Study Title:

Modulation of the FOLFIRI-based standard 1st-line therapy with cetuximab, controlled by monitoring the *RAS* mutation load by liquid biopsy in *RAS*-mutated mCRC patients: a randomized phase II study with FOLFIRI-based 1st-line therapy with or without intermittent cetuximab

Name of the test products: Cetuximab
Indication: Metastatic colorectal Cancer
Clinical trial phase II

Short Title / Acronym: MoLiMoR
Protocol number: Version 3.0, March 9th, 2021
EudraCT Number / EUCT number: 2019-003714-14

[redacted] 2021 – [redacted] 2024/December 6th, 2024

Clinical Study Report

Sponsor's Responsible Medical Officer:

TheraOp gGmbH
Represented by [redacted], Winchesterstr. 3, 35394 Gießen, Germany
Phone: [redacted]
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Principal / Coordinating Investigator(s):

Prof Dr. med. Alexander Baraniskin, Evangelisches Krankenhaus Hamm
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The study was performed in compliance with International Council for Harmonisation (ICH) guideline on Good clinical Practice (GCP), including archiving of essential documents

Version / Date: v2.0 / October 21st, 2025

SYNOPSIS

Name of Sponsor: TheraOp gGmbH, Winchesterstr. 3, 35394 Gießen, Germany	
Name of Finished product: Erbitux®	
Active Ingredient: Cetuximab	
Study title:	Modulation of the FOLFIRI-based standard 1st-line therapy with cetuximab, controlled by monitoring the *RAS* mutation load by liquid biopsy in *RAS*-mutated mCRC patients Anpassung einer FOLFIRI-Erstlinientherapie mit Cetuximab durch Kontrolle des *RAS*-Mutationsstatus mittels ‚Liquid Biopsy' bei Patienten mit *RAS*-mutiertem, metastasiertem Kolorektalkarzinom
Trial registry name:	Modulation of the FOLFIRI-based standard 1st-line therapy with cetuximab, controlled by monitoring the *RAS* mutation load by liquid biopsy in *RAS*-mutated mCRC patients
EudraCT-No.:	2019-003714-14
Protocol number:	42130002 (Version 3.0 dated March 9th, 2021; last version including Amendment 1) Amendment 1 includes: - *Adaptation of some inclusion / exclusion criteria (i.e., Permission of 1-2 cycles FOLFIRI before enrolment. exclusion of patients with complete DPD deficiency confirmed by genotyping or phenotyping)* - *Permission to administer FOLFIRI without 5-FU bolus (mFOLFIRI)* - *Update of table 'Schedule of visits and assessments'* - *Update of section 7.1 and 7.2 accordingly* - *Correction of a mistake in section 3.1.2 Randomization* - *Specification of monitoring of vital signs during study treatment in section 6.0* *Specification of start date for analysis of efficacy parameters*
Principal / Coordinating Investigator: Prof. Dr. med. Alexander Baraniskin, Evangelisches Krankenhaus Hamm, Werler Stra. 110, 59063 Hamm, Germany	
Study sites: A total of 21 German and 1 Austrian site participated in the study. Between [redacted] 2020 and [redacted] 2021, 20 sites in Germany and 1 site in Austria screened patients. Of these, 4 sites randomized patients(*). 1) [redacted] Onkologisches Zentrum Donauwörth, Onkologisches Zentrum (Dachau II), Hochstr. 27, 85221 Dachau, Germany* 2) [redacted] Kliniken Essen-Mitte Evang. Huyssens-Stiftung, Klinik für internistische Onkologie/Hämatologie, Henrichstr. 92, 45136 Essen, Germany* 3) [redacted] Medizinische Universitätsklinik Bochum, In der Schornau 23-25, 44892 Bochum, Germany* 4) [redacted] Evangelisches Krankenhaus Hamm, Werler Str. 110, 39063 Hamm, Germany* 5) [redacted] Landeskrankenhaus Feldkirch, Carinagasse 47, 6800 Feldkirch, Austria 6) [redacted] Universitätsklinikum Leipzig, Liebigstraße 20, 04103 Leipzig, Germany 7) [redacted] Klinikum Magdeburg, Birkenallee 34, 39130 Magdeburg, Germany	

