

First-Line Cetuximab Monotherapy in *KRAS/NRAS/BRAF* Mutation-Negative Colorectal Cancer Patients

Vladimir M. Moiseyenko¹ · Fedor V. Moiseyenko^{1,2,3} · Grigoriy A. Yanus^{2,4} · Ekatherina Sh. Kuligina² · Anna P. Sokolenko^{2,4} · Ilya V. Bizin⁵ · Alexey A. Kudriavtsev¹ · Svetlana N. Aleksakhina² · Nikita M. Volkov¹ · Vyacheslav A. Chubenko¹ · Kseniya S. Kozyreva¹ · Mikhail M. Kramchaninov^{1,4} · Alexandr S. Zhuravlev¹ · Kseniya V. Shelekhova¹ · Denis V. Pashkov⁶ · Alexandr O. Ivantsov^{2,4} · Aigul R. Venina² · Tatyana N. Sokolova² · Elena V. Preobrazhenskaya^{2,4} · Natalia V. Mitiushkina² · Alexandr V. Togo^{2,4} · Aglaya G. Iyevleva^{2,4} · Evgeny N. Imyanitov^{1,2,3,4,7}

© Springer International Publishing AG, part of Springer Nature 2018

Abstract

Background Colorectal carcinomas (CRCs) are sensitive to treatment by anti-epidermal growth factor receptor (EGFR) antibodies only if they do not carry activating mutations in down-stream EGFR targets (*KRAS/NRAS/BRAF*). Most clinical trials for chemo-naïve CRC patients involved combination of targeted agents and chemotherapy, while single-agent cetuximab or panitumumab studies

included either heavily pretreated patients or subjects who were not selected on the basis of molecular tests. We hypothesized that anti-EGFR therapy would have significant efficacy in chemo-naïve patients with *KRAS/NRAS/BRAF* mutation-negative CRC.

Methods Nineteen patients were prospectively included in the study.

Results Two (11%) patients experienced partial response (PR) and 11 (58%) subjects showed stable disease (SD). Median time to progression approached 6.1 months (range 1.6–15.0 months). Cetuximab efficacy did not correlate with RNA expression of *EGFR* and insulin-like growth factor 2 (*IGF2*). Only one tumor carried *PIK3CA* mutation, and this CRC responded to cetuximab. Exome analysis of patients with progressive disease (PD) revealed 1 CRC with high-level microsatellite instability and 1 instance of *HER2* oncogene amplification; 3 of 4 remaining patients with PD had allergic reactions to cetuximab, while none of the subjects with PR or SD had this complication. Comparison with 19 retrospective *KRAS/NRAS/BRAF* mutation-negative patients receiving first-line fluoropyrimidines revealed no advantages or disadvantages of cetuximab therapy.

Conclusions Cetuximab demonstrates only modest efficacy when given as a first-line monotherapy to *KRAS/NRAS/BRAF* mutation-negative CRC patients. It is of question, why meticulous patient selection, which was undertaken in the current study, did not result in the improvement of outcomes of single-agent cetuximab treatment.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s40261-018-0629-1>) contains supplementary material, which is available to authorized users.

✉ Evgeny N. Imyanitov
evgeny@imyanitov.spb.ru

¹ City Cancer Center, Saint Petersburg 197758, Russia

² Laboratory of Molecular Oncology, N.N. Petrov Institute of Oncology, Pesochny-2, Saint Petersburg 197758, Russia

³ I.I. Mechnikov North-Western Medical University, Saint Petersburg 191015, Russia

⁴ St. Petersburg Pediatric Medical University, Saint Petersburg 194100, Russia

⁵ St. Petersburg State Polytechnical University, Saint Petersburg 195251, Russia

⁶ S.M. Kirov Military Medical Academy, Saint Petersburg 194044, Russia

⁷ St. Petersburg State University, Saint Petersburg 199034, Russia

Key Points

This study evaluated upfront cetuximab monotherapy in 19 *KRAS/NRAS/BRAF* mutation-negative CRC patients; 2 (11%) partial responses and 11 (58%) instances of disease stabilization were observed.

Median time to progression approached 6.1 months.

Exome sequencing of tumors from patients with progressive disease suggested that microsatellite instability and HER2 amplification may render resistance to cetuximab.

1 Introduction

There is a diversity of drugs utilized for the therapy of metastatic colorectal cancer (CRC), including fluoropyrimidines, oxaliplatin, irinotecan, antiangiogenic agents and anti-epidermal growth factor receptor (EGFR) antibodies. These agents are usually applied in combination, especially in the early lines of treatment, given that the drug doublets or triplets produce significantly higher response rates (RR) and longer progression-free survival (PFS) as compared to single agents [1]. An alternative approach implies sequential use of the CRC-specific drugs, at least in some clinical circumstances [2]. Proponents of sequential therapy acknowledge its good safety profile, potential availability of distinct medical options during almost the entire duration of the treatment, and non-inferior results of overall outcomes demonstrated in some clinical trials. Although there are some clinical criteria guiding the choice between different CRC drugs, acting either in combination or alone, the selection of optimal treatment, especially in the first line, remains largely empirical [3].

