



# Efficacy of Regorafenib and Trifluridine/Tipiracil According to Extended *RAS* Evaluation in Advanced Metastatic Colorectal Cancer Patients: A Multicenter Retrospective Analysis

Michele Basso · Carlo Signorelli · Maria Alessandra Calegari · Jessica Lucchetti · Ina Valeria Zurlo · Emanuela Dell'Aquila, et al. [full author details at the end of the article]

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## Abstract

**Background** There are few molecular markers driving treatment selection in later lines of treatment for advanced colorectal cancer patients. The vast majority of patients who progress after first- and second-line therapy undergo chemotherapy regardless of molecular data.

**Objective** We aimed to assess the prognostic and predictive effects of specific *RAS* mutations on overall survival of patients receiving regorafenib (rego), trifluridine/tipiracil (TFD/TPI), or both.

**Patients and methods** This was a retrospective observational study based on data from a previous study of our research network, involving nine Italian institutions over a 10-year timeframe (2012–2022). Extended *RAS* analysis, involving *KRAS* exon 2–4 and *NRAS* exon 2–4, and *BRAF* were the main criteria for inclusion in this retrospective evaluation. Patients with *BRAF* mutation were excluded. Patients were classified according to treatment (rego- or TFD/TPI-treated) and *RAS* mutational status (wild-type [WT], *KRAS* codon 12 mutations, *KRAS* codon 13 mutations, *KRAS* rare mutations and *NRAS* mutations, *KRAS* G12C mutation and *KRAS* G12D mutation).

**Results** Overall, 582 patients were included in the present analysis. Overall survival did not significantly differ in rego-treated patients according to *RAS* extended analysis, although a trend toward a better median survival in patients carrying G12D mutation (12.0 months), Codon 13 mutation (8.0 months), and Codon 12 mutation (7.0 months) has been observed, when compared with WT patients (6.0 months). Overall survival did not significantly differ in TFD/TPI-treated patients according to *RAS* extended analysis, although a trend toward a better median survival in WT patients had been observed (9.0 months) in comparison with the entire population (7.0 months). Patients receiving both drugs displayed a longer survival when compared with the population of patients receiving rego alone ( $p = 0.005$ ) as well as the population receiving TFD/TPI alone ( $p < 0.001$ ), suggesting a group enriched for favorable prognostic factors. However, when each group was analyzed separately, the addition of TFD/TPI therapy to the rego-treated group improved survival only in all-*RAS* WT patients ( $p = 0.003$ ). Differently, the addition of rego therapy to TFD/TPI-treated patients significantly improved OS in the Codon 12 group ( $p = 0.0004$ ), G12D group ( $p = 0.003$ ), and the rare mutations group ( $p = 0.02$ ), in addition to all-*RAS* WT patients ( $p = 0.002$ ). The rego-TFD/TPI sequence, compared with the reverse sequence, significantly improved OS only in the *KRAS* codon 12 group ( $p = 0.003$ ).

**Conclusions** Our data demonstrate that *RAS* mutations do not affect outcome in rego-treated patients as well as TFD/TPI-treated patients. Nevertheless, a trend toward a higher efficacy of rego in *RAS*-mutated (in particular codon 12, rare *RAS* mutations, and G12D) patients has been recorded. The rego-TFD/TPI sequence seems to be superior to the reverse sequence in patients carrying an *RAS* codon 12 mutation, although the impact of other factors as disease burden or performance status cannot be excluded.

## Key Points

There are few molecular markers driving treatment selection in later lines of advanced colorectal cancer patients, despite a growing commitment toward molecular characterization aimed at treatment personalization.

Regorafenib, trifluridine/tipiracil, fruquintinib, and the combination of trifluridine/tipiracil (TFD/TPI) with bevacizumab demonstrated effectiveness in third-/fourth-line of therapy in randomized, phase III studies, irrespective of molecular characterization.

The vast majority of patients progressing after first- and second-line therapy undergo chemotherapy regardless of molecular data.

We aimed to assess the prognostic and predictive effects of specific RAS mutations on overall survival of patients receiving regorafenib, trifluridine/tipiracil, or both.

## 1 Introduction

Despite a growing commitment toward precision medicine, there are few molecular markers driving treatment selection in later lines of treatment for advanced colorectal cancer (CRC) patients [1]. A small percentage of patients (6–7%) who carry a clinically actionable *BRAFV600E* mutation are eligible for targeted therapy with a combination of encorafenib and cetuximab [2]. Patients with microsatellite instable tumors (about 5%) are eligible for immune checkpoint inhibitors [3, 4]. Moreover, small molecule inhibitors (sotorasib and adagrasib) showed activity in *KRAS* G12C-mutated CRCs (3–4%), in monotherapy or in combination with anti-epidermal growth factor receptor (EGFR) agents [5, 6]. Regorafenib (rego), trifluridine/tipiracil (TFD/TPI), fruquintinib, and the combination of TFD/TPI with bevacizumab demonstrated effectiveness in third-/fourth-line of therapy in randomized, phase III studies [7–10], irrespective of molecular characteristics. Therefore, the vast majority of patients with an advanced CRC progressing after first- and second-line therapy will be treated regardless of molecular data.

In Italy, as in many Western Countries, the combination of TFD/TPI and bevacizumab and fruquintinib are not yet available in clinical practice. For this reason, most patients receive

rego or TFD/TPI based on physician's judgment. Many studies attempted to demonstrate the superiority of one drug over the other [11–15], but no significant differences have been documented in terms of efficacy. On the other hand, the two drugs differ in their adverse effects. Rego displays more symptomatic adverse events such as asthenia, hand-foot syndrome, and skin reactions, while TFD/TPI-related adverse effects are generally more asymptomatic (neutropenia, anemia) [16]. Therefore, TFD/TPI is more often prescribed in older patients or in patients with worse performance status [16, 17].

A previous publication from our research network [17] aimed to assess the outcomes in a large retrospective series of metastatic CRC (mCRC) patients treated with rego, TFD/TPI, or both, in late lines. Even in our study, overall survival (OS) displayed no significant differences between patients receiving rego or TFD/TPI. A subgroup analysis in patients treated with the rego-TFD/TPI sequence showed a significantly higher OS when compared with patients receiving the reverse sequence. At multivariate analysis, better performance status, male sex, lower disease burden (liver metastases only) and wild-type (WT) *RAS* status were the factors affecting OS.

Recently, *KRAS* G12 mutations have been reported to be associated with reduced OS in patients with mCRC treated with TFD/TPI [18]. This evidence was obtained in a discovery cohort and replicated in a large real-world cohort and in a retrospective analysis of RECURSE patients [7].

Unfortunately, there are no studies properly aimed at assessing the efficacy of rego according to *RAS* mutational status. The *RAS* gene is the most commonly mutated oncogene in CRC, occurs in 45% of cases, and its mutations result in a constitutive activation of *RAS* protein [1, 8]. Rego is a multikinase inhibitor that blocks the activity of several protein kinases, including those involved in the downstream signaling cascade of EGFR.

