ESMO Open Cancer Horizons

Clinical outcome and molecular characterisation of chemorefractory metastatic colorectal cancer patients with long-term efficacy of regorafenib treatment

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To cite: Martinelli E, Sforza V, Cardone C, *et al*. Clinical outcome and molecular characterisation of chemorefractory metastatic colorectal cancer patients with long-term efficacy of regorafenib treatment. *ESMO Open* 2017;**2**:e000177. doi:10.1136/esmoopen-2017-000177

► Prepublication history and additional material are available. To view these files please visit the journal online (http://dx.doi.org/10.1136/esmoopen-2017-000177).

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Received 21 February 2017
Revised 14 March 2017
Accepted 15 March 2017

Please click here to see linked paper

ABSTRACT

Background To investigate the potential predictors of response to regorafenib, in chemorefractory metastatic colorectal cancer (mCRC) patients with long-term efficacy from regorafenib treatment.

Methods Retrospective, single institution analysis of patients with chemorefractory mCRC treated with regorafenib, in clinical practice setting. 123 patients were treated and stratified into two groups according to number of cycles received (<7 and ≥7). Overall survival (OS), progression-free survival (PFS) and safety were evaluated. 20 tumour samples (10 poor and 10 long responders) were analysed with the OncoMine Comprehensive Assay for 143 genes.

Results A good Eastern Cooperative Oncology Group performance status, a lung limited metastatic disease and a long history of metastatic disease were significantly associated with better OS and PFS from treatment with regorafenib. Mutations were mostly found in *TP53*, *KRAS* and *PIK3CA* as well as in *NRAS*, *ERBB2, SMAD4* and *PTEN* genes. BCL2L1, ERBB2, KRAS, MYC, GAS6 gene amplifications were detected as well as ALK rearrangement. No significant correlation between molecular alterations and response to regorafenib was observed. However, HER2 gene alterations were found in three poor responder patients, suggesting a potential role in regorafenib resistance. Conversely, GAS6 amplification and SMAD4 mutation, detected in two long responder patients, might suggest a role of epithelial–mesenchymal transition phenotype in regorafenib response.

Conclusion A subgroup of long responder patients to regorafenib treatment was identified and a comprehensive molecular characterisation was performed; however, further research efforts are essential to confirm our data.

Key questions

What is already known about this subject?

Regorafenib displayed a survival benefit in unselected patients with metastatic colorectal cancer refractory to standard treatments according to two randomised phase III clinical trials. However, considering the toxicity profile and the lack of predictive biomarkers, a better definition of patients who might derive a benefit from regorafenib treatment, avoiding unnecessary adverse events, is needed.

What does this study add?

In this study, we have evaluated potential clinical and molecular predictors of regorafenib efficacy in a subgroup of patients with different outcome following regorafenib treatment. Our analysis supports the idea that a good Eastern Cooperative Oncology Group performance status, the presence of a lung-limited metastatic disease and a long history of metastatic disease (≥18 months) are significantly associated with better clinical outcome in patients with metastatic colorectal cancer treated with regorafenib. No significant correlation between molecular alterations, found by next-generation sequencing analysis, and response to regorafenib was observed. However, HER2 gene alterations and GAS6 amplification-SMAD4 mutation seem to play a role in regorafenib resistance and response, respectively.

How might this impact on clinical practice?

Our data support the importance of careful selection of patients and intensive clinical monitoring. Further research efforts are essential to confirm our molecular data.

Linked
► http://dx.doi.org/10.1136/esmoopen-2017-000205

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INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and a leading cause of cancer-related death worldwide.[1] Nearly 25%–30% of patients have evidence of metastases at the time of disease diagnosis and almost 25% will subsequently develop metastases. For patients with metastatic colorectal cancer (mCRC), the standard of treatment consists of medical therapy with palliative intent. However, over the last two decades, the median overall survival (OS) of patients with mCRC has increased from 12 months, with the only 5-fluorouracil-based chemotherapy, to roughly 30 months due to the improvements in the number and efficacy of systemic therapies.[2]

Regorafenib is an oral multikinase inhibitor that blocks several kinases involved in the regulation of angiogenesis (VEGFR1/3, TIE-2), oncogenesis (*KIT, RET, RAF1, BRAF* and mutant *V600E BRAF*) and also tumour microenvironment (PDGFR and FGFR).[3] Two phase III clinical trials, CORRECT and CONCUR, demonstrated a significant improvement of OS and progression-free survival (PFS) in regorafenib plus best supportive care (BSC) arm over placebo and BSC in patients with mCRC who have previously been treated with fluoropyrimidine, oxaliplatin, irinotecan, anti-VEGF therapy and anti-EGFR drugs if *KRAS exon-2* wild-type (WT).[4–6] As a result, regorafenib was approved in several countries for patients with chemorefractory mCRC.

