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The prevalent *KRAS* exon 2 c.35 G > A mutation in metastatic colorectal cancer patients: A biomarker of worse prognosis and potential benefit of bevacizumab-containing intensive regimens?

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Abstract

Bevacizumab-containing chemotherapy differently predict increased efficacy in *KRAS* exon 2 mutant and wild-type metastatic colorectal cancer (MCRC) patients. Mutant compared to wild-type status did not significantly affect progression-free survival (PFS) and overall survival (OS) in patients fit for first line bevacizumab-containing FIr-B/FOx regimen, and after progression. In patients unfit for intensive regimens, mutant status significantly affected PFS, while not OS. Codon 12 *KRAS* mutations differentially affect GTPase function, and confer worse clinical behaviour. Prognostic relevance of the prevalent c.35 G > A *KRAS* mutation was retrospectively evaluated. Fit c.35 G > A mutant patients showed significantly worse OS compared to wild-type and to other mutant. After progression and in unfit patients, c.35 G > A

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mutation affected significantly worse PFS and OS. c.35 G > A mutant status does not significantly affect worse PFS in patients fit for first line FIr-B/FOx, and it may depend upon effectiveness of anti-VEGF-containing intensive regimen.

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This review comprehensively evaluate prognostic and predictive relevance of *KRAS* exon 2 genotype, depending from biological aggressiveness and differential treatment effectiveness, and specifically of the prevalent c.35 G > A mutation, that we retrospectively evaluated in MCRC. We discuss biological insights concerning specific alteration of KRAS GTPase activity and angiogenesis induction, that can be "hypothesis generating" for prospective trials evaluating the efficacy of VEGF-inhibitors in MCRC, specifically harbouring c.35 G > A mutation.

1. *KRAS* mutations: different biological effect and clinical behaviour in colorectal cancer patients

Most colorectal cancers (CRC) show mutations of *RAS (KRAS, NRAS)*, *BRAF*, *PIK3CA* genes, or PTEN inactivation, activating the RAS-MAPK or PI3 K pathways [1–3]. *KRAS* exon 2 mutations occur, as early event [4,5], in 35–45% CRC, mostly codon 12 (80%) c.35 G > A (G12D) and c.35 G > T (G12 V) transversions, representing 32.5% [4,5], and 22.5% [4,6], respectively, and codon 13, prevalently c.38 G > A (G13D) mutations [7]. RAS proteins are small guanine-nucleotide binding proteins (p21ras) with GTPase activity, differently reduced by mutations of exons 2–4 (codons 12, 13; 59, 61; 117, 146). Codon 12 and 13 mutations lead to substitution of glycine residues adjacent to the GDP/GTP binding pocket, impairing the GTPase activity of KRAS after guanine nucleotide activating protein (GAP) binding, thus locking the protein in a constitutively active, GTP bound state, and leading to constitutive, growth-factor-receptor independent activation of downstream signalling [8–10]. Codon 12 *KRAS* mutations may impair the hydrolysis of GTP, leading to a KRAS protein permanent GTP-bound state that activates downstream effectors, leading to unregulated signaling, enhanced and unregulated cell proliferation and transformation [11]. Both the prevalent *KRAS* mutations G12D and G12 V greatly impair the KRAS GTPase activity [12], but they differently increase aggressiveness [13–15], affect downstream effectors [16], angiogenesis [13–15], confer worse clinical behaviour, and influence response to anti-vascular endothelial growth factor (anti-VEGF) [13–15].

KRAS codon 12 mutations increase transforming capacity and aggressiveness more than codon 13 mutations, by differentially regulating KRAS downstream pathways that lead to inhibition of apoptosis, enhanced loss of contact inhibition, and increased predisposition to anchorage-independent growth [17,18]. *KRAS* codon 12 mutations may differentially correlate with AKT/protein kinase B activation, bcl-2, E-catherin, β-catenin, and focal adhesion kinase overexpression, and RhoA underexpression. Codon 13 *KRAS* mutations correlate with activation of the c-Jun-NH$_2$-terminal kinase I pathway that confer increased sensitivity [17].

KRAS codon 12 and 13 mutations correlate with different clinical outcome in colorectal cancer patients, significantly increasing the risk of death by 26% [6,19]; c.35 G > T (G12 V) mutation represented an independent risk factor for recurrence and significantly increased the risk of death by 44% [19]. It also had a significant worse impact, increasing the risk of recurrence or death by 30% [5], and up to 50% in Dukes' C cancers [6]. *KRAS* codon 12 mutations were associated with reduced survival compared to *KRAS*-wild-type/*BRAF*-wild-type patients [20]. Worse prognosis conferred by codon 12 *KRAS* mutations was not confirmed in other studies [21,22].

2. *RAS* genotype in metastatic colorectal cancer (MCRC) patients

Clinical factors, related to the patient's fitness for more effective treatment strategies (according to age and comorbidity status) [23,24], to the extension of metastatic disease (liver-limited or other/multiple metastatic) [25,26], to the proper treatment strategy (integrated medical and surgical, different medical regimens, lines of effective treatments) [25,27], and biological factors, in particular *BRAF* and *KRAS* genotype status [28–31], contribute to determine the clinical outcome in the individual MCRC patient. The proper definition of a bioclinical algorithm could help address tailored clinical management of individual patients.