Thus, the current analysis was mainly designed to assess the prognostic and predictive effects of specific *RAS* mutations on patients with refractory mCRC receiving rego, TFD/TPI, or both, hypothesizing that rego could work better in patients carrying *RAS* mutation(s). This kind of information could help physicians to select treatments in later lines, based on efficacy data in addition to the pattern of adverse effects.

## 2 Patients and Methods

The present analyses are based on data from a previous study of our research network, which was presented in a recent publication [17]. Nine Italian institutions contributed to this 10-year (2012–2022) retrospective observational study. The study was approved by the Ethics Committee (2022-no.1021/

CE Lazio 1) and was carried out in conformity with the Declaration of Helsinki. To protect sensitive information, all data were anonymized and patients were identified only by their initials and a number. In accordance with the law, the lead investigator served as the data manager and had access to the complete database.

## 2.1 Patients

Criteria for study inclusion have been extensively described in the former publication [17]. Extended *RAS* analysis, involving *KRAS* exon 2–4 and *NRAS* exon 2–4, and *BRAF* was the main criteria for inclusion in this retrospective evaluation. Patients with *BRAF* mutation were excluded. If data about codon mutation were available, even in the absence of specific information concerning amino acid substitution, patients were considered eligible. Patients were considered WT if no mutation involving *KRAS* exon 2–4, *NRAS* exon 2–4, and *BRAF* was detected. Briefly, inclusion criteria were histologically confirmed stage IV adenocarcinoma of the colon or rectum with unresectable metastatic disease; age >18 years; Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2; progression to at least two prior regimens of standard chemotherapy using fluoropyrimidine, irinotecan, oxaliplatin, anti-vascular endothelial growth factor (VEGF) antibodies (bevacizumab and aflibercept), or anti-EGFR antibodies (cetuximab or panitumumab); known *RAS* mutation status; adequate organ function at the start of treatment.

## 2.2 Treatment Details

Study details have been extensively described in the former publication [17]. Briefly, TFD/TPI was administered orally twice daily at a dose of 35 mg/m<sup>2</sup> on days 1–5 and 8–12 of a 28-day cycle [7], and rego was administered at a standard dose of 160 mg once daily for 21 days of a 28-day cycle. The ReDos dose-escalation strategy of rego (starting dose 80 mg/day orally with weekly escalation, per 40 mg increment, to 160 mg/day, permitted if there were no significant drug-related adverse events) was used according to the physician decision [8, 19]. Eligible patients were deemed to be treated until disease progression or unacceptable toxicity.

## 2.3 Endpoints and Statistical Analysis

The objective of the study was to investigate the prognostic and predictive effect of specific *RAS* mutations on patients with refractory CRC receiving rego, TFD/TPI, or both. OS was defined as the interval time between the start of treatment (rego or TFD/TPI) and death from any cause, or last follow-up in the case of patients lost to follow-up. Time to progression (TTP) was defined as the interval time between

the start of treatment and disease progression (clinical, radiological, or both) or death. Patients not experiencing an event were censored at the time of last follow-up. Analyses were based on aggregated retrospective data comparing outcome by *RAS* extended analysis (provided by the investigators). Patients were classified according to treatment in two groups (rego- or TFD/TPI-treated) and stratified in six groups according to *RAS* mutational status: (1) patients with tumor harboring no mutations involving *KRAS* exon 2–4 and *NRAS* exon 2–4 (WT); (2) patients with tumor harboring a mutation involving *KRAS* codon 12 (*KRAS* codon 12); (3) patients with tumor harboring a mutation involving codon 13 (*KRAS* codon 13); (4) patients with tumor harboring a rare *KRAS* mutation or an *NRAS* mutation (rare mutations); (5) patients with tumor harboring a *KRAS* G12C mutation (*KRAS* G12C); (6) patients with tumor harboring a *KRAS* G12D mutation (*KRAS* G12D). Patients carrying G12C or G12D mutations were considered distinctly, since these two were the only mutations for which a targeted treatment is available [5, 6, 20].

The pertinent data were compiled using descriptive statistics. Possible relationships were assessed using the Chi-square and Fisher's exact tests. The Kaplan–Meier product limit approach was used to compute both progression-free survival (PFS) and OS, and the log-rank test was used to evaluate differences between subgroups. Significance was established at  $p = 0.05$ . All of the statistical analyses were performed using MedCalc Statistical software, version 14.8.1 (MedCalc Software, Ostend Belgium).

## 3 Results

### 3.1 Patients

Overall, 866 patients were included in the former study [17], of whom 10 (1.1%) carried a *BRAF* mutation and were excluded from further analysis. For 274 patients (31.6%), data about *RAS* extended analysis were not available and thus these patients were excluded from the present study. Overall, 582 patients were included in the present analysis (Online Resource Table 1), of whom 270 (46.6%) were WT, 212 (36.4%) carried a codon 12 *KRAS* mutation, 49 (8.4%) carried a codon 13 mutation, and 51 (8.7%) carried other *KRAS* rare mutations or *NRAS* mutations. Among codon 12 *KRAS* mutations, the G12D mutation was the most frequent. Details on detected mutations of our population are summarized in Table 1; patient characteristics are summarized in Table 2 and Table 3. In particular, when considering rego-treated patients (Table 2), characteristics were well-balanced between groups, with the exception of number of previous lines of therapy (>4 lines was less frequent in the G12C and G12D groups) and previous administered drugs, since

anti-EGFRs were restricted to WT patients. About 50% of patients in each group received TFP/TPI in addition to rego (before or after, with a well-balanced distribution). Even for TFD/TPI-treated patients, characteristics were well-balanced between groups, with the exception of number of previous lines of therapy (>4 lines was less frequent in the G12C and G12D groups) and previously administered drugs, since anti-EGFRs were restricted to all WT patients. About 50% of patients in each group received rego in addition to TFD/TPI (before or after, with a well-balanced distribution).

**Table 1** RAS assessment (582 patients)

RAS mutation	Patients (%)
No mutation (all wild-type)	270 (46.3)
Kras Codon 12	212 (36.4)
<b>G12D</b>	63 (10.8)
G12V	53
<b>G12C</b>	17 (2.9)
G12R	3
G12S	9
G12A	14
G12V/D/A	19
G12X	34
Kras Codon 13	49 (8.4)
G13D	40
G13C	1
G13A	1
G13X	7
Rare mutations	51 (8.7)
Kras A146X	8
Kras A146T	6
Kras A146V	1
Kras G61X	2
Kras Q61K	1
Kras K117X	2
Kras A146G	1
Kras A59T	1
Kras Q22K	1
Kras codon 61 not further specified	3
Nras G12A	3
Nras G61L	3
Nras Q61H	3
Nras Q61X	4
Nras Q61L	2
Nras Q61R	2
Nras Q61K	2
Nras G61A	2
Nras A117X	2
Nras G61C	1
Nras A11P	1

### 3.2 Efficacy Outcome According to RAS Extended Analysis in Regorafenib-Treated Patients

OS did not significantly differ in rego-treated patients according to RAS extended analysis ( $p = 0.69$ ) (Fig. 1), although a trend toward a better median survival in patients carrying G12D mutation (12.0 months), Codon 13 mutation (8.0 months) and Codon 12 mutation (7.0 months) has been observed, when compared with WT patients (6.0 months). Accordingly, TTP did not differ significantly in rego-treated patients according to RAS extended analysis ( $p = 0.71$ ) (Online Resource Fig. 1). However, a non-statistically significant benefit was observed in patients carrying a Codon 12 mutation (3.6 months), a rare mutation (3.9 months), or a G12C mutation (4.5 months), when compared with WT patients (3.0 months).

