

20. News, no authors listed. Checkpoint IDO draws \$1 billion. *Nat Biotechnol* 2014; 32(12): 1177.
21. Gourley C, McCavigan A, Perren T et al. Molecular subgroup of high-grade serous ovarian cancer (HGSOC) as a predictor of outcome following bevacizumab. *J Clin Oncol* 2014; 32 (15 Suppl): 5502.
22. Perren TJ, Swart AM, Pfisterer J et al. A phase 3 trial of bevacizumab in ovarian cancer. *N Engl J Med* 2011; 365: 2484–2496.
23. Melichar B, Kalábová H, Krčmová L et al. Urinary neopterin, serum retinol, α -tocopherol and homocysteine in breast cancer patients during treatment with bevacizumab and chemotherapy. *Pteridines* 2010; 21: 87–93.

Annals of Oncology 27: 1746–1753, 2016
doi:10.1093/annonc/mdw261
Published online 29 June 2016

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

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Received 9 May 2016; revised 20 June 2016; accepted 20 June 2016

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*

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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 KKR 0604 [18], AIO KKR 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 KKR 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 KKR 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, <i>n</i> = 664 (%)	<i>KRAS</i> mutation, <i>n</i> = 462 (%)	<i>NRAS</i> mutation, <i>n</i> = 39 (%)	<i>BRAF</i> mutation, <i>n</i> = 74 (%)
FIRE-1 (2000–2004) [<i>n</i> = 479]	FUFI (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) [<i>n</i> = 362]	FOLFIRI plus bevacizumab (n = 283, 100%)	177 (62.5)	69 (24.4)	12 (4.2)	25 (8.8)
AIO KKR 0604 (2005–2006) [<i>n</i> = 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 KKR 0207 ^b (2009–2013) [<i>n</i> = 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) [<i>n</i> = 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). FUFI, 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.

^aOnly tested for *KRAS* exon 2 mutations.

^bTwenty-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 (Dunnnett'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)	<i>KRAS</i> mutation (n = 462)	<i>NRAS</i> mutation (n = 39)	<i>BRAF</i> 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. Metastatic 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.