RAS genotype, wild-type or mutant, addresses the selection of medical treatment of fit MCRC patients (triplet regimens associating chemotherapeutic drugs, or doublets plus targeted agents) [32]. *KRAS* exon 2 wild-type genotype significantly predicts favourable clinical outcome of anti-epidermal growth factor receptor (anti-EGFR) or anti-VEGF drugs added to doublet chemotherapy [30,33–35]. In *KRAS* exon 2 mutant genotype, bevacizumab (BEV) addition to irinotecan, 5-fluorouracil and leucovorin (IFL) significantly prolonged progression-free survival (PFS) up to 9.3 months, while not overall survival (OS) and activity, compared to IFL [30,33]. Addition of anti-EGFR treatment is not effective in mutant patients, and detrimental if associated to oxaliplatin [34,35].

The comparison of clinical outcome (PFS, OS) in equivalently treated MCRC patients, according to wild-type and mutant genotype, and also according to different *KRAS* mutations, evaluates prognostic relevance of *KRAS* genotype, depending from the balance between tumor aggressiveness, conferred by specific mutations, and treatment effectiveness, related to medical regimens and secondary liver surgery, further lines of treatment [31].

Retrospective analysis showed that *KRAS* exon 2 mutant status did not significantly affect median OS (19.9 and 27.7 months, in *KRAS* mutant and wild-type patients, respectively) of MCRC patients treated with BEV added to IFL [29,30]. Prognostic relevance of *KRAS* exon 2 or *BRAF* mutant genotype was not significantly different compared to *KRAS* or *BRAF* wild-type status. Significantly, worse prognosis was reported only when patients harbouring mutations in *KRAS* exon 2 or *BRAF* gene were compared with *KRAS/BRAF* wild-type (HR 0.51) [33].

In the second line setting, a significant interaction was demonstrated between *KRAS* exon 2 wild-type genotype and effectiveness of cetuximab compared to best supportive care, with increased PFS 3.7 months and OS 9.5 months [36]. Panitumumab confirmed the same significantly positive predictive effect, with objective response rate (ORR) 17%, median PFS 12.3 weeks, median OS 8.1 months, compared to exon 2 mutant genotype [37,38]. In *KRAS* exon 2 wild-type patients, the addition of panitumumab to FOLFIRI significantly increased ORR 35% and PFS 5.9 months, with a trend toward increased OS [39].

In MCRC patients, specific mutations of different genes involved in the same signalling pathway (*BRAF* and *RAS* mutations) can confer different biological aggressiveness and effectiveness of treatment strategies. The prevalent *BRAF* exon 15 c.1799 T > A (V600E) mutation, characterizing 4.7–8.7% CRC, demonstrated worse prognostic effect in MCRC patients treated with doublet chemotherapy alone or added to cetuximab, BEV, and cetuximab plus BEV, with median PFS 5.6–8 months and median OS 10.3–15.9 months [28,29,40]. The favourable predictive effect of cetuximab or BEV addiction to chemotherapy in *KRAS* exon 2 wild-type patients was not significantly confirmed in *BRAF* mutant MCRC patients [29,40,41].

Cetuximab addiction to chemotherapy conferred worse clinical outcome in patients harbouring *KRAS* c.35 G > T mutation and other mutations [42].

KRAS exon 2, codon 13, c.38 G > A mutation (G13D) was significantly associated with worse prognosis in MCRC patients pre-treated with chemotherapy [43]. Cetuximab or cetuximab addiction to chemotherapy significantly predicted increased OS (median 7.6 and 10.6 months, respectively) and PFS (median 4.0 and 4.1 months, respectively) compared to other *KRAS* exon 2 mutations [43], not significantly different from wild-type patients [43]. This favourable predictive effect was confirmed in first line setting [42], with a significantly improved PFS (median, 7.4 versus 6.0 months) and ORR (40.5% versus 22.0), but not OS (median, 15.4 versus 14.7

months). Moreover, systematic reviews and meta-analyses confirmed that *KRAS* c.38 G > A (G13D) mutation significantly predict a favourable effect of cetuximab-containing associations, with no significantly different PFS and OS compared to wild-type patients [44,45]. In patients with MCRC treated with panitumumab added to chemotherapy in first- or second-line settings, no consistent associations were found between specific *KRAS* mutations and clinical outcome: opposite findings when combined with first line oxaliplatin, while similar data when combined with second-line FOLFIRI [46].

No evaluation of the prognostic and predictive relevance of the prevalent exon 2, codon 12, c.35 G > A *KRAS* mutation is reported in MCRC patients, specifically treated with conventional first line triplet regimens, prevalently consisting of VEGF-inhibitors associated to doublet chemotherapy.

Recently retrospective analysis of clinical outcome according to *KRAS/NRAS* exons 2–4 genotype in PRIME, FIRE-3, PEAK, TRIBE randomized studies showed that EGFR- and VEGF-inhibitors are much more, and equivalently, effective in *KRAS/NRAS* exons 2–4 wild-type patients [47–52]. In the TRIBE study, intensive medical treatment including VEGF-inhibitors can predict a favourable effect in *BRAF* mutant patients [52].

3. Prognostic relevance of c.35 G > A (G12D) *KRAS* mutation in MCRC patients

We recently evaluated the prognostic relevance of *KRAS* exon 2 genotype and of the prevalent codon 12 c.35 G > A (G12D) *KRAS* mutation in 59 MCRC patients enrolled in a previously published phase II study and in an expanded clinical program proposing BEV added to triplet chemotherapy, FIr-B/FOx intensive regimen, as first line treatment [25,26,53]: 31 (53%) *KRAS* wild-type and 28 (47%) *KRAS* mutant [31]. *KRAS* exon 2 mutations prevalently affected codon 12 (24 patients, 40.6%), specifically c.35 G > A (G12D) 15 (25.4%), c.35 G > T (G12 V) 7 (11.8%); codon 13, 4 (6.7%), c.38 G > A (G13D), 3 (5%) (Table 1). After progression, among 38 second line treated patients [27], 21 were wild-type (55.3%) and 17 mutant (44.7%).

