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ORIGINAL RESEARCH

Lack of *KRAS*, *NRAS*, *BRAF* and *TP53* mutations improves outcome of elderly metastatic colorectal cancer patients treated with cetuximab, oxaliplatin and UFT

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Abstract There is conflicting evidence on the predictive role of *KRAS* status when cetuximab is added to oxaliplatin-based regimens. This study investigated the impact of *KRAS*, *NRAS*, *BRAF*, *PI3KCA* and *TP53* status on outcome of elderly metastatic colorectal cancer patients enrolled in TEGAFOX-E (cetuximab, oxaliplatin and oral uracil/ftorafur—UFT) phase II study. Twenty-eight patients were enrolled and all were evaluable for safety and activity. Twenty-three specimens were analysed for *KRAS*, *BRAF*, *NRAS*, *PI3KCA* and *TP53* mutational status by means of polymerase chain reaction and correlated with objective response, progression-free survival and overall survival. An evident increase of response rate was noted in *KRAS*/*NRAS* wild-type cases (70 versus 33 %, $P=0.198$). *KRAS*/*NRAS* wild-type status showed an independent association with a longer progression-free survival (44 versus 9 weeks, $P=0.009$). Considering the combined assessment of *BRAF*, *KRAS*/*NRAS* and *TP53*, a trend towards an increase of response rate was noted in patients without mutations (83 versus 33 %, $P=0.063$). Moreover, patients with all wild-type genes had significantly longer progression-free survival than patients with any mutation (48 versus 10 weeks, $P=0.007$). As a single biomarker, only *KRAS*/*NRAS* proteins maintained an independent value for outcome prediction. Patients with *KRAS*/*NRAS*, *BRAF* and *TP53* wild-type tumours could derive the maximal benefits from treatment with cetuximab, oxaliplatin and UFT.

Keywords Colorectal cancer · Cetuximab · Chemotherapy · Biomarkers · KRAS · BRAF · TP53

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Introduction

Colorectal cancer (CRC) is one of the commonest human malignant diseases and a leading cause of cancer-related deaths worldwide for both genders [1]. Over the past years, the treatment approaches to CRC in the adjuvant and palliative settings have seen dramatic changes, mainly driven by the availability of new combination therapies. These include not only conventional chemotherapeutics, such as irinotecan, oxaliplatin and the oral fluoropyrimidines capecitabine and uracil/ftorafur (UFT), but also new targeted therapies, such as the anti-angiogenic agent bevacizumab and the anti-epidermal growth factor receptor (EGFR) antibodies cetuximab and panitumumab [2, 3]. In

particular, the addition of cetuximab to irinotecan-based regimens has improved metastatic CRC (mCRC) patient outcome in terms of response rate and survival, in both chemorefractory disease and first-line setting [4–6]. The role of cetuximab in addition to oxaliplatin-containing regimens remains more controversial. Despite a minimal increase of response rate, the recent phase III MRC COIN trial failed to demonstrate an advantage in terms of progression-free survival (PFS) and overall survival (OS) from the addition of cetuximab to first-line oxaliplatin-based chemotherapy [7]. Notably, this unexpected absence of benefit was particularly relevant when cetuximab and oxaliplatin were combined with a fluoropyrimidine backbone including bolus 5-FU [8] and oral capecitabine [7]. This seems to be ascribable, at least for capecitabine combined with cetuximab, to the increase of gastrointestinal and skin toxicity that could lead to decreased dose intensity and consequent impairment of efficacy endpoint. However, when anti-EGFR agents were combined with infusional 5-FU and oxaliplatin (FOLFOX-4 regimen), the primary endpoints were significantly improved in the KRAS wild-type population, as evidenced by the randomised phase II OPUS study, the phase III PRIME study and the subgroup analysis of the MRC COIN trial [7, 9, 10].

