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Original Research

Relevance of baseline carcinoembryonic antigen for first-line treatment against metastatic colorectal cancer with FOLFIRI plus cetuximab or bevacizumab (FIRE-3 trial)

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Abstract ***Purpose:*** Increased baseline carcinoembryonic antigen (CEA) serum level is associated with inferior overall survival (OS) in metastatic colorectal cancer (mCRC). However, limited data exist on its predictive relevance for targeted therapies. Therefore, we analysed its relevance in FIRE-3, a randomised phase III study.
Experimental design: FIRE-3 evaluated first-line FOLFIRI plus cetuximab (FOLFIRI/Cet) versus FOLFIRI plus bevacizumab (FOLFIRI/Bev) in mCRC patients with *RAS*-WT tumour (i.e. wild-type in *KRAS* and *NRAS* exons 2–4). Herein, the impact of CEA on patient outcome was investigated.
Results: Of 400 patients, 356 (89.0%) were evaluable for CEA. High CEA (>10 ng/ml; $N = 237$) compared to low CEA (≤10 ng/ml; $N = 119$) was associated with shorter OS in the FOLFIRI/Bev arm (hazard ratio [HR] = 1.50; $P = 0.036$), while no significant OS difference was observed in the FOLFIRI/Cet arm (HR = 1.07; $P = 0.74$). In patients with high CEA, FOLFIRI/Cet compared to FOLFIRI/Bev showed a greater OS benefit (HR = 0.56; $P < 0.001$) than in patients with low CEA (HR = 0.78; $P = 0.30$). Furthermore, FOLFIRI/Cet exhibited significantly superior objective response rate in patients with high CEA (odds ratio = 2.21; $P = 0.006$) in contrast to patients with low CEA (odds ratio = 0.90; $P = 0.85$).
Conclusion: In patients with *RAS*-WT mCRC receiving first-line chemotherapy with FOLFIRI/Cet versus FOLFIRI/Bev, elevated CEA was associated with inferior survival in the bevacizumab arm, while this was not the case when cetuximab was applied. Comparison of OS and objective response rate according to treatment arms indicated that cetuximab was greatly superior to bevacizumab in patients with elevated CEA, while this effect was markedly lower and lost statistical significance in patients with low CEA.

1. Introduction

Worldwide, colorectal cancer (CRC) is the third most common cancer in men and the second in women [1]. About 50% of patients develop distant metastases [2,3]. Most of them succumb to the metastatic disease (mCRC) with a 5-year survival rate of only 12.5% [1,4]. With the introduction of modern chemotherapy in combination with monoclonal antibodies such as bevacizumab targeting the vascular endothelial growth factor (VEGF) and cetuximab or panitumumab targeting the epithelial growth factor receptor (EGFR), median overall survival (OS) exceeding 30 months has been reached [5–7]. Presently, the only established biomarker for optimal patient selection in first-line treatment is the mutation status in *KRAS*- and *NRAS* oncogenes. The benefit from first-line anti-EGFR medication plus standard chemotherapy compared to chemotherapy alone or in combination with anti-VEGF treatment is restricted to patients with wild-type *KRAS* and *NRAS* (*RAS*-WT) tumours [5,6,8–10].

Unlike anti-EGFR-directed therapies, no validated biomarkers exist to predict benefit from treatment against VEGF. This needs to be seen in the context that not all patients benefit from anti-VEGF treatment [11,12]. Additionally, given the significant medical burden of mCRC and the socio-economic costs of a treatment with monoclonal antibodies, there remains an urgent need to identify predictive biomarkers. Here, angiogenic factors like angiopoetin-2 [12] or VEGF [13] came into the focus. Furthermore, with a study demonstrating that carcinoembryonic antigen (CEA) might contribute to tumour angiogenesis, CEA has been evaluated as a readily available biomarker to predict benefit from anti-VEGF therapy [14–17]. Under the idea of CEA as stimulus for angiogenesis, increased CEA appeared to counteract treatment against VEGF. However, these retrospective analyses are limited by heterogeneity of chemotherapy and molecular subgroups, as well as sample size. Hence, we investigated the prognostic and predictive relevance of CEA in *RAS*-WT mCRC patients who were treated with first-line FOLFIRI combined with either cetuximab (FOLFIRI/Cet) or bevacizumab (FOLFIRI/Bev) in FIRE-3, a randomised phase III clinical trial. New response-related outcome parameters such as early tumour shrinkage (ETS) ≥20% and depth of response (DpR) were explored in this context [5,18].

2. Methods

2.1. Patients and methods

2.1.1. Study design

FIRE-3/AIO KRK0306 was a prospective, multicentre, randomised, open-label, phase III study (NCT00433927). The study design has been described elsewhere [12]. Briefly, FIRE-3 compared FOLFIRI/Cet

to FOLFIRI/Bev as first-line treatment of patients with unresectable mCRC and *KRAS*-WT tumours. In a post hoc analysis, patients with *RAS*-WT tumours were identified [5]. Details regarding the conduct of the trial, the full study population, treatment schedules, concordance with the Declaration of Helsinki and approval of ethics committees were reported [12].

2.1.2. Patient population

In light of the adoption of *RAS* analyses as an improved biomarker of response to cetuximab therapy and its evaluation in FIRE-3, we decided to perform the present analyses in the *RAS*-WT population as previously described [5,19]. ETS and DpR were assessed in patients accessible for independent, centralised radiological review [5,18].

