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Outcome according to KRAS-, NRAS- and BRAF-mutation as well as KRAS mutation variants: pooled analysis of five randomized trials in metastatic colorectal cancer by the AIO colorectal cancer study group

D. P. Modest[1,2*], I. Ricard[3], V. Heinemann[1,2], S. Hegewisch-Becker[4], W. Schmiegel[5], R. Porschen[6], S. Stintzing[1,2], U. Graeven[7], D. Arnold[8], L. F. von Weikersthal[9], C. Giessen-Jung[1], A. Stahler[1,10], H. J. Schmoll[11], A. Jung[2,10], T. Kirchner[2,10], A. Tannapfel[12] & A. Reinacher-Schick[13]

[1]Department of Medical Oncology and Comprehensive Cancer Center, University Hospital Grosshadern, Ludwig-Maximilians-Universität, Munich; [2]German Cancer Consortium (DKTK), German Cancer Research Centre (DKFZ), Heidelberg; [3]Institute of Medical Informatics, Biometry and Epidemiology, University of Munich, Munich; [4]HOPE-Practice for Oncology, Hamburg; [5]Medizinische Klinik, Knappschaftskrankenhaus Ruhr-Universität Bochum, Bochum; [6]Klinik für Innere Medizin, Klinikum Bremen-Ost, Bremen; [7]Kliniken Maria Hilf GmbH, Department of Hematology, Oncology and Gastroenterology, Mönchengladbach, Germany; [8]Instituto CUF de Oncologia, Lisboa, Portugal; [9]Gesundheitszentrum St Marien, Amberg; [10]Institute of Pathology University of Munich, Munich; [11]Department of Hematology/Oncology, University Hospital Halle, Halle (Saale); [12]Institute for Pathology, Ruhr-University Bochum, Bochum; [13]Department of Hematology, Oncology and Palliative Care, St Josef-Hospital, Ruhr University Bochum, Bochum, Germany

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Background: To explore the impact of *KRAS*, *NRAS* and *BRAF* mutations as well as *KRAS* mutation variants in patients with metastatic colorectal cancer (mCRC) receiving first-line therapy.

Patients and methods: A total of 1239 patients from five randomized trials (FIRE-1, FIRE-3, AIOKRK0207, AIOKRK0604, RO91) were included into the analysis. Outcome was evaluated by the Kaplan–Meier method, log-rank tests and Cox models.

Results: In 664 tumors, no mutation was detected, 462 tumors were diagnosed with KRAS-, 39 patients with NRAS- and 74 patients with BRAF-mutation. Mutations in KRAS were associated with inferior progression-free survival (PFS) and overall survival (OS) [multivariate hazard ratio (HR) for PFS: 1.20 (1.02–1.42), $P = 0.03$; multivariate HR for OS: 1.41 (1.17–1.70), $P < 0.001$]. BRAF mutation was also associated with inferior PFS [multivariate HR: 2.19 (1.59–3.02), $P < 0.001$] and OS [multivariate HR: 2.99 (2.10–4.25), $P < 0.001$]. Among specific KRAS mutation variants, the KRAS G12C-variant ($n = 28$) correlated with inferior OS compared with unmutated tumors [multivariate HR 2.26 (1.25–4.1), $P = 0.001$]. A similar trend for OS was seen in the KRAS G13D-variant [$n = 71$, multivariate HR 1.46 (0.96–2.22), $P = 0.10$]. More frequent KRAS exon 2 variants like G12D [$n = 152$, multivariate HR 1.17 (0.86–1.6), $P = 0.81$] and G12V [$n = 92$, multivariate HR 1.27 (0.87–1.86), $P = 0.57$] did not have significant impact on OS.

Conclusion: Mutations in KRAS and BRAF were associated with inferior PFS and OS of mCRC patients compared with patients with non-mutated tumors. KRAS exon 2 mutation variants were associated with heterogeneous outcome compared with unmutated tumors with KRAS G12C and G13D (trend) being associated with rather poor survival.

Key words: BRAF, colorectal cancer, mutation, prognostic factor, RAS

**Correspondence to:* Dr med. Dominik Paul Modest, Department of Medical Oncology and Comprehensive Cancer Center, University Hospital Grosshadern, Marchioninistrasse 15, D-81377 Munich, Germany. Tel: +49-89-4400-72208; Fax: +49-89-4400-75256; E-mail: dominik.modest@med.uni-muenchen.de

introduction

KRAS exon 2–4 and *NRAS* exon 2–4 mutations (=*RAS* mutations) are found in ~50% of metastatic colorectal cancer (mCRC) tumors and exclude affected patients from epidermal growth factor receptor (EGFR)-directed therapy [1–3]. Besides their negative predictive value, *RAS* mutations may also carry distinct prognostic information [4–6]. Some studies suggest that EGFR inhibition may even be detrimental in patients with *RAS*-mutant mCRC [1, 7] maybe due to interaction with the chemotherapeutic backbone [8–10]. Furthermore, low prevalence of the different *RAS* mutation variants limits conclusions concerning the impact of different subtypes of *RAS* mutation on prognosis so far.