Over the same frame of time, it became apparent that the efficacy of EGFR-targeted treatment is influenced by mechanisms of primary resistance. Several recent studies in mCRC patients have established that *KRAS* mutations are independently predictive of resistance to anti-EGFR antibodies when combined with irinotecan [11]. The response to this drug regimen is also negatively affected by *NRAS*, *BRAF* and *PI3KCA* mutations [12–14], as well as by a wild-type *TP53* [15], even if, possibly due to the heterogeneity of this neoplasm, some of *KRAS* wild-type patients failed to achieve a response and a little subgroup of patients hosting *KRAS* mutant disease achieved a prolonged stabilisation. The addition of cetuximab to capecitabine-based regimens may not improve treatment efficacy in patients with *KRAS* wild-type compared to *KRAS* mutant, as evidenced in the AIO KRK-0104 and the MRC COIN trials [7, 16], and the identification of predictive biomarkers to this combinations is still needed.

Furthermore, some phase III studies demonstrated that UFT, an oral third-generation fluoropyrimidine, is equivalent in efficacy and has a more favourable toxicity profile than bolus 5-FU [17]. In the light of these data, we previously conducted a randomised phase II study to evaluate the safety profile of UFT/leucovorin (LV) combined with irinotecan (TEGAFIRI) or oxaliplatin (TEGAFOX) in the first-line treatment of mCRC [18]. The results of this trial demonstrated that the two regimens obtained response rates comparable to the corresponding infusional fluoropyrimidines combinations and that TEGAFOX regimen showed a better tolerability and feasibility in older patients with age ≥65 years. In keeping with these findings, we consequently investigated the combination of TEGAFOX regimen with cetuximab among elderly mCRC patients in an open-label, multicentre, phase II trial of TEGAFOX-E (UFT/LV and oxaliplatin combined with cetuximab) regimen as first-line treatment of patients aged ≥70 years, with the aims to evaluate the safety profile of this drug combination, as well as the therapeutic efficacy in terms of response rate, duration of response, time to progression and OS, and to explore the impact of *KRAS*/*NRAS*, *BRAF*, *PI3KCA* and *TP53* mutational status reviewed retrospectively on surgical tumour specimen. The results about the biological study are here reported.

Materials and methods

Patients and study design

Elderly patients, aged ≥70 years old, previously untreated for advanced/metastatic adenocarcinoma of the colon or rectum, were eligible for this study. Adjuvant chemotherapy, if administered, was to be completed at least 6 months before enrollment in the study. Histological confirmation of colorectal adenocarcinoma and the presence of at least one unidimensionally measurable lesion was requested. The patients had to be ≥70 years of age, with ECOG performance status 0–1. Other eligibility criteria were constituted by adequate bone marrow, liver and renal functions. The study was conducted according to the Good Clinical Practices and Declaration of Helsinki. Written informed consent for the treatment and for biologic tumour evaluation was required. The study and all current amendments were approved by the ethics committees of all of the participating centres. Patients were not included if they had a history of other cancer except cured basal cell carcinoma of the skin and carcinoma in situ of the uterine cervix, or if they had not fully recovered from recent, major surgery (within 4 weeks). Other exclusion criteria were presence of organ allograft, central nervous system involvement or neurological or psychiatric disorders that could interfere with treatment compliance, severe cardiac disease or a myocardial infarction within the previous 12 months, uncontrolled metabolic disorders or active serious infections, inflammatory bowel disease, bowel obstruction or history of chronic diarrhoea and malabsorption syndrome. Patients were also excluded from the study if they had active neuropathy or previous fluoropyrimidines toxicity. Therapy consisted of UFT (250 mg/m^2 day) and LV (45 mg total dose daily), given for 14 days, combined with a 3-h infusion of oxaliplatin (120 mg/m^2 on day 1) and cetuximab (loading dose 400 mg/m^2, then 250 mg/m^2 weekly). The total daily UFT dose was divided to be given every 8 h, and if the dose could not be equally divided, the greatest dose was administered in the morning. The treatment was given for a maximum of six cycles in the presence of disease stabilisation or eight cycles in case of objective responses;

subsequently, in case of disease control, cetuximab could be continued as maintenance for a maximum of 1 year. The therapy was interrupted for progressive disease, unacceptable toxicity or consent withdrawal. Clinical response was assessed every 9 weeks with radiological examination (computerised tomodensitometry or magnetic resonance imaging). The Response Evaluation Criteria in Solid Tumors were adopted for evaluation, and objective tumour response was classified into complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) [19]. Patients with SD or PD were defined as non-responders. Response to therapy was also evaluated retrospectively by independent radiologists.

