

Loss of Chromosome 18q11.2-q12.1 Is Predictive for Survival in Patients With Metastatic Colorectal Cancer Treated With Bevacizumab

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ABSTRACT

Purpose

Patients with metastatic colorectal cancer (mCRC) have limited benefit from the addition of bevacizumab to standard chemotherapy. However, a subset probably benefits substantially, highlighting an unmet clinical need for a biomarker of response to bevacizumab. Previously, we demonstrated that losses of chromosomes 5q34, 17q12, and 18q11.2-q12.1 had a significant correlation with progression-free survival (PFS) in patients with mCRC treated with bevacizumab in the CAIRO2 clinical trial but not in patients who did not receive bevacizumab in the CAIRO trial. This study was designed to validate these findings.

Materials and Methods

Primary mCRC samples were analyzed from two cohorts of patients who received bevacizumab as first-line treatment; 96 samples from the European multicenter study Angiopredict (APD) and 81 samples from the Italian multicenter study, MOMA. A third cohort of 90 samples from patients with mCRC who did not receive bevacizumab was analyzed. Copy number aberrations of tumor biopsy specimens were measured by shallow whole-genome sequencing and were correlated with PFS, overall survival (OS), and response.

Results

Loss of chromosome 18q11.2-q12.1 was associated with prolonged PFS most significantly in both the cohorts that received bevacizumab (APD: hazard ratio, 0.54; $P = .01$; PFS difference, 65 days; MOMA: hazard ratio, 0.55; $P = .019$; PFS difference, 49 days). A similar association was found for OS and overall response rate in these two cohorts, which became significant when combined with the CAIRO2 cohort. Median PFS in the cohort of patients with mCRC who did not receive bevacizumab and in the CAIRO cohort was similar to that of the APD, MOMA, and CAIRO2 patients without an 18q11.2-q12.1 loss.

Conclusion

We conclude that the loss of chromosome 18q11.2-q12.1 is consistently predictive for prolonged PFS in patients receiving bevacizumab. The predictive value of this loss is substantiated by a significant gain in OS and overall response rate.

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INTRODUCTION

Standard chemotherapeutic first-line treatment of patients with metastatic colorectal cancer (mCRC) consists of a fluoropyrimidine combined with either irinotecan or oxaliplatin and a targeted agent. Targeted agents are either cetuximab or panitumumab, which are both epidermal

growth factor receptor (EGFR) inhibitors, or bevacizumab, a vascular endothelial growth factor (VEGF) inhibitor.¹ The addition of bevacizumab to first-line combination chemotherapy in two large randomized phase III mCRC clinical trials, by Hurwitz et al² and Saltz et al,³ showed inconsistent results on median progression-free survival (PFS) and overall survival (OS).^{2,3} Two explanations for this inconsistency have been

ASSOCIATED CONTENT



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presented: (1) the use of a regimen with bolus fluorouracil (FU) plus irinotecan in Hurwitz et al,² which, because of its toxicity, is now considered obsolete; and (2) the inconsistent treatment continuation until progressive disease in Saltz et al.³ The importance of bevacizumab continuation was demonstrated recently by the results of the CAIRO3 trial.⁴ The benefit of bevacizumab, however, is associated with a high cost and an appreciable toxicity profile.^{2,5-7} Consequently, and despite international guidelines,¹ bevacizumab is not available in all countries.⁸ A predictive biomarker for bevacizumab is thus an urgent clinical need, as acknowledged by the European Society for Medical Oncology.¹

Several candidate biomarkers for bevacizumab, including tumor VEGF isoforms (VEGF-A, VEGF-B, VEGF-C, and VEGF-D) and neuropilin-1, a coreceptor for VEGF, have been proposed. These markers have yielded promising results in small exploratory studies, but validation in larger independent trials has failed.⁹⁻¹¹

In a genome-wide screen of 119 well-annotated mCRC tumors from patients treated with bevacizumab from the CAIRO2 phase III clinical trial,¹² losses at chromosomes 5q, 17q, and 18q correlated significantly with prolonged PFS, with the highest significance at the chromosomal regions 18q11.2-q12.1, 5q34, and 17q12.¹³ Although the strongest trend was observed for region 18q11.2-q12.1, almost all patients with a loss of region 18q11.2-q12.1 lost the entire chromosomal arm 18q, rendering this finding comparable to that of previous publications in which loss of chromosomal arm 18q was found to be a prognostic marker.¹⁴⁻¹⁷ In 186 patients from the CAIRO phase III clinical trial,¹⁸ in which patients received sequential or combination chemotherapy but no bevacizumab, the same chromosomal regions did not correlate with PFS.¹³ It could not be concluded whether the biomarker candidates would be predictive for oxaliplatin or bevacizumab, because the CAIRO2 patients had received bevacizumab combined with capecitabine and oxaliplatin, whereas the CAIRO patients had received either capecitabine or capecitabine and irinotecan but neither bevacizumab nor oxaliplatin. Here we present validation data from three additional multicenter cohorts showing that chromosomal region 18q11.2-q12.1 has predictive value for bevacizumab in mCRC.

