REACT

Adjuvant chemotherapy for prevention of recurrence in patients with detectable ctDNA after surgery in high-risk rectal cancer.
A phase III, multicentre study.

CLINICAL TRIAL TITLE

'Adjuvant chemotherapy for prevention of recurrence in patients with detectable ctDNA after surgery in high-risk rectal cancer.'

Protocol ID	***NL82006.078.22***
Short title	**REACT**
EU trial number	***2024-517700-12-00***
Protocol version	**4.0**
Protocol date	***18-06-2025***
Sponsor (in Dutch: verrichter/opdrachtgever)	***Erasmus MC, University Medical Center Rotterdam*** Department of Surgery Dr. Molewaterplein 40 3015 GD Rotterdam, The Netherlands
Legal representative(s) of the sponsor:	***Erasmus MC, University Medical Center Rotterdam*** Prof. dr. J.M. Hendriks Head of Department of Surgery Dr. Molewaterplein 40 3015 GD Rotterdam, The Netherlands
Principal investigator (in Dutch: hoofdonderzoeker/uitvoerder)	***Erasmus MC, University Medical Center Rotterdam*** Prof. dr. C. Verhoef Department of Surgery Rotterdam, The Netherlands Phone: 0031 10 704 1902 E-mail: c.verhoef@erasmusmc.nl
Co-investigator	***Erasmus MC, University Medical Center Rotterdam*** Prof. dr. H.M.W. Verheul Department of Medical Oncology Rotterdam, The Netherlands Phone: 0031 10703 0776 E-mail: h.verheul@erasmusmc.nl
Multicenter research: per site	***Reinier de Graaf Hospital*** Dr. A. Verschoor Department of Medical Oncology Delft, The Netherlands Phone: 015 2604080 E-mail: a.verschoor@rdgg.nl ***Bravis Hospital*** Dr. S. Boudewijns Department of Medical Oncology Roosendaal, The Netherlands Phone: 088 7066820 E-mail: s.boudewijns@bravis.nl

Franciscus Gasthuis
Dr. M. Verseveld
Department of Surgical Oncology
Rotterdam, The Netherlands
Phone: 010 4616163
E-mail: m.verseveld@franciscus.nl

Albert Schweitzer Hospital
Dr. E. Belt
Department of Surgical Oncology
Dordrecht, The Netherlands
Phone: 078 65232350
E-mail: e.j.t.belt@asz.nl

ZorgSaam Hospital
Dr. M.P.S. Sie
Department of Medical Oncology
Terneuzen, The Netherlands
Phone: 0115 688202
E-mail: m.sie@zzv.nl

Ikazia Hospital
Dr. J. Nonner
Department of Surgical Oncology
Rotterdam, The Netherlands
Phone: 010 2975220
E-mail: j.nonner@ikazia.nl

Jeroen Bosch Hospital
Dr. H. Schut
Department of Medical Oncology
's-Hertogenbosch, The Netherlands
Phone: 073 5538225
E-mail: h.schut@jbz.nl

Elisabeth Tweesteden Hospital
Dr. L. Beerepoot
Department of Medical Oncology
Tilburg, The Netherlands
Phone: 013 2213069
E-mail: l.beerepoot@etz.nl

Maasstad Hospital
Dr. B. Haberkorn
Department of Medical Oncology
Rotterdam, The Netherlands
Phone: 010 2911840

	E-mail: haberkornb@maasstad.nl ***Admiraal de Ruyter Hospital*** Dr. H. van Halteren Department of Medical Oncology Goes, The Netherlands Phone: 088 1254510 E-mail: hk.vanhalteren@adrz.nl
Coordinating investigator/project leader	***Erasmus MC, University Medical Center Rotterdam*** Prof. Dr. C. Verhoef Department of Surgery Rotterdam, The Netherlands Phone: 0031 10 704 1902 E-mail: c.verhoef@erasmusmc.nl
Study coordinators	***Erasmus MC, University Medical Center Rotterdam*** Drs. M. Ubink, MD Department of Surgery Rotterdam, The Netherlands E-mail: m.ubink@erasmusmc.nl ***Erasmus MC, University Medical Center Rotterdam*** Drs. D.M. Mens, MD Department of Surgery Rotterdam, The Netherlands E-mail: d.mens@erasmusmc.nl
Funding party	***Erasmus MC Foundation***
Independent expert	***Erasmus MC, University Medical Center Rotterdam*** Dr. J.L. Boormans Department of Urological Oncology Rotterdam, The Netherlands Tel. 0031 (0)107030538
Laboratory sites	***Erasmus MC, University Medical Center Rotterdam*** Medical Oncology Laboratory Dr. Molewaterplein 40 3015 GD Rotterdam
Pharmacy	***Not applicable***

