Clinical Study Protocol

Sponsor's Protocol Code No.	4213000
EudraCT-No.	2019-003714-14
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
Short Title	**MoLiMoR**
Protocol Version	**3.0 (including Amendment 1)**
Date of Protocol	**09.03.2021**
Study Phase	**II**
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Investigator Signature Page

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I confirm that I have read this study protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practice, or the applicable laws and regulations of the country of the study site for which I am responsible, whichever provides the greater protection of the individual. I am aware of my responsibilities as an Investigator / Representative under the GCP national regulations and trial protocol. I agree to appropriately direct and assist the staff under my control, who will be involved in this clinical trial. This is documented in a training log.

Site Number: _____

Site Name: _____

Site Address: _____

_____

_____	_____	_____
Date	Investigator, Print Name	Signature

_____	_____	_____
Date	Representative, Print Name	Signature

Protocol Versions

Version	Date	Comments	Changes in Patient information / informed consent
Version 1.0	28.08.2019	Final version for competent authorities and ethics committee	First version: Version 1.0, 28.08.2019
Version 1.1	20.02.2020	Revision according to deficiency letter of competent authority	No
Version 2.0	18.05.2020	Revision according to deficiency letter of ethics committee including changes required for competent authority	Version 2.0, 18.05.2020
Version 2.1	03.07.2020	Inserting information according to appraisal letter of ethics committee	Version 2.1, 03.07.2020
Version 3.0	09.03.2021	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	Version 3.0, 09.03.2021

List of Abbreviations Used in the Text

AE	Adverse event
ALAT	Alanine-aminotransferase (= SGPT = serum glutamate pyruvate transaminase)
ASAT	aspartate-aminotransferase (= SGOT = serum glutamate oxalacetate transaminase)
BRAF	Human gene that encodes B-Raf
CA19-9	Carbohydrate-Antigen 19-9
CEA	Carcinoembryonic antigen
Cq	Cycle quantification value
CR	Complete response
CRC	Colorectal cancer
CRF	Case report form
CRO	Clinical research organization
CRP	C-reactive protein
CRS	Cytokine release syndrome
CT	Computer tomography
CTC	Circulating tumor cells
CTCAE	Common Terminology Criteria of Adverse Events
cfDNA	Circulating cell-free DNA
ctDNA	Circulating cell-free tumor DNA
CTFG	Clinical Trial Facilitation Group
ddPCR	Droplet Digital PCR
DNA	Deoxyribonucleic acid
DPD	Dihydropyrimidine dehydrogenase
DpR	Depth of response
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
ECOG (PS)	Eastern Cooperative Oncology Group (Performance Status)
EDC	Electronic data capture
eCRF	electronic case report form
e.g.	for example
EGFR	Epidermal growth factor receptor
FFPE	Formalin-fixed paraffin-embedded
FPI	First patient in
5-FU	Fluorouracil
GCP	Good Clinical Practice
GCP-V	German Good Clinical Practice Ordinance
GGT	Gamma-glutamyltransferase
h	Hour
ICH-GCP	International Conference on Harmonisation - Good Clinical Practice
INR	International normalized ratio
ISF	Investigator site file
ITT	Intent to treat
KRAS	Kirsten Rat Sarcoma Virus
LKP	National Coordinating Investigator (Leiter Klinische Prüfung)
LP	last patient
LPI	Last patient in
LVEF	Left ventricular ejection fraction
mAB	monoclonal antibodies
mCRC	Metastatic colorectal cancer
mFOLFIRI	modified FOLFIRI
MedDRA	Medical dictionary for regulatory activities
MRI	Magnetic resonance imaging
NCI	National Cancer Institute

NPY	Neuropeptide Y
ORR	Overall response rate
OS	Overall survival
OTC	Over-the-counter
PCR	Polymerase chain reaction
PD	Progressive disease
PFS	Progression free survival
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PP	Per protocol
PR	Partial response
PT	Preferred term
(a)PTT	(activated) Partial thromboplastin time
RAID	Redundant Array of Independent Disks
RAS	Rat sarcoma
RECIST	Response Evaluation Criteria In Solid Tumors V 1.1
SAE	Serious adverse event
SD	Stable disease
SmPC	*Produkt-/ Fachinformation* [Summary of medicinal Product Characteristics]
SOC	System organ class
SP	Safety population
SUSAR	Suspected unexpected serious adverse reaction
TFTS	Time to failure of treatment strategy
TLS	Transport Layer Security
UICC	Union for International Cancer Control
ULN	Upper limit of normal
VEGF	Vascular endothelial growth factor
WBDC	Web Based Data Capture
WIF1	Wnt inhibitory factor 1
WOCBP	Women of childbearing potential

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1 Synopsis

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 MoLiMoR
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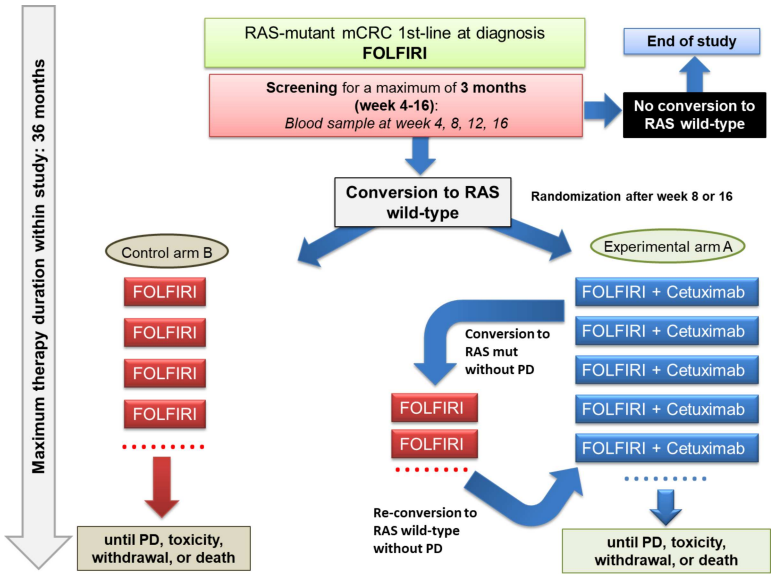
Study phase	Clinical phase II
Study under IND	☐yes ☒ no
FDA “covered study”	☐yes ☒ no
Study sites	Up to 30 sites in Germany and Austria
Planned study period	Duration of recruitment: 18 months at a rate of 8 patients/month (counted from first patient in (FPI)). Follow-up from last patient in (LPI) to end of safety follow up: 1 month after last administration of up to 36 months of study treatment. Further follow-up for survival until trial termination: 4.5 years after FPI. Expected total trial duration: 4.5 years
Study objectives	The aim of this randomized study is to evaluate whether patients with left sided RAS-mutant mCRC at diagnosis will derive a benefit from the adaptation of adding cetuximab to first-line therapy (FOLFIRI) after RAS-mutation status has changed to wild-type and changing back to FOLFIRI, as required if RAS-mutation status has changed to mutant, depending upon, and monitored by longitudinal ctDNA liquid biopsies. **Primary objective:** The primary objective is 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 tumor mass 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 (optional):** • 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 both ddPCR and BEAMing with those patients showing conversion to *RAS* wild-type in ddPCR but not in BEAMing
Study design	This is an open-label, prospective, randomized, multicenter phase II trial that will evaluate the efficacy and safety of intermittent addition of cetuximab to a FOLFIRI-based 1st-line therapy to patients with *RAS*-mutant mCRC at diagnosis who convert to *RAS* wild-type using monitoring of the *RAS* mutation status by liquid biopsy. Patients with left-sided *RAS*-mutated metastatic colorectal cancer (mCRC) eligible for study participation and receiving standard FOLFIRI as 1st line treatment will be screened for a maximum for 3 months (from week 4 to week 16) for conversion to *RAS* wild-type. At week 8, for patients converting at week 4 or 8, and at week 16 for patients converting at week 12 or 16 (directly after follow-up CT scan in each case), patients without progressive disease will be randomized 1:1 into experimental Arm A (switch arm) or control Arm B. For patients without conversion to RAS wild-type by week 16, the 'end of study'-form will be completed. Patients in Arm A will receive FOLFIRI + cetuximab until progressive disease (PD), unacceptable toxicity, withdrawal of informed consent or death, whatever occurs first. The recurrence of *RAS*-mutation without PD leads to switch back to FOLFIRI. In case of repeated conversion to *RAS* wild-type without PD, treatment will shift to FOLFIRI + cetuximab again, and so on. Switches of treatment will proceed until progressive disease (PD), unacceptable toxicity, withdrawal of informed consent or death, whatever occurs first. Patients in Arm B will continue therapy with FOLFIRI until PD, unacceptable toxicity, withdrawal of informed consent or death, whatever occurs first.

	An overview of study design is shown in the following figure.  Patients terminating therapy due to PD, unacceptable toxicity, or treatment withdrawal for other reasons, prior to maximum study duration of 36 months, will be followed for survival data every 3 months. As a 'proof of concept model', the recruitment will be paused after randomization of 20 patients. In the first 20 patients with conversion to ctDNA *RAS* wild-type, an additional tumor biopsy will be taken (if feasible), at the time point of conversion for RAS analysis, locally within 10 days; if the *RAS* results are concordant the patient will be randomized. Overall, in at least 80% of patients a biopsy should be taken to confirm RAS status in tissue of respective patients. Blood samples will be taken at more frequent time points after randomization (at weeks 2, 4, 6 and 8) to be analyzed for *RAS* status. Results of these analyses will be reviewed by a Data and Safety Monitoring Board (DSMB) and notified to the ethics committee together with recommendations of the DSMB regarding study conduct. In addition, after 50 screened patients, the proportion of patients with conversion from *RAS* mutant to *RAS* wild-type will be analyzed and evaluated by the DSMB. If less than 50% of analyzed patients show conversion to *RAS* wild-type, the DSMB will make recommendations regarding the conduct of the study. These will be notified to the ethics committee.
Planned number of patients	Estimated number patients to be screened (conversion rate of 80%): 144 Total number of randomized patients: 116 The screening will be continued until randomization of 116 patients.

	After randomization of 20 patients: Randomization will be paused and results of *RAS* analyses in blood samples and tumor biopsies will be reviewed by a Data and Safety Monitoring Board (DSMB) and notified to the ethics committee together with recommendations of the DSMB regarding study conduct. After screening of 50 patients: The proportion of patients with conversion from *RAS* mutant to *RAS* wild-type will be analyzed and evaluated by the DSMB. If less than 50% of analyzed patients show conversion to *RAS* wild-type, the DSMB will make recommendations regarding the conduct of the study, which will be notified to the ethics committee.
Patient selection	**Inclusion Criteria:** Patients may be **included** in the study only if they meet **all** the following criteria: • Histologically confirmed, UICC stage IV adenocarcinoma of the left-sided colon or rectum with metastases (metastatic colorectal cancer), primarily non-resectable, confirmed *RAS* mutations proven in the primary tumor or metastasis (*KRAS* and *NRAS* exon 2, 3, 4) • Age ≥ 18 years on day of signing informed consent • No previous chemotherapy for metastatic disease (1- 2 cycles FOLFIRI or mFOLFIRI are permitted before enrolment until RAS status is determined) • Patients suitable for chemotherapy administration • ECOG performance status 0-1 • Consent to liquid biopsy and mutation analysis • Estimated life expectancy > 3 months • 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 enrollment) • Adequate bone marrow function defined as: o Leukocytes 3.0 x 10^9/L with neutrophils 1.5 x 10^9/L o Thrombocytes 100 x 10^9/L o Hemoglobin 9 g/dL • Adequate hepatic function defined as: o Serum bilirubin 1.5 x ULN o ALAT and ASAT 2.5 x ULN (in the presence of hepatic metastases, ALAT and ASAT 5 x ULN) • Adequate renal function: Creatinine clearance ≥50 mL/min • Adequate cardiac function defined as o Normal ECG and echocardiogram with a left ventricular ejection fraction (LVEF) of 55% • INR < 1.5 and aPTT < 1.5 x ULN (patients without anticoagulation). Therapeutic anticoagulation is allowed if INR and aPTT have remained stable within the therapeutic range for at least 2 weeks. • 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 to start of 1st line treatment

	• Any relevant toxicities of prior treatments must have resolved to grade ≤ 1 according to the CTCAE (version 5), except alopecia • Women of childbearing potential (WOCBP) should have a negative urine pregnancy test within 72 hours prior to receiving the first dose of study medication. • Highly effective contraception for both male and female patients throughout the study and for at least 3 months after last dose of study medication administration if the risk of conception exists. Highly effective contraception has to be in line with the definition of the CTFG (Clinical Trial Facilitation Group) recommendation • Signed written informed consent and capacity of understanding the informed consent **Exclusion Criteria:** Patients will be **excluded** from the study for **any** of the following reasons: • Right sided mCRC • Primarily resectable metastases • Previous chemotherapy for the colorectal cancer with the exception of adjuvant treatment, completed at least 6 months before entering the study (1- 2 cycles FOLFIRI or mFOLFIRI are permitted before enrolment) • Patients with known brain metastases • Symptomatic peritoneal carcinosis • Progressive disease before randomization • History of acute or subacute intestinal occlusion, inflammatory bowel disease, immune colitis or chronic diarrhea • 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 enrollment, unstable angina pectoris, serious cardiac arrhythmia according to investigator's judgment requiring medication • Active infection with hepatitis B or C • Medical or psychological impairments associated with restricted ability to give consent or not allowing conduct of the study • 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 • Uncontrolled hypertension • Marked proteinuria (nephrotic syndrome) • Arterial thromboembolism or severe hemorrhage within 6 months prior to randomization (with the exception of tumor bleeding before tumor resection surgery) • Hemorrhagic diathesis or tendency towards thrombosis • Participation in a clinical study or experimental drug treatment within 30 days prior to study • Known hypersensitivity or allergic reaction to any of the study medications

	• Severe, non-healing wounds, ulcers, bone fractures or an infection requiring systemic therapy • Known history of alcohol or drug abuse • Complete dihydropyrimidine dehydrogenase (DPD) deficiency (phenotype and/or genotype test) (Patients with partial DPD deficiency may be included in this clinical trial at the discretion of the investigator and should receive a reduced starting 5-FU dose) • Known glucuronidation deficiency (Gilbert's syndrome) (specific screening not required) • Absent or restricted legal capacity • For female patients only: Pregnancy (absence to be confirmed by ß-HCG test) or lactating
	The 1st-line treatment is defined as therapy until progressive disease (PD), unacceptable toxicity, withdrawal of informed consent, loss to follow-up, patient preference, or death. **FOLFIRI (q14d):** o Irinotecan 180 mg/m² iv, 30 - 90 min, day 1 o Folinic acid (racemic) 400 mg/m² iv, 120 min, day 1 o Fluorouracil (5-FU) 400 mg/m² bolus, day 1 o 5-FU 2400 mg/m² iv over 46 h, day 1-2 OR **modified FOLFIRI (mFOLFIRI) (q14d):** o Irinotecan 180 mg/m² iv, 30 - 90 min, day 1 o Folinic acid (racemic) 400 mg/m² iv, 120 min, day 1 o 5-FU 2400 mg/m² iv over 46 h, day 1-2 **FOLFIRI** or **mFOLFIRI (q14d) + cetuximab (q7d):** o Irinotecan 180 mg/m² iv, 30 - 90 min, day 1 o Folinic acid (racemic) 400 mg/m² iv, 120 min, day 1 o [5-FU 400 mg/m² bolus, day 1] o 5-FU 2400 mg/m² iv over 46 h, day 1-2 o Cetuximab initially 400 mg/m² as a 120 min infusion (≤ 5 mg/min); subsequently 250 mg/m² iv as a 60 min infusion (≤ 10 mg/min), day 1 + 8 **One cycle consists of 14 days**
Planned treatment duration per patient	Treatment of randomized patients will be continued for a maximum of 36 months from start of first line therapy or until PD, unacceptable toxicity, withdrawal of informed consent, patient preference or death, whatever occurs first.

Statistical hypothesis and sample size calculation	It is assumed that the difference in PFS at 12 months between the experimental and control arms will amount to 20%, which means an increase from 30% to 50% (Hazard ratio = 0.5757). Data from historical studies suggest the 12-months PFS rate of FOLFIRI in *RAS*-mutant mCRC to be in a range of 25-27%. In the CRYSTAL study PFS-rate at 12 months of FOLFIRI + Cetuximab in left-sided *RAS* wild-type mCRC was 50% (HR = 0.56). With a two-sided alpha error of 5% (0.05) and beta error of 20% (0.2) 2 x 58 patients are to be randomized and 107 PFS events are needed to achieve a power of 80%. With a recruitment period of 18 months, a drop-out rate of 0.25% per month the 107 events are expected after a total follow-up time of 52 months assuming an exponential distribution.