Molecular analysis

Formalin-fixed paraffin-embedded tumour tissues were reviewed for quality and tumour content. A tissue containing at least 80 % of neoplastic cells was selected for each case. Microscopic dissection of 7 μm methylene blue-stained sections allowed the separation of neoplastic and normal cells. Genomic DNA was extracted using the Qiamp FFPE DNA kit (Qiagen, Chatsworth, CA, USA) following the manufacturer's instructions. Mutational analysis of *KRAS* exons 2 and 3 was performed as previously described [20]. *KRAS* exon 2 status was further confirmed through a specific mutant enriched PCR, known to be a more sensitive approach [21]. The *KRAS* coding sequence of exon 4 was amplified using the following primers: sense 5′-TTGTGGACAGGTTTTGAAAGA-3′ and antisense 5′-TTGCAGAAAACAGATCTGTATTT-3′ with an annealing temperature of 58 °C.

BRAF (exon 15), *NRAS* (exon 2), *PI3KCA* (exons 9 and 20) and *TP53* (exons 5 to 8) mutational analysis was performed by means of PCR using specific primers previously described [20, 22, 23]. The PCR products were subjected to direct sequencing using an ABI Prism 3500 DX Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and then evaluated by means of the ChromasPro software.

Statistical analysis

The primary study endpoint was the proportion of patients who responded to TEGAFOX-E regimen (CR+PR). The secondary study endpoints were OS and PFS. Patients who received at least three cycles of chemotherapy were evaluated for response. Regarding the biomarker analyses, the associations between *KRAS*/*NRAS* and *TP53* status with dichotomous parameters were evaluated using the Fisher's exact test. PFS and OS rates were calculated by the method of Kaplan–Meier from the date of enrollment to clinical events [24]. The univariate Cox proportional hazards model was applied to assess the effect of covariates on PFS and OS from the first day of TEGAFOX-E treatment.

Results

Patients' characteristics

Between October 2008 and July 2010, 28 patients were enrolled by five Italian institutions. Tumour tissue was available for 26 patients who provided a specific written consent for biological analyses. We successfully analysed 23 formalin-fixed paraffin-embedded surgical specimens or biopsies of the primary tumour; the surgical material from three patients was not evaluable because of its exiguity. Table 1 shows the main demographic and baseline characteristics of the 23 patients included in this study. Most patients had received no prior adjuvant therapy. At the time of final analysis on June 2012, all patients were dead.

Antitumour activity

Overall, in all 28 patients enrolled in the clinical study, one CR and 12 PR were observed for an objective response rate of 47 % (13/28 patients), and median duration of response was 41 weeks (range, 10–99 weeks); only 10 % (3/28) presented with SD, while 43 % (12/28) with PD. The median PFS was 23 weeks and the median OS was 52 weeks.

Safety evaluation

The percentage of patients who experienced at least one adverse event was 89 % (grades 3–4, 11 %). The most common grade 3 to 4 toxicities were diarrhoea and acneiform rash and only four patients interrupted treatment for side effects. Table 2 depicts the frequency of the reported adverse events.

Correlation between gene status as a single biomarker evaluation and response rate and outcome

The results of mutational analysis of the 23 evaluable patients are detailed in Table 3. *BRAF* mutation (V600E) was detected in 1 of 20 (5 %) cases. *KRAS* mutations involving codons 12 (eight cases) and 13 (three cases) were found in 11 of 23 (48 %) cases. No mutation was detected in exons 3 and 4 of the *KRAS* gene except for the new nonsense mutation at residue Q43. The G12D *NRAS* mutation was observed in one *KRAS* wild-type non-responder patient (4 %). Since it is well known that *BRAF* mutation is associated with poorer survival in mCRC both in terms of overall prognosis [25, 26] and possible prediction of cetuximab efficacy [13], the unique *BRAF* mutated case was excluded from the analyses regarding the predictive and prognostic role of the single biomarkers (*RAS* or *TP53*). Comparing *KRAS*/*NRAS* wild-type and mutated tumours, an increase of response rate in KRAS/NRAS wild-type cases was noted (70 versus 33 %), although this difference did not reach statistical significance