However, the identification of useful predictive factors for the treatment response is currently lacking. Many parameters have been investigated, both clinical and biological, such as Eastern Cooperative Oncology Group (ECOG) performance status (PS) (ie, 0 vs 1), lactic dehydrogenase, neutrophil:lymphocyte ratio, platelet count, the rs2010963 SNP of VEGF-A, ANG-2, interleukin-6 (IL-6), IL-8, PIGF, sTie-1, sVEGFR-1, VEGF-A, VEGF-C, VEGF-D, VEGF-A-121, BMP-7, M-CSF, SDF-1, TIMP-2 and VWF without conclusive results.[7–9]

Herein, we reported data on efficacy and safety of a consecutive cohort of 123 patients treated with regorafenib, as per labelling, in our institution. We also performed an explorative analysis evaluating potential clinical and molecular predictors of regorafenib efficacy, by performing an extensive next generation sequencing (NGS) analysis, in 20 patients selected from the whole population.

PATIENTS AND METHODS

We performed a retrospective, single institution analysis in patients with mCRC treated with regorafenib, in a clinical practice setting, after failure of standard therapies including fluoropyrimidine, oxaliplatin, irinotecan, anti-VEGF therapy and anti-EGFR agents if *KRAS* WT. The study population consisted of a consecutive cohort of 123 patients, older than 18 years with histological confirmed adenocarcinoma of the colon or rectum, ECOG PS of 0–2. Data were collected from patients who received at least one regorafenib dose. Baseline demographical and clinical characteristics are listed in table 1. All patients

Table 1 Patients' characteristics

	Regorafenib	
	n=123	IQR, %
Age		
Median (years)	62.1	54.9–70.0
Gender		
Male	76	62%
Female	47	38%
Race		
Caucasian	123	100%
Eastern Cooperative Oncology Group performance status		
0	103	84%
1	14	11%
2	6	5%
Primary site of disease		
Right colon	62	50%
Left colon/ rectum	61	50%
KRAS exon-2 mutation		
Yes	65	53%
No	58	47%
Histology		
Adenocarcinoma	123	100%
No of previous systemic anticancer therapies (from diagnosis of metastatic disease)		
1	2	2%
2	36	29%
3	43	35%
4	23	19%
≥5	16	13%
No of metastatic sites		
1	16	13%
2	59	48%
3	33	27%
4	12	10%
5	2	2%
6	1	1%
Lung only metastatic disease		
Yes	9	7%
No	114	93%
Liver only metastatic disease		
Yes	5	4%
No	121	96%
Time from diagnosis of metastatic disease		
Median months	33.2	(20.2–46.8)
<18 months	25	20%
>18 months	98	80%

provided informed consent before receiving the first dose of regorafenib. Patients also consented to collect their data and to analyse the tumour samples. Regorafenib was administered at a dose of 160 mg/day for the first 3 weeks of each 4-week cycle.

Severity of adverse events (AEs) was graded using National Cancer Institute Common Terminology Criteria for Adverse Events, V.4. We performed a weekly clinical visit during the first month with a physical and biochemistry assessment. Moreover, to prevent the HFSR, we suggested the daily prophylactic use of moisturising creams.[10]

The observational period of treatment with regorafenib comprised May 2012 to December 2016. Data cut-off was 31 December 2016. We stratified patients into two groups according to number of cycles received (<7 cycles and ≥7 cycles) and evaluated for each group the OS, PFS and safety. Tumour response was evaluated every 8 weeks and assessed according to the Response Evaluation Criteria in Solid Tumours (RECIST 1.1).