2.1.3. Statistical analysis

The primary end-point of FIRE-3 was objective response rate (ORR). Secondary end-points were OS, progression-free survival (PFS) and DpR. The statistical design of FIRE-3 has been described elsewhere [12]. CEA subgroups ($\leq$10 and $>$ 10 ng/ml) were based on twofold upper limit of normal at 5 ng/ml, as described previously [15,20]. Alternative cutoff thresholds of CEA were evaluated by time-dependent receiver operating characteristic curves and graphs representing martingale residuals from the null model against logarithmic (log) CEA. To further describe the relationship between CEA and OS, fractional polynomials were used. Fisher exact test was applied to compare dichotomous variables. Continuous variables were described as medians and compared by Mann–Whitney *U* tests. Survival curves by CEA level and treatment arm were estimated using the Kaplan–Meier method; differences were assessed using a log-rank test. A Cox regression model was used to estimate hazard ratios (HRs). Interactions between treatment arms and CEA level were assessed by Cox models for OS and PFS as well as a logistic model for ORR and ETS. Regarding DpR, a non-parametric bootstrap technique and the Mann–Whitney *U* test were used. To analyse CEA as a continuous parameter, it was integrated as log-transformed variable in logistic regressions, where indicated. To evaluate independent prognostic factors for OS, multivariate Cox proportional hazard models were fitted to estimate the effect of log-transformed CEA on OS adjusted for possibly prognostic baseline parameters [21]. To examine how CEA cutoff thresholds other than 10 ng/ml would influence treatment efficacy, HR and median OS times for both treatment arms were plotted for subgroups of patients with CEA levels below or equal to varying CEA thresholds. The significance level was 0.05 and all considered tests were two-sided. Statistical analyses were implemented in R version 3.2.2. The packages survival, Forest plot and mfp (fractional polynomial) were used.

3. Results

3.1. Patients and CEA data distribution

Of 400 patients with *RAS*-WT tumours in the FIRE-3 study, baseline CEA data were available for 356 patients (89.0%). Of those, 295 patients (82.9%) were evaluable for centralised radiological review to determine ETS and DpR (Supplementary Fig. S1). Supplementary Fig. S2 summarises the baseline CEA serum level distribution in the patient cohort. Patients were divided into CEA subgroups of $\leq$10 ng/ml (N = 119, 33.4%) and $>$10 ng/ml (N = 237 patients, 66.6%). Within each CEA subgroup, baseline patient and tumour characteristics were analysed for both treatment arms and are listed in Table 1. In the CEA subgroup $\leq$10 ng/ml, median value for CEA was 3.2 ng/ml in FOLFIRI/Cet arm and 3.6 ng/ml in FOLFIRI/Bev arm (P = 0.51). In the CEA subgroup $>$10 ng/ml, median value for CEA was 69.7 ng/ml in FOLFIRI/Cet arm and 99.85 ng/ml in FOLFIRI/Bev arm (P = 0.18).

3.2. The prognostic relevance of baseline CEA

The prognostic relevance of baseline CEA serum level within the treatment arms of FIRE-3 was evaluated (Table 2 and Supplementary Fig. S3). CEA $>$10 ng/ml was significantly associated with inferior OS in patients treated with FOLFIRI/Bev (median OS 23.8 versus 26.1 months; HR = 1.50; P = 0.036). By contrast, baseline CEA had no significant impact on OS in patients treated with FOLFIRI/Cet, albeit those with CEA $>$10 ng/ml showed a numerically longer median OS (37.1 versus 28.7 months; HR = 1.07; P = 0.74). However, no formal interaction between CEA and treatment arm was observed (P = 0.28). With regard to PFS, no significant impact of CEA was evident in both treatment arms. Regarding ORR, CEA $>$10 ng/ml was associated with significantly increased response rate in patients receiving FOLFIRI/Cet (ORR 74.4% versus 55.0%; P = 0.011), but not in patients receiving FOLFIRI/Bev (P = 1.0). A significant interaction between CEA and treatment arm was observed for ORR (P = 0.05). Also, the response-related outcome parameters ETS and DpR both appeared to be beneficial in patients with CEA $>$10 ng/ml only when treated with FOLFIRI/Cet, albeit not reaching the level of significance.

3.3. Cutoff optimisation for baseline CEA

With regard to the prognostic relevance of baseline CEA, we evaluated whether an alternative cutoff threshold (other than 10 ng/ml) would improve sensitivity and specificity by using time-dependent receiver operating characteristic curves and graphs representing martingale residuals from the null model against log CEA. Here, results did not reveal a superior threshold as fractional polynomials suggest rather a continuous log-

Table 1
Baseline characteristics in CEA subgroups.