After a median follow-up of 21.5 months (Table 2) [25,31], among *KRAS* exon 2 wild-type patients, ORR was 90%, liver metastasectomies 35% (83% in liver-limited (L-L)), median PFS 14 months, median OS 38 months; among *KRAS* exon 2 mutant patients, ORR was 67%, liver metastasectomies 25% (54% in L-L), median PFS 11 months, median OS 20 months. *KRAS* exon 2 mutant compared with wild-type patients did not show significantly different PFS nor OS, even if OS was trendly worse in *KRAS* mutant patients (Fig. 1A).

We verified previously reported findings of significantly different outcome (PFS and OS) according to extension of metastatic disease (L-L compared to other/multiple metastatic (O/MM) patients) [26,31], in exon 2 *KRAS* wild-type and mutant patients (Fig. 1B). Among *KRAS*

Table 1
KRAS mutations.

				Fit patients		Unfit patients		Total
				No. of patients	%	No. of patients	%	
Overall				59		36		95
Wild-type				31	53	21	58	52
Mutant				28	47	15	42	43
Exon	**Codon**	**Hot spot site**	**Amino acid**					
2	12			24	40.6	13	36.1	37
		c.34 G > A	p.Gly12Ser	1	1.6	–	–	1
		c.35 G > A	p.Gly12Asp	15	25.4	7	19.4	22
		c.35 G > T	p.Gly12Val	7	11.8	6	16.6	13
		c.35 G > C	p.Gly12Ala	1	1.6	–	–	1
	13			4	6.7	2	5.5	6
		c.37_39 dupl	p.Gly13dupl	1	1.6	–	–	1
		c.37 G > T	p.Gly13Val	–	–	1	2.7	1
		c.38 G > A	p.Gly13Asp	3	5	1	2.7	4

exon 2 wild-type patients, significantly different clinical outcome was confirmed in L-L compared to O/MM, respectively (Fig. 1C): median PFS 21 months versus 12 months ($p = 0.044$); median OS 47 months versus 28 months ($p = 0.017$). Among *KRAS* exon 2 mutant patients, the comparison of PFS and OS in L-L and O/MM was not significantly different: median PFS 11 months, equivalently; median OS 39 months versus 19 months, respectively (Fig. 1D).

Among c.35 G > A *KRAS* exon 2 mutant patients (Table 2), ORR was 71%, median PFS 9 months, median OS 14 months. Among other than c.35 G > A *KRAS* exon 2 mutant patients, ORR was 61%, median PFS 12 months, median OS 39 months [53]. PFS of c.35 G > A *KRAS* exon 2 mutant compared to wild-type patients was not significantly different (Fig. 2A), while OS was significantly worse ($p = 0.002$) (Fig. 2B). More, PFS of c.35 G > A *KRAS* mutant compared to other than c.35 G > A *KRAS* exon 2 mutant patients was not significantly different, while OS was significantly worse ($p = 0.05$) (Fig. 2C and D). Other than c.35 G > A *KRAS* exon 2 mutant compared to wild-type patients did not show different PFS and OS (Fig. 2E, 2F). *KRAS* c.35 G > A mutant patients also showed significantly worse OS compared to: other than c.35 G > A *KRAS* mutant plus *KRAS* wild-type patients ($p = 0.002$); *KRAS* exon 2/*BRAF* wild-type patients

($p = 0.03$); other codon 12 mutant patients ($p = 0.03$). Clinical outcome was not significantly different compared to c.35 G > T *KRAS* mutant patients ($p = 0.142$).

After progression to FIr-B/FOx intensive regimen, among *KRAS* exon 2 mutant patients, ORR was 29%, median PFS 10 months, median OS 12 months. Among *KRAS* exon 2 wild-type patients [27], ORR was 50%, median PFS 10 months, median OS 17 months (Table 2). *KRAS* exon 2 mutant compared with wild-type patients, prevalently treated with triplet regimens or re-challenge of triplet chemotherapy plus targeted agent (80%), did not show significantly different PFS nor OS (Fig. 3A and B). c.35 G > A *KRAS* mutant patients showed significantly worse PFS and OS compared to *KRAS* exon 2 wild-type ($p = 0.000$, and $p = 0.000$, respectively) (Fig. 3C and D), and to other than c.35 G > A *KRAS* mutant patients ($p = 0.007$, and $p = 0.002$, respectively) (Fig. 3E and F). PFS and OS were also significantly worse in c.35 G > A *KRAS* mutant compared to other than c.35 G > A *KRAS* mutant plus *KRAS* wild-type patients ($p = 0.000$, and $p = 0.000$, respectively). No different clinical outcomes were reported in other than c.35 G > A *KRAS* mutant compared to wild-type patients (Fig. 3G and H).

Prognostic relevance of *KRAS* exon 2 genotype and of c.35 G > A (G12D) mutation was also evaluated in 36 MCRC patients unfit for intensive first line FIr-B/FOx regimen, due

Table 2
KRAS genotype and c.35 G > A *KRAS* mutation in MCRC.