MOLECULAR CHARACTERISATION

We have analysed samples by using a targeted high-multiplex PCR-based NGS panel (OncoMine Comprehensive Assay) coupled with high-throughput sequencing using Ion Proton sequencer for routine screening of solid tumours. The panel screens 143 genes using low amounts of formalin-fixed, paraffin-embedded (FFPE) DNA (20 ng) and RNA (10 ng). The capability of the panel is to detect 148 single-nucleotide variants, 49 insertions or deletions, 40 copy number aberrations and a subset of gene fusions. The OncoMine Comprehensive Assay analysed 73 hotspot genes (hotspot coverage): *ABL1, AKT1, ALK, AR, ARAF, BRAF, BTK, CBL, CDK4, HEK2, CSF1R, CTNNB1, DDR2, DNMT3A, EGFR, ERBB2, ERBB3, ERBB4, ESR1, EZH2, FGFR1, FGFR2, FGFR3, FLT3, FOXL2, GATA2, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, IFITM1, IFITM3, JAK1, JAK2, JAK3, KDR, KIT, KNSTRN, KRAS, MAGOH, MAP2K1, MAP2K2, MAPK1, MAX, MED12, MET, MLH1, MPL, MTOR, MYD88, NFE2L2, NPM1, NRAS, PAX5, PDGFRA, PIK3CA, PPP2R1A, PTPN11, RAC1, RAF1, RET, RHEB, RHOA, SF3B1, SMO, SPOP, SRC, STAT3, U2AF1, XPO1;* CDS, n=26 (full gene)*: APC, ATM, BAP1, BRCA1, BRCA2, CDH1, CDKN2A, FBXW7, GATA3, MSH2, NF1, NF2, NOTCH1, PIK3R1, PTCH1, PTEN, RB1, SMAD4, SMARCB1, STK11, TET2, TP53, TSC1, TSC2, VHL, WT1;* copy gain, n=49*: ACVRL1, AKT1, APEX1, AR, ATP11B, BCL2L1, BCL9, BIRC2, BIRC3, CCND1, CCNE1, CD274, CD44, CDK4, CDK6, CSNK2A1, DCUN1D1, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GAS6, IGF1R, IL6, KIT, KRAS, MCL1, MDM2, MDM4, MET, MYC, MYCL, MYCN, MYO18A, NKX2-1, NKX2-8, PDCD1LG2, PDGFRA, PIK3CA, PNP, PPARG, RPS6KB1, SOX2, TERT, TIAF1, ZNF217* and fusion drivers, n=22*: ALK, RET, ROS1, NTRK1, ABL1, AKT3, AXL, BRAF, CDK4, EGFR, ERBB2, ERG, ETV1, ETV4, ETV5, FGFR1, FGFR2, FGFR3, NTRK3, PDGFRA, PPARG, RAF1.*

Only for 20 patients out of 123 (16.3%), 10 long responders and 10 poor responders, tumour samples from primitive CRC were available and selected for the analysis together with four CRC cancer cell lines (HT29, HT29 regorafenib resistant, HCT116 and HCT116 regorafenib and cetuximab resistant).

CELL LINES

The human HT29 and HCT116 colon cancer cell lines were obtained and authenticated from IRCCS 'Azienda Ospedaliera Universitaria San Martino-IST Istituto Nazionale per la Ricerca sul Cancro, Genova' Italy. HT29 regorafenib resistant and HCT116 regorafenib and cetuximab resistant were obtained after continuous exposure (6 and 8 months, respectively) to the drugs. Resistant clones had an IC_{50}100-fold higher than parental cells. HT29 cell lines were grown in McCoy (Lonza), whereas HCT116 cells were grown in RPMI-1640 (Lonza), supplemented with 10% foetal bovine serum (FBS; Lonza) and 1% penicillin/streptomycin (Lonza). All cell lines were grown in a humidified incubator with 5% of carbon dioxide (CO_2) and 95% air at 37°C.

STATISTICAL ANALYSIS

We used the Kaplan-Meier method to estimate median PFS time, and p values were calculated using log-rank tests at a significance level of 5%. Differences between categorical data within subgroups were measured using parametrical tests, χ^2 and Fisher's exact tests, when adequate. All statistical analyses were performed using IBM-SPSS statistics V.22.0.