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21) [redacted] Klinikum Frankfurt (Oder) GmbH, Müllroser Chaussee 7, 15236 Frankfurt/Oder, Germany
22) [redacted] Gefos Dortmund mbH - Standort Hörde, Am Oelpfad 12, 44263 Dortmund, Gefos Dortmund mbH - Standort Kirchlinde, Zollernstr. 40, 44379 Dortmund, Gefos Dortmund mbH - Standort Brackel, Am Knappschaftskrankenhaus 1, 44309 Dortmund, Germany

Publication: not applicable

Study Period:	**Phase of development: II**
First patient in: [redacted] 2021	
Last patient out: [redacted] 2024	
Data base hard lock: December 6th, 2024	

Result analysis stage: final

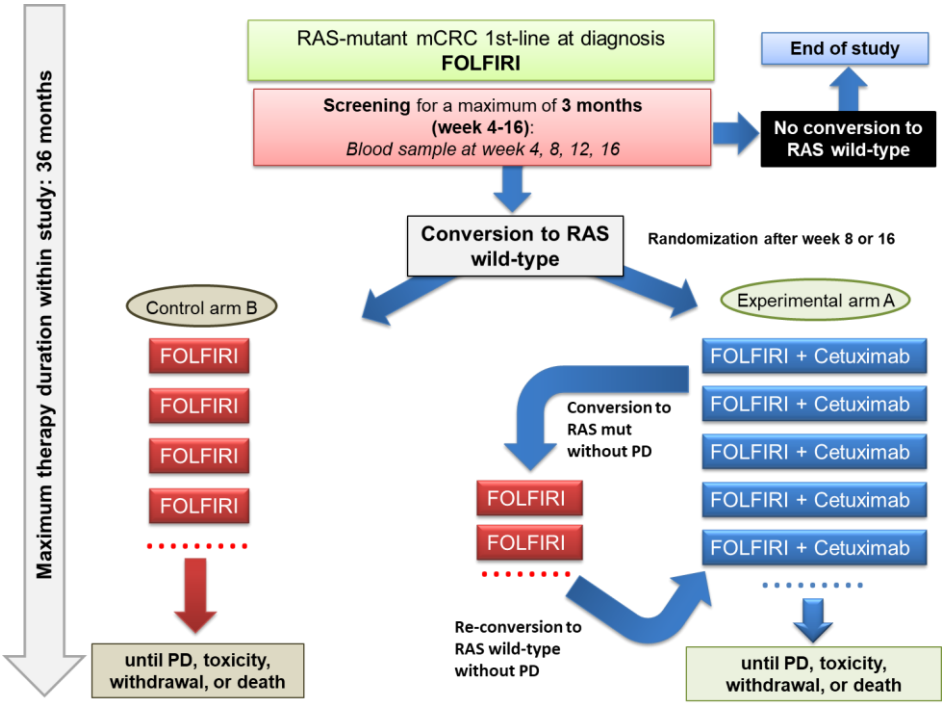
Background and rationale for the study:

Colorectal Cancer (CRC) is the third most common cancer worldwide. 20-25% of patients are initially diagnosed with metastatic disease (UICC stage IV). The therapeutic management of CRC is strongly dependent on the disease stage. Next to surgery and chemotherapy, targeted therapies are available.

The use of EGFR-Inhibitors such as cetuximab is used in the therapy of *RAS* wild-type mCRC with left sided primary tumors, since it has been shown that cetuximab is ineffective in CRC with *KRAS* mutation and location of the primary tumors in the right side is associated with minor response.

A major limitation in the treatment of *RAS* wild-type patients with cetuximab is the development of resistance during anti-EGFR treatment that is predictive for reduced benefit from this therapy. After discontinuation of EGFR inhibition, *RAS* mutational load rapidly decreases. Therefore, an adaption of the therapy in accordance to regular monitoring of *RAS*-status should be used to adjust therapy. Patients with mutant *RAS* received standard 1st line therapy with FOLFIRI until conversion of *RAS* to wild-type was observed. Patients were randomised to receive adjusted treatment according to

RAS status in the experimental arm or FOLFIRI monotherapy in the control arm. The intermittent addition of cetuximab to FOLFIRI until re-conversion to mutant *RAS* was compared to FOLFIRI alone.



