	0 (0%)	0 (0%)	3 (%)

Interval of metastases and primary tumor resection status							
Synchronous[c], resection	180 (32%)	16 (29%)	17 (30%)	30 (52%)	23 (41%)	22 (39%)	18 (32%)
Synchronous, no resection	230 (41%)	15 (27%)	20 (35%)	18 (31%)	18 (32%)	15 (26%)	20 (35%)
Metachronous	147 (26%)	24 (44%)	20 (35%)	10 (17%)	15 (27%)	20 (35%)	19 (33%)
Synchronous vs metachronous mCRC							
Synchronous	410 (74%)	31 (56%)	37 (65%)	48 (83%)	41 (73%)	37 (65%)	38 (67%)
Metachronous	147 (26%)	24 (44%)	20 (35%)	10 (17%)	15 (27%)	20 (35%)	19 (33%)
Resection primary tumor							
Yes	327 (59%)	40 (73%)	37 (65%)	40 (69%)	38 (68%)	42 (74%)	37 (65%)
Platelet count at start induction treatment							
< 400 x 10^9/L	346 (62%)	37 (67%)	37 (65%)	33 (57%)	37 (67%)	35 (61%)	35 (61%)
≥ 400 x 10^9/L	163 (29%)	17 (31%)	17 (30%)	16 (28%)	18 (33%)	14 (25%)	15 (26%)
Unknown	48 (9%)	1 (2%)	3 (5%)	9 (16%)	0 (0%)	8 (14%)	7 (12%)
Treatment arm							
Observation	279 (50%)	26 (47%)	28 (49%)	34 (59%)	23 (41%)	33 (58%)	32 (56%)
Maintenance	278 (50%)	29 (53%)	29 (51%)	24 (41%)	33 (59%)	24 (42%)	25 (44%)
Tissue source							
Primary tumor	-	55 (100%)	55 (97%)	53 (91%)	55 (98%)	56 (98%)	52 (91%)
Metastasis	-	0 (0%)	2 (4%)	5 (9%)	1 (2%)	1 (2%)	5 (9%)
Tumor purity							
Median (range)	-	20 (10-80)	30 (10-70)	40 (10-70)	43 (10-80)	30 (10-60)	25 (10-70)
Mismatch repair status							
dMMR	4 (0.7%)	2 (4%)	0 (0%)	0 (0%)	0 (0%)	2 (4%)	0 (0%)
pMMR	275 (49%)	31 (56%)	35 (61%)	40 (69%)	32 (57%)	40 (70%)	34 (60%)
unknown	278 (50%)	22 (40%)	22 (39%)	18 (31%)	24 (43%)	15 (26%)	23 (40%)

Patient characteristics in total study population and in *KRAS* MAF and *KRAS* adjMAF tertiles (0 – 33rd, 33rd-66th, 66th–100th percentile, respectively). Data are *n* (%) unless otherwise specified. Due to rounding, not all percentages total 100. dMMR = deficient mismatch repair. pMMR = proficient mismatch repair. [a] Right colon: cecum to transverse colon. [b] Left colon: splenic flexure to rectum. [c] Synchronous metastases: distant metastases discovered ≤ 6 months after diagnosis of the primary tumor.

Survival analyses

Patients with *KRAS* MAFs in the second tertile (MAF>23.9%-35.6%) had a lower median OS (16.3 months [95%CI 12.5-20.1]) compared with patients with *KRAS* MAFs in the first tertile (MAF up to 23.9%; 18.7 months [13.4-24.1]) and last tertile (MAF>35.6%; 18.2 months [14.2-22.2]). These differences were not statistically significant (Table 2). In both univariable (p=0.466) and multivariable (p=0.424) Cox regression analyses, we found no significant association between *KRAS* MAF and OS. Although univariable Cox regression analyses with RCS showed a significant association between *KRAS* MAF and OS (p=0.035)(Supplementary Figure 2A), this was not observed after adjustment for potential confounders in multivariable Cox regression analyses with RCS (p=0.585; Figure 2A). There was no significant interaction between *KRAS* MAF and tumor purity in relation to OS (p=0.453). Furthermore, *KRAS* MAFs ≤30% or >30% (median value) were not associated with significant differences in median OS (p=0.513; data not shown).

Table 2. Kaplan-Meier survival estimates

	***KRAS* MAF (n=170)**		
	First tertile (lowest – 23.9%)	**Second tertile (>23.9% – 35.6%)**	**Last tertile (>35.6% – highest)**
	***n* =55**	***n* =57**	***n* =58**
Overall survival			
Events	53	57	57
Median (months)	18.7	16.3	18.2
95% CI	13.4-24.1	12.5-20.1	14.2-22.2
Log-Rank *p*-value		0.540	
	***KRAS* adjusted MAF (n=170)**		
	First tertile (lowest – 0.77)	**Second tertile (>0.77-1.16)**	**Last tertile (>1.16 – highest)**
	***n* =56**	***n* =57**	***n* =57**
Overall survival			
Events	53	57	57
Median (months)	15.8	21.9	18.2
95% CI	11.0-20.6	16.9-27.0	13.3-23.1
Log-Rank *p*-value		0.958	

Association between KRAS adjMAF and OS

KRAS adjMAF distribution and patient characteristics

The distribution of *KRAS* adjMAFs varied among patients with *KRAS*-mutant tumors (Figure 1B), with a median of 0.96 (IQR 0.69-1.32). Patient characteristics according to *KRAS* adjMAF tertiles are shown in Table 1. Patients with *KRAS* adjMAFs in the last tertile had a lower proportion of elevated LDH compared with the first and second tertiles. Subsequent treatments for mCRC were comparable between *KRAS* adjMAF tertiles, except for a higher percentage of irinotecan received in patients within the second *KRAS* adjMAF tertile (Supplementary Table 1).

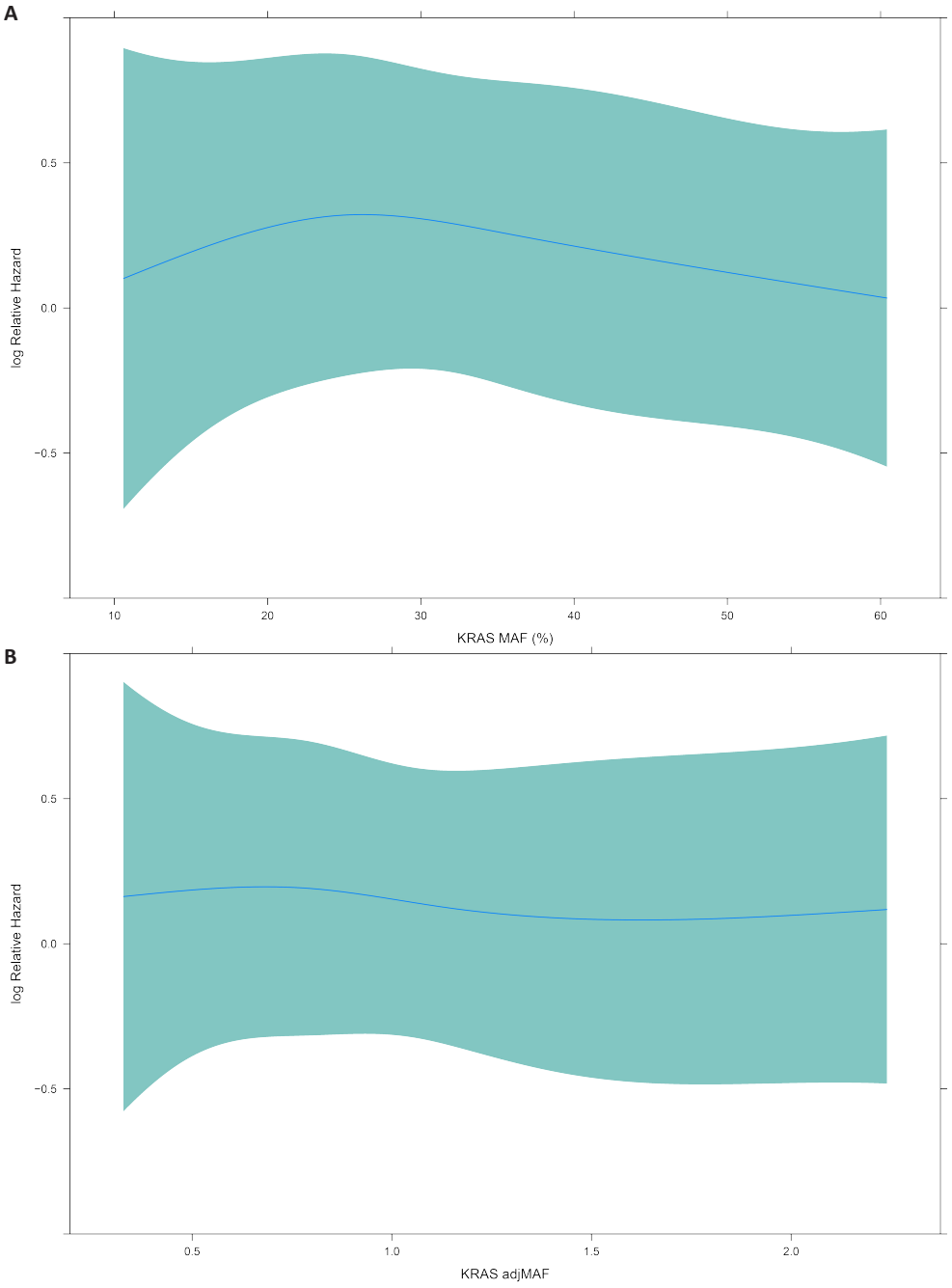

Figure 2. Multivariable RCS modeling of the association between **(A)** *KRAS* MAF or **(B)** *KRAS* adjMAF and overall survival. The log of the HRs derived from the multivariable Cox model is shown on the y-axis. The 95% CIs of the adjusted HRs are represented by the shaded area.

Survival analysis

Patients with *KRAS* adjMAFs in the second tertile (adjMAF>0.77-1.16) had a higher median OS (21.9 months [95%CI 16.9-27.0]) compared to patients with *KRAS* adjMAFs in the first tertile (adjMAF up to 0.77; 15.8 months [11.0-20.6]) and last tertile (adjMAF>1.16; 18.2 months [13.3-23.1]). These differences were not statistically significant (Table 2). In univariable (p=0.651) and multivariable (p=0.898) Cox regression analyses, we found no significant association between *KRAS* MAF and OS. Likewise, univariable and multivariable Cox regression analyses with RCS did not show a significant association between *KRAS* adjMAF and OS (univariable: p=0.875; multivariable: p=0.901)(Supplementary Figure 2B; Figure 2B).

Differences between KRAS MAFs and adjMAFs

We observed a heterogeneity in median OS when comparing *KRAS* MAF with adjMAF tertiles, particularly among the first and second tertiles (Table 2). While patients with *KRAS* MAFs in the second tertile had a lower median OS compared with other *KRAS* MAF tertiles, patients with *KRAS* adjMAFS in the second tertile had a higher median OS compared with other *KRAS* adjMAF tertiles. Besides variations in median tumor purity, patient characteristics were comparable between *KRAS* MAF and *KRAS* adjMAF tertiles (Table 1). The percentage of patients that received irinotecan was slightly higher in the second *KRAS* adjMAF tertile compared with the second *KRAS* MAF tertile.

Discussion

In this exploratory analysis of the CAIRO3 study, we investigated the distribution and independent prognostic significance of *KRAS* MAFs and *KRAS* adjMAFs in patients with *KRAS*-mutant tumors. Among these patients, there was a marked variation of *KRAS* MAFs and adjMAFs. Using different statistical methods, we found no association between *KRAS* MAF and OS. Since MAFs are influenced by tumor purity, we also analyzed the prognostic value of *KRAS* MAFs with normalization for tumor purity (*KRAS* adjMAF). Again, we observed no association between *KRAS* adjMAF and OS. Our findings show that within CAIRO3 trial patients with *KRAS*-mutant tumors, *KRAS* MAFs or adjMAFs were not independently associated with OS.

We observed marked heterogeneity in the distribution of *KRAS* MAFs and adjMAFs among patients with *KRAS*-mutant tumors. The phase 3 CAPRI-GOIM trial also demonstrated substantial heterogeneity in MAFs among mCRC patients with tumors carrying *KRAS, NRAS, PIK3CA* and *BRAF* mutations[13]. Moreover, median OS varied among *KRAS* MAF and adjMAF tertiles, though these differences were not statistically significant. Median OS also varied

between *KRAS* MAF and adjMAF tertiles. Of note, these were unadjusted analyses in small patient subgroups.

Several phase 3 mCRC trials have demonstrated that *KRAS* mutations have a negative prognostic value[5,6]. Our recent report showed that mCRC patients with *KRAS*-mutant tumors had a worse prognosis compared to patients with *KRAS* wild-type tumors[8]. In the current analysis, we investigated whether *KRAS* MAFs or adjMAFS are candidates to optimize personalized medicine in mCRC through more accurate prediction of prognosis. Our findings show that *KRAS* MAFs or adjMAFS cannot be used to predict OS in mCRC patients treated with observation versus CAP-B maintenance treatment following 6 cycles of CAPOX-B.

In contrast with our findings, Vincenzi et al. reported a significant association between *KRAS* mutation rate and prognosis in a multicenter cohort of 263 mCRC patients treated with bevacizumab-containing first-line therapy[7]. The investigators found that a *KRAS* mutation rate of >40% was a significant predictor of worse PFS and OS, both in univariable and multivariable analyses. However, their cut-off of 40% (median value) was arbitrarily chosen and not independently validated. Furthermore, only samples with a tumor purity of ≥60% were included for *KRAS* mutation analysis using pyrosequencing[7], although a minimum tumor purity of ≥10% is considered sufficient for pyrosequencing and other NGS technologies[10,14]. In our analysis, we used RCS for objective identification of a threshold by visualization of the model output. No significant OS differences were found when we used a 30% cut-off based on the median value of *KRAS* MAF. In addition, we included all patients with *KRAS*-mutant tumors in our analysis, provided that the sample used for NGS had a tumor purity of ≥10%.

Our results regarding *KRAS* adjMAFs are in line with the study of Dienstmann et al.[1] Although the investigators found *RAS* and *BRAF*^{V600E} mutations to have a negative effect on survival in a single-center cohort of 763 mCRC patients, the adjMAFs of these driver mutations did not impact on survival and did not help predict benefit with matched targeted therapy[1]. Similar to their study, we performed quantitative assessment of *KRAS* MAFs with normalization for tumor purity (*KRAS* adjMAF). This approach suffers from the approximate estimation of the neoplastic cell count[15]. In order to improve this in our analysis, estimation of tumor purity was done by two experienced pathologists and always in the same section used for NGS. Although ideally, estimation of tumor purity should be done by one pathologist to prevent interobserver variability[15], this was not possible for logistic reasons. Although an adjMAF should be no more than 0.5 in a pure tumor sample with a heterozygous mutation, a large proportion of our *KRAS*-mutant study population had *KRAS* adjMAF values exceeding 1.0. Possible explanations could be PCR amplification bias of the mutant allele, genomic amplification of the mutant allele, loss of the wild-type allele, or a structural underestimation of tumor purity.

Following two studies with conflicting results[1,7], this is the first study to investigate the prognostic implications of *KRAS* MAFs or adjMAFS within a phase 3 trial population. Mutational analyses were performed in a single certified Molecular Pathology laboratory. Potential confounders were adjusted for in multivariable analyses, including tumor purity when analyzing *KRAS* MAFs. Out of 170 patients with *KRAS*-mutant tumors, only two had dMMR. Although MSI is a poor prognostic factor in mCRC patients, numbers were too small to adjust for this variable in multivariable analyses. One patient had a concomitant $^{non\text{-}V600}$*BRAF* mutation. However, $^{non\text{-}V600}$*BRAF* mutations represent a distinct molecular subtype of mCRC with better outcome compared to patients with ^{V600E}*BRAF* mutations[16].