MATERIALS AND METHODS

Patient Samples

Samples from patients with histologically proven mCRC or locally irresectable colorectal cancer treated with standard first-line combination chemotherapy with or without bevacizumab with extensive clinical follow-up were included in this study.

Formalin-fixed and paraffin-embedded primary tumor samples from three cohorts were assembled for clinical validation purposes: (1) the Angiopredict (APD) cohort (96 tumor samples from patients receiving chemotherapy supplemented with bevacizumab in first-line treatment)¹⁹; (2) the Maintenance Bevacizumab Only or Bevacizumab Plus Metronomic Chemotherapy in Advanced Colorectal Cancer (MOMA) cohort (81 tumor samples from the MOMA clinical trial of patients receiving chemotherapy supplemented with bevacizumab in first-line treatment [ClinicalTrials.gov identifier: NCT02271464])²⁰; and (3) the NoBev cohort (90 tumor samples from patients who did not receive bevacizumab and received standard combination chemotherapy regimens similar to that of the APD cohort¹⁹; Table 1). Of the 90 NoBev samples, 80 are also included in Neerincx et al (manuscript in preparation) and 38 of those are included

in Smeets et al (manuscript in preparation). T and N classification, grading, and tumor location were collected by reviewing patient records. T and N classifications and grading were evaluated routinely by pathologists from the participating centers using the American Joint Committee Classification 7th edition cancer staging manual classification.²¹ TNM staging and age were not significantly correlated with bevacizumab supplementation in either the APD or the NoBev cohort (Table 2).

Chemotherapy in the APD and NoBev cohorts was variable yet balanced and consisted of capecitabine or FU and leucovorin combined with either oxaliplatin, irinotecan, or both (Table 1). Combination chemotherapy in the MOMA cohort was FU and leucovorin, oxaliplatin, and irinotecan supplemented with bevacizumab in first-line treatment.

Array comparative genomic hybridization (arrayCGH) copy number data from the CAIRO and CAIRO2 cohorts were retrieved from the gene expression omnibus (data set GSE3684). Samples from patients receiving fewer than three cycles of chemotherapy and samples with microsatellite instability (MSI) were excluded by Haan et al.¹³ However, these data were included here to render the CAIRO and CAIRO2 cohorts more comparable to the APD and NoBev cohorts, in which MSI status and the number of cycles to progression were not considered¹⁹ because they were not always available. This added arrayCGH data from a total of 44 samples to the CAIRO and CAIRO2 data sets. In the CAIRO cohort, seven samples were MSI positive²² and 24 patients received fewer than three cycles of chemotherapy (total expanded data set [n = 216 samples]). In the CAIRO2 cohort, 12 samples were MSI positive²² and two patients received fewer than three cycles of chemotherapy (total expanded data set [n = 133 samples]).

The conclusions by Haan et al¹³ remained unaffected after expansion of the data set (Fig 1A). The overall median PFS in the CAIRO2 cohort was 326 days in Haan et al,¹³ and it became 323 days in the expanded data set. Significance for 5q34 remained unchanged and was reduced from 0.004 to 0.045 for 17q12. In the CAIRO cohort, median PFS was 224 days in Haan et al,¹³ and PFS was 249 days in the expanded data set.

For technical validation, arrayCGH results by Haan et al¹³ were compared with shallow next generation sequencing (NGS) results. Therefore, DNA previously isolated and used for the arrayCGH experiments¹³ from 96 tumor samples from the CAIRO2 trial were subjected to shallow NGS.

Power Calculation and Statistics

Power analysis was performed using the proportional hazards cure model.²³ Data from the CAIRO2 trial¹³ were used to estimate the hazard

Table 1. Chemotherapy Regimens for All Three Validation Cohorts and the CAIRO and CAIRO2 Cohorts of the Extended Data Set

Cohort	APD	NoBev	MOMA	CAIRO	CAIRO2
CAPOX/FOLFOX	56 (58)	45 (50)	0 (0)	0 (0)	133 (100)
CAPIRI/FOLFIRI	30 (31)	16 (18)	0 (0)	111 (51)	0 (0)
CAP/FU*	5 (5)	17 (19)	0 (0)	105 (49)	0 (0)
FOLFOXIRI	0 (0)	3 (3)	81 (100)	0 (0)	0 (0)
Other†	5 (5)	9 (10)	0 (0)	0 (0)	0 (0)
Total	96 (100)	90 (100)	81 (100)	216 (100)	133 (100)

NOTE. Data are presented as No. (%).

Abbreviations: APD, Angiopredict; CAP/FU, capecitabine or fluorouracil alone; CAPIRI/FOLFIRI, capecitabine or fluorouracil in combination with irinotecan; CAPOX/FOLFOX, capecitabine or fluorouracil in combination with oxaliplatin; FOLFOXIRI, fluorouracil in combination with oxaliplatin and irinotecan; NoBev, cohort of patients who did not receive bevacizumab and received standard combination chemotherapy regimens similar to that of the APD cohort.