Schedule of visits and assessments

Patients	***All patients***		***Randomized patients (Experimental arm)***			***Randomized patients (Control arm)***			**End of therapy**	**Follow-up**
Trial period	Pre-Screening / Baseline	Pre-randomization (Screening) Phase (for conversion to *RAS* wild-type)	Switch arm (FOLFIRI/mFOLFIRI + cetuximab -> FOLFIRI/mFOLFIRI -> FOLFIRI/mFOLFIRI + cetuximab -> etc)			FOLFIRI/mFOLFIRI			Final Assessment	FU**
Scheduling window	-28 - -1 days	From Months 1 to 4	Every 2 weeks ± 3 days	Every 4 weeks ± 3 days	Every 8 / 12 weeks ± 3 days*	Every 2 weeks ± 3 days	Every 4 weeks ± 3 days	Every 8 / 12 weeks ± 3 days*	30± 2 days after last treatment	3 months ± 7 days
Procedures / Assessments										
Written informed consent	X									
Inclusion/Exclusion Criteria	X	X (before randomization)								
RAS mutation analysis on tumor tissue	X	X (New tumor biopsy at conversion, if possible, for the first 20 patients with conversion to *RAS* wild-type in liquid biopsy)***								
20 ml blood sample for liquid biopsy with *RAS* mutation analysis	X[1]	X[2]	X[2] (until week 8 for the first 10 patients)	X[2] (until week 8)	X[2] (from week 16 on)			X (week 8 / 24 only)		
Vital signs[3]	X	X (before study treatment administration)	X			X				

Patients	***All patients***		***Randomized patients (Experimental arm)***			***Randomized patients (Control arm)***			**End of therapy**	**Follow-up**
Trial period	Pre-Screening / Baseline	Pre-randomization (Screening) Phase (for conversion to *RAS* wild-type)	Switch arm (FOLFIRI/mFOLFIRI + cetuximab -> FOLFIRI/mFOLFIRI -> FOLFIRI/mFOLFIRI + cetuximab -> etc)			FOLFIRI/mFOLFIRI			Final Assessment	FU**
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Procedures / Assessments										
Demographics and medical history / tumor characterization	X									
Previous and concomitant medication[4]	X									
ECOG performance status	X	X (before study treatment administration)	X			X				
ECG[5]	X	If clinically indicated	If clinically indicated			If clinically indicated				
Echocardiography	X									
Tumor imaging examination[6]	X	X[7]			X			X		
Blood count[8]	X	X	X			X			X	
Biochemistry[9]	X	X	X			X			X	

Patients	***All patients***		***Randomized patients (Experimental arm)***			***Randomized patients (Control arm)***			**End of therapy**	**Follow-up**
Trial period	Pre-Screening / Baseline	Pre-randomization (Screening) Phase (for conversion to *RAS* wild-type)	Switch arm (FOLFIRI/mFOLFIRI + cetuximab -> FOLFIRI/mFOLFIRI -> FOLFIRI/mFOLFIRI + cetuximab -> etc)			FOLFIRI/mFOLFIRI			Final Assessment	FU**
Scheduling window	-28 - -1 days	From Months 1 to 4	Every 2 weeks ± 3 days	Every 4 weeks ± 3 days	Every 8 / 12 weeks ± 3 days*	Every 2 weeks ± 3 days	Every 4 weeks ± 3 days	Every 8 / 12 weeks ± 3 days*	30± 2 days after last treatment	3 months ± 7 days
Procedures / Assessments										
Test for DPD deficiency	X[15]									
Coagulation parameters (INR, aPTT)	X									
Hepatitis B and C serology	X									
CEA and CA 19-9	X	X (every 2 weeks)	X			X			X	
ß-HCG-test (for WOCBP)[10]	X (urine)	X (urine) (every 4 weeks)		X (urine)			X (urine)		X (urine)	
Administration of study treatment	X[11] (FOLFIRI/ mFOLFIRI)	X[11] (FOLFIRI/mFOLFIRI)	X[12]			X				
Adverse Events (AEs) / Serious adverse Events (SAEs)[13]	X	X (after every cycle)	X[14]			X			X	X (SAEs related to study treatment

Patients	***All patients***		***Randomized patients (Experimental arm)***			***Randomized patients (Control arm)***			**End of therapy**	**Follow-up**
Trial period	Pre-Screening / Baseline	Pre-randomization (Screening) Phase (for conversion to *RAS* wild-type)	Switch arm (FOLFIRI/mFOLFIRI + cetuximab -> FOLFIRI/mFOLFIRI -> FOLFIRI/mFOLFIRI + cetuximab -> etc)			FOLFIRI/mFOLFIRI			Final Assessment	FU**
Scheduling window	-28 - -1 days	From Months 1 to 4	Every 2 weeks ± 3 days	Every 4 weeks ± 3 days	Every 8 / 12 weeks ± 3 days*	Every 2 weeks ± 3 days	Every 4 weeks ± 3 days	Every 8 / 12 weeks ± 3 days*	30± 2 days after last treatment	3 months ± 7 days
Procedures / Assessments										
Change in or new concomitant medication		X (before study treatment administration)	X			X				
New tumor therapy									X	X
Survival data									X	X

* Every 8 weeks during the first year, thereafter every 12 weeks up to end of study duration of 36 months

** 3-monthly follow-up visits until end of study duration of 36 months; phone calls will count as study visits.

*** The results of *RAS* status, assessed locally, in tumor biopsy must be available within 10 days after the results of liquid biopsy become known. No randomization in case of different results for *RAS* status in tumor and liquid biopsy. 'End of study' form must be filled in for this case. Overall, in at least 80% of patients a biopsy should be taken to confirm *RAS* status in tissue of respective patients.

[1)] Blood samples from pre-screening/baseline will be analyzed only in cases with *RAS* wild-type in liquid biopsy at week 4 after starting FOLFIRI/mFOLFIRI. In patients who receive 1 -2 cycles FOLFIRI/mFOLFIRI in the pre-screening phase, the blood sample should be taken before administration of these cycles.

[2a)] Before randomization: Blood samples for liquid biopsy until detection of *RAS* wild-type at week 4, 8, 12 and 16 after starting FOLFIRI/mFOLFIRI .

[2b)] After randomization into the experimental arm: For the first 10 randomized patients only, blood samples for liquid biopsy at week 2, 4, 6 and 8 after starting FOLFIRI/mFOLFIRI; for subsequent patients at weeks 4 and 8; thereafter, for all patients, every 8 weeks for the first year, and every 12 weeks for the remaining study period. If receiving FOLFIRI/mFOLFIRI + cetuximab and disease progression is detected on CT and prior ctDNA analysis was wild-type, a single ctDNA analysis should be performed at the time of disease progression.

[3)] Body temperature, heart rate, blood pressure, weight and height (only at baseline)

[4)] Previous medication taken within the last 2 weeks before date of informed consent has to be recorded.

[5)] If clinically indicated, more ECGs should be performed during study

[6)] CT scan: abdomen, chest and pelvis. In cases of contraindicated CT scan (e.g. contrast medium allergy) the MRI should be performed. The same method should be used throughout the study.

[7)] Every 8 weeks ± 3 days

[8)] Hemoglobin, thrombocytes, leukocytes, neutrophils (within 2 days before administration of study treatment)

[9)] Na^+, K^+, Mg^{2+}, Ca^{2+}, ASAT, ALAT, alkaline phosphatase, Gamma-glutamyl transferase (GGT), bilirubin, urea, creatinine, creatinine-clearance and C-reactive protein (CRP) (within 2 days before administration of study treatment)

[10)] Urine pregnancy test must be conducted for WOCBP; a negative result must be confirmed within 72 hours of first administration of study treatment

[11)] Every 2 weeks ± 3 days. 1-2 cycles of FOLFIRI / mFOLFIRI may be administered in the pre-screening period before the result of the RAS analysis is available. Patients with RAS mutation who fulfill all eligibility criteria will be enrolled into the pre-randomization phase. Patients with RAS wild-type are considered screening-failures and the blood sample will be discarded.

[12)] Cetuximab is administered every week

[13)] All (S)AEs that occur between the patient´s first administration of study treatment until 30 days after discontinuation of study treatment, must be reported. All adverse events should be followed until resolution or stabilization. After the aforementioned time period the investigator should report any SAE which is believed to be related to study drug

[14)] Weekly during cetuximab therapy

[15)] Genotyping and/or phenotyping according to the ‘red-hand-letter’ for 5-FU (i.v.), capecitabine- and tegafur-containing drugs, dated 04.06.2020

2 Introduction

2.1 Metastatic Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer worldwide with 1.8 million new cases in 2018 [1]. In Germany, about 61,000 persons, 33,100 men and 27,900 women, were diagnosed with CRC in 2014 and more than 25,500 patients died of CRC [2].

The therapeutic management of CRC is strongly dependent on the disease stage and the genetic profile of the tumor. Surgical resection represents the cornerstone of therapy for early-stage CRC. In UICC stages I-III surgery alone may be curative; patients at high risk for recurrence in UICC stage II and all patients in UICC stage III should receive adjuvant chemotherapy additionally. Overall prognosis for patients with CRC in UICC stages I-II is favorable, with a 5-year survival rate of up to 90% [3]. However, due to often vague symptoms of CRC, 20-25% of patients are initially diagnosed with metastatic disease (UICC stage IV) [4]. In addition, 35-45% of patients in UICC stages II-III relapse within 5 years after surgery [5]. Patients in UICC stage IV have a dramatically reduced 5-year survival rate of about 15% [6]. Due to improvements in diagnosis and introduction of new therapies including targeted therapies with anti-epidermal growth factor receptor (EGFR) and anti-vascular endothelial growth factor (VEGF) antibodies, colorectal cancer death rates declined by approximately 2% per year during the 1990s and by approximately 3% per year during the past decade [7]. The addition of targeted agents to chemotherapy also raised the median OS of mCRC significantly to approximately 30 months [8].

In recent years retrospective studies demonstrated worse outcomes for patients with right-sided CRC compared to left sided CRC. Right-sided CRC can arise out of the transverse colon, ascending colon or cecum. Primary tumors that originated from the descending colon, sigmoid colon or rectum are classified as the left-sided CRC.
Recently, multiple randomized clinical trials have consistently shown that primary tumor location in the right side is associated with minor response to anti-EGFR therapy despite wild-type *RAS* status. In light of this evidence, the European Society for Medical Oncology (ESMO) and the National Comprehensive Cancer Network (NCCN) have revised their recommendations for the use of anti-EGFR therapy for first-line treatment in mCRC. Treatment with anti-EGFR antibodies in first line is now recommended only for wild-type RAS and left-sided primary tumors. However, stratification by primary tumor location has not been extended to subsequent treatment [9, 10].

2.2 Epidermal growth factor receptor

The epidermal growth factor receptor (EGFR) is a commonly expressed transmembrane glycoprotein of the tyrosine kinase growth factor receptor family. EGFR is normally expressed in many human tissues. Binding of its natural ligands ‘epidermal growth factor’ and ‘transforming growth factor α’ results in activation with auto-phosphorylation of the receptor tyrosine kinase thereby initiating a cascade of downstream protein phosphorylations which finally lead to cell proliferation and differentiation.
The implication of EGFR signaling in tumor pathogenesis has been demonstrated in a variety of human cancers, such as head and neck cancer, colorectal cancer, lung cancer, ovarian cancer, cervical cancer, and gastric cancer. Constitutive activation of EGFR leads to stimulation of tyrosine kinase-dependent signal transduction pathways that can contribute to neoplastic transformation and tumor growth.

Consequently, inhibition of EGFR signaling has been tested as treatment for various tumor types.
Among the EGFR inhibitors approved so far for tumor therapy are monoclonal antibodies (mAB) (cetuximab, panitumumab) as well as small molecule kinase inhibitors (erlotinib, gefitinib). While kinase inhibitors bind to the intracellular domain of the EGFR and block kinase activity, antibodies target the extracellular part of the receptor, thereby preventing ligand binding, conformational activation, and/or receptor dimerization.

2.3 Cetuximab

Cetuximab is a chimeric human/mouse monoclonal antibody of the immunoglobulin G (IgG1) subclass that targets specifically the extracellular domain human tyrosine kinase EGFR. Cetuximab binds to EGFR on both normal and tumor cells with a 5- to 10-fold higher affinity than endogenous ligands thereby competitively blocking the binding of endogenous EGFR ligands. This results in inhibition of the ligand-induced, tyrosine kinase-dependent phosphorylation and downstream signaling of the receptor.

The use of cetuximab was initially restricted to Kirsten Rat Sarcoma Virus (*KRAS*) wild-type in CRC since it had been shown that cetuximab is ineffective in colorectal cancers with *KRAS* mutation, which causes constant oncogenic activation of RAS/MEK/ERK signal transduction at the EGFR downstream independent of EGFR-mediated signaling [11]; resulting in unregulated downstream signaling that leads to tumor growth and survival. Consequently, inhibition of EGFR signaling by cetuximab does not have an inhibitory effect on signaling events that are downstream of constitutively activated mutant *KRAS*. Further retrospective and post-hoc analyses of clinical trials revealed that not only KRAS mutation negatively influences response to cetuximab but also other *RAS* mutations. Thus approval has been restricted to *RAS* wild-type mCRC.

2.3.1 Preclinical Efficacy Pharmacology

In vitro assays and in vivo animal studies have shown that cetuximab inhibits the growth and survival of tumor cells that express EGFR. No antitumor effects of cetuximab were observed in human tumor xenografts lacking EGFR expression. In animal studies, the addition of cetuximab to irinotecan or irinotecan plus 5-fluorouracil (5-FU), or the platinum-containing drugs, cisplatin and oxaliplatin, resulted in an increase in antitumor effects compared to chemotherapy alone [12].

2.3.2 Clinical Efficacy Summary and *RAS* status

Cetuximab as a single agent or in combination with chemotherapy was investigated in 5 randomized controlled clinical studies and several supportive studies. The 5 randomized studies investigated a total of 3734 patients with metastatic colorectal cancer, in whom EGFR expression was detectable and who had an ECOG performance status of ≤ 2. In all of these studies, cetuximab was administered once a week with an initial dose of 400 mg/m^2 and 250 mg/m^2 subsequently.
CRYSTAL, a randomized study in patients with metastatic colorectal cancer who had not received prior treatment for metastatic disease, compared the combination of cetuximab and irinotecan plus infusional 5-fluorouracil/folinic acid (FOLFIRI) to the same chemotherapy (FOLFIRI) alone. Median PFS and OS was 8.9 and 19.9 months, respectively, for patients in the cetuximab arm, and 8.0 and 18.6 months, respectively, for patients receiving chemotherapy only. Retrospective efficacy analysis according to *RAS* status (wild-type or mutant) is shown in Table 1.

Variable/ statistic	RAS wild-type population		RAS mutant population	
	Cetuximab plus FOLFIRI	FOLFIRI	Cetuximab plus FOLFIRI	FOLFIRI
	(N=178)	(N=189)	(N=246)	(N=214)
OS				
months, median	28.4	20.2	16.4	17.7
(95% CI)	(24.7, 31.6)	(17.0, 24.5)	(14.9, 18.4)	(15.4, 19.6)
Hazard Ratio (95% CI)	0.69 (0.54, 0.88)		1.05 (0.86, 1.28)	
p-value	0.0024		0.6355	
PFS				
months, median	11.4	8.4	7.4	7.5
(95% CI)	(10.0, 14.6)	(7.4, 9.4)	(6.4, 8.0)	(7.2, 8.5)
Hazard Ratio (95% CI)	0.56 (0.41, 0.76)		1.10 (0.85, 1.42)	
p-value	0.0002		0.4696	
ORR				
%	66.3	38.6	31.7	36.0
(95% CI)	(58.8, 73.2)	(31.7, 46.0)	(25.9, 37.9)	(29.6, 42.8)
Odds Ratio (95% CI)	3.1145 (2.0279, 4.7835)		0.8478 (0.5767, 1.2462)	
p-value	<0.0001		0.3970	

Table 1: Efficacy according to *RAS* status

The importance of *RAS* as a predictive marker for the efficacy of cetuximab treatment in mCRC was supported by other clinical trials.