While the indications for the use of cytotoxic drugs and antiangiogenic compounds remained relatively constant in the past, there was a remarkable progress with regard to personalization of anti-EGFR therapy. First clinical trials on cetuximab and panitumumab involved CRC patients selected on the basis of tumor immunohistochemical (IHC) staining for EGFR. This inclusion criteria later turned out to be erroneous, given that EGFR IHC-positive CRC patients have the same treatment outcomes as the EGFR non-expressors [4–6]. It was subsequently revealed that CRC with mutated *KRAS* oncogene have almost null probability of response to EGFR-specific therapy, and the appropriate testing was quickly approved for the clinical

use [7]. This allowed the exclusion of approximately 40% of CRC cases from anti-EGFR therapy, thus significantly enriching the patient population by potential responders. Early genetic tests considered only the most frequent *KRAS* mutations, i.e. nucleotide substitutions affecting codons 12 and 13. Additional studies revealed that other mutations in *KRAS* oncogene, including hot-spots in codons 61 and 146, as well as *NRAS* mutations (codons 12, 13, 61, 146, etc.), also contribute to resistance to cetuximab and panitumumab. Current treatment guidelines require comprehensive *KRAS/NRAS* testing, by which more than a half of CRC patients turn out to be illegible to receive anti-EGFR therapy [8]. In addition, a couple of studies demonstrated the role of *BRAF* mutations in determining non-response to EGFR-specific antibodies [9]. Although not included in minimal diagnostic standards, *BRAF* testing is likely to allow further enrichment of patient population by potential responders.

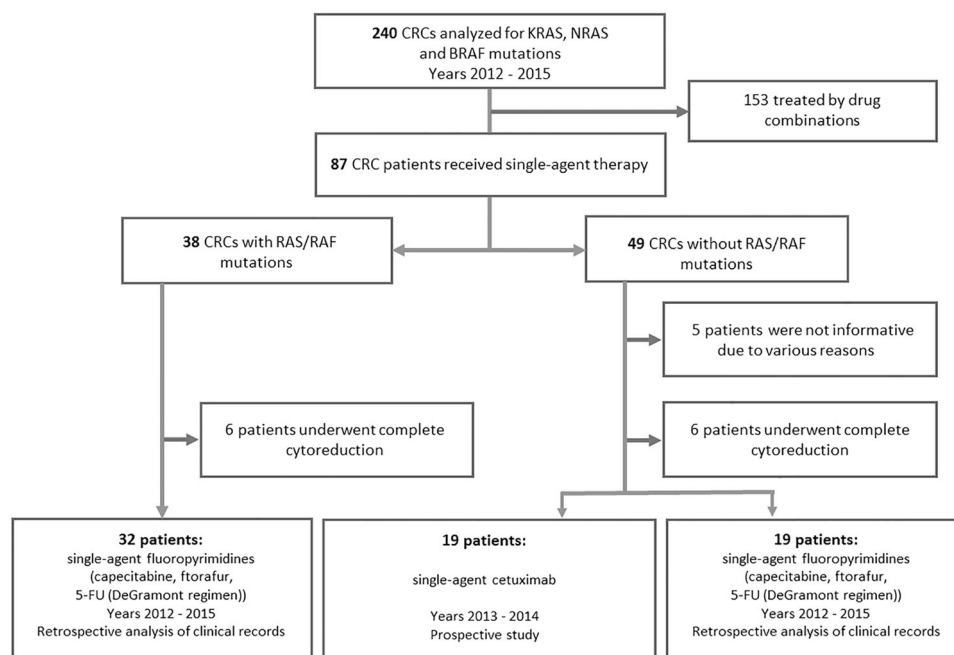
Early trials on cetuximab or panitumumab involved heavily pretreated, genetically unselected patients, however demonstrated response rates close to 10% [10]. Given that comprehensive *KRAS/NRAS/BRAF* analysis may increase the proportion of potential responders by approximately three times, and that many known cancer drugs demonstrate higher efficacy in early lines of therapy, it appears feasible to investigate the activity of anti-EGFR monotherapy in the first-line setting. Upfront monotherapy by targeted agents allows to delay administration of cytotoxic drugs and is frequently used in the treatment of breast, prostate, lung and some other cancer types [11–13]. Here, we present the results of a prospective trial of first-line cetuximab in *KRAS/BRAF/NRAS* mutation-negative patients with metastatic colorectal cancer, coupled with additional molecular analysis of tumor tissue.

2 Patients and Methods

2.1 Patients

Single-agent cetuximab was offered to 19 patients with treatment-naïve, asymptomatic, metastatic, *KRAS/BRAF/NRAS* mutation-negative CRC, who were referred to the Cancer City Center (St. Petersburg, Russia) between August 2013 and October 2014. Patients, who experienced dramatic disease manifestation or looked to be potential candidates for subsequent cytoreductive surgery, were excluded from the screening and subjected to combination therapy. Cetuximab was administered intravenously at the loading dose of 400 mg/m² followed by 250 mg/m² weekly. All patients provided informed consent. The study was approved by the local Ethics Committee. In addition to prospective study of CRC patients receiving cetuximab, we

Fig. 1 Flow-chart diagram describing patient selection. 5-FU 5-fluoropyrimidines, CRC colorectal cancer



also performed a retrospective analysis of treatment outcomes for patients receiving other single-agent therapies (Fig. 1).