BRAF V600E mutation occurs in ~5%–10% of mCRC tumors [1, 5, 11]. Despite the limitation of sample size in single trials, *BRAF* mutation represents a consistently poor prognostic marker in the context of mCRC treatment [1, 11, 12], associated with rapid clinical deterioration after progression to initial therapy [12]. However, promising data with combination regimens as well as experimental treatment options may lead to routine assessment of this mutation in mCRC in the near future [5, 13].

This analysis was designed to explore the prognostic impact of mutations in RAS genes, their subtypes and *BRAF* on outcome of mCRC patients treated within randomized trials of the AIO colorectal cancer study group. With respect to potentially confounding factors of EGFR-based treatment, patients receiving EGFR-targeted agents as first-line therapy were not included.

patients and methods

studies

This analysis is based on individual patient data from five first-line trials in mCRC: FIRE-1 [14, 15], FIRE-3 (only bevacizumab-arm) [2, 16, 17], AIO KRK 0604 [18], AIO KRK 0207 [19] and RO91 [20]. Protocols, responsibilities, declarations of Helsinki, ethical approvals, definitions, treatment schedules and results of the studies were reported previously [2, 14, 18–20].

molecular assessment

Patients were derived from molecularly characterized subsets of the original study-populations (that were evaluated for *KRAS* exon 2 mutations and *BRAF* V600E mutation). FIRE-1, FIRE-3 and AIO KRK 0207 were additionally analyzed for mutations in *KRAS* exon 3–4 as well as *NRAS* exon 2–4. Methods of testing have been reported in previous publications [15–19, 21]. Patients were only included into the analysis if a single specified (i.e. including base-exchange) *RAS/BRAF* mutation or no *RAS/BRAF* mutation was present.

patient data

The following information was assessed for all patients: sex, age, mutation information, treatment, ECOG, location of primary tumor (colon versus rectum), metastatic spread, prior adjuvant chemotherapy, progression-free survival (PFS), overall survival (OS) and response information.

PFS and OS

PFS was defined as interval between randomization or registration and progression or death from any cause. OS was defined as interval between randomization or registration and death from any cause. For AIO KRK 0207, PFS and OS were calculated from the initial registration (start of induction

Table 1. Patients and studies

Original study (recruiting years) [full population]	Evaluable subset	No mutation, *n* = 664 (%)	*KRAS* mutation, *n* = 462 (%)	*NRAS* mutation, *n* = 39 (%)	*BRAF* mutation, *n* = 74 (%)
FIRE-1 (2000–2004) [*n* = 479]	FUFIRI (*n* = 108, 100%)	45 (41.7)	55 (50.9)	7 (6.5)	1 (0.9)
	mIROX (*n* = 100, 100%)	48 (48)	41 (41.0)	4 (4.0)	7 (7.0)
FIRE-3 (2007–2012) [*n* = 362]	FOLFIRI plus bevacizumab (*n* = 283, 100%)	177 (62.5)	69 (24.4)	12 (4.2)	25 (8.8)
AIO KRK 0604 (2005–2006) [*n* = 255]	CAPOX plus bevacizumab (*n* = 110, 100%)	65 (59.0)	40 (36.4)[a]	n.a	5 (4.5)
	CAPIRI plus bevacizumab (*n* = 103, 100%)	72 (70.0)	30 (29.1)[a]	n.a.	1 (1.0)
AIO KRK 0207[b] (2009–2013) [*n* = 472]	Observation (*n* = 115, 100%)	47 (40.9)	53 (46.1)	8 (7.0)	7 (6.1)
	bevacizumab (*n* = 109, 100%)	45 (41.3)	53 (48.6)	4 (3.7)	7 (6.4)
	FP plus bevacizumab (*n* = 109, 100%)	48 (44.0)	49 (45.0)	4 (3.7)	8 (7.3)
RO91 (2002–2004) [*n* = 474]	CAPOX/FUFOX (*n* = 202)	117 (57.9)	72 (35.6)[a]	n.a.	13 (6.4)

n.a., not assessed; FP, fluoropyrimidine; percentages in parentheses indicate percentage of molecular subgroups within the respective study(-arm). FUFIRI, infusional 5-FU, folinic acid, irinotecan; mIROX, irinotecan plus oxaliplatin; FOLFIRI, infusional and bolus 5-FU, folinic acid, irinotecan; CAPOX, capecitabine, oxaliplatin; CAPIRI, capecitabine, irinotecan; FUFOX, infusional 5-FU, oxaliplatin.
[a]Only tested for KRAS exon 2 mutations.
[b]Twenty-four weeks fluoropyrimidine, oxaliplatin plus bevacizumab.

therapy, and not from randomization for maintenance treatment arms) in order to enable comparison of efficacy parameters [19].

