Clinical Trial Report

Optimal anti-EGFR Treatment of mCRC Patients with Low-frequency RAS Mutation

Investigational medicinal product:	Panitumumab (Vectibix®)
Sponsor	Klinikum der Ludwig-Maximilians-Universität München, Marchioninistr. 15, 81377 München, Germany
Short title:	FIRE-5
Protocol code:	AIO TF-0118
EudraCT number:	2018-002849-11
Phase of development:	II
Date of first enrolment:	26 May 2020
Date of completion:	16 June 2021

This clinical trial was conducted in compliance with the International Council for Harmonisation (ICH) guidelines for Good Clinical Practice (GCP), all applicable national regulations as well as the requirements of the appropriate Independent Ethics Committee, any other institutional requirements and the clinical trial protocol.

Report Version 1.0 dated 09 May 2022

2 Synopsis

Name of sponsor/company: Klinikum der Universität München (represented by the managing medical director), Ludwig-Maximilians-Universität München, Marchioninistr. 15, 81377 München, Germany

Name of finished product: Vectibix® 20 mg/mL concentrate for solution for infusion

Name of active ingredient: Panitumumab

Title of the trial: Optimal anti-EGFR Treatment of mCRC Patients with Low-frequency RAS Mutation
Protocol code: FIRE-5 (AIO TF-0118)
EudraCT no.: 2018-002849-11

This report is based on version 4.0 of the protocol, dated 07 July 2020, including the modifications of two amendments to the study protocol (Amendment 1, dated 29 January 2020; Amendment 2, dated 07 July 2020), see Annex I, Table A.1.

Principal/coordinating investigator: Prof. Dr. med. Volker Heinemann, Medizinische Klinik III, Campus Großhadern, Klinikum der Ludwig-Maximilians-Universität München, Marchioninistr. 15, 81377 München, Germany

Number of trial centre(s):
Planned: 50 trial sites in Germany
Initiated: 35 trial sites in Germany
Overall, of 52 centres receiving a favourable opinion of the ethics committees, 35 centres were initiated and 28 activated, only three of which included one patient each:

- Klinikum der Universität München-Großhadern, Med. Klinik III, Marchioninistraße 15, 81377 München
- Kathol. Marienkrankenhaus gGmbH, Zentrum f. Innere Medizin, Alfredstr. 9, 22087 Hamburg
- Onkologische Schwerpunktpraxis Kurfürstendamm, Kurfürstendamm 65, 10707 Berlin.

Publications (reference): –

Studied period:
Date first patient enrolled: 26 May 2020
Date last patient completed: 16 June 2021

On 30 September 2020, patient recruitment had to be put on hold since the pharmaceutical entrepreneur withdrew his financial support for the study because of poor recruitment; all participating study centres were informed on the same day. The competent authority and the responsible and involved ethics committees were informed of this temporary interruption on 02 October 2020. The three patients who had been included in the trial up to this point, two of whom were still in treatment and one in follow-up, were continued to be treated according to protocol.
Since study progress as planned in the protocol could not realistically be expected and the pharmaceutical entrepreneur had withdrawn his funding, the study was terminated early on 22 June 2021 because public research funding could not be obtained, either. At that time, the three patients who had been treated in the study had been off treatment for considerably longer than 28 days (last dose on 04 February 2021), and no unexpected adverse events or toxicities had been observed; all patients had last been contacted in June 2021, i.e. shortly before study termination. The competent authority and responsible ethics committee were informed and confirmed their acknowledgement on 24 June and 28 July 2021, respectively.

Phase of development: II

Background and rationale for the trial

1. RAS mutation in colorectal cancer

RAS mutations (KRAS and NRAS, exons 2-4) were expected to occur at a rate of 50% in metastatic colorectal cancer (mCRC). Typically, RAS mutation is associated with a more unfavorable outcome compared to RAS wild-type. The notion was that tumours with RAS mutation are resistant to anti-EGFR agents (van Cutsem et al. 2015; for bibliographic references, see Annex II).

2. Limited treatment options in patients with RAS mutant tumours

Since treatment options are limited in patients with RAS mutant tumours, all treatment options should be exploited even if remissions are of limited duration. The addition of a further treatment option including anti-EGFR treatment in patients with low-level RAS mutation may therefore prove to add to the continuum of treatment and may accordingly contribute to prolonged overall survival.