RESULTS

From May 2012 to April 2016, we treated 123 mCRC patients with regorafenib 160 mg/day orally for the first 3 weeks of each 4-week cycle. Ninety-five patients out of 123 (77%) received <7 cycles of therapy and were defined as poor responders, whereas 28 patients out of 123 (23%) were treated for ≥7 cycles and defined as long responders. It is worth noting that 14 patients out of 28 long responders received more than 12 cycles of treatment. In the overall population, the median duration of treatment was 13.9 weeks (0.7–150.3 weeks); the median PFS was 3.41 months (95% CI 3.2 to 3.6 months) and median OS was 7.9 months (95% CI 6.8 to 9.0 months) (figures 1 and 2). Among patients treated for less than 7 cycles, the median duration of treatment was 12.3 weeks (7.6–15.1 weeks); conversely long responders were treated for a median of 50.5 weeks (35.6–65.9 weeks). For the poor responders subgroup, we reported a median PFS of 3.0 months (95% CI 2.9 to 3.2 months), whereas the long responders subgroup achieved a PFS of 11.6 months (95% CI 9.2 to 14.1 months) (p<0.0001) (figure 1). Regarding the OS, poor responders achieved 6.3 months (95% CI 5.5 to 7.0 months) of survival and

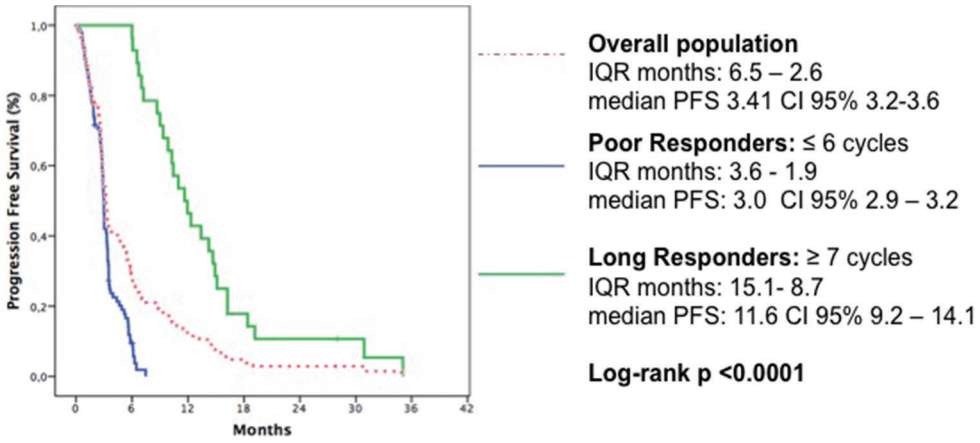

Figure 1 Kaplan-Meier of progression-free survival (PFS).

18.7 months (95% CI 14.3 to 23.0 months) (p<0.0001) were reported for long responders (figure 2).

Analysing the patients' baseline characteristics, according with the duration of treatment, we found that the subgroup of patients who received ≥7 cycles, the so-called long responders, were predominantly with ECOG PS 0 (p=0.03), had lung limited metastases (p=0.02) and had a long course of metastatic disease (time from diagnosis of metastatic disease of ≥18 months) (p=0.01) (table 2). Regarding treatment response, 42 patients (34%) achieved a stable disease (SD) as best response, 21 (50%) of them received less than 7 cycles and 16 patients (38%) were treated for ≥7 cycles. Five out of 42 patients (12%) who achieved an SD, presented lung metastases excavation at CT scan evaluation, four of them being treated for ≥7 cycles. Only 10 patients (8%) achieved a partial response (PR), and eight of them received ≥7 cycles.

Sixty-five patients (52.8%) out of 123 required a dose modification due to AEs, in particular 40 (61%) reduced the dose during the first six cycles of treatment, 25 of them required one dose level reduction (120 mg) mainly for hyperbilirubinemia, hypertransaminasemia and fatigue, while 15 patients required two dose levels reduction (80 mg), mainly due to fatigue and hyperb ilirubinemia (table 3). Twenty-five patients required a dose adjusting from ≥7 cycles, 14 of them required one dose level reduction (120 mg) mainly for HFSR, hyperbilirubinemia and fatigue while 11 patients received two dose levels reduction (80 mg) mainly for HFSR. Hyperbilirubinemia occurred more frequently during the first six cycles, whereas HFSR was mainly reported in patients receiving more than six cycles and did not cause treatment discontinuation. Notably, 51 out of 65 patients (78.4%) required a dose reduction during the first three cycles of treatment (table 3). The reason of discontinuation from treatment was radiological progression of disease in 72 patients out of 123 (58%), clinical PD in 38 patients (31%), while nine (8%) patients were discontinued for toxicity exclusively during the first six cycles of treatment. Finally, four patients (3%) refused to continue treatment.