Objectives:

Primary objective:

To evaluate efficacy in terms of progression free survival (PFS) from the date of randomization in the study according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria in experimental and control arms

Secondary objectives:

- Overall survival (OS) in experimental and control arms from date of randomization
- Time to failure of treatment strategy (TFTS) in experimental and control arms after randomization
- PFS rate 1 year after date of randomization
- Depth of response in terms of reduction of tumormass in experimental and control arms after start of 1st-line treatment
- Metastasis resections in experimental and control arms after start of 1st line treatment
- Objective response rate (ORR) defined as patients with partial or complete response (CR + PR) in experimental and control arms after start of 1st-line treatment
- Safety profile according to CTCAE, Version 5.0 criteria in experimental and control arms recorded from the date of signature of Informed Consent

Exploratory objectives:

- To identify driver mutations (e.g. BRAF, PI3K-AKT-mTOR etc.) in patients with progressive disease (PD) under cetuximab therapy who remain *RAS* wild-type in liquid biopsy
- To compare the efficacy in terms of progression free survival (PFS) in patients with conversion to *RAS* wild-type in ddPCR and BEAMing, both sensitive digital Polymerase Chain Reaction methods, with those patients showing conversion to *RAS* wild-type in ddPCR but not in BEAMing

Methodology: This was a prospective, open-label, randomized, multicentre phase II trial conducted in Germany and Austria to evaluate the efficacy and safety of intermittent addition of

cetuximab to a FOLFIRI-based 1st-line therapy in patients with *RAS*-mutant mCRC at diagnosis who converted to *RAS* wild-type.

Number of patients (planned and analysed):

It was planned to include a total of 144 patients with left-sided *RAS*-mutated mCRC into pre-randomization phase, the expected number of patients eligible to randomization due to conversion to *RAS* wild-type until week 16 was 116: 58 patients in the experimental arm, 58 patients in the control arm.

129 patients were screened; six patients were randomized (experimental arm: N=4; control arm: N=2).

Diagnosis and main criteria for inclusion and exclusion:

Diagnosis: UICC stage IV adenocarcinoma of the left-sided colon or rectum with metastases primarily non-resectable, confirmed *RAS* mutations proven in the primary tumor or metastasis (*KRAS* and *NRAS* exon 2, 3, 4)

Inclusion criteria:

1. Age ≥ 18 years on day of signing informed consent
2. No previous chemotherapy for metastatic disease (1 - 2 cycles FOLFIRI or mFOLFIRI are permitted before enrolment until *RAS* status is determined)
3. Patients suitable for chemotherapy administration
4. ECOG performance status 0 - 1
5. Consent to liquid biopsy and mutation analysis
6. Estimated life expectancy > 3 months
7. Presence of at least one measurable reference lesion according to the RECIST 1.1 criteria (chest CT and abdominal CT 4 weeks or less before enrolment)
8. Adequate organ system function
9. Time interval of at least 6 months since last administration of any previous neoadjuvant/adjuvant chemotherapy or radiochemotherapy of the primary tumor in curative treatment intention
10. Any relevant toxicities of prior treatments must have resolved to grade ≤ 1 according to the CTCAE (version 5), except alopecia
11. Women of childbearing potential (WOCBP) should have a negative urine pregnancy test within 72 hours prior to receiving the first dose of study medication, and agrees to use adequate contraception
12. Signed written informed consent and capacity to understand the informed consent

Exclusion criteria:

1. Right sided mCRC
2. Primarily resectable metastases
3. Previous chemotherapy for the colorectal cancer except for adjuvant treatment, completed at least 6 months before entering the study (1-2 cycles of FOLFIRI or mFOLFIRI are permitted before enrolment)
4. Patients with known brain metastases
5. Symptomatic peritoneal carcinosis
6. Progressive disease before randomization
7. History of acute or subacute intestinal occlusion, inflammatory bowel disease, immune colitis or chronic diarrhea
8. Grade II heart failure (NYHA classification), Myocardial infarction, balloon angioplasty (PTCA) with or without stenting, and cerebral vascular accident/stroke within the past 12 months before enrolment, unstable angina pectoris, serious cardiac arrhythmia according to investigator's judgment requiring medication
9. Medical or psychological impairments associated with restricted ability to give consent or not allowing conduct of the study

10. Active infection with hepatitis B or C
11. Additional cancer (exceptions include adequately treated basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy without evidence of recurrence)
12. Uncontrolled hypertension
13. Marked proteinuria (nephrotic syndrome)
14. Arterial thromboembolism or severe hemorrhage within 6 months prior to randomization (except for tumor bleeding before tumor resection surgery)
15. Hemorrhagic diathesis or tendency towards thrombosis
16. Participation in a clinical study or experimental drug treatment within 30 days prior to study
17. Known hypersensitivity or allergic reaction to any of the study medications
18. Severe, non-healing wounds, ulcers, bone fractures or an infection requiring systemic therapy
19. Known history of alcohol or drug abuse
20. Complete dihydropyrimidine dehydrogenase (DPD) deficiency (phenotype and/or genotype test)
21. Known glucuronidation deficiency (Gilbert's syndrome) (specific screening not required)
22. Absent or restricted legal capacity
23. For female patients only: Pregnancy (absence to be confirmed by ß-HCG test) or lactating

Test product (dosage, method of administration, batch number(s))

FOLFIRI + cetuximab

- Irinotecan 180 mg/m^2 iv, 30-90 min
- Folinic acid (racemic) 400mg/m^2 iv, 120 min
- 5-FU 400 mg/m^2 bolus
- 5-FU 2400 mg/m^2 iv, over 46 h
- Cetuximab: initially 400 mg/m² iv, 120 min (≤ 5 mg/min), subsequently 250 mg/m² iv, 60 min infusion every week (≤ 10 mg/min)

OR

mFOLFIRI + cetuximab:

- Irinotecan 180 mg/m² iv, 30 - 90 min
- Folinic acid (racemic) 400 mg/m² iv, 120 min
- 5-FU 2400 mg/m² iv over 46 h
- Cetuximab initially 400 mg/m^2 as a 120 min infusion (≤ 5 mg/min), subsequently 250 mg/m^2 iv as a 60 min infusion every week (≤ 10 mg/min)

Batch numbers cetuximab:

G00T4B
G00SY1
G00X1K
G010XY
G012F4
G012F5
G013DH
G0157D
GO15RU
GO17AM
G00XGL

Duration of treatment:

Patients continued study treatment for a maximum of 36 months or until disease progression, unacceptable toxicity, withdrawal of informed consent, patient preference or death, whichever occurred first

Reference product (dosage, method of administration, batch number(s))

FOLFIRI

- Irinotecan 180 mg/m^2 iv, 30-90 min
- Folinic acid (racemic) 400mg/m^2 iv, 120 min
- 5-FU 400 mg/m^2 bolus
- 5-FU 2400 mg/m^2 iv, over 46 h

OR

mFOLFIRI

- Irinotecan 180 mg/m² iv, 30 - 90 min
- Folinic acid (racemic) 400 mg/m² iv, 120 min
- 5-FU 2400 mg/m² iv over 46 h

Batch numbers Irinotecan:

Pre-randomization phase:	Randomization phase:
M2006116	M2010732
M2010732	M2014001
AC0283S	AC0283S
M2014001	AC0284S
CF41	AC0292AS
CF45	AC0303S
CM94	AC291
	M2015432
	AC0921
	CF41

Batch number Folinic Acid

Prerandomization phase	Randomization phase
OH125HO	9N109N9
BK81U	0N138N0
BR90	BK81U
CB21	CB21
BL10U	CH89
CL13	BL10U
	CL13
	0N139C1
	CL10U
	10CL13
	CL24
	16QG1971
	OG124H0

Batch number 5-FU

Prerandomization phase	Randomization phase
PY06909	F200304A
E200264A	F200322AA
E200270A	F200306A
F200304A	F200307A