3. Longer survival of patients with RAS mutant tumours treated with anti-EGFR agents compared to anti VEGF agents

Three studies (FIRE-3: Stintzing et al. 2017, PEAK: Rivera et al. 2017, CALGB: Lenz et al. 2014) were available to compare the first-line use of targeted therapy with either anti-EGFR- or anti-VEGF directed agents. In an analysis of patients with KRAS exon-2 wild-type other RAS mutant mCRC, two of these studies predominantly using an oxaliplatin-based chemotherapy showed a superior survival in patients receiving first-line therapy with an anti-EGFR agent. A subsequent meta-analysis of the available studies showed an overall survival (OS) related hazard ration (HR) of 0.70 (p=0.0426) favoring the anti-EGFR arm (Heinemann et al. 2016).

This finding is surprising since it was expected that anti-EGFR agents should not be effective in RAS mutant tumours. While multiple considerations may explain this observation, an important hypothesis is that the group of RAS mutant mCRC may in fact be heterogeneous.

4. Low-frequency RAS mutation

CRC tumours are characterized by high intra-tumour heterogeneity. A CRC tumour grows from a single expansion to a diverse population of tumour subclones during carcinogenesis. Each tumour cell is potentially contributing to the heterogeneity by introducing new mutations with its next cell division. According to Sottoriva's Big Bang growth model (Sottoriva et al., 2015), new mutations arise over time and become detectable only when the clone has expanded to a sufficient size regardless of any growth advantages i.e. malignancy. Here, the frequency of a mutation refers to the ratio between DNA molecules with a mutation and DNA molecules without this mutation in a biopsy sample. Because of the lower sensitivity of direct sequencing, the former gold standard technology for genotyping, the cut-off sensitivity was about 20% in the past to differentiate between RAS wild-type and mutant tumours. Mutations with frequencies lower than 20% were thus probably not detected. Hence, patients with mutation frequencies lower than 20% were likely considered RAS wild-type, qualified for anti-EGFR treatment and possibly benefited from this treatment although a clinically relevant number of patients may have had low-frequency RAS mutations. This subgroup of patients bearing tumours with low-frequency RAS mutations has not been characterized in detail with regard to efficacy of anti-EGFR therapies. Hikosaka and coworkers used a cut-off of 10% when using pyrosequencing for their trial (Hikosaka et al., 2013). 217 of 358 analyzed mCRC patients had a KRAS wildtype with routinely used methods of determining KRAS mutational status. However, if analyzing the KRAS genotype by pyrosequencing with the defined cut-off, a further 93 patients who had been analyzed as KRAS wildtype before were detected to have a low-frequency KRAS mutation (26% of all patients analyzed (93/358) or 42.9% (93/217) of those previously analyzed as KRAS wildtype), whereas in 124 patients no KRAS mutation was detected.

50 of these patients with low-frequency KRAS mutation and 47 patients with no detectable KRAS mutation by pyrosequencing were treated with an anti-EGFR antibody. Patients with low-frequency KRAS mutation benefited from this treatment as well (Hikosaka et al., 2013).

	RR	**DCR**	**PFS**
no KRAS mutation detectable by means of pyrosequencing (n=47)	32%	70%	158 days
low-frequency KRAS mutation (n=50)	42%	74%	145 days

RR = response rate; DCR = disease control rate; PFS = progression-free survival.

The focus of the present trial was on mCRC patients with RAS mutation frequencies ≤ 20%. These patients were defined as "low RAS mutant" and included in the trial.

5. Detection of low-frequency RAS mutation

With modern genotyping methods, the sensitivity of detecting a mutation has been improved to sensitivity cut-offs of 0.1% to 5%. However, the pathology reports state the RAS mutation status only qualitatively as RAS wild-type tumour or RAS mutant tumour. Accordingly, the treating physician does

not receive quantitative information on the extent of RAS mutation of a tumour. Depending on the cut-off level of sensitivity (typically ≤ 5%), the frequency of RAS mutation may range from very low levels such as ≤ 5% to 100% in the evaluated tumours. At present, the true incidence of low-level RAS mutation in the population of mCRC patients is unclear.

Detection of tumours with low-level RAS mutation requires a quantitative readout that provides the proportion of cells with a RAS mutation normalized to the proportion of wildtype cells. To generate optimal results, screening for low-level RAS mutation should be performed in an experienced central pathology laboratory.