*Because bevacizumab is typically given in addition to combination chemotherapy, the group receiving CAP/FU monotherapy is slightly underrepresented in the APD cohort compared with the NoBev cohort (χ^2 test, $P = .007$).

†Other refers to patients receiving some treatment in the Table supplemented by mitomycin (n = 1), panitumumab (n = 2), cetuximab (n = 3), aflibercept (n = 1), imatinib (n = 1), or raltitrexed (n = 1), monotherapy irinotecan (n = 2), or monotherapy bevacizumab (n = 3).

Table 2. Available Clinical and Pathologic Variables in the APD, NoBev, and MOMA Cohorts at Time of Diagnosis/Surgery

Cohort	APD	NoBev	P	MOMA
Age, years	61.6	61.1	.40	61
Sex				
Male	68 (71)	58 (64)	.20	49 (61)
Female	28 (29)	32 (36)		31 (38)
Not available	0	0		1 (1)
Total patients	96 (100)	90 (100)		81 (100)
T stage				
T1	1 (1)	2 (2)	.43	0 (0)
T2	11 (11)	6 (7)		0 (0)
T3	61 (64)	50 (56)		0 (0)
T4	22 (23)	29 (32)		0 (0)
Not available	5 (5)	3 (3)		81 (100)
N stage				
N0	24 (25)	24 (27)	.67	0 (0)
N1	28 (29)	29 (32)		0 (0)
N2	37 (39)	34 (38)		0 (0)
Not available	7 (7)	3 (3)		81 (100)
M stage				
M0	34 (35)	38 (42)	.42	0 (0)
M1	61 (54)	52 (58)		0 (0)
Not available	1 (1)	0 (0)		81 (100)
Metastasis				
To one organ	0 (0)	0 (0)	—	34 (42)
To multiple organs	0 (0)	0 (0)		47 (58)
Not available	96 (100)	90 (100)		0 (0)
Adjuvant treatment				
Received	0 (0)	0 (0)	—	10 (12)
Did not receive	0 (0)	0 (0)		71 (88)
Not available	96 (100)	90 (100)		0 (0)

NOTE. Data are presented as No. (%) unless indicated otherwise. M0 corresponds to patients with metachronous cancer, and M1 corresponds to patients with synchronous cancer. A χ^2 test was used to calculate *P* values for an association between the clinical variables and the addition of bevacizumab to chemotherapy between the APD and NoBev cohorts. No significant associations were found.

Abbreviations: APD, Angiopredict; NoBev, cohort of patients who did not receive bevacizumab and received standard combination chemotherapy regimens similar to that of the APD cohort.

ratio for patients with and without a loss, and to estimate the proportion of patients with a chromosomal aberration. For all three candidate biomarkers, loss of chromosomal regions 5q34, 17q12, and 18q11.2-q12.1, the minimal sample size was calculated for $\alpha = .05$ and 80% power. This demonstrated that a minimum number of 69 patient samples for the 18q11.2-q12.1 loss, 115 for the 5q34 loss, and 339 for the 17q12 loss were required to validate the results obtained by Haan et al.¹³ All cohorts were thus sufficiently powered to validate the 18q11.2-q12.1 loss. However, none of the cohorts was sufficiently powered to validate either the 5q34 or the 17q12 losses; these losses were not considered for additional analysis.

Using the statistical programming language R (v 3.4.0) and package CGHtest (v 1.1), a two-sided log-rank test was used to calculate a *P* value for the association between copy number aberrations and PFS.²⁴ A *P* value threshold of .05 was used to test the significance of the association between 18q11.2-q12.1 loss and PFS. In these cohorts, only chromosome 18q11.2-q12.1 loss was tested; hence, no multiple testing correction was applied. The interaction *P* value was calculated for the interaction of PFS with bevacizumab. OS and the overall response rate (ORR) were evaluated as secondary end points.

Correlations between loss of 18q11.2-q12.1 and standard prognostic factors serum lactate dehydrogenase elevation (LDH; normal v abnormal), age, sex, number of affected organs (one v more), and previously received adjuvant treatment were investigated for the CAIRO and CAIRO2 cohorts. These correlations were not calculated by Haan et al.¹³ Equivalent calculations were not possible for the APD and NoBev cohorts because the number of affected organs, previously received adjuvant treatment, and LDH status were not available. For the MOMA trial, this calculation was

performed for age, sex, number of affected organs and previously received treatment, but not for LDH status because that status was not available.

Sequencing and Processing

Copy number profiles of samples from all three validation cohorts and the 96 matched CAIRO2 samples were obtained for technical validation using shallow NGS as described by Scheinin et al.²⁵ Details are summarized in the Data Supplement. Algorithms and settings to call chromosomal copy numbers gains and losses and to determine chromosomal regions were performed according to Haan et al.^{13,26-29} Shallow NGS data have been deposited at the European Genome-phenome Archive (EGA; <http://www.ebi.ac.uk/ega/>), which is hosted by the European Bioinformatics Institute, under accession numbers EGAS00001002724 (APD and NoBev cohorts) and EGAS00001002617 (MOMA cohort and CAIRO2 technical validation samples).