CEA subgroups	CEA ≤ 10 ng/ml		p-value[1]	CEA > 10 ng/ml		p-value[1]	p-value[2]
Treatment	**FOLFIRI + bev**	**FOLFIRI + cet**		**FOLFIRI + bev**	**FOLFIRI + cet**		
N (%)	59 (16.6%)	60 (16.8%)		120 (33.7%)	117 (32.9%)		
Age							
	64 (31–77)	62 (42–76)	0.64	67 (40–77)	67 (41–77)	0.12	**0.03**
Sex							
Male	45 (76.3%)	45 (75%)	1.00	70 (58.3%)	87 (74.4%)	**0.01**	0.09
Female	14 (23.7%)	15 (25%)		50 (41.7%)	30 (25.6%)		
Performance status							
ECOG 0	38 (64.4%)	33 (55%)	0.35	61 (50.8%)	63 (53.8%)	0.70	0.21
ECOG 1-2	21 (35.6%)	27 (45%)		59 (49.2%)	54 (46.2%)		
Primary tumour location							
Colon	36 (61%)	37 (61.7%)	1.00	77 (64.2%)	67 (57.3%)	0.30	0.61
Rectum	22 (37.3%)	22 (36.7%)		37 (30.8%)	46 (39.3%)		
Colon and rectum	1 (1.7%)	1 (1.7%)		6 (5%)	3 (2.6%)		
NA	0 (0%)	0 (0%)		1 (0.8%)	0 (0%)		
Primary tumour sidedness							
Left	41 (69.5%)	43 (71.7%)	0.84	93 (77.5%)	99 (84.6%)	0.08	**0.008**
Right	18 (30.5%)	17 (28.3%)		25 (20.8%)	14 (12%)		
NA	0 (0%)	0 (0%)		4 (3.3%)	2 (1.7%)		
Resection of primary tumour							
No	4 (6.8%)	17 (28.3%)	**0.003**	19 (15.8%)	8 (6.8%)	**0.04**	0.10
Yes	54 (91.5%)	42 (70%)		101 (84.2%)	107 (91.5%)		
NA	1 (1.7%)	1 (1.7%)		2 (1.7%)	0 (0%)		
T-stage of primary							
1–2	1 (1.7%)	10 (16.7%)	**0.004**	9 (7.5%)	9 (7.7%)	1.0	0.68
3–4	55 (93.2%)	44 (73.3%)		102 (85%)	97 (82.9%)		
NA	6 (10.2%)	3 (5%)		11 (9.2%)	9 (7.7%)		
N-stage of primary							
Negative	12 (20.3%)	8 (13.3%)	0.46	21 (17.5%)	20 (17.1%)	1.0	0.76
Positive	41 (69.5%)	44 (73.3%)		78 (65%)	76 (65%)		
Missing	8 (13.6%)	6 (10%)		21 (17.5%)	21 (17.9%)		
Prior radiotherapy							
No	48 (81.4%)	48 (80%)	1.00	109 (90.8%)	109 (93.2%)	0.46	**0.002**
Yes	11 (18.6%)	12 (20%)		11 (9.2%)	7 (6%)		
NA	0 (0%)	0 (0%)		1 (0.8%)	0 (0%)		
Prior adjuvant treatment							
No	41 (69.5%)	42 (70%)	1.00	107 (89.2%)	102 (87.2%)	0.84	**0.0001**
Yes	18 (30.5%)	18 (30%)		13 (10.8%)	14 (12%)		
NA	0 (0%)	0 (0%)		1 (0.8%)	0 (0%)		
Development of metastases							
Synchronous	36 (61%)	39 (65%)	0.85	97 (80.8%)	95 (81.2%)	0.87	**0.0004**
Metachronous	22 (37.3%)	21 (35%)		23 (19.2%)	21 (17.9%)		
NA	0 (0%)	1 (1.7%)		1 (0.8%)	0 (0%)		
Liver-limited disease							
No	49 (83.1%)	38 (63.3%)	0.02	75 (62.5%)	79 (67.5%)	0.50	0.15
Yes	10 (16.9%)	22 (36.7%)		45 (37.5%)	38 (32.5%)		
Lung-limited disease							
No	52 (88.1%)	57 (95%)	0.20	118 (98.3%)	115 (98.3%)	1.00	**0.003**
Yes	7 (11.9%)	3 (5%)		2 (1.7%)	2 (1.7%)		
BRAF mutation							
No	53 (89.8%)	50 (83.3%)	0.42	108 (90%)	106 (90.6%)	0.65	0.20
Yes	6 (10.2%)	10 (16.7%)		12 (10%)	9 (7.7%)		
NA	0 (0%)	0 (0%)		2 (1.7%)	0 (0%)		
Alkaline phosphatase							
≤300 U/l	57 (96.6%)	58 (96.7%)	1.00	96 (80%)	98 (83.8%)	0.39	**0.0001**
>300 U/l	2 (3.4%)	2 (3.3%)		23 (19.2%)	17 (14.5%)		
NA	0 (0%)	0 (0%)		2 (1.7%)	1 (0.9%)		
LDH							
≤250 U/l	26 (44.1%)	36 (60%)	0.58	29 (24.2%)	21 (17.9%)	0.49	**0.0001**
>250 U/l	8 (13.6%)	8 (13.3%)		49 (40.8%)	46 (39.3%)		
NA	16 (27.1%)	25 (41.7%)		50 (41.7%)	42 (35.9%)		
White blood cell count							
≤10 G/l	53 (89.8%)	56 (93.3%)	0.53	89 (74.2%)	93 (79.5%)	0.28	**0.0007**
>10 G/l	6 (10.2%)	4 (6.7%)		31 (25.8%)	23 (19.7%)		
NA	0 (0%)	0 (0%)		1 (0.8%)	0 (0%)		

Table 1 (*continued*)