FIRE-3, an investigator-sponsored randomized clinical phase-III study, compared the treatment of FOLFIRI in combination with either cetuximab or bevacizumab in the first-line treatment of patients with *KRAS* exon 2 wild-type mCRC. The primary endpoint was objective response analyzed by intention to treat. No significant difference was observed between the 2 treatment arms regarding the primary endpoint as well as PFS. However, median OS was 28.7 months (95% CI 24.0-36.6) in the cetuximab group compared with 25.0 months (22.7-27.6) in the bevacizumab group (HR 0.77, 95% CI 0.62-0.96; p=0.017) [13]. Further post-hoc analyses on mutations other than *KRAS* exon 2 have been evaluated. Formalin-fixed paraffin-embedded (FFPE) tumor material of FIRE 3 patients was analyzed for *KRAS* and *NRAS* exon 2, 3 and 4 mutations and for *BRAF* mutations. Taking into account only patients as wild-type without newly identified *RAS* and *BRAF* mutations (n=400), median overall survival was higher in the FOLFIRI plus cetuximab group than the FOLFIRI plus bevacizumab group (33.1 months (95% CI 24.5–39.4) vs 25·0 months (CI 23.0–28.1); HR 0.70 (0.54–0.90); p=0.0059). The objective response rate was higher in the cetuximab versus the bevacizumab arm (72.0% (95% CI 64.3–78.8) vs. 56.1% (48.3–63.6); p=0.0029) [14].

Retrospective analyses of tumor material from different clinical trials investigating cetuximab concerning *KRAS* and *NRAS* exon 2, 3 and 4 mutations were performed. A majority of analysed tumor biopsies revealed a mutation in the exon 2 of the *KRAS* gene. However, it was shown retrospectively that patients with mCRC whose tumors had somatic mutations beyond *KRAS* exon 2, including *KRAS* exon 3 and 4 and *NRAS* exon 2, 3, and 4 mutations (new *RAS* mutation), also did not benefit from therapy with cetuximab [15, 16].

Nevertheless, it should be considered that there is an association between the proportion of RAS-mutated cancer cells in a tumor and the level of EGFR-targeted therapy resistance and that patients with other tumor RAS mutation signals between 0.1% and 5% may have benefited from the addition of cetuximab to FOLFIRI [17].

Recently, retrospective analyses were conducted in patients with wild-type *RAS* mCRC from two phase III trials, CRYSTAL and FIRE-3, in which mCRC was subclassified as left-sided or right-sided. Patients in both clinical trials with left-sided CRC had a markedly better prognosis than those with right-sided CRC, especially if receiving FOLFIRI plus cetuximab [18].

2.3.3 Clinical Safety Summary

Skin reactions (acneiform rash/acneiform dermatitis) are observed as the specific adverse reactions in the majority of patients, occurring at grade 3 or 4 in 15.7% of patients [19]. Further notable side effects are hypomagnesaemia which occurs in more than 10% of patients (grade 3-4 hypomagnesaemia in 3% [19]) and infusion related reactions, which occur with mild to moderate symptoms in more than 10% of patients and with severe symptoms in more than 1% of patients. An increased frequency of severe and sometimes fatal cardiovascular events and treatment emergent deaths has been observed in the treatment of non-small cell lung cancer, squamous cell carcinoma of the head and neck and colorectal carcinoma.

Please refer to the current Summary of medicinal Product Characteristics (SmPC).

2.4 Conversion of RAS Status

A major limitation of treatment of *RAS* wild-type patients with cetuximab is the development of resistance. It has been acknowledged that newly detected *RAS* mutations evolve during anti-EGFR mAb treatment and are predictive for reduced benefit from this therapy [20-26]. More than half of patients with acquired resistance to first-line anti-EGFR containing therapy revealed conversion from RAS wild-type to RAS mutated status [20-26]. By examination of circulating, cell-free DNA, Diaz et al. discovered that acquired resistance to treatment was the result of selection of clonal existing subpopulations [27]. There is predominant suppression of RAS wild-type clones during anti-EGFR mAb therapy and, in this manner, indirect selection of RAS mutated clones. However, after the discontinuation of EGFR inhibition, RAS mutational load rapidly decreases within a few weeks, probably due to the lack of selective pressure from the anti-EGFR mAb therapy [20]. In addition, new *RAS* mutations can arise without a direct selection pressure by an anti-EGFR mAb. In this case, modifications of therapy agents may lead to renewed disappearance of *RAS*-mutated clones [20].

Recently, longitudinal analyses of cell-free tumor DNA of mCRC patients with *RAS* mutation at diagnosis demonstrated that they convert to wild-type *RAS* during systemic 1st line chemotherapy in most cases. Remarkably, the conversion was observed even after just one cycle of chemotherapy. The conversion to *RAS* wild-type occurred independent of type and intensity of chemo- and anti-VEGF therapy and amounted to about 70% of patients with initially *RAS* mutated mCRC who showed conversion to *RAS* wild-type [28, 29]. Additional examination of tumor-specific epigenetic biomarkers (hypermethylation of promoter regions of Wnt inhibitory factor 1 (*WIF1*) and neuropeptide Y (*NPY*)) revealed that these were detected in samples with disappearance of *RAS* mutation, thus being a hint, that in these samples tumor-DNA was still present [29]. Aberrant methylation of the promotor regions of *NPY* and *WIF1* has been demonstrated to be involved in decreased expression which promotes development of cancer. Thus, methylation analysis of these promotor regions in liquid biopsy samples can serve as a surrogate marker for cancer cells [30, 31].

Positive effects of therapy adaptation to *RAS* conversion were shown by Bouchahda et al. [32]. Examination of plasma samples of patients with *RAS* mutated CRC and progressive disease after 1st - 4th therapy line yielded 9 of 16 samples with evidence of *RAS* wild-type. Patients with conversion to *RAS* wild-type were switched to treatment with FOLFIRI + cetuximab whereas patients showing no conversion were treated with standard therapy. Observed outcome parameters such as remission rate (55.6% in converted vs. 42.9% in non-converted patients) and median PFS (9 months vs. 3.5 months) were improved in *RAS* wild-type patients treated with FOLFIRI + cetuximab compared to *RAS* mutated patients treated with standard treatment.

2.5 Liquid Biopsy

In contrast to invasive conventional biopsy which is performed on solid tumors, liquid biopsy uses biological fluids (mainly blood) of cancer patients for molecular DNA analysis. This non-invasive method has emerged in the last few years and is based on the detection of circulating tumor cells (CTCs) and circulating cell-free tumor DNA (ctDNA) in blood.

The analysis of ctDNA has become the established technology for detection of mutations. Furthermore, ctDNA analysis outperforms CTCs for *RAS* mutations in both diagnostic sensitivity and specificity, enabling real-time sampling of multifocal clonal evolution [33]. The development of highly sensitive analysis methods enables the quantification of small amounts of biological entities [34, 35]. It is suspected, that ctDNA in the blood mainly originates from apoptotic or necrotic tumor cells [36]. Also, a correlation of ctDNA release with the ratio of cells in G1 phase was shown. The enhanced release of circulating free DNA from differentiated cells might be due to the active release of circulating free DNA packaged inside exosomes or in other forms that are protected from degradation in the blood [37]. However, ctDNA harbors the same mutations as the original tumor cell [38].

Detection of ctDNA depends on tumor type and stage; however in nearly 100% of CRC patients, ctDNA can be found [35].
High concordance of *RAS* mutation analysis between ctDNA samples and FFPE tumor tissue of about 90-95% of CRC patients has been shown by several research groups [39, 40].
Therefore especially for this cancer entity the analysis of ctDNA provides opportunities for clinical monitoring of results of anti-cancer treatment.

Due to the overall small amount of ctDNA in blood, analysis was only possible after development of high gain amplification techniques which use digitization of signals. Among these techniques are the BEAMing (stands for ‘beads, emulsion, amplification, magnetics’) digital polymerase chain reaction (PCR) and the Droplet Digital PCR (ddPCR). Both BEAMing and ddPCR are available and established in the study lab. Within this clinical trial ddPCR will be preferentially used for the monitoring of *RAS* status during treatment. An optional comparison of results of ddPCR and BEAMing PCR is planned.

2.5.1 Mutation Analysis using ddPCR

As with ‘conventional’ quantitative PCR (qPCR), ddPCR technology utilizes the primer-probe amplification in a standard PCR reaction to amplify a target DNA fragment from a complex sample using pre-validated primer or primer/probe assays. However, there are two distinct differences:
1) For ddPCR, the partitioning of the PCR reaction into thousands of individual reaction vessels based on water-oil emulsion droplet technology to ensure that each partition contains a discrete number of nucleic acid sequences (e.g. 1 or 2) prior to amplification and
2) the acquisition of data at the end of reaction.

These factors offer the advantage of direct and independent quantification of DNA without standard curves giving more precise and reproducible data versus qPCR especially in the presence of sample contaminants that can partially inhibit Taq polymerase and/or primer annealing. In addition, end-point measurement enables nucleic acid quantification independently of the reaction efficiency, resulting in a positive-negative call for every droplet and greater amenability to multiplexed detection of target molecules [41].
Only the difference between a positive (contains respective target nucleic acid sequence) and negative partition (does not contain the target nucleic acid sequence) is measured.
The ratio of positive to negative partitions can then be related to the number of molecules in the sample, using Poisson statistics.
Due to the concentration effect, the limit of detection is improved because a small reaction volume increases the effective concentration of the target molecules. Additionally, the enrichment effect improves the analysis of complex mixtures by purifying the target of interest from interfering compounds [42].
Thereby, ddPCR technology can be used for extremely low-target quantification from variably contaminated samples where the sample dilution requirements to assure consistent and acceptable reaction efficiency, primer annealing and cycle quantification value (Cq) values for qPCR would likely lead to undetectable target levels [41].
Since 2011 ddPCR is commercially available for in vitro use.
Within this clinical trial the validated ddPCR technology developed by BIORad is used according to the manufacturer's instructions in the Immunological-Molecular Biological Laboratory of the Knappschaftskrankenhaus, Ruhr University Bochum. The laboratory participates in yearly proficiency testing of isolation of DNA from plasma, for *RAS* (and *BRAF* V600E) mutation analysis, by assessing 3 unknown samples with BEAMing as well as with ddPCR technology.
In general, the procedure of ddPCR will be performed as described below:
First, cell-free DNA is isolated from blood plasma. Target regions are fractionated into several thousands of water-oil droplets, so that statistically only 1 target sequence together with primers, PCR enzyme and specific fluorescence-labelled probes is included in a single droplet.

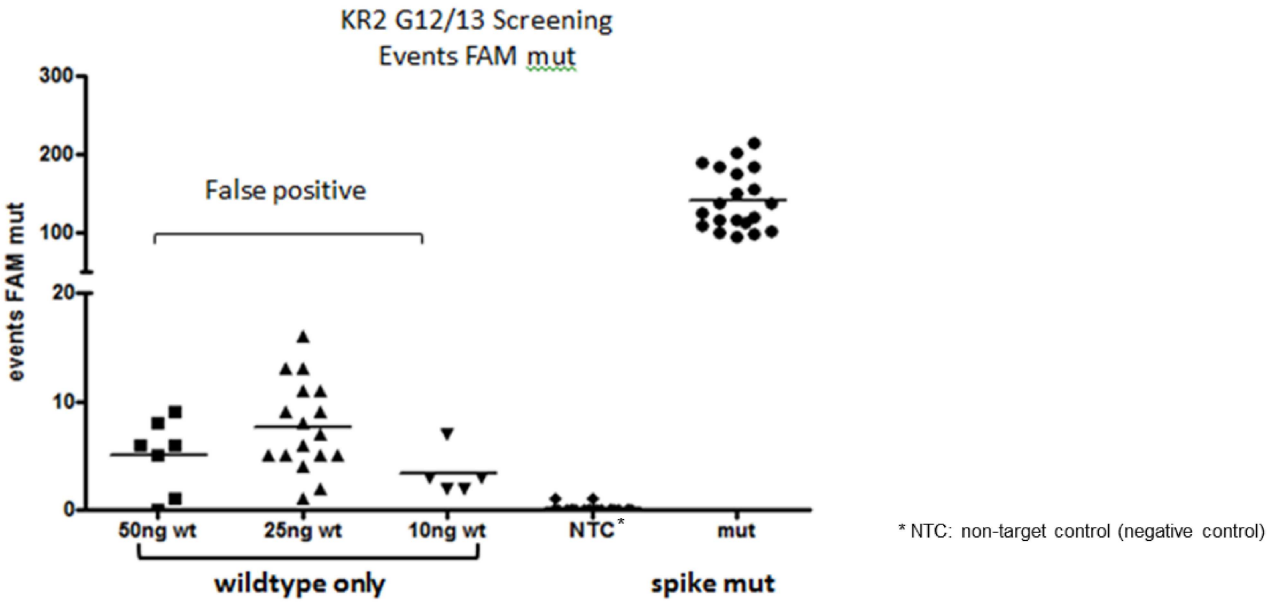
The following *KRAS* and *NRAS* mutations on exon 2, 3, 4 will be tested:

KRAS		***NRAS***	
Exon	**Mutation**	**Exon**	**Mutation**
2	G12S G12R G12C G12D G12A G12V G13D	2	G12S G12R G12C G12D G12A G12V G13R G13D G13V
3	A59T Q61L Q16H* Q16H*	3	A59T Q61K Q61R Q61L Q61H* Q61H*
4	K117N* K117N* A146T A146V	4	K117N* K117N* A146T

*Two separate mutations detected for each of these codons

As quality control, standardized samples will be used additionally in each analysis.

The cutoff value is determined with 'wild-type only' samples (fragmented genomic DNA of a human cell line) and titrations of defined mutated samples and samples assessed as positive or negative by BEAMing technique.
Conversely, the cutoff for detection of a mutation is determined with regard to the false positive signals detected in several 'wild-type only' analyses. As shown below, the proportion of false positive signals depends on the amount of wild-type DNA.



The percentage of false positive signals of 30 'wild-type only' samples analyzed amounted to a mean of 0.09 ± 0.06%. To ensure detection of mutation the cutoff was set to 0.27% (mean including standard deviation).
The mean number of false positive events in these 30 analyzed samples was 6.3 ± 4. Thus, a definite detection of the mutation is possible in case of >12 events.
With these determined cutoff values a mutation can be safely distinguished from false positive wild-type signals within low amount of DNA.

27 samples determined as wild-type or mutated by BEAMing technique which is approved for *RAS* mutation analysis, were analyzed with ddPCR using the before established cutoffs additionally. It could be shown that no sample detected as wild-type (=negative) with BEAMing was identified as mutated (=positive) with ddPCR:

Cutoff 0.27%	**BEAMing pos**	**BEAMing neg**	**Total**
ddPCR pos	11	0	**11**
ddPCR neg	3	13	**16**
Total	**14**	**13**	**27**

Thus, the ddPCR analysis determined with a sensitivity of 79% and specificity of 100% a mutation in KR2-12/13 with 89% certainty [False discovery rate ((false negative +false positive) / all) = 11%].

2.6 Study Rationale

As mentioned before, it has been shown, that *RAS* mutated status of mCRC present at diagnosis mostly converts to wild-type status during the first line chemotherapy in liquid biopsy. Since treatment options for patients with mCRC are limited, especially for patients with *RAS* mutations, which are not be considered eligible for therapy with EGFR antibodies, this study will use regular monitoring of *RAS* status by liquid biopsy to treat patients according to their actual *RAS* status. Thus, mCRC patients with mutant *RAS* will receive standard first line chemotherapy (FOLFIRI) according to guidelines recommending a double or triple chemotherapy in case of *RAS* mutated mCRC until the conversion of *RAS* to wild-type is observed [43]. Wild-type patients will then be randomized to continue the existing treatment regimen (Control Arm) or to switch to the EGFR antibody cetuximab in combination with FOLFIRI (Experimental Arm) until re-conversion to mutant *RAS*. In both arms the treatment strategy will be followed until PD, unacceptable toxicity, withdrawal of informed consent or death, whichever occurs first.

This study design of therapy adaptations as performed in the experimental arm has 2 advantages:

1. Patients with initially mutant *RAS* can benefit from anti-EGFR treatment due to conversion of *RAS* status during standard first line therapy and to the data that the treatment with an EGFR antibody is the most effective treatment option for patients with left-sided *RAS* wild-type mCRC.

2. Emerging resistance to treatment with cetuximab which may limit the clinical benefit is bypassed by early switching to chemotherapy (FOLFIRI) without cetuximab before PD. This ensures that patients with *RAS* mutations in liquid biopsy do not receive EGFR antibodies according to approval.

3. Steps 1 and 2 may be repeated as long as the patient does not experience disease progression.

Therefore, it is expected that patients in the experimental switch arm will have a longer PFS compared to control arm.