Since first-line anti-EGFR treatment is well established and unquestioned in patients with RAS-wildtype tumours, the present study focused on mCRC patients with previously determined RAS mutation. In this subpopulation, the study aimed to define the incidence of low-level RAS mutation and to prospectively explore the efficacy of anti-EGFR directed therapy in patients with low-frequency RAS mutation in the setting of first-line treatment.

Low-frequency RAS mutation was defined as follows:

Proportion of frequency of RAS-mutated alleles to frequency of wildtype alleles in the microdissected tissue sample ≤ 20%.

After central re-testing and determination of a low-frequent RAS mutation, patients of one of the following three groups could be included:

1. **Group A:** patients with low-frequency RAS mutation ≤ 5%
2. **Group B:** patients with low-frequency RAS mutation > 5% to ≤ 10%
3. **Group C:** patients with low-frequency RAS mutation > 10% to ≤ 20%)

Patients with a frequency of a RAS mutation of more than 20% were not enrolled for treatment.

Objectives:

Primary objective: The primary objective of the study was to define an optimal cut-off for anti-EGFR treatment with panitumumab in combination with FOLFIRI of patients with low-frequency RAS mutation defined by digital Next Generation Sequencing (dNGS) using the Oncomine cfDNA pan-cancer gene assay on an Ion Torrent (S5 Prime) platform.

Secondary objectives:

- To analyse efficacy parameters (progression-free survival [PFS], overall survival [OS], early tumour shrinkage [ETS], Depth of response [DpR]) in patients with low-frequency RAS-mutation treated with panitumumab in combination with FOLFIRI
- To determine retrospectively a cut-off frequency for low-frequency RAS mutation as limit for treatment with anti EGFR agents
- To analyse safety and tolerance of the first-line treatment.

Translational research objectives

- Within each biopsy sample the actual amount of tumour tissues in relation to healthy tissue (i.e. from stoma or vascular tissues) is unknown. For an ideal quantitative analysis, the RAS mutation frequencies will be normalized to the ratio of actual tumour tissue to normal tissue.
- Analysis of gene expression parameters allowing classification according to Consensus Molecular Subtypes; only in patients with low-frequency RAS-mutation
- Treatment given as 2nd line therapy as well as investigator reported PFS and best-overall response in 2nd-line therapy
- Investigation of EGFR pathways related biomarkers for prediction of sensitivity and secondary resistance to an anti-EGFR treatment (including tumour biopsies and liquid biopsies from blood samples).

Methodology:

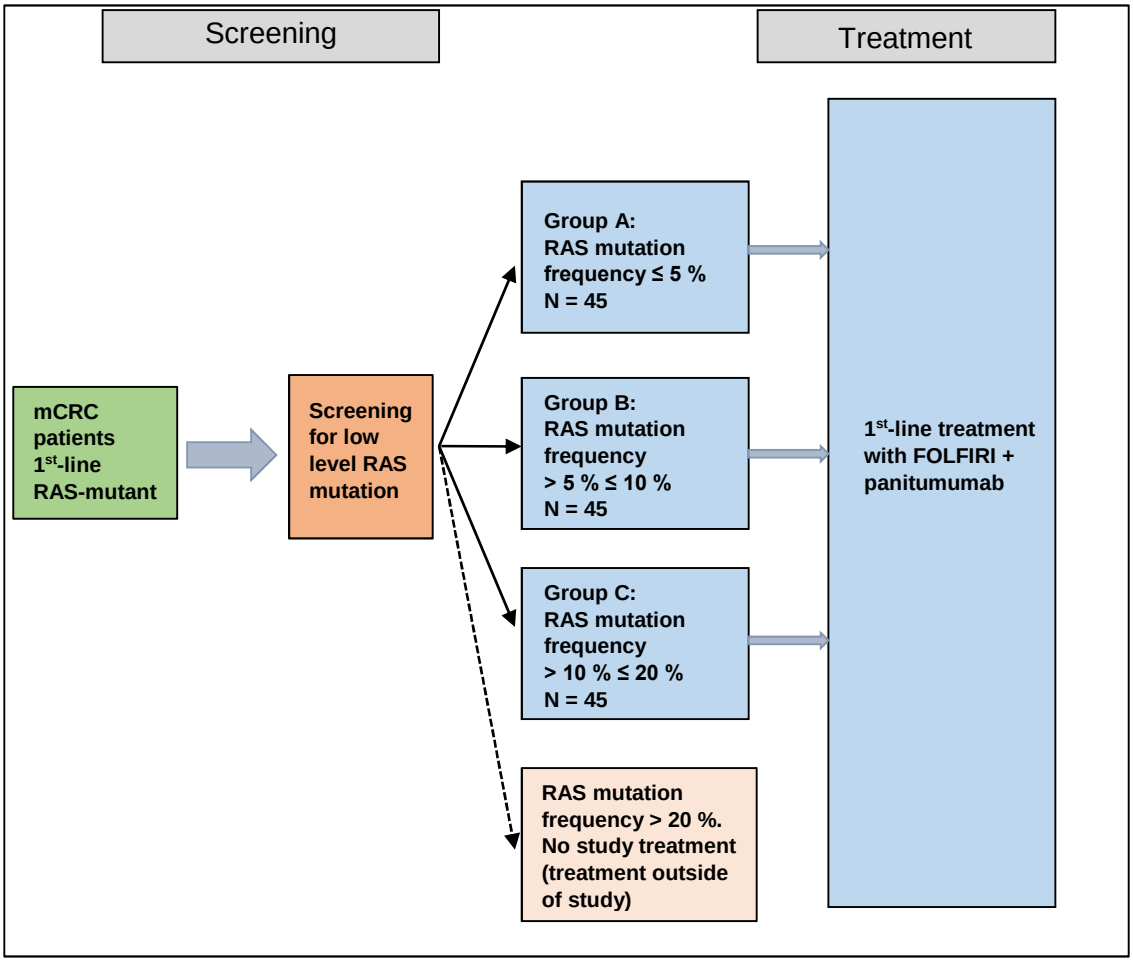
An open, non-randomized multicentre phase II trial with three treatment groups according to the frequency of RAS mutant cells within the tumourous tissue in first-line treatment of patients suffering from mCRC with low-frequency RAS mutation.

The study screened patients with known RAS mutation as determined decentrally by the local pathologist. Tumour probes of participating patients were submitted to a central NGS-based analysis

of RAS mutation status including RAS mutation frequency at the Institute of Pathology, Ludwig-Maximilians-Universität München, Marchioninistr. 15, 81377 München, Germany; Professor Andreas Jung`s laboratory.

In addition, patients could be included to the study prior to results of the central RAS mutation frequency testing if the frequency of the RAS mutation (number of mutant RAS alleles/number of analyzed RAS alleles) analyzed by a digital NGS method was <20% (or <0.2) according to the local pathology report. The coverage of the NGS method had to be >200 copies with respect to the RAS gene. Therefore, the number of analyzed RAS genes, the number of RAS mutant alleles, and the coverage had to be evident in the pathological report. Low-frequency RAS mutation has been defined above (see Background and rationale, no. 5).

The study design is displayed in the following figure:



FOLFIRI =	Infusional 5-fluorouracil + folinic acid + irinotecan
mCRC =	Metastatic colorectal cancer
N =	Number of patients
RAS mutation =	Rat sarcoma oncogene mutation

Only patients with low-frequency RAS mutation (Group A, B or C) were treated within the study and received first-line therapy with FOLFIRI plus panitumumab. FOLFOX was avoided as a treatment option, since a negative interaction of anti-EGFR agents and oxaliplatin-based chemotherapy in RAS mutant tumours could not be excluded. Patients with high-frequency RAS mutation (>20%) were considered as screening failures and thus did not receive study treatment. They were treated according to their treating physician's decision outside of study.

Treatment was planned to be performed until progression or when toxicity required termination (for the actual reasons for treatment termination, see result section below). Toxicity-related de-escalation from FOLFIRI plus panitumumab to FUFA (5-FU and folinic acid) plus panitumumab, to irinotecan

plus panitumumab, or to panitumumab monotherapy was allowed within the trial. Re-escalation was allowed. Treatment within the study ended once a new agent, not contained in the study regimen, was used.

Number of patients:
Planned: 500 screened, 135 included (45 per RAS mutation group)
Screened: N=25
Enrolled: N=3
Analyzed: N=3.