CEA subgroups	CEA ≤ 10 ng/ml		*p*-value[1]	CEA > 10 ng/ml		*p*-value[1]	*p*-value[2]
Treatment	**FOLFIRI + bev**	**FOLFIRI + cet**		**FOLFIRI + bev**	**FOLFIRI + cet**		
Haemoglobin							
≤11 g/dl	4 (6.8%)	7 (11.7%)	0.53	28 (23.3%)	26 (22.2%)	0.88	**0.01**
>11 g/dl	55 (93.2%)	53 (88.3%)		92 (76.7%)	90 (76.9%)		
NA	0 (0%)	0 (0%)		1 (0.8%)	0 (0%)		
Platelets							
≤400 G/l	54 (91.5%)	54 (90%)	1.00	83 (69.2%)	87 (74.4%)	0.38	**0.0001**
>400 G/l	5 (8.5%)	6 (10%)		37 (30.8%)	29 (24.8%)		
NA	0 (0%)	0 (0%)		1 (0.8%)	0 (0%)		

CEA = carcinoembryonic antigen; bev = bevacizumab; cet = cetuximab; ECOG = Eastern Cooperative Oncology Group; LDH = lactate dehydrogenase; NA = not assessable.
Fisher exact test was used to compare proportions, and Mann–Whitney test was used to compare age between treatment arms (*p*-value[1]) and between subgroups CEA ≤ 10 ng/ml and >10 ng/ml (*p*-value[2]).

Table 2
Impact of baseline CEA serum level on survival and objective response per treatment arm.

Treatment	FOLFIRI + bevacizumab		FOLFIRI + cetuximab	
CEA subgroups (ng/ml)	**CEA ≤ 10**	**CEA > 10**	**CEA ≤ 10**	**CEA > 10**
N (patients)	59	120	60	117
Median OS, months (95% CI)	26.1 (23.6–43.7)	23.8 (20.8–28.0)	28.7 (23.8–51.3)	37.1 (24.5–40.9)
Hazard ratio (95% CI)	Reference	1.50 (1.02–2.19)	Reference	1.07 (0.71–1.62)
p-value (log-rank test)		**0.036**		0.74
Interaction test		$p = 0.28$		
Median PFS, months (95% CI)	10.7 (9.2–13.5)	10.4 (9.5–11.8)	10.0 (7.9–12.2)	11.5 (10.3–13.3)
Hazard ratio (95% CI)	Reference	1.15 (0.82–1.62)	Reference	0.99 (0.70–1.41)
p-value (log-rank test)		0.41		0.98
Interaction test		$p = 0.64$		
ORR	57.6% (34/59)	56.7% (68/120)	55.0% (33/60)	74.4% (87/117)
Odds ratio (95% CI)	Reference	0.96 (0.49–1.89)	Reference	2.36 (1.17–4.81)
p-value (Fisher's test)		1.0		**0.011**
Interaction test		$\mathbf{p = 0.05}$		
ETS rate	58.3% (28/48)	46.7% (49/105)	70.5 (31/44)	77.6% (76/98)
Odds ratio (95% CI)	Reference	0.57 (0.29–1.32)	Reference	1.41 (0.59–3.45)
p-value (Fisher test)		0.40		0.16
Interaction test		$p = 0.12$		
DpR, % reduction	28.36 (0–43.86)	27.68 (7.04–43.71)	32.28 (0–58.41)	43.75 (0–64.4)
p-value (Mann–Whitney *U* test)		0.98		0.25
Interaction test		$p = 0.64$		

CEA = carcinoembryonic antigen; OS = overall survival; CI = confidence interval; PFS = progression-free survival; ORR = objective response rate; ETS = early tumour shrinkage; DpR = depth of response.

linear relationship between CEA and OS (results not shown). Hence, the analyses were performed with CEA both as a dichotomous variable (≤10 and > 10 ng/ml) and a log-transformed continuous parameter.

3.4. Evaluation of baseline CEA as continuous parameter

As categorisation of a parameter implies loss of information, we also evaluated baseline CEA as log-transformed continuous variable. Comparable to its categorical evaluation, a differential prognostic impact of CEA between the treatment arms in FIRE-3 was also observed for the continuous evaluation (Supplementary Table S1). Baseline log CEA was inversely correlated with OS in bevacizumab-treated patients (HR = 1.1; $P = 0.014$), but not in the cetuximab arm (HR = 1.03; $P = 0.50$). However, higher CEA was associated with increased ORR in the cetuximab arm, albeit not reaching the level of significance (odds ratio [OR] = 1.13; $P = 0.11$). No significant impact of log CEA on ORR was observed in bevacizumab-treated patients (OR = 0.99; $P = 0.97$).

3.5. Evaluation of baseline CEA in a multivariate Cox regression analysis

To adjust for other potentially influencing baseline parameters and also to account for imbalances between the treatment arms in the CEA subgroups, we evaluated the differential prognostic relevance of log-transformed CEA

Table 3
Impact of baseline CEA serum level on treatment efficacy comparing FOLFIRI plus either bevacizumab (bev) or cetuximab (cet).