The randomized study design allows direct comparison of standard therapy and switch treatment without bias by investigator or patient characteristics.

2.7 Risk/Benefit Assessment

All mCRC patients enrolled into this randomized trial will receive therapy according to the current *RAS* status and approval status of the drugs employed. Thus, it is not expected that study participants are under-treated or are exposed to additional risks from the treatment. Tumor staging will also be performed in line with the guidelines for stage IV patients in both randomization arms.
Blood sampling for liquid biopsy is collected during routine blood sampling before chemotherapeutic treatment. No additional invasive methods are used.

Patients in the experimental arm additionally may have a benefit of treatment adaptations compared to control arm.

However, due to limited clinical data supporting this innovative study design, two stopping rules are included to increase patient safety:

1. After randomization of 20 patients, further enrollment into the study will be paused. Liquid biopsy samples from the first 20 screened patients will be analyzed in parallel by 2 laboratories. In case of concordant results, the study will continue. In case of discordant results, DSMB will make recommendations for further progress of the study. Additionally, tumor-specific genes will be analyzed to define ctDNA in the samples. This should primarily be performed by measurement of “house-keeping genes” that are identified by Next Generation Sequencing (NGS) of baseline tumor tissue or if not applicable by methylation analysis of promotor region of the genes WIF1 and NPY which serve as surrogate markers for tumor cells.
Also for the first 20 patients showing conversion to *RAS* wild-type in liquid biopsy during screening, at two time points, tumor material assessed locally, as well as a liquid biopsy sample will be analyzed comparatively for *RAS* mutation / wild-type to assess agreement between the analysis methods. This will be done at baseline and once during screening when *RAS* wild-type is detected in liquid biopsy. For the second comparison tumor material of a new biopsy assessed locally has to be used, if possible. In cases of discordant *RAS* results between liquid and tumor biopsy, patients will not be randomized. Overall, in at least 80% of patients a biopsy should be taken to confirm *RAS* status in tissue of respective patients. However, if a patient refuses a new biopsy, he/she will still be randomized and can continue the study.
In addition, in the first 8 weeks after randomization, blood samples of the first 10 patients randomized into experimental arm A will be taken more frequently: at weeks 2, 4, 6 and 8 instead of just weeks 4 and 8. If ≥5 of 10 patients in the experimental arm A show *RAS* mutation within 4 weeks after randomization by liquid biopsy, the study may be terminated prematurely, because, in this case, the effect of *RAS* conversion does not seem to be lasting long enough.

Results of these analyses will be reviewed by a Data and Safety Monitoring Board (DSMB) and notified to the ethics committee together with recommendations of the DSMB regarding study conduct.

2. After 50 patients have been screened, the proportion of patients with conversion from *RAS* mutant to *RAS* wild-type will be analyzed and evaluated by the DSMB. If less than 50% of analyzed patients show conversion to *RAS* wild-type, the DSMB will make recommendations regarding the conduct of the study. These will be notified to the ethics committee.

3 Investigational Plan

3.1 Overall Study Design and Plan

This is an open-label, prospective, randomized, multicenter phase II trial 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 convert to RAS wild-type by monitoring the RAS mutation status by liquid biopsy.
Patients with left-sided RAS-mutated metastatic colorectal cancer (mCRC) eligible for study participation and receiving standard FOLFIRI as 1st line treatment will be screened for a maximum of 3 months (week 4 to 16) for conversion to *RAS* wild-type. As soon as *RAS* wild-type is detected, no further blood samples for screening will be taken. Conversion to *RAS* wild-type at week 4 or 8 and at week 12 or 16 will lead to randomization at weeks 8 or 16 (directly after follow-up CT scan), respectively. Patients will be randomized 1:1 into experimental Arm A (switch arm) or control Arm B.

For patients without conversion to *RAS* wild-type by week 16, the 'end of study' form must be completed.

Figure 1 gives on overview of study design.

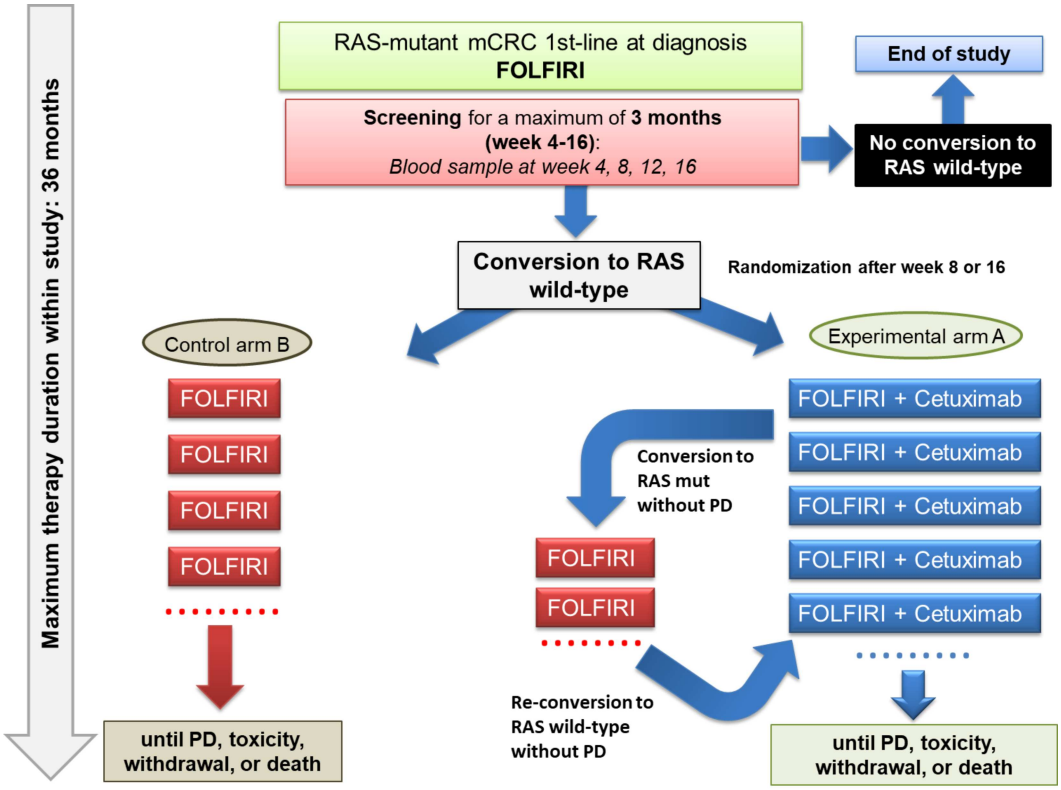


Figure 1: Overview of study design

For the safety of the patients, 2 stopping rules are included:

1. After randomization of 20 patients, further enrollment into the study will be paused. For the first 20 patients with *RAS* wild-type result in liquid biopsy during screening this result should be confirmed by new tumor biopsy assessed locally, if feasible. If results between the methods are discordant, the patient cannot be randomized and the 'end of study' form must be filled in. Overall, in at least 80% of patients a biopsy should be taken to confirm *RAS* status in tissue of respective patients. However, if a patient refuses a new biopsy, he/she will be randomized and can continue the study. Also, analysis of blood samples from the first 20 screened patients will be analyzed in parallel by 2 laboratories. An overview of planned examinations for the first 20 patients s given in Figure 2 (before randomization) and Figure 3.
   In addition, in the first 8 weeks after randomization, blood samples of the first 10 patients randomized into experimental arm A will be taken more frequently: at weeks 2, 4, 6 and 8 instead of just weeks 4 and 8. If ≥5 of 10 patients in the experimental arm A show *RAS* mutation within 4 weeks after randomization by liquid biopsy, the study may be terminated prematurely, because, in this case, the effect of RAS conversion does not seem to be lasting long enough.

Results of all analyses will be forwarded to the DSMB which will assess the data and will provide recommendations for study conduct. Results and recommendations of the DSMB will be notified to the ethics committee.

2. After 50 patients have been screened, proportion of patients with conversion from RAS mutant to *RAS* wild-type will be analyzed and evaluated by the DSMB. If less than 50% of analyzed patients show conversion to *RAS* wild-type, the DSMB will make recommendations regarding the conduct of the study. These will be notified to the ethics committee.

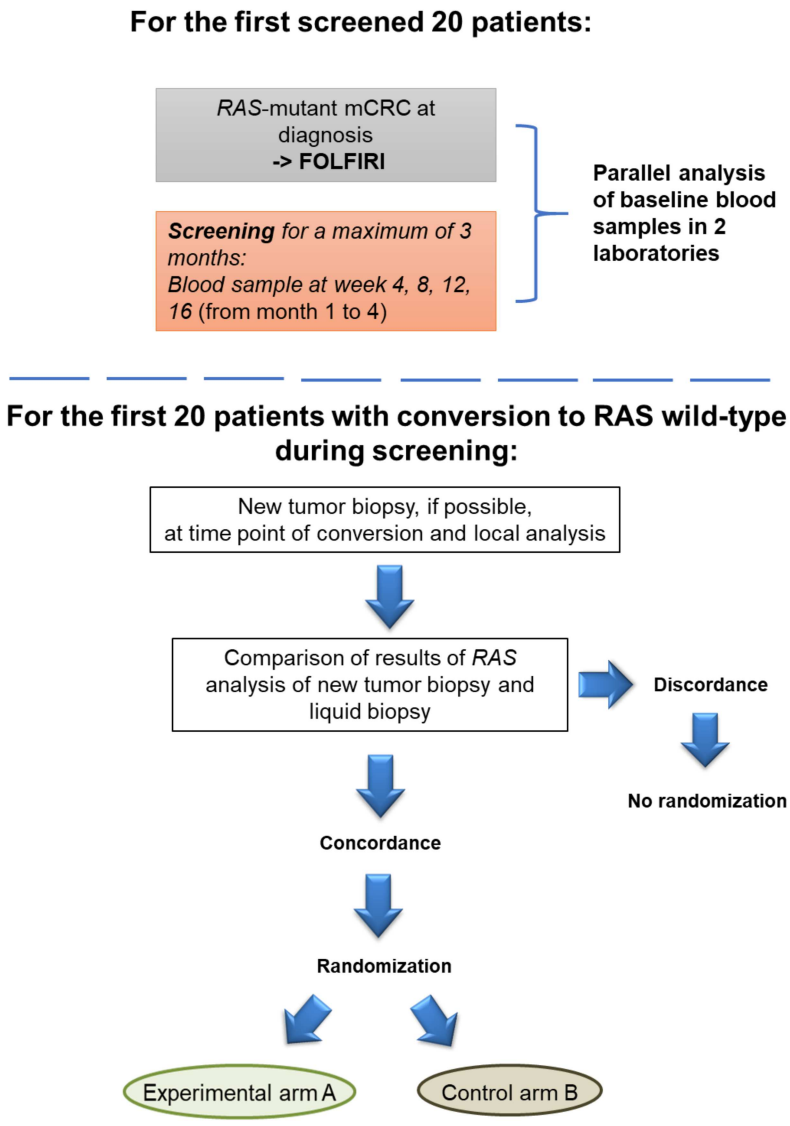


Figure 2: Overview of *RAS* analysis for the first 20 screened patients and for the first 20 patients with conversion to *RAS* wild-type

3.1.1 Blood Sampling for *RAS* Analysis

All *RAS* analysis by liquid biopsy is performed at a central laboratory by ddPCR.
Blood samples for liquid biopsy will be collected at the time points indicated in Figure 3 and in the 'Schedule of visits and assessments'. At each time point, 20 ml blood will be taken into STRECK BCT tubes and sent for central analysis to:

Immunologisch-Molekularbiologisches Labor
Medizinische Universitätsklinik, Ruhr-Universität Bochum
Universitätsklinikum Knappschaftskrankenhaus Bochum GmbH
In der Schornau 23-25
D-44892 Bochum
Germany

The central laboratory will forward cfDNA from blood samples of the first 20 screened patients for additional analysis to the second laboratory (Institut für Klinische Chemie, Prof. Neumaier, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Haus 17 und 22). However, randomization will be based on results of central laboratory only.

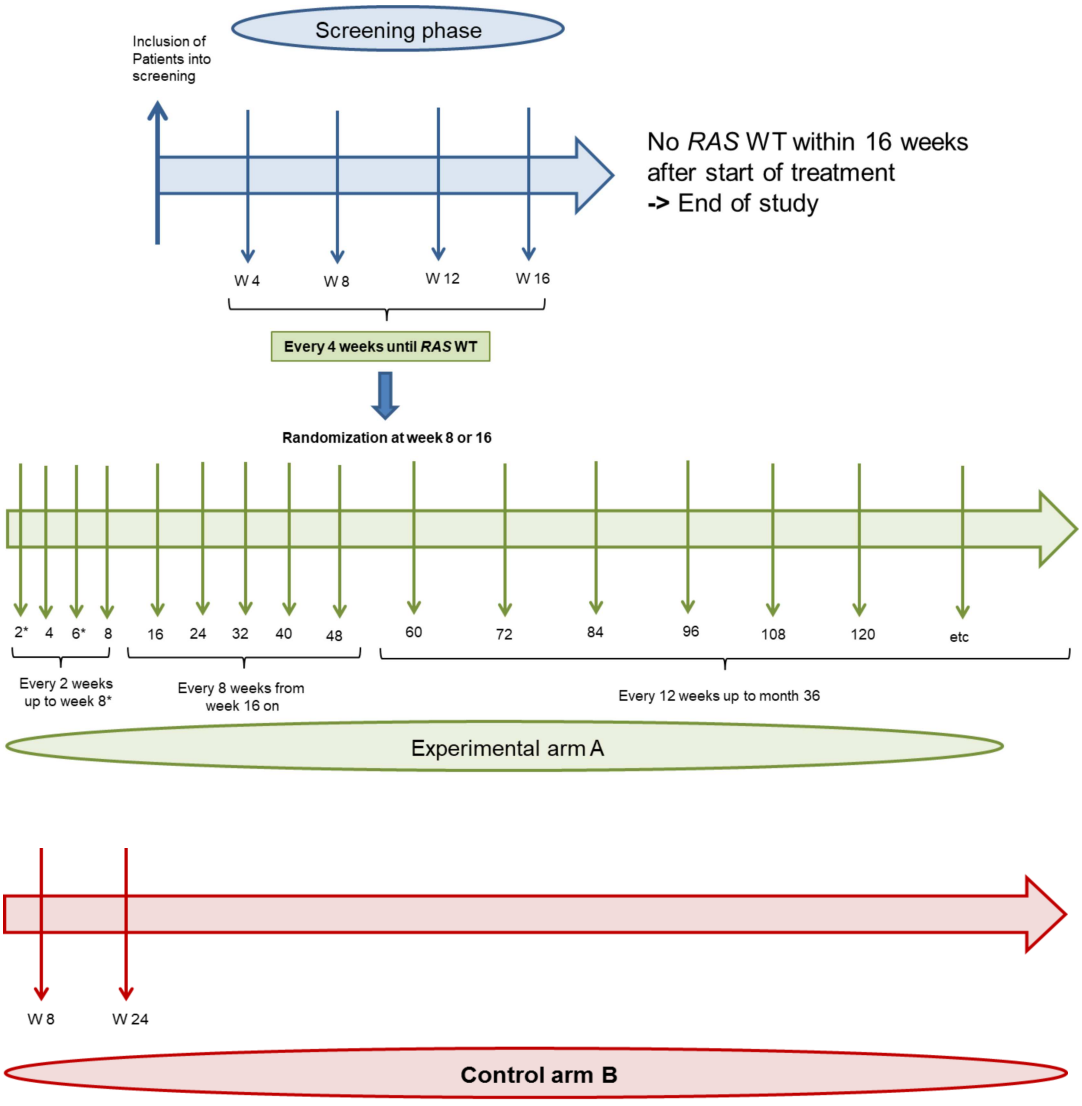


Figure 3: Time points of blood sampling
* At first, blood sampling at week 2 and 6 will be performed for the first 10 patients in Arm A; However, it may be continued for further patients due to recommendations of the DSMB.

Participating centers will be provided with STRECK BCT tubes, labels and packaging material. Shipment is free of charge for the participating centers and will be carried out by express delivery "GO! Express & Logistics".

Shipment of blood samples is possible from Monday to Thursday to ensure RAS analysis in a timely manner.
Instructions for blood sampling, labelling and shipment will be given to the centers with the investigator site file.

The central laboratory will document the results of the *RAS* analysis in the eCRF within 3-4 days for blood samples from the pre-randomization phase and within 1 week for blood samples taken after randomization.