For 22 of 123 (18%) patients, 11 poor responders and 11 long responders, FFPE tumour tissues were available and analysed with the OncoMine Comprehensive Assay. High-quality DNA was extracted from 20 samples (10 poor

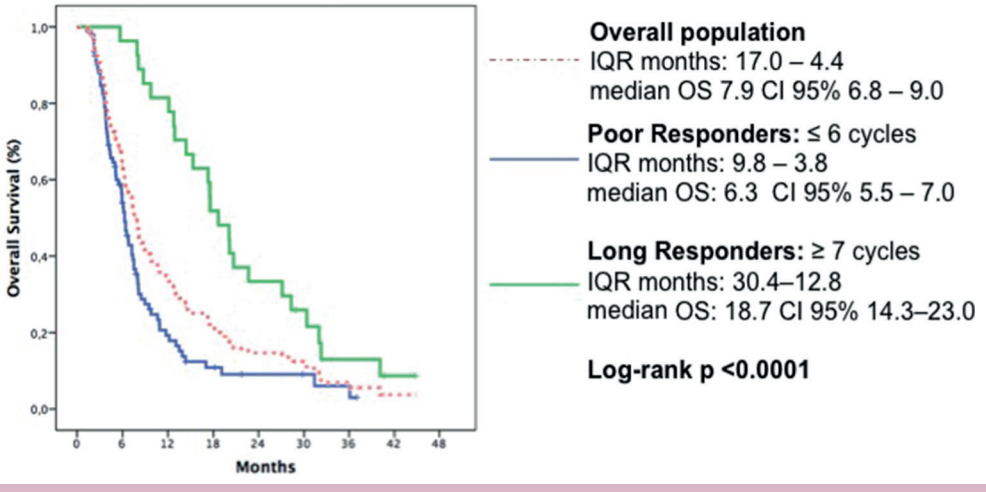

Figure 2 Kaplan-Meier of overall survival (OS).

Table 2 Correlation between clinical characteristics and duration of treatment

Patients characteristics	Poor responders <7 cycles		Long responders ≥7 cycles		p-Value
	n=95	IQR %	n=28	IQR%	
Median age (years)	61	52.8–66.9	63	56.8–71.2	0.48
Gender					
Male	58	61%	18	64%	0.5
Female	37	39%	10	36%	
Race					
White	95	100	28	100%	–
Eastern Cooperative Oncology Group performance status					
0	75	79%	28	100%	0.03*
1	14	15%	096352	0%	
2	6	6%	0	0%	
Primary site of disease					
Right colon	46	48%	16	57%	0.52
Left colon/rectum	49	52%	12	43%	
KRAS exon-2 mutation					
Yes	54	57%	11	39%	0.13
No	41	43%	17	61%	
Histology					
Adenocarcinoma	95	100%	28	100%	-
No of previous systemic anticancer therapies (from the diagnosis of metastatic disease)					
1	2	2%	0	0%	0.32
2	30	32%	6	21%	
3	35	37%	8	29%	
4	16	17%	10	36%	
≥5	12	13%	4	14%	
No of metastatic sites					
1	11	12%	5	18%	0.37
2	44	46%	15	54%	
3	25	26%	8	29%	
4	12	13%	0	0%	
5	2	2%	0	0%	
6	1	1%	0	0%	
Lung-limited metastatic disease					
Yes	4	4%	5	18%	0.02*
No	91	96%	23	82%	
Liver-limited metastatic disease					
Yes	5	5%	0	0%	0.56
No	93	98%	25	89%	
Time from diagnosis of metastatic disease					
Median (months)	29.6	18.0–42.21	41.4	31.6–55.0	
<18 months	24	25%	1	4%	
>18 months	71	71%	27	96%	0.01*

*$p < 0.05$.