CEA subgroups (ng/ml)	**CEA ≤ 10**		**CEA > 10**	
Treatment	**FOLFIRI + bev**	**FOLFIRI + cet**	**FOLFIRI + bev**	**FOLFIRI + cet**
N (patients)	59	60	120	117
Median OS, months (95% CI)	26.1 (23.6–43.7)	28.7 (23.8–51.3)	23.8 (20.8–28.0)	37.1 (24.5–40.9)
Hazard ratio (95% CI)	Reference	0.78 (0.49–1.24)	Reference	0.56 (0.40–0.77)
p-value (log-rank test)	0.30			**< 0.001**
Interaction test			$p = 0.28$	
Median PFS, months (95% CI)	10.7 (9.2–13.5)	10.0 (7.9–12.2)	10.4 (9.5–11.8)	11.5 (10.3–13.3)
Hazard ratio (95% CI)	Reference	1.0 (0.67–1.48)	Reference	0.84 (0.64–1.1)
p-value (log-rank test)	1.0			0.21
Interaction test			$p = 0.64$	
ORR	57.6% (34/59)	55.0% (33/60)	56.7% (68/120)	74.4% (87/117)
Odds ratio (95% CI)	Reference	0.90 (0.41–1.97)	Reference	2.21 (1.24–4.00)
p-value (Fisher test)	0.85			**< 0.006**
Interaction test			$\mathbf{p = 0.05}$	
ETS rate	58.3% (28/48)	70.5% (31/44)	46.7 (49/105)	77.6% (76/98)
Odds ratio (95% CI)	Reference	1.69 (0.66–4.46)	Reference	3.92 (2.06–7.65)
p-value (Fisher test)	0.28			**< 0.0001**
Interaction test			$p = 0.12$	
DpR, % reduction	28.36 (0–43.86)	32.28 (0–58.41)	27.68 (7.04–43.71)	43.75 (0–64.4)
p-value (Mann–Whitney *U* test)	0.65			**0.006**
Interaction test			$p = 0.64$	

CEA = carcinoembryonic antigen; OS = overall survival; CI = confidence interval; PFS = progression-free survival; ORR = objective response rate; ETS = early tumour shrinkage; DpR = depth of response.

by using a multivariate Cox regression analysis for OS stratified by treatment (supplementary Fig. S4). Again, a clear prognostic relevance of baseline CEA was observed only in patients treated with FOLFIRI/Bev (HR = 1.2; $P < 0.001$). In patients treated with FOLFIRI/Cet, no significant impact on OS was evident (HR = 1.0; $P = 0.95$).

3.6. The predictive relevance of baseline CEA

With a differential prognostic relevance of baseline CEA in the two treatment arms of FIRE-3, we next examined whether it could predict treatment efficacy comparing FOLFIRI/Cet to FOLFIRI/Bev. To this end, the treatment effects in patients with CEA ≤10 and > 10 ng/ml were evaluated (Table 3 and Fig. 1).

In patients with CEA >10 ng/ml, treatment with FOLFIRI/Cet compared to FOLFIRI/Bev resulted in a significant improvement in median OS by 13.3 months (37.1 versus 23.8 months; HR = 0.56; $P < 0.001$). In contrast, increase in median OS was only 2.6 months in patients with CEA ≤10 ng/ml and this difference did not reach statistical significance (HR = 0.78; $P = 0.30$).

Additionally, in patients with CEA >10 ng/ml, treatment with FOLFIRI/Cet compared to FOLFIRI/Bev significantly increased the response rate resulting in an ORR of 74.4% versus 56.7% (OR = 2.21; $P < 0.006$). In contrast, no significant ORR benefit was exhibited when comparing the treatment arms in patients with CEA ≤10 ng/ml ($P = 0.85$). Furthermore, only patients with elevated baseline CEA receiving FOLFIRI/Cet showed superior ETS rates ($P < 0.0001$) and DpR ($P = 0.006$) compared to patients receiving FOLFIRI/Bev. No significant association of CEA with PFS was observed in any of the CEA subgroups. Evaluation of CEA as a continuous parameter yielded essentially comparable results for OS (HR = 1.07; $P = 0.023$) and ETS (OR = 3.01; $P < 0.0001$) (Supplementary Table S2).

To further characterise the predictive relevance of baseline CEA with regard to survival, we evaluated OS HRs and median OS for both treatment arms in populations defined as less than or equal to varying CEA thresholds (Fig. 2). With a decreasing median OS in the bevacizumab arm and a sigmoidal increase in the cetuximab arm along with increasing baseline levels of CEA, a significant treatment benefit of FOLFIRI/Cet compared to FOLFIRI/Bev was only evident above the threshold of 35.3 ng/ml ($P = 0.05$). The corresponding analysis was also performed for the outcome parameters PFS, ORR, ETS and DpR (Supplementary Fig. S5). These analyses indicate an increasing treatment benefit of FOLFIRI/Cet over FOLFIRI/Bev along with increasing baseline levels of CEA with regard to ORR, ETS and DpR.