3.1.2 Randomization

All enrolled patients with conversion of mutant *RAS* as estimated centrally by liquid biopsy of cfDNA within the first 4 months (weeks 4 to 16) and without PD as determined by CT scan will be randomized at week 8, for patients converting at week 4 or 8, and at week 16 for patients converting at week 12 or 16 directly after the CT scan in a 1:1 ratio to either experimental Arm A (switch arm) or control Arm B. Patients with progressive disease before the randomization will not be considered for randomization.

The randomization lists will be kept in safe and confidential custody at Alcedis GmbH.

Stratification factors for randomization are:

- Conversion to *RAS*-wt: ≤ 8 weeks vs. > 8 weeks

Randomization of a patient during pre-randomization phase will be performed automatically, at weeks 8 or 16, if centrally assessed *RAS* wild-type in liquid biopsy is documented by the central analysis laboratory before 8 weeks or after 8 weeks, respectively, and there is no disease progression at that time. Randomization result is notified immediately by an automatically generated fax to the investigator.

3.1.3 Blinding

Not applicable.

3.1.4 Number of Patients

Estimated number of patients enrolled into pre-randomization phase (conversion rate of 80%): 144

Number of randomized patients: 116 (58 patients in each arm) with 107 required events.
The screening will be continued until randomization of 116 patients.

3.1.5 Planned Study Timelines

Duration of recruitment: 18 months at a rate of 8 patients/month (counted from first patient in (FPI)).
Follow-up from last patient in (LPI) to end of safety follow up: 1 month after last administration of up to 36 months of study treatment.
Further follow-up for survival until trial termination: 4.5 years after FPI.
Expected total trial duration: 4.5 years

3.2 Study Objectives

The aim of this randomized study is to evaluate whether patients with left sided *RAS*-mutant mCRC at diagnosis will derive a benefit from the adaptation of adding cetuximab to 1st line therapy (FOLFIRI) after *RAS*-mutation status has changed to wild-type and changing back to

FOLFIRI, as required if RAS-mutation status has changed to mutant, depending upon, and monitored by, longitudinal ctDNA liquid biopsies.

3.2.1 Primary Objective

The primary objective is 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

3.2.2 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 tumor mass 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

3.2.3 Exploratory objectives (optional)

- 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 with those patients showing conversion to *RAS* wild-type in ddPCR but not in BEAMing

3.3 Study Population and Justification of Choice of Gender

The selection of patients occurs through the investigator according to the inclusion and exclusion criteria after informing the patient written and orally about the study and after the patient has signed the informed consent. No gender specific differences are expected concerning the efficacy and safety of the study treatment, hence there is no preferred enrolment of men or women within this study. However, pregnant or breast-feeding women are excluded from participation.

3.3.1 Inclusion Criteria

Patients may be **included** in the study only if they meet **all** the following criteria:

- Histologically confirmed, UICC stage IV adenocarcinoma of the left-sided colon or rectum with metastases (metastatic colorectal cancer), primarily non-resectable, confirmed RAS mutations proven in the primary tumor or metastasis (KRAS and NRAS exon 2, 3, 4)
- Age ≥ 18 years on day of signing informed consent
- No previous chemotherapy for metastatic disease (1 - 2 cycles FOLFIRI or mFOLFIRI are permitted before enrolment until RAS status is determined)

- Patients suitable for chemotherapy administration
- ECOG performance status 0-1
- Consent to liquid biopsy and mutation analysis
- Estimated life expectancy > 3 months
- 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 enrollment)
- Adequate bone marrow function defined as:
  - Leukocytes 3.0 x 10^9/L with neutrophils 1.5 x 10^9/L
  - Thrombocytes 100 x 10^9/L
  - Hemoglobin 9 g/dL
- Adequate hepatic function defined as:
  - Serum bilirubin 1.5 x ULN
  - ALAT and ASAT 2.5 x ULN (in the presence of hepatic metastases, ALAT and ASAT 5 x ULN)
- Adequate renal function: Creatinine clearance ≥50 mL/min
- Adequate cardiac function defined as
  - Normal ECG and echocardiogram with a left ventricular ejection fraction (LVEF) of 55%
- INR < 1.5 and aPTT < 1.5 x ULN (patients without anticoagulation). Therapeutic anticoagulation is allowed if INR and aPTT have remained stable within the therapeutic range for at least 2 weeks.
- 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
- Any relevant toxicities of prior treatments must have resolved to grade ≤ 1 according to the CTCAE (version 5), except alopecia
- Women of childbearing potential (WOCBP) should have a negative urine pregnancy test within 72 hours prior to receiving the first dose of study medication.
- Highly effective contraception for both male and female patients throughout the study and for at least 3 months after last dose of study medication administration if the risk of conception exists. Highly effective contraception has to be in line with the definition of the CTFG recommendation (see 17.5)
- Signed written informed consent and capacity to understand the informed consent

3.3.2 Exclusion Criteria

Patients will be **excluded** from the study for **any** of the following reasons:

- Right sided mCRC
- Primarily resectable metastases
- Previous chemotherapy for the colorectal cancer with the exception of adjuvant treatment, completed at least 6 months before entering the study (1-2 cycles of FOLFIRI or mFOLFIRI are permitted before enrolment)
- Patients with known brain metastases
- Symptomatic peritoneal carcinosis
- Progressive disease before randomization
- History of acute or subacute intestinal occlusion, inflammatory bowel disease, immune colitis or chronic diarrhea

- 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 enrollment, unstable angina pectoris, serious cardiac arrhythmia according to investigator's judgment requiring medication
- Medical or psychological impairments associated with restricted ability to give consent or not allowing conduct of the study
- Active infection with hepatitis B or C
- 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
- Uncontrolled hypertension
- Marked proteinuria (nephrotic syndrome)
- Arterial thromboembolism or severe hemorrhage within 6 months prior to randomization (with the exception of tumor bleeding before tumor resection surgery)
- Hemorrhagic diathesis or tendency towards thrombosis
- Participation in a clinical study or experimental drug treatment within 30 days prior to study
- Known hypersensitivity or allergic reaction to any of the study medications
- Severe, non-healing wounds, ulcers, bone fractures or an infection requiring systemic therapy
- Known history of alcohol or drug abuse
- Complete dihydropyrimidine dehydrogenase (DPD) deficiency (phenotyp and/or genotype test)
  (Patients with partial DPD deficiency may be included and should receive the first cycle with a reduced 5-FU dose. Dose reduction and escalation are at the discretion of the investigator and must be determined in the best interest of the patient.)
- Known glucuronidation deficiency (Gilbert's syndrome) (specific screening not required)
- Absent or restricted legal capacity
- For female patients only: Pregnancy (absence to be confirmed by ß-HCG test) or lactating

3.3.3 Enrolment of Patients

Only patients who meet the inclusion and exclusion criteria are selected and enrolled by online registration.

Online registration is possible 24 hours a day. After the registration form has been saved, a registration number is notified immediately by an automatically generated fax to the investigator.

Patients who are not registered, but sign informed consent and undergo at least some of the selection procedures will be considered screening failures. A record of these patients will be maintained by the study site.

After registration, treatment with FOLFIRI/mFOLFIRI should be instituted within a maximum interval of 14 days.

Patients who are registered but who are not treated within 14 days can be treated under the same patient number after further examinations to make sure that all inclusion and exclusion criteria are still met.

4 Study Treatment

All medications used as study treatment will be prescribed according to routine clinical practice. The drugs will be stored, prepared and administered according to the respective SmPC. Except for cetuximab (Erbitux®, Merck), all other drugs are specified by active substance only within this study.

5 Treatment Plan

5.1 Treatment Regimen

The 1st-line therapy is defined as all treatment from start of FOLFIRI / mFOLFIRI (date of the 1st cycle either in the pre-screening or the pre-randomization phase) until progressive disease (PD), unacceptable toxicity, withdrawal of informed consent, loss to follow-up, patient preference, or death.

One cycle consists of 14 days. Drugs will be administered according to each center's routine.

Pre-randomization phase:

Patients will be treated with FOLFIRI. The 5-FU bolus may be omitted at the investigator's discretion to achieve better therapy tolerability (mFOLFIRI).

FOLFIRI (q14d):

- Irinotecan 180 mg/m² iv, 30 - 90 min on day 1
- Folinic acid (racemic) 400 mg/m² iv, 120 min on day 1
- 5-FU 400 mg/m² bolus on day 1
- 5-FU 2400 mg/m² iv over 46 h on day 1-2

OR

mFOLFIRI (q14d):

- Irinotecan 180 mg/m² iv, 30 - 90 min on day 1
- Folinic acid (racemic) 400 mg/m² iv, 120 min on day 1
- 5-FU 2400 mg/m² iv over 46 h on day 1-2

Patients with partial DPD deficiency should receive the first cycle of FOLFIRI with a reduced 5-FU dose. Dose reduction and escalation are at the discretion of the investigator and must be determined in the best interest of the patient.

One or two cycles of FOLFIRI/mFOLFIRI may be administered in the pre-screening period before the result of the RAS analysis is available. Patients with any RAS mutation who fulfill all the eligibility criteria will be enrolled into the pre-randomization phase. Patients with RAS wild-type status will be considered screening-failures.

Randomized phase

Control arm:

FOLFIRI (q14d):

- Irinotecan 180 mg/m² iv, 30 - 90 min on day 1
- Folinic acid (racemic) 400 mg/m² iv, 120 min on day 1

- 5-FU 400 mg/m² bolus on day 1
- 5-FU 2400 mg/m² iv over 46 h on day 1-2

OR

mFOLFIRI (q14d):

- Irinotecan 180 mg/m² iv, 30 - 90 min on day 1
- Folinic acid (racemic) 400 mg/m² iv, 120 min on day 1
- 5-FU 2400 mg/m² iv over 46 h on day 1-2

Experimental arm (switch arm):

FOLFIRI (q14d) + cetuximab (q1w):

- Irinotecan 180 mg/m² iv, 30 - 90 min on day 1
- Folinic acid (racemic) 400 mg/m² iv, 120 min on day 1
- 5-FU 400 mg/m² bolus on day 1
- 5-FU 2400 mg/m² iv over 46 h on day 1-2
- Cetuximab initially 400 mg/m² as a 120 min infusion (≤ 5 mg/min) on day 1; subsequently 250 mg/m² iv as a 60 min infusion every week (≤ 10 mg/min)

OR

mFOLFIRI (q14d) + cetuximab (q1w):

- Irinotecan 180 mg/m² iv, 30 - 90 min on day 1
- Folinic acid (racemic) 400 mg/m² iv, 120 min on day 1
- 5-FU 2400 mg/m² iv over 46 h on day 1-2
- Cetuximab initially 400 mg/m² as a 120 min infusion (≤ 5 mg/min) on day 1; subsequently 250 mg/m² iv as a 60 min infusion every week (≤ 10 mg/min)

If *RAS* wild-type converts to *RAS* mutant:
FOLFIRI or mFOLFIRI (q14d) as stated above

If *RAS* mutant converts to *RAS* wild-type again:
FOLFIRI or mFOLFIRI (q14d) + cetuximab q1w as stated above

In case one of the substances has to be stopped due to toxicity, the others should be continued, if tolerable for the patient.

5.2 Concomitant Medication /Therapy

Any medications (other than those excluded by the clinical trial protocol) that are considered necessary for the patients' welfare and will not interfere with the trial drug may be given at the investigator's discretion.
All concomitant medication or medication administered within the 2 weeks preceding date of informed consent, during the study and 30 days after the last dose of trial treatment must be recorded in the electronic case report forms (eCRF) including all prescription, over-the-counter (OTC), herbal supplements, and intravenous medications and fluids. The generic name of the medication must be specified along with the dose, duration, and indication of each drug.
The patient must notify the investigational site about any new medications he/she takes after the start of the study drug. Patients taking concomitant medications chronically should maintain the same dose and dose schedule throughout the study if medically feasible.

Any additional concomitant therapy that becomes necessary during the trial and any relevant change to concomitant drugs must be recorded in the corresponding section of the eCRF, noting the name, dose, duration, and indication of each drug.

5.2.1 Permitted concomitant medication / therapy

All treatments that the investigator considers necessary for a patient's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. This includes resection of primary tumor / metastases in patients becoming eligible for surgery during study treatment.
Radiotherapy for treatment of bone metastases and drugs for treatment of adverse reactions of study treatment, anthroposophical and homeopathic medicinal products (e.g. mistletoe therapy) are permitted.

5.2.2 Prohibited Therapy

Any concomitant therapy intended for the treatment of primary tumor (except surgery) is prohibited during study therapy, including:

- Chemotherapy
- Immunotherapy
- Targeted treatment (e.g. with small molecules)
- Radiation of primary tumor
- OTC medication
- Any other cytotoxic drugs

5.3 Planned Treatment Duration per Patient

Each randomized patient will be treated for maximum of 36 months (time period includes pre-randomization phase plus randomized phase) or until PD, unacceptable toxicity, withdrawal of informed consent, patient preference or death, whatever occurs first.

5.4 Removal of Patients from Study

Each patient remains in the study until either the patient or the investigator determines discontinuation to be in the patient's best interest.

Patients who discontinue participation in the clinical study on their own by withdrawal of informed consent (at any time), patient preference or patients who are withdrawn by the investigator, for reasons other than disease progression, will be defined as premature withdrawals.

5.4.1 Permanent Treatment Discontinuation

Patients will receive study treatment until any of the following occur:

- 3 years of study treatment
- Patient experiences PD according to RECIST v 1.1 criteria. Individual decision concerning discontinuation should be performed at the discretion of the treating physician
- Patient experiences unacceptable toxicity or an adverse experience that would, in the investigator's or sponsor's judgment, make continued administration of the study regimen an unacceptable risk

- Situations requiring a therapeutic intervention that is not permitted by the treatment plan
- Development of an intercurrent illness or situation which would, in the judgement of the investigator, affect assessments of clinical status and study endpoints to a significant degree
- For WOCBP: Pregnancy
    - All WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.
    - The investigator must immediately notify the delegated CRO (who will inform the sponsor / coordinating investigator) in the event of a confirmed pregnancy in a patient or in a partner of a patient participating in the study.
- Request by the patient to stop the treatment
- Patient is considered by the investigator or the sponsor to be significantly noncompliant with the requirements of the protocol (e.g., patient becomes pregnant)
- Study is closed or terminated
- Patient is lost to follow-up
- Investigator’s decision
- At the specific request of the sponsor

In case of prolongation of any treatment delay beyond 28 days after the last administration of the chemotherapy as a result of delayed hematological recovery or/and non-hematological adverse events (AEs), permanent treatment discontinuation should be decided after consultation with the coordinating investigator.
Note: A temporary interruption in study medication due to an AE is not considered to be permanent discontinuation of investigational product.

The reason for discontinuing study treatment must be clearly documented in the patient's medical record and recorded on the case report forms (CRF). Patients for whom the study treatment has been permanently discontinued will continue in the study unless they meet the criteria for study withdrawal (below).

All patients with permanent treatment discontinuation (except for withdrawal of consent or loss to follow-up or death) will be followed up as described.

5.4.2 Withdrawal from the Study

Patients will be encouraged to complete the study; however, they may voluntarily withdraw at any time. The investigator may also, at his/her discretion, withdraw the patient from participating in this study at any time, or the sponsor may discontinue the study.

Reasons for early withdrawal from the study should be documented in the eCRF as:

- Study closed/terminated
- Patient is lost to follow-up
- Patient died
- Investigator’s decision
- Patient withdrew consent

Date of withdrawal from the study, with reason for withdrawal (if applicable), will be documented in the patient's medical record and recorded on the eCRF. In the case of death, a death certificate should be obtained if possible, with the cause of death evaluated and documented.

5.4.3 Study Discontinuation

The sponsor may discontinue the study upon 30 days prior written notice.

Study discontinuation is at the discretion of the sponsor in any of the following events:

- Medical or ethical reasons affecting the continued performance of the study (e.g. recommendations of DSMB)
- Difficulties in the recruitment of patients

In addition, the study may be discontinued at the discretion of the sponsor in the event of any or all of the following:

- Inefficacy of the study treatment
- Occurrence of AEs previously unknown in respect of their nature, severity and duration, or unexpected incidence of known AEs

Safety data from the study will be reviewed by the sponsor, the DSMB and the Coordinating Investigator on a regular and ongoing basis in order to ensure the safety of the patients.

5.4.4 Definition of the End of Study

The end of study is defined as the last visit of the last patient, either at the end of study treatment or in the follow-up-period. Phone contact during the follow-up-period will be regarded as a visit. A patient will be considered as lost to follow-up if no contact could be established for 3 consecutive time points.