	Fit, first line			Second line			Unfit, first line		
	No. pts	PFS (months)	OS (months)	No. pts	PFS (months)	OS (months)	No. pts	PFS (months)	OS (months)
Overall	59	12	28	38	10	14	36	7	13
***KRAS* mutant**	28	11	20	17	10	12	15	6	8
P		NS	NS		NS	NS		0.043	NS
***KRAS* wild-type**	31	14	38	21	10	17	21	8	13
P		NS	0.002		0.000	0.000		0.000	0.049
c. 35 G > A *KRAS* mutant	15	9	14	8	3	6	7	3	6
P		NS	0.05		0.007	0.002		0.020	0.048
Other *KRAS* mutations	13	12	39	9	10	38	8	9	13

Abbreviations: pts, patients; PFS, progression-free survival; OS, overall survival; NS, not statistically significance difference.
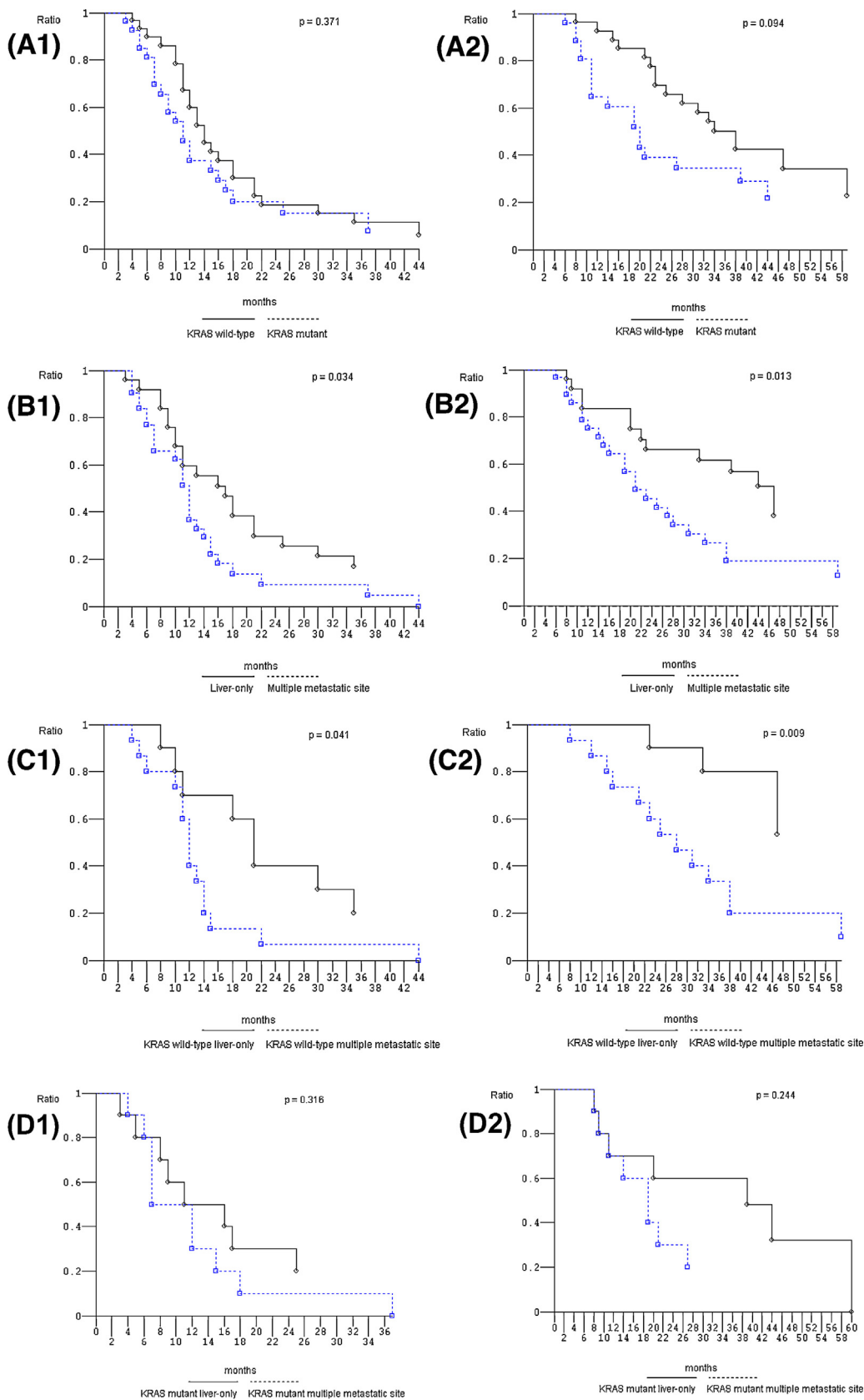
Fig. 1. Fit patients, first line FIr-B/FOx intensive regimen, Kaplan–Meier survival estimate. A, overall population, *KRAS* wild-type versus *KRAS* mutant; B, liver-only versus multiple metastatic sites; C, liver-only