Patients receiving TFD/TPI (before or after, 184 patients) in addition to rego showed a statistically significant gain in OS compared with those not exposed to TFD/TPI (9 vs. 5 months;  $p = 0.005$ ) (Online Resource Fig. 2a), suggesting an enrichment for favorable prognostic factors. When focusing on rego-treated patients who were exposed to TFD/TPI, a trend toward statistical significance was observed for WT patients (10 months), Codon 13 (12 months), and G12D (14 months;  $p = 0.05$ ) (Online Resource Fig. 2b).

### 3.3 Efficacy Outcome According to RAS Extended Analysis in Trifluridine/Tipiracil-Treated Patients

OS did not significantly differ in TFD/TPI-treated patients according to RAS extended analysis ( $p = 0.09$ ) (Fig. 2), although trend toward a better median survival was observed in WT patients (9 months) in comparison with the whole population (7.0 months). Accordingly, TTP did not significantly differ in TFD/TPI-treated patients according to RAS extended analysis ( $p = 0.86$ ) (Online Resource Fig. 3). However, a non-statistically significant trend was observed in favor of WT patients (4.0 months) when compared with the whole population (3.7 months).

Patients receiving rego in addition to TFD/TPI (before or after, 188 patients) showed a statistically significant gain in OS when compared with those not exposed to rego (10 vs. 5 months;  $p < 0.001$ ) (Online Resource Fig. 4a), suggesting an enrichment for favorable prognostic factors. According to RAS extended analysis, a survival gain was equally distributed among groups, without statistically significant differences (Online Resource Fig. 4b).

**Table 2** Patient characteristics of regorafenib-treated patients [ $n = 363$ ]

	WT [ $n = 174$ ]	Codon 12 [ $n = 89$ ]	Codon 13 [ $n = 29$ ]	Rare [ $n = 30$ ]	G12C [ $n = 10$ ]	G12D [ $n = 31$ ]
Median age, years ( $p = 0.99$ )	64.6	66.4	67.2	66.9	62.8	65.1
Sex [M/F] ( $p = 0.75$ )	100/74	57/32	14/15	18/12	7/3	19/12
ECOG PS ( $p = 0.22$ )						
0	50 (28.7)	23 (25.8)	4 (13.7)	8 (26.6)	4 (40.0)	10 (32.2)
1	98 (56.3)	58 (65.1)	24 (82.7)	16 (53.3)	5 (50.0)	19 (61.2)
2	26 (14.9)	8 (8.9)	1 (3.4)	6 (20.1)	1 (10.0)	2 (6.4)
Primary tumor location ( $p = 0.62$ )						
Right	47 (27.0)	33 (37.0)	10 (34.4)	9 (30.0)	2 (20.0)	11 (35.5)
Left	99 (57.0)	43 (48.4)	16 (55.2)	18 (60.0)	5 (50.0)	13 (41.9)
Rectum	28 (16.0)	13 (14.6)	3 (10.4)	3 (10.0)	3 (30.0)	7 (22.6)
Metastatic synchronous disease ( $p = 0.28$ )	145 (83.3)	70 (78.6)	20 (68.9)	25 (83.3)	6 (60.0)	25 (80.6)
Site of metastatic disease ( $p = 0.16$ )						
Liver only	19 (10.9)	6 (6.7)	5 (17.2)	–	2 (20.0)	4 (12.9)
Liver + other	86 (49.5)	46 (51.6)	18 (62.2)	19 (63.3)	7 (70.0)	17 (54.8)
Other sites	69 (39.7)	37 (41.5)	6 (20.6)	11 (36.7)	1 (10.0)	10 (32.3)
Previous lines of therapy ( $p < 0.004$ )						
2	69 (39.6)	56 (62.9)	16 (55.1)	20 (66.6)	7 (70.0)	18 (58.0)
3	63 (36.2)	14 (15.7)	6 (20.6)	3 (10.0)	2 (20.0)	10 (32.2)
4	19 (10.9)	4 (4.4)	2 (6.8)	2 (6.6)	1 (10.0)	3 (9.6)
>4	23 (13.2)	15 (16.8)	5 (17.2)	5 (16.6)	–	–
Previous drugs administered ( $p < 0.001$ ; $p = 0.99$ [excluding anti-EGFRs])						
Fluoropyrimidine	131 (75.2)	65 (73.0)	21 (72.4)	23 (76.6)	7 (70.0)	25 (80.6)
Oxaliplatin	127 (72.9)	58 (65.1)	22 (75.8)	22 (73.3)	7 (70.0)	23 (74.1)
Irinotecan	119 (68.3)	52 (58.4)	23 (79.3)	20 (66.6)	6 (60.0)	22 (70.9)
Bevacizumab	125 (71.8)	59 (66.2)	19 (65.5)	17 (56.6)	7 (70.0)	23 (74.1)
Anti-EGFRs	129 (74.1)	–	–	–	–	–
Immunotherapy	4 (2.2)	1 (1.1)	1 (3.4)	–	–	–
Aflibercept	12 (6.8)	7 (6.8)	2 (6.8)	6 (20.0)	2 (20.0)	4 (12.9)
Unknown	43 (24.7)	22 (24.7)	8 (27.5)	7 (23.3)	2 (20.0)	6 (19.3)
Receiving TFD/TPI ( $p = 0.88$ )	79 (45.4)	47 (52.8)	17 (58.6)	16 (53.3)	6 (60.0)	19 (61.2)
Before rego	46 (26.4)	26 (29.2)	8 (27.5)	10 (33.3)	4 (40.0)	9 (29.0)
After rego	33 (18.9)	21 (23.5)	9 (31.0)	6 (20.0)	2 (20.0)	10 (32.2)

ECOG PS Eastern Cooperative Oncology Group performance status, EGFRs epidermal growth factor receptors, F female, M male, rego regorafenib, TFD/TPI trifluridine/tipiracil, WT wild-type

### 3.4 Efficacy Outcome According to Each Subgroup

Since no differences concerning efficacy of rego and TFD/TPI according to RAS extended analysis were documented, we analyzed each subgroup separately. These analyses are to be considered exploratory.

Patient characteristics were well-balanced in each population. Statistically significant differences in previously administered drugs for WT, Codon 12, and G12D patients were registered, with missing data being significantly more frequent for the rego-treated population, likely due to the issue

that some patients in the rego group had been treated previously. Details of the comparison of patient characteristics in each group have been reported in Online Resource Table 2.