Diagnosis and main criteria for inclusion and exclusion:
Adult patients with histologically confirmed, UICC stage IV metastatic adenocarcinoma of the colon or rectum with primarily non-resectable metastases (or refusing surgical resection) suitable for chemotherapy administration and with a life expectancy >3 months.
Documented low-level RAS mutation in the tumour; presence of at least one measurable reference lesion according to the RECIST 1.1 criteria; tumour tissue from primary tumour or metastasis available; no previous chemotherapy for metastatic disease (with the exception of two cycles of FOLFIRI in patients in need of immediate treatment e.g. while waiting for the result of RAS genotyping). Further inclusion criteria included ECOG performance status 0–2, and adequate bone marrow, hepatic, and renal function.

The major exclusion criteria included Grade II or IV heart failure (NYHA classification) and any other severe concomitant disease or disorder which could have influenced the safety of the patient during the clinical trial (such as, but not restricted to, history of uncontrolled bronchial asthma; interstitial pneumonitis or pulmonary fibrosis; known brain metastases; HIV, HBV, or HCV infection; complete DPD deficiency; history of acute or subacute intestinal occlusion or chronic inflammatory bowel disease or chronic diarrhoea; symptomatic peritoneal carcinomatosis), relevant hypersensitivities and/or allergies, and excluded previous and concomitant therapy.

Test product, dose and mode of administration, batch number:
Panitumumab was the investigational medicinal product. Panitumumab has no marketing authorization for administration in patients with RAS mutant tumours. It was supplied by Amgen.
Batch numbers: 1102131
1111180
1127784.
All patients received background chemotherapy with the FOLFIRI regimen, consisting of 5-FU, folinic acid, and irinotecan. All medicinal products of the FOLFIRI regimen have been authorized for many years. FOLFIRI is a standard chemotherapeutic first-line treatment option for patients with mCRC irrespective of their RAS mutation status. Thus, the medicinal products were used within the scope of their respective authorisations and had to be prescribed.

Patients received panitumumab in addition to background therapy in 14-day cycles until progression or unacceptable toxicity. Treatment was administered as an intravenous infusion via an infusion pump. Prior to infusion, panitumumab was diluted in sodium chloride 9 mg/mL (0.9%) solution to a final concentration not exceeding 10 mg/mL.

A 14-day cycle consisted of

- Panitumumab 6 mg/kg BW as 60-min i.v. infusion* D 1

followed by

- Irinotecan 180 mg/m² BSA iv infusion over 30 – 90 min D 1
- Folinic acid (racemic) 400 mg/m² BSA iv over 30 – 120 min according to local trial centre standard D 1
- 5-FU 400 mg/m² BSA bolus D 1
- 5-FU 2400 mg/m² BSA iv over 46 h Days 1-2 of the respective cycle.

*If the 1st infusion was well tolerated, all subsequent infusions could be applied over 30-60 minutes.

Duration of treatment: In 14-day cycles until the time of progression under first-line treatment or unacceptable toxicity. The anticipated duration of study treatment in the individual patient was 10 months.

Reference therapy, dose and mode of administration, batch number:
This was an open, non-randomized trial. Placebos or active comparators were not used.

Criteria for evaluation:

Primary endpoint

ORR according to RECIST 1.1, evaluated separately for each group of patients with defined low-frequency RAS mutation (Groups A, B and C, see above).

Secondary endpoints

Efficacy

- PFS, separately for each group of patients with defined low-frequency RAS mutation (Groups A, B and C, see above)
- OS, separately for each group of patients with defined low-frequency RAS mutation (Groups A, B and C, see above)
- ETS, separately for each group of patients with defined low-frequency RAS mutation (Groups A, B and C, see above)
- DpR, separately for each group of patients with defined low-frequency RAS mutation (Groups A, B and C, see above)
- Retrospectively determined optimal cut-off frequency for low-frequency RAS mutation leading to RR, PFS and OS in ranges comparable to that of RAS-wildtype first-line patients treated with panitumumab in combination with chemotherapy
- Prospective analysis of tumour marker level evolution (CEA and CA 19-9)

Safety

- Type, incidence, relatedness, and severity of adverse events (severity according to NCI CTCAE version 5.0)

Statistical methods:
Due to poor recruitment and early trial termination, only three patients were included and treated in the clinical trial. Statistical analyses in such a small patient sample can hardly provide meaningful results; therefore, all data are presented purely descriptively in by-patient listings. Only safety data (treatment-emergent adverse events, TEAE) are presented by System Organ Class (SOC) and Preferred Term (PT).