This study has some limitations. First, the CAIRO3 study consists of a selected subgroup of patients with SD or better after 6 cycles of CAPOX-B, which limits the generalizability of our subgroup analyses to the broader population of mCRC. Second, this was a retrospective subgroup analysis in a relatively small sample size of 170 patients. We chose OS as primary endpoint to investigate the prognostic value of *KRAS* MAFs and adjMAFS. Although this endpoint is being influenced by subsequent treatment lines, we found no clear imbalances in subsequent treatments between *KRAS* MAF or adjMAF tertiles, except for a somewhat higher percentage of irinotecan received by patients in the second *KRAS* adjMAF tertile. Another limitation is that *KRAS* MAFs and adjMAFS were determined based on a single FFPE tumor sample. Several studies have demonstrated a high concordance of *KRAS* mutation status in tissue derived from the primary tumor or metastasis[17–19]. However, the relevance of intra-tumor heterogeneity as a result of accumulation of genetic aberrations and selective pressure has become increasingly apparent[20,21]. Therefore, a single FFPE tumor sample is likely to underestimate the complexity of the genomic landscape of the tumor[22]. Furthermore, extended periods between sampling and clinical application of the results may result in an altered genetic tumor composition. In addition, targeted NGS focuses on a selected set of hotspot mutations. As a result, other potentially relevant mutations could have been missed, as well as epigenetic changes and copy number variations. As a promising alternative, repeated blood sampling, or 'liquid biopsies', offer the opportunity to systematically monitor tumor-associated genetic aberrations (including *KRAS*) in the blood, and to track genomic evolution of the tumor[22].

In conclusion, this exploratory analysis of the CAIRO3 study shows a heterogeneous distribution of *KRAS* MAFs and adjMAFs in mCRC patients treated with CAP-B maintenance treatment versus observation after 6 cycles CAPOX-B. Although previous studies have demonstrated the negative prognostic value of *KRAS* mutations in mCRC, we did not observe an association between *KRAS* MAFs or adjMAFS and OS. Our findings suggest that *KRAS* MAFs or adjMAFS are not independently associated with prognosis in mCRC patients with *KRAS*-mutant tumors.

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Supplementary Material

Supplementary Table 1. *KRAS* mutation variants

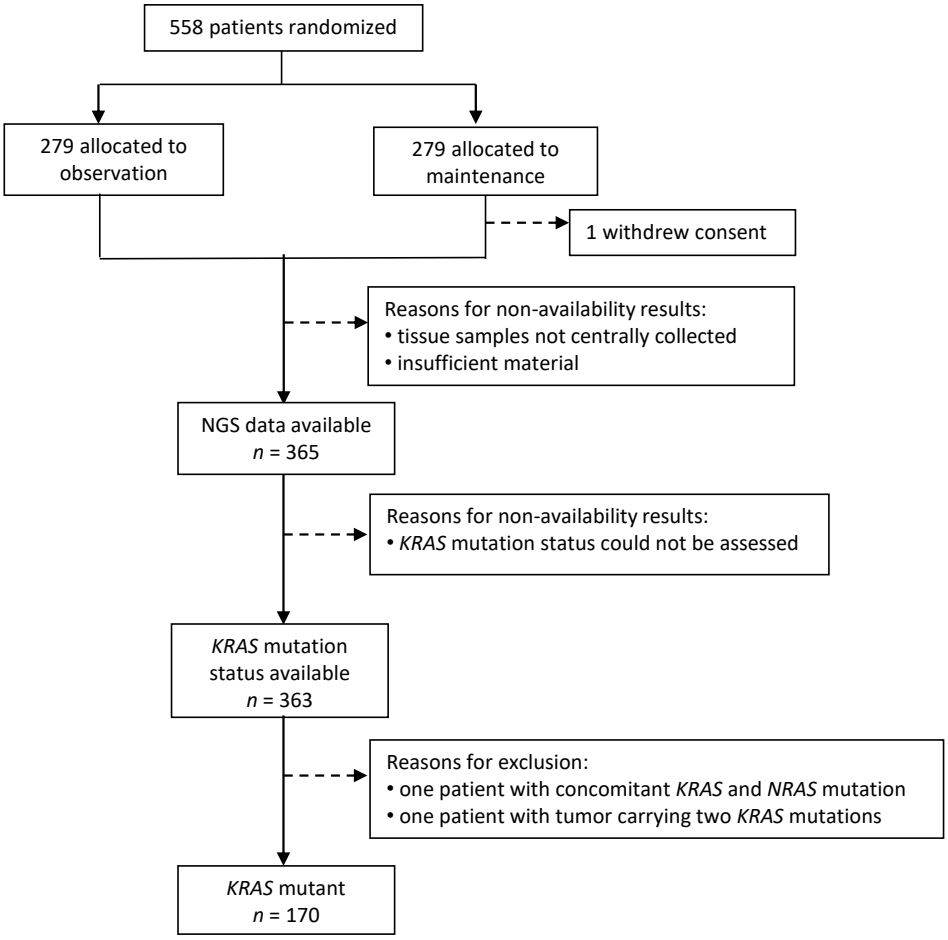
	Number of variants
***KRAS* exon 2 (*n*=154)**	
G12D	43
G12V	50
G12S	9
G12C	11
G12A	10[a]
G12F	1
G12L	1
G13D	27
G13C	1
AG11-12AV	1
***KRAS* exon 3 (*n*=3)**	
Q61H	2
Q61L	1
***KRAS* exon 4 (*n*=13)**	
A146T	8
A146V	4
A146QQ	1

Frequencies based on analyzed population with *KRAS*-mutant tumors. [a] One patient had a tumor with a *KRAS* G12A and *BRAF* G460V mutation.

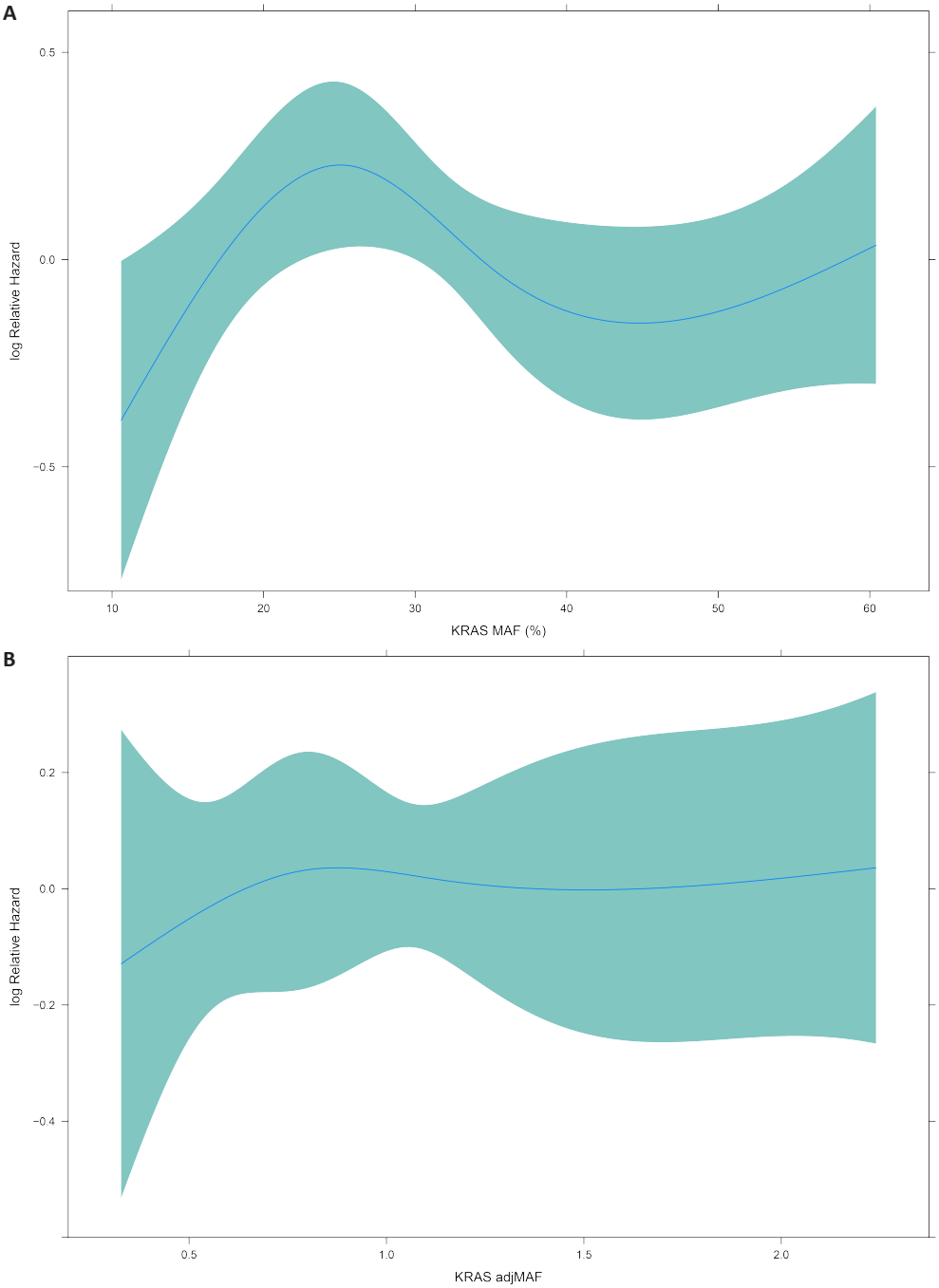
Supplementary Table 2. Subsequent treatments received during the course of metastatic disease in patients with *KRAS*-mutant tumors

		***KRAS* MAF (n=170)**		
	all *KRAS* MT	**First tertile (lowest – 23.9%)**	**Second tertile (>23.9% – 35.6%)**	**Last tertile (>35.6% – highest)**
	***n*=170**	***n* =55**	***n* =57**	***n* =58**
Irinotecan	104 (61%)	35 (64%)	35 (61%)	34 (59%)
Anti-EGFR therapy	10 (6%)	2 (4%)	4 (7%)	4 (7%)
Tegafur-Uracil	3 (2%)	0 (0%)	0 (0%)	3 (5%)
Aflibercept/Placebo	2 (1%)	1 (2%)	1 (2%)	0 (0%)
Regorafenib	2 (1%)	2 (4%)	0 (0%)	0 (0%)
MEK-I	1 (1%)	1 (2%)	0 (0%)	0 (0%)
Metastasectomy	5 (3%)	1 (2%)	3 (5%)	1 (2%)
		***KRAS* adjusted MAF (n=170)**		
	all *KRAS* MT	**First tertile (lowest – 0.77)**	**Second tertile (>0.77-1.16)**	**Last tertile (>1.16 – highest)**
	***n* =170**	***n* =56**	***n* =57**	***n* =57**
Irinotecan	104 (61%)	31 (55)	41 (72%)	32 (56%)
Anti-EGFR therapy	10 (6%)	4 (7%)	3 (5%)	3 (5%)
Tegafur-Uracil	3 (2%)	0 (0%)	1 (2%)	2 (4%)
Aflibercept/Placebo	2 (1%)	0 (0%)	2 (4%)	0 (0%)
Regorafenib	2 (1%)	0 (0%)	2 (4%)	0 (0%)
MEK-I	1 (1%)	0 (0%)	1 (2%)	0 (0%)
Metastasectomy	5 (3%)	2 (4%)	1 (2%)	2 (4%)

Data are *n* (%) unless otherwise specified. MAF = mutant allele fraction. MT = mutant.



Supplementary Figure 1. Flow chart of study design



Supplementary Figure 2. Univariable RCS modeling of association between (A) *KRAS* MAF or (B) *KRAS* adjMAF and overall survival. The log of the HRs derived from the univariable Cox model is shown on the y-axis. The 95% CIs of the HRs are represented by the shaded area.

Chapter 7

Clinicopathological factors influencing outcome in metastatic colorectal cancer patients treated with fluoropyrimidine and bevacizumab maintenance treatment *vs* observation: an individual patient data meta-analysis of two phase 3 trials

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Abstract

Background

The CAIRO3 and AIO 0207 trials demonstrated the efficacy of fluoropyrimidine plus bevacizumab (FP+Bev) maintenance treatment in metastatic colorectal cancer (mCRC) patients. In this individual patient data meta-analysis with updated follow-up, we aim to provide more precise estimates of treatment effects and to identify subgroups that benefit most from maintenance treatment or observation.

Methods

In 871 patients, randomised to FP+Bev maintenance treatment or observation, we investigated whether treatment effect was modified by sex, age, performance status, response to induction treatment, primary tumour location, number of metastatic sites, disease stage and primary tumour resection, serum LDH, platelet count, CEA, and *RAS/BRAF* mutation status. Primary endpoint was time to second progression after reintroduction of the induction regimen (PFS2). Secondary endpoints were first progression-free survival (PFS1) and overall survival (OS).

Results

At a median follow-up of 68.5 months (IQR 54.6-87.0 months), maintenance treatment was more effective compared with observation in PFS1 (HR 0.40[95%CI 0.34-0.47]) and PFS2 (HR 0.70[0.60-0.81]). No subgroups were identified that did not benefit from maintenance treatment in PFS1 and PFS2; no clinically relevant subgroup effects were observed. Regarding OS, pooled results were not significant (HR 0.91[0.78-1.05]), and the trials showed marked heterogeneity in overall treatment effect and subgroup effects.

Conclusions

FP+Bev maintenance treatment is effective in all patients, regardless of the investigated subgroups.

Introduction

The clinical outcome of patients with metastatic colorectal cancer (mCRC) has significantly improved during the last decade, partly due to the increased availability of targeted drugs. The addition of bevacizumab to fluoropyrimidine-based chemotherapy has resulted in a prolonged overall and progression-free survival, and is considered a standard option in first-line treatment of mCRC[1–5]. Until recently, the optimal duration of systemic therapy including bevacizumab in first-line treatment of mCRC was not well established.

The phase 3 CAIRO3 and AIO 0207 trials showed that maintenance treatment with fluoropyrimidine and bevacizumab is the preferred strategy in mCRC patients with stable disease or better after induction treatment with a fluoropyrimidine, oxaliplatin and bevacizumab, as it maintains disease control and quality of life without relevant toxicity[6–8]. However, not all patients may benefit from this strategy. The ability to identify subgroups of patients in which a treatment break is safe and on the other hand those in which continuous treatment is prerequisite for better survival, would improve clinical decision-making and reduce therapy costs.

In this individual patient data (IPD) meta-analysis of the CAIRO3 and AIO 0207 trials with updated follow-up, we aim to provide more precise estimates of treatment effects regarding the use of fluoropyrimidine plus bevacizumab maintenance treatment after induction treatment with combination chemotherapy and bevacizumab. In addition, we aim to identify patient subgroups according to clinical and pathological characteristics that benefit most from fluoropyrimidine and bevacizumab maintenance treatment or observation.