5.4.5 Plan for Treatment after the End of Study

Following the end of treatment evaluation or the end of treatment for any other cause, patients will be treated and followed according to the German guidelines for colorectal carcinoma.

6 Adaptation

According to SmPC, patients in experimental arm receiving cetuximab must receive premedication with an antihistamine and a corticosteroid at least 1 hour prior to administration of cetuximab. Patients should be closely monitored during the infusion and for at least 1 hour after the end of the infusion. Immediate emergency treatment of an infusion-related reaction or a severe hypersensitivity reaction according to institutional standards must be assured.

The first dose of cetuximab should be administered slowly and the speed must not exceed 5 mg/min whilst all vital signs (body temperature, heart rate, blood pressure) are closely monitored during the infusion time. If during the first infusion, an infusion-related reaction occurs within the first 15 minutes, the infusion should be stopped. A careful benefit/risk assessment should be undertaken including consideration whether the patient may have preformed IgE antibodies before a subsequent infusion is given.

Patients should be instructed to report any reactions to the investigator immediately, even if they are delayed.

6.1 General Notes Regarding Dose Modifications for Therapy-Related Toxicity

In case of therapy-related toxicities, dose modifications will be performed according to specifications in SmPC of used drugs and to routine practice. The patient must be treated according to the best available medical practice.

Cetuximab:

Mild or moderate infusion-related reactions are very common comprising symptoms such as fever, chills, dizziness, or dyspnea that occur in a close temporal relationship mainly to the first cetuximab infusion. If the patient experiences a mild or moderate infusion-related reaction, the infusion rate may be decreased. It is recommended to maintain this lower infusion rate in all subsequent infusions.

Severe infusion-related reactions, including anaphylactic reactions, may commonly occur during cetuximab therapy, in some cases with fatal outcome. Occurrence of a severe infusion-related reaction requires immediate and permanent discontinuation of cetuximab therapy and may necessitate emergency treatment. Some of these reactions may be anaphylactic or anaphylactoid in nature or represent a cytokine release syndrome (CRS). Symptoms may occur during the first infusion and for up to several hours afterwards or with subsequent infusions. It is recommended to warn patients of the possibility of such a late onset and instruct them to contact their physician if symptoms or signs of an infusion-related reaction occur. Symptoms may include bronchospasm, urticaria, increase or decrease in blood pressure, loss of consciousness or shock. In rare cases, angina pectoris, myocardial infarction or cardiac arrest have been observed.

Anaphylactic reactions may occur as early as within a few minutes of the first infusion e.g. due to preformed IgE antibodies cross-reacting with cetuximab. These reactions are commonly associated with bronchospasm and urticaria. They can occur despite the use of premedication.

A CRS typically occurs within one hour after infusion and is less commonly associated with bronchospasm and urticaria. CRS is normally most severe in relation to the first infusion.

6.2 Dose Modification and Treatment Alteration of Cetuximab

Infusion-related reactions

The first dose of cetuximab should be administered slowly and the speed must not exceed 5 mg/min whilst all vital signs (body temperature, heart rate, blood pressure) are closely monitored during infusion time. If during the first infusion, an infusion-related reaction occurs within the first 15 minutes, the infusion should be stopped. A careful benefit/risk assessment should be undertaken including consideration whether the patient may have preformed IgE antibodies before a subsequent infusion is given.

If an infusion-related reaction develops later during the infusion or at a subsequent infusion further management will depend on its severity:

a) Grade 1:	Continue slow infusion under close supervision
b) Grade 2:	Continue slow infusion and immediately administer treatment for symptoms
c) Grade 3 and 4:	Stop infusion immediately, treat symptoms vigorously and permanently discontinue cetuximab.

Eye disorders

Occurrence of conjunctivitis is to be expected in approx. 5% of patients.
Keratitis, in some cases ulcerative keratitis, has been reported uncommonly. Patients presenting with signs and symptoms suggestive of keratitis such as acute or worsening: eye inflammation, lacrimation, light sensitivity, blurred vision, eye pain and/or red eye should be referred promptly to an ophthalmology specialist. If the diagnosis of ulcerative keratitis is

confirmed, the cetuximab treatment should be interrupted or discontinued. If keratitis is diagnosed, the benefits and risks of continuing treatment should be carefully considered.
Cetuximab should be used with caution in patients with a history of keratitis, ulcerative keratitis or severe dry eye. Contact lens use is also a risk factor for keratitis and ulceration.

Interstitial lung disease

Cases of interstitial lung disease, including fatal cases, have been reported in patients treated with cetuximab, with the majority in Japanese patients. Confounding or contributing factors, such as concomitant chemotherapy known to be associated with ILD, and pre-existing pulmonary diseases were frequent in fatal cases. Such patients should be closely monitored. All study patients must undergo a suitable imaging procedure for visualising the chest prior to the start of cetuximab administration in the study. This is a precautionary measure in order to document pulmonary status at baseline. If respiratory tract symptoms are present on inclusion in the study, lung function tests and further diagnostic procedures must be additionally conducted in order to be able to diagnose pre-existing pulmonary fibrosis or interstitial pneumonitis. Furthermore, all patients will be regularly questioned about occurrence of any respiratory tract symptoms during the course of the study. If respiratory tract symptoms occur or worsen during or after treatment with cetuximab, a detailed description is necessary and the investigators should perform diagnostic procedures that, according to the best of their knowledge, allow for a definitive diagnosis. If interstitial lung disease is diagnosed, the treatment with cetuximab must be discontinued and the patient be treated appropriately.

Skin reactions:

Main adverse reactions of cetuximab are skin reactions which may become severe, especially in combination with chemotherapy. The risk for secondary infections (mainly bacterial) is increased and cases of staphylococcal scalded skin syndrome, necrotising fasciitis and sepsis, in some cases with fatal outcome, have been reported.
Skin reactions are very common and treatment interruption or discontinuation may be required. According to clinical practice guidelines, prophylactic use of oral tetracyclines (6 – 8 weeks) and topical application of 1% hydrocortisone cream with moisturiser should be considered. Medium to high-potency topical corticosteroids or oral tetracyclines have been used for the treatment of skin reactions.

a) 1st occurrence of skin reaction ≥ grade 3:
- Interrupt cetuximab treatment.
- If the reaction has resolved to ≤ grade 2, resume treatment without any change in dose level.

b) 2nd and 3rd occurrence of skin reaction ≥ grade 3:
- Interrupt cetuximab therapy.
- If the reaction has resolved to ≤ grade 2, resume at a lower dose level (200 mg/m² after the 2nd occurrence and 150 mg/m² after the 3rd occurrence).
- If the reaction does not resolve to ≤ grade 2, permanently discontinue cetuximab treatment.

c) 4th occurrence of severe skin reaction:
- Permanently discontinue cetuximab treatment.

If cetuximab has to be discontinued permanently patients will receive FOLFIRI chemotherapy until PD or conversion to *RAS*-mutant without PD. In this case patients will receive FOLFIRI.

When new conversion to *RAS*-wild-type is observed, patients will start again with FOLFIRI + cetuximab, as long as there are no reasons for permanent discontinuation of cetuximab.

7 Clinical Examinations

7.1 Clinical Examinations and Procedures prior to Therapy (Pre-Screening / Baseline)

The baseline examinations should be performed within 28 days before start of treatment, except laboratory parameters and urine pregnancy test (ß-hCG), which should be assessed within 3 days before registration:

- Informed Consent
- Demographic data, medical history
- Previous and concomitant medication within the last 2 weeks before signing of informed consent
- *RAS* mutation analysis on tumor tissue
- Blood sample (20 ml) for liquid biopsy
- TNM-staging at first diagnosis
- CT-scan of abdomen, chest and pelvis to document baseline tumor status (in case of contraindications to CT scan, e.g. allergy to contrast medium, MRI should be performed); current TNM staging
- Vital signs: body temperature, heart rate, blood pressure, height and weight
- ECG
- Echocardiography
- ECOG performance status assessment
- Hematological and biochemical laboratory assessment including hemoglobin, thrombocytes, neutrophils, Na^+, K^+, Mg^{2+}, Ca^{2+}, ASAT, ALAT, alkaline phosphatase, GGT, bilirubin, urea, creatinine, creatinine-clearance, C-reactive CRP, CEA, CA-19-9
- Test for DPD deficiency as specified in the ‘red-hand-letter’ for 5-FU (i.v.), capecitabine- and tegafur-containing drugs, dated 04.06.2020:
    - Genotyping (4 variants of the DPYD genotype, namely c.1905+1G>A, c.1679T>G, c.2846A>T und c.1236G>A/HapB3, are associated with a high risk for severe toxicities)

  and/or
    - Phenotyping (a blood uracil level between ≥ 16 and < 150 ng/ml is an indicator of partial DPD deficiency, a blood uracil level ≥ 150 ng/ml is an indicator of complete DPD deficiency)
- Coagulation parameters: INR, aPTT
- Hepatitis B and C serology
- Females of childbearing potential: urine pregnancy test (ß-hCG)
  Female patients of childbearing potential as well as all men must be informed that they are obliged to practice medically accepted contraception throughout the study.
- Adverse events
- Administration of 1 or 2 cycles of study treatment (FOLFIRI or mFOLFIRI) are permitted before RAS status is available. In case of RAS wild-type, patients are defined as screening failures and will not be treated further in the study.

7.2 Clinical Examinations and Procedures during Pre-randomization (Screening) Phase for conversion to RAS wild-type (maximum time period of 16 weeks from start of FOLFIRI)

Prior to every cycle (within 48 h before administration of study treatment):

- Hematological and biochemical laboratory assessment including hemoglobin, thrombocytes, neutrophils, Na^+, K^+, Mg^{2+}, Ca^{2+}, ASAT, ALAT, alkaline phosphatase, GGT, bilirubin, urea, creatinine, creatinine-clearance, C-reactive CRP, CEA, CA-19-9

Prior the first cycle (within 72 h before administration of study treatment):

- Females of childbearing potential: urine pregnancy test (ß-hCG)

Prior to administration of study treatment:

- Assessment of ECOG performance
- Survey of concomitant medication
- Vital signs: body temperature, heart rate, blood pressure, and weight

Every 2 weeks:

- Administration of study treatment (FOLFIRI or mFOLFIRI)
  Patients with partial DPD deficiency should receive the first cycle with a reduced 5-FU dose. Dose reduction and escalation are at the discretion of the investigator and must be determined in the best interest of the patient.

Every 4 weeks:

- Females of childbearing potential: urine pregnancy test (ß-hCG)

After every cycle:

- Assessment of adverse events. Adverse events are recorded according to NCI-CTCAE v.5.0. Serious adverse events have to be reported within 24 h to Alcedis GmbH (section 9.6)

Every 8 weeks:

- Tumor assessment using CT-scan (in case of contraindications to CT scan, e.g. allergy to contrast medium, MRI should be performed) and classification of the response according to RECIST-Criteria V 1.1; the same method should be used throughout the study

At week 4, 8, 12 and 16 (until conversion to RAS wild-type):

- Blood sample (20 ml) for liquid biopsy

If clinically indicated: ECG

For patients without conversion to *RAS* wild-type by week 16, the ‘end of study’-form must be completed.

Patients with conversion of *RAS* mutation to *RAS* wild-type, by ≤8 weeks or by >8 weeks, will be randomized at week 8 or 16 respectively. Before randomization, the inclusion / exclusion criteria have to be checked.

For the first 20 patients with *RAS* wild-type in liquid biopsy: New tumor biopsy, if possible, with *RAS* analysis. Overall, in at least 80% of patients a biopsy should be taken to confirm *RAS* status in tissue of respective patients.

7.3 Clinical Examinations during randomized Treatment Phase (Experimental Arm)

Prior to every cycle (within 48 h before administration of study treatment):

- Hematological and biochemical laboratory assessment including hemoglobin, thrombocytes, neutrophils, Na^+, K^+, Mg^{2+}, Ca^{2+}, ASAT, ALAT, alkaline phosphatase, GGT, bilirubin, urea, creatinine, creatinine-clearance, C-reactive CRP, CEA, CA-19-9

Prior to administration of study treatment:

- Assessment of ECOG performance
- Survey of concomitant medication
- Vital signs: body temperature, heart rate, blood pressure, and weight

Administration of study treatment:

- Cetuximab (every week) on day 1 and 8
- FOLFIRI (every 2 weeks) on day 1

After every administration of study treatment (d1 and d8):

- Assessment of adverse events. Adverse events are recorded according to NCI-CTCAE v.5.0. Serious adverse events have to be reported within 24 h to Alcedis GmbH (section 9.6)

Every 4 weeks:

- Females of childbearing potential: urine pregnancy test (ß-hCG)

Every 2 weeks until week 8 (for the first 10 patients randomized to Arm A):

- Blood sample (20 ml) for liquid biopsy

Every 4 weeks until week 8 (for all other patients, unless otherwise recommended by DSMB):

- Blood sample (20 ml) for liquid biopsy

Every 8 weeks during the first year:

- Tumor assessment using CT-scan (in case of contraindications to CT scan, e.g. allergy to contrast medium, MRI should be performed) and classification of the response according to RECIST-Criteria v1.1; the same method should be used throughout the study.
  If progression of disease is suspected for any reason at or between the 8-weekly evaluation visits, radiological confirmation is necessary and a new scan must be performed unless a scan taken no more than 14 days earlier is available.
- Blood sample (20 ml) for liquid biopsy

Every 12 weeks after the first year until end of study treatment:

- Tumor assessment using CT-scan (in case of contraindications to CT scan, e.g. allergy to contrast medium, MRI should be performed) and classification of the

response according to RECIST-Criteria; the same method should be used throughout the study.
If progression of disease is suspected for any reason at or between the 12-weekly evaluation visits, radiological confirmation is necessary and a new scan must be performed unless a scan taken no more than 14 days earlier is available.
- Blood sample (20 ml) for liquid biopsy

If clinically indicated: ECG

7.4 Clinical Examinations during randomized Treatment Phase (Control Arm)

Prior to every cycle (within 48 h before administration of study treatment):
- Hematological and biochemical laboratory assessment including hemoglobin, thrombocytes, neutrophils, Na^+, K^+, Mg^{2+}, Ca^{2+}, ASAT, ALAT, alkaline phosphatase, GGT, bilirubin, urea, creatinine, creatinine-clearance, C-reactive CRP, CEA, CA-19-9

Prior to administration of study treatment:
- Assessment of ECOG performance
- Survey of concomitant medication
- Vital signs: body temperature, heart rate, blood pressure, and weight

Every 2 weeks:
- Administration of study treatment (FOLFIRI)

After every cycle:
- Assessment of adverse events. Adverse events are recorded according to NCI-CTCAE v.5.0. Serious adverse events have to be reported within 24 h to Alcedis GmbH (section 9.6)

Every 4 weeks:
- Females of childbearing potential: urine pregnancy test (ß-hCG)

Every 8 weeks during the first year:
- Tumor assessment using CT-scan (in case of contraindications to CT scan, e.g. allergy to contrast medium, MRI should be performed) and classification of the response according to RECIST-Criteria; the same method should be used throughout the study.
If progression of disease is suspected for any reason at or between the 8-weekly evaluation visits, radiological confirmation is necessary and a new scan must be performed unless a scan taken no more than 14 days earlier is available.

Every 12 weeks after the first year until end of study treatment:
- Tumor assessment using CT-scan (in case of contraindications to CT scan, e.g. allergy to contrast medium, MRI should be performed) and classification of the response according to RECIST-Criteria; the same method should be used throughout the study.
If progression of disease is suspected for any reason at or between the 12-weekly evaluation visits, radiological confirmation is necessary and a new scan must be performed unless a scan taken no more than 14 days earlier is available.

Week 8 and 24:

- Blood sample (20 ml) for liquid biopsy

If clinically indicated: ECG

7.5 Final examinations after the treatment phase (End of Treatment Evaluation visit)

Final examinations should be performed **30±2 days** after last given medication according to protocol.

- Hematological and biochemical laboratory assessment including hemoglobin, thrombocytes, neutrophils, Na^+, K^+, Mg^{2+}, Ca^{2+}, ASAT, ALAT, alkaline phosphatase, GGT, bilirubin, urea, creatinine, creatinine-clearance, C-reactive CRP, CEA, CA-19-9
- Assessment of adverse events. Adverse events are recorded according to NCI-CTCAE v.5.0. Serious adverse events have to be reported within 24 h to the Alcedis GmbH (section 9.6)
- If applicable: new tumor therapy
- Survival data
- Females of childbearing potential: urine pregnancy test (ß-hCG)

7.6 Examinations during Follow-up Period

The follow-up period will last until the defined end of the study of a maximum of 36 months after last patient recruited.