versus multiple metastatic sites, *KRAS* wild-type; D, liver-only versus multiple metastatic sites, *KRAS* mutant; 1, progression-free Survival; 2, overall survival.
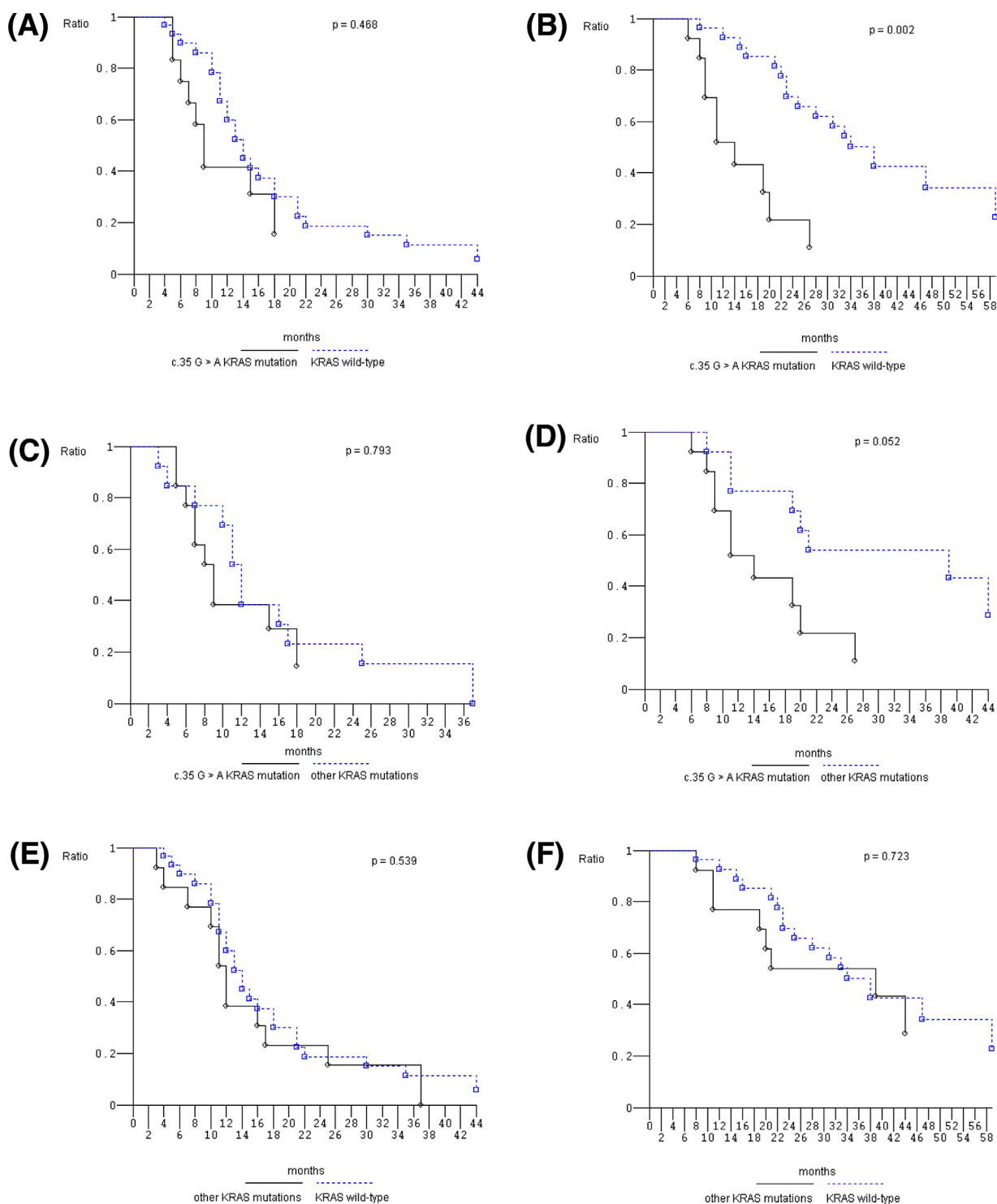
Fig. 2. Fit patients, first line FIr-B/FOx intensive regimen, Kaplan–Meier survival estimate. A, c.35 G > A *KRAS* mutant patients versus *KRAS* wild-type patients, progression-free survival; B, c.35 G > A *KRAS* mutant patients versus *KRAS* wild-type patients, overall survival; C, c.35 G > A *KRAS* mutant patients versus other *KRAS* mutant patients, progression-free survival; D, c.35 G > A *KRAS* mutant patients versus other *KRAS* mutant patients, overall survival; E, other *KRAS* mutant patients versus *KRAS* wild-type patients, progression-free survival; F, other *KRAS* mutant patients versus *KRAS* wild-type patients, overall survival.