Methods

Study design and participants

This analysis is based on individual patient data from two open-label, randomised phase 3 trials on maintenance treatment *vs* observation in first-line treatment of mCRC: CAIRO3 (NCT00442637) and AIO 0207 (NCT00973609)[6,7]. The CAIRO3 study, a superiority trial done by the Dutch Colorectal Cancer Group, was conducted in 64 hospitals in the Netherlands between May 30, 2007 and October 15, 2012. The AIO 0207 study, a non-inferiority trial conducted by the AIO Studien gGmbH, enrolled patients from 106 institutions (55 hospitals and 51 private practices) in Germany between September 17, 2009 and February 21, 2013. Detailed eligibility criteria, ethical approvals, treatment protocols and outcomes have been reported elsewhere[6,7]. In brief, eligible patients in both trials were older than 18 years, had WHO/ECOG performance status (PS) 0-2, histologically proven colorectal adenocarcinoma with distant metastases, previously untreated for metastatic disease, with stable disease, partial or complete response according to Response Evaluation Criteria in Solid Tumours

(RECIST, version 1.1) after induction treatment with a fluoropyrimidine, oxaliplatin and bevacizumab.

In the two-armed CAIRO3 study, patients with stable disease or better after 6 cycles (18 weeks) induction treatment with capecitabine, oxaliplatin and bevacizumab (CAPOX-B) in whom reintroduction of oxaliplatin appeared feasible were randomised (1:1) to either observation or maintenance treatment with capecitabine and bevacizumab (CAP-B). Patients were not enrolled if they had experienced toxicity from the fluoropyrimidine, oxaliplatin, or bevacizumab during induction treatment that would prevent its safe continuation or reintroduction. Induction treatment was not an integral part of the trial. Randomisation was stratified by previous adjuvant chemotherapy (yes *vs* no), response to induction treatment (stable disease *vs* complete or partial response), WHO/ECOG PS (0 *vs* 1), serum lactate dehydrogenase (LDH) concentrations (normal *vs* abnormal), and treatment centre.

In the three-armed AIO 0207 study, eligible patients were registered prior to the start of a 24-week induction treatment with a fluoropyrimidine (infusional fluoropyrimidine or capecitabine), oxaliplatin and bevacizumab. The choice of a standard protocol (i.e. FOLFOX, CAPOX-B) was left to the local investigator's discretion. Patients with stable disease or better and without option for metastasectomy after 24 weeks of induction treatment were randomised (1:1:1) to either maintenance treatment with any fluoropyrimidine and bevacizumab, bevacizumab monotherapy, or observation. Preliminary discontinuation of oxaliplatin or other drugs (e.g. due to toxicity) during induction treatment was allowed. Randomisation was stratified by response to induction treatment (stable disease *vs* complete or partial response), treatment with oxaliplatin (stopped before termination of induction treatment *vs* ongoing until end of induction phase), previous adjuvant therapy (with oxaliplatin *vs* without oxaliplatin *vs* no adjuvant treatment), and WHO/ECOG PS (0-1 *vs* 2). Patients from the bevacizumab monotherapy arm were excluded from the present analysis. All patients in both trials provided written informed consent.

Study treatments

In the CAIRO3 study, maintenance treatment consisted of capecitabine 625 mg/m^2 orally twice daily continuously, plus bevacizumab 7.5 mg/kg intravenously every 3 weeks. Patients with progressive disease in either the observation or maintenance arm were to receive reintroduction of the induction treatment regimen, i.e. CAPOX-B. Reintroduced CAPOX-B was to be continued until progression, death, or an unacceptable adverse event, whichever occurred first. If CAPOX-B reintroduction was not possible after all due to persisting sensory neuropathy (grade ≥2) or any other reason, the treatment choice was left to the local investigator's discretion.

In the AIO 0207 study, randomised patients received either continuation of a fluoropyrimidine (infusional every 2 weeks, or capecitabine every 3 weeks in standard

dosages; the fluoropyrimidine could be switched between induction and maintenance treatment) plus bevacizumab (7.5 mg/kg every 3 weeks, or 5 mg/kg every 2 weeks), or bevacizumab monotherapy (same dosage), or no treatment. Maintenance treatment was continued until disease progression, unacceptable toxicity, surgical resection, other ablative treatment, at patient's request, or local investigator's decision. If either the fluoropyrimidine or bevacizumab was discontinued before progression, the remaining drug was continued as monotherapy in the fluoropyrimidine plus bevacizumab arm. At first progression, all patients were to receive reintroduction of the induction treatment regimen (i.e. any fluoropyrimidine, oxaliplatin plus bevacizumab) according to protocol. Reintroduction included all drug components of the induction treatment, except for those that could not be used due to persistent toxicity or contraindications. If reintroduction of the induction treatment regimen was not possible for any reason, the choice of treatment was at the local investigator's discretion.

Outcomes

Patients in both trials were assessed for disease status according to RECIST criteria. The primary endpoints in both trials (time to second progression upon reintroduction of the induction treatment regimen in CAIRO3, and time to failure of strategy in AIO 0207) were comparable in definition. The primary endpoint in this IPD meta-analysis was second progression-free survival (PFS2), defined as the interval between randomisation and second progression (for those who had a first progression) while under treatment with reintroduction of a fluoropyrimidine, oxaliplatin and bevacizumab, or until the beginning of another treatment (including a new drug), death or end of trial for patients who did not have a second progression. PFS2 was regarded as equal to PFS1 if patients did not receive reintroduction of the induction regimen for any reason, or if a valid response evaluation was not performed. Secondary endpoints in both trials included time until first progression (PFS1), and overall survival (OS). PFS1 was defined as the interval between randomisation and first progression while under maintenance treatment or observation, or until death or end of trial for patients without progression. OS was defined as the time from randomisation to death from any cause or date of last follow-up, at which point patients who were still alive were censored. Cut-off dates for the present analysis were March, 2017 for CAIRO3, and December 2016 for AIO 0207.

Statistical analysis

This pooled analysis was based on individual patient data of the intention-to-treat population of the CAIRO3 and AIO 0207 trials, comprising all patients who were randomised to fluoropyrimidine plus bevacizumab maintenance treatment or observation. Patients from the bevacizumab monotherapy arm of the AIO 0207 study were excluded from the analyses, since the CAIRO3 study did not include this treatment option.

First, the median duration of follow-up was calculated for the pooled study population using the reverse Kaplan-Meier method. Survival curves were estimated with the Kaplan-Meier method and compared with the log-rank test. Next, we performed subgroup analyses including the following parameters: age (< or ≥ 70 years at randomisation), sex (male *vs* female), primary tumour location (colon *vs* rectum or rectosigmoid), response to induction treatment (stable disease *vs* complete or partial response), WHO/ECOG PS (0 *vs* 1-2), number of metastatic sites (1 *vs* >1), stage of disease and primary tumour resection status (synchronous, resected *vs* synchronous, non-resected *vs* metachronous disease), serum LDH at randomisation (normal *vs* elevated), platelet count (<400 *vs* ≥400 x10^9/L) and serum CEA (≤ 20 *vs* >20 ng/mL) at start of induction treatment, and *RAS/BRAF* mutation status (*RAS* plus ^{V600E}*BRAF* wild-type *vs RAS* mutant *vs* ^{V600E}*BRAF* mutant). No power, sample size, or sensitivity calculations were done as these subgroup analyses were exploratory in nature. We analysed overall and subgroup treatment effects using mixed effect Cox models with study as random intercept to take clustering of patients within studies into account, and treatment (and any co-variables) as fixed effects to calculate hazard ratios (HRs) and 95% confidence intervals (CIs). We refrained from including a random treatment slope per study as none of the models significantly improved upon such extension. Analyses were stratified for prior adjuvant chemotherapy, response to induction treatment, and WHO/ECOG PS, and adjusted for the following potential confounders by including as co-variable: age, sex, stage, primary tumour location, primary tumour resection, number of metastatic sites, LDH at randomisation, and the interval between primary diagnosis and randomisation. Subgroup analyses regarding stage of disease combined with primary tumour resection status were not adjusted for stage and primary tumour resection. Patients with missing values in variables relevant for a particular analysis were excluded from that analysis. Interaction terms between treatment and each subgroup variable were used to assess and test heterogeneity of treatment effects. Inspection of Schoenfeld residuals showed that the proportionality of the hazard assumption was not violated. We report nominal, two-sided *P*-values (significance level set to 0.05), without taking multiple testing into account. Statistical analyses were performed using IBM SPSS Statistics, version 21.0 (Armonk, NY: IBM Corp) and R version 3.0.3 (particularly library coxme version 2.2-5).

Results

Patients

By pooling individual patient data from both trials, including both treatment arms of CAIRO3 and two out of three treatment arms of AIO 0207, we obtained data of 871 patients: 437 assigned to the observation group and 434 assigned to the fluoropyrimidine plus bevacizumab maintenance treatment group (Figure 1). Patient characteristics were

comparable between treatment groups, except for a higher percentage of patients with age ≥70 years in the observation group (Table 1). Differences in overall patient characteristics between CAIRO3 and AIO 0207 (bevacizumab monotherapy arm excluded) were found regarding WHO/ECOG PS, prior adjuvant chemotherapy, primary tumour location, stage of disease combined with primary tumour resection status, and serum LDH at randomisation (Supplementary Table 1).

Table 1. Patient characteristics

	Observation (*n*=437)	FP+Bev (*n*=434)
Study		
CAIRO3	279 (64%)	278 (64%)
AIO 0207	158 (36%)	156 (36%)
Age		
≥ 70	130 (30%)	101 (23%)
Sex		
Male	278 (64%)	288 (66%)
WHO/ECOG performance status		
0	236 (56%)	249 (59%)
1	178 (42%)	170 (40%)
2	11 (3%)	5 (1%)
Best response to induction treatment		
Complete or partial response (CR/PR)	290 (66%)	282 (65%)
Stable disease (SD)	147 (34%)	152 (35%)
Prior adjuvant chemotherapy		
Yes	111 (25%)	112 (26%)
Site primary tumour		
Colon	244 (56%)	236 (54%)
Rectum	134 (31%)	138 (32%)
Rectosigmoid	59 (14%)	60 (14%)
Number of metastatic sites		
1	171 (41%)	188 (44%)
>1	249 (59%)	235 (56%)
Stage of disease and primary tumour resection status		
Synchronous[a], resection	171 (39%)	182 (42%)
Synchronous, no resection	154 (35%)	163 (38%)
Metachronous	112 (26%)	89 (21%)
LDH elevated at randomisation		
Yes	212 (50%)	224 (54%)
Platelets at start induction treatment		
< 400 x 10^9/L	265 (65%)	287 (70%)
≥ 400 x 10^9/L	144 (35%)	122 (30%)
CEA at start induction treatment		
≤ 20 ng/mL	121 (35%)	142 (41%)
> 20 ng/mL	227 (65%)	208 (59%)
***RAS/BRAF* mutation status**		
RAS / ^{V600E}*BRAF* wild-type	110(34%)	129 (40%)
RAS mutant	189 (59%)	167 (52%)
^{V600E}*BRAF* mutant	22 (7%)	23 (7%)

Data are *n* (%) unless otherwise stated. Due to rounding, not all percentages total 100. Bev = bevacizumab. FP = fluoropyrimidine. [a] Synchronous disease was defined as distant metastases discovered ≤ 6 months of the primary CRC diagnosis.

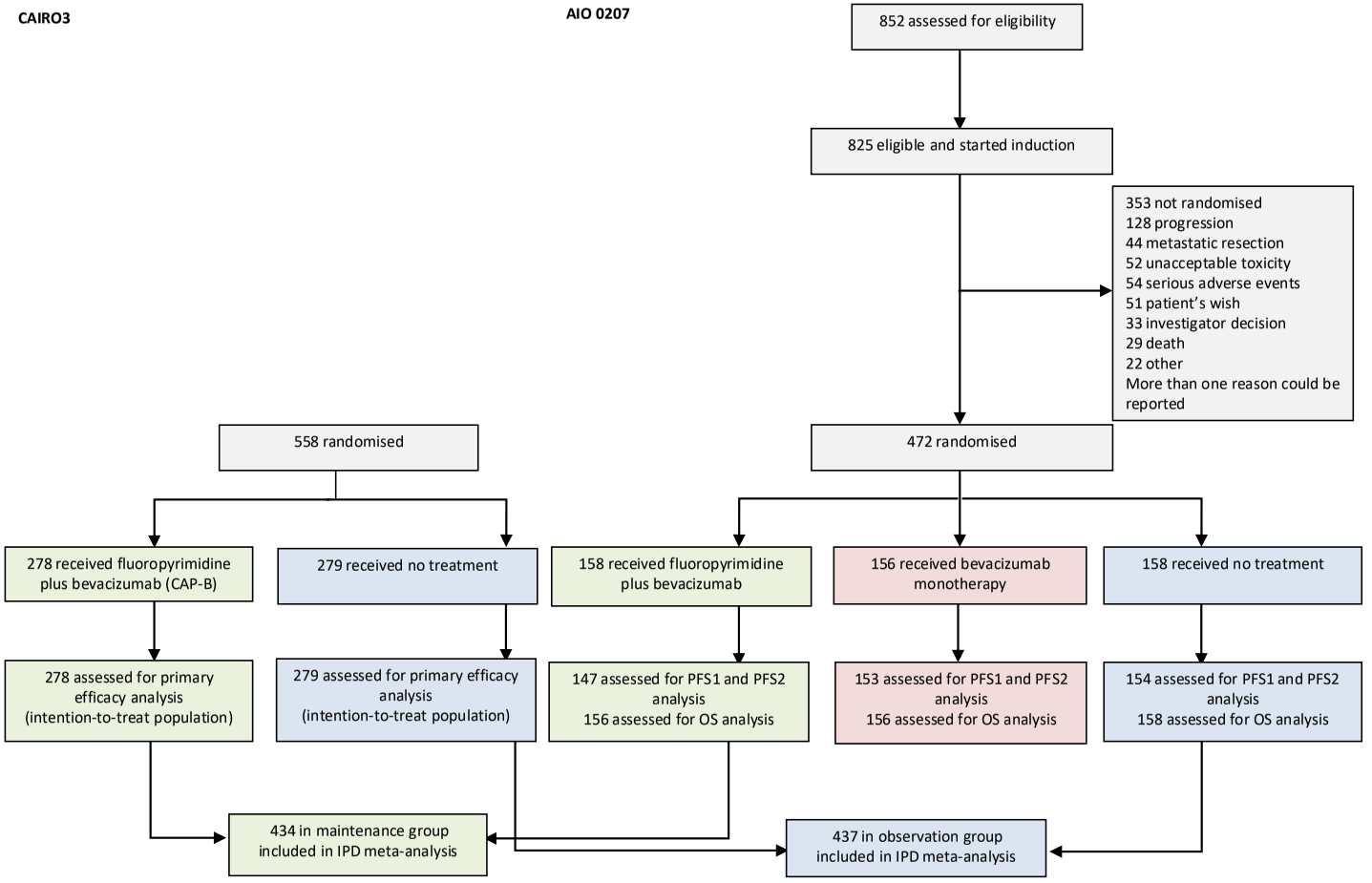

Figure 1. Trial profiles for CAIRO3 and AIO 0207.

Efficacy

Median follow-up time for all patients was 68.5 months (IQR 54.6 - 87.0 months). Overall, there was a significant benefit from maintenance treatment compared with observation for PFS1 (HR 0.40 [95% CI 0.34-0.47]) and the primary endpoint PFS2 (HR 0.70 [0.60-0.81]). The benefit of maintenance treatment was observed in all subgroups that were investigated (Figure 2 and 3), although for patients with metachronous disease this was non-significant in PFS2 (at a nominal *P*-value for significance of 0.05). In particular, primary tumour location was not predictive of the benefit of maintenance treatment or observation. Patients with elevated compared to normal platelet count at start of induction treatment showed a significant interaction in favour of maintenance treatment regarding PFS1 (HR 0.32 [95% CI 0.24-0.42] *vs* HR 0.45 [0.37-0.55], nominal *P*-value for interaction [$P_{interaction}$] = 0.042), and PFS2 (HR 0.55 [95% CI 0.42-0.72] *vs* HR 0.77 [0.64-0.93], nominal $P_{interaction}$=0.040), respectively. Supplementary Table 2 shows efficacy outcomes in the pooled study population and individual studies for PFS1 and PFS2. Supplementary Table 3 and 4 show individual study results regarding subgroup analyses for PFS1 and PFS2.