influence of treatment on outcome

The outcome of molecular subgroups was also analyzed in the context of different treatment regimens (oxaliplatin- versus irinotecan-based therapy as well as bevacizumab versus non-bevacizumab therapy). For the assessment of irinotecan- versus oxaliplatin-based treatment, the mIROX arm of the FIRE-1 trial was excluded from the dataset.

statistical analysis

PFS and OS were assessed by the Kaplan–Meier method and compared with log-rank tests. Hazard ratios (HRs) were calculated by the Cox regression models stratified by study and treatment if appropriate. Multivariate tests were carried out using the Cox models adjusted for study treatment, ECOG, sex, adjuvant chemotherapy, liver-limited disease and number of involved organs. Comparisons of patients with mutation variants to patients with wild-type mCRC were adjusted for multiplicity (Dunnett's test). The significance level was set to 0.05. All statistical analyses were carried out using SAS 9.2 (SAS Institute Inc., Cary, NC), IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY) and R (version 3.2.2).

results

For this analysis, data of 1239 patients were available. Distribution of patients across studies according to molecular characteristics is summarized in Table 1.

mutations

Of 1239 analyzed tumors, in 664 tumors (53.6%), no mutation was detected, whereas 462 tumors harboring *KRAS* (37.3%) mutations and 39 *NRAS* (3.1%) mutations were found. Additionally, a total of 74 tumors (6.0%) were carrying *BRAF* V600E mutations (supplementary Table S1, available at *Annals of Oncology* online).

Table 2 Baseline characteristics according to molecular subgroups

	No mutation ($n = 664$)	*KRAS* mutation ($n = 462$)	*NRAS* mutation ($n = 39$)	*BRAF* mutation ($n = 74$)	*P*-value
Age					
Median (range)	65 (25–82)	64 (25–83)	64 (32–81)	62 (29–82)	0.17
Missing data	0	0	0	0	
Sex					
Male (%)	460 (69.3)	292 (63.2)	21 (53.8)	37 (50.7)	0.002
Female (%)	204 (30.7)	170 (36.8)	18 (46.2)	36 (49.3)	
Missing data	0	0	0	1	
Primary tumor site					
Colon (%)	414 (63.1)	286 (61.9)	23 (59.0)	56 (77.8)	0.06
Rectum (%)	236 (36.0)	175 (37.9)	15 (38.5)	15 (20.8)	
Colon + rectum (%)	6 (0.9)	1 (0.2)	1 (2.6)	1 (1.4)	
Missing data	8	0	0	2	
ECOG performance status					
0 (%)	340 (51.3)	225 (49.7)	18 (46.2)	33 (45.8)	0.64
1 (%)	297 (44.8)	206 (45.5)	20 (51.3)	33 (45.8)	
2 (%)	26 (3.9)	22 (4.9)	1 (2.6)	6 (8.3)	
Missing data	1	9	0	2	
Prior adjuvant treatment					
Adjuvant treatment (%)	140 (21.1)	87 (18.9)	10 (25.6)	11 (15.1)	0.43
Missing data	2	1	0	1	
Metastatic lesions					
Liver (%)	550 (83.2)	366 (80.6)	33 (84.6)	57 (78.1)	0.54
Missing data	3	8	0	1	
Liver limited (%)	290 (43.9)	164 (36.1)	15 (38.5)	22 (30.1)	0.02
Missing data	3	8	0	1	
Lung (%)	196 (29.7)	184 (40.5)	13 (33.3)	17 (23.3)	<0.001
Missing data	3	8	0	1	
Peritoneum	30 (5.5)	20 (5.2)	5 (12.8)	12 (20.0)	<0.001
Missing data	120	80	0	14	
Lymph nodes	80 (29.7)	29 (17.8)	9 (39.1)	13 (40.6)	0.005
Missing data	395	299	16	42	
>2 organs involved	99 (15.0)	77 (17.0)	11 (28.9)	15 (20.5)	0.10
Missing data	4	9	1	1	

P values by χ^2 tests, except for age: Wilcoxon's test. Calculations based on non-missing data. Metastastic spread reported to different extent in studies with evaluable data for all trials concerning liver and lung metastases and no of involved organs. Karnofsky performance status was translated into ECOG for the FIRE-1 study: Karnofsky 100 = ECOG 0; Karnofsky 80–90 = ECOG 1; Karnofsky 70 = ECOG 2.