2.2 Molecular Analysis

Patient selection was based on the exclusion of CRCs carrying *KRAS*, *NRAS* or *BRAF* mutations. Nucleic acid isolation was performed according to a standard protocol [14]. Nucleotide substitutions in hot-spot codons were detected as described by Yanus et al [15]. *KRAS* and *NRAS* exons 2–4 were further analyzed by the high-resolution melting (HRM) and Sanger sequencing in CRC samples lacking the recurrent mutations in the above genes (see primers in supplementary Table S1)

All colorectal carcinomas included in the cetuximab study were tested for a number of potential predictive markers. *PIK3CA* analysis was performed as reported in Yanus et al [15]. *EGFR* expression and *IGF2* expression were tested using quantitative real-time PCR (see primers in supplementary Table S1). In addition, based on the results obtained by the whole exome sequencing (WES), cetuximab-treated CRCs were analyzed for microsatellite instability [15] and *HER2* amplification (supplementary Table S1).

WES analysis of tumor DNA was performed for those CRCs that demonstrated the disease progression on cetuximab monotherapy. Preparation of libraries was done using Nextera Rapid Capture Exome kit. Sequencing runs were performed on the MiSeq instrument (Illumina, USA) with at least 50× coverage. Bioinformatic analysis utilized

MuTect2 and GATK software tools for the identification of somatic mutational events [16]. Consideration of potentially significant (driver) mutation relied on the known functions of involved genes as well as on the Combined Annotation Dependent Depletion (CADD) scores [17].

2.3 Statistical Analysis

Fisher's exact test and chi-squared test for linear trend were used to assess the association between objective response and clinical parameters of CRC (primary CRC location, characteristics of metastatic lesions, allergic reactions, skin toxicity, etc.). Continuous variables (time to progression, overall survival, RNA expression, etc.) were evaluated by Independent-Sample Median Test. The $P < 0.05$ was set at a threshold for statistical significance. All statistical analyses were performed using SPSS Statistics software (Version 23.0, Armonk, NY 10504-1722, USA).

3 Results

Front-line cetuximab was applied to 19 *KRAS/NRAS/BRAF* mutation-negative patients with metastatic CRC (Table 1). No patients demonstrated complete response (CR). Two (11%) subjects (#11 and #20) experienced partial response (PR); the duration of response was 6.1 and 7.9 months, respectively. Eleven (58%) patients showed disease stabilization and 6 (32%) had progressive disease. The total rate of clinical benefit was 68%. Median time to progression approached 6.1 months (range 1.6–15.0 months).

Table 1 Characteristics of *KRAS/NRAS/BRAF* mutation-negative CRC patients treated by first-line cetuximab

Patient characteristics				Front-line cetuximab treatment							
ID	Age, gender	Primary CRC location	Prior surgery	Metastatic lesions	Clinical data				Response		
					Tolerability						
					Maximal toxicity grade (except allergy)	Skin toxicity grade	Paronychia or skin fissures (grade)	Allergic reactions	Dose reduction	RECIST (%)	Time to progression, months
Partial response											
11	Female, 62	Left	Palliative	Liver, lungs	2	2	1	0	No	PR (− 33%)	6.1
20	Male, 72	Right	Radical	Liver, peritoneum	2	2	0	0	No	PR (− 31%)	7.9
Stable disease											
2	Female, 62	Left	Palliative	Liver, lymph nodes	2	1	0	0	No	SD (+ 15%)	7.8
7	Female, 63	Left	Radical	Lymph nodes, lungs	2	2	2	0	No	SD (+ 15.5%)	9.8
12	Female, 77	Right	None	Liver, lungs	2	1	2	0	No	SD (− 29%)	9.2
21	Male, 60	Left	Palliative	Liver	2	2	0	0	No	SD (− 10%)	7.4
22	Female, 65	Left	Radical	Liver, lymph nodes, lungs	2	2	0	0	No	SD (+ 5%)	6.0
27	Male, 76	Left	Radical	Lymph nodes	1	1	0	0	No	SD (− 12%)	15.0
29	Female, 68	Left	Palliative	Liver	2	2	0	0	No	SD (− 25%)	5.7
36	Male, 82	Right	Radical	Lungs, pleura	2	1	2	0	Yes	SD (− 5%)	7.8
41	Male, 65	Left	Palliative	Liver, lymph nodes	2	2	0	0	No	SD (+ 13%)	6.4
44	Female, 70	Right	Radical	Liver, lungs	1	1	0	0	No	SD (+ 18%)	2.8
45	Male, 85	Left	None	Liver, lungs, soft tissues	2	2	0	0	No	SD (− 4%)	9.0
Progressive disease											
8	Female, 73	Left	Radical	Liver, lymph nodes	2 (pruritis)	1	0	3	No	PD (+ 40%)	1.6
14	Male, 49	Left	Radical	Lungs, lymph nodes	3	3	0	0	No	PD (+ 27%; new lesions)	1.7
24 ^b	Female, 78	Right	Radical	Lymph nodes	1	1	0	0	No	PD (+ 48%)	3.8*
31	Male, 62	Right	None	Peritoneum	1	1	0	3	No	PD (+ 45%; new lesions)	1.7
34	Male, 60	Left	None	Liver	2	2	0	3	Yes	PD (+ 29%; new lesions)	1.7
40	Female, 66	Left	None	Peritoneum	3	3	0	0	No	PD (+ 45%; new lesions)	1.8