RESULTS

Comparison Between Next-Generation Sequencing and arrayCGH

Technical validation of the shallow NGS procedures for copy number analysis was performed for 96 of the CAIRO2 samples analyzed previously by arrayCGH in Haan et al.¹³

Frequency plots for arrayCGH and shallow NGS copy number aberrations were comparable (Data Supplement). Genome-wide, the 30-kbp bins used in our shallow NGS analysis that are covered by an arrayCGH probe showed a 92% concordance in copy number calling, which once again technically validates the copy number results obtained by shallow NGS compared with arrayCGH.²⁵ For the 18q11.2-q12.1 region, 91 of the 96 samples have the same copy number calls. Despite these five discordant calls, survival analysis for the 96 samples by shallow NGS shows that the conclusions remained the same and are in concordance with the arrayCGH results (Data Supplement).^{25,30}

Landscape of Chromosomal Aberrations in the Different mCRC Cohorts

We performed shallow NGS for 96 tumor samples from the APD cohort, 90 from the NoBev cohort, and 81 from the MOMA cohort. The frequency distribution of chromosomal gains and losses was similar for all three cohorts and was comparable with the frequency distributions presented previously for the CAIRO and CAIRO2 cohorts¹³ (Data Supplement) and with the mCRC copy number data from The Cancer Genome Atlas.¹⁷

Association With Survival

Losses at three chromosomal regions, 5q34, 17q12, and 18q11.2-q12.1, were found previously to be the most significantly associated with PFS in the CAIRO2 treatment arm that was supplemented with bevacizumab¹³; these associations were not observed in the CAIRO data set. Given the power requirements, only chromosomal region 18q11.2-q12.1 could be tested in the APD, NoBev, and MOMA cohorts. However, these cohorts were too small to validate the 5q34 and 17q12 losses, because these copy number aberrations occur less frequently (Data Supplement) and would require cohorts of $n = 115$ and $n = 339$ or larger, respectively. A log-rank test was performed to determine the

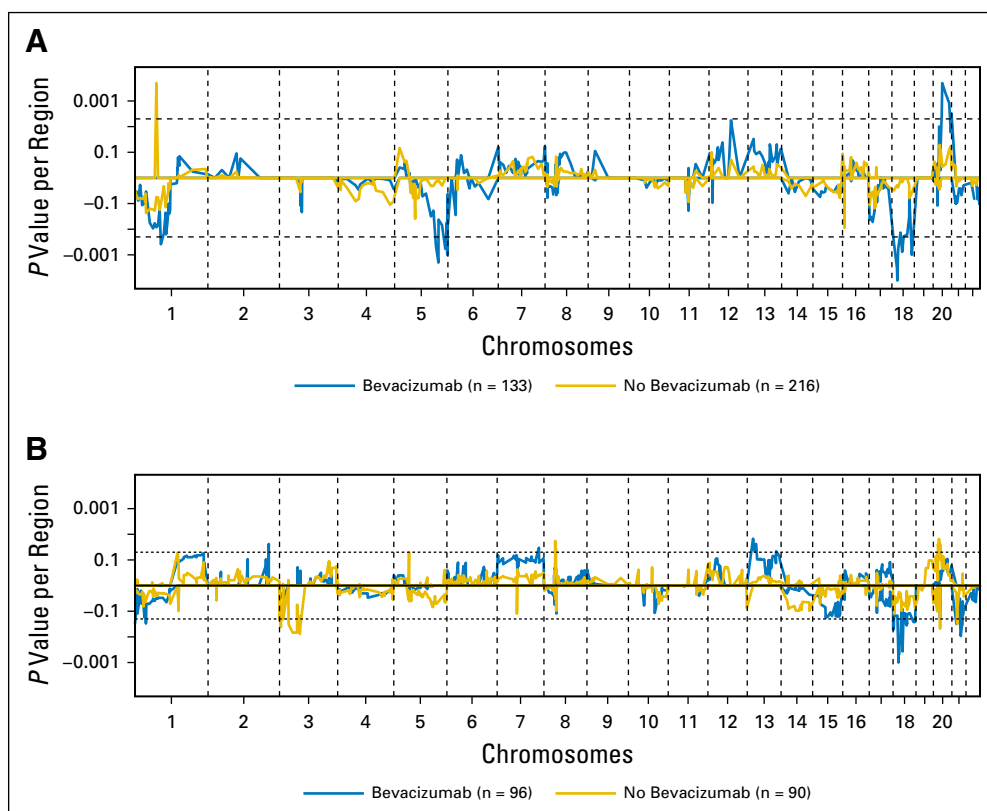


Fig 1. Genome-wide association of progression-free survival with either gain or loss in four cohorts. (A) CApecitabine, IRinotecan, and Oxalipatin in advanced colorectal cancer (CAIRO) and CAIRO2 (extended) cohorts. (B) Angiopredict cohort (APD) and NoBev cohort (patients who did not receive bevacizumab and received standard combination chemotherapy regimens similar to that of the APD cohort). *P* values of gains (positive values) or losses (negative values) are shown as a function of chromosomal regions sorted by chromosomal position (*x*-axis). Vertical dotted lines show the chromosomal borders. Horizontal dotted lines show the significance thresholds ($P = .005$ for the CAIRO cohorts and $P = .05$ for the APD and NoBev cohorts). In both cohorts with bevacizumab treatment (CAIRO2 and APD, blue lines), the chromosomal region in 18q is significantly associated with survival. In both cohorts treated without bevacizumab (CAIRO and NoBev, gold lines), there is no significant association. Even though only chromosomal region 18q11.2-q12.1 has a *P* value below the significance threshold, the same trend is observed for the entire chromosomal arm 18q for all cohorts treated with bevacizumab.