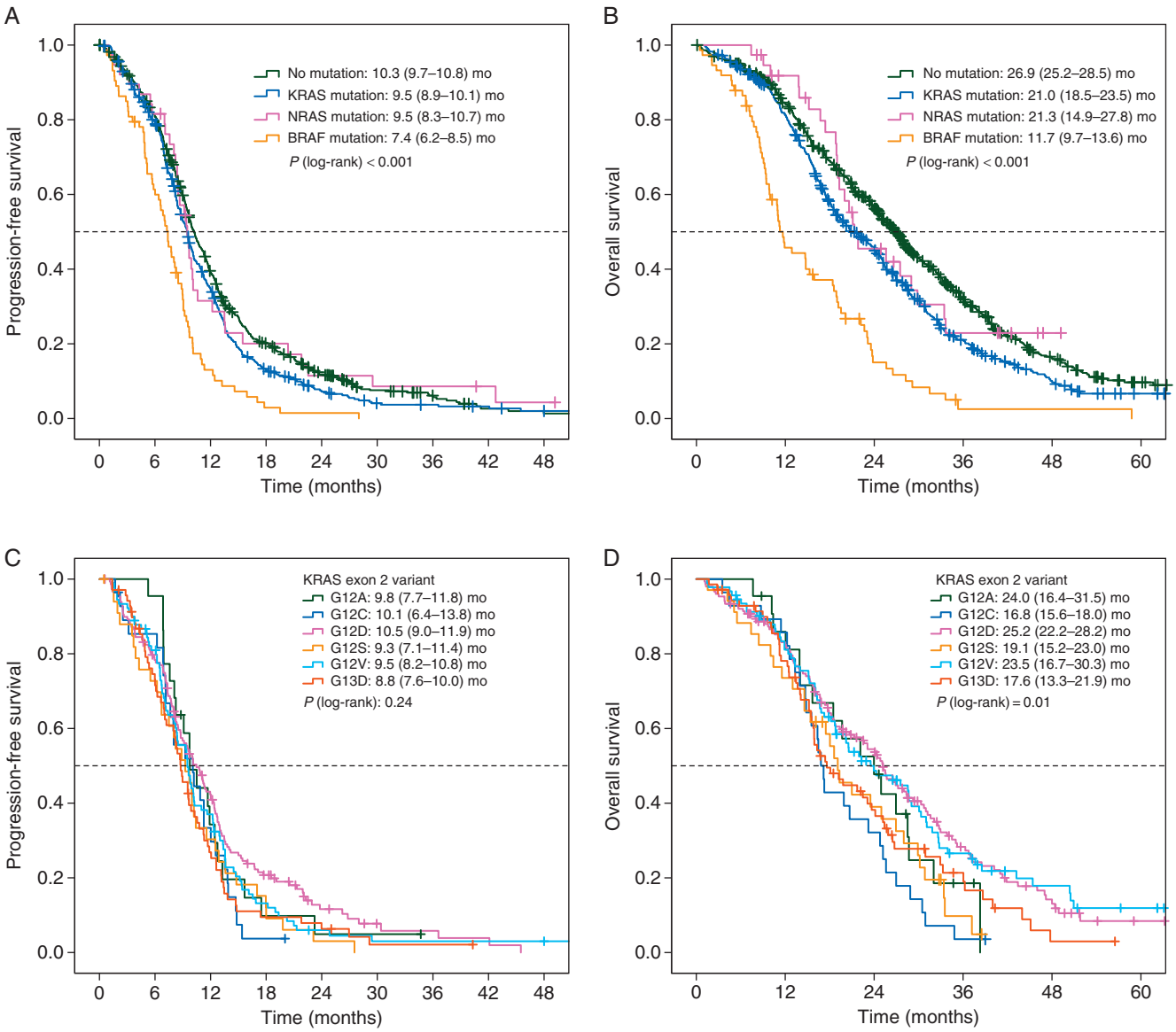

Figure 1. Prognostic role of alterations in *KRAS*-, *NRAS*- and *BRAF*-genes. (A) Progression-free survival (PFS) according to molecular subgroups. (B) Overall survival (OS) according to molecular subgroups. (C) PFS in *KRAS* exon 2 variants. (D) OS in *KRAS* exon 2 variants, *P* values below 0.05 by log-rank test indicate at least one significant difference between two groups.

baseline characteristics

Distributions of baseline characteristics in molecular subgroups are summarized in Table 2.

prognostic role of KRAS, NRAS and BRAF mutation

PFS and OS were significantly influenced by molecular subgroups (Figures 1A and B and 2A and B). Univariate and multivariate comparisons of PFS and OS in patients with mutant tumors (*KRAS*, *NRAS*, *BRAF*) versus patients with non-mutated tumors revealed a negative prognostic effect of *KRAS* and *BRAF* mutations (Figure 2A and B). Interestingly, the negative prognostic role of *KRAS* and *BRAF* mutations was consistently observed across different treatment regimens (subgroups of irinotecan- and oxaliplatin-treated as well as in bevacizumab- and non-bevacizumab-treated) (Figure 2A and B).

prognostic role of single RAS mutation variants

The median PFS of patients with *KRAS* exon 2 mutant tumor subtypes ranged from 8.8 [95% confidence interval (CI) 7.6–10.0] months (G13D mutation) to 10.5 (95% CI 9.0–11.9) months in (G12D variants). The median OS widely ranged between 16.8 (95% CI 15.6–18.0) months (G12C) and 25.2 (95% CI 22.2–28.2) (G12D variants) (Figure 1C and D). Besides *KRAS* exon 2 variants, *KRAS* mutations A146T ($n = 18$) and Q61H ($n = 17$) as well as *NRAS* mutation G12D ($n = 11$) were separately evaluated for efficacy end points, all other variants were less frequent (supplementary Table S1, available at *Annals of Oncology* online).