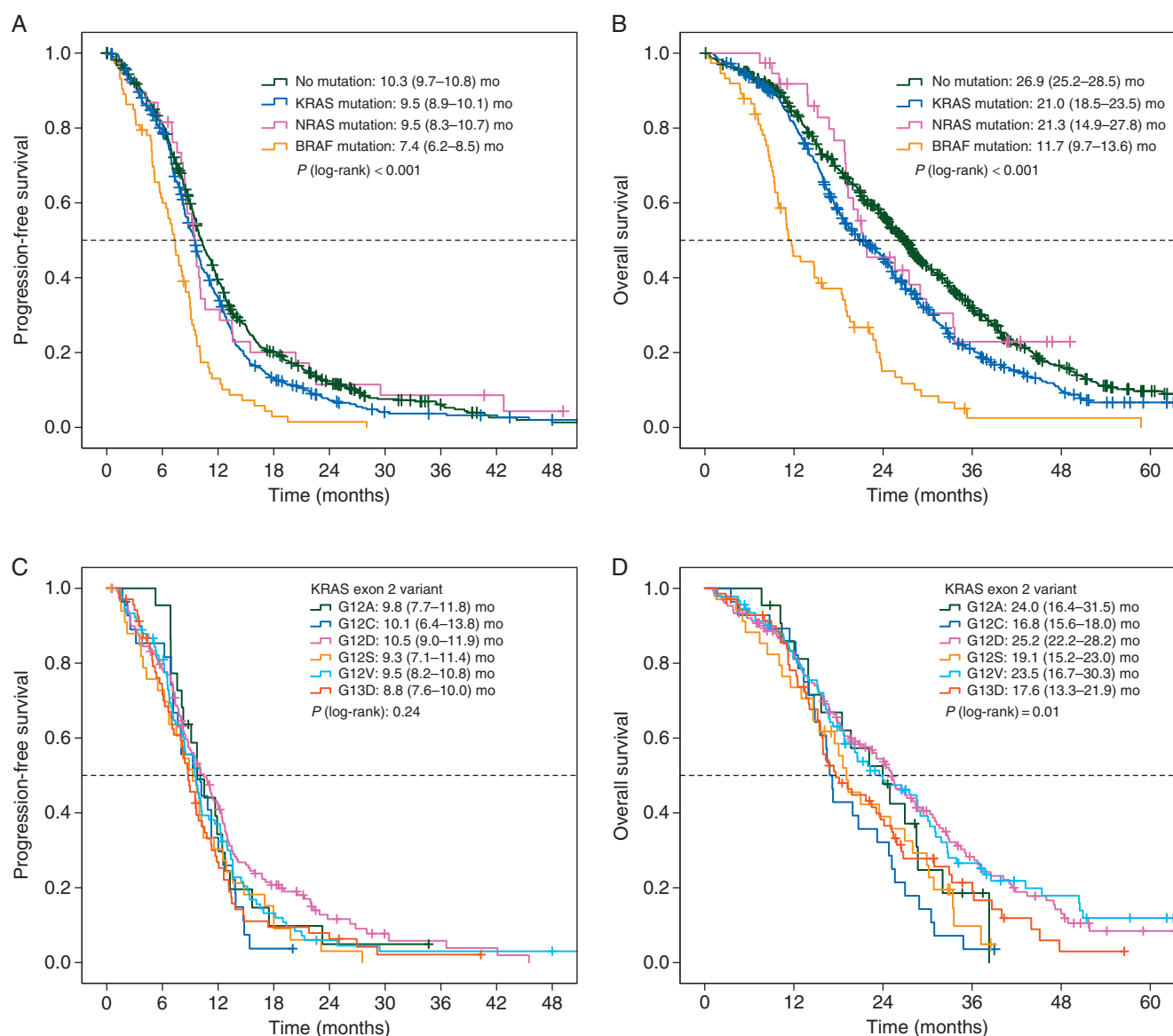


Figure 1. Prognostic role of alterations in *KRAS*-, *NRAS*- and *BRAF*-genes. (A) Progression-free survival (PFS) according to molecular subgroups. (B) Overall survival (OS) according to molecular subgroups. (C) PFS in *KRAS* exon 2 variants. (D) OS in *KRAS* exon 2 variants, *P* values below 0.05 by 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-