Table 1 continued

Patient characteristics					Front-line cetuximab treatment						
ID	Age, gender	Primary CRC location	Prior surgery	Metastatic lesions	Molecular analysis		Whole exome sequencing (WES)		Post-WES tests		
					Candidate gene tests						
					<i>PIK3CA</i>	<i>EGFR</i> expression ^a	<i>IGF2</i> expression ^a			<i>HER2</i> amplification	MSI
Partial response											
11	Female, 62	Left	Palliative	Liver, lungs	E542K	0.17	9.2	No	No	No	MSS
20	Male, 72	Right	Radical	Liver, peritoneum	wt	0.06	13034.0	No	No	No	MSS
Stable disease											
2	Female, 62	Left	Palliative	Liver, lymph nodes	wt	0.08	32.0	No	No	No	MSS
7	Female, 63	Left	Radical	Lymph nodes, lungs	wt	0.05	42.2	No	No	No	MSS
12	Female, 77	Right	None	Liver, lungs	wt	0.05	2.1	No	No	No	MSS
21	Male, 60	Left	Palliative	Liver	wt	0.08	1024.0	No	No	No	MSS
22	Female, 65	Left	Radical	Liver, lymph nodes, lungs	wt	0.05	3327.0	No	Yes	Yes	MSS
27	Male, 76	Left	Radical	Lymph nodes	wt	0.09	256.0	No	No	No	MSS
29	Female, 68	Left	Palliative	Liver	wt	0.14	512.0	No	No	No	MSS
36	Male, 82	Right	Radical	Lungs, pleura	wt	nd	nd	No	No	No	MSS
41	Male, 65	Left	Palliative	Liver, lymph nodes	wt	0.03	45.2	No	No	No	MSS
44	Female, 70	Right	Radical	Liver, lungs	wt	nd	nd	No	No	No	MSS
45	Male, 85	Left	None	Liver, lungs, soft tissues	wt	0.11	3.0	No	No	No	MSS
Progressive disease											
8	Female, 73	Left	Radical	Liver, lymph nodes	wt	nd	17.8	Yes	No	No	MSS
14	Male, 49	Left	Radical	Lungs, lymph nodes	wt	0.03	2.8	Yes	Yes	Yes	MSS
24 ^b	Female, 78	Right	Radical	Lymph nodes	wt	0.14	0.7	Yes	No	No	MSI
31	Male, 62	Right	None	Peritoneum	wt	0.22	33.8	Yes	No	No	MSS
34	Male, 60	Left	None	Liver	wt	0.44	nd	Yes	No	No	MSS
40	Female, 66	Left	None	Peritoneum	wt	0.17	29.9	Yes	No	No	MSS
Patient characteristics					Post-cetuximab treatment				Overall survival, months		
ID	Age, gender	Primary CRC location	Prior surgery	Metastatic lesions	Overall number of lines		Oxaliplatin and/or irinotecan		Aggressive second line (mono vs polychemo)		
Partial response											
11	Female, 62	Left	Palliative	Liver, lungs	3		Yes	Yes	Yes	17.0	
20	Male, 72	Right	Radical	Liver, peritoneum	1		Yes	Yes	Yes	22.2	
Stable disease											
2	Female, 62	LEFT	Palliative	Liver, lymph nodes	2		Yes	Yes	Yes	30.6	
7	Female, 63	Left	Radical	Lymph nodes, lungs	1		No	No	No	14.9	
12	Female, 77	Right	None	Liver, lungs	1		Yes	Yes	Yes	13.4	
21	Male, 60	Left	Palliative	Liver	1		Yes	Yes	Yes	21.6	
22	Female, 65	Left	Radical	Liver, lymph nodes, lungs	1		Yes	Yes	Yes	18.7	
27	Male, 76	Left	Radical	Lymph nodes	2		Yes	Yes	Yes	36+	

Table 1 continued

Patient characteristics				Post-cetuximab treatment		Overall survival, months		
ID	Age, gender	Primary CRC location	Prior surgery	Metastatic lesions	Overall number of lines			
29	Female, 68	Left	Palliative	Liver	2	Yes	Yes	25.3
36	Male, 82	Right	Radical	Lungs, pleura	1	No	No	14.8
41	Male, 65	Left	Palliative	Liver, lymph nodes	1	No	No	15.3
44	Female, 70	Right	Radical	Liver, lungs	2	Yes	No	10.9
45	Male, 85	Left	None	Liver, lungs, soft tissues	0	–	–	14.1
Progressive disease								
8	Female, 73	Left	Radical	Liver, lymph nodes	1	No	No	18.9
14	Male, 49	Left	Radical	Lungs, lymph nodes	1	No	No	7.7
24 ^b	Female, 78	Right	Radical	Lymph nodes	2	No	No	11.6
31	Male, 62	Right	None	Peritoneum	2	Yes	Yes	8.2
34	Male, 60	Left	None	Liver	1	No	No	4.0
40	Female, 66	Left	None	Peritoneum	2	No	No	10.7

CRC colorectal cancer, *MSI* microsatellite instability, *MSS* microsatellite stability, *nd* no data, *PD* progressive disease, *PR* partial response, *RECIST* response evaluation criteria in solid tumors, *SD* stable disease, *WES* whole exome sequencing

^aExpression levels of *EGFR* and *IGF2* are presented as $2^{\Delta\Delta CT}$ (ref – target)

^bPatient #24 missed scheduled disease assessments, and underwent first tumor imaging only at 3.8 months after the beginning of cetuximab therapy; however, this case was classified as PD based on the large extent of tumor enlargement