Comparisons of PFS and OS (univariate and multivariate) of patients with mutation variants to patients with non-mutated tumors revealed the *KRAS* exon 2 G12C-variant ($n = 28$) to correlate with inferior OS compared with non-

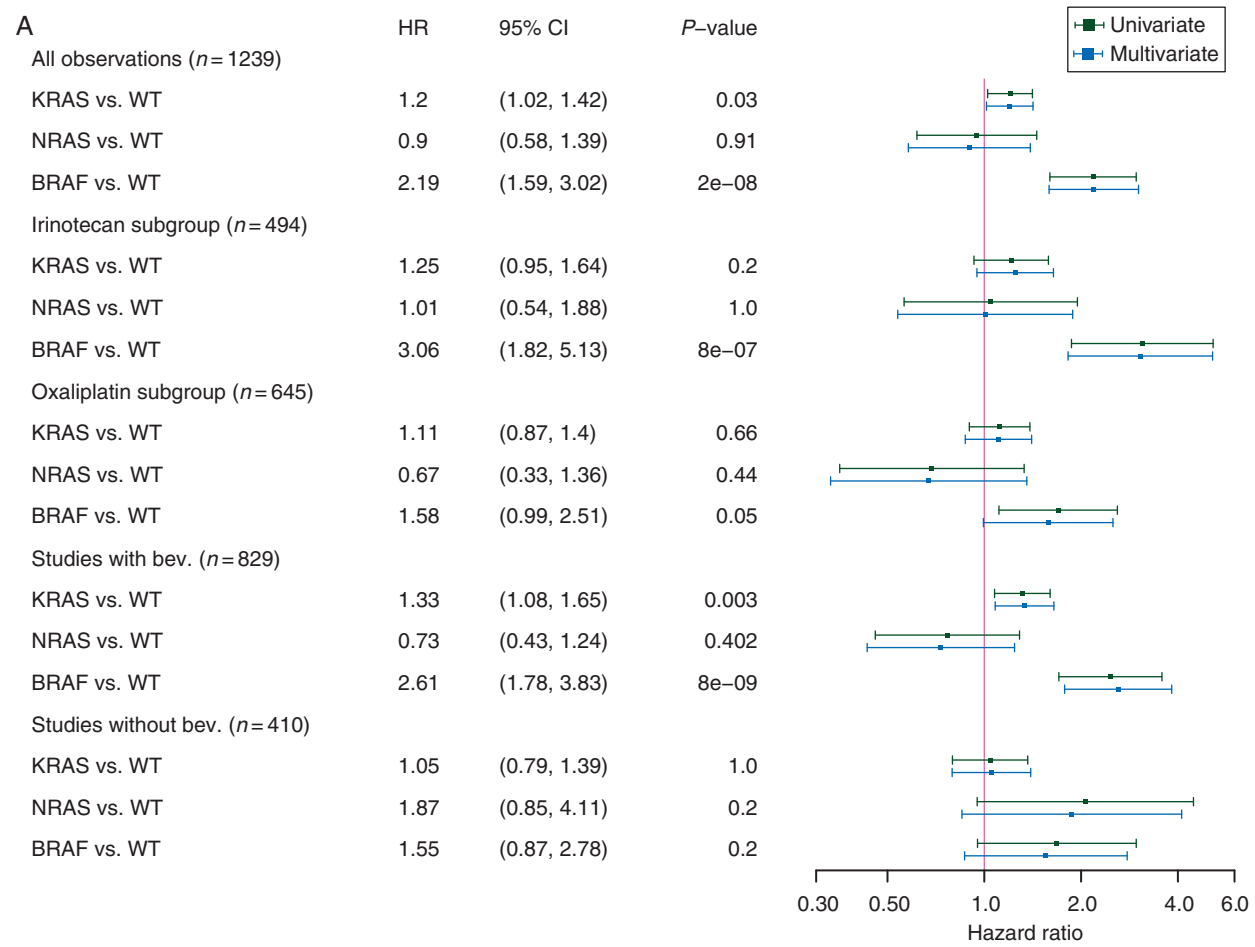

Figure 2. Forest plots of metastatic colorectal cancer (mCRC) molecular subgroups as well as mutation variants compared with *KRAS*/*NRAS*/*BRAF* wild-type mCRC. (A) Progression-free survival (PFS) according to molecular subgroups. (B) Overall survival (OS) according to molecular subgroups. (C) PFS according to mutation variants. (D) OS according to mutation variants; hazard ratios (HR) with 95% confidence intervals (95% CI) adjusted for multiplicity indicate results drawn from the multivariate model. An HR >1 indicates a higher hazard rate for death or progression in patients with mutated tumors compared with patients with unmutated tumors. Only mutation variants with >10 patients were included into the analysis in C and D. All variants in C and D represent respective *KRAS* mutations except NG12D, *NRAS* G12D; V600E, *BRAF* V600E; bev., bevacizumab; WT, unmutated tumors.