significance of associations between chromosome 18q11.2-q12.1 loss and PFS. This loss presented with a significant association with PFS for both patient cohorts receiving combination chemotherapy supplemented with bevacizumab: the APD cohort of 96 patients ($P = .01$; hazard ratio [HR], 0.54; median PFS, 290 days with loss and 226 days without loss) and the MOMA cohort of 81 patients ($P = .019$; HR, 0.55; median PFS, 275 days with loss and 231 days without loss), consistent with the 133 patients included in the expanded CAIRO2 cohort ($P = .0086$; HR, 0.57; median PFS, 330 days with loss and 196 days without loss; Figs 2A-2C).

No significant association was found for the 90 patients in the NoBev cohort who received combination chemotherapy treatment without bevacizumab ($P = .47$; HR, 0.85; median PFS, 206 days with loss and 206 days without loss), consistent with the 216 patients included in the CAIRO cohort ($P = .82$; HR, 0.96; median PFS, 225 days with loss and 192 days without loss; Figs 2A and 2B).

A combined analysis of all cohorts shows that chromosome 18q11.2-q12.1 loss provides an increase in median PFS of 109 days for patients treated with bevacizumab, when compared with patients without that loss or with patients who did not receive bevacizumab supplementation (Fig 2D).

The same analysis was performed for OS using a log-rank test to determine the significance of an association with chromosome 18q11.2-q12.1 loss. Of course, to calculate the association with OS, the heterogeneity of treatment modalities in second and subsequent treatment lines could not be considered, and this is a confounding factor. Nevertheless, for all cohorts, OS showed a trend similar to that of PFS. For the APD and MOMA

cohorts, these trends were not statistically significant, but in a combined analysis that included the CAIRO2 cohort, a significant association was observed ($P = .039$; HR, 0.73; median OS, 584 days with loss and 465 days without loss; Fig 3). No significant association between OS and 18q11.2-q12.1 loss was found for either the NoBev or the CAIRO cohorts, either alone or combined (Fig 3).

ORR was available for 566 of 616 patients; it was higher in patients with a loss of chromosomal region 18q11.2-q12.1 in all three cohorts treated with bevacizumab, consistent with the results for PFS and OS. This association was statistically significant in the CAIRO2 cohort, but not in the MOMA or APD cohort, both of which suffer from lower patient numbers. In a combined analysis, a significant association was found for patients treated with bevacizumab ($P = .0033$) but not for patients who did not receive bevacizumab ($P = .20$; Data Supplement).

Association of 18q11.2-q12.1 Loss With Known Prognostic Variables

In a combined analysis of the CAIRO and CAIRO2 cohorts,^{12,18} the number of affected organs and LDH status were significant prognostic factors. In the CAIRO cohort, in a multivariate Cox model, loss of 18q11.2-q12.1 was not significantly associated with PFS. In the CAIRO2 cohort, in a multivariate Cox model, loss of 18q11.2-q12.1 remained significantly associated with PFS. In the MOMA cohort, LDH status was not available. In a multivariable Cox model, loss of 18q11.2-q12.1 remained significantly associated with PFS (Data Supplement).