Table 2 Correlations between CRC characteristics and outcome of cetuximab therapy

Clinical and molecular parameters	Response (RECIST)		<i>P</i> value	Time to progression, (median, months)	<i>P</i> value
	Progressive disease (PD), %	Clinical benefit (OR + SD), %			
Allergic reactions			0.021		0.004
No (<i>n</i> = 16)	3 (18.8%)	13 (81.3%)		6.9 [1.7–15.0]	
Yes (<i>n</i> = 3)	3 (100%)	0 (0.0%)		1.7 [1.6–1.7]	
Primary CRC location			1.000		0.898
Left (<i>n</i> = 13)	4 (30.8%)	9 (69.2%)		6.1 [1.6–15.0]	
Right (<i>n</i> = 6)	2 (33.3%)	4 (66.7%)		5.8 [1.7–9.2]	
Metastatic lesions			0.046		0.701
Liver (<i>n</i> = 13)	2 (15.4%)	11 (84.6%)		6.4 [1.6–9.2]	
Other (<i>n</i> = 6)	4 (66.7%)	2 (33.3%)		2.8 [1.7–15.0]	
<i>EGFR</i>	0.17 [0.03–0.44]	0.08 [0.03–0.17]	0.090	Spearman's ρ correlation: – 0.420	0.105
RNA expression, median					
<i>IGF2</i>	17.8 [0.73–33.8]	45.2 [2.14–13034.0]	0.069	Spearman's ρ correlation: 0.227	0.399
RNA expression, median					

CRC colorectal cancer, PD progressive disease, OR objective response, RECIST response evaluation criteria in solid tumors, SD stable disease

We considered clinical predictors of cetuximab efficacy. Allergic reactions to cetuximab were significantly associated with treatment failure as all three patients demonstrating this adverse effect showed progressive disease ($P = 0.021$). Patients with metastatic spread to the liver tended to have a higher probability of clinical benefit as compared to other CRC cases ($P = 0.046$). There were no differences in cetuximab sensitivity for the left-sided versus right-sided tumors (Table 2).

The response to anti-EGFR antibodies is known to be independent of the level of *EGFR* expression as determined by immunohistochemical (IHC) analysis [4–6]. IHC diagnostic antibodies usually recognize a certain epitope of the target molecule and may therefore miss some protein isoforms. We hypothesized that the measurement of *EGFR* expression on the level of RNA may be of value in predicting the response to cetuximab; however, the amount of *EGFR* transcript did not correlate with the treatment outcome (Tables 1, 2).

PIK3CA mutations may contribute to the EGFR-independent activation of mTOR signaling pathways and were long considered predictive for resistance to EGFR-directed therapy. The analysis of predictive value of *PIK3CA* mutation is compromised by their frequent coexistence with *RAS* mutations [15]. In agreement with this, only 1 out of 19 *KRAS/NRAS/BRAF*-mutation-negative tumors included in the study contained *PIK3CA* mutation. This patient was characterized by a partial response to cetuximab (Table 1).

Insulin-like growth factor 2 (*IGF2*) overexpression was shown to be associated with poor therapeutic efficacy of

EGFR inhibition [18]. However, the amount of *IGF2* did not correlate with cetuximab efficacy in the present data set (Tables 1, 2).

Tumors from all six patients characterized by the disease progression upon first-line cetuximab treatment were subjected to the whole exome sequencing. Plausible explanation for the lack of response to cetuximab was obtained in 2 cases (CRC #14 and CRC #24). CRC #14 with upfront progression on anti-EGFR therapy was shown to carry *HER2* amplification (Table 1), which was accompanied by evident *HER2* overexpression (data not shown). We further analyzed *HER2* status in the remaining CRCs. One additional case of *HER2* amplification and overexpression was identified; this patient (#22, Table 1) was characterized by stable disease lasting for 6 months. Another CRC progressing on cetuximab therapy was shown to have a high-level microsatellite instability, MSI-H (patient #24, Table 1). None of other tumors had microsatellite instability-high (MSI-H) phenotype (Table 1). The exome analysis of tumor DNA from the remaining four patients did not reveal overt candidates for determinants of cetuximab resistance (data not shown); it is essential to keep in mind that three of these four patients showed allergic reactions to cetuximab, which could significantly compromise the response to the treatment.

We further retrieved retrospective data on patients receiving single-agent cytotoxic fluoropyrimidine therapy as an upfront treatment. Treatment outcomes were similar between *KRAS/NRAS/BRAF* wild-type patients receiving either anti-EGFR antibody or 5-fluorouracil (5-FU) based regimens (Table 3; supplementary Tables S2, S3).