No differences in terms of the response rate were registered between the rego-treated population and the TFD/TPI-treated population in each RAS subgroup. Details about response rate have been reported in Online Resource Table 3. Moreover, concerning dose intensity, dose reductions were more frequent in rego-treated patients (compared with TFD/TPI patients), with statistically significant differences in WT, Codon 12 and Rare subgroups. Details

**Table 3** Patient characteristics of TFD/TPI-treated patients [ $n = 408$ ]

	WT [ $n = 177$ ]	Codon 12 [ $n = 90$ ]	Codon 13 [ $n = 38$ ]	Rare [ $n = 37$ ]	G12C [ $n = 14$ ]	G12D [ $n = 52$ ]
Median age, years ( $p = 0.99$ )	66.8	68.0	68.6	69.0	66.0	68.3
Sex [M/F] ( $p = 0.13$ )	97/80	55/35	16/22	17/20	11/3	27/25
ECOG PS ( $p = 0.29$ )						
0	48 (27.1)	26 (28.8)	7 (18.4)	12 (32.4)	4 (28.5)	9 (17.3)
1	102 (57.6)	56 (62.2)	29 (76.3)	18 (48.6)	9 (64.2)	34 (65.3)
2	27 (15.2)	8 (8.8)	2 (5.2)	7 (18.9)	1 (7.1)	9 (17.3)
Primary tumor location ( $p = 0.82$ )						
Right	47 (26.5)	26 (28.8)	14 (36.8)	15 (40.5)	4 (28.5)	13 (25.0)
Left	90 (50.8)	41 (45.5)	16 (42.1)	14 (37.8)	8 (57.1)	27 (51.9)
Rectum	40 (22.5)	23 (25.5)	8 (21.0)	8 (21.6)	2 (14.2)	12 (23.1)
Synchronous metastatic disease ( $p = 0.59$ )	130 (73.4)	64 (71.1)	23 (60.5)	29 (78.3)	10 (71.4)	39 (75.0)
Site of metastatic disease ( $p = 0.65$ )						
Liver only	22 (12.4)	11 (12.2)	6 (15.7)	3 (8.1)	3 (21.4)	6 (11.5)
Liver + other	89 (50.2)	46 (51.1)	19 (51.3)	23 (62.1)	8 (57.1)	34 (65.3)
Other sites	66 (37.2)	33 (36.6)	13 (34.2)	11 (29.7)	3 (21.4)	12 (23.0)
Previous lines of therapy ( $p = 0.0001$ )						
2	77 (43.5)	61 (67.7)	16 (42.1)	19 (51.3)	9 (64.2)	26 (50.0)
3	71 (40.1)	18 (20.0)	12 (31.5)	8 (21.6)	4 (28.5)	17 (32.6)
4	9 (5.0)	2 (2.2)	2 (5.2)	3 (8.1)	1 (7.1)	9 (17.3)
>4	20 (11.2)	9 (10.0)	8 (21.0)	7 (18.9)	–	–
Previous drugs administered ( $p < 0.0001$ ; $p = 0.91$ [with exclusion of anti-EGFRs])						
Fluoropyrimidine	173 (97.7)	86 (95.5)	36 (94.7)	36 (97.2)	14 (100.0)	52 (100.0)
Oxaliplatin	165 (93.2)	81 (90.0)	32 (84.2)	34 (91.8)	13 (92.8)	43 (82.6)
Irinotecan	166 (93.7)	82 (91.1)	35 (92.1)	34 (91.8)	14 (100.0)	45 (86.5)
Bevacizumab	95 (53.6)	75 (83.3)	28 (73.6)	28 (75.6)	9 (64.2)	42 (80.7)
Anti-EGFRs	154 (87.0)	–	–	–	–	–
Immunotherapy	2 (1.1)	2 (2.2)	2 (5.2)	–	–	–
Aflibercept	9 (5.0)	12 (13.3)	6 (15.7)	12 (32.4)	4 (28.5)	8 (15.3)
Unknown	4 (2.2)	3 (3.3)	2 (5.2)	1 (2.7)	–	–
Receiving rego ( $p = 0.65$ )	80 (45.1)	49 (54.4)	17 (44.7)	16 (43.2)	7 (50.0)	19 (36.5)
Before TFD/TPI	32 (18.0)	19 (21.1)	9 (23.6)	5 (13.5)	2 (14.2)	10 (19.2)
After TFD/TPI	48 (27.1)	30 (33.3)	8 (21.0)	11 (29.7)	5 (35.7)	9 (17.3)

ECOG PS Eastern Cooperative Oncology Group performance status, EGFRs epidermal growth factor receptor, F female, M male, rego regorafenib, TFD/TPI trifluridine/tipiracil, WT wild-type

on dose reductions have been reported in Online Resource Table 4, and details of comparison of patient characteristics according to the treatment sequence in each group have been reported in Online Resource Table 5.

**Efficacy outcome in the WT group:** In WT patients, median rego-related OS was significantly longer in patients receiving TFD/TPI (overall 79 patients [45.0%]; 46 before, 33 after) in addition to rego ( $p = 0.003$ ) (Fig. 3a). At univariate analysis, including age, sex, number of previous lines, synchronous disease, PS, and sites of disease, no statistically significant differences in terms

of OS were observed, with the exception of patients with PS  $< 2$  ( $p = 0.001$ ) and patients with a synchronous disease ( $p = 0.002$ ). Multivariate analysis was not performed because of the low sample size.

At the same time, median TFD/TPI-related OS was significantly longer in patients receiving rego (overall 80 patients [45.1%]; 32 before, 48 after) in addition to TFD/TPI ( $p = 0.002$ ) (Fig. 3b). At univariate analysis, including age, sex, number of previous lines, synchronous disease, PS, and sites of disease, no statistically significant differences in terms of OS were observed.