The following examinations will be performed every 3 months after last treatment administration (if visit at the site is not possible, phone calls count as visit):

- If applicable: new tumor therapy
- Survival data
- Adverse events: Final documentation of outcome of adverse events still ongoing at the End of Treatment Evaluation visit. Documentation of SAEs related to study treatment, until resolution or stabilization.

8 Criteria for Tumor Assessment and Response

8.1 Eligibility for Evaluation

Patients with a measurable disease according to RECISTv1.1 criteria before the start of study treatment are eligible for response evaluation according to RECISTv1.1 criteria (refer to section 8.5 and Appendix 17.2) [44].

Note: The method of tumor response evaluation must not be changed during the course of the study!

8.2 Criteria for Measurable and Non-Measurable Lesions

Measurable lesions are lesions that can be accurately measured in at least one dimension with longest at least 10 mm with CT scan, MRI techniques or spiral CT scan.

Non-measurable lesions are all other lesions, including uni-dimensionally measurable disease and small lesions (lesions without at least one diameter ≥ 10 mm), and any of the following bone lesions, ascites, pleural effusion, cysts and abdominal masses that are not confirmed by imaging techniques.
All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (in other words the lesions with the longest diameter) and their suitability for accurate repeated measurements. A sum of the longest diameter for all target lesions will be calculated and reported as the baseline sum of the longest diameter. This will be used as reference to objectively characterize the tumor. All other lesions should be identified as non-target lesions and are not measured at baseline but presence should be noted.

8.3 Definition of Measurable Lesion Response Using RECIST Criteria Version 1.1

Target lesions will be measured in a dimension using the longest diameter is used. The same investigational method must be used throughout.

Complete response (CR): Disappearance of all lesions and appearance of no new lesions.

Partial response (PR): At least a 30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum diameters. No new lesions may occur or individual lesions progress.

Progressive disease (PD): At least a 20% increase in the total of the longest diameters of target lesions, taking as reference the smallest sum on study since the treatment started (this includes the baseline sum if that is the smallest on study) or the appearance of new lesions.
If the marker lesion disappears and the progression of a lesion or the appearance of new lesions is observed elsewhere at the same time, this is also documented as a progressive disease.

Stable disease (SD): Neither a partial nor a complete response in the absence of progression.

8.4 Definition of Non-Measurable Lesion Response Using RECIST Criteria Version 1.1

Any other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥10 to < 15 mm short axis) as well as truly non-measurable lesion are considered non-measurable or non-target lesions. Truly non-measurable lesions include: leptomeningeal disease, ascites, pleural and pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses / abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Evaluation of non-target lesions:
Complete Response (CR): Disappearance of all non-target lesions
Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesion(s)
Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

8.5 Best Overall Response According to RECIST Criteria

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	SD	No	PR
PR	SD	No	PR
SD	SD	No	SD
PD	any	Yes or No	PD
any	PD	Yes or No	PD
any	any	Yes	PD

9 Assessment of Safety

Toxicities will be defined according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (Appendix 17.3).
The investigator is responsible for documenting all adverse events that occur during the study. Adverse events have to be reported after informed consent until 30 days after last dose of study treatment. Then only adverse events which are causally related to study treatment have to be reported.

9.1 Adverse Events

Adverse events (AEs) are any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

This also includes deterioration in pre-existing diseases or events, diseases that occur in the intervening period, changes of medication or a significant deterioration in the disease studied. Expected daily variations in the disease under study that do not represent a clinically significant deterioration should not be considered an AE.

Unexpected adverse event is defined as:
an experience not previously reported (in nature, severity or incidence) in the current Investigator's Brochure, SmPC, the protocol or elsewhere (ICH E.G. (R1) guideline, July 2002).

A **non-serious adverse event** is an AE not classified as serious.

Laboratory test abnormalities

All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported to Alcedis GmbH as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the patient to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (e.g., anemia versus low hemoglobin value).

9.2 Serious Adverse Events

A serious adverse event (SAE) is any event that

- Results in death
- Results in a life-threatening condition (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Results in in persistent or significant disability/incapacity (defined as impairment of the ability of the patient to go about his/her daily life normally)
- Results in congenital abnormalities or birth defects
- Requires hospitalization or prolongation of existing hospitalization
- Any important medical event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, it may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Although pregnancy is not always serious by regulatory definition, however, these events must be reported within the SAEs timeline.

EXCEPTIONS:

Within this study, the following serious events are not considered SAEs will be excluded from compulsory notification.

- A visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- Elective surgery, planned prior to signing consent
- Hospitalization associated with therapeutic measures exclusively (administration of the trial substances, blood transfusions, elective procedures and surgery for tumor removal).
- Overnight hospitalizations occurring exclusively for logistical reasons (e.g., no transportation available for the patient to return home on the same day).
- Progression of the underlying disease only, even if life-threatening or results in death.

9.3 Follow-Up Examination in the Case of Adverse Events

The investigator should follow up patients with AEs until the event regresses or the condition has stabilized or until end of therapy (for AEs) and until end of therapy plus 30 days (for SAEs). After the safety observation period, the investigator shall report to Alcedis GmbH only serious adverse events that are related to the study medication.

9.4 Causal Assessment

The degree of certainty of the relationship between AE and drug is determined by how well the event can be explained by the following factors: (1) known pharmacological properties, (2) comparable reactions observed previously with the drug or another member of its class, (3) an event with comparable substances commonly described in the literature as drug-related, (4) a chronological relationship between the event and drug intake, disappearance on withdrawal or recurrence on re-institution of the drug.
The investigator should make every effort to elucidate any AE and where necessary to assess its relationship to the study medication. The causal relationship should be assessed on the basis of the following categories:
Related: There is a reasonable causal relationship between study drug administration and the AE (definitely, probably, or possibly)

Not related: There is not a reasonable causal relationship between study drug administration and the AE (unlikely, no correlation).

9.5 Adverse Event Collection and Reporting Information

The following information should be captured for all AEs in the respective form of the eCRF: onset, duration, intensity, seriousness, relationship to study drugs, action taken, and treatment required. If treatment for the AE is administered, it should be recorded in the medical record.

All SAEs that occur following the date of signature of informed consent until 30 days after discontinuation of dosing must be reported to Alcedis GmbH, whether related or not related to study drug. Afterwards the investigator should report any SAE occurring after these aforementioned time periods, which is believed to be related to study drug.

9.6 Notification of (Serious) Adverse Events

All adverse events that occur with the study therapy must be entered in the case report form. Similarly, the duration and estimated severity of each adverse event must be noted. The assessment of an adverse event is made on the basis of the Common Terminology Criteria of Adverse Events (CTCAE) in Version 5.0 (Appendix 17.3)

If a CTCAE classification is not applicable, the adverse event must be described on the basis of the following classification:

Grade 1 = mild toxicity

Grade 2 = moderate toxicity

Grade 3 = severe toxicity

Grade 4 = life-threatening toxicity

Grade 5 = fatal toxicity

Each investigator must **inform Alcedis GmbH** (study office, address see below) **within 24 hours after becoming known** of the SAE that occur during the clinical study or within 30 days of the last dose of the study medication being received, irrespective of whether or not they are attributable to the preparations.

Each serious adverse event must be documented on the electronically available "**Serious adverse events report**" form. The SAE form must be printed and kept on file at the study site.

The completed form is automatically faxed (fax no. is filed automatically in the system) **to the sponsor and coordinating investigator.** In the event that electronic reporting is not possible, paper SAE forms in the investigator's file handed out at the beginning of the study are at the doctor’s disposal for notification by conventional fax:

Study office:
Alcedis GmbH
Winchesterstraße 3
D- 35394 Gießen
Germany
Phone: +49 (0)641 / 94436-0
Fax: +49 (0)641 / 94436-70

These data must be entered in the eCRF afterwards.

Complete data on these events, including safety laboratory data, vital signs and efficacy data necessary to assess and monitor the safety of the funder’s studied medicinal product, shall be collected and provided by the sponsor to Merck Healthcare Global Patient Safety as specified in the respective ISS Agreement.

If only limited information is initially available, follow-up reports are required.

The initial report must be followed **within 5 days** by a detailed written communication (in English) containing a precise description of the side effects, all countermeasures taken and their outcome. In the event of death, if an autopsy has been performed, a copy of the autopsy report should be attached.

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours using the same procedure used for transmitting the initial SAE report.

The Sponsor will send appropriate safety notifications to Health Authorities in accordance with applicable laws and regulations.
When specifically required by regulations and guidelines, the Sponsor/designee will provide appropriate Safety Reports directly to the concerned lead IEC/IRB and will maintain records of these notifications. When direct reporting is not clearly defined by national or site-specific regulations, the Investigator will be responsible for promptly notifying the concerned IEC/IRB of any Safety Reports provided by the Sponsor/designee and of filing copies of all related correspondence in the Investigator Site File.
For studies covered by the European Directive 2001/20/EC, the Sponsor’s responsibilities regarding the reporting of SAEs/SUSARs/Safety Issues will be carried out in accordance with that Directive and with the related Detailed Guidance documents.

9.7 Pregnancy

WOCBP and male patients with partners of childbearing potential must agree to always use a highly effective form of contraception according to CTFG during the course of this study and for at least 3 months after completion of study therapy. Birth control methods which may be considered as highly effective can achieve a failure rate of less than 1% per year when used consistently and correctly. Pregnancy tests should be performed at baseline, prior to the first cycle and subsequently every 4 weeks during pre-randomization part and randomized part.

Before study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during study participation and the potential risk factors for an unintentional pregnancy. All WOCBP should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. The patient must sign an informed consent form documenting this discussion.

The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of hCG. If the pregnancy test is positive, the patient must not receive study treatment and must not be enrolled in the study.

If, following initiation of the clinical trial, it is subsequently discovered that a study patient is pregnant or may have been pregnant at the time of investigational product exposure the study treatment will be permanently discontinued in an appropriate manner. The investigator must immediately notify Alcedis GmbH of a pregnancy occurring during the conduct of the clinical trial including at least 2 months after last study administration and record the pregnancy on the Clinical Trial Pregnancy Reporting Form (not an SAE form). Initial information on a pregnancy must be reported within 24 hours of investigator/site awareness of the event to Alcedis GmbH and Merck, and the outcome information provided once the outcome is known. Completed Pregnancy Surveillance Forms must be forwarded to Alcedis GmbH according to SAE reporting procedures.

10 Statistical Methods

10.1 Statistical Objectives

The primary objective is to evaluate efficacy in terms of PFS according to RECIST version 1.1 criteria in experimental and control arms.

Secondary Objectives are evaluation of:

- OS in experimental and control arms after the date of randomization
- TFTS in experimental and control arms after the date of randomization
- PFS rate 1 year after the date of randomization
- Depth of response in terms of reduction of tumor mass in experimental and control arms after start of 1st line treatment
- Percentage of patients with metastasis resections in experimental and control arms after start of 1st line treatment
- 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 to be recorded from the date of signature of Informed Consent

Optional exploratory objectives are

- To identify driver mutations (e.g. BRAF, PI3K-AKT-mTOR etc.), in patients with PD under cetuximab therapy which remain RAS wild-type in liquid biopsy
- To compare PFS in patients with conversion to *RAS* wild-type in ddPCR and BEAMing with those patients showing conversion to *RAS* wild-type in ddPCR but not in BEAMing

10.1.1 Primary Variable

PFS, defined as being the 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 occurs first. Any change of *RAS* mutation status without disease progression according to RECIST v.1.1 is not considered a PFS event. PFS should be censored, in patients without a PFS event, on the last date they were known to be alive and progression-free (last valid tumor assessment) or at the start of new anti-tumor therapy. Patients becoming eligible for surgery during study treatment, will not be censored for PFS at this event (resection).

10.1.2 Secondary Variables

- OS, defined as time from date of randomization until death from any cause; OS should be censored, in patients without an OS event, on the last date they were known to be alive.
- TFTS, is defined as time from date of randomization to failure of treatment strategy defined as treatment discontinuation for any reason, including disease progression, withdrawal of consent, treatment toxicity, patient preference, loss to follow up or death from any cause.
- PFS rate 1 year after date of randomization, derived by Kaplan-Meier method (defined as the estimated rate of patients with progression or death from any cause 1 year after date of randomization)
- Depth of response (DpR) in terms of reduction of tumor mass in experimental and control arms, defined as the percentage of tumor shrinkage, based on longest diameters of all target lesions or reconstructed volume, observed at the lowest point (nadir) compared to baseline. The time for DpR assessment varies between patients relating to the individual achievement of maximal tumor shrinkage, which typically occurs 4–6 months after the start of 1st-line therapy. DpR varies between 100% (= all target tumor lesions have disappeared) and 0% (=no change in tumor size) and has a negative value in cases of increased tumor volume.
- Percentage of patients with metastasis resections, defined as percentage of patients with complete resection of primary tumor and metastases (R0-resection), at any time after the start of 1st line therapy until the end of the treatment strategy.
- ORR, defined as the proportion of randomized patients with CR or PR as best response since start of 1st line therapy.
- Safety, defined as all SAEs regardless of causality, and non-serious adverse events according to CTCAE Version 5.0 criteria, that are definitely, probably, or possibly related to the administration of any study treatment, or that cause permanent discontinuation of treatment, recorded from the date of signature of Informed Consent.

10.2 Study Populations

The following study populations will be examined:

Modified Intention to Treat (mITT): All randomized patients who received at least 1 dose, complete or incomplete, of study medication

Safety population (SP) = mITT population

Per protocol (PP) population: All randomized patients who received study treatment according to randomization and did not have major disqualifying protocol violations.

Major protocol violations will be stated in the statistical analysis plan (SAP).

10.3 Statistical Methods

In general summary statistics (n, mean, standard deviation, median, minimum and maximum for continuous variables, and number of patients and percentage in each category for categorical variables) will be provided.
Time to event variables will be summarized using Kaplan-Meier methods as well as a Cox-regression model taking into account the following stratification factors: conversion to RAS wildtype within the first 8 weeks from start of 1st line therapy or later. Other factors (such as e.g. ECOG PS or location of metastases) will be taken into account in a multivariable Cox regression as well. Details will be specified in the SAP.

All analyses except for the log-rank test for the primary endpoint will be of exploratory manner. Hence no adjustment of the type I error rate is necessary.
All efficacy variables (primary and secondary endpoints) will be analyzed in the mITT and the PP set.
The SP set will be used for the analyses of the safety variables. Safety analysis includes:

- System Organ Class (SOC) and Preferred Terms (PT) according to the Medical Dictionary for Regulatory Activities (MedDRA). The most current MedDRA version, which is valid at the time of analysis, will be used.
- Causality of AE
- Outcome of AE
- AEs leading to death
- AEs leading to treatment/study discontinuation
- All SAEs

Details of the analyses will be given in the SAP.

10.4 Sample Size

Data from historical studies suggest the 12-months PFS rate of FOLFIRI in RAS-mutant mCRC to be in a range of 25-27%. In CRYSTAL study PFS-rate at 12 months of FOLFIRI + Cetuximab in left-sided *RAS* wild-type mCRC was 50% (HR = 0.56).
Assuming that the difference in PFS at 12 months between the experimental and control arms will amount to 20%, which means an increase from 30% to 50% (Hazard ratio = 0.5757), 2 x 58 patients are to be randomized and 107 PFS events are needed if alpha error is 5% (0.05) and beta error is 20% (0.2). With a recruitment period of 18 months, a drop-out rate of 0.25% per month the 107 events are expected after a total follow-up time of 52 months assuming an exponential distribution.
Based on our data, the conversion rate is 80%. Assuming this conversion rate, 144 patients need to be screened to achieve 116 patients for the randomization. The screening will be continued until 116 patients are randomized.

10.5 Interim Analysis

Two interim proof of concept analyses are planned, the first after randomization of 20 patients and the second after screening of 50 patients.
In these interim analyses, data of *RAS* analysis as well as safety data will be included. Data will be reviewed by an independent DSMB.
Both analyses as well as recommendations of the DSMB will be notified to ethics committee.