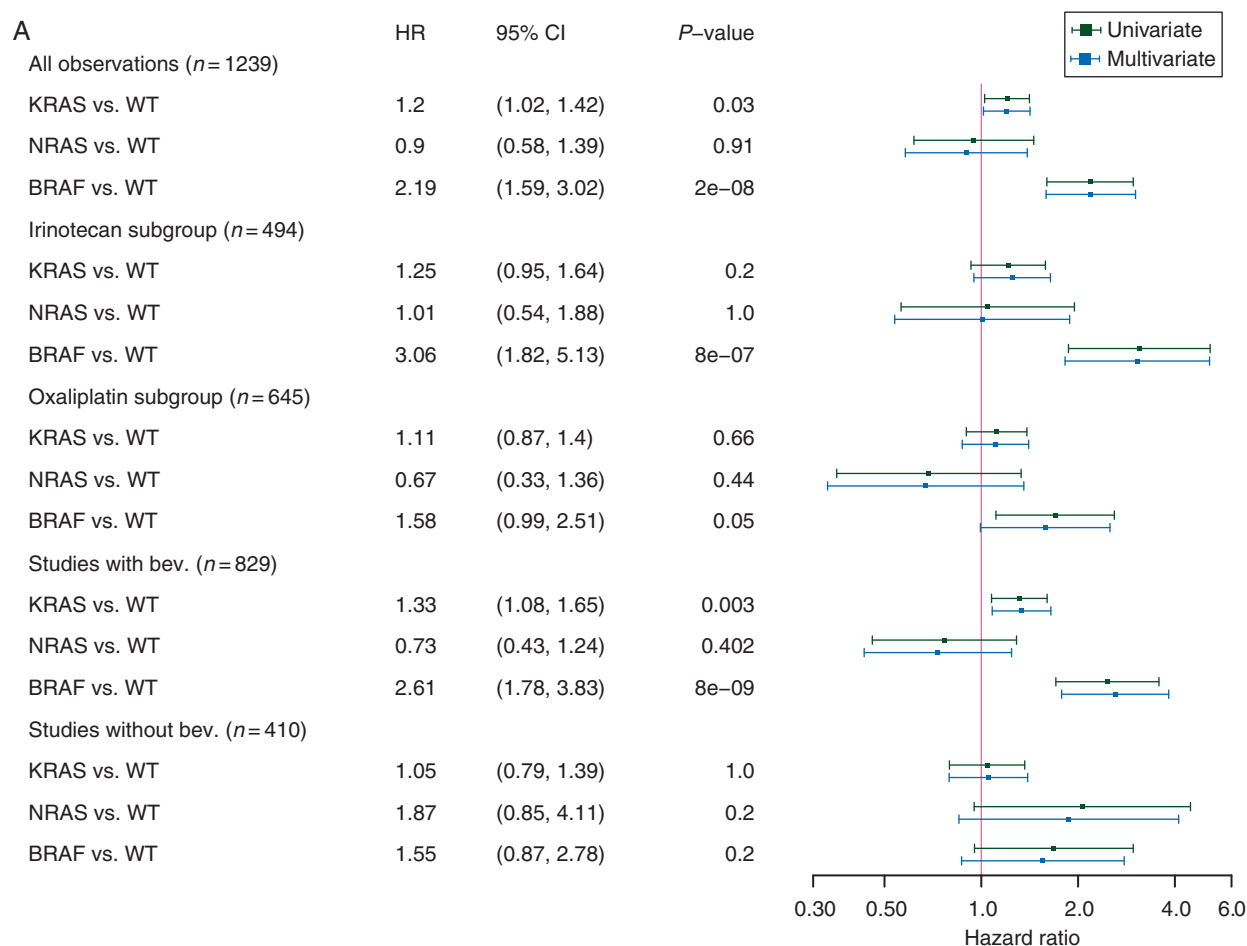


Figure 2. Forest plots of metastatic colorectal cancer (mCRC) molecular subgroups as well as mutation variants compared with *KRAS/NRAS/BRAF* wild-type mCRC. (A) Progression-free survival (PFS) according to molecular subgroups. (B) Overall survival (OS) according to molecular subgroups. (C) PFS according 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 KKR 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

