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ORIGINAL ARTICLE

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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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.

Patients and 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: hazard ratio (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- 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.

ClinicalTrials.gov number: NCT00442637.

Key words: colorectal cancer, metastatic disease, bevacizumab, maintenance treatment, *RAS* mutation status, *BRAF* mutation status

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 (MSI), 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 bevacizumab (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 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: caecum-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 out 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 S1, available at *Annals of Oncology* online). 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 S1, available at *Annals of Oncology* online).

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 S2, available at *Annals of Oncology* online). 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 S3, available at *Annals of Oncology* online. 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 S4, available at *Annals of Oncology* online).

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).

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 S5, available at *Annals of Oncology* online). 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) versus 21.8 months (20.2–23.5), $P = 0.010$; supplementary Table S6, available at *Annals of Oncology* online]. 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 S7, available at *Annals of Oncology* online). 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 S8, available at *Annals of Oncology* online). 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.

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,

Table 1. Patients' 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				
$<400\times10^9$/l	346 (62%)	92 (66%)	150 (63%)	13 (43%)
$\geq400\times10^9$/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.
[a]Two patients with concomitant *BRAF* MT tumour excluded.
[b]One patient with concomitant *RAS* MT tumour excluded.
[c]Right colon: caecum to transverse colon.
[d]Left colon: splenic flexure to rectum.
[e]Synchronous metastases: distant metastases discovered ≤6 months after diagnosis of the primary tumour.
MT, mutant; WT, wild-type.

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.

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	

[a]Two patients with concomitant *BRAF* MT tumour excluded.

[b]One patient with concomitant *RAS* MT tumour 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: $P = 0.028$.

CI, confidence interval; HR, hazard ratio for maintenance treatment versus observation; Maint, maintenance; MT, mutant; Obs, observation; WT, wild-type.

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, 7]. 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.

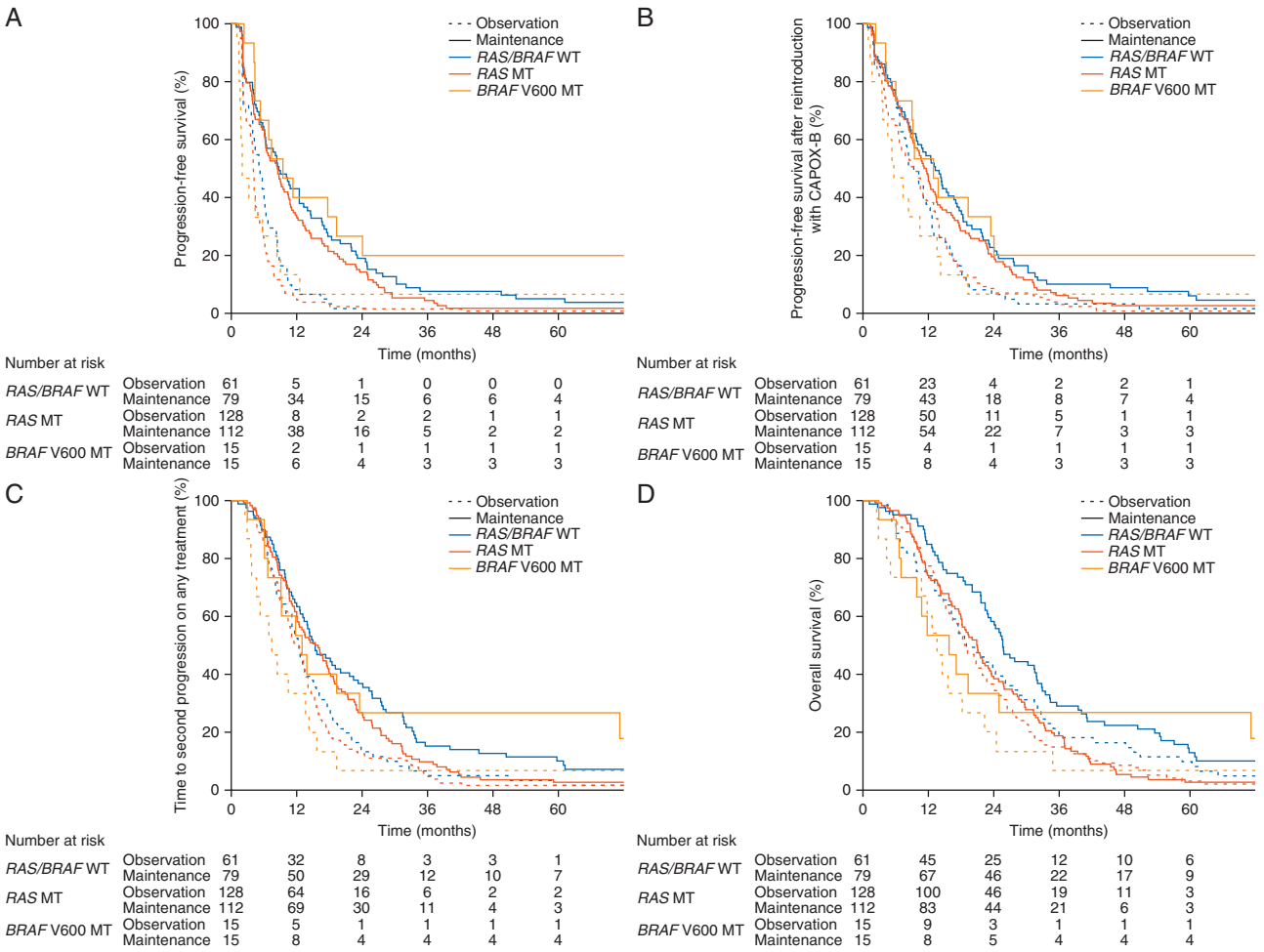

Figure 1. Kaplan–Meier curves for progression-free and overall survival according to *RAS* and *BRAF* mutation status. (A) Progression-free survival, (B) progression-free survival after CAPOX-B reintroduction, (C) time to second progression on any treatment, (D) overall survival. MT, mutant WT, wild-type.

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 AIO0207 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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Disclosure

CJAP has acted in an advisory role for Amgen, Roche, Bayer, Nordic Pharma and Merck-Serono. MK has acted in an advisory role for Amgen, Roche, Bayer, Servier and Merck. SMW has acted in an advisory role for Pfizer, Roche and MSD. All remaining authors have declared no conflicts of interest.

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