Summary of results and conclusions:
See “statistical methods” above: all data are presented purely descriptively by patient and by SOC/PT, respectively.

Demography and baseline characteristics:

Only three patients were included and treated in the clinical study. Patient 1-002 and 36-002 were included in Group B, and 14-001 in Group C. Of the 25 patients screened, 21 were excluded because of their mutation status.

Demographic data are shown in Table 1, disease characteristics in Table 2, and information on previous treatment of the colorectal cancer in Table 3.

Table 1: Demographic and baseline data

Patient	Age (years)	Sex	Ethnicity	ECOG
1-002	50	Female	Caucasian	0
14-001	58	Male	Caucasian	0
36-002	71	Male	Caucasian	0

Table 2: Disease characteristics

Patient	Date of first diagnosis	Date of first diagnosis of MD	Localisation primary tumour	Localisation metastases
1-002	12 May 2020	20 May 2020	Sigmoid	Liver, Lung, Other (Spleen)
14-001	18 May 2020	18 May 2020	Sigmoid, Rectum	Liver, Lung
36-002	15 January 2019	31 August 2020	Rectum	Liver, Lung

MD = metastatic disease

Table 3: Previous treatment of colorectal cancer

Patient	Previous cycle FOLFIRI[1]	Chemotherapy	Radiation	Resection	
				Prim. tumour	Metastases
1-002	Yes	No	No	No	No
14-001	Yes	No	No	No	No
36-002	No	Yes	No	No	No

[1] Previous chemotherapy of the metastatic disease was an exclusion criterion, with the exception of up to two cycles of the background therapy. Patients 1-002 and 14-001 both received two cycles of FOLFIRI until two weeks before start of study therapy, see Table 4.

Extent of exposure:

Table 4: Treatment with study medication

Patient	First dose (date)	Last dose (date)	Treatment duration (days)[1]	No. of cycles
1-002	03 July 2020	17 July 2020	15	2
14-001	25 June 2020	04 February 2021	225	14
36-002	28 September 2020	04 January 2021	99	8

[1] Day of last dose – day of first dose + 1

Patients 1-002 and 14-001 received 2 cycles of FOLFIRI pretreatment from 05–19 June 2020, and 27 May – 10 June 2020, respectively. The reasons for end of therapy were specified as progression (Pat. 01-002), Other: Resection (Pat. 14-001), and Other: Patient wants metastasis surgery (Pat. 36-002).

No relevant protocol deviations occurred; the majority were time window violations that were considered minor by the sponsor/study coordinator. Moreover, some specified procedures were omitted at the end of treatment visit in patients 1-002 and 14-001. These omissions were rated minor as well.

Efficacy results:

Early termination and the small sample size do not allow for any efficacy analyses. Table 5 shows a by-patient display of response and survival data.

Table 5: Efficacy results

Patient	Best response[1]	PFS event[2]	PFS (days)[3]	OS event[2]	OS (days)[4]
1-002	NE	Yes	53	No	347
14-001	PR	No	357	No	357
36-002	PR	Yes	254	No	254

[1] Best response evaluation result (according to RECIST 1.1) in the time from first dose to last dose + 28 days
[2] Yes = patient experienced the event (progression / death)
[3] Calculated as day of PD/last date progression-free – day of first dose + 1
[4] Calculated as day of death/last contact – day of first dose + 1

Safety results:

All three patients experienced a total of 27 treatment-emergent adverse events (TEAE) that are displayed by SOC and PT in Table 6. With the exception of rash and fatigue (n=2 each), all events occurred in individual patients only. N=19 events (70.4%) were considered related to study therapy (IMP or background therapy). All PTs of SOC Skin and subcutaneous tissue disorders (7 events in n=3 patients) were considered related to panitumumab.