mutated tumors [multivariate model HR 2.26 (1.25–4.1), $P = 0.001$] (Figure 2C and D). A similar trend was seen in the *KRAS* exon 2 G13D-variant [$n = 71$, multivariate model HR 1.46 (0.96–2.22), $P = 0.10$]. More frequent *KRAS* exon 2 variants like G12D [$n = 152$, multivariate model HR 1.17 (0.86–1.6), $P = 0.81$] and G12V [$n = 92$, multivariate model HR 1.27 (0.87–1.86), $P = 0.57$] did not have significant impact on OS. The G12V mutation variant had a negative prognostic effect on PFS in the multivariate analysis (Figure 2C).

discussion

The present analysis was motivated by the limited clinical data regarding the prognostic impact of *RAS* mutation variants in patients with mCRC receiving first-line systemic treatment without EGFR-targeted therapy. Our analysis comprises data of 1239 patients and therefore represents one of the largest datasets available.

KRAS (37.3%) and *NRAS* (3.1%) mutations were a little less frequent in our cohort compared with other series. [1, 22]. Selection of *KRAS* exon 2 wild-type for inclusion in the FIRE-3 trial as well as lack of testing for *KRAS* exon 3–4 and *NRAS* exon 2–4 in AIO KRK 0604 and RO91 may have contributed to this result. The lack of testing in these two studies might cause a small negative bias on outcome of patients with unmutated tumors.

Baseline characteristics compared between molecular subgroups reflected more aggressive disease in patients with mutated tumors (in particular in patients with *BRAF*-mutant mCRC). *BRAF* mutation seemed associated with female sex and tumor location (colon). These results confirm previous observations [23].

PFS of patients evaluated by molecular subgroups demonstrated a strong negative prognostic effect of *BRAF* mutation (HR 2.19, $P < 0.0001$) as well as a smaller, but also significant negative effect of *KRAS* mutation, both compared with non-mutated tumors (HR 1.2, $P = 0.03$). The differences in outcome associated with molecular subtype were pronounced in OS. Of note, the median OS reported in patients with non-mutated tumors was 26.9 (95% CI 25.2–28.5) months. Taking into account that not all patients had access to EGFR-targeted agents since these were partly unavailable at the time of study conduct of FIRE-1/RO91, this result compares