Table 3 Treatment outcomes in CRC patients receiving single-agent first-line therapy

Characteristics of CRC patients	<i>KRAS/NRAS/BRAF</i> wild-type		<i>KRAS/NRAS/BRAF</i> mutated	<i>P</i> value
	Cetuximab (<i>n</i> = 19)	Fluoropyrimidines (<i>n</i> = 19)	Fluoropyrimidines (<i>n</i> = 32)	
Age (median [range], years)	66 [49–85]	64 [44–84]	68 [56–87]	0.814
Gender				0.546
Males	9 (47.4%)	9 (47.4%)	11 (34.4%)	
Females	10 (52.6%)	10 (52.6%)	21 (65.6%)	
Number of metastatic zones				0.053
0	0 (0%)	1 (5.3%)	1 (3.1%)	
1	7 (36.8%)	16 (84.2%)	21 (65.6%)	
2	10 (52.6%)	2 (10.5%)	8 (25.0%)	
3	2 (10.5%)	0 (0.0%)	2 (6.3%)	
CRC location				0.753
Left	13 (68.4%)	15 (78.9%)	24 (75.0%)	
Right	6 (31.6%)	4 (21.2%)	8 (25.0%)	
Tumor response				0.928
Objective response	2 (10.5%)	1 (5.2%)	2 (6.3%)	
Stable disease	11 (57.9%)	13 (68.4%)	22 (68.8%)	
Progressive disease	6 (31.5%)	5 (26.3%)	8 (25.0%)	
Time to progression (median [range], months)	6.1 [1.6–15.0]	6.7 [1.4–18.0]	3.8 [0.5–21.2]	0.150
Overall survival (median [range], months)	14.9 [4.0–36.0]	18.4 [3.8–68.5]	12.7 [2.4–42.5]	0.169

CRC colorectal cancer

Fluoropyrimidine-treated patients with mutated *KRAS* or *NRAS* showed numerically shorter time to progression and overall survival as compared to patients with the wild-type *RAS*, although this difference did not reach statistical significance (Table 3).

4 Discussion

There are a significant number of studies assessing anti-EGFR monotherapy CRC patients, but most involved heavily pretreated patients and/or did not utilize mutation tests for exclusion of potential non-responders [5, 6, 10, 19–26]. Despite these apparent limitations, objective tumor response was observed in approximately 10% of analyzed subjects. Few studies utilized a distinct design. Pessino et al [27] administered cetuximab to 31 chemo-naïve patients with advanced CRC. Despite the fact that *RAS* testing was not exercised at the time of this study, 1 patient had CR, 3 experienced PR and 13 subjects had disease stabilization; the time-to-progression in 4 responders approached clinically meaningful estimates, being 12, 9, 9 and 6 months, respectively. Similar investigation involving 41 elderly patients was undertaken by Sastre et al [28]; 18 patients were *KRAS* wild-type, 5 had *KRAS* mutation and 18 had unknown *KRAS* status. Five of 18

patients with wild-type *KRAS* experienced objective tumor response, and 7 patients remained progression-free at 12 weeks since the beginning of cetuximab therapy. Contrary to other single-agent anti-EGFR therapy studies, Pietrantonio et al [29] implemented comprehensive molecular testing for the patient selection and excluded subjects with *KRAS*, *NRAS* and *BRAF* mutations from the treatment. This study included 40 elderly subjects, 10 of whom were chemo-naïve and 30 who had experienced prior chemotherapy. Among 10 patients receiving upfront cetuximab, PR was documented in 4 (40%) cases, and 3 (30%) subjects had stable disease (all lasting for more than 4 months); progression-free and overall survival approached 7.0 and 12.3 months, respectively. Similar estimates were obtained in 30 subjects receiving cetuximab after chemotherapy failure.

Our patient series is apparently the largest study utilizing both the administration of anti-EGFR single-agent therapy in treatment-naïve patients and the use of comprehensive molecular testing for exclusion of potentially resistant patients. Despite these apparent strengths, the clinical outcomes of this study are modest at best, demonstrating a relatively low response rate (11%) and a limited number of patients experiencing disease control (68%). However, the duration of the time to progression in patients with clinical benefit from cetuximab appears to be

clinically acceptable, being 6 months or longer in 11/13 (85%) cases. It is important to acknowledge, that some of the patients, who were included in this study and progressed on cetuximab, would have been likely to have received other treatment options if they were diagnosed today (#14 and # 24, see Table 1). For example, MSI-H CRCs benefit significantly from immune checkpoint modulators [30], and HER2-driven CRCs are likely to be considered for HER2-specific therapy [31]. Three of six patients with progressive disease suffered from cetuximab-induced grade 3 allergy, and all of these three patients lacked known molecular determinants associated with anti-EGFR therapy resistance. One may speculate that the lack of benefit from EGFR-directed treatment in these patients is related, not to the tumor resistance but to systemic reaction to the drug.

5 Conclusion

This investigation considered colorectal cancer patients receiving single-agent therapy as an upfront treatment. The observed outcomes were relatively poor, irrespective of whether these patients received cetuximab (prospective study) or fluoropyrimidines (retrospective analysis) (Table 3). This can be attributed both to the limited efficacy of monotherapy per se and by negative selection of the patients; indeed, current guidelines encourage more effective combination regimens to virtually all subjects with newly diagnosed metastatic CRC, who may be considered as potential candidates for cytoreductive surgery and/or likely to tolerate intensive systemic treatment [1, 3]. Cetuximab demonstrated only modest therapeutic activity when given as a monotherapy to treatment-naïve *KRAS/NRAS/BRAF* mutation-negative metastatic CRC patients. The efficacy of cetuximab in this category of CRC cases was comparable to that of single-agent fluoropyrimidines. It remains to be explained why meticulous selection of the patients, which was carried out within this study, did not result in remarkable improvement of cetuximab efficacy as compared to historical controls.

Acknowledgements The authors cordially thank Mrs. Olga S. Yatsuk and Olga A. Zaitseva for their technical assistance.