Table 6: TEAEs by SOC and PT

System Organ Class/ Preferred Term	Total N=3		NCI grade			Related to			
	AE	n	1	2	3	5-FU	FA	Iri	Pan
Blood and lymphatic system disorders									
Neutropenia	1	1	-	1	-	1	-	1	-
Gastrointestinal disorders									
Abdominal pain	1	1	1	-	-	-	-	-	-
Diarrhoea	1	1	-	-	1	1	-	-	-
Mechanical ileus	1	1	-	-	1	-	-	-	-
Nausea	1	1	1	-	-	1	1	1	1
Stomatitis	3	1	3	-	-	3	-	3	-
Subileus	1	1	-	-	1	-	-	-	-
General disorders and administration site conditions									
Fatigue[1]	2	2	1	1	-	1	-	1	1
Mucosal inflammation	1	1	1	-	-	1	-	-	-
Pain	1	1	-	1	-	-	-	-	-
Infections and infestations									
Paronychia	1	1	-	1	-	-	-	-	1
Rash pustular	1	1	-	1	-	-	-	-	1
Staphylococcal infection	2	1	-	2	-	-	-	-	-
Injury, poisoning and procedural complications									
Wound dehiscence	1	1	1	-	-	-	-	-	-
Investigations									
Weight decreased	1	1	1	-	-	1	-	1	-
Musculoskeletal and connective tissue disorders									
Flank pain	1	1	-	-	1	-	-	-	-
Skin and subcutaneous tissue disorders									
Dermatitis acneiform	2	1	1	-	1	-	-	-	2
Dry skin	1	1	-	1	-	-	-	-	1
Palmar-plantar erythrodysaesthesia syndrome	1	1	-	1	-	-	-	-	1
Rash	2	2	1	1	-	-	-	-	2
Skin fissures	1	1	-	1	-	-	-	-	1

[1] Fatigue: one NCI grade 2 event suspected to be related to 5-FU, irinotecan; one grade 1 event suspected to be related to panitumumab.

A total of n=5 events of NCI grade 3 occurred, two of which (one case each of diarrhea and dermatitis acneiform) were considered related to 5-FU and panitumumab, respectively.
Three dose reductions due to TEAEs were reported: the dose of 5-FU was reduced due to grade 2 neutropenia in patient 1-002 and due to grade 3 diarrhoea in patient 36-001; the doses of 5-FU, folinic acid, and irinotecan were reduced due to weight decreased in patient 14-001. In patient 14-001, temporary discontinuation / dose delay of all study medication was reported due to the simultaneous events subileus, staphylococcal infection, and rash pustular. Moreover, panitumumab was temporarily discontinued / delayed twice in this patient due to wound dehiscence and dermatitis acneiform, respectively. Two events in this patient, PTs fatigue grade 2 related to 5-FU and irinotecan, and dermatitis acneiform grade 3 related to panitumumab, had not recovered at the end of study.

Two treatment-emergent serious adverse events (TESAEs) were reported, see Table 7. Both events were not considered related to study treatment. No deaths were reported during the study.

Table 7: TESAEs

Patient	Preferred term	Start date	Stop date	NCI Grade	Related (y/n)	Action taken	Outcome
1-002	Mechanical ileus	22 Jul 20	17 Aug 20	3	no	None	Recovered / resolved
14-001	Subileus	24 Sep 20	06 Nov 20	3	no	Temporary discontinuation / dose delay[1]	Recovered / resolved

[1] In patient 14.001, both IMP and background medication were temporarily discontinued in spite of the fact that the event was not related to study treatment because of the concurrent occurrence of several events, some of which were considered related to IMP.

It should be noted that in patient 1-002, some adverse events of grade 1 were reported during FOLFIRI pretreatment, three of which (nausea, mucosal inflammation, and decreased appetite) were considered related to treatment. Since pretreatment is not considered study treatment as defined in the protocol, these events are not included in the above tables of **TE**(S)AEs. All these events had resolved before the start of study treatment as reported in Table 4.

Translational research results:
The study centre at the University of Munich reported receipt of one blood sample (PaxGene) for pharmacogenetics analyses for each of the patients included in the study; no tumour samples were received. The Laboratory for Immunological Molecular Biology, PD Dr. A. Baraniskin, Medizinische Klinik Knappschaftskrankenhaus Bochum GmbH, Bochum, Germany, reported receipt of 4 blood samples (2 Streck tubes each) for liquid biopsy for patients 1-002, 14-001 (2 samples), and 36-001. No translational research objectives were pursued.

Conclusions:
The small sample size due to poor recruitment and subsequent early study termination does not allow for any conclusions. Most importantly, there is insufficient data for the primary objective of the study, the definition of an optimal cut-off for anti-EGFR treatment with panitumumab in combination with FOLFIRI of patients with low-frequency RAS mutation.
As regards safety, no unexpected adverse events or toxicities were observed. No serious adverse events related to treatment nor deaths were reported.