Table 1 Patient demographics, disease characteristics and prior therapy at baseline

	Patients (total 23) *N* (%)
Gender	
Male	13
Female	10
Median age (range), years	77 (70–87)
ECOG performance status	
0	15 (65)
1	8 (35)
Primary tumour site	
Colon	12 (52)
Rectum	11 (48)
No. of metastatic sites	
1	14 (61)
2	9 (39)
Onset of metastases	
Synchronous	15 (65)
Metachronous	8 (35)
Prior adjuvant therapy	
Yes	5 (22)
No	18 (78)

ECOG Eastern Cooperative Oncology Group

(*P*=0.19). On the contrary, patients with *KRAS*/*NRAS* wild-type tumours had significantly longer median PFS (44 versus 9 weeks, *P*=0.003 by log-rank test; *P*=0.009 by Wilcoxon's test; HR=4.68 [95 % CI, 1.65–13.27], Fig. 1a). This outcome was similar when considering *KRAS* mutated versus wild-type tumours (data not shown). In the *KAS* mutant group, 2 out of 11 patients discontinued treatment after only one cycle for oxaliplatin allergic reaction and worsening clinical condition. Carriers of *KRAS*/*NRAS* wild-type gene did not show a significantly different OS compared to carriers of mutation (76 versus 54 weeks, *P*=0.478 by log-rank test; HR=1.47 [95 % CI, 0.5–4.31]).

The results of *PI3KCA* mutational analysis are detailed in Table 3. *PI3KCA* activating mutations involving exon 9 (E542K and Q546R in three cases) and exon 20 (H1047R and T1025A in three cases) occurred in 6 of 21 (28 %) cases. All but one *PI3KCA* mutations were coupled with *KRAS* mutations: 5 of 11 (45 %) in *KRAS* mutants versus 1 of 10 (10 %) in *KRAS* wild type. This association was particularly true for *PI3KCA* exon 9 mutations (3/11 in KRAS mutants versus 0/10 in *KRAS* wild type), in keeping with the literature [12]. The median PFS (10 weeks) and OS (45 weeks) of five patients with tumour showing both *KRAS*/*NRAS* and *PI3KCA* mutation was similar to the median PFS (9 weeks) and OS (52 weeks) of sic patients showing both *KRAS* mutant and *PI3KCA* wild-type tumour. Thus, neither *PI3KCA* exon 9 nor exon 20 mutations had a significant additional effect on PFS and OS among *KRAS*/*NRAS* mutant patients. Unexpectedly, the unique *KRAS* wild-type case harbouring the activating H1047R *PI3KCA* exon 20 mutation, previously reported to be associated with resistance to cetuximab [12, 14], in our series resulted to be s responder.

The results of *TP53* mutational analysis are detailed in Table 3. *TP53* mutations, including one non-in-frame deletion of nucleotides 12711 and 12712 (involving the codons 214 and 215), and four missense mutations classified as non-functional [27] were found in 5 of 21 (24 %). No statistically significant differences were noted between *TP53* wild type and mutated tumours in terms of response rate (53 versus 40 %, *P*=1.0), median PFS (34 versus 11 weeks, *P*=0.764) (Fig. 1b) or OS (54 versus 41 weeks, *P*=0.961). Overall, our results based on a single biomarker evaluation (*KRAS*/*NRAS* or *TP53*) suggest that only *RAS* proteins maintained a significant value for outcome prediction in patients treated with TEGAFOX-E regimen.

Correlation between combined BRAF/KRAS/NRAS/TP53 gene status and response rate and outcome

On the basis of the combined assessment of *BRAF*, *KRAS*/*NRAS* and *TP53* status, all 21 evaluable samples were molecularly classified in two categories: the first group (group of patients with any mutation) comprised 15 patients with *BRAF*, *KRAS*/*NRAS* and/or *TP53* mutations; the second group (group of patients without mutations) was constituted by six patients with wild-type status for *BRAF*, *KRAS*/*NRAS* and *TP53*. There was a trend towards an increase of response rate in patients without mutations as compared to patients with *BRAF*, *KRAS*/*NRAS* and/or *TP53* mutations (83 versus 33 %, *P*=0.063). Moreover, patients with any mutation had significantly shorter PFS (median, 10 weeks) than patients without mutations (median,