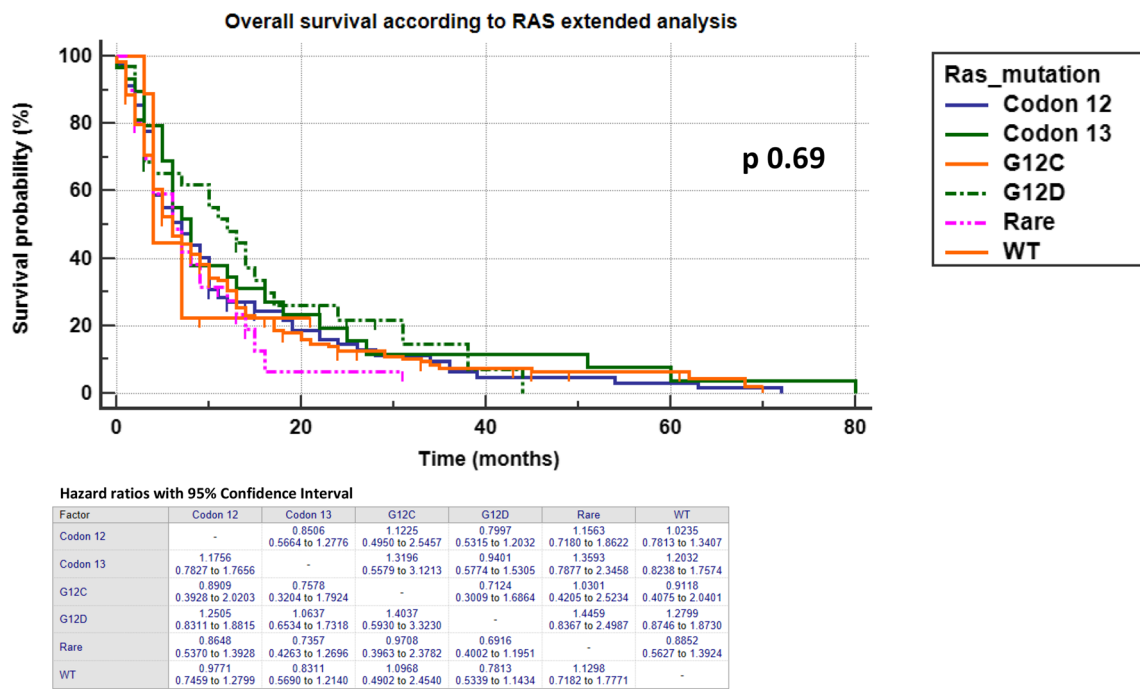


Fig. 1 Overall survival of Rego-treated patients according to RAS extended analysis

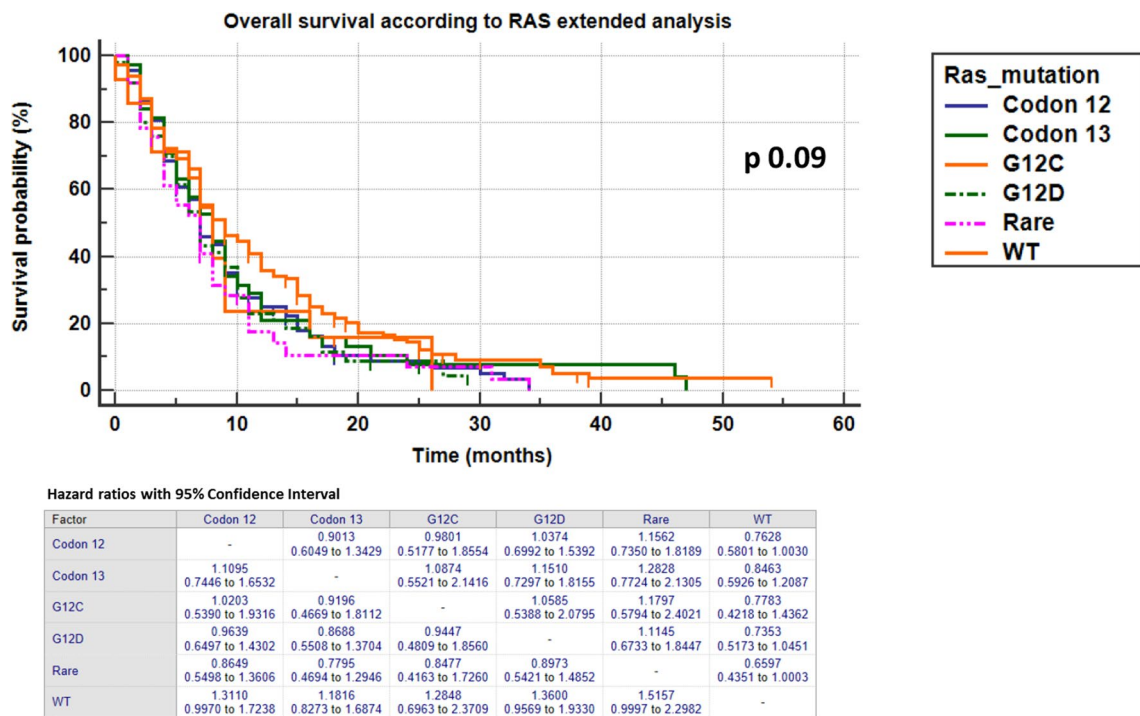


Fig. 2 Overall survival of TFD/TPI-treated patients according to RAS extended analysis

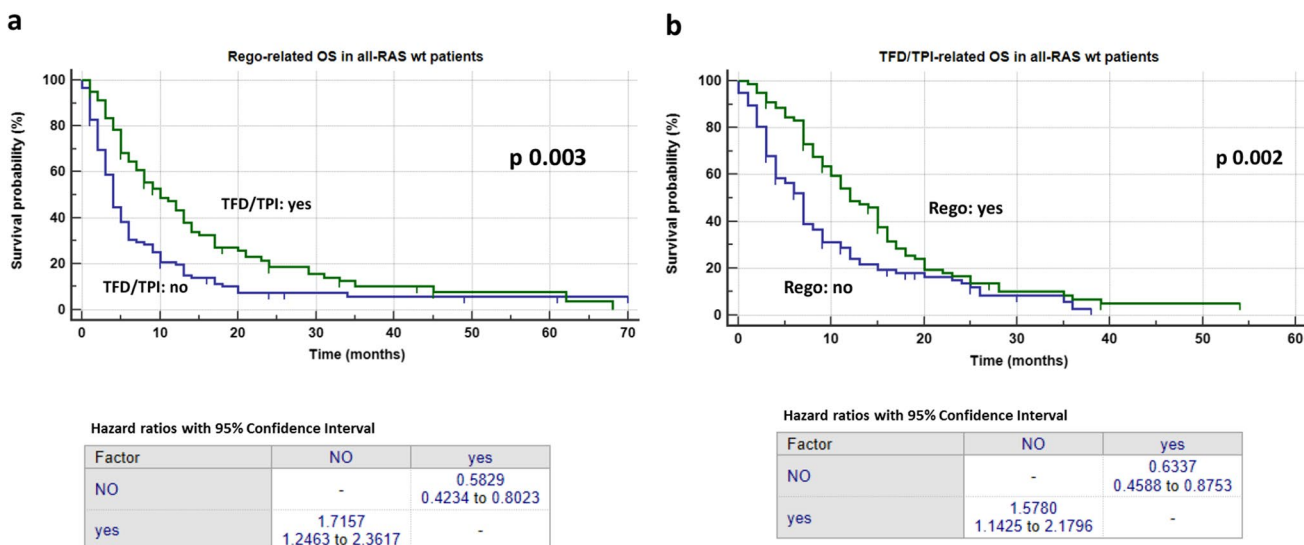


Fig. 3 Rego-related and TFD/TPI-related OS of all-RAS wt patients

When focusing on the treatment administration sequence (comparing patients who received rego followed by TFD/TPI or the reverse sequence), no statistically significant differences were documented ( $p = 0.13$ ) (Online Resource Fig. 5).

*Efficacy outcome in the Codon 12 group:* In Codon 12-mutated patients, median rego-related OS showed no differences between patients receiving TFD/TPI (overall 47 patients [52.8%]; 26 before, 21 after) in addition to rego ( $p = 0.16$ ) (Fig. 4a). Conversely, median TFD/TPI-related OS was significantly longer in patients receiving rego (overall 49 patients [54.4%]; 19 before, 30 after) in addition to TFD/TPI ( $p = 0.0004$ ) (Fig. 4b). At univariate analysis, including age, sex, number of previous lines,

synchronous disease, PS, and sites of disease, no statistically significant differences in terms of OS were observed.