Overall treatment effect in OS did not reach statistical significance, neither in the individual trials, nor when data were pooled (HR 0.91 [95% CI 0.78-1.05])(Figure 4). In fact, overall treatment effect for OS was significantly different between the two trials (likelihood ratio *P*-value=0.008). While maintenance treatment versus observation resulted in a clinically relevant increase in median OS in CAIRO3, this was not observed in AIO 0207 (Supplementary Table 2). Subgroup analyses for OS showed a marked heterogeneity with opposite results between the two trials (Supplementary Table 5). Despite this, the combined data suggested that maintenance treatment improved OS for female sex (nominal $P_{interaction}$ = 0.003) and complete or partial response as best response on induction treatment (nominal $P_{interaction}$ = 0.035)(Figure 4).

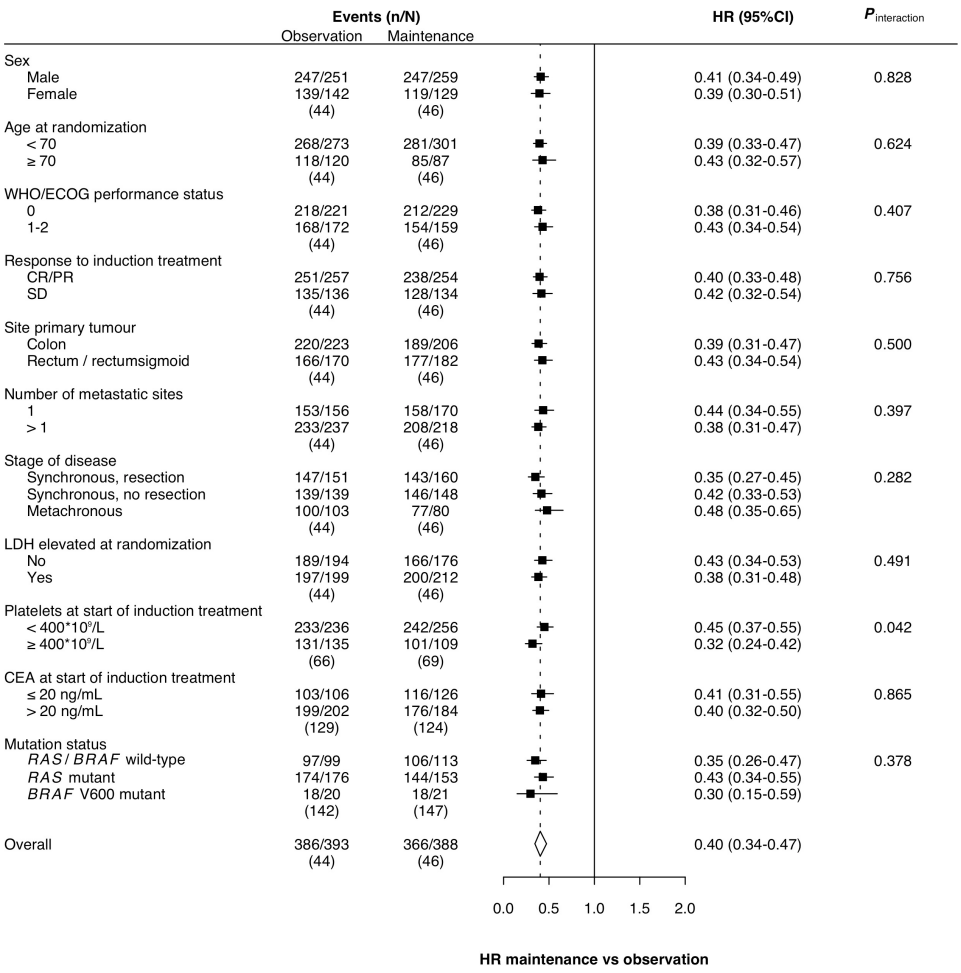

Figure 2. Forest plot showing adjusted treatment effects for **PFS1** in subgroups with *P*-values for heterogeneity across subgroups. Analyses were performed using a mixed effect Cox model with study as random intercept and treatment (and any co-variables) as fixed effects. Subgroup analyses were stratified for prior adjuvant chemotherapy, response to induction treatment, WHO/ECOG PS, and adjusted for age, sex, stage, primary tumour location, primary tumour resection, number of metastatic sites, LDH at randomisation, and interval between primary diagnosis and randomisation. Subgroup analyses for 'stage of disease and primary tumour resection status' were not adjusted for stage and primary tumour resection. CR/PR = complete or partial response. SD = stable disease.

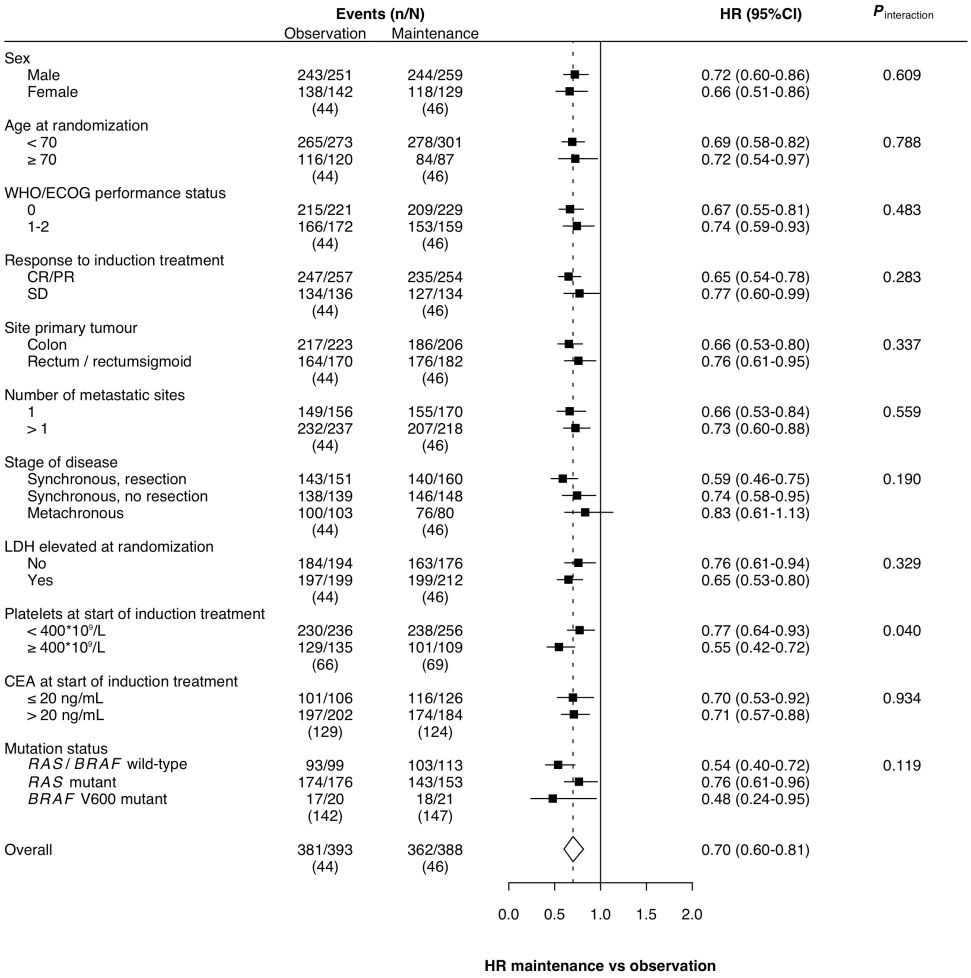

Figure 3. Forest plot showing adjusted treatment effects for **PFS2** in subgroups with *P*-values for heterogeneity across subgroups. Analyses were performed using a mixed effect Cox model with study as random intercept and treatment (and any co-variables) as fixed effects. Subgroup analyses were stratified for prior adjuvant chemotherapy, response to induction treatment, WHO/ECOG PS, and adjusted for age, sex, stage, primary tumour location, primary tumour resection, number of metastatic sites, LDH at randomisation, and interval between primary diagnosis and randomisation. Subgroup analyses for 'stage of disease and primary tumour resection status' were not adjusted for stage and primary tumour resection. CR/PR = complete or partial response. SD = stable disease.

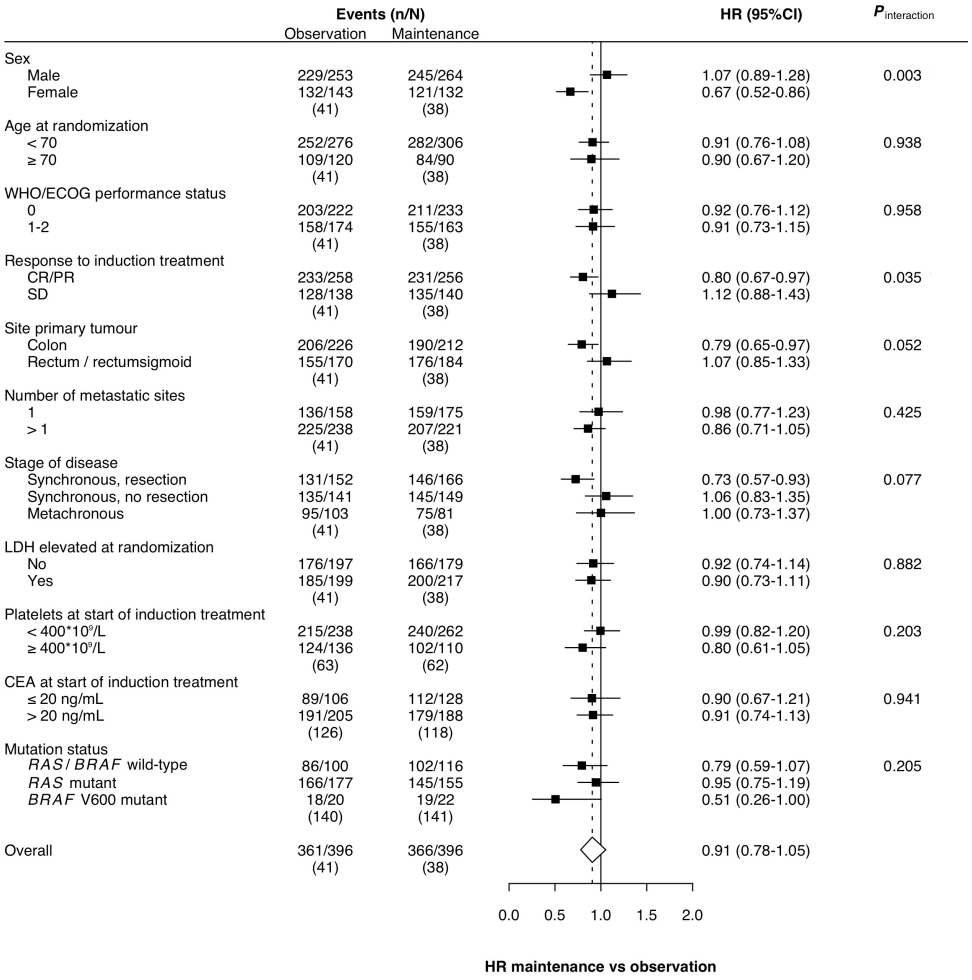

Figure 4. Forest plot showing adjusted treatment effects for **OS** in subgroups with *P*-values for heterogeneity across subgroups. Analyses were performed using a mixed effect Cox model with study as random intercept and treatment (and any co-variables) as fixed effects. Subgroup analyses were stratified for prior adjuvant chemotherapy, response to induction treatment, WHO/ECOG PS, and adjusted for age, sex, stage, primary tumour location, primary tumour resection, number of metastatic sites, LDH at randomisation, and interval between primary diagnosis and randomisation. Subgroup analyses for 'stage of disease and primary tumour resection status' were not adjusted for stage and primary tumour resection. CR/PR = complete or partial response. SD = stable disease.

Treatment upon first progression

After first progression, 407 (47%) of 871 patients underwent reintroduction of the induction treatment regimen. Out of these 407 patients, 377 (93%) received reintroduction of all components, i.e. fluoropyrimidine, oxaliplatin and bevacizumab. The percentage of patients that underwent reintroduction according to protocol was significantly lower in the fluoropyrimidine plus bevacizumab group compared with the observation group (165/429 [38%] *vs* 242/437 [55%], respectively, *P*<0.001). The percentage of patients that received reintroduction of the induction treatment regimen was significantly higher in CAIRO3 compared with AIO 0207 (304/557 [54%] *vs* 103/309 [33%], respectively, *P*<0.001). Subsequent therapies received during the course of metastatic disease were comparable between the two trials and within treatment groups, although anti-EGFR therapy was more frequently received by patients in AIO 0207 compared with CAIRO3 (84/314 [27%] *vs* 102/557 [18%], respectively; Table 2).

Table 2. Treatment upon first progression

	CAIRO3			AIO 0207		
	Observation (*n*=279)	**FP+Bev (*n*=278)**	**Total (*n*=557)**	**Observation (*n*=158)**	**FP+Bev (*n*=156)**	**Total (*n*=314)**
Anti-EGFR therapy	48 (17%)	54 (19%)	102 (18%)	40 (25%)	44 (28%)	84 (27%)
Irinotecan	165 (59%)	162 (58%)	327 (59%)	96 (61%)	85 (54%)	181 (58%)
Mitomycin	1 (0.4%)	1 (0.4%)	2 (0.4%)	13 (8%)	13 (8%)	26 (8%)
Regorafenib	2 (0.7%)	1 (0.4%)	3 (0.5%)	9 (6%)	13 (8%)	22 (7%)
Aflibercept/placebo*	8 (3%)	4 (1.4%)	12 (2%)	12 (8%)	6 (4%)	18 (6%)
Ramucirumab/placebo	2 (0.7%)	2 (0.7%)	4 (0.7%)	1 (1%)	2 (1%)	3 (1%)
Tegafur-uracil	3 (1%)	5 (2%)	8 (1%)	-	-	-
Cisplatin	1 (0.4%)	-	1 (0.2%)	-	-	-
Paclitaxel	-	1 (0.4%)	1 (0.2%)	-	-	-
Experimental study drug	9 (3%)	4 (1%)	13 (2%)	-	1 (1%)	1 (0.3%)
Dendritic cell vaccinations	1 (0.4%)	-	1 (0.2%)	-	-	-
No other agent than FP, Ox, Bev	104 (37%)	101 (36%)	205 (37%)	60 (38%)	65 (42%)	125 (40%)

Data are *n* (%) unless otherwise specified. * No placebo for AIO 0207. Bev = bevacizumab. FP = fluoropyrimidine. Ox = oxaliplatin.

Discussion

This IPD meta-analysis of the CAIRO3 and AIO 0207 trials with updated follow-up confirms the benefit of fluoropyrimidine plus bevacizumab maintenance treatment compared with observation in first-line treatment of mCRC. Despite differences in the study design of CAIRO3 and AIO 0207, our pooled results show that fluoropyrimidine plus bevacizumab maintenance treatment is more effective compared with no treatment for PFS1 and the primary endpoint PFS2, regardless of the investigated subgroups.