Compliance with Ethical Standards

Funding This work has been supported by the Russian Foundation for Basic Research (Grants 15-04-01744, 16-04-01141 and 17-54-12007). Exome sequencing has been supported by the Russian Science Foundation (Grant 17-75-30027).

Conflict of interest Vladimir M. Moiseyenko, Fedor V. Moiseyenko, Grigoriy A. Yanus, Ekatherina Sh. Kuligina, Anna P. Sokolenko, Ilya V. Bizin, Alexey A. Kudriavtsev, Svetlana N. Aleksakhina, Nikita M.

Volkov, Vyacheslav A. Chubenko, Kseniya S. Kozyreva, Mikhail M. Kramchaninov, Alexandr S. Zhuravlev, Kseniya V. Shelekhova, Denis V. Pashkov, Alexandr O. Ivantsov, Aigul R. Venina, Tatyana N. Sokolova, Elena V. Preobrazhenskaya, Natalia V. Mitushkina, Alexandr V. Togo, Aglaya G. Iyevleva, Evgeny N. Imyanitov declare that they have no conflicts of interest.

Ethical approval The study was approved by the local Ethics Committee.

Informed consent All patients provided written informed consent.

References

- Stein A, Bokemeyer C. How to select the optimal treatment for first line metastatic colorectal cancer. *World J Gastroenterol*. 2014;20:899–907.
- Shah U, Goldberg RM. Sequential versus combination therapy in the treatment of patients with advanced colorectal cancer. *Clin Colorectal Cancer*. 2008;7:315–20.
- Van Cutsem E, Cervantes A, Nordlinger B, Arnold D, ESMO Guidelines Working Group. Metastatic colorectal cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2014;25(Suppl 3):iii1–9.
- Chung KY, Shia J, Kemeny NE, Shah M, Schwartz GK, Tse A, Hamilton A, Pan D, Schrag D, Schwartz L, Klimstra DS, Fridman D, Kelsen DP, Saltz LB. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol*. 2005;23:1803–10.
- Hecht JR, Mitchell E, Neubauer MA, Burris HA 3rd, Swanson P, Lopez T, Buchanan G, Reiner M, Gansert J, Berlin J. Lack of correlation between epidermal growth factor receptor status and response to Panitumumab monotherapy in metastatic colorectal cancer. *Clin Cancer Res*. 2010;16:2205–13.
- Wierzbiński R, Jonker DJ, Moore MJ, Berry SR, Loehrer PJ, Youssoufian H, Rowinsky EK. A phase II, multicenter study of cetuximab monotherapy in patients with refractory, metastatic colorectal carcinoma with absent epidermal growth factor receptor immunostaining. *Investig New Drugs*. 2011;29:167–74.
- Bouché O, Beretta GD, Alfonso PG, Geissler M. The role of anti-epidermal growth factor receptor monoclonal antibody monotherapy in the treatment of metastatic colorectal cancer. *Cancer Treat Rev*. 2010;36:S1–10.
- Pillai RK, Lopategui JR, Dhall D, Guindi M, Slavin T, Lofton-Day CE, Patterson SD. The state of the art in colorectal cancer molecular biomarker testing. *Adv Anat Pathol*. 2016;23:92–103.
- Pietrantonio F, Petrelli F, Coinu A, Di Bartolomeo M, Borgonovo K, Maggi C, Cabiddu M, Iacovelli R, Bossi I, Lonati V, Ghilardi M, de Braud F, Barni S. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. *Eur J Cancer*. 2015;51:587–94.
- Peeters M, Price T, Van Laethem JL. Anti-epidermal growth factor receptor monotherapy in the treatment of metastatic colorectal cancer: where are we today? *Oncologist*. 2009;14:29–39.
- Zimmermann S, Peters S. Present standards and future perspectives in the treatment of metastatic non-small cell lung cancer. *Cancer Metastasis Rev*. 2015;34:173–82.
- Perez EA. Treatment strategies for advanced hormone receptor-positive and human epidermal growth factor 2-negative breast cancer: the role of treatment order. *Drug Resist Update*. 2016;24:13–22.