to age and/or comorbidities, and treated with tailored first line treatments [24]: 21 (58%) wild-type and 15 (42%) mutant. *KRAS* exon 2 mutations detected were prevalently codon 12, 13 (36.1%), specifically c.35 G > A (G12D), 7 (19.4%), c.35

G > T (G12 V), 6 (16.6%); codon 13, 2 (5.5%), c.38 G > A (G13D), 1 (2.7%) (Table 1). Among *KRAS* exon 2 mutant patients, ORR was 25%, median PFS 6 months, median OS 8 months (Table 2). Among *KRAS* exon 2 wild-type patients,
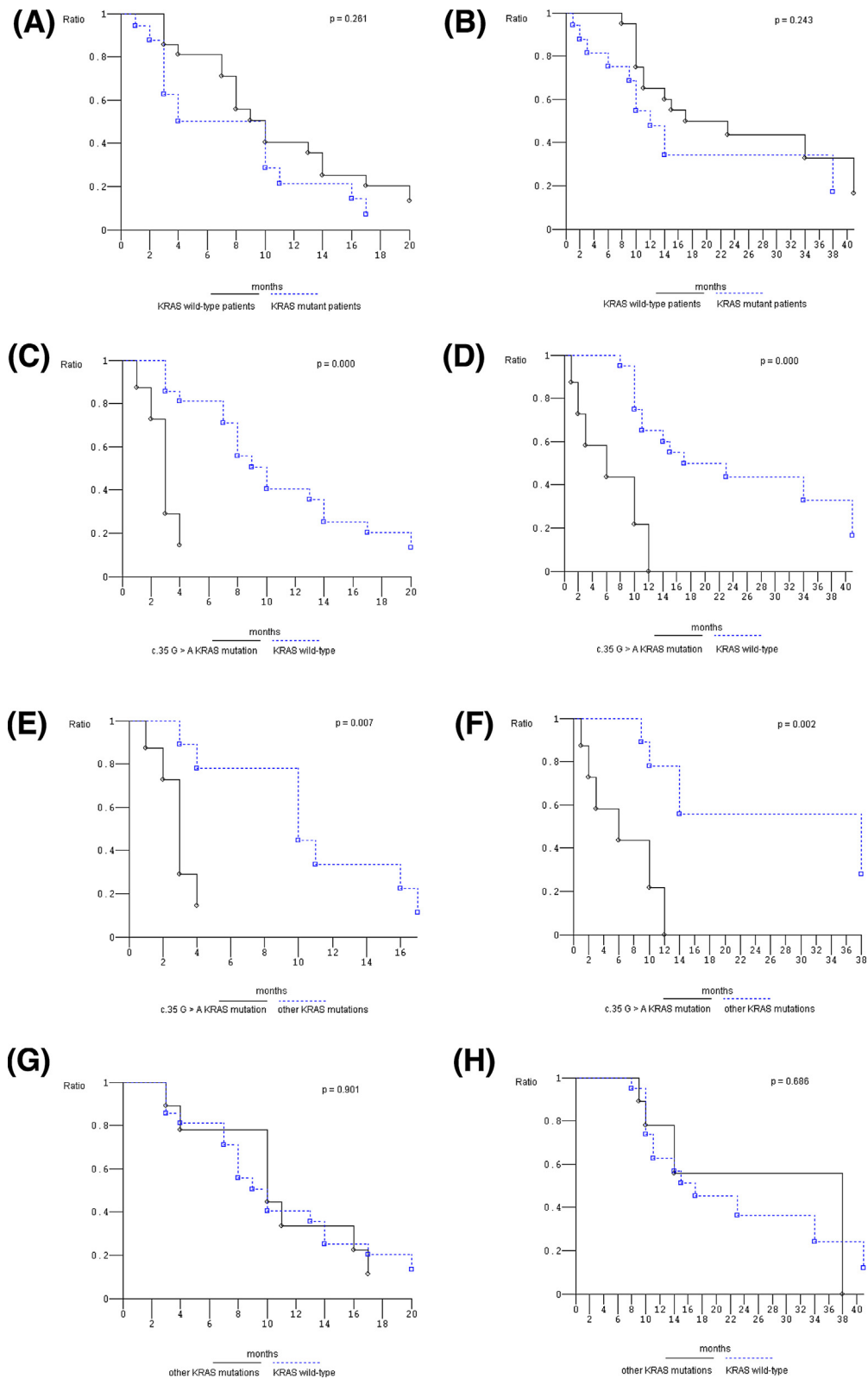
Fig. 3. Second line, Kaplan–Meier survival estimate. A, second line treatment, *KRAS* wild-type versus *KRAS* mutant patients, progression-free survival; B, second line treatment, *KRAS* wild-type versus *KRAS* mutant patients, overall survival; C, progression-free survival c.35 G > A *KRAS* mutant patients versus *KRAS* wild-type patients; D, overall survival c.35 G > A *KRAS* mutant patients versus *KRAS* wild-type patients; E, progression-free survival c.35 G > A *KRAS* mutant patients versus other *KRAS* mutant patients; F, overall survival c.35 G > A *KRAS* mutant patients versus other *KRAS* mutant patients; G, progression-free survival other *KRAS* mutant patients versus *KRAS* wild-type patients; H, overall survival other *KRAS* mutant patients versus *KRAS* wild-type patients.

ORR was 50%, median PFS 8 months, median OS 13 months. *KRAS* exon 2 mutant compared with wild-type patients, treated with conventional medical treatments, showed significantly different PFS ($p = 0.043$), but not OS (Fig. 4A and B). *KRAS* c.35 G > A mutant patients showed significantly worse PFS and OS compared to wild-type ($p = 0.000$, and $p = 0.049$, respectively) (Fig. 4C and D), and to other mutant patients ($p = 0.020$, and $p = 0.048$, respectively) (Fig. 4E and F). PFS and OS were also significantly worse in c.35 G > A *KRAS* mutant patients compared to other mutant plus wild-type patients ($p = 0.000$, and $p = 0.021$, respectively). No different clinical outcomes were reported in other than c.35 G > A *KRAS* mutant compared to wild-type patients (Fig. 4G and H).

Our retrospective data are "hypotesis generating" and require further prospective validation in fit, also after progression, and in unfit MCRC patients.

4. c.35 G > A *KRAS* mutation in MCRC patients: prognostic relevance and predictive implications for intensive treatment adding VEGF-inhibitors to triplet chemotherapy.

In *KRAS* exon 2 mutant patients BEV addition to IFL compared to IFL significantly increased PFS up to 9.3 months, while not OS and activity [4,30]. *KRAS* exon 2 status, wild-type or mutant, does not significantly affect clinical outcome of MCRC patients treated with BEV-containing chemotherapy. Reported median OS ranges between 29.9–38 months in *KRAS* wild-type and 19.9–21 months in *KRAS* mutant patients [29–31,54].

Retrospective analysis showing that median OS of MCRC patients treated with BEV-containing chemotherapy was significantly worse when a gene (*KRAS* or *BRAF*) involved in the RAS/RAF/MEK/ERK pathway was mutated, raised the question of the relationship of this pathway with angiogenesis. In patients treated with BEV added to IFL, median PFS reported in *KRAS* exon 2 mutant patients was 9.3 months, not significantly lower than in *KRAS* wild-type (13.5 months) [4,30]. Prospective trials will evaluate the benefit of treatment with VEGF inhibitors associated to chemotherapy in *KRAS* mutant MCRC patients.