By using individual patient data, this pooled analysis distinguishes itself from study-level meta-analyses[9–12]. Our pooled subgroup analyses provide the best available evidence on predictors of response to fluoropyrimidine and bevacizumab maintenance treatment compared with observation thus far. All investigated subgroups showed a significant benefit from maintenance treatment regarding PFS1 and PFS2, except for patients with metachronous disease in PFS2. The latter may be partly due to the small number of patients with metachronous disease assigned to maintenance treatment (*n*=76). Another possible explanation could be a (partial) chemoresistance due to previous adjuvant treatment [13], since 108 out of 201 patients (54%) with metachronous disease received prior adjuvant chemotherapy. There is growing evidence that primary tumour sidedness (right colon: cecum-transverse colon; left colon: splenic flexure-rectum) influences prognosis and therapy response in mCRC patients[14,15]. Although specific data on sidedness were lacking in the present analysis, our findings do not suggest a predictive role of primary tumour location (colon *vs* rectosigmoid or rectum) for the benefit of maintenance treatment or observation. Patients with elevated compared to normal platelet count at start of induction treatment showed a significant interaction in favour of maintenance treatment regarding PFS1 and PFS2. Given the exploratory nature of our subgroup analyses, these findings do not allow definitive conclusions. Nonetheless, our results are in line with the MRC COIN trial, which previously showed that patients with elevated baseline platelet count had inferior survival and quality of life with intermittent chemotherapy, and should therefore not receive a treatment break[16].

Regarding OS, it should be noted that both trials were not designed or powered to show a difference in this endpoint. Overall treatment effect for OS differed significantly between CAIRO3 and AIO 0207, which limits the credibility of subgroup analyses regarding this endpoint. There was no significant difference in overall treatment effect when data were pooled. Although subgroup analyses for OS showed a marked heterogeneity between the two trials, significant interactions with OS and maintenance treatment were observed for females, and patients with complete or partial response as best response to induction treatment. The latter subgroup was also a significant predictor for the effect size of maintenance treatment in OS in the initial subgroup analyses of CAIRO3[6]. This may be partly explained by the fact that pooled OS results were more influenced by CAIRO3 due to a larger sample size per arm.

There are several reasons that could explain the diverging overall treatment effect in OS between CAIRO3 and AIO 0207. For instance, OS can be highly influenced by subsequent treatment lines[17]. In our analysis, therapies received during subsequent treatment lines were comparable between both trials, except for a higher rate of patients that received anti-EGFR therapy in AIO 0207 compared with CAIRO3. Data on systematic differences in the sequence of agents used or in the total number of agents received were beyond the scope

of the present analysis, since data are likely to be too limited for a proper investigation on the impact of these differences. Furthermore, several important differences exist between CAIRO3 and AIO 0207 regarding patient registration (after *vs* before start induction therapy), fluoropyrimidine maintenance protocols (capecitabine *vs* any fluoropyrimidine), duration of induction treatment (18 *vs* 24 weeks), and exclusion of patients who experienced toxicity from oxaliplatin during induction treatment that precluded reintroduction of this agent (yes *vs* no). These differences in study designs, together with varying study populations, could have influenced treatment outcomes, especially regarding OS.

The rate of reintroduction according to protocol was significantly higher in CAIRO3 (54%) compared with AIO 0207 (33%). This is likely to be related to the exclusion of patients who were not eligible for oxaliplatin reintroduction in CAIRO3. It may also be related to a higher cumulative oxaliplatin dose resulting from the longer induction period in AIO 0207, suggesting that a 24-week induction period may be too long. These differences between CAIRO3 and AIO 0207 in number of cycles and cumulative doses administered during the induction and reintroduction phase may have influenced OS outcomes.

Our findings support the ESMO consensus guidelines recommendation that a combination of a fluoropyrimidine plus bevacizumab is the optimal maintenance treatment following induction treatment with fluoropyrimidine, oxaliplatin and bevacizumab[18]. Our results suggest that both patients with poor prognostic characteristics and patients with favourable prognostic characteristics derive a significant benefit from maintenance treatment. Clearly, alternative outcome measures and factors should be considered in the treatment decision-making process, such as quality of life (QoL) and a patient's cultural and social preferences. Although inclusion of QoL measures in this IPD meta-analysis was difficult due to differences in time points of assessment and compliance rates, the individual trials reported comparable findings in the QoL analyses. Both trials showed that active maintenance treatment was not associated with a detrimental effect on QoL when compared with no treatment[6,8]. Most importantly, treatment decisions should be individualised after a thorough discussion with the patient. This should include discussion of the estimated survival time, time free from cancer-related symptoms, side effects and treatment constraints, and the impact on career and family life (social and financial), as stated in the ESMO consensus guidelines[18].

In conclusion, this IPD meta-analysis shows that fluoropyrimidine plus bevacizumab maintenance treatment is effective in mCRC patients with stable disease or better after induction treatment with a fluoropyrimidine, oxaliplatin, and bevacizumab, with a significant benefit in PFS1 and PFS2. Subgroup analyses did not identify any subpopulations that derived comparable benefit from observation after induction treatment.

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Supplementary Material

Supplementary Table 1. Patient characteristics according to study and treatment arm

	CAIRO3			AIO 0207		
	Obs (*n*=279)	FP+Bev (*n*=278)	Total (*n*=557)	Obs (*n*=158)	FP+Bev (*n*=156)	Total (*n*=314)
Age						
≥ 70	76 (27%)	61 (22%)	137 (25%)	54 (34%)	40 (26%)	94 (30%)
Sex						
Male	179 (64%)	182 (66%)	361 (65%)	99 (63%)	106 (68%)	205 (65%)
WHO/ECOG performance status						
0	173 (62%)	172 (62%)	345 (62%)	62 (43%)	77 (53%)	140 (48%)
1	106 (38%)	106 (38%)	212 (38%)	72 (49%)	64 (44%)	136 (47%)
2	0 (0%)	0 (0%)	0 (0%)	11 (8%)	5 (3%)	16 (6%)
Best response to induction treatment						
Complete or partial response	184 (66%)	182 (66%)	366 (66%)	106 (67%)	100 (64%)	206 (66%)
Stable disease	95 (34%)	96 (35%)	191 (34%)	52 (33%)	56 (36%)	108 (34%)
Prior adjuvant chemotherapy						
Yes	95 (34%)	93 (34%)	188 (34%)	16 (10%)	19 (12%)	35 (11%)
Primary tumour location						
Colon	143 (51%)	134 (48%)	277 (50%)	100 (63%)	102 (65%)	202 (64%)
Rectum	77 (28%)	84 (30%)	161 (29%)	58 (37%)	54 (35%)	112 (36%)
Rectosigmoid	59 (21%)	60 (22%)	119 (21%)	0 (0%)	0 (0%)	0 (0%)
Number of metastatic sites						
1	111 (42%)	118 (44%)	229 (43%)	60 (38%)	70 (45%)	130 (42%)
>1	152 (58%)	150 (56%)	302 (57%)	97 (62%)	85 (55%)	182 (58%)
Stage of disease and primary tumour resection status						
Synchronous[a], resection	84 (30%)	96 (35%)	180 (32%)	87 (55%)	86 (55%)	173 (55%)
Synchronous, no resection	107 (38%)	123 (44%)	230 (41%)	47 (30%)	40 (26%)	87 (27%)
Metachronous	88 (32%)	59 (21%)	147 (26%)	24 (15%)	30 (19%)	54 (17%)
LDH elevated at randomisation						
Yes	157 (56%)	155 (56%)	312 (56%)	55 (39%)	69 (51%)	124 (44%)
Platelets at start induction treatment						
< 400 x 10^9/L	167 (66%)	179 (70%)	346 (68%)	98 (63%)	108 (70%)	206 (67%)
≥ 400 x 10^9/L	87 (34%)	76 (30%)	163 (32%)	57 (37%)	46 (30%)	103 (33%)
CEA at start induction treatment						
≤ 20 ng/mL	78 (38%)	91 (43%)	169 (41%)	43 (30%)	51 (37%)	94 (33%)
> 20 ng/mL	126 (62%)	120 (57%)	246 (59%)	101 (70%)	88 (63%)	189 (67%)
***RAS/BRAF* mutation status**						
RAS / ^{V600E}*BRAF* wild-type	63 (31%)	81 (39%)	144 (35%)	47 (41%)	48 (44%)	95 (42%)
RAS mutant	128 (62%)	113 (54%)	241 (58%)	61 (53%)	54 (49%)	115 (51%)
^{V600E}*BRAF* mutant	15 (7%)	15 (7%)	30 (7%)	7 (6%)	8 (7%)	15 (7%)

Data are *n* (%) unless otherwise stated. Due to rounding, not all percentages total 100. [a] Synchronous disease was defined as distant metastases discovered ≤ 6 months of the primary CRC diagnosis. Bev = bevacizumab. FP = fluoropyrimidine. Obs = observation.

Supplementary Table 2. Median PFS1, PFS2, OS and 6-month / 1-year / 2-year PFS1, PFS2 and OS rates

	CAIRO3 *n*=557		AIO 0207 *n*=314		Pooled study population *n*=871	
	Obs (*n*=279)	**FP+Bev (*n*=278)**	**Obs (*n*=158)**	**FP+Bev (*n*=156)**	**Obs (*n*=437)**	**FP+Bev (*n*=434)**
PFS1						
Events	275	268	150	131	425	399
Median (months)	4.1	8.5	3.5	6.3	4.0	7.3
95% CI	3.9-4.2	6.6-10.3	2.7-4.3	5.4-7.3	3.8-4.2	6.3-8.3
Log-rank P-value	<0.001		<0.001		<0.001	
PFS2						
Events	274	266	145	129	419	395
Median (months)	8.6	11.6	6.4	6.9	7.6	9.9
95% CI	7.0-10.1	10.0-13.3	4.9-7.9	5.8-8.0	6.7-8.5	8.5-11.2
Log-rank P-value	<0.001		0.056		<0.001	
OS						
Events	268	263	124	134	392	397
Median (months)	18.2	21.6	22.4	20.2	19.0	21.4
95% CI	16.1-20.3	19.5-23.7	19.3-25.5	16.9-23.5	17.1-20.8	19.6-23.1
Log-rank P-value	0.118		0.431		0.444	
PFS1 rate						
6-month	30.1%	62.2%	26.0%	51.7%	28.6%	58.6%
1-year	6.5%	34.5%	3.9%	23.1%	5.5%	30.6%
2-year	2.5%	14.0%	0.6%	4.1%	1.8%	10.6%
PFS2 rate[a]						
6-month	88.8%	91.1%	75.3%	76.7%	84.7%	88.5%
1-year	52.7%	60.7%	39.7%	30.0%	48.8%	55.2%
2-year	8.3%	17.0%	4.1%	3.3%	7.0%	14.5%
OS rate						
6-month	90.0%	92.8%	91.8%	86.5%	90.6%	90.6%
1-year	72.0%	74.1%	72.2%	73.1%	72.1%	73.7%
2-year	36.6%	43.2%	39.2%	40.4%	37.5%	42.2%

CI = confidence interval. Bev = bevacizumab. FP = fluoropyrimidine. Obs = observation. [a] Only patients that received reintroduction of the induction treatment regimen were assessed in the calculation of PFS2 rates.

Supplementary Table 3. Individual study results - Adjusted treatment effects for PFS1 in subgroups

	CAIRO3					AIO 0207				
Subgroup	**Obs**	**FP+Bev**	**HR**	***P*-value**	$P_{interaction}$	**Obs**	**FP+Bev**	**HR**	***P*-value**	$P_{interaction}$
Sex										
Male	168/169	170/174	0.36 (0.29-0.46)	<0.001	0.882	79/82	77/85	0.48 (0.34-0.69)	<0.001	0.776
Female	92/94	87/93	0.37 (0.27-0.51)	<0.001		47/48	33/37	0.44 (0.27-0.72)	0.001	
	(16)	(12)				(28)	(34)			
Age										
< 70	188/189	199/209	0.37 (0.29-0.46)	<0.001	0.979	80/84	82/92	0.44 (0.32-0.62)	<0.001	0.527
≥ 70	72/74	57/57	0.37 (0.25-0.53)	<0.001		46/46	28/30	0.54 (0.31-0.93)	0.026	
	(16)	(12)				(28)	(34)			
WHO/ECOG performance status										
0	163/164	158/166	0.38 (0.30-0.48)	<0.001	0.405	55/57	54/63	0.37 (0.24-0.56)	<0.001	0.109
1-2	97/99	98/100	0.33 (0.24-0.44)	<0.001		71/73	56/59	0.58 (0.40-0.84)	0.004	
	(16)	(12)				(28)	(34)			
Response to induction treatment										
Complete or partial response	165/168	163/173	0.35 (0.28-0.45)	<0.001	0.583	86/89	75/81	0.48 (0.34-0.67)	<0.001	0.671
Stable disease	95/95	93/93	0.39 (0.29-0.53)	<0.001		40/41	35/41	0.43 (0.26-0.70)	0.001	
	(16)	(12)				(28)	(34)			
Primary tumour location										
Colon	136/137	120/127	0.34 (0.26-0.45)	<0.001	0.502	84/86	69/79	0.43 (0.30-0.62)	<0.001	0.453
Rectum / rectosigmoid	124/126	136/139	0.39 (0.30-0.51)	<0.001		42/44	41/43	0.54 (0.34-0.85)	0.008	
	(16)	(12)				(28)	(34)			
Number of metastatic sites										
1	109/111	112/117	0.45 (0.34-0.59)	<0.001	0.058	44/45	46/53	0.41 (0.26-0.66)	<0.001	0.522
>1	151/152	144/149	0.31 (0.24-0.40)	<0.001		82/85	64/69	0.50 (0.35-0.72)	<0.001	
	(16)	(12)				(28)	(34)			
Stage of disease and primary tumour resection status										
Synchronous, resection	76/77	84/91	0.28 (0.20-0.39)	<0.001	0.090	71/74	59/69	0.48 (0.33-0.70)	<0.001	0.971
Synchronous, no resection	103/103	115/117	0.38 (0.28-0.50)	<0.001		36/36	31/31	0.47 (0.28-0.79)	0.004	
Metachronous	81/83	57/58	0.48 (0.33-0.68)	<0.001		19/20	20/22	0.43 (0.21-0.90)	0.025	
	(16)	(12)				(28)	(34)			

LDH elevated at randomisation										
No	114/117	113/116	0.34 (0.25-0.45)	<0.001	0.416	75/77	53/60	0.58 (0.40-0.86)	0.006	0.095
Yes	146/146 (16)	143/150 (12)	0.39 (0.30-0.50)	<0.001		51/53 (28)	57/62 (34)	0.35 (0.23-0.55)	<0.001	
Platelet count at start induction treatment										
< 400 x 10^9/L	155/156	164/171	0.39 (0.31-0.50)	<0.001	0.144	78/80	78/85	0.52 (0.37-0.75)	<0.001	0.353
≥ 400 x 10^9/L	84/86 (37)	69/72 (35)	0.29 (0.20-0.41)	<0.001		47/49 (29)	32/37 (34)	0.40 (0.25-0.64)	<0.001	
CEA at start induction treatment										
≤ 20 ng/mL	69/71	79/85	0.36 (0.25-0.52)	<0.001	0.989	34/35	37/41	0.48 (0.28-0.82)	0.007	0.902
> 20 ng/mL	119/120 (88)	112/114 (79)	0.36 (0.27-0.48)	<0.001		80/82 (41)	64/70 (45)	0.47 (0.32-0.67)	<0.001	
Mutation status										
RAS / ^{V600E}*BRAF* wild-type	58/58	74/77	0.32 (0.22-0.47)	<0.001	0.456	39/41	32/36	0.37 (0.21-0.65)	0.001	0.605
RAS mutant	122/123	108/110	0.38 (0.28-0.51)	<0.001		52/53	36/43	0.53 (0.33-0.85)	0.009	
^{V600E}*BRAF* mutant	13/14 (84)	11/14 (77)	0.22 (0.10-0.52)	<0.001		5/6 (58)	7/7 (70)	0.46 (0.13-1.66)	0.234	

Numbers between brackets: number of patients with missing values per analysis. $P_{interaction}$ = *P*-value for heterogeneity across subgroups. Analyses performed using a mixed effect Cox model with study as random intercept and treatment (and any co-variables) as fixed effects. Subgroup analyses were stratified for prior adjuvant chemotherapy, response to induction treatment, WHO/ECOG PS, and adjusted for age, sex, stage, primary tumour location, primary tumour resection, number of metastatic sites, LDH at randomisation, and interval between primary diagnosis and randomisation. Subgroup analyses for 'stage of disease and primary tumour resection status' were not adjusted for stage and primary tumour resection. Bev = bevacizumab. FP = fluoropyrimidine. Obs = observation.