Table 2 Adverse events in all 28 patients enrolled in the TEGAFOX-E study

	No. of grade 1–2 adverse events (%)	No. of grade ≥3 adverse events (%)
Diarrhoea	10 (36)	2 (7)
Nausea/vomiting	4 (14)	–
Rash	16 (57)	2 (7)
Fatigue	2 (7)	–
Deep venous thrombosis	1 (3.5)	–
Neurotoxicity	4 (14)	–
Anaemia	1 (3.5)	–
Thrombocytopenia	3 (11)	–
Neutropenia	1 (3.5)	–
Mucositis	1 (3.5)	–

Table 3 BRAF, KRAS, NRAS and TP53 mutational analysis

Case	Response	BRAF	KRAS	NRAS	PI3KCA	TP53
1	PR	WT	G12V	WT	H1047R	WT
2	PR	WT	WT	WT	WT	E285K
3	PR	WT	WT	WT	H1047R	WT
4	PR	WT	WT	WT	WT	WT
5	PR	WT	G12D	WT	E542K	WT
6	PR	WT	WT	WT	WT	R273H
7	PR	WT	G13D	WT	WT	WT
8	PR	WT	WT	WT	WT	WT
9	PR	N.E.	G12S	WT	N.E.	N.E.
10	PR	N.E.	WT	N.E.	N.E.	WT
11	CR	WT	WT	WT	WT	WT
12	SD	WT	G12D	WT	WT	Δ12711–12712 nucleotides
13	SD	WT	WT	WT	WT	WT
14	SD	WT	G13D	WT	E542K	WT
15	PD	WT	G12D	WT	WT	WT
16	PD	WT	G12D	WT	Q546R	R175H
17	PD	WT	G13D	WT	WT	WT
18	PD	N.E.	WT	WT	WT	N.E.
19	PD	WT	WT	WT	WT	R213L
20	PD	WT	WT	G12D	WT	WT
21	PD	V600E	WT	WT	WT	WT
22	PD	WT	G12V	WT	T1025A	WT
23	PD	WT	G12V	WT	WT	WT
Total		5 %	48 %	4 %	28 %	24 %

PR partial response, *CR* complete response, *SD* stable disease, *PD* progression disease, *WT* wild type

48 weeks; P=0.043 by log-rank test; P=0.007 by Wilcoxon's test; HR=2.65 [95 % CI, 1.02–6.86], Fig. 2). Nevertheless, OS was not significantly different for patients with any mutation (median, 51 weeks) than for patients with wild-type status (median, 86 weeks; P=0.376 by log-rank test; HR=1.54 [0.58–4.06]).

Discussion

This study investigated the impact of *KRAS*, *NRAS*, *BRAF*, *PI3KCA* and *TP53* gene status on response rate and outcome of the combination of cetuximab, oxaliplatin and UFT/LV among elderly mCRC patients enrolled in the TEGAFOX-E phase II trial. The results contribute to the knowledge of some major aspects.

The TEGAFOX-E regimen induced a response rate of 48 % when the overall population was considered and a median PFS and OS of 23 and 52 weeks, respectively. Importantly, our results were obtained from a prospectively enrolled, but biomolecularly unselected elderly patient population; nowadays, *KRAS* mutational analysis is recommended in the clinical practice prior to cetuximab administration, and routinary patient selection will not allow to obtain future cohorts similar to our study population. Concerning our mutational analysis, *KRAS*/*NRAS* wild-type status was evidenced as the most important predictor of efficacy in terms of longer PFS (44 versus 9 weeks, P=0.009). An evident increase of response rate was observed in *KRAS*/*NRAS* wild-type patients (70 versus 33 %), which however did not reveal statistical power possibly due to the limited sample size (P=0.19). *KRAS* and *NRAS* genes were lumped together in the analysis of endpoints due to the similar prognostic effect reported in the MRC COIN trial [7]. However, it was well established that multiple mutation testing can improve patient selection and treatment outcome: KRAS/NRAS/BRAF/exon20PI3KCA wild-type patients with chemorefractory disease responded to cetuximab with higher rates as compared to the ones with KRAS wild type and mutations of other downstream genes [12]. Thus, we widened the mutational status analysis to *TP53* and *BRAF* and found out that combined wild-type status for *KRAS*/*NRAS*, *BRAF* and *TP53* was significantly associated with a longer PFS (48 versus 10 weeks, P=0.009) and that it might predict more accurately treatment responses (83 versus 33 %, P=0.063). Our results, suggesting a predominant impact of *KRAS* mutation on the lack of response and PFS benefit from anti-EGFR agents plus oxaliplatin-based regimens, are in