BEV addition to triplet chemotherapy, according to FIr-B/FOx or FOLFOXIRI/BEV schedules, reported high activity and efficacy in *KRAS* exon 2 mutant and wild-type MCRC patients [31,54]. In the FOLFOXIRI plus BEV study, median PFS was equivalent 12.6 and 13.6 months, respectively [54]. In the FIr-B/FOx study, *KRAS* mutant compared with wild-type patients did not show significantly different PFS nor OS, even if OS was lower in *KRAS* mutant patients (Fig. 1A). Among *KRAS* exon 2 mutant patients, the comparison of PFS and OS in L-L and O/MM was not significantly different (Fig. 1D).

Intensive regimens adding BEV to triplet chemotherapy can further increase clinical outcome of MCRC patients.

In overall MCRC patients treated with BEV added to irinotecan/5-fluorouracil, or with more intensive regimens (FIr-B/FOx, FOLFOXIRI/BEV), PFS and OS were not significantly different in *KRAS* exon 2 wild-type and mutant patients [23,29–31,54]. Recently *KRAS* exon 2 genotype was reported as significantly affecting PFS and OS in patients treated with XelOx/BEV [55].

The prevalent c.35 G > A (G12D) *KRAS* mutation characterizes 22.4% MCRC patients, representing approximately 50% *KRAS* exon 2 mutations [6]. MCRC patients harbouring c.35 G > A *KRAS* mutation reported high activity of FIr-B/FOx intensive regimen (ORR 71%); median PFS and OS were 9 and 14 months, respectively. In *KRAS* c.35 G > A mutant patients, PFS was not significantly different, putatively due to effectiveness of BEV addition to triplet chemotherapy, while OS was significantly worse compared to *KRAS* exon 2 wild-type, to *KRAS/BRAF* wild-type, and to other codon 12 and 13 mutant patients. Thus, FIr-B/FOx intensive regimen may potentially limit this worse prognostic effect.

KRAS genotype failed to significantly differentiate clinical outcome also after progression to FIr-B/FOx regimen [27,29–31]. In *KRAS* exon 2 mutant patients harbouring the prevalent c.35 G > A transversion, median PFS and OS were significantly worse compared to wild-type and/or other than c.35 G > A *KRAS* mutant patients, maybe due to increased aggressiveness and resistance to medical treatment [17]. In patients unfit for FIr-B/FOx, treated with conventional medical regimens, *KRAS* exon 2 mutant compared to wild-type patients showed a significantly different PFS, and not OS [24]. Furthermore, *KRAS* c.35 G > A mutant genotype affected significantly worse PFS and OS, compared to wild-type and/or other mutant.

KRAS c.35 G > A (G12D) mutant genotype may represent a biomarker of unfavourable clinical outcome in MCRC patients, at first, second line of treatment or in patients unfit for first line intensive regimens. Activity and efficacy of first line intensive regimen adding BEV to triplet chemotherapy (FIr-B/FOx) may overcome resistance of *KRAS* c.35 G > A mutant MCRC patients, thus limiting its worse predictive effect, potentially due to VEGF inhibition.

Present data confirm that *KRAS* mutant genotype, particularly c.35 G > A mutant, confers different biological aggressiveness [17] and resistance to medical treatments. Further prospective studies will evaluate the prognostic and predictive value of c.35 G > A *KRAS* mutation in MCRC patients, fitting for intensive regimens, or unfit, and after progression to first line treatments [29,53].

5. c.35 G > A *KRAS* mutation: specific aggressiveness and potential effect of VEGF-inhibitors

KRAS undergoes conformational changes when its P-loop, that includes Glycin 12 and 13, binds GTP, to exert its GTPase function. The specificity of GTPase binding to its
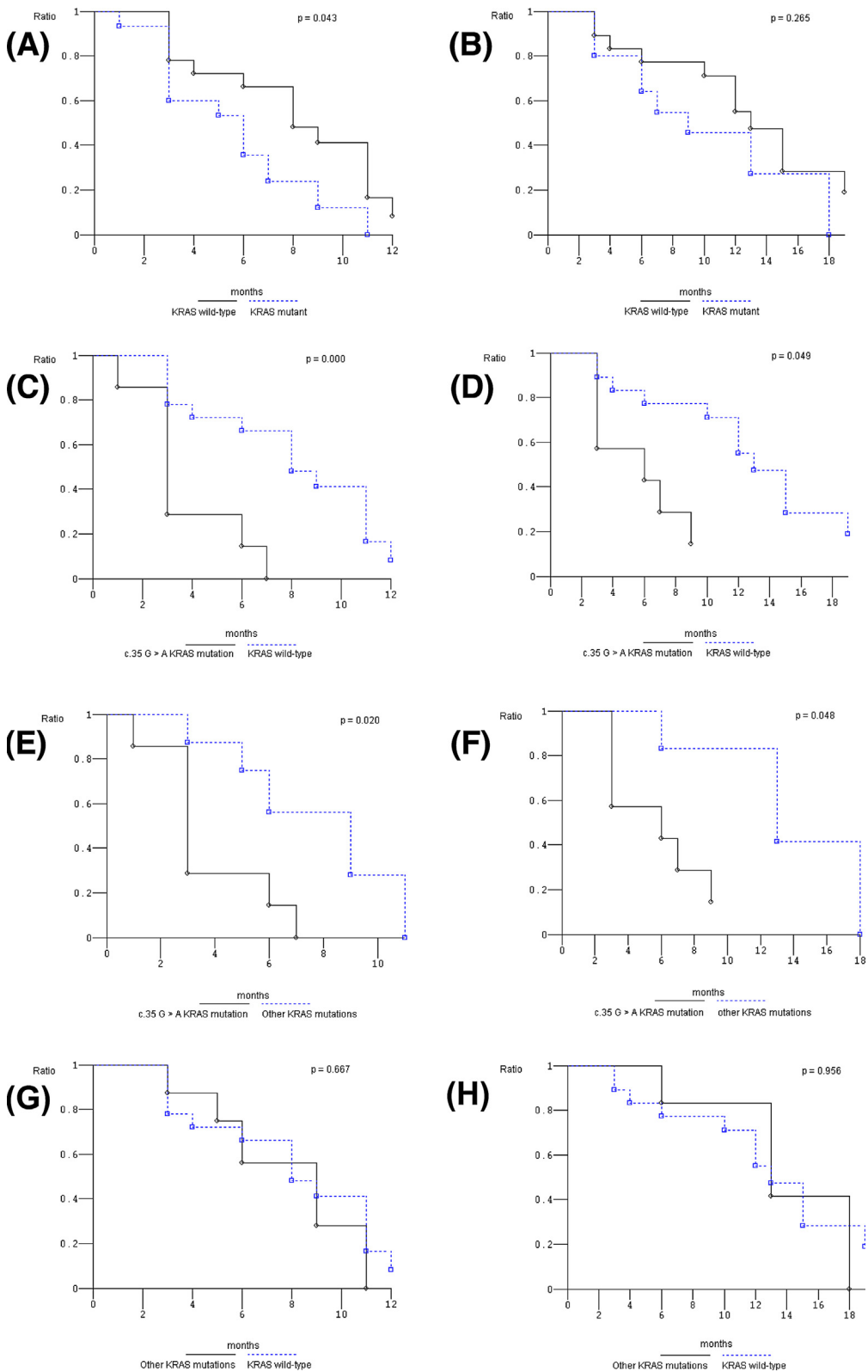
Fig. 4. Unfit patients, first line, Kaplan–Meier survival estimate. A, Overall population, *KRAS* wild-type versus *KRAS* mutant, progression-free survival; B, overall population, *KRAS* wild-type versus *KRAS* mutant, overall survival; C, progression-free survival c.35 G > A *KRAS* mutant versus *KRAS* wild-type patients; D, overall survival c.35 G > A *KRAS* mutant versus *KRAS* wild-type patients; E, progression-free survival c.35 G > A *KRAS* mutant versus other *KRAS* mutant patients; F, overall survival c.35 G > A *KRAS* mutant versus other *KRAS* mutant patients; G, progression-free survival other *KRAS* mutant versus *KRAS* wild-type patients; H, overall survival other *KRAS* mutant versus *KRAS* wild-type patients.