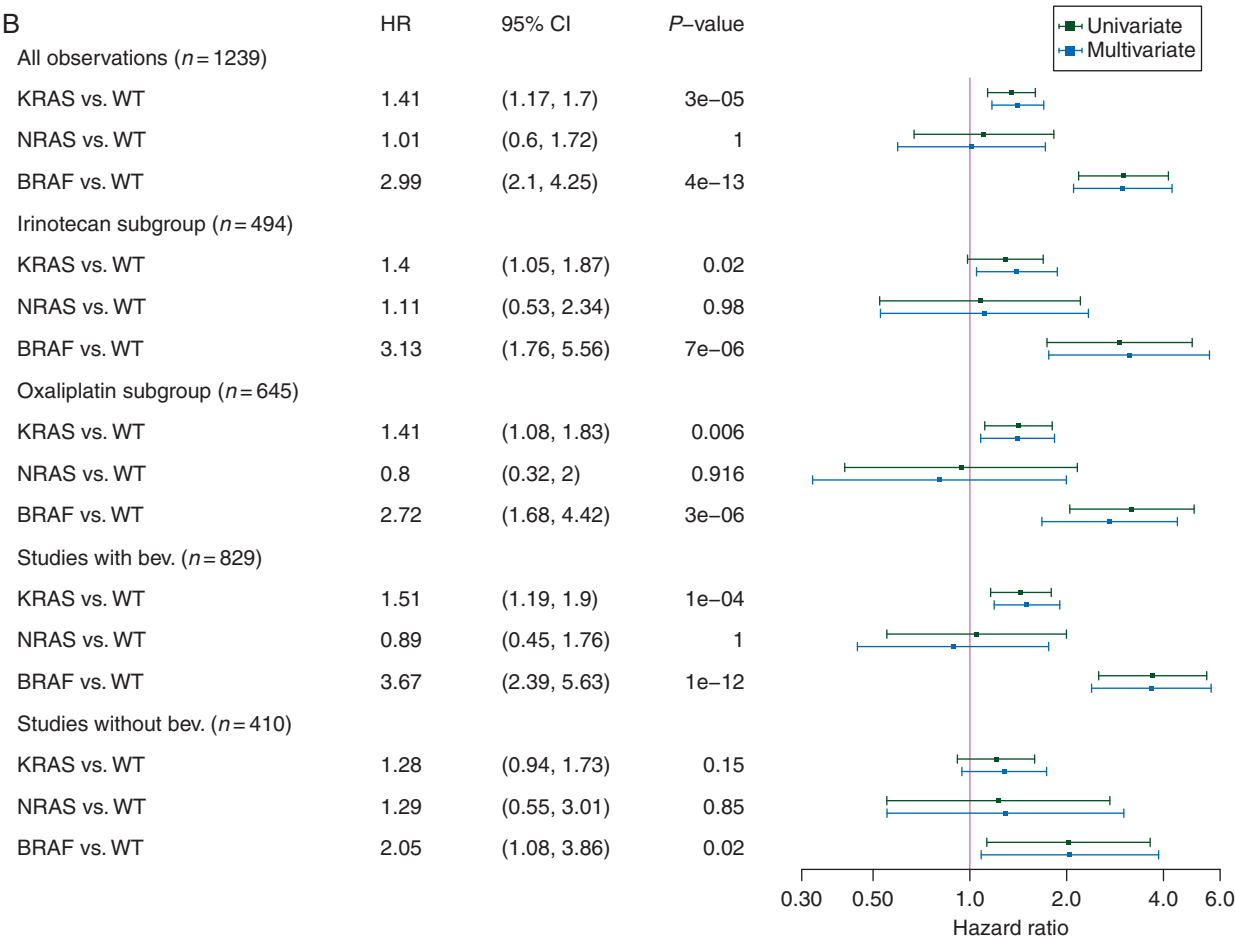


Figure 2. Continued

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-unstable 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-unstable) 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