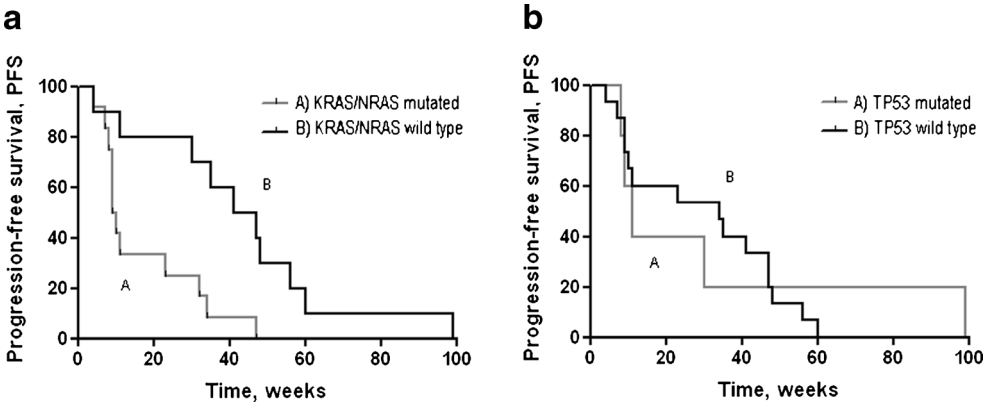

Fig. 1 Effect of *KRAS*/*NRAS* and *TP53* mutational status on progression-free survival (PFS). Kaplan–Meier graphs showing the effect of **a** mutated versus wild-type *KRAS*/*NRAS*; **b** mutated versus wild-type *TP53*

keeping with the randomised phase II study demonstrating the activity of cetuximab plus FOLFOX-4 and with the randomised phase III trial showing efficacy of panitumumab added to the same regimen in *KRAS* wild-type mCRC [9, 10]. Interestingly, the predictive value of *KRAS* in terms of PFS and OS was not confirmed in recently published data of the phase III MRC COIN trial, which failed to show any significant improvement of outcome from the addition of cetuximab to oxaliplatin-based regimens. In the subgroup analysis, these results were confirmed when the fluoropyrimidine backbone was constituted by capecitabine, while in the FOLFOX-4 cohort, the benefit of cetuximab was still demonstrable in *KRAS* wild-type patients [7].