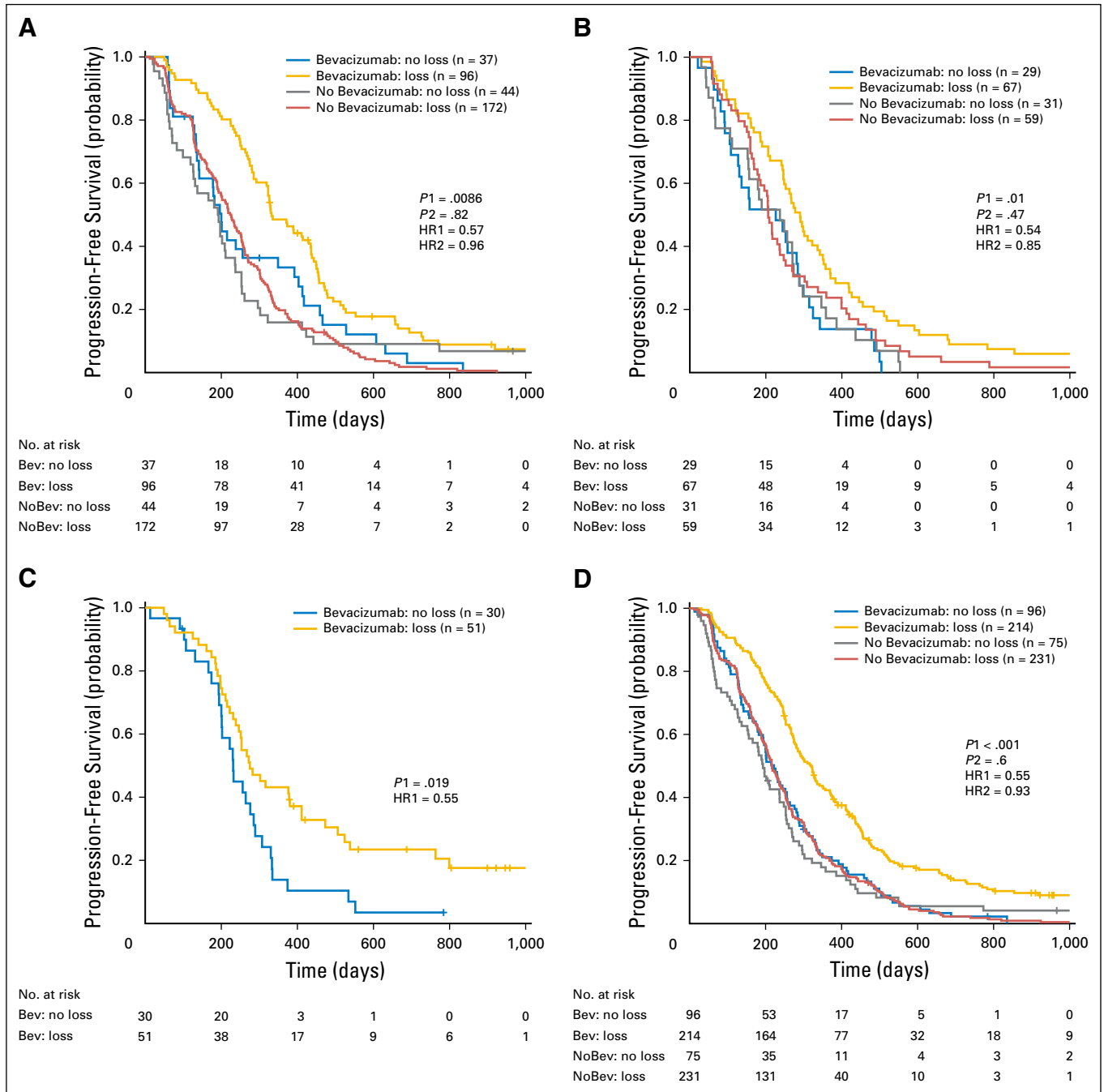


Fig 2. Kaplan-Meier survival analysis for loss versus no loss of 18q11.2-q12.1 with respect to progression-free survival for all cohorts, and a meta-analysis of the combined data. (A) Capecitabine, Irinotecan, and Oxaliplatin in Advanced Colorectal Cancer (CAIRO) and CAIRO2 cohorts. (B) Angiopredict cohort (APD) and NoBev cohort (patients who did not receive bevacizumab [Bev] and received standard combination chemotherapy regimens similar to that of the APD cohort). (C) Maintenance Bevacizumab Only or Bevacizumab Plus Metronomic Chemotherapy in Advanced Colorectal Cancer (MOMA) cohort. (D) Combined data sets. P_1 and HR_1 indicate P values and hazard ratios for patients treated with Bev (blue lines v gold lines). P_2 and HR_2 indicate significance and hazard ratios for patients treated without Bev (red lines v gray lines).

For the clinical variables available, the same analysis was performed in the APD and NoBev cohorts. There was no significant bias for age ($P = .40$), sex ($P = .20$), T stage ($P = .43$), N stage ($P = .67$), or M stage ($P = .42$) in the selection criteria for the APD versus the NoBev cohort (Table 2). Loss of chromosomal region 18q11.2-q12.1 was also compared with standard prognostic factors. Again, no significant bias for sex ($P = .16$), T stage ($P = .08$), N stage ($P = .79$), or M stage ($P = .22$) was found (Table 3). No multivariable Cox model was

used for this cohort, because there were no significant associations between clinical variables and PFS (Data Supplement).

The interaction P value of response to bevacizumab (P_{int}) with respect to loss of chromosomal region 18q11.2-q12.1 was not significant for the expanded CAIRO and CAIRO2 cohort pair ($P_{int} = .06$) or for the APD and NoBev cohort pair ($P_{int} = .14$). Both cohort pairs were underpowered to detect an interaction P value. Taken across all five cohorts, P_{int} was .006. We could not consider