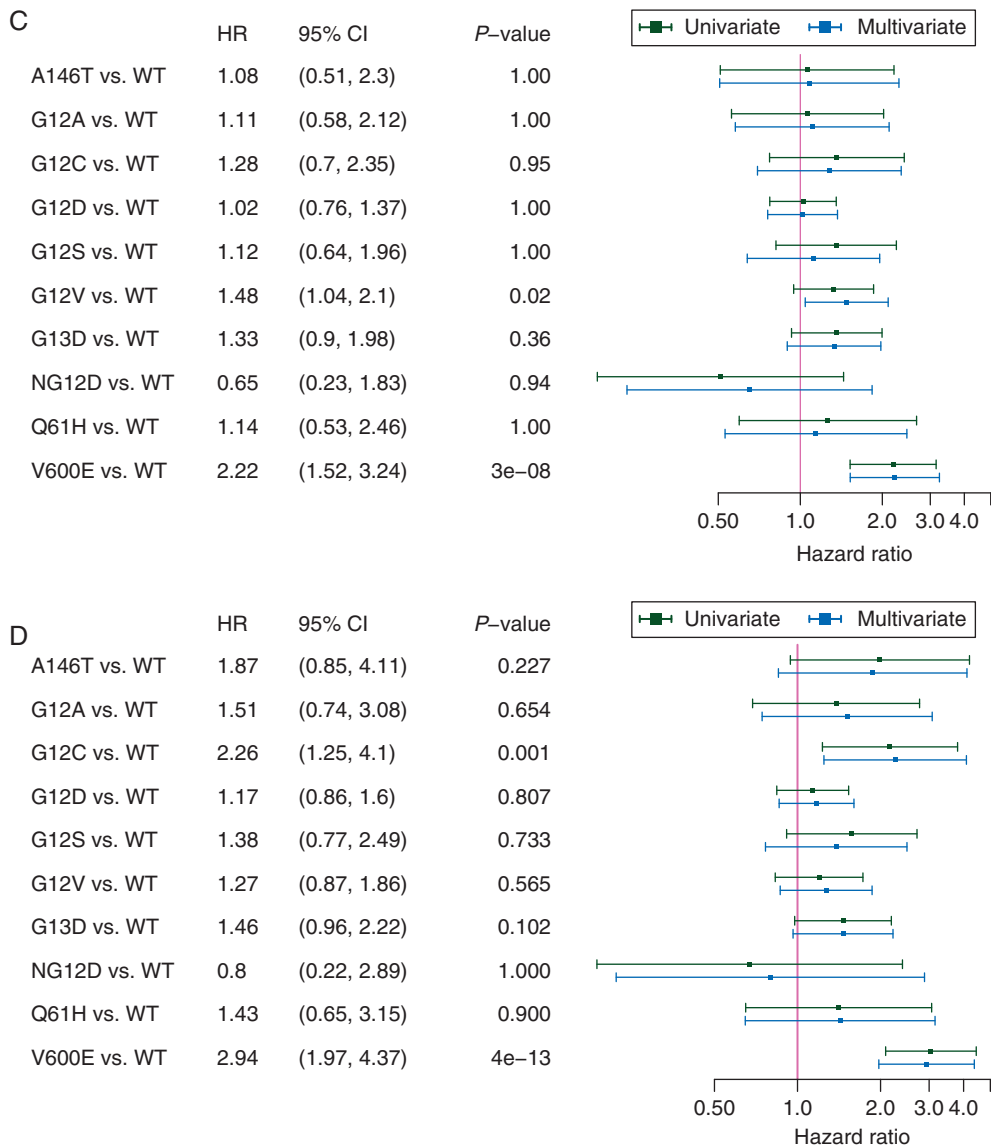


Figure 2. Continued

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.

acknowledgements

The authors thank all patients and families for participation in the studies, as well as all involved study centers, colleagues and nurses.

funding

FIRE-1 was supported by Pfizer; FIRE-3 was supported by Merck and Pfizer; RO91 and AIOKRK0604 were supported by Roche and Sanofi; AIOKRK0207 was supported by Roche. The present evaluation did not receive specific funding and the funding sources did not contribute to this evaluation.

disclosure

DPM: honoraria: Merck, Roche, Amgen, Bayer; advisory role: Merck, Bayer, Amgen; research grant: Amgen (inst), Merck (inst), Roche (inst); travel support: Amgen, Merck, Sanofi, Bayer.

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, Roche, Amgen, Bayer, Sanofi-aventis; 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.