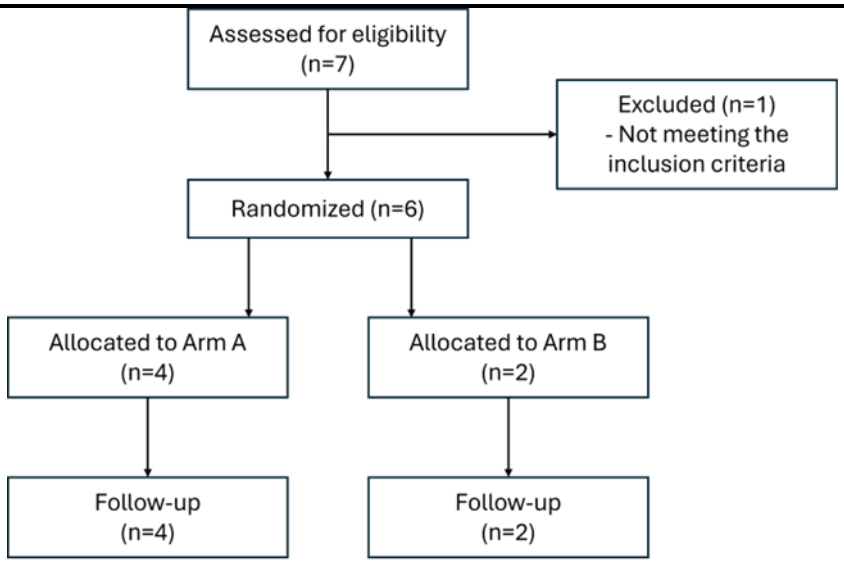
2004009S	F200328A
2004010S	P2001386
L190730AA	F200324A
C200111AA	F200333A
F200323AA	H210313A
G200389AA	P2000331
F200331AA	2004010S
	C200152AA
	AF0074S
	AF0077
	D200202AA
	AF0087F
	E200259AA
	AF0087S
	AF0101S
	F210313A
	H210305A
	H210307A
	H210309A
	H210312A
	K210463A

Endpoints: not applicable

Statistical methods:
Due to the small number of patients included in the study, most analyses were presented as listings. Summary statistics of continuous variables like mean, standard deviation, and quantiles were not provided. Event-related data like ORR, OS, and PFS were estimated by the Kaplan-Meier method. A swimmer plot showing the treatment and response timeline for each patient was calculated. Each listing showed the patient number, the arm into which the patient was randomized, and all relevant variables.
There was no differentiation between the analysis populations mITT (modified Intention to treat population), SP (Safety Population) and PP (per protocol population).

Summary of results and Conclusion*:*

Subject Disposition: Between [redacted] 2020 and [redacted] 2021, 129 patients were screened by 20 sites in Germany and 1 site in Austria. Six patients were enrolled by four sites in Germany.
Four patients were randomized into the experimental arm and two patients the control arm. All six patients received at least one dose of study treatment.
The following figure gives an overview of the patient distribution.



Demographic and baseline characteristics: Patients enrolled in the study were between [redacted] and [redacted] years old. One female and five male patients were included in the study. All patients were Caucasian. Four out of six randomized patients had an ECOG of 0 at Baseline. Two Patients had ECOG 1 at Baseline.
Regarding previous therapies five out of six patients received a resection of the primary tumor before study inclusion, all with a Resection status of R0.
The time from baseline sampling until conversion to *RAS* wild-type ranged between 21 days and 64 days in the experimental arm and between 21 and 29 days in the control arm..

Primary efficacy endpoint: PFS of patients
PFS of patients, defined as time between date of randomization until the date of progression according to RECIST v.1.1 criteria or date of death from any cause, whichever occurred first, ranged from 1.97 to 9.77 months in the experimental treatment group. In the control group PFS ranged from 3.68 to 8.95 months.