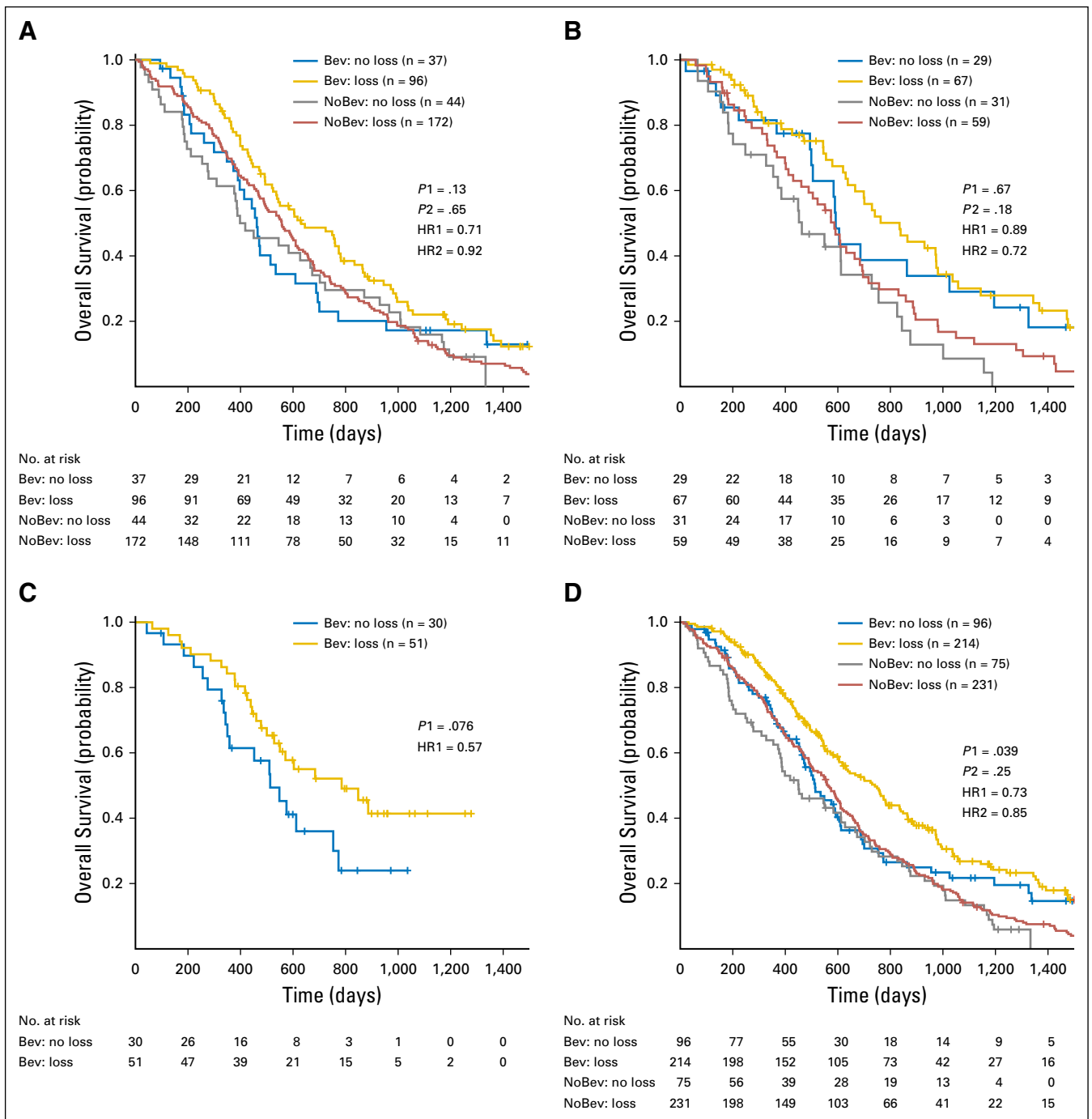


Fig 3. Kaplan-Meier survival analysis for loss versus no loss of 18q11.2-q12.1 with respect to overall survival for all cohorts, and a meta-analysis of the combined data. (A) Capecitabine, Irinotecan, and Oxaliplatin in Advanced Colorectal Cancer (CAIRO) and CAIRO2 cohorts. (B) Angiopredict cohort (APD) and NoBev cohort (patients who did not receive bevacizumab [Bev] and received standard combination chemotherapy regimens similar to that of the APD cohort). (C) Maintenance Bevacizumab Only or Bevacizumab Plus Metronomic Chemotherapy in Advanced Colorectal Cancer (MOMA). (D) Combined data sets. P_1 and HR_1 indicate P values and hazard ratios for patients treated with Bev (blue lines v gold lines). P_2 and HR_2 indicate significance and hazard ratio for patients treated without Bev (red lines v gray lines).

two potential confounding factors, MSI status and *KRAS* mutations. *KRAS* status is used as predictive marker for response to anti-EGFR inhibition, which was given to a small minority of patients in the NoBev and APD cohorts (n = 3). Together, these data demonstrate that the loss of chromosome 18q11.2-q12.1 has independent predictive value for prolonged PFS in patients with mCRC treated with bevacizumab (Table 3).