Table 3 Toxicities and dose modification			
No of cycles	<7	≥7	
No of patients	95	28	N=123
No of patients with reduced dose	40	25	65 (52,8%)
Dose reduced from the first to third cycle	37	14	51
Dose reduced after the third cycle	3	11	14
Patients requiring one dose level reduction: 120 mg	25	14	39
Toxicities:	Hyperbilirubinemia (43%)	HFSR (44%)	
	Hypertransaminasemia (28%)	Hyperbilirubinemia (28%)	
	Fatigue (15%)	Fatigue (28%)	
	Rash (14%)		
Patients requiring two dose levels reduction: 80 mg	15	11	26
Toxicities:	Hyperbilirubinemia (56%)	HSFR (70%)	
	Fatigue (44%)	Fatigue (30%)	

responders and 10 long responders), allowing multiple gene mutation assessment possible in all these cases. As shown in table 4, no mutations in all tested genes were found in 2 of 20 (10%) cases, whereas one or more genes were mutated or amplified in 18 (90%) samples. The most frequently mutated gene was *TP53* (13/20, 65%), that was found mutated in seven long responder patients and six poor responders, respectively. In 6 (2 long responders and 4 poor responders) out of 20 (30%) samples, *KRAS* gene mutations were detected, with one tumour sample having two different *KRAS* mutations. *PIK3CA* mutations were found in 6 samples out of 20 (30%) samples (3 long responders and 3 poor responders) (table 4). Less frequent mutations were found in other 10 genes including *NRAS*, *ERBB2* (one poor responder patient), *SMAD4* (one long responder) and *PTEN* (see table 4 and online supplementary table S1 for more details).

The molecular analyses also revealed amplifications in the following genes: BCL2L1, ERBB2, KRAS, MYC and GAS6. One case of ALK rearrangement was found

Table 4 Molecular alterations in patients treated with regorafenib				
Molecular alterations	**Long responders (10)**	**Poor responders (10)**	**Total**	**p-Value (Fisher's exact test)**
ALK rearrangement	0	1	1	1
BCL2L1 amplification	1	0	1	1
DNMT3A mutation	1	0	1	1
EGFR mutation	1	0	1	1
ERBB2 amplification	0	2	2	0.47
ERBB2 mutation	0	1	1	1
FBXW7 mutation	0	1	1	1
IDH2 mutation	0	1	1	1
JAK1 mutation	1	0	1	1
KRAS mutation	4	2	6	0.62
KRAS amplification	0	1	1	1
MYC amplification	0	1	1	1
NOTCH1 mutation	1	0	1	1
NRAS mutation	1	0	1	1
PIK3CA mutation	3	3	6	1
PTEN mutation	1	0	1	1
TP53 mutation	7	6	13	1
GAS6 amplification	1	0	1	1
SMAD4 mutation	1	0	1	1
Wild-type for all analysed genes	1	1	2	1

(table 4). No significant correlation between molecular alteration and response to regorafenib was found.

Regarding the human CRC cell lines, no different molecular profile was detected between parental and regorafenib-resistant clones (see online supplementary table S2).

DISCUSSION

Regorafenib displayed a survival benefit in unselected patients with mCRC refractory to standard treatments according to two randomised phase III clinical trials. However, considering the toxicity profile and the lack of predictive biomarkers, a better definition of patients who might derive a benefit from regorafenib treatment, avoiding unnecessary AEs, is needed.

In this study, we have evaluated potential clinical and molecular predictors of regorafenib efficacy, by performing an extensive NGS analysis in a subgroup of patients with different outcome following regorafenib treatment. We have treated a consecutive cohort of 123 mCRC patients with regorafenib, reporting a median OS (7.9 months) and PFS (3.4 months) in the overall population in line with the reported data from CORRECT and CONCUR phase III trials, in which the OS and PFS were 6.4 months and 1.9 months and 8.8 months and 3.2 months, respectively.

We stratified patients into two groups according to the number of cycles received (<7 and ≥7 cycles); the cut-off of 7 cycles was selected considering the median duration of treatment (3.3 cycles) and quartile stratification (IQR 6.51–2.01). Our data showed that the outcome in the so-called long responders group was significantly better. In particular, our analysis supports the idea that a good ECOG PS, the presence of a lung limited metastatic disease and a long history of metastatic disease (≥18 months) are significantly associated with better clinical outcome in patients with mCRC treated with regorafenib.

We did not find unexpected AEs as compared with previously reported data. Hyperbilirubinemia occurred more frequently during the first six cycles, whereas HFSR was mainly found in patients receiving more than six cycles and did not cause treatment discontinuation. Although most of patients required a regorafenib dose reduction, even during the first cycle of treatment, the efficacy of the drug was not impaired. The dose adjustment would have avoided the withdrawal from the treatment due to AEs. Taken together, our data support the importance of an intensive clinical monitoring (weekly clinical visit during the first month with physical and biochemistry assessment) and the prophylactic use of moisturising creams in order to prevent and promptly recognise the AEs and obtain the maximum benefit from regorafenib.