Of note, preclinical evidence demonstrated that *KRAS* mutation, coupled with a wild-type *TP53*, increases the sensitivity of CRC cell lines to oxaliplatin [28–30]. The significance of *TP53* as a biomarker of chemotherapy outcome in mCRC is controversial [31], although *TP53* mutations are thought to confer treatment resistance, particularly to DNA-damaging agents such as platinum derivatives [32, 33]. The predictive value of *TP53* in terms of efficacy of anti-EGFR antibodies has been explored less extensively. In a previous retrospective study of 64 chemorefractory mCRC patients treated with cetuximab and irinotecan-based regimens, it has been reported that disease control and time to progression were significantly increased in *KRAS* wild-type patients with *TP53* mutation [15]. In a larger data set of 100 *KRAS* and *BRAF* wild type, irinotecan-refractory patients treated with cetuximab-based regimens, PFS was significantly longer in patients harbouring *TP53* mutations [34]. This is the only first-line study focussing on the potential prognostic role of *TP53* in mCRC patients treated with an oxaliplatin-based regimen plus cetuximab. Moreover, patients with silent *TP53* mutation were aprioristically considered as wild type, and different from previous analyses, all *TP53* mutations in our data set resulted as non-functional [25]. In fact, inactivation of p53 function is the most important factor in the spectrum of *TP53* mutation and that sequence-specific transactivation is the critical function in p53-dependent tumour suppression. Our retrospective analysis of the TEGAFOX-E study showed that response rate, PFS and OS were not influenced by *TP53* mutational status when considered as a single biomarker. However, patient selection based not only on *KRAS/NRAS* status, but also on *TP53* and *BRAF* status, could identify a subgroup of wild-type subjects who most likely will benefit from the combination of cetuximab, oxaliplatin and oral fluoropyrimidines. This finding was confirmed by Wilcoxon test demonstrating that the addition of *BRAF* and *TP53* to *KRAS*/*NRAS* genes status had a significant statistical power for the identification of patients most likely to gain a PFS advantage from TEGAFOX-E treatment. Regarding *PI3KCA*, the mutations resulted to be mostly associated with *KRAS* mutations in keeping with the literature [12] and did not have an independent effect on PFS and OS of our study patients. Although the predictive value of exon 20 *PI3KCA* mutations is greater than that of exon 9 mutations [14], in this report, the separate analysis of *PI3KCA* mutation subtypes was considered futile due to association with *KRAS* mutation in all but one case.

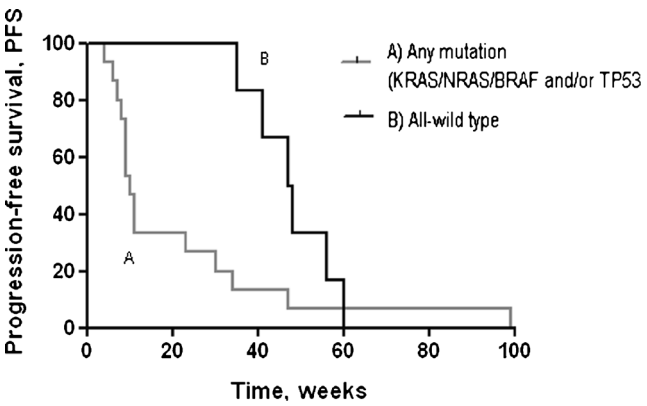

Fig. 2 Effect of *BRAF*/*KRAS*/*NRAS* and *TP53* mutational status on progression-free survival (PFS). Kaplan–Meier graphs showing the effect of any mutation (*BRAF*/*KRAS*/*NRAS* and/or *TP53*) versus all wild-type genes

Capecitabine is not currently considered as an optimal chemotherapy backbone for oxaliplatin and cetuximab-based combinations, even in KRAS wild-type CRC patients [7, 16]. We hypothesised that the administration of the well-tolerated UFT/LV could improve treatment feasibility and allow the maintenance of an adequate dose intensity. In our study, the relatively short median PFS observed in patients with *KRAS*/*NRAS* mutation might be explained by the toxicity-driven reduction of dose intensity of the triplet combination in an elderly population unresponsive to anti-EGFR treatment. Retrospective data suggest that, in *KRAS*-mutated CRC, the addition of cetuximab to an oxaliplatin-containing regimen may be detrimental [9, 10]. The combination of UFT/LV with oxaliplatin and cetuximab determined a significantly inferior outcome in terms of PFS when compared to the standard FOLFOX plus cetuximab regimen, particularly regarding the *KRAS*-mutated subgroup [35].

The main limitation of this study is the small sample size and the lack of a control group, which leaves open the possibility that multiple oncogenic mutations may be a prognostic factor rather than a predictive one. However, the encouraging median PFS observed in patients with *KRAS*/*NRAS*, *BRAF* and *TP53* wild-type tumours suggested the possibility of a better prognosis of all wild-type mCRC elderly patients treated with the TEGAFOX-E regimen. Due to the small data set and the possible bias derived from the mixed prognostic–predictive value of multiple mutations, carrying out a multivariate analysis was judged as not accurate by our statisticians. Our data may prompt both retrospective validation on larger trials and proposal for future studies investigating the role of the considered biomarkers on the efficacy of infusional 5-fluorouracil, oxaliplatin and anti-EGFR antibodies combinations.

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