Supplementary Table 4. Individual study results - Adjusted treatment effects for PFS2 in subgroups

	CAIRO3					AIO 0207				
Subgroup	**Obs**	**FP+Bev**	**HR**	***P*-value**	$P_{interaction}$	**Obs**	**FP+Bev**	**HR**	***P*-value**	$P_{interaction}$
Sex										
Male	168/169	169/174	0.64 (0.51-0.80)	<0.001	0.936	75/82	75/85	0.81 (0.57-1.16)	0.251	0.934
Female	91/94	85/92	0.63 (0.46-0.86)	0.004		47/48	33/37	0.83 (0.52-1.34)	0.446	
	(16)	(12)				(28)	(34)			
Age										
< 70	188/189	197/209	0.63 (0.51-0.79)	<0.001	0.989	77/84	81/92	0.80 (0.57-1.12)	0.186	0.765
≥ 70	71/74	57/57	0.64 (0.44-0.91)	0.014		45/46	27/30	0.88 (0.51-1.51)	0.640	
	(16)	(12)				(28)	(34)			
WHO/ECOG performance status										
0	162/164	157/166	0.70 (0.56-0.88)	0.002	0.174	53/57	52/63	0.59 (0.38-0.90)	0.015	0.028
1-2	97/99	97/100	0.54 (0.41-0.73)	<0.001		69/73	56/59	1.11 (0.77-1.60)	0.584	
	(16)	(12)				(28)	(34)			
Response to induction treatment										
Complete or partial response	164/168	162/173	0.57 (0.45-0.71)	<0.001	0.134	83/89	73/81	0.85 (0.61-1.20)	0.358	0.606
Stable disease	95/95	92/93	0.75 (0.56-1.01)	0.061		39/41	35/41	0.73 (0.45-1.19)	0.210	
	(16)	(12)				(28)	(34)			
Primary tumour location										
Colon	136/137	119/127	0.58 (0.45-0.75)	<0.001	0.322	81/86	67/79	0.76 (0.53-1.09)	0.136	0.507
Rectum / rectosigmoid	123/126	135/139	0.70 (0.54-0.90)	0.006		41/44	41/43	0.93 (0.58-1.50)	0.771	
	(16)	(12)				(28)	(34)			
Number of metastatic sites										
1	108/111	110/117	0.68 (0.51-0.89)	0.006	0.566	41/45	45/53	0.63 (0.39-1.02)	0.061	0.190
>1	151/152	144/149	0.61 (0.48-0.77)	<0.001		81/85	63/69	0.95 (0.66-1.37)	0.786	
	(16)	(12)				(28)	(34)			
Stage of disease and primary tumour resection status										
Synchronous, resection	75/77	83/91	0.49 (0.36-0.69)	<0.001	0.129	68/74	57/69	0.74 (0.50-1.11)	0.142	0.557
Synchronous, no resection	103/103	115/117	0.66 (0.50-0.88)	0.004		35/36	31/31	1.04 (0.62-1.74)	0.896	
Metachronous	81/83	56/58	0.81 (0.57-1.16)	0.256		19/20	20/22	0.71 (0.35-1.43)	0.335	
	(16)	(12)				(28)	(34)			

LDH elevated at randomisation										
No	113/117	112/116	0.60 (0.46-0.79)	<0.001	0.611	71/77	51/60	1.02 (0.69-1.50)	0.923	0.106
Yes	146/146	142/150	0.66 (0.52-0.85)	0.001		51/53	57/62	0.62 (0.40-0.96)	0.033	
	(16)	(12)				(28)	(34)			
Platelet count at start induction treatment										
< 400 x 10^9/L	15/156	162/171	0.69 (0.54-0.87)	0.002	0.065	75/80	76/85	0.89 (0.63-1.26)	0.512	0.666
≥ 400 x 10^9/L	83/86	69/72	0.47 (0.33-0.66)	<0.001		46/49	32/37	0.78 (0.49-1.26)	0.312	
	(37)	(35)				(29)	(34)			
CEA at start induction treatment										
≤ 20 ng/mL	69/71	79/85	0.62 (0.44-0.88)	0.007	0.783	32/35	37/41	0.67 (0.39-1.17)	0.158	0.297
> 20 ng/mL	119/120	112/114	0.58 (0.44-0.77)	<0.001		78/82	62/70	0.95 (0.67-1.35)	0.782	
	(88)	(79)				(41)	(45)			
Mutation status										
RAS / ^{V600E}*BRAF* wild-type	57/58	73/77	0.50 (0.35-0.73)	<0.001	0.096	36/41	30/36	0.61 (0.34-1.10)	0.100	0.461
RAS mutant	122/123	107/110	0.72 (0.54-0.95)	0.021		52/53	36/43	0.83 (0.51-1.36)	0.470	
^{V600E}*BRAF* mutant	13/14	11/14	0.32 (0.14-0.73)	0.007		4/6	7/7	1.33 (0.35-5.01)	0.675	
	(84)	(77)				(58)	(70)			

Numbers between brackets: number of patients with missing values per analysis. $P_{interaction}$ = *P*-value for heterogeneity across subgroups. Analyses performed using a mixed effect Cox model with study as random intercept and treatment (and any co-variables) as fixed effects. Subgroup analyses were stratified for prior adjuvant chemotherapy, response to induction treatment, WHO/ECOG PS, and adjusted for age, sex, stage, primary tumour location, primary tumour resection, number of metastatic sites, LDH at randomisation, and interval between primary diagnosis and randomisation. Subgroup analyses for 'stage of disease and primary tumour resection status' were not adjusted for stage and primary tumour resection. Bev = bevacizumab. FP = fluoropyrimidine. Obs = observation.

Supplementary Table 5. Individual study results - Adjusted treatment effects for OS in subgroups

	CAIRO3					AIO 0207				
Subgroup	**Obs**	**FP+Bev**	**HR**	***P*-value**	$P_{interaction}$	**Obs**	**FP+Bev**	**HR**	***P*-value**	$P_{interaction}$
Sex										
Male	165/169	167/174	0.92 (0.74-1.15)	0.470	0.035	64/84	78/90	1.51 (0.96-2.16)	0.026	0.130
Female	90/94	84/92	0.61 (0.45-0.84)	0.002		42/49	37/40	0.95 (0.59-1.52)	0.829	
	(16)	(12)				(25)	(26)			
Age										
< 70	184/189	195/209	0.82 (0.67-1.02)	0.074	0.629	68/87	87/97	1.20 (0.86-1.68)	0.280	0.552
≥ 70	71/74	56/57	0.74 (0.52-1.06)	0.105		38/46	28/33	1.46 (0.86-2.48)	0.166	
	(16)	(12)				(25)	(26)			
WHO/ECOG performance status										
0	159/164	156/166	0.93 (0.74-1.17)	0.525	0.070	44/58	55/67	1.03 (0.67-1.58)	0.909	0.166
1-2	96/99	95/100	0.66 (0.49-0.88)	0.005		62/75	60/63	1.54 (1.06-2.22)	0.023	
	(16)	(12)				(25)	(26)			
Response to induction treatment										
Complete or partial response	162/168	159/173	0.69 (0.55-0.86)	0.001	0.024	71/90	72/83	1.27 (0.90-1.78)	0.168	0.843
Stable disease	93/95	92/93	1.05 (0.78-1.41)	0.752		35/43	43/47	1.34 (0.84-2.15)	0.217	
	(16)	(12)				(25)	(26)			
Primary tumour location										
Colon	134/137	117/127	0.68 (0.52-0.89)	0.004	0.088	72/89	73/85	1.08 (0.76-1.54)	0.651	0.131
Rectum / rectosigmoid	121/126	134/139	0.94 (0.72-1.21)	0.607		34/44	42/45	1.74 (1.06-2.85)	0.029	
	(16)	(12)				(25)	(26)			
Number of metastatic sites										
1	105/111	108/117	0.89 (0.67-1.18)	0.408	0.361	31/47	51/58	1.35 (0.84-2.16)	0.219	0.763
>1	150/152	143/149	0.75 (0.58-0.95)	0.018		75/86	64/72	1.23 (0.85-1.77)	0.272	
	(16)	(12)				(25)	(26)			
Stage of disease and primary tumor resection status										
Synchronous, resection	74/77	81/91	0.48 (0.34-0.67)	<0.001	0.001	57/75	65/75	1.34 (0.92-1.95)	0.130	0.574
Synchronous, no resection	103/103	114/117	0.99 (0.75-1.31)	0.951		32/38	31/32	1.39 (0.83-2.33)	0.208	
Metachronous	78/83	56/58	1.02 (0.71-1.45)	0.930		17/20	19/23	0.89 (0.42-1.86)	0.754	
	(16)	(12)				(25)	(26)			

LDH elevated at randomisation										
No	113/117	110/116	0.76 (0.58-1.00)	0.048	0.606	63/80	56/63	1.35 (0.92-1.99)	0.124	0.636
Yes	142/146	141/150	0.84 (0.65-1.08)	0.165		43/53	59/67	1.17 (0.76-1.81)	0.473	
	(16)	(12)				(25)	(26)			
Platelet count at start induction treatment										
< 400 x 10^9/L	151/156	160/171	0.84 (0.67-1.07)	0.158	0.500	64/82	80/91	1.58 (1.10-2.26)	0.012	0.119
≥ 400 x 10^9/L	83/86	68/72	0.73 (0.52-1.03)	0.073		41/50	34/38	0.99 (0.61-1.59)	0.956	
	(37)	(35)				(26)	(27)			
CEA at start induction treatment										
≤ 20 ng/mL	66/71	77/85	0.73 (0.51-1.04)	0.082	0.691	23/35	35/43	1.37 (0.76-2.46)	0.289	0.760
> 20 ng/mL	119/120	111/114	0.80 (0.61-1.05)	0.107		72/85	68/74	1.23 (0.87-1.76)	0.244	
	(88)	(77)				(38)	(39)			
Mutation status										
RAS / ^{V600E}*BRAF* wild-type	56/58	71/77	0.64 (0.44-0.93)	0.020	0.032	30/42	31/39	1.51 (0.86-2.56)	0.150	0.367
RAS mutant	120/123	107/110	0.94 (0.71-1.25)	0.681		46/54	38/45	0.96 (0.59-1.56)	0.867	
^{V600E}*BRAF* mutant	13/14	11/14	0.32 (0.14-0.76)	0.010		5/6	8/8	1.80 (0.54-5.97)	0.336	
	(84)	(77)				(56)	(64)			

Numbers between brackets: number of patients with missing values per analysis. $P_{interaction}$ = *P*-value for heterogeneity across subgroups. Analyses performed using a mixed effect Cox model with study as random intercept and treatment (and any co-variables) as fixed effects. Subgroup analyses were stratified for prior adjuvant chemotherapy, response to induction treatment, WHO/ECOG PS, and adjusted for age, sex, stage, primary tumour location, primary tumour resection, number of metastatic sites, LDH at randomisation, and interval between primary diagnosis and randomisation. Subgroup analyses for 'stage of disease and primary tumour resection status' were not adjusted for stage and primary tumour resection. Bev = bevacizumab. FP = fluoropyrimidine. Obs = observation.

Chapter 8

Summary

General Discussion

Summary

Colorectal cancer (CRC) is one of the most frequent cancer types and a leading cause of death worldwide. Approximately half of all CRC patients will develop metastatic disease. Although surgical resection of metastases offers the best chance for cure, this is only reserved for a minority of patients with metastatic colorectal cancer (mCRC). The majority of mCRC patients will present with more advanced, unresectable metastases. Due to new developments in systemic treatment, surgery and local treatment modalities, the prognosis of patients with unresectable mCRC has increased dramatically during the past decades, reaching median overall survival (OS) of more than 30 months. Nonetheless, only a subset of patients experience benefit from systemic treatment. As a result, many patients are unnecessarily exposed to toxic effects of often very expensive treatments. Ongoing research focuses on a shift from one-size-fits-all to tailor-made treatment for mCRC. Prognostic and predictive factors are essential components in the treatment decision-making process and development of precision medicine. Prognostic factors have the ability to identify patient subgroups with different clinical outcomes, regardless of treatment. Predictive factors allow selection of patients that may or may not benefit from a specific treatment. The aim of the research described in this thesis is to gain insight in prognostic and predictive factors in order to optimise treatment outcomes of patients with mCRC.

Chapter 2

Although synchronous and metachronous metastases are considered as separate entities of mCRC with different outcomes, their distribution and prognostic impact are not frequently reported in mCRC studies. Chapter 2 describes our findings on trends in inclusion and survival of patients with synchronous versus metachronous metastases in randomised controlled trials (RCTs), cohort studies and population-based studies investigating initial systemic therapy or surgical treatment of mCRC, published between 2004 and 2016. Only a small number of mCRC studies was found to report the proportion of synchronous versus metachronous metastases. In the 46 studies enrolled, 17 different definitions for synchronous mCRC were used. This confirms that there is no consensus on what constitutes synchronous or metachronous disease. In first-line systemic therapy RCTs, the proportion of patients with synchronous mCRC significantly increased during recent years. In these RCTs, estimated median OS of the total study population slightly increased over time, though no differentiation was made for survival among subgroups of patients with synchronous or metachronous mCRC. No significant results were observed in the included cohort or population-based studies. This systematic review argues that uniform definitions and consistent reporting of the proportion of synchronous versus metachronous metastases in mCRC studies are essential to gain more knowledge on differences in clinical outcome, and to improve cross-study comparisons.

Chapter 3

In 2007, standardised reporting of patient characteristics and use of stratification in trials investigating systemic treatment of mCRC has been proposed[1]. However, the adoption of these recommendations in mCRC trials published in more recent years has not been evaluated. Chapter 3 describes our findings on the reporting of patient characteristics and use of stratification factors in phase 3 trials investigating first-line systemic treatment of mCRC, published between 2005 and 2016. In this systematic review of 67 phase 3 trials including more than 35,000 patients, we observed marked heterogeneity with respect to reported patient characteristics and stratification factors. In studies published from 2005-2008 compared to 2009-2016, there was only a slight improvement in the reporting of patient characteristics as proposed in 2007[1]. Therefore, we must conclude that the proposed standardisation of these items has not been widely implemented. In addition, novel prognostic factors that have become relevant in the light of new targeted drugs were infrequently reported. This systematic review highlights how little attention is given to prognostic factors, although many of these factors have the potential to determine the survival of patients to a greater extent than any available treatment regimen. It stresses the importance of reaching consensus on a standardised set of prognostic factors to use as patient characteristics and stratification factors in future mCRC trials, in order to improve interpretation of trial results and cross-study comparisons.