13. Shiota M, Eto M. Current status of primary pharmacotherapy and future perspectives toward upfront therapy for metastatic hormone-sensitive prostate cancer. *Int J Urol*. 2016;23:360–9.
14. Mitiushkina NV, Iyevleva AG, Poltoratskiy AN, Ivantsov AO, Togo AV, Polyakov IS, Orlov SV, Matsko DE, Novik VI, Imyanitov EN. Detection of *EGFR* mutations and *EML4-ALK* rearrangements in lung adenocarcinomas using archived cytological slides. *Cancer Cytopathol*. 2013;121:370–6.
15. Yanus GA, Belyaeva AV, Ivantsov AO, Kuligina ESh, Suspitsin EN, Mitiushkina NV, Aleksakhina SN, Iyevleva AG, Zaitseva OA, Yatsuk OS, Gorodnova TV, Strelkova TN, Efremova SA, Lepenchuk AY, Ochir-Garyaev AN, Paneyah MB, Matsko DE, Togo AV, Imyanitov EN. Pattern of clinically relevant mutations in consecutive series of Russian colorectal cancer patients. *Med Oncol*. 2013;30:686.
16. MuTect2. https://software.broadinstitute.org/gatk/documentation/tooldocs/3.8-0/org_broadinstitute_gatk_tools_walkers_cancer_m2_MuTect2.php. Assessed 7 Oct 2017.
17. Combined Annotation Dependent Depletion (CADD). <http://cadd.gs.washington.edu/>. Assessed 7 Oct 2017.
18. Zanella ER, Galimi F, Sassi F, Migliardi G, Cottino F, Leto SM, Lupo B, Erriquez J, Isella C, Comoglio PM, Medico E, Tejpar S, Budinská E, Trusolino L, Bertotti A. IGF2 is an actionable target that identifies a distinct subpopulation of colorectal cancer patients with marginal response to anti-EGFR therapies. *Sci Transl Med*. 2015;7:272ra12.
19. Muro K, Yoshino T, Doi T, Shirao K, Takiuchi H, Hamamoto Y, Watanabe H, Yang BB, Asahi D. A phase 2 clinical trial of panitumumab monotherapy in Japanese patients with metastatic colorectal cancer. *Jpn J Clin Oncol*. 2009;39:321–6.
20. Mizota A, Shitara K, Kondo C, Nomura M, Yokota T, Takahari D, Ura T, Inaba Y, Yamaura H, Sato Y, Kato M, Muro K. Retrospective analysis of cetuximab monotherapy for patients with irinotecan-intolerant metastatic colorectal cancer. *Int J Clin Oncol*. 2011;16:416–20.
21. Mekata E, Endo Y, Sonoda H, Shimizu T, Kawai Y, Umeda T, Shiomi H, Naka S, Kubota Y, Murata S, Yamamoto H, Abe H, Tani T. Cetuximab as salvage monotherapy in chemotherapy-refractory metastatic colorectal cancer: a single-center report. *Oncol Lett*. 2013;6:1011–4.
22. Gasparini G, Buttitta F, D'Andrea MR, Tumolo S, Buonadonna A, Pavese I, Cordio S, De Tursi M, Mosconi S, Stumbo L, Felicioni L, Marchetti A. Optimizing single agent panitumumab therapy in pre-treated advanced colorectal cancer. *Neoplasia*. 2014;16:751–6.
23. van Hellemond IE, Creemers GJ, van Warmerdam LJ, et al. Panitumumab monotherapy as a second-line treatment in metastasised colorectal cancer: a single centre experience. *Clin Oncol (R Coll Radiol)*. 2014;26:135–41.
24. Lakomy R, Rogowski W, Piko B, Mihaylova Zh, Pritzova E, Kvocekova L. Prospective noninterventional study on the use of panitumumab monotherapy in patients with recurrent or progressive colorectal cancer: the VECTIS study. *Cancer Manag Res*. 2015;7:311–8.
25. Pietrantonio F, Cremolini C, Petrelli F, Di Bartolomeo M, Loupakakis F, Maggi C, Antoniotti C, de Braud F, Falcone A, Iacovelli R. First-line anti-EGFR monoclonal antibodies in panRAS wild-type metastatic colorectal cancer: a systematic review and meta-analysis. *Crit Rev Oncol Hematol*. 2015;96:156–66.
26. Daoud MA, Aboelnaga EM, Mohamed WM. Second-line panitumumab as a triweekly dose for patients with wild-type *KRAS* exon 2 metastatic colorectal cancer: a single-institution experience. *Cancer Biol Med*. 2016;13:136–41.
27. Pessino A, Artale S, Sciallero S, Guglielmi A, Fornarini G, Andreotti IC, Mammoliti S, Comandini D, Caprioni F, Bennicelli E, Andretta V, Siena S, Sobrero A. First-line single-agent cetuximab in patients with advanced colorectal cancer. *Ann Oncol*. 2008;19:711–6.
28. Sastre J, Aranda E, Grávalos C, Massutí B, Varella-Garcia M, Rivera F, Soler G, Carrato A, Manzano JL, Díaz-Rubio E, Hidalgo M. First-line single-agent cetuximab in elderly patients with metastatic colorectal cancer. A phase II clinical and molecular study of the Spanish group for digestive tumor therapy (TTD). *Crit Rev Oncol Hematol*. 2011;77:78–84.
29. Pietrantonio F, Cremolini C, Aprile G, Lonardi S, Orlandi A, Mennitto A, Berenato R, Antoniotti C, Casagrande M, Marsico V, Marmorino F, Cardellino GG, Bergamo F, Tomasello G, Formica V, Longarini R, Giommoni E, Caporale M, Di Bartolomeo M, Loupakakis F, de Braud F. Single-agent panitumumab in frail elderly patients with advanced *ras* and *braf* wild-type colorectal cancer: challenging drug label to light up new hope. *Oncologist*. 2015;20:1261–5.
30. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA Jr. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372:2509–20.
31. Sartore-Bianchi A, Trusolino L, Martino C, Bencardino K, Lonardi S, Bergamo F, Zagonel V, Leone F, Depetris I, Martinelli E, Troiani T, Ciardiello F, Racca P, Bertotti A, Siravegna G, Torri V, Amatu A, Ghezzi S, Marrapese G, Palmeri L, Valtorta E, Cassingena A, Lauricella C, Vanzulli A, Regge D, Veronese S, Comoglio PM, Bardelli A, Marsoni S, Siena S. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, *KRAS* codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicenter, open-label, phase 2 trial. *Lancet Oncol*. 2016;17:738–46.