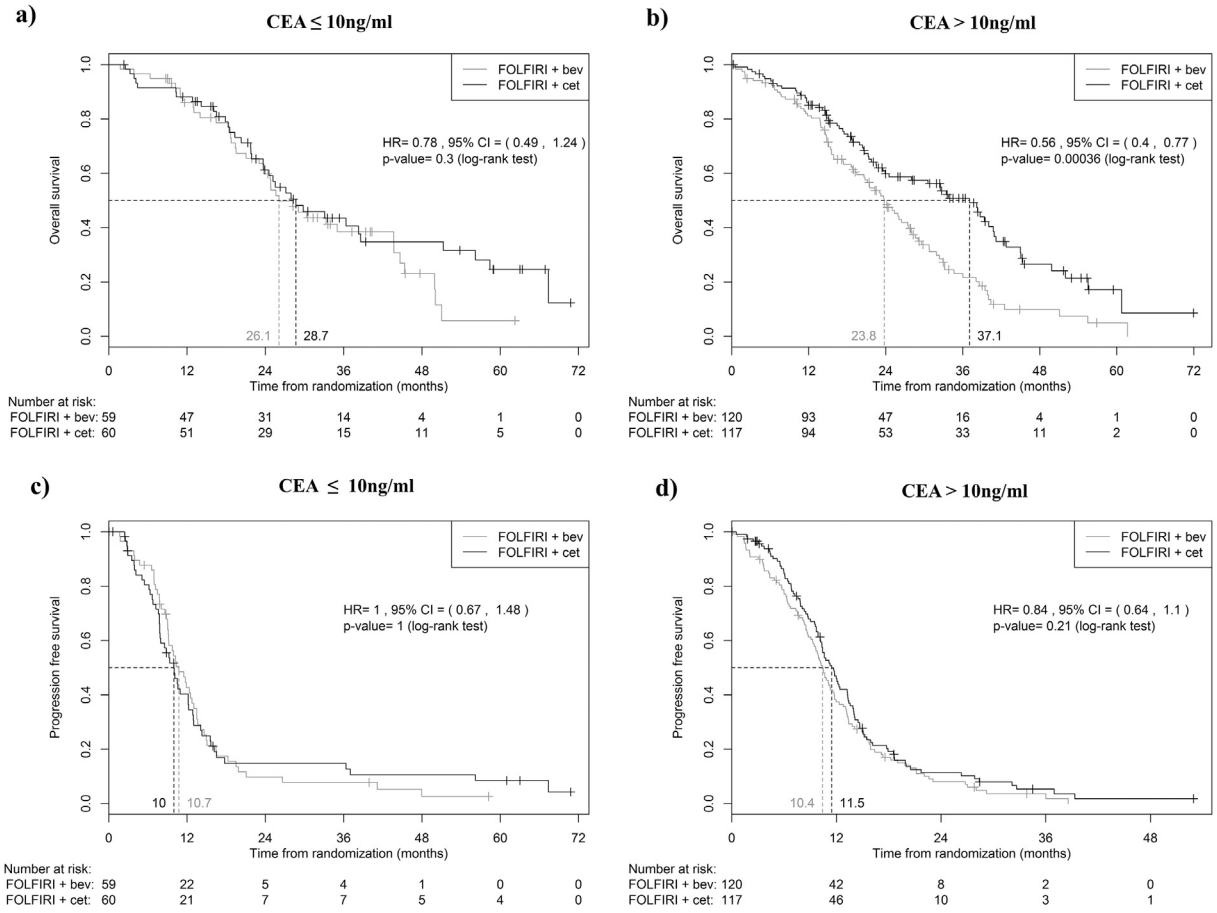

Fig. 1. Impact of baseline CEA serum level on treatment efficacy comparing FOLFIRI plus either bevacizumab or cetuximab. The Kaplan–Meier graphs of OS (upper row) and PFS (lower row) evaluate the impact of CEA baseline level ≤ 10 ng/ml (a and c) or > 10 ng/ml (b and d) on the treatment efficacy comparing FOLFIRI plus either bevacizumab (bev) or cetuximab (cet).

4. Discussion

We investigated the prognostic and predictive relevance of baseline CEA serum level in mCRC patients (*RAS*-WT) treated with first-line FOLFIRI/Cet or FOLFIRI/Bev in FIRE-3. An important finding of FIRE-3 was prolonged OS favouring FOLFIRI/Cet in the absence of significant differences in PFS and ORR [5,12]. The response-related outcome parameters ETS and DpR obtained by independent radiological review clearly related to the OS benefit conferred by FOLFIRI/Cet in patients with *RAS*-WT tumours [5].

Consistent with several reports indicating a prognostic role of baseline CEA in mCRC [14,22,23], we also observed that CEA baseline level was inversely correlated with OS in patients treated with FOLFIRI/Bev in FIRE-3. This association was, however, not evident in the cetuximab arm where median OS in patients with >10 ng/ml was even 8.4 months longer than in patients with CEA <10 ng/ml. Nevertheless, the corresponding survival curves (Fig. S3b) illustrate the HR of 1.07 as they overlap substantially. Considering CEA as continuous parameter, however, reveals a prolonged OS with increasing baseline levels of CEA following a sigmoidal correlation (Fig. 2b). The data are further supported by significantly higher ORR observed in patients with elevated (>10 ng/ml) versus lower (≤10 ng/ml) baseline CEA level treated with FOLFIRI/Cet. Baseline CEA showed no correlation with PFS. Formal interaction tests between CEA and treatment arm were non-significant with regard to OS. Regarding ORR however, the interaction test was significant for CEA subgroups ($P = 0.05$).

The findings in FIRE-3 need to be discussed in view of a previous report by Prager *et al.* [14]. In this retrospective cohort study, the authors observed that baseline CEA predicted PFS in patients receiving first-line treatment with bevacizumab, whereas this was not observed for cetuximab-based treatment in *KRAS* wild-type patients. In accordance with FIRE-3, also Prager *et al.* found a negative prognostic effect of elevated baseline CEA serum levels on OS.