Secondary efficacy endpoint: Objective response rate (RECIST v1.1 criteria)
The ORR was defined as patients with partial or complete response (PR + CR).
No patient irrespective of treatment received a CR. Three patients in the experimental arm achieved PR. For the control arm, one patient showed PR

Secondary efficacy endpoint: Overall survival
OS was calculated from the date of randomization until death from any cause. OS in the experimental arm ranged from 10.86 to 35.07 months, in the control arm from 10.23 to 28.36 months.

Secondary efficacy endpoint: Time to failure of treatment strategy
TFTS was defined as time from date of randomization to failure of treatment strategy defined as treatment discontinuation for any reason. In the experimental arm the shortest TFTS was 1.88 months, the maximum TFTS was 7.83. The median was 4.9.
In the control arm one patient had a failure of treatment strategy after 4.05 months, the other patient after 7.83 months. The calculated median was 4.9; however, the small sample size of patients limited the statistical validity of this calculation.

Safety results:
All six patients who received at least one cycle of the study treatment reported a total of 78 adverse events (AE). Thereof, 47 AEs were classified as treatment-related. Three patients had at least one

AE with maximal grade of 2, one patient had at three AEs of grade 3. Two patients with AEs died as result of an AE. 50% of the patients experienced a serious AE (SAE).

Most frequently reported AEs were nausea (N=5), diarrhoea (N=4), fatigue and alopecia (N=3 each).

AEs were defined as special if they involved pregnancy or medication error. There were two cases of medication error reported as special AE. Once medication was overdosed, the other time medication dose was changed.

Conclusion(s*):*
This multi-centre, randomized phase-II clinical study aimed to investigate the efficacy and safety of the adaption of adding cetuximab to 1st-line therapy with FOLFIRI after *RAS*-mutation status changed to wild-type and changing back to FOLFIRI, as required if *RAS*-mutation status changed to mutant.
The planned number of patients was not reached. The recruitment phase was terminated early. Only six patients were randomized (experimental arm: N=4, control arm: N=2). All randomized patients were evaluable for analysis of efficacy and safety. Due to the small number of evaluable patients the explanatory power of these results is limited.

The primary efficacy endpoint was PFS. In the experimental arm, PFS ranged from 1.97 to 9.77 months, in the control arm PFS ranged from 3.68 to 8.95 months.

Analysis of the secondary efficacy objectives (ORR, OS, TFTS) Was not able to show differences between experimental and control group due to limited data.
78 AEs in six patients were recorded during the study. NCI grade of the AEs ranged from 1 (N=57) to 5 (N=2). Four SAEs occurred in three patients. Mainly AEs in the SOC ‘gastrointestinal disorders’ and ‘Investigations’ were experienced which is in line with the toxicity profile of FOLRIRI and Cetuximab. No new safety issues for FOLFIRI and Cetuximab were identified in the study.

In 2014, 61.000 people were diagnosed with CRC and a total of 25.5000 patients died due to CRC. In 2022, the number of people affected was roughly constant. The numbers are trending upwards, making clear that there is still a great unmet need for further development of CRC treatment, especially for advanced stages.
The development of resistance to targeted therapies is a major limitation in treatment of mCRC, thus the development of new strategies to overcome secondary drug resistance is very important. The approach of liquid biopsy-guided therapy, in which *RAS* mutation status is analysed at regular intervals and the therapy is adjusted, as investigated in this study could be one solution for this problem. Analysis of ctDNA is component of further studies that investigate re-challenge therapy with anti-EGRF after development of resistance in previous therapies.
Future studies should continue to investigate the value of regular re-evaluation of *RAS* mutation status by liquid biopsy and subsequent adjustment of anti-EGFR therapy in patients with mCR. The optimal timing for sample collection should also be an aspect of the research.
To address the problem of low patient recruitment in future studies, the study design should be kept as simple as possible. Consideration should be given to administering cetuximab at a once every-second-week dose of 500 mg/m^2 instead of 250 mg/m^2 on day 1 and day 8. The safety of this administration of cetuximab was assessed in a phase-I dose escalation study.
Another problem in this study was the time required to determine the RAS mutation status in patients with high therapy pressure. Faster molecular pathological analysis of samples could make these patients eligible for the study.

Date and version of this report: v2.0, October 21st, 2025