Furthermore, in order to identify potential molecular alterations associated with regorafenib activity, we analysed 143 cancer genes by NGS of genomic DNA from 20 patient tumour specimens and 4 human cancer cell lines. One or more genes were mutated or amplified in the majority of samples. As expected, the most frequently mutated genes were *TP53*, *KRAS* and *PIK3CA*. Less frequent mutations included *NRAS*, *ERBB2*, *SMAD4* and *PTEN* genes together with BCL2L1, ERBB2, KRAS, MYC, GAS6 gene amplification. One case of ALK rearrangement was found. No significant correlation between molecular alterations and response to regorafenib was observed. However, HER2 gene alterations (one mutation and two amplifications) were found in three patients that were rapidly progressing on regorafenib, suggesting a potential HER2 involvement in regorafenib resistance. Of note, GAS6 (the AXL receptor ligand) amplification and SMAD4 (an intracellular transducer of downstream TGF-β-receptor activation) mutation were detected in two long responder patients, respectively.[11] In particular, the patient with SMAD4 mutation was treated with regorafenib for 33 cycles, and to date he is still alive. The activation of AXL and/or TGF-β pathways suggests the epithelial–mesenchymal transition (EMT) phenotype of these tumours, confirming the previous observations of a greater PFS benefit for regorafenib in patients defined as 'high-risk' subgroup, according to Marisa molecular subtypes (C4 and C6), corresponding with an upregulation of EMT pathway.[12] Regarding the human CRC cell lines, no different molecular profile was detected between parental and regorafenib resistant clones (see online supplementary table S2).

Although this is a retrospective, exploratory and hypothesis generating analysis, in which we molecularly characterised 20 out of 123 patients treated with regorafenib at our institution, the data presented are interesting and deserve farther investigation.

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Funding EM had financial support from Associazione Italiana per la Ricerca sul cancro (MFAG-2015-ID:7778).

Competing interests None declared.

Patient consent Patients signed the local informed consent form.

Ethics approval Local Ethic Committee.

Provenance and peer review Not commissioned; internally peer reviewed.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. *CA: A Cancer Journal for Clinicians* 2016;66:7–30.
2. Van Cutsem E, Cervantes A, Adam R, *et al*. ESMO consensus guidelines for the management of patients with metastatic colorectal Cancer. *Ann Oncol* 2016;27:1386–422.
3. Wilhelm SM, Dumas J, Adnane L, *et al*. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. *Int J Cancer* 2011;129:245–55.
4. 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.
5. Li J, Qin S, Xu R, *et al*. Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2015;16:619–29.
6. Van Cutsem E, Ciardiello F, Seitz JF, *et al*. Results from the large, open-label phase 3b CONSIGN study of regorafenib in patients with previously treated metastatic colorectal cancer [abstract]. *Ann Oncol* 2015.
7. Giampieri R, Salvatore L, Del Prete M, *et al*. Angiogenesis genotyping and clinical outcome during regorafenib treatment in metastatic colorectal Cancer patients. *Sci Rep* 2016;6:25195.
8. Del Prete M, Giampieri R, Loupakis F, *et al*. Prognostic clinical factors in pretreated colorectal Cancer patients receiving regorafenib: implications for clinical management6:33982–92, 10.18632/. *Oncotarget* 2015;6:591.
9. Tabernero J, Lenz HJ, Siena S, *et al*. Analysis of circulating DNA and protein biomarkers to predict the clinical activity of regorafenib and assess prognosis in patients with metastatic colorectal Cancer: a retrospective, exploratory analysis of the CORRECT trial. *Lancet Oncol* 2015;16:937–48.
10. McLellan B, Ciardiello F, Lacouture ME, *et al*. Regorafenib-associated hand-foot skin reaction: practical advice on diagnosis, prevention, and management. *Ann Oncol* 2015;26:2017–26.
11. Martinelli E, Martini G, Cardone C, *et al*AXL is an oncotarget in human colorectal Cancer. *Oncotarget* 2015;6:23281–96.
12. Teufel M, Schwenke S, Seidel H, *et al*. Molecular subtypes and outcomes in regorafenib-treated patients with metastatic colorectal Cancer (mCRC) enrolled in the CORRECT trial. [abstr 3558]. *J Clin Oncol* 2015.