1. Interim analysis:
After randomization of 20 patients, further enrollment into the study will be paused. Liquid biopsy samples from the first 20 screened patients will be analyzed in parallel by 2 laboratories. In case of concordant results, the study will continue. In case of discordant results, DSMB will make recommendations for further progress of the study. Additionally, tumor-specific genes will be analyzed to define ctDNA in the samples. This should primarily be performed by measurement of “house-keeping genes” that are identified by Next Generation Sequencing (NGS) of baseline tumor tissue or if not applicable by methylation analysis of promotor region of the genes WIF1 and NPY which serve as surrogate markers for tumor cells.
Also, for the first 20 patients showing conversion to *RAS* wild-type in liquid biopsy during screening, at two time points, tumor material assessed locally, as well as a liquid biopsy sample will be analyzed comparatively for *RAS* mutation / wild-type to assess agreement between the analysis methods. This will be done before start of 1st line therapy and once during screening when *RAS* wild-type is detected in liquid biopsy. For the second comparison tumor material of a new biopsy assessed locally has to be used, if possible.
In addition, in the first 8 weeks after randomization, blood samples of the first 10 patients randomized into experimental arm A will be taken more frequently: at weeks 2, 4, 6 and 8 instead of just weeks 4 and 8. If $\geqslant$5 of 10 patients in the experimental arm A show *RAS* mutation within 4 weeks after randomization by liquid biopsy, the study may be terminated prematurely, because, in this case, the effect of *RAS* conversion does not seem to be lasting long enough.
2. Interim analysis:
After 50 patients have been screened, the proportion of patients with conversion from *RAS* mutant to *RAS* wild-type will be analyzed and evaluated by the DSMB. If less than 50% of analyzed patients show conversion to *RAS* wild-type, the DSMB will make recommendations regarding the conduct of the study.

11 Translational Project

We expect that most of patients will develop PD simultaneously or shortly after the recurrence of RAS mutations. In these cases, it can reasonably be concluded, that RAS mutations are driver mutations for the resistance to cetuximab containing treatment. In cases with PD without recurrence of RAS mutations, the driver mutation leading to PD remains unclear. To close this gap, the analysis of further genes whose proteins act downstream of *RAS* (e.g. *BRAF*) and other pathways regulated by EGFR (e.g. PI3K-AKT-mTOR pathway which is frequently activated in cancer) will be performed in patients with PD under cetuximab therapy who remain *RAS* wild-type in liquid biopsy. DdPCR or NGS in cases of sufficient amount of ctDNA will be performed to examine whether mutations downstream or in other pathways have an impact on the benefit of cetuximab.

RAS wild-type patients in ddPCR will be additionally analyzed by BEAMing. For randomization, only the result of ddPCR will be used. However, PFS of patients who show conversion to *RAS* wild-type as determined by both analysis procedures will be compared to PFS in patients who

only have conversion to *RAS* wild-type as determined by ddPCR but with remaining detection of RAS mutation in BEAMing.

12 Data Management

12.1 Patient Identification

All data related to patients will be assessed pseudonymously. Each patient will be clearly identified through the patient number given in the enrolment procedure. At the center site the investigator compiles a confidential list, in which the patient name and address is assigned to the patient number.

12.2 Data Collection

The data management for this study will be performed by Alcedis GmbH, Winchesterstr. 3, D-35394 Gießen.
The eCRF is part of the EDC system which allows documentation of the relevant study variables by all participating sites in a standardized way. The following chapters describe the software employed and measures applied for data security. Data are recorded, processed and stored using the following software tools.
CRF database (Location: Alcedis GmbH, Winchesterstr. 3, 35394 Giessen, Germany)
Wherever applicable, current GCP guidelines, actual technical standards and guidelines are observed.

12.2.1 Employed Software

CRF database

For data capturing and data management of this clinical trial, a web-based validated software (WBDC) will be employed. The software consists of the following modules:

a) **Administration:** Administration of sites (clinics / office based physicians) by system administrator and project management. Within the individual sites the following system users are defined: Investigator and study nurse. All access rights are administered in a role-based security system.

b) **Forms / Form validator:** Electronic Case Report Forms (eCRFs) for data capture including online validation of CRFs during data capture, e. g. check on range, plausibility, type mismatch.
In addition to the system based plausibility checks, a formal query process will be implemented to resolve inconsistencies in SAE data.

c) **Reports:** Dynamic report generator, e.g. reports for investigators on CRF status.

d) **Database:** Relational database for data management. The data from the relational database will be retrieved using the export engine of *Alcedis Med* and thereafter converted into data sets for further validation and analysis.

The employed technology and technical requirements for data entry on site are as follows:

a) The used software is completely server-based, i.e. all program processes are executed centrally on a web or database server.
b) Data are saved exclusively in the central database server. This server is located in the facilities of Alcedis GmbH, Winchesterstr. 3, D-35394 Gießen.
c) For system access, users require a conventional desktop computer with internet access.

12.2.2 Data Security and Storage

For client / server communication via the Internet only encrypted transmissions are applied. State of the art encryption technology is used exclusively. For data transmission in this clinical trial an encryption algorithm (sha256 RSA) is employed by means of the Transport Layer Security (TLS) version 1.2.
In addition, the server identifies itself to the client workstation by means of a digital server certificate issued by an authorized certification authority. By this, it is ensured that data are sent to the server of Alcedis GmbH only.
Data are protected from potential virtual attacks and physical damage.
Views on data or reports as well as edit or read only rights are controlled with individual passwords. Access authorization to the eCRF database is granted individually to participating physicians and data entry personnel by means of user accounts.
The CRO project management has a read-only access to all patient data pseudonymously stored in the CRF database.
Assurance of data will be made by RAID-Systems (Redundant Array of Independent Disks), thereby ensuring data security even if one hard disc failed.
Furthermore an encrypted backup onto magnetic tape is performed according to the following scheme:

- daily back-up over a period of 32 days;
- monthly back-up over a period of continuance of the study.

Participating sites will receive an encrypted data medium after the end of the study for archiving purposes; this medium will contain all pseudonymized data of the patients the respective sites have documented in the eCRF database during the study.

12.2.3 Data retrieval and Query Procedure

Data will be entered into the eCRF at the participating site. The participating physician will be responsible for entering data into the WBDC system in accordance with data entry instructions handed out to the site. As soon as data have been entered into the EDC-system, all changes and user information will be saved in an audit trail.
Electronic edit checks are performed automatically and directly when entering data in the eCRF. Here, the user is notified on missing data entries or implausibilities. This is done by warning and error messages, whereby the latter prevents to save the eCRF form until the error is corrected by the user.

13 Monitoring

13.1 Monitoring

Study monitoring is undertaken by monitors appointed by Alcedis GmbH on behalf of the sponsor. The responsible monitor will be allowed, on request, to inspect the various records of the trial (Case Report Forms and other pertinent data).

Due to the electronic documentation system checks for range and plausibility are performed during data entry. The monitor gets an access to read the data only.
In line with ICH GCP guidelines, monitoring will include verification of data entered in the eCRF against original patient records. This verification will be performed by direct access to the original patient records, and the Sponsor guarantees that patient confidentiality will be respected at all times. Participation in this study will be taken as agreement to permit direct source data verification.
Within this study a risk-based monitoring will be performed. Details will be specified in the monitoring plan.
In case that onsite visits are not possible for a long time period (e.g. due to a pandemic situation) remote monitoring will be performed. The monitor will contact sites at an appointed date and will discuss study data remotely with the responsible study personal at the site. Further details will be included in the monitoring plan.

13.2 Audit, Inspections

The sponsor retains the right to undertake a quality audit in accordance with ICH-GCP guidelines at any time, particularly if any doubt should arise about the quality of the documentation.
In such an audit, as with an audit by the competent supervisory or health authorities, the authorized representatives involved should be granted access to the originals of the patient files. The investigator guarantees his full co-operation in the event of an audit.

14 Ethical Considerations

14.1 Declaration of Helsinki

The study is conducted in accordance with the Declaration of Helsinki in its recent version (Fortaleza, Brazil, 2013, Appendix 17.1).

14.2 Patient Information

An unconditional prerequisite for a patient participating in the study is his/her written informed consent. Adequate information must therefore be given to the patient by the investigator before informed consent is obtained. A person designated by the investigator may give the information, if permitted by local regulations. A patient information sheet in the local language will be provided for the purpose of obtaining informed consent. In addition to this written information, the investigator or his designate will inform the patient verbally. In doing so, the wording used will be chosen so that the information can be fully and readily understood by laypersons.
The patient information sheet will be revised whenever important new information becomes available that may be relevant to the consent of patients.

The written informed consent of the patient to participate in the clinical study has to be given before any study-related activities are carried out. It must be signed and personally dated by the patient and by the investigator / person designated by the investigator to conduct the informed consent discussion. Patients are also asked to give consent to additional analysis of collected blood samples to investigate the explorative objectives. This approval is not a precondition for participation in the study.

Provision of consent will be confirmed in the eCRF by the investigator. The signed and dated declaration of informed consent will remain at the investigator's site and must be safely archived by the investigator so that the forms can be retrieved at any time for monitoring, auditing and inspection purposes. A copy of the signed and dated information and consent should be provided to the patient prior to participation.

14.3 Consent to Participate

Each patient must give his/her written consent to participate in the study. At the same time, the patient must be given sufficient time and opportunity to decide on their participation and to clarify any outstanding questions before the institution of any study procedures.

The declaration of consent is signed by the patient and the investigator.

The original signed and dated patient informed consent will remain at the investigator's site. A signed and dated duplicate must be given to the patient.

14.4 Use, Storage and Transmission of Data

Patients will be informed that data, related to their illness, will be saved and in anonymous form used for scientific analysis and publications.
Patients are entitled to get information about the saved data.

The informed consent to data security and data transfer will be given apart of the informed consent to study participation.

15 Legal and Administrative Regulations

15.1 Coordinating Investigator according to Local Law

PD Dr. med. Alexander Baraniskin

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The coordinating investigator can demonstrate at least 2 years’ experience in the clinical testing of medicinal products.

15.2 Compliance with the Protocol and Good Clinical Practice Guidelines

The treatment and assessments should be conducted exactly as described in the protocol. The Investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the ethics committee of an amendment. Exceptions to this are emergency situations in which the concern for the safety

and well-being of the patients makes alternative treatment necessary. Any protocol deviation must be reported.

The recommendations of Good Clinical Practice (see ICH-GCP: International Conference on Harmonisation - Good Clinical Practice), valid since 17.1.1997, must be observed.

15.3 Protocol Supplements

Supplements or changes to the protocol may only be made by the coordinating investigator after consultation with the sponsor and submitted to the Ethics Committees and the national competent authorities as an amendment to the protocol.

15.4 Study Monitoring

The sponsor and the investigators must ensure the clinical trial is conducted correctly. The state of progress of the study must be presented at study meetings.

15.5 Specialist Qualification of Investigators and Suitability of Study-Centres

All participating investigators must demonstrate the suitability of the study center in accordance with the local law and the GCP Ordinance.

15.6 Maintenance of a Study File

Prior to study start each site will receive an Investigator Site File (ISF). The investigator will keep all study-relevant documents (e.g. study plan, curriculum vitae, ethics committee approval) and correspondence in the ISF.

15.7 Authorization by the Competent Authorities

The documents on the pharmacological and toxicological testing of study medication are filed with the Competent Authorities in accordance with local law. The application for approval of a clinical trial with a medicinal product for human use is made on behalf of the sponsor by Alcedis GmbH.

15.8 Ethics Committee Approval

Before the beginning of the study, an application for approval will be submitted to the Ethics Committees of the coordinating investigator and to the ethics committees of the participating investigators.

15.9 Notification to the Competent Supervisory Authorities

Participating investigators are notified to the competent supervisory authorities according to local law by Alcedis GmbH.

15.10 Archival

The investigator is legally obliged to keep the patient identification list for at least 10 years after the end of the study. The patient data recorded, including the original or copies of test results, the informed consents, the ethics committee approval and the correspondence and other original documents associated with the study must also be stored by the investigator for a period of 10 years. This precondition also applies if the doctor transfers the documents (and the associated obligation of storage) to a successor.

Original data from the study patients (patient records) must be stored in accordance with the archiving period applicable in the study centers, but for not less than 10 years.

15.11 Confidentiality

The contents of the study protocol and case report form must be treated confidentially and may not be disclosed to unauthorized parties either verbally or in writing.

15.12 Patient Insurance

The study patients are insured at the Chubb European Group SE in accordance with the local law up to a sum of €500,000.00.

Policy Number: DELSCA33426

15.13 Final Report / Publications

After completion of the study, the final biometric report will be provided by Alcedis GmbH on behalf of the sponsor.
Once the final biometric report is available, the final medical report will be written.

The results of the study will be published in general publications. The order in which the authors are listed will be based on the number of patients they have included. A co-author will be included in the list of authors for each center in which more than 4 patients are included.

15.14 Third Party Financing

This study will entail no additional financial expenditure either for the organizations funding the hospital or for the health insurance companies in association with the clinical trial. Third party funds will be provided by Merck for study co-ordination, documentation, monitoring and analysis.

This study entails no additional financial expenditure for supplementary laboratory analyses or additional diagnostic measures associated with the therapy, as the study design is deliberately based on the procedure for the therapy administered in the previous standard.

15.15 Data and Safety Monitoring Board (DSMB)

A Data and Safety Monitoring Committee will be established, consisting of two medical experts in treatment of CRC, and a statistical expert. All members are completely independent of the trial, there are no financial, scientific or other conflicts of interest. The DSMB will receive safety and *RAS* analysis results for the interim analyses. Details on the work of the board will be described in a DSMB charter, to be jointly agreed upon by the board and the sponsor.

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17 Appendices

17.1 Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI 2013

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
   The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.
2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”
4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
5. Medical progress is based on research that ultimately must include studies involving human subjects.
6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.
8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.
10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.
11. Medical research should be conducted in a manner that minimizes possible harm to the environment.
12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.
13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.
14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

    Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.
17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

    Measures to minimize the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.
18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

    When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

    All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

    The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

    In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

    The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study’s findings and conclusions.

Privacy and Confidentially

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.
26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study

provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.
28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.
29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorized representative.
31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.
32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

    Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provision

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.
36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

17.2 RECIST v1.1 Criteria

Definition of a measurable lesion:

- At least one lesion which can be measured in at least one dimension
- In case only a solitary lesion is present, cytology and histology are recommended
- Measurement of the largest diameter, e. g. using a chest X-ray ≥ 2cm or with a CT scan, MRI techniques or spiral CT ≥ 1cm

Definition of a non-measurable lesion:

All other lesions, including small lesions (longest diameter < 1 cm or pathological lymph nodes with ≥ 1 cm to < 1.5 cm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

General guidelines:

- During the study and follow-up, use of the same investigations and the same measuring techniques
- Measurements with pictures are preferred to clinical measurements
- Clinical measurements are to include the surfaces involved e. g. cutaneous nodes (color photo including a measuring staff)
- Lesions which are not measurable: “unclear” → control after 1 month of further therapy
- Splitting of a lesion → addition

Baseline of measurable and non-measurable lesions:

- Estimation of the total tumor mass
- As shortly as possible prior to therapy start (≤ 4 weeks)
- A maximum of 2 per organ, a maximum of 5 in all, which are defined as target lesions
- Representative of all organs
- For largest lesions: a repeat of the results has to be possible
- Sum of all of the largest diameters (this sum is the reference for objective tumor response)
- All non-measurable lesions are to be considered and during the course noted as present or absent, i. e. defined as being non target lesions

17.3 Common Toxicology Criteria for Adverse Events (CTCAE) v.5.0

It is recommended to follow the “Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0” that can be found online at the following address:

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50

17.4 ECOG / Karnofsky-Index

ECOG Grade	ECOG Status	Karnofsky Grade	Karnofsky Status
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (e.g. light housework, office work).	90	Able to carry on normal activities. Minor signs or symptoms of disease
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (e.g. light housework, office work).	80	Normal activity with effort
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	70	Care for self. Unable to carry on normal activity or to do active work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but able to care for most of his needs
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	50	Requires considerable assistance and frequent medical care
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled. Requires special care and assistance
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	30	Severly disabled. Hospitalisation indicated though death nonimminent
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick. Hospitalisation necessary. Active supportive treatment necessary
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	10	Moribund
5	Dead	0	Dead

Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982 Dec; 5(6):649-55.

17.5 CTFG (Clinical Trial Facilitation Group) recommendation for highly effective Contraception

Birth control methods which may be considered as highly effective:

For the purpose of this guidance, methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[1]:
  - oral
  - intravaginal
  - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation[1]:
  - oral
  - injectable
  - implantable[2]
- intrauterine device (IUD)[2]
- intrauterine hormone-releasing system (IUS)[2]
- bilateral tubal occlusion[2]
- vasectomised partner[2,3]
- sexual abstinence[4]

[1] Hormonal contraception may be susceptible to interaction with the investigational medicinal product, which may reduce the efficacy of the contraception method

[2] Contraception methods that in the context of this guidance are considered to have low user dependency.

[3] Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.

[4] Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments.
The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.