Different findings between studies may, at least in part, be explained by the fact that treatment in the report by Prager consisted of various chemotherapy regimens combined with bevacizumab in molecularly

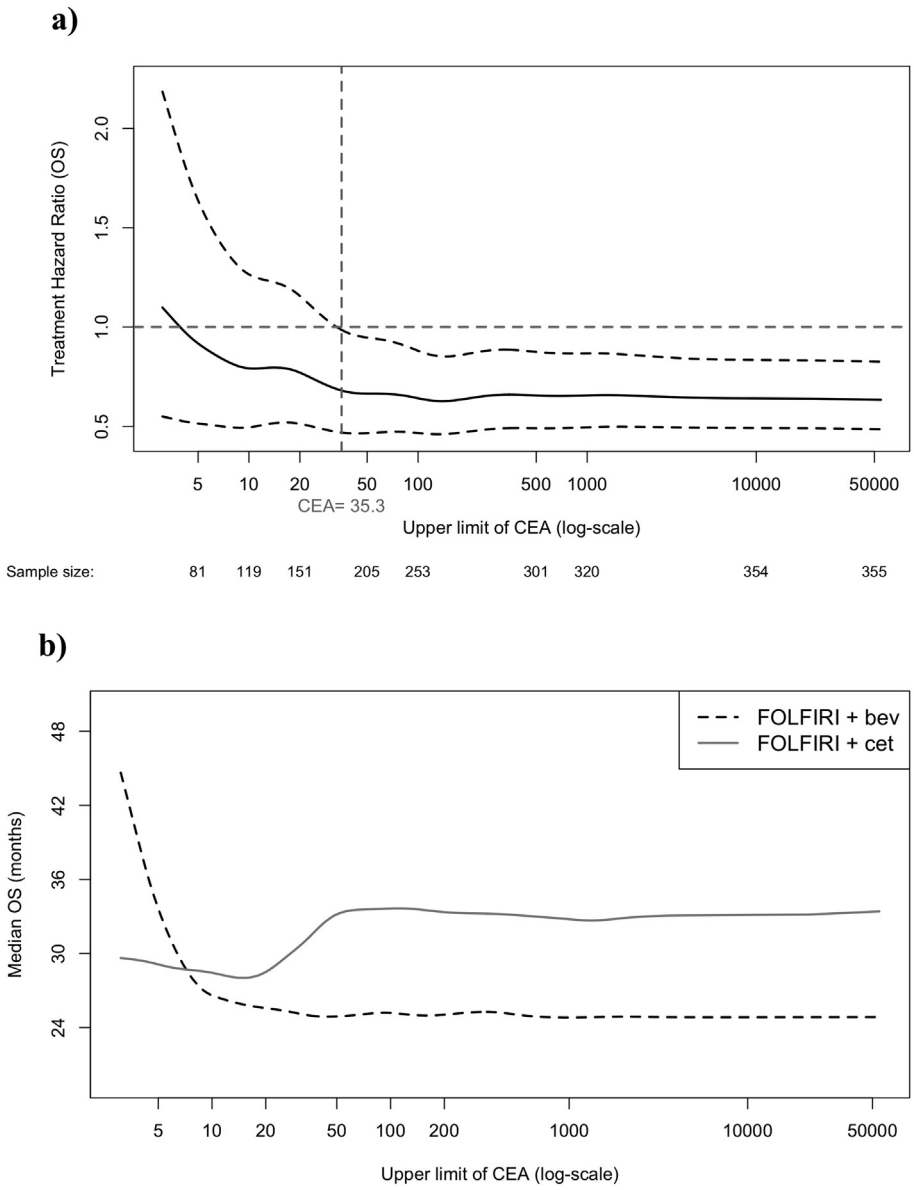

Fig. 2. Impact of different baseline CEA thresholds on treatment efficacy (OS) comparing FOLFIRI plus either bevacizumab or cetuximab. (a) OS HR and 95% CI (dashed lines) and (b) median OS for FOLFIRI plus either bevacizumab (bev) or cetuximab (cet) are graphed with smoothing for populations defined as less than or equal to a varying baseline CEA threshold (displayed in log-scale on *x*-axis). The first considered threshold is 3.08 ng/ml, with a corresponding sample size of 30 patients. The vertical line (in a) illustrates the CEA level, above which a significant difference in the efficacy of the two treatment arms with regard to OS is observed.

unselected patients and with cetuximab in *KRAS*-WT patients. Hence, our findings are the first to describe a differential prognostic relevance of baseline CEA for *RAS*-WT patients treated with first-line FOLFIRI/Cet or FOLFIRI/Bev in a prospective, randomised, phase III trial.

In line with the conclusions by Prager *et al.* [14], the findings of FIRE-3 suggest that elevated baseline CEA serum levels may be regarded as a negative predictor of survival in patients receiving first-line chemotherapy plus bevacizumab. A different result was obtained by the analysis of *RAS*-WT patients receiving FOLFIRI/Cet in FIRE-3. In cetuximab-treated patients, data support the hypothesis that elevated baseline CEA serum levels might constitute a positive predictor of survival and tumour response.

To further analyse the predictive relevance of baseline CEA, we evaluated its impact on treatment efficacy comparing FOLFIRI/Cet to FOLFIRI/Bev in FIRE-3. In patients from both CEA subgroups, an OS benefit was observed for treatment with FOLFIRI/Cet. However, this was markedly more pronounced in patients with CEA >10 ng/ml (HR = 0.56; $P < 0.001$) compared to CEA ≤10 ng/ml (HR = 0.78; $P = 0.30$). Furthermore, we found that ORR was significantly more favourable in patients with CEA >10 ng/ml receiving

treatment with FOLFIRI/Cet. Also, a significant benefit with regard to ETS and DpR was only evident in patients with elevated baseline CEA. In contrast, no significant difference for PFS between treatment arms was observed in both CEA subgroups. As ETS and DpR were supposed to function as more appropriate surrogate marker for OS than PFS, we think that this congruent finding with data from intention-to-treat and *RAS*-WT populations indicates that baseline CEA may easily identify a subgroup of patients who gain a higher benefit from first-line FOLFIRI/Cet compared to FOLFIRI/Bev [5,12]. Comparing populations with varying CEA thresholds, we found that a significant survival benefit of FOLFIRI/Cet over FOLFIRI/Bev was restricted to patients with baseline CEA of at least 35.3 ng/ml.