When focusing on the treatment administration sequence, patients receiving rego before TFD/TPI displayed a significantly longer OS ( $p = 0.003$ ) (Fig. 5). At univariate analysis, including age, sex, number of previous lines, synchronous disease, PS, and sites of disease, no statistically significant differences in terms of OS were observed, with the exception of patients with liver involvement alone ( $p = 0.0002$ ). Multivariate analysis was not performed because of the low sample size.

*Efficacy outcome in the Codon 13 group:* In Codon 13-mutated patients, median rego-related OS showed no differences between patients receiving TFD/TPI (overall

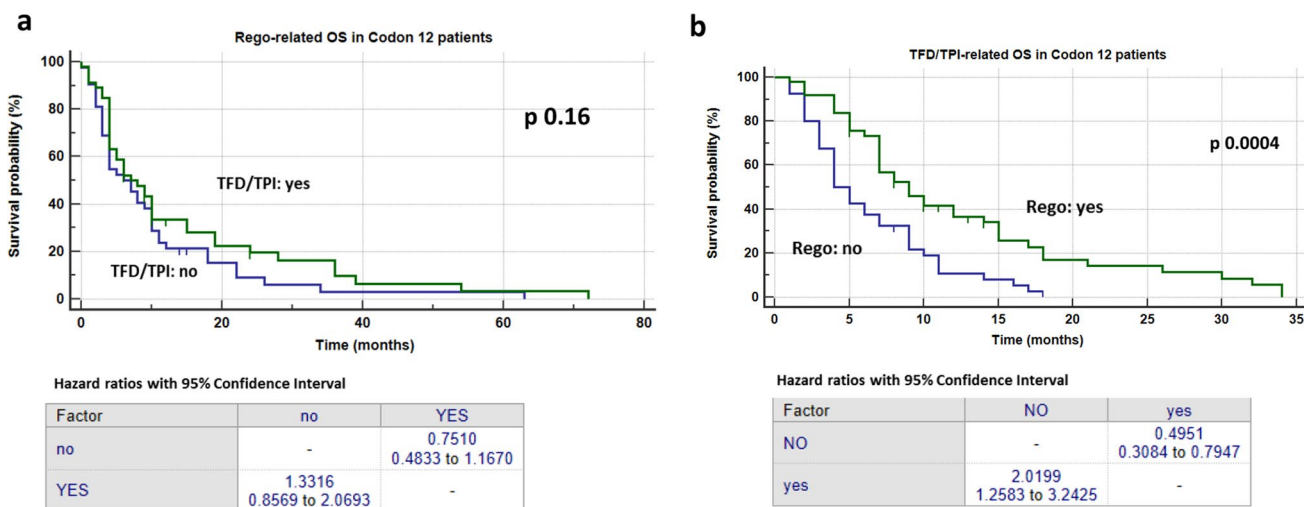
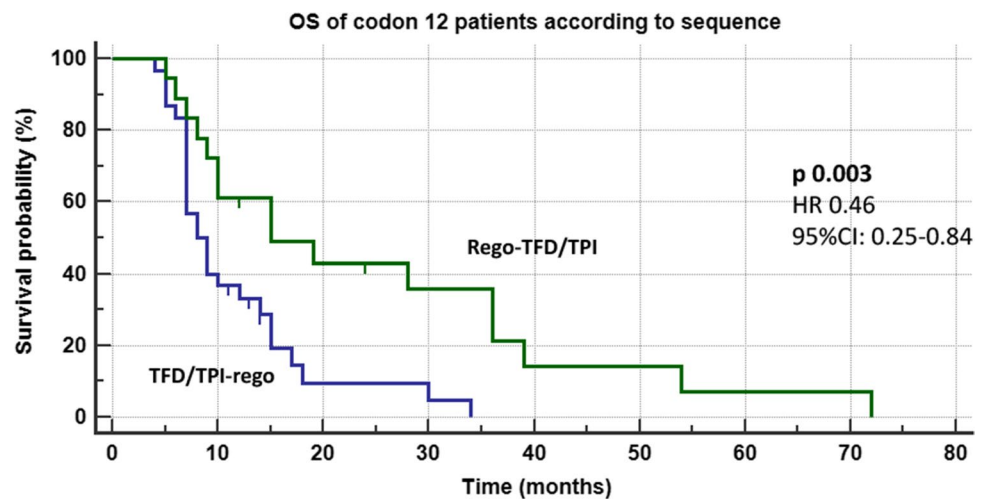


Fig. 4 Rego-related and TFD/TPI-related OS of Codon 12 patients

**Fig. 5** OS of Codon 12 patients according to sequence (49 pts)



17 patients [58.6%]; 8 before, 9 after) in addition to rego ( $p = 0.35$ ) (Online Resource Fig. 6a). Accordingly, median TFD/TPI-related OS showed no differences between patients receiving rego (overall 17 patients [44.7%]; 9 before, 8 after) in addition to TFD/TPI ( $p = 0.11$ ) (Online Resource Fig. 6b).

When focusing on the treatment administration sequence, no statistically significant differences were documented ( $p = 0.12$ ) (Online Resource Fig. 7), although a trend toward a better performance in patients receiving rego followed by TFD/TPI was observed.

**Efficacy outcome in the rare mutations group:** In the Rare group, median rego-related OS showed no differences between patients receiving TFD/TPI (overall 16 patients [53.3%]; 10 before, 6 after) in addition to rego ( $p = 0.80$ ) (Online Resource Fig. 8a). Conversely, median TFD/TPI-related OS was significantly longer in patients receiving rego (overall 16 patients [43.2%]; 5 before, 11 after) in addition to TFD/TPI ( $p = 0.02$ ) (Online Resource Fig. 8b). At univariate analysis, including age, sex, number of previous lines, synchronous disease, PS, and sites of disease, no statistically significant differences in terms of OS were observed, with the exception of patients with liver involvement alone ( $p = 0.0002$ ). Multivariate analysis was not performed because of the low sample size.

When focusing on the treatment administration sequence, a trend toward a better performance in patients receiving rego first was observed ( $p = 0.09$ ) (Online Resource Fig. 9).

**Efficacy outcome in the G12C group:** In the G12C group, median rego-related OS showed no differences between patients receiving TFD/TPI (overall 6 patients [60.0%]; 4 before, 2 after) in addition to rego ( $p = 0.98$ ) (Online Resource Fig. 10a). Accordingly, median TFD/TPI-related OS was not significantly longer in patients receiving rego (overall 7 patients [50.0%]; 2 before, 5

after) in addition to TFD/TPI ( $p = 0.65$ ) (Online Resource Fig. 10b).

When focusing on the treatment administration sequence, no statistically significant differences were documented ( $p = 0.73$ ) (Online Resource Fig. 11).

**Efficacy outcome in the G12D group.** In patients in the G12D group, median rego-related OS showed no differences between patients receiving TFD/TPI (overall 19 patients [61.2%]; 9 before, 10 after) in addition to rego ( $p = 0.42$ ) (Online Resource Fig. 12a). Conversely, median TFD/TPI-related OS was significantly longer in patients receiving rego (overall 19 patients [36.5%]; 10 before, 9 after) in addition to TFD/TPI ( $p = 0.03$ ) (Online Resource Fig. 12b). At univariate analysis, including age, sex, number of previous lines, synchronous disease, PS, and sites of disease, no statistically significant differences in terms of OS were observed.