Date and version of report: Final report version 1.0 dated 09 May 2022.

ANNEX I:

Protocol Changes

There were 2 amendments to the study protocol version 2.0 of 09 May 2019 as first authorized by the German competent authority on 22 May 2019 and receiving a positive vote of the ethics committee on 17 June 2019 (version 1.0 had been submitted on 20 December 2018 and supplemented by version 2.0 due to objections raised by the authority). Key changes are summarized in Table A.1 for each amendment. A few minor editorial changes (not shown) were made.

Table A.1 Key Protocol Changes in Study FIRE-5

Protocol version version date	**Key Changes**
The following amendments to the original Protocol V 2.0 (from 09 May 2019, approved on 22 May 2019, EC vote 17 June 2019) were performed during the study period:	
Amendment 1 Protocol V 3.0/ 29 January 2020 Approved on 27 February 2020 EC vote: 16 March 2020	• Adjustment of inclusion and exclusion criteria (Sections 1, 2.2, 6.2, and 6.3 of the protocol) to allow the availability of tumor tissue from the metastasis as inclusion criterion (previously, tissue from the primary tumour had to be available); moreover, the allowed previous chemotherapy was restricted to two cycles (instead of only one application) of FOLFIRI. Accordingly, the note regarding the primary endpoint (ORR) in Section 8.2 had to be reworded to allow for two cycles of FOLFIRI as well. • Adjustment of the study duration (Sections 1 and 5.4 of the protocol): planned start and end of the study were delayed by one year (FPFV QI 2020 instead of QI 2019; LPLV QI 2027 instead of QI 2026). • Adjustment of time intervals for baseline examinations and for restaging (Sections 2.2 and 8.1 of the protocol): baseline time window was extended to 35 (from 28) days, restaging was to be performed in 12-week (i.e. 6-cycle) intervals from week 8/cycle 4 (previously in 8-week intervals until week 24 and in 12-week intervals thereafter). • Liquid biopsies were now rendered optional (Sections 2.2, 8.1, 8.4.3 of the protocol). • For clarification, the translational research project (of pharmacogenetics factors) was also explicitly designated as optional in Section 8.4.4. • Administration of panitumumab (Section 7.4.6 of the protocol) was amended in line with SmPC version September 2019, adding instructions for dilution (in sodium chloride 9 mg/mL (0.9%) solution for injection for the final concentration not to exceed 10 mg/mL). • The bibliography (Section 16) was updated to include the new SmPC version September 2019 (from previous January 2018 version).
Amendment 2 Protocol V 4.0/ 07 July 2020 Approved on 03 August 2020 EC vote: 25 August 2020	• RAS mutation frequency testing by the local pathologist was allowed (instead of obligatory central testing) provided the testing requirements could be met (Sections 1, 2.1, 2.2, and 5.1 of the protocol). The central laboratory was named, and the requirements for decentralized testing were defined. • Accordingly, the procedures for shipment of tissue samples to the central laboratory were clarified (Section 1 of the protocol) and the inclusion criteria

	for the treatment phase were amended to allow for local RAS mutation frequency testing as well (Sections 1, 6.1, and 6.2). • The recommendations of the Rote-Hand-Brief dated 04 June 2020 regarding DPD deficiency and 5-FU treatment were incorporated in the protocol. This regarded exclusion criterion 20 (Sections 1 and 6.3 of the protocol), the treatment regimen (including recommendation of a reduced starting dose in Sections 1, 7.2, and 7.5.2), toxicity management (Section 7.5.5), and a recommendation of screening for DPD deficiency in the study schedule (Section 2.2).

ANNEX II:

Bibliography

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Signatures

Title of the Study:

Optimal anti-EGFR Treatment of mCRC Patients with Low-frequency RAS Mutation

EudraCT No.: 2018-002849-11

The signatories have read this clinical study report and hereby confirm that, to the best of their knowledge, it accurately describes the conduct and the results of the study

Munich, 10-May-2022
Place, date

Principal investigator & sponsor's representative
Prof. Dr. V. Heinemann

Berlin, 10 May 2022
Place, date

Study Coordinator
Prof. Dr. S. Stintzing

Lauterbach, 11.05.2022
Place, date

ClinAssess GmbH
Dr. B. Deuß

Leverkusen, 11. May 2022
Place, date

Biometrician, ClinAssess GmbH
Frau S. Held