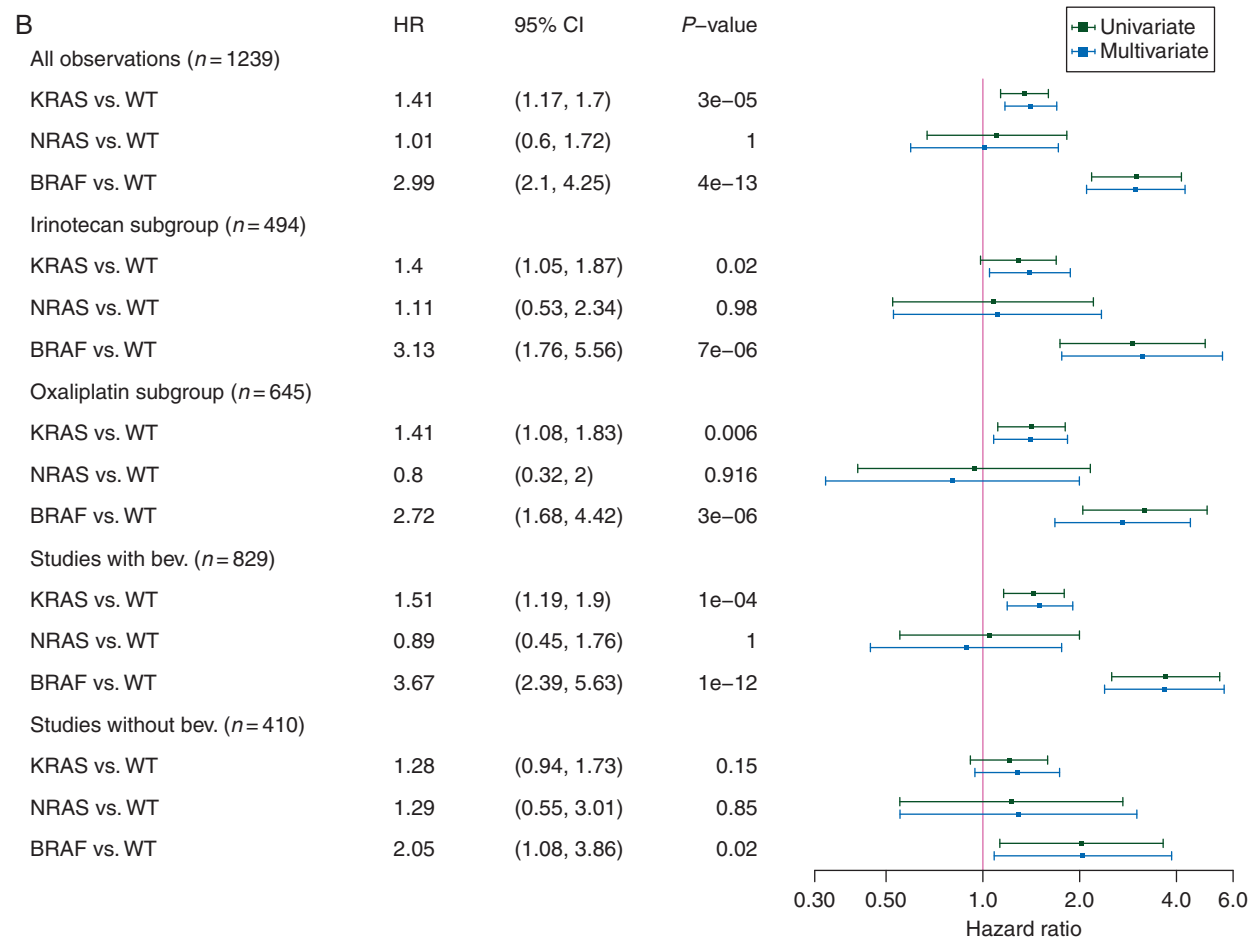

Figure 2. *Continued*

well with recent reports of first-line treatment in mCRC [5, 24]. Outcome of patients with *KRAS* or *BRAF*-mutant mCRC demonstrated significantly shorter medians of OS: 21.0 (18.5–23.5) and 11.7 (9.7–13.6) months, respectively, translating to HRs of 1.41 ($P < 0.001$) for KRAS and 2.99 ($P < 0.001$) for *BRAF*. Availability of later-line treatment (i.e. EGFR-targeted agents) in patients with non-mutated tumors might have impacted on OS for those patients. However, the also present differences in PFS in patients with non-mutated tumors compared with patients with *KRAS*-mutant mCRC support the hypothesis that *KRAS* is a prognostic factor *per se* and differences in outcome are not only mediated by a subset of patients receiving later-line EGFR-inhibitors. The number of patients with *NRAS*-mutant tumors in this dataset was probably too small to allow for significant effects on outcome.

In this pooled dataset, the prognostic effect of molecular subgroups (i.e. *KRAS* and *BRAF* mutation) in comparison with non-mutated tumors was consistently observed in all subsets of patients being treated with irinotecan- or oxaliplatin combinations as well as in bevacizumab- or non-bevacizumab-treated patient. Considering that microsatellite-instable tumors are rare in stage IV mCRC, these findings compare well with a recent analysis of the adjuvant PETACC-8-trial that identified *KRAS* and *BRAF* mutations as prognostic markers in microsatellite-stable (but not microsatellite-instable) tumors [25]. Further classification of mCRC might be seen in differentiation of left-sided versus right-sided primary tumor location, probably being a surrogate for molecular profiles that have not been understood in full extent [26]. Unfortunately, primary tumor location was not recorded during study conduct for the majority of patients in this cohort and cannot be taken into account for our analysis.

KRAS exon 2 mutation variants were associated with heterogeneous outcome concerning OS as well as PFS. The G12V mutation variant, representing one of the most frequent subtypes, was associated with a significantly worse PFS compared with patients without any mutation (HR = 1.48, $P = 0.02$). OS was also inferior —however not significant—in G12V and G13D 1 subtypes, and significantly inferior in G12C mutations variants compared with patients with non-mutated tumors. This observation supports the hypothesis that *KRAS* exon 2 mutation variants are associated with a differing spectrum of clinical outcome [4, 8, 27]. It might be speculated that the reason for differing outcomes could be mediated by differing activation of *KRAS*-depending pathways by distinct mutation variants, as suggested previously with high baseline activation and potentially aggressive biology in G12C variants [28]. In addition, the poor outcome of patients with G12C mutant mCRC might be of clinical relevance as allele-specific inhibitors may provide therapeutic options in the future [29, 30]. In this context, also the mutation rate of *KRAS* could be a factor that