When focusing on the treatment administration sequence, no statistically significant differences were documented ( $p = 0.73$ ) (Online Resource Fig. 13).

## 4 Discussion

To our knowledge, this is the first report evaluating the efficacy of rego and TFD/TPI in later lines of real-life treatment of CRC patients, according to extended RAS analysis.

Our study suggests that extended RAS mutation status does not affect the outcome of heavily pretreated CRC patients, neither when receiving TFD/TPI nor when receiving rego. Nevertheless, a trend toward a higher efficacy of rego in RAS-mutated patients has been documented (in particular in patients carrying G12D and Codon 12 mutations), when compared with RAS WT patients. Our study also demonstrated that about 50% of patients are able to receive both drugs independently of molecular characteristics. Patients receiving both drugs surely represent a favorable prognostic

factor-enriched population, since the median OS is significantly higher when compared with monotherapy, both in our study and in other papers [9–15]. Rego-related OS was significantly longer when considering patients receiving TFD/TPI in addition to rego, and, accordingly, TFD/TPI-related survival was significantly longer when considering patients receiving rego in addition to TFD/TPI. Interestingly enough, the scenario changed when each molecular subgroup was considered separately. While, in *RAS* WT patients, both rego-related OS and TFD/TPI-related OS were longer in patients receiving both drugs, in mutated groups (Codon 12, Codon 13, Rare, G12C and G12D), rego-related survival was not significantly improved in patients receiving TFD/TPI in addition to rego. Differently, TFD/TPI-related OS was significantly longer when considering patients receiving rego in addition to TFD/TPI, not only in *RAS* WT group but also in the Codon 12, Rare and G12D groups. In particular, these data were highly significant in Codon 12 ( $p = 0.0003$ ). Accordingly, only in this group (Codon 12), the rego followed by TFD/TPI sequence was statistically superior to the reverse sequence. Interestingly enough, the G12C subgroup displayed a different clinical behavior, probably due to the worse prognosis of patients carrying this mutation [21].

A recent paper [18] analyzing two independent real-world datasets (960 patients overall) and an independent validation cohort based on the global phase III RECURSE trial (800 patients) demonstrated that *KRAS* codon 12 mutations are predictive of reduced TFD/TPI efficacy. On the contrary, all other patients benefited from TFD/TPI therapy compared with placebo. Unfortunately, no data about patients receiving rego in addition to TFD/TPI were available for this population. Our data are consistent with the data reported above since rego-related OS of the Codon 12 group did not benefit from the addition of TFD/TPI.

A Japanese study [16] comparing rego and TFD/TPI efficacy in a large (7279 patients) nationwide database showed a significantly longer survival in patients receiving TFD/TPI first compared with those receiving rego first. However, 39% of patients in the rego-first group received subsequent TFD/TPI treatment, whereas only 25% of the patients in the TFD/TPI-first group received subsequent rego treatment. This imbalance likely affected the outcome, since patients receiving both drugs displayed a significantly higher survival. This evidence, reported in this study, is in line with our results as well as with other reports [9–17]. In this regard, the percentage of patients receiving both drugs (about 50% in our study, much higher than other reports) deserves further attention since, considering the high variability among reports [22], we suspect it could be attributed to factors other than patient-related or disease-related factors.

Last, but not least, our study provides a picture of the molecular landscape of patients reaching later lines of treatment in advanced CRC. The proportion of patients carrying

a *BRAF* mutation is very low. The percentage of patients with no *RAS* mutation (WT) is 46.3% and is in line with literature data [21] and with patients treated in the first-line. G12D is the most common codon 12 mutation and G13D is the most common codon 13 mutation, in line with available evidence on CRC *RAS* mutation epidemiology [23].

The main limitation of our study is represented by its retrospective nature, which might have affected the results due to its intrinsic selection bias. Another important limitation is the long interval time over which patients have been included. Last, but not least, the sample size of the subgroups analysis is quite limited.

Although our study demonstrates that efficacy of rego and TFD/TPI in later lines of therapy of advanced CRC patients is not significantly affected by extended *RAS* mutational status, it identifies subgroups of patients in whom rego seems to work better. In patients carrying *KRAS* codon 12 mutations, rarer *KRAS* mutations and *NRAS* mutations and *KRAS* G12D mutation, the addition of rego (before or after) to TFD/TPI significantly improves TFD/TPI-related survival, but is not the same when adding TFD/TPI to rego. Moreover, only for patients with CRC harboring *KRAS* codon 12 mutations, the rego followed by TFD/TPI sequence seems significantly superior to the reverse sequence. Because of the small sample size of some subgroups, we cannot exclude that other factors such as disease burden or PS could play an important role in our results.

## 5 Conclusion

Although our results cannot be considered conclusive, they are in line with previous evidence [18] investigating the efficacy of TFD/TPI in relation to *KRAS* mutational status, and add another important piece to the puzzle of treatment personalization of advanced CRC patients. Bearing in mind that no more than 50% of patients will receive both drugs in the history of their disease, we think that available data could suggest to consider rego anticipation (in comparison with TFD/TPI) in patients carrying a codon 12 mutation.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11523-024-01050-3>.

## Declarations

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**Ethics Approval** The study was approved by the Ethics Committee (2022-no.1021/CE Lazio 1) and was carried out in conformity with the Declaration of Helsinki.

**Consent to Participate** Informed consent was obtained from all patients prior to participating in this study.

**Consent to Publish** Not applicable.

**Availability of Data and Materials** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Code Availability** Not applicable.

**Authors' Contributions** The authors met the criteria for authorship as recommended by the International Committee of Medical Journal Editors.