Chapter 4

The results of the systematic review presented in Chapter 3 prompted us to address the marked heterogeneity in reporting of patient characteristics and use of stratification factors in mCRC trials. With the use of a two-round modified Delphi survey, we have developed the first consensus recommendation among 30 international mCRC experts on essential patient characteristics and stratification factors in phase 3 trials investigating systemic treatment of mCRC. This consensus recommendation, presented in Chapter 4, is supported by the Aide et Recherche Cancérologie Digestive (ARCAD) group[2]. The recommended set consists of 14 patient characteristics: age, performance status, primary tumour location, primary tumour resection, prior chemotherapy, number of metastatic sites, liver-only disease, liver involvement, surgical resection of metastases, synchronous versus metachronous metastases, *(K)RAS* and *BRAF* mutation status, microsatellite instability (MSI) / mismatch repair (MMR) status, and number of prior treatment lines. A total of five patient characteristics are considered the most relevant stratification factors: *RAS/BRAF* mutation status, performance status, primary tumour sidedness and liver-only disease. Inclusion of these essential baseline characteristics and stratification factors in study protocols and final reports of future mCRC trials will greatly improve interpretation of study results, cross-study comparisons and meta-analyses.

Chapter 5

The phase 3 CAIRO3 study previously showed that in mCRC patients, capecitabine plus bevacizumab (CAP-B) maintenance treatment after six cycles capecitabine, oxaliplatin and bevacizumab (CAPOX-B) is effective, without compromising quality of life[3]. However, maintenance treatment may not be considered as cost-effective, and better patient selection would improve clinical decision-making and reduce therapy costs[4]. Chapter 5 presents the results of a post hoc analysis of the CAIRO3 study with updated follow-up and data regarding primary tumour sidedness, in which we defined subgroups according to *RAS*, *BRAF* mutation status and mismatch repair (MMR) status, and investigated their influence on treatment efficacy. *RAS*, *BRAF* mutations and MMR deficiency were prevalent in 58%, 9% and 1% of patients, respectively. Maintenance treatment was more effective compared with observation across *RAS/BRAF* wild-type, *RAS*-mutant and ^{V600E}*BRAF*-mutant subgroups for the primary endpoint PFS2 (second progression-free survival after reintroduction of CAPOX-B) and secondary endpoints, except for the *RAS*-mutant subgroup regarding OS. When mutational subgroup analyses were adjusted for sidedness instead of primary tumour location, comparable efficacy results were observed. Both patients with right- and left-sided tumours showed significant benefit from maintenance treatment. Our findings suggest that CAP-B maintenance treatment after six cycles of CAPOX-B is effective in first-line treatment of mCRC across all mutational subgroups. The benefit of maintenance treatment was most pronounced in patients with *RAS/BRAF* wild-type or ^{V600E}*BRAF*-mutant tumours.

Chapter 6

Next generation sequencing (NGS) has become a routine procedure to guide personalised medicine in mCRC. Next to sensitive detection of mutations in putative driver genes, NGS allows digital quantification of the mutational burden. The mutant allele fraction (MAF), defined as the number of mutant reads divided by the total number of reads at a specific genomic position of interest[5], may have important clinical implications in the management of mCRC. Chapter 6 describes our findings on the distribution and independent prognostic value of *KRAS* MAFs or MAFs normalised for tumour purity (adjMAFs) in 170 mCRC patients with *KRAS*-mutant tumours enrolled in the CAIRO3 study. Among these patients, we observed marked heterogeneity in the distribution of *KRAS* MAFs and adjMAFs. Median OS varied among tertiles of *KRAS* MAF and adjMAF, though differences were not statistically significant. In multivariable Cox regression analysis with and without restricted cubic splines, we observed no significant (non-)linear associations between either *KRAS* MAFs or adjMAFs and OS. This exploratory analysis suggests that *KRAS* MAFs or adjMAFs are not independently associated with OS in mCRC patients with *KRAS*-mutant tumours treated with CAP-B maintenance treatment versus observation after six cycles CAPOX-B.

Chapter 7

The phase 3 CAIRO3 and AIO 0207 trials demonstrated that in mCRC patients with stable disease or better after induction treatment with a fluoropyrimidine (either 5-fluorouracil or capecitabine), oxaliplatin and bevacizumab, maintenance treatment with a fluoropyrimidine plus bevacizumab is more effective compared with observation, while preserving the quality of life[3,6,7]. Since not all patients may benefit from this strategy, better patient selection would improve precision medicine and reduce therapy costs. Chapter 7 concerns an individual patient data (IPD) meta-analysis with updated follow-up of the CAIRO3 and AIO 0207 trials. In 871 mCRC patients, randomised to fluoropyrimidine plus bevacizumab maintenance treatment versus observation, we investigated whether treatment effect was modified by sex, age, performance status, response to induction treatment, primary tumour location, number of metastatic sites, disease stage and primary tumour resection, serum LDH, platelet count, CEA and *RAS/BRAF* mutation status. Fluoropyrimidine plus bevacizumab maintenance treatment was more effective compared with observation, with significant results for PFS1 (first progression-free survival) and PFS2 (primary endpoint). Subgroup analyses did not identify subpopulations that did not benefit from maintenance treatment for PFS1 and PFS2, and no clinically relevant subgroup effects were observed. Pooled results for OS were not statistically significant, and the trials showed marked heterogeneity in overall treatment effect and subgroup effects. This IPD meta-analysis shows that fluoropyrimidine plus bevacizumab maintenance treatment is effective in mCRC patients with stable disease or better after induction treatment with a fluoropyrimidine, oxaliplatin and bevacizumab, with a significant benefit in PFS1 and PFS2. Subgroup analyses did not identify any subpopulations that derived comparable benefit from observation after induction treatment.

General Discussion

In recent years, it has become evident that metastatic colorectal cancer (mCRC) is a heterogeneous and molecularly complex disease[8,9]. Prognosis and treatment response are being influenced by a combination of clinical, pathological and molecular features. Since few biomarkers are currently available, almost all systemic treatments are administered with a one-size-fits-all approach, with only a subset of patients experiencing benefit. The research presented in this thesis focuses on prognostic and predictive markers in patients with mCRC in order to better predict treatment outcomes in mCRC.

Although randomised controlled trials (RCTs) are considered the gold standard to evaluate the efficacy of new treatment strategies, they often show heterogeneity in response and survival rates. This could be partly explained by differences in patient characteristics, since many characteristics are of prognostic value. Therefore, uniform trial reporting of patient characteristics and use of stratification factors is essential to enable a valid comparison of treatment arms, to facilitate meta-analyses, and to evaluate whether study populations are representative of the general patient population. However, there is marked inconsistency in the reporting of patient characteristics in mCRC trials, which was first described by Sorbye et al. in 2007[1]. For example, although several studies have reported that synchronous metastases are associated with worse outcome compared with metachronous metastases[10,11], mCRC studies often do not report the distribution, prognostic impact and a (uniform) definition of synchronous versus metachronous metastases. We observed persistent heterogeneity in the reporting of patient characteristics and use of stratification factors in 67 first-line phase 3 trials published between 2005 and 2016. With the use of a two-round Delphi survey, we have developed the first consensus recommendation among 30 mCRC experts from 15 different countries on essential patient characteristics and stratification factors in mCRC trials. Implementation of this minimum set of essential baseline characteristics and stratification factors in study protocols and final reports of future mCRC trials will greatly improve trial reporting, interpretation of results, and cross-study comparisons. Clearly, this recommendation will evolve over time. Therefore, plans are being made to update the consensus recommendation every 2-3 years in a continuing subprogram of the ARCAD group, a worldwide collaboration of clinicians, statisticians and scientists specialised in colorectal cancer (CRC), whose ultimate goal is to develop more efficient clinical trials[2].

The phase 3 CAIRO3 and AIO 0207 trials showed that maintenance treatment with fluoropyrimidine and bevacizumab is the preferred strategy in mCRC patients with stable disease (SD) or better after induction treatment with a fluoropyrimidine, oxaliplatin and bevacizumab, as it maintains disease control and quality of life without relevant toxicity[3,6,7]. However, not all patients may benefit from this strategy. To improve personalised medicine, we aimed to identify patient subgroups according to clinical, pathological and molecular

characteristics that benefit most from fluoropyrimidine and bevacizumab maintenance treatment or observation. We combined individual patient data (IPD) of the only two large phase 3 trials that investigated the efficacy of fluoropyrimidine plus bevacizumab maintenance treatment versus observation[3,6], and found that maintenance treatment was effective, regardless of several relevant clinical and pathological subgroups. Likewise, in a post hoc analysis of the CAIRO3 study, we found that capecitabine and bevacizumab (CAP-B) maintenance treatment was more effective compared with observation, regardless of *RAS/BRAF* wild-type, *RAS*-mutant or ^{V600E}*BRAF*-mutant subgroups. Within the CAIRO3 study, we also investigated whether quantification of the mutational burden, i.e. the mutant allele fraction (MAF), could be used as independent prognostic factor in mCRC patients with *KRAS*-mutant tumours. Our findings suggest that *KRAS* MAFs or MAFs adjusted for tumour purity (adjMAFS) are not independently associated with prognosis in mCRC patients with *KRAS*-mutant tumours.

Future perspectives

Molecular testing has significantly expanded our knowledge on CRC development. In the future, the progressive understanding of tumour biology will lead to more complex sub-classifications of CRC. Since molecular subtyping substantially impacts on prognosis and on treatment selection for different stages of CRC, clinical trials will increasingly focus on specific molecular subgroups of CRC patients, which will make patient recruitment challenging. In addition, results of RCTs influence treatment guidelines, although only 5%-15% of patients participate in clinical trials[12,13]. To improve tailor-made treatment of (m)CRC, novel study designs, methods for data acquisition and large (inter)national collaborations are needed to collect data from a large cohort of CRC patients in order to facilitate basic, translational and clinical research. In the Netherlands, the multidisciplinary Prospective Dutch ColoRectal Cancer cohort (PLCRC) was initiated to gather longitudinal clinical data, biomaterial and patient reported outcome measures of CRC patients under a broad informed consent[14]. By sharing data with other researchers upon request, PLCRC anticipates on the growing need for comprehensive data collection and sharing. Of note, next to defining molecular subgroups, the importance of reporting routine clinical and pathological parameters should not be neglected, since not all patients expressing a certain molecular marker will respond to a specific therapy.

The CAIRO3 molecular subgroup analyses were performed on a single tumour sample. Since the relevance of intra-tumour heterogeneity has become increasingly apparent[8,15], a single tumour sample likely underestimates the complexity of the genomic landscape of the tumour[16]. Due to the evolutionary nature of cancer and therapy resistance as a result of selective pressure, extended periods between sampling and clinical application of the results may result in an altered genetic tumour composition. Therefore, multiple biopsies

may be needed to adequately determine biological characteristics of the evolving tumour. A promising alternative for tissue biopsies is repeated blood sampling. These 'liquid biopsies' focus on circulating tumour DNA, circulating tumour cells or exosomes, and offer the opportunity to monitor tumour-associated genetic aberrations in the blood, and to track genomic evolution of the tumour[16]. In addition to biomarker studies, preclinical cancer models, such as organoid cultures and xenograft models[17–20], may be used to gain understanding of the complex CRC biology and to better guide therapeutic decision making.

Overall, we did not discover predictive markers to guide selection of patients that may or may not benefit from maintenance treatment. Our findings suggest that fluoropyrimidine plus bevacizumab maintenance treatment is more effective compared with observation in first-line treatment of mCRC, irrespective of several relevant clinical, pathological and mutational subgroups. These findings support the ESMO consensus guidelines recommendation that a combination of a fluoropyrimidine plus bevacizumab is the optimal maintenance treatment following induction treatment with a fluoropyrimidine, oxaliplatin, and bevacizumab[21]. Alternative outcome measures and factors should also be considered in the treatment decision-making process, such as the quality of life and a patient's cultural and social preferences. Most importantly, treatment decisions should be individualised and made after discussion with the patient, which should include discussion of the estimated survival time, time free from cancer-related symptoms, side effects, treatment constraints, and the impact on career and family life[21].

Conclusion

In conclusion, the research described in this thesis supports the use of fluoropyrimidine plus bevacizumab maintenance treatment after induction treatment with a fluoropyrimidine, oxaliplatin and bevacizumab in first-line treatment of mCRC, regardless of relevant clinical, pathological and mutational subgroups. Furthermore, we provide a tool for uniform reporting of patient characteristics and use of stratification factors in future mCRC studies, which is essential to improve interpretation of study results and cross-study comparisons. In the future, the role of tumour heterogeneity and molecular subtyping will become increasingly important. Novel approaches for biomarker research and preclinical cancer models are pivotal to unravel the complexity of CRC biology. In addition, large (inter)national consortia, such as PLCRC, are essential to better integrate research into clinical practice. The growing need for international collaboration on sharing individual patient data, including data on biomaterial and patient-reported outcome measures, requires standardised data collection and reporting. This enables us to better answer clinical questions concerning a specific subgroup of (m)CRC patients, with the ultimate goal to tailor treatment for each individual patient based on clinical, pathological and genomic characteristics.

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8

Chapter 9

Samenvatting
(Dutch summary)

Samenvatting

Dikke darm- en endeldarmkanker (colorectaalcarcinoom, CRC) is een van de meest voorkomende kankersoorten en een van de belangrijkste doodsoorzaken wereldwijd. Het aantal patiënten met de diagnose CRC is sinds 1990 verdubbeld, wat deels te maken heeft met de groei en vergrijzing van de bevolking. Ongeveer de helft van alle patiënten ontwikkelt uitzaaiingen (metastasen) tijdens het ziektebeloop. Deze metastasen kunnen aanwezig zijn op het moment van het vaststellen van de kanker ('synchrone metastasen') of zich later ontwikkelen na eerdere verwijdering van de darmtumor ('metachrone metastasen'). Chirurgische verwijdering (resectie) van alle metastasen biedt de beste kans op genezing. Echter, slechts een klein deel van de patiënten met een gemetastaseerd CRC komt hiervoor in aanmerking, gezien de meerderheid zich presenteert met vergevorderde metastasen die niet chirurgisch te verwijderen (niet resectabel) zijn. Voor deze patiënten is genezing niet mogelijk, en heeft medicamenteuze, ofwel systemische therapie (chemotherapie en 'targeted' therapie) de voorkeur. Nieuwe ontwikkelingen op het gebied van systemische therapie, chirurgie en lokale behandelmethoden hebben ertoe geleid dat de prognose van CRC patiënten met niet resectabele metastasen de afgelopen decennia drastisch is toegenomen, waarbij een mediane totale overleving (OS) van meer dan 30 maanden kan worden bereikt. Echter, slechts een minderheid van de patiënten met een gemetastaseerd CRC heeft baat bij systemische therapie. Als gevolg hiervan worden veel patiënten onnodig blootgesteld aan bijwerkingen en toxiciteit van - vaak zeer dure - behandelingen. Lopende onderzoeken richten zich meer en meer op een behandeling op maat voor patiënten met een gemetastaseerd CRC. Prognostische en predictieve factoren zijn essentieel om tot een weloverwogen besluit te komen voor een optimale behandelstrategie. Prognostische factoren geven informatie over het ziektebeloop, onafhankelijk van de behandeling. Predictieve factoren voorspellen hoe een tumor op een bepaalde behandeling reageert, waardoor patiënten beter voor deze behandeling kunnen worden geselecteerd. Het doel van het onderzoek zoals beschreven in dit proefschrift is om inzicht te verkrijgen in prognostische en predictieve factoren om daarmee de behandeluitkomsten van patiënten met een gemetastaseerd CRC te verbeteren.