Our data nicely complement post hoc findings from the RAISE study [15]. This phase III trial compared second-line FOLFIRI plus placebo to FOLFIRI plus ramucirumab targeting the VEGF receptor-2. The authors report that the survival benefit of adding ramucirumab to FOLFIRI was more pronounced in patients with baseline CEA $\leq$10 compared to CEA >10 ng/ml (HR, 0.68 versus 0.90). A treatment-by-subgroup interaction was evident for OS ($P = 0.088$).

Several mechanisms might contribute to the described observations regarding the predictive relevance of CEA. Firstly, CEA appears to exert angiogenic properties independent of VEGF [14,20]. Hence, increased serum levels of CEA might counteract the inhibition of VEGF and its receptor [15]. Secondly, CEA correlates with tumour load [14,15,24]. In this scenario, an earlier and deeper response as achieved by anti-EGFR compared to anti-VEGF treatment when combined with chemotherapy might be more important with high tumour burden in *RAS*-WT patients [5]. To address this issue, an analysis of tumour burden in FIRE-3 is currently under way and will be reported elsewhere. Thirdly, evidence suggests that high serum levels of CEA may be indicative of aggressive tumour biology [22,25,26]. This could also affect treatment efficacy of targeted therapies. In line with findings of a previous report [27], patients in FIRE-3 with baseline CEA >10 ng/ml was associated with primarily adverse baseline characteristics such as increased lactate dehydrogenase or alkaline phosphatase [21].

Furthermore, these mechanisms might contribute to the differential prognostic relevance seen in the treatment arms of FIRE-3. In the FOLFIRI/Bev arm, the adverse prognostic tumour biology associated with increased CEA might lead to inferior survival, which cannot be compensated using an anti-VEGF antibody as CEA appears to exert angiogenic properties. In contrast, the prognostic relevance of the adverse tumour biology associated with increased CEA appears to recede into the background when patients receive FOLFIRI/Cet, probably due to the effective early tumour shrinkage of increased baseline tumour [5].

The relevance of the present analysis is certainly limited by its retrospective nature. Accordingly, imbalances between subgroups could not be avoided. Adjusting for the imbalances did not alter the differential prognostic relevance of baseline CEA, however. Furthermore, baseline CEA data were evaluable only for part of the study population, but from the majority of study patients (89.0%). However, sample size was too limited to address the relevance of CEA specifically in further patient subgroups, e.g. right-sided mCRC. Of note, the observed effect of CEA was independent of tumour sidedness as evaluated by multivariate Cox regression analysis. Taken together, the presented results are hypothesis generating and need to be validated in other clinical trials and in a prospective manner.

In conclusion, the presented analysis of FIRE-3 revealed a differential prognostic relevance of baseline CEA serum level between the treatment arms. Increased baseline CEA was associated with inferior survival only in patients receiving FOLFIRI/Bev, while this negative correlation was not observed in the FOLFIRI/Cet arm. Elevated baseline CEA appears to be a readily available biomarker that may predict greater benefit from first-line chemotherapy plus cetuximab as compared to bevacizumab. This finding may help to optimise the sequence of anti-VEGF and anti-EGFR application in patients with *RAS*-WT mCRC.

Conflict of interest statement

None declared.

Role of the funding source

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Disclosure

Julian W. Holch served on advisory board for Roche, has received honoraria from Roche and travel support from Novartis. Sebastian Stintzing has received honoraria from Merck, Roche, Amgen, Bayer and Sanofi-Aventis and has served on advisory boards for Merck Serono, Roche. Travel support: Roche, Merck Serono and Sanofi-Aventis. Alexander Kiani has received honoraria and travel support from Merck,

Roche and Amgen and has served on advisory boards for Amgen. Frank Kullmann has received honoraria (speakers engagements) from Roche. Markus Moehler has received honoraria from Merck KgaA, Pfizer, Roche, Baxalta, Amgen, Nordic BMS, Sanofi-Aventis and MSD and travel support from Merck KgaA, Amgen, Roche, BMS and MSD. Dominik P. Modest has received honoraria and advisory boards: Amgen, Merck, Roche, BMS, MSD, Servier, Pfizer, Sirtex. Travel support: Merck, Roche, BMS, Bayer and Amgen. C. Benedikt Westphalen served on advisory boards for Roche and Shire, has received honoraria from Celgene, Ipsen and Roche and has received travel support from Celgene, Halozyme, RedHill, Roche and Shire. Marlies Michl has received honoraria from Sirtex and has received travel support from Sirtex, Amgen and Merck. Volker Heinemann has received honoraria from Merck KgaA, Roche AG, Amgen, Sanofi, Sirtex and Baxalta and has received travel support from Merck KgaA, Roche AG, Amgen, Sirtex and Baxalta and has served on advisory boards for Merck KgaA, Roche AG, Amgen, Sanofi, Lilly, Sirtex, Böhringer Ingelheim, Baxalta, Taiho and Merrimack. The remaining authors declare no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2018.10.001.

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