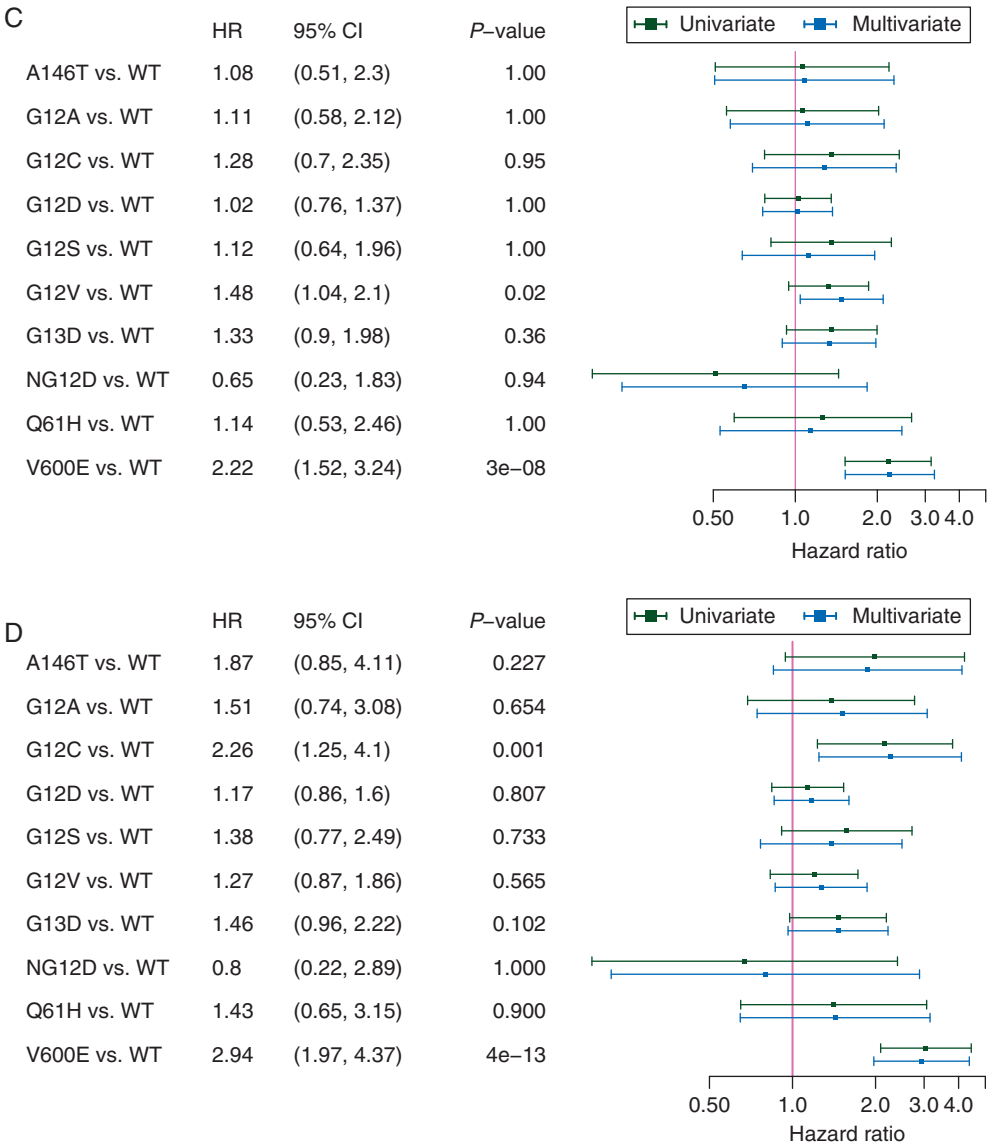

Figure 2. *Continued*

impacts significantly on prognosis of *KRAS*-mutant mCRC [31]. Unfortunately, this information is not available for our cohort.

In general, despite high data quality, pooled datasets of different randomized trials may always lead to cohorts with study-specific bias. Although multivariate models can adjust calculations for some (obvious) factors, retrospectively evaluated, pooled data invoke uncertainties. Pooling data from five studies has enlarged the number of some mutation variants (i.e. *NRAS* as well as *KRAS* exon 2 mutation variants) to a level that consecutively enabled survival analysis. However, absolute numbers in these subgroups are still unsatisfactory and the analyses appear underpowered to allow for definite conclusions, especially in rare mutation variants. In particular, false-negative results cannot be excluded as potential limitations in this setting. Given that some biomarkers (i.e. *KRAS* mutation variants) were identified as potential prognostic markers, validation of our findings within alternative study-sets appears justified.

In conclusion, our data suggest that mutations in *KRAS* and *BRAF* are associated with inferior PFS and OS of mCRC patients compared with patients with non-mutated tumors. Whereas role of chemotherapy and treatment with or without bevacizumab did not affect these findings, *KRAS* exon 2 mutation variants differed, with G12C being associated with shorter OS when compared with patients with non-mutated tumors, while G13D mutations were showing a similar trend.

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disclosure

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VH: honoraria: Merck, Roche, Amgen, Sirtex, Sanofi-aventis; advisory role: Merck, Roche, Amgen, Sirtex, Sanofi-aventis; research funding: Amgen, Merck, Roche, Sanofi-Aventis; travel support: Merck, Roche. SS: honoraria: Merck, Roche, Amgen, Bayer, Sanofi-aventis; advisory role: Merck Serono, Roche; travel support: Roche, Merck Serono, Sanofi-aventis. UG: honoraria: Amgen, Roche, Merck, Sanofi, Bayer. DA: honoraria: Bayer, Merck, Roche, Sanofi; advisory role: Roche, Bayer, Merck, Servier, Sandoz ARS: honoraria: Pfizer, Sanofi-Aventis, Merck, Celgene, Amgen, Roche; advisory role: Amgen, Roche, Pfizer, Sanofi-Aventis, Merck, Celgene; research support: Roche, Sanofi-Aventis, Celgene. All remaining authors have declared no conflicts of interest.

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