DISCUSSION

A correlation between PFS and chromosome 18q11.2-q12.1 loss for patients with mCRC treated with bevacizumab, first reported by Haan et al,¹³ was validated in two large independent cohorts of patients treated with bevacizumab. Because the correlation of PFS

Table 3. Comparison of Available Clinical and Pathologic Variables for Loss of 18q11.2-q12.1 v No Loss of 18q11.2-q12.1 at Time of Diagnosis/Surgery

18q11.2-q12.1 Status	APD and NoBev Loss	APD and NoBev No Loss	<i>P</i>	MOMA Loss	MOMA No Loss	<i>P</i>
Age, years	58.4	63.0	.07	60	61	.76
Sex						
Male	90 (71)	36 (60)	.16	31 (61)	18 (60)	1.00
Female	36 (29)	24 (40)		19 (37)	12 (40)	
Not available	0 (0)	0 (0)		1 (1)	0 (0)	
T stage						
T1	0 (0)	3 (5)	.08	0 (0)	0 (0)	—
T2	11 (9)	2 (3)		0 (0)	0 (0)	
T3	76 (60)	35 (58)		0 (0)	0 (0)	
T4	33 (26)	18 (30)		0 (0)	0 (0)	
Not available	6 (5)	2 (3)		51 (100)	30 (100)	
N stage						
N0	31 (25)	17 (28)	.79	0 (0)	0 (0)	—
N1	38 (30)	19 (32)		0 (0)	0 (0)	
N2	51 (40)	20 (3)		0 (0)	0 (0)	
Not available	6 (5)	4 (7)		51 (100)	30 (100)	
M stage						
M0	52 (41)	20 (33)	.22	0 (0)	0 (0)	—
M1	74 (59)	39 (65)		0 (0)	0 (0)	
Not available	0	1 (2)		51 (100)	30 (100)	
Affected organs						
1	0 (0)	0 (0)	—	24 (47)	10 (33)	.32
Multiple	0 (0)	0 (0)		27 (53)	20 (67)	
Not available	126 (100)	60 (100)		0 (0)	0 (0)	
Previous adjuvant treatment						
Yes	0 (0)	0 (0)	—	6 (12)	4 (13)	1.00
No	0 (0)	0 (0)		45 (88)	26 (87)	
Not available	126 (100)	60 (100)		0 (0)	0 (0)	

NOTE. Data are presented as No. (%) unless indicated otherwise. M0 corresponds to patients with metachronous cancer, and M1 corresponds to patients with synchronous cancer. A χ^2 test was used to calculate *P* values for an association between the loss of chromosomal region 18q11.2-q12.1 and the clinical variables. No significant associations were found.

Abbreviations: APD, Angiopredict; NoBev, cohort of patients who did not receive bevacizumab and received standard combination chemotherapy regimens similar to that of the APD cohort.

with 18q11.2-q12.1 loss was not observed in tumors of two large cohorts of patients with mCRC who received chemotherapy alone, we conclude that 18q11.2-q12.1 loss has predictive value for treatment with bevacizumab. The predictive value of this loss is supported by the statistical significance of an association with both OS and ORR in a combined analysis of all 310 patients treated with bevacizumab, which was not observed in the 306 patients treated with chemotherapy alone.

Loss of the entire chromosomal arm 18q has been observed previously to be a prognostic marker in several studies that considered non-mCRC primarily and hence, there was no bevacizumab-containing treatment.¹⁴⁻¹⁷ In a systematic review with a meta-analysis, it was concluded that although the loss of 18q is a poor prognostic indicator, results are variable among studies.³¹ Loss of the entire 18q arm was shown to correlate strongly with the vascularization of tumors in a cohort of 70 patients with non-mCRC.¹⁵ Hence, it is possible that the higher degree of vascular invasion in tumors with an 18q loss leads to a better response to the anti-angiogenic properties of bevacizumab. In our analysis, which considered only patients with mCRC, loss of the subcentromeric region 18q11.2-q12.1 had a slightly greater beneficial effect than loss of the entire chromosomal arm; in 91% of these samples, the entire chromosome 18q arm was lost. The effects of bevacizumab treatment can therefore not be attributed necessarily to 18q11.2-q12.1 alone; its effect may be multifactorial. There are 20 protein-coding genes located on 18q11.2-q12.1. Haan et al¹³ reported

LAM3 as the only gene significantly correlated with expression in colorectal cancer. *LAM3* encodes for epiligrin, which is also expressed at wound sites of human skin.³² *SMAD4*³³ and *DCC*³⁴ are both located outside and proximal to the 18q11.2-q12.1 region. Both genes have been suggested as tumor suppressor genes; in addition, loss of *SMAD4* has been linked to FU resistance³⁵ and is mutated in 14% of mCRC tumors.³⁶

Various approaches toward copy number analysis by shallow NGS have been introduced into the clinical diagnostic setting for formalin-fixed and paraffin-embedded solid tumor specimens.³⁷ Shallow NGS copy number analysis may be combined cost effectively with NGS mutation analysis,^{25,30} necessary for *KRAS* and *EGFR* screening of samples from patients with mCRC,¹⁸ in a clinical diagnostic setting.

The data presented here, which are supported by a robust and mature NGS copy number analysis platform, demonstrate that the loss of 18q should be considered in mCRC clinical decision making when considering supplementing combination chemotherapy with bevacizumab.

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Disclosures provided by the authors are available with this article at jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Loss of Chromosome 18q11.2-q12.1 Is Predictive for Survival in Patients With Metastatic Colorectal Cancer Treated With Bevacizumab

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