effector molecules is controlled by the effector loop that consists of two regions of the protein, the switch I and switch II [8]. This conformational change drives its interactions with transducers, the GTPase-activating proteins (GAPs) that optimize its GTPase function and activate downstream signaling effectors [56]. The conformational changes of the c.35 G > A (p.G12D) mutant, when compared with the wild-type and the mutant c.38 G > A (p.G13D), significantly impair the interactions with GAPs and the binding to its effector molecules, thus maintaining KRAS active conformation. The larger amino acid side chains inserted into the GDP/GTP binding pocket, due to the KRAS glycine substitutions, specifically aspartate (c.35 G > A) and valine (c.35 G > T) at codon 12 and aspartate (c.38 G > A) at codon 13 [19], interferes with the GTP hydrolysis [57].

Exon 2 codon 12 mutations differently modify mutated KRAS protein, specifically in the GTP-bound state, thus limiting GTPase activity and affinity of GTPase-activating proteins [6,17].

Expression of VEGF and/or of negative regulators of angiogenesis (thrombospondin-1 repression through phosphorylated Myc, hyperactivation of PIK3/Rho pathway) have been reported to be regulated by RAS/RAF/MEK/ERK signalling, suggesting that aberrations in KRAS and/or BRAF may differentially stimulate tumor angiogenesis and influence the response to antiangiogenic therapy [13–15,58]. In codon 12 mutant tumours, showing a high glycolytic phenotype through HIF-1α-dependent induction of glycolytic enzymes, a dense microvascular network is observed. Cells harbouring codon 12 KRAS mutations compared with cells containing codon 13 mutations showed increased anaerobic glycolytic metabolism [59]. Codon 13 mutant tumours, through RAS/RAF/ERK transcriptional activation of the VEGF-A promoter, induce a vascular network associated with VEGF-A expression, without induction of HIF-1α, determining a less aggressive phenotype and a less effective vascularization.

Pro-angiogenetic balance through VEGF up-regulation and down-regulation of angiogenesis repressors has also been associated with P53 dysfunction. Thus, RAS/RAF/MEK/ERK or P53 pathway dysfunctions may influence the efficacy of anti-VEGF drugs, such as BEV [60–62].

6. Conclusion

Retrospective comparative evaluation of clinical outcome according to KRAS exon 2 genotype, depending from differential biological aggressiveness and effectiveness of medical treatment, shows that mutant KRAS exon 2 status determined by different codon 12 and 13 mutations does not significantly affect worse clinical outcome of MCRC patients treated with first line BEV-containing intensive regimens; the prevalent KRAS c.35 G > A (G12D) mutant genotype may affect significantly worse OS compared to wild-type or to different other KRAS mutations. Clinical outcome of MCRC progressing to first line FIr-B/FOx regimen may be significantly worse in c.35 G > A mutant compared to wild-type and other KRAS mutant patients.

In MCRC patients unfit for first line intensive FIr-B/FOx regimen, KRAS exon 2 genotype may affect significantly different PFS, and c.35 G > A KRAS mutant, a significantly worse PFS and OS, compared to wild-type and other mutant.

The differential prognosis and predictive effect of VEGF-inhibitors in MCRC patients harbouring different KRAS mutations, specifically the prevalent KRAS c.35 G > A mutation (G12D), should be prospectively confirmed.

Conflict of interest

The authors declare that they have no competing interests.

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