references

- Douillard JY, Oliner KS, Siena S et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013; 369: 1023–1034.
- Heinemann V, von Weikersthal LF, Decker T et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. *Lancet Oncol* 2014; 15: 1065–1075.
- Van Cutsem E, Lenz HJ, Kohne CH et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. *J Clin Oncol* 2015; 33: 692–700.
- Bazan V, Migliavacca M, Zanna I et al. Specific codon 13 K-ras mutations are predictive of clinical outcome in colorectal cancer patients, whereas codon 12 K-ras mutations are associated with mucinous histotype. *Ann Oncol* 2002; 13: 1438–1446.
- Cremolini C, Loupakis F, Antoniotti C et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. *Lancet Oncol* 2015; 16: 1306–1315.
- De Roock W, Claes B, Bernasconi D et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010; 11: 753–762.
- Bokemeyer C, Bondarenko I, Hartmann JT et al. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol* 2011; 22: 1535–1546.
- Modest DP, Brodowicz T, Stintzing S et al. Impact of the specific mutation in KRAS codon 12 mutated tumors on treatment efficacy in patients with metastatic colorectal cancer receiving cetuximab-based first-line therapy: a pooled analysis of three trials. *Oncology* 2012; 83: 241–247.
- Peeters M, Douillard JY, Van Cutsem E et al. Mutant KRAS codon 12 and 13 alleles in patients with metastatic colorectal cancer: assessment as prognostic and predictive biomarkers of response to panitumumab. *J Clin Oncol* 2013; 31: 759–765.
- Tejpar S, Celik I, Schlichting M et al. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. *J Clin Oncol* 2012; 30: 3570–3577.
- Van Cutsem E, Kohne CH, Lang I et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011; 29: 2011–2019.
- Seligmann J. Exploring the poor outcomes of BRAF mutant (BRAF mut) advanced colorectal cancer (aCRC): analysis from 2,530 patients (pts) in randomized clinical trials (RCTs). *J Clin Oncol* 2015; 33 (Suppl): abstr 3509.
- Corcoran RB, Atreya CE, Falchook GS et al. Combined BRAF and MEK inhibition with dabrafenib and trametinib in BRAF V600-mutant colorectal cancer. *J Clin Oncol* 2015; 33: 4023–4031.
- Fischer von Weikersthal L, Schalthorn A, Stauch M et al. Phase III trial of irinotecan plus infusional 5-fluorouracil/folinic acid versus irinotecan plus oxaliplatin as first-line treatment of advanced colorectal cancer. *Eur J Cancer* 2011; 47: 206–214.
- Stahler A, Heinemann V, Giessen-Jung C et al. Influence of mRNA expression of epiregulin and amphiregulin on outcome of patients with metastatic colorectal cancer treated with 5-FU/LV plus irinotecan or irinotecan plus oxaliplatin as first-line treatment (FIRE 1-trial). *Int J Cancer* 2016; 138: 739–746.
- Stintzing S, Fischer von Weikersthal L, Decker T et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer-subgroup analysis of patients with KRAS: mutated tumours in the randomised German AIO study KRK-0306. *Ann Oncol* 2012; 23: 1693–1699.
- Stintzing S, Jung A, Rossius L, Modest DP et al. Analysis of KRAS/NRAS and BRAF mutations in FIRE-3 A randomized phase III study of FOLFIRI plus cetuximab or bevacizumab as first-line treatment for wild-type KRAS (exon 2) metastatic colorectal cancer patients. In ESMO/ECCO, 2013; Abstract E17-7073.
- Schmiegel W, Reinacher-Schick A, Arnold D et al. Capecitabine/irinotecan or capecitabine/oxaliplatin in combination with bevacizumab is effective and safe as first-line therapy for metastatic colorectal cancer: a randomized phase II study of the AIO colorectal study group. *Ann Oncol* 2013; 24: 1580–1587.
- Hegewisch-Becker S, Graeven U, Lerchenmuller CA et al. Maintenance strategies after first-line oxaliplatin plus fluoropyrimidine plus bevacizumab for patients with metastatic colorectal cancer (AIO 0207): a randomised, non-inferiority, open-label, phase 3 trial. *Lancet Oncol* 2015; 16: 1355–1369.
- Porschen R, Arkenau HT, Kubicka S et al. Phase III study of capecitabine plus oxaliplatin compared with fluorouracil and leucovorin plus oxaliplatin in metastatic colorectal cancer: a final report of the AIO Colorectal Study Group. *J Clin Oncol* 2007; 25: 4217–4223.
- Reinacher-Schick A, Schulmann K, Modest DP et al. Effect of KRAS codon13 mutations in patients with advanced colorectal cancer (advanced CRC) under oxaliplatin containing chemotherapy. Results from a translational study of the AIO colorectal study group. *BMC Cancer* 2012; 12: 349.
- Neumann J, Zeindl-Eberhart E, Kirchner T, Jung A. Frequency and type of KRAS mutations in routine diagnostic analysis of metastatic colorectal cancer. *Pathol Res Pract* 2009; 205: 858–862.
- Yokota T, Ura T, Shibata N et al. BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. *Br J Cancer* 2011; 104: 856–862.
- Simkens LH, van Tinteren H, May A et al. Maintenance treatment with capecitabine and bevacizumab in metastatic colorectal cancer (CAIRO3): a phase 3 randomised controlled trial of the Dutch Colorectal Cancer Group. *Lancet* 2015; 385: 1843–1852.
- Taieb J, Zaanan A, Le Malicot K et al. Prognostic effect of BRAF and KRAS mutations in patients with stage iii colon cancer treated with leucovorin, fluorouracil, and oxaliplatin with or without cetuximab: a post hoc analysis of the PETACC-8 trial. *JAMA Oncol* 2016; 1–11.
- Missiaglia E, Jacobs B, D'Ario G et al. Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features. *Ann Oncol* 2014; 25: 1995–2001.
- De Roock W, Jonker DJ, Di Nicolantonio F et al. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. *JAMA* 2010; 304: 1812–1820.
- Camaj P, Primo S, Wang Y et al. KRAS exon 2 mutations influence activity of regorafenib in an SW48-based disease model of colorectal cancer. *Future Oncol* 2015; 11: 1919–1929.
- Lito P, Solomon M, Li LS et al. Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. *Science* 2016; 351: 604–608.
- Ostrem JM, Peters U, Sos ML et al. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* 2013; 503: 548–551.
- Vincenzi B, Cremolini C, Sartore-Bianchi A et al. Prognostic significance of K-Ras mutation rate in metastatic colorectal cancer patients. *Oncotarget* 2015; 6: 31604–31612.