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## References

- Benson AIB, Venook AP, Al-Hawary MM, et al. NCCN clinical practice guidelines in oncology: colon cancer, version 2.2021. *J Natl Compr Canc Netw*. 2021;19:329–59.
- Tabernero J, Grothey A, Van Cutsem E, et al. Encorafenib plus cetuximab as a new standard of care for previously treated BRAF V600E-mutant metastatic colorectal cancer: updated survival results and subgroup analyses from the BEACON study. *J Clin Oncol*. 2021;39:273–84.
- Overman MJ, McDermott R, Leach JJ, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol*. 2017;18(9):1182–91.
- André T, Shiu KK, Kim TW, et al. Pembrolizumab in microsatellite-Instability-high advanced colorectal cancer. *N Engl J Med*. 2020;383(23):2207–18.
- Fakih MG, Kopetz S, Kuboki Y, et al. Sotorasib for previously treated colorectal cancers with KRASG12C mutation (CodeBreaK100): a prespecified analysis of a single-arm, phase 2 trial. *Lancet Oncol*. 2022;23(1):115–24. [https://doi.org/10.1016/S1470-2045\(21\)00605-7](https://doi.org/10.1016/S1470-2045(21)00605-7).
- Yaeger R, Weiss J, Pelster MS, et al. Adagrasib with or without cetuximab in colorectal cancer with mutated KRAS G12C. *N Engl J Med*. 2023;388(1):44–54. <https://doi.org/10.1056/NEJMoa2212419>.
- Mayer RJ, Van Cutsem E, Falcone A, et al. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. *N Engl J Med*. 2015;372:1909–19.
- Grothey A, Van Cutsem E, Sobrero A, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet*. 2013;381:303–12.
- Dasari NA, Lonardi S, Garcia-Carbonero R, et al. LBA25 FRESCO-2: a global phase III multiregional clinical trial (MRCT) evaluating the efficacy and safety of fruquintinib in patients with refractory metastatic colorectal cancer [abstract]. *Ann Oncol*. 2022;33(Suppl 7):S1391–2.
- Prager GW, Tajeb J, Fakih M, et al. Trifluridine-Tipiracil and bevacizumab in refractory metastatic colorectal cancer. *N Engl J Med*. 2023;388:1657–67. <https://doi.org/10.1056/NEJMoa2214963>.
- Moriwaki T, Fukuoka S, Taniguchi H, et al. Propensity score analysis of regorafenib versus trifluridine/tipiracil in patients with metastatic colorectal cancer refractory to standard chemotherapy (REGOTAS): a Japanese Society for Cancer of the Colon and Rectum multicenter observational study. *Oncologist*. 2018;23:7–15.
- Masuishi T, Taniguchi H, Hamauchi S, et al. Regorafenib versus trifluridine/tipiracil for refractory metastatic colorectal cancer: a retrospective comparison. *Clin Colorectal Cancer*. 2017;16:e15–22.
- Tanaka A, Sadahiro S, Suzuki T, et al. Retrospective study of regorafenib and trifluridine/tipiracil efficacy as a third-line or later chemotherapy regimen for refractory metastatic colorectal cancer. *Oncol Lett*. 2018;16:6589–97.
- Ogata M, Kotaka M, Ogata T, et al. Regorafenib vs trifluridine/tipiracil for metastatic colorectal cancer refractory to standard chemotherapies: a multicenter retrospective comparison study in Japan. *PLoS One*. 2020;15: e0234314.
- Patel AK, Abhyankar R, Brais LK, et al. Trifluridine/tipiracil and regorafenib in patients with metastatic colorectal cancer: a retrospective study at a tertiary oncology center. *Oncologist*. 2021;26:e2161–2169.
- Nakashima M, Takeuchi M, Kawakami K. Effectiveness and safety of regorafenib vs. Trifluridine/Tipiracil in unresectable colorectal cancer: a retrospective cohort study. *Clin Colorectal Cancer*. 2020;19(4):e208–25.
- Signorelli C, Calegari MA, Basso M, et al. Treatment settings and outcomes with regorafenib and trifluridine/tipiracil at third-line and beyond in metastatic colorectal cancer: a real-world multicenter retrospective study. *Curr Oncol*. 2023;30(6):5456–69. <https://doi.org/10.3390/curroncol30060413>.
- van de Haar J, Ma X, Ooft SN, et al. Codon-specific KRAS mutations predict survival benefit of trifluridine/tipiracil in metastatic colorectal cancer. *Nat Med*. 2023;29:605–14. <https://doi.org/10.1038/s41591-023-02240-8>.
- Bekaii-Saab TS, Ou FS, Ahn DH, et al. Regorafenib dose-optimization in pts with refractory metastatic colorectal cancer (ReDOS): a randomized, multicentre, open-label, phase 2 study. *Lancet Oncol*. 2019;20(8):1070–82. [https://doi.org/10.1016/S1470-2045\(19\)30272-4](https://doi.org/10.1016/S1470-2045(19)30272-4).
- Hallin J, Bowcut V, Calinisan A, et al. Anti-tumor efficacy of a potent and selective non-covalent KRASG12D inhibitor. *Nat Med*. 2022;28(10):2171–82. <https://doi.org/10.1038/s41591-022-02007-7>.
- Koulouridi P, Karagianni M, Messaritakis I, et al. Prognostic value of KRAS mutations in colorectal cancer patients. *Cancers*. 2022;14:3320. <https://doi.org/10.3390/cancers14143320>.

22. Nevala-Plagemann C, Sama S, Ying J, et al. A real-world comparison of regorafenib and trifluridine/tipiracil in refractory metastatic colorectal cancer in the United States. *Natl Compr Canc Netw*. 2023;21(3):257–64. <https://doi.org/10.6004/jncn.2022.7082>.
23. Timar J, Kashofer K. Molecular epidemiology and diagnostics of KRAS mutations in human cancer. *Cancer Metastasis Rev*. 2020;39:1029–38. <https://doi.org/10.1007/s10555-020-09915-5>.

## Authors and Affiliations

Michele Basso<sup>1</sup>  · Carlo Signorelli<sup>2</sup> · Maria Alessandra Calegari<sup>1</sup> · Jessica Lucchetti<sup>3</sup> · Ina Valeria Zurlo<sup>4</sup> · Emanuela Dell'Aquila<sup>5</sup> · Giulia Arrivi<sup>6</sup> · Federica Zoratto<sup>7</sup> · Fiorenza Santamaria<sup>8,9</sup> · Rosa Saltarelli<sup>10</sup> · Giovanni Trovato<sup>11</sup> · Giulia Caira<sup>11</sup> · Lorenzo Angotti<sup>3</sup> · Marta Schirripa<sup>2</sup> · Annunziato Anghelone<sup>11</sup> · Francesco Schietroma<sup>11</sup> · Mario Giovanni Chillelli<sup>2</sup> · Lisa Salvatore<sup>1,11</sup> · Carmelo Pozzo<sup>1</sup> · Giampaolo Tortora<sup>1,11</sup>

✉ Michele Basso  
michele.basso@policlinicogemelli.it

<sup>1</sup> Comprehensive Cancer Center, Fondazione Policlinico Universitario “A Gemelli” – IRCCS, Largo Agostino Gemelli n 8, 00168 Rome, Italy

<sup>2</sup> Medical Oncology Unit, Belcolle Hospital, ASL Viterbo, Viterbo, Italy

<sup>3</sup> Operative Research Unit of Medical Oncology, Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy

<sup>4</sup> Medical Oncology, “Vito Fazzi” Hospital, Lecce, Italy

<sup>5</sup> Medical Oncology 1, IRCCS Regina Elena National Cancer Institute, Rome, Italy

<sup>6</sup> Department of Clinical and Molecular Medicine, Sapienza University of Rome, Oncology Unit, Sant’ Andrea University Hospital, Rome, Italy

<sup>7</sup> Medical Oncology Unit, ASL Latina, Latina, Italy

<sup>8</sup> Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy

<sup>9</sup> Medical Oncology A, Department of Hematology, Oncology and Dermatology, Policlinico Umberto I, Sapienza University of Rome, Rome, Italy

<sup>10</sup> UOC Oncology, San Giovanni Evangelista Hospital, ASL RM5 Tivoli, RM, Italy

<sup>11</sup> Medical Oncology, Catholic University of Sacred Heart, Rome, Italy