Hoofdstuk 2

Uit de literatuur is bekend dat CRC patiënten met synchrone metastasen een slechtere prognose hebben dan patiënten met metachrone metastasen. Desondanks wordt het aantal geïncludeerde patiënten met synchrone en metachrone metastasen, evenals hun invloed op de overleving, niet frequent gerapporteerd in studies naar gemetastaseerd CRC. In Hoofdstuk 2 worden de resultaten beschreven van onderzoek naar de inclusie en overleving van patiënten met synchrone en metachrone metastasen in gerandomiseerde

gecontroleerde trials (RCTs), cohort- en populatiestudies naar eerstelijns systemische therapie of chirurgische behandeling van gemetastaseerd CRC. In slechts een klein deel van de publicaties verschenen tussen 2004 en 2016 was het aantal patiënten met synchrone of metachrone metastasen gerapporteerd. Verder bleek er geen consensus te bestaan over de definitie van 'synchrone metastasen', gezien het feit dat in 46 studies 17 verschillende definities werden gebruikt. In de RCTs nam het percentage patiënten met synchrone metastasen de laatste jaren aanzienlijk toe ten opzichte van het percentage patiënten met metachrone metastasen. In deze RCTs nam de mediane OS van de totale onderzoekspopulatie in de loop der tijd toe. Echter, er werd geen onderscheid gemaakt in de mediane OS tussen patiënten met synchrone of metachrone metastasen. Analyse van de cohort- en populatiestudies leverde geen significante resultaten op. Deze studie benadrukt het belang van een algemeen geaccepteerde definitie van 'synchrone metastasen' en het consistent rapporteren van het aantal patiënten met synchrone en metachrone metastasen in studies naar gemetastaseerd CRC. Uniformiteit op dit gebied kan leiden tot meer kennis over verschillen in overleving tussen CRC patiënten met synchrone en metachrone metastasen, en zal het maken van vergelijkingen tussen studies vereenvoudigen.

Hoofdstuk 3

In 2007 is een voorstel gedaan voor het gestandaardiseerd rapporteren van patiëntkenmerken en gebruik van stratificatiefactoren in studies over systemische therapie bij patiënten met een gemetastaseerd CRC. Echter, het is onbekend in hoeverre dit voorstel is opgevolgd in publicaties van RCTs over gemetastaseerd CRC die in meer recente jaren zijn verschenen. Hoofdstuk 3 beschrijft onze bevindingen over de rapportage van patiëntkenmerken en het gebruik van stratificatiefactoren in RCTs in eerstelijns systemische therapie van gemetastaseerd CRC, gepubliceerd tussen 2005 en 2016. In deze studie van 67 RCTs met meer dan 35.000 patiënten troffen wij veel heterogeniteit aan met betrekking tot de gerapporteerde patiëntkenmerken en gebruikte stratificatiefactoren. In studies gepubliceerd tussen 2009 tot 2016 was er slechts een lichte vermindering van deze heterogeniteit ten opzichte van eerder gepubliceerde studies. Hieruit blijkt dat het voorstel voor het gestandaardiseerd rapporteren van patiëntkenmerken en gebruik van stratificatiefactoren niet op grote schaal wordt toegepast. Bovendien viel op dat nieuwe prognostische factoren die relevant zijn geworden door de komst van 'targeted' therapieën zelden werden gerapporteerd. Deze studie laat zien dat er weinig aandacht wordt besteed aan prognostische factoren die de uitkomst van patiënten in belangrijke mate kunnen voorspellen. Daarnaast benadrukt deze studie de noodzaak om internationale consensus te bereiken over een gestandaardiseerde set van prognostische factoren om als patiëntkenmerken en stratificatiefactoren te gebruiken in studies bij patiënten met een gemetastaseerd CRC, hetgeen de interpretatie van studieresultaten kan vergemakkelijken als ook een onderlinge vergelijking van studieresultaten kan verbeteren.

Hoofdstuk 4

De resultaten gepresenteerd in Hoofdstuk 3 vormden voor ons de aanleiding om het rapporteren van patiëntkenmerken en gebruik van stratificatiefactoren in RCTs naar gemetastaseerd CRC te verbeteren. Met behulp van de Delphi-procedure hebben wij in samenwerking met 30 internationale experts op het gebied van CRC een consensusstuk geschreven over essentiële patiëntkenmerken en stratificatiefactoren in RCTs over systemische behandeling van patiënten met een gemetastaseerd CRC. Dit consensusstuk, gepresenteerd in Hoofdstuk 4, wordt gesteund door de Aide et Recherche Cancérologie Digestive (ARCAD) groep, een internationaal samenwerkingsverband tussen clinici, statistici en wetenschappers gespecialiseerd in CRC, die als doel voor ogen hebben om efficiëntere klinische trials te ontwikkelen. Onze aanbevolen set van essentiële patiëntkenmerken bestaat uit 14 factoren: leeftijd, performance status, primaire tumorlocatie, primaire tumorresectie, eerdere chemotherapie, aantal organen met metastasen, metastasen beperkt tot de lever, metastasen met betrokkenheid van de lever, chirurgische resectie van metastasen, synchrone versus metachrone metastasen, *(K)RAS* en *BRAF* mutatiestatus, microsatelliet instabiliteit (MSI) / mismatch repair (MMR) status en het aantal eerdere lijnen van systemische therapie. De vijf meest relevante stratificatiefactoren zijn: *RAS/BRAF* mutatiestatus, performance status, lateraliteit (links- versus rechtszijdige lokalisatie van de primaire tumor), en of metastasen beperkt zijn tot de lever. Het verzamelen en gebruiken van deze essentiële patiëntkenmerken en stratificatiefactoren in onderzoeksprotocollen en finale publicaties van toekomstige studies naar gemetastaseerd CRC zal de interpretatie van onderzoeksresultaten, het vergelijken van studieresultaten en het uitvoeren van betrouwbare meta-analyses aanzienlijk verbeteren.

Hoofdstuk 5

De gerandomiseerde fase 3 CAIRO3-studie heeft aangetoond dat capecitabine en bevacizumab (CAP-B) onderhoudsbehandeling na zes cycli capecitabine, oxaliplatine en bevacizumab (CAPOX-B) effectief is bij patiënten met een gemetastaseerd CRC, met behoud van kwaliteit van leven. Echter, onderhoudsbehandeling is mogelijk niet kosteneffectief, en een betere selectie van patiënten zou de klinische besluitvorming verbeteren en de therapiekosten verminderen. In Hoofdstuk 5 worden de resultaten beschreven van een post-hoc analyse van de CAIRO3-studie met bijgewerkte gegevens over follow-up en lokalisatie van de primaire tumor, waarin wij subgroepen hebben gedefinieerd volgens *RAS*, *BRAF*-mutatiestatus en MMR-status en hun invloed op de effectiviteit van de behandeling hebben onderzocht. *RAS* mutaties, *BRAF* mutaties en MMR-deficiëntie kwamen voor bij respectievelijk 58%, 9% en 1% van de patiënten. Onderhoudsbehandeling is effectiever vergeleken met observatie in de *RAS/BRAF* wild-type, *RAS*-gemuteerde en ^{V600E}*BRAF*-gemuteerde subgroepen voor het primaire eindpunt PFS2 (tweede progressievrije overleving na herintroductie van CAPOX-B) en de secundaire eindpunten, behalve in de *RAS*-gemuteerde

subgroep voor OS. Het corrigeren van de moleculaire subgroep-analyses voor rechts- versus linkszijdige lokalisatie van de primaire tumor in plaats van de oorspronkelijke indeling voor primaire tumorlocatie (colon versus rectosigmoid versus rectum) levert vergelijkbare resultaten op. Zowel patiënten met rechts- als linkszijdige tumoren hebben significant baat bij onderhoudsbehandeling. Deze studie laat zien dat CAP-B onderhoudsbehandeling na zes cycli CAPOX-B effectief is als eerstelijnsbehandeling bij patiënten met een gemetastaseerd CRC. Het voordeel van onderhoudsbehandeling wordt waargenomen in alle moleculaire subgroepen en is het meest uitgesproken in patiënten met *RAS/BRAF* wild-type of ^{V600E}*BRAF*-gemuteerde tumoren.

Hoofdstuk 6

'Next generation sequencing' (NGS) speelt een belangrijke rol om te komen tot een behandeling op maat voor patiënten met een gemetastaseerd CRC. Naast sensitieve detectie van mutaties stelt NGS ons ook in staat om mutante allelen te kwantificeren. De fractie mutante allelen ('mutant allele fraction', MAF) wordt gedefinieerd als het aantal mutante allelen gedeeld door het totale aantal allelen op een specifieke genomische positie. Deze MAF-waarde kan van toegevoegde waarde zijn in de behandeling van patiënten met een gemetastaseerd CRC. In Hoofdstuk 6 staan de resultaten beschreven van onderzoek naar de verdeling en de prognostische waarde van *KRAS* MAFs en *KRAS* MAFs genormaliseerd voor het percentage tumorcellen in het sample gebruikt voor NGS (adjMAFs) in 170 patiënten met een gemetastaseerd CRC en *KRAS*-gemuteerde tumoren uit de CAIRO3 studie. Er is een duidelijke heterogeniteit waarneembaar in de verdeling van *KRAS* MAFs en adjMAFs. De mediane OS varieert tussen tertielen van *KRAS* MAF en adjMAF, maar deze verschillen zijn niet statistisch significant. In multivariabele Cox-regressieanalyse met en zonder 'restricted cubic splines' hebben wij geen significante (non-)lineaire associaties waargenomen tussen *KRAS* MAFs of adjMAFs en OS. Deze exploratieve analyse suggereert dat *KRAS* MAFs en adjMAFs geen onafhankelijke prognostische factoren zijn voor patiënten met een gemetastaseerd CRC en *KRAS*-gemuteerde tumoren die behandeld zijn met CAP-B onderhoudsbehandeling versus observatie na zes cycli CAPOX-B.

Hoofdstuk 7

De gerandomiseerde fase 3 CAIRO3 en de AIO 0207 studies hebben aangetoond dat onderhoudsbehandeling met een fluoropyrimidine (5-fluorouracil of capecitabine) en bevacizumab effectiever is dan observatie bij patiënten met een gemetastaseerd CRC met stabiele ziekte of respons na inductiebehandeling met een fluoropyrimidine, oxaliplatine en bevacizumab, terwijl de kwaliteit van leven behouden blijft. Gezien niet alle patiënten baat zullen hebben bij onderhoudsbehandeling, is een betere patiëntselectie van belang om te komen tot behandeling op maat, waardoor therapiekosten zullen verminderen.

Hoofdstuk 7 betreft een meta-analyse op basis van individuele patiëntdata met bijgewerkte follow-up van de CAIRO3 en AIO 0207 studies. In 871 patiënten met een gemetastaseerd CRC, gerandomiseerd voor fluoropyrimidine en bevacizumab onderhoudsbehandeling versus observatie, onderzochten we of het behandeleffect werd beïnvloed door geslacht, leeftijd, performance status, respons op inductiebehandeling, primaire tumorlocatie, aantal organen met metastasen, synchrone versus metachrone metastasen in combinatie met resectiestatus van de primaire tumor, serum LDH, trombocyten, CEA en *RAS/BRAF* mutatiestatus. Onderhoudsbehandeling met fluoropyrimidine en bevacizumab is effectiever vergeleken met observatie, met significante resultaten voor PFS1 (eerste progressievrije overleving) en PFS2 (primair eindpunt). De subgroep-analyses tonen geen subpopulaties die geen baat hadden bij onderhoudsbehandeling voor PFS1 en PFS2; ook zijn er geen klinisch relevante subgroep-effecten. Gepoolde resultaten voor OS zijn niet statistisch significant, en de CAIRO3 en AIO 0207 studies tonen heterogeniteit in zowel het algemene behandeleffect als in subgroep-effecten. Deze studie laat zien dat onderhoudsbehandeling met een fluoropyrimidine en bevacizumab effectief is bij patiënten met een gemetastaseerd CRC met stabiele ziekte of respons na inductiebehandeling met een fluoropyrimidine, oxaliplatine en bevacizumab, met significante resultaten voor PFS1 en PFS2. Subgroep-analyses tonen geen subpopulaties van patiënten voor wie observatie een vergelijkbare uitkomst geeft ten opzichte van onderhoudsbehandeling.

Conclusie

Het onderzoek beschreven in dit proefschrift ondersteunt het gebruik van onderhoudsbehandeling met een fluoropyrimidine en bevacizumab na inductiebehandeling met een fluoropyrimidine, oxaliplatine en bevacizumab in de eerstelijnsbehandeling van patiënten met een gemetastaseerd CRC, ongeacht relevante klinische, pathologische en moleculaire subgroepen. Ons consensus voorstel biedt een hulpmiddel voor het uniform rapporteren van patiëntkenmerken en gebruik van stratificatiefactoren in toekomstige studies naar gemetastaseerd CRC, wat essentieel is om de interpretatie van studieresultaten te verbeteren en het onderling vergelijken van studies beter mogelijk te maken. In de toekomst zal de rol van tumorheterogeniteit en moleculaire subtypen steeds meer terrein winnen. Nieuwe strategieën voor biomarker-onderzoek en preklinische kankermodellen zijn essentieel om de complexiteit van de biologie van het CRC te ontrafelen. Daarnaast zijn grote (inter)nationale consortia, zoals het Prospectief Landelijk ColoRectaal Carcinoom cohort (PLCRC), van belang om onderzoek beter te kunnen integreren in de klinische praktijk. Er is steeds meer behoefte aan internationale samenwerking bij het delen van individuele patiëntgegevens, inclusief gegevens over biomateriaal en patiënt-gerapporteerde uitkomsten. Dit vereist een gestandaardiseerde aanpak voor het verzamelen en rapporteren van patiëntgegevens. Op deze manier kunnen wij in de toekomst klinische vragen over een specifieke subgroep van

patiënten met een (gemetastaseerd) CRC beter beantwoorden, met als uiteindelijk doel om te komen tot een behandeling op maat voor elke individuele patiënt op basis van klinische, pathologische en genetische kenmerken.

Appendices

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Curriculum Vitae

List of Publications

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Kaitlyn

Curriculum Vitae

Kaitlyn Goey was born on the 15th of February 1989 in Amsterdam, the Netherlands. She was raised in Ridderkerk and moved to Goirle in 1999. In 2007, she graduated cum laude from the Mill-Hill College in Goirle (Gymnasium). Subsequently, she started studying Medicine at the University of Utrecht in September 2007.

Early in medical school, she became interested in doing research. In her first research internship, she studied circadian rhythms in stem-cell derived cardiomyocytes at the Department of Medical Physiology of the University Medical Center (UMC) Utrecht, supervised by dr. L.W. van Laake, dr. M.K.B. Jonsson and dr. T.A.B. van Veen. In her final year, she investigated the predictive value of serum microRNAs in early breast cancer detection at the Department of Pathology of the UMC Utrecht, supervised by prof. dr. P.J. van Diest and dr. C.B. Moelans.

In December 2013, she obtained her medical degree and decided to pursue her scientific ambitions in her field of interest: medical oncology. In February 2014, she started her PhD project described in this thesis at the Department of Medical Oncology of the UMC Utrecht, supervised by prof. dr. M. Koopman, prof. dr. C.J.A. Punt and dr. M.G.H. van Oijen. In September 2017, she started working as a resident Internal Medicine at the Diakonessenhuis in Utrecht. Her ambition is to combine patient care with scientific research.

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