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor or Legal representative: Prof. dr. J.M. Hendriks, Vascular Surgeon Erasmus MC, University Medical Center Rotterdam **Head of Department:** Prof. dr. J.M. Hendriks, Vascular Surgeon Erasmus MC, University Medical Center Rotterdam		
Coordinating Investigator: Prof. dr. C. Verhoef, Surgeon Erasmus MC, University Medical Center Rotterdam **Co-investigator:** Prof. dr. H.M.W. Verheul, Medical Oncologist Erasmus MC, University Medical Center Rotterdam		
Principal Investigators per site: Dr. A. Verschoor, Medical Oncologist Reinier de Graaf Hospital Delft Dr. S. Boudewijns, Medical Oncologist Bravis Hospital Roosendaal Dr. M. Verseveld, Surgeon Franciscus Gasthuis Hospital Rotterdam Dr. E. Belt, Surgeon Albert Schweitzer Hospital Dordrecht Dr. M.P.S. Sie, Medical Oncologist ZorgSaam Hospital Terneuzen Dr. J. Nonner, Surgeon Ikazia Hospital Rotterdam Dr. H. Schut, Medical Oncologist		

Jeroen Bosch Hospital 's-Hertogenbosch Dr. L. Beerepoot, Medical Oncologist Elisabeth Tweesteden Hospital Tilburg Dr. B. Haberkorn, Medical Oncologist Maasstad Hospital Rotterdam Dr. H. van Halteren, Medical Oncologist Admiraal de Ruyter Hospital Goes		

DOCUMENT HISTORY

Document	Date of version	Summary of Changes
Protocol version no. 4.0	*06-03-2025*	- Addition of 10 centres for multicenter study - Added the results of the PRODIGE-23 study in the background information - Updated preliminary research - Added additional ctDNA at first follow-up and at progression as secondary endpoints - Described overall study duration and follow-up - Described the patient participation in the design of the trial - Updated the in- and exclusion criteria - Described the considerations of in-vitro diagnostics - Described the lifestyle restrictions - Described the screening procedure - Updated the ctDNA analysis procedure - Elaborated on the data collection procedure - Described the efficacy and safety assessments - Updated the safety reporting according to the CTR regulations - Described the analysis sets - Elaborated on the randomisation procedure (stratification and block sizes) - Described potential reasons for early termination of the trial - Described procedures for accounting for missing data and potential deviations from statistical plan - Elaborated on the monitoring plan - Described the collection and storage of biological samples
Original protocol	*10-07-2024*	Not applicable

CONFIDENTIALITY STATEMENT

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigative team, regulatory authorities, and members of the Research Ethics Committee.

TABLE OF CONTENTS

1. ABBREVIATIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
AxMP	**Auxiliary Medicinal Product**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CtDNA	**Circulating tumour DNA**
CfDNA	**Cell-free DNA**
CHIP	**Clonal haematopoiesis of indeterminate potential**
CRO	**Contact Research Organisation**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
e-CRF	**Electronic Case Report Form**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation**
IB	**Investigator's Brochure**
IC	**Informed Consent**
ICF	**Informed Consent Form**
ICH	**International Conference on Harmonisation**
IKNL	**Integraal Kankercentrum Nederland**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
MS	**Member State**
NCR	**Netherlands Cancer Registry**
NGS	**Next generation sequencing**

PCR	**Polymerase chain reaction**
PI	**Principal Investigator**
PLCRC	**Prospective Dutch ColoRectal Cancer**
PROMs	**Patient Reported Outcomes**
RSI	**Reference Safety Information**
SAE	**Serious Adverse Event**
SmPC	**Summary of Product Characteristics**
SOP	**Standard Operating Procedure**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
TME	**Total mesorectal excision**
TwiCs	**Trial within cohorts**
UMIs	**unique molecule identifiers**
WBP	**Wet Basisregistratie Personen**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

2. SYNOPSIS

2024-517700-12-00 - 'Adjuvant chemotherapy for prevention of recurrence in patients with detectable ctDNA after surgery in high-risk rectal cancer.'

Rationale

Rectal cancer is a worldwide cause of cancer related mortality.(1) The incidence of rectal cancer in the Netherlands is approximately 3500 patients per year.(2) The introduction of combined neoadjuvant (chemo)radiotherapy and total mesorectal excision (TME) has significantly reduced the local recurrence rate, but distant recurrence rates remain around 30%.(3-5) Recurrences are likely to derive from residual local disease or subclinical metastatic disease (minimal residual disease). These micro metastases are undetectable by the currently used imaging techniques but still present after surgery. Adjuvant chemotherapy might be beneficial for patients at high risk for recurrence. However, there are only a few randomised controlled trials on perioperative chemotherapy available. Studies on adjuvant chemotherapy in rectal cancer yielded conflicting results.(6, 7) As a consequence, treatment with adjuvant chemotherapy in patients with rectal cancer is not evidence based and therefore not standard of care in the Netherlands. Recent studies suggest that preoperative intensive chemotherapy with radiotherapy, compared to standard chemotherapy and radiotherapy, resulted in a prolonged disease-free survival (8, 9) and overall survival. (9) However, this was at the cost of increased toxicity, and whether the observed improvement in overall survival can be attributed to the addition of neo-adjuvant intensive chemotherapy is under debate.(10) Consequently, there is an urgent need for biomarkers to identify those patients at high risk to recur after standard treatment, to select the patients that might benefit the most from adjuvant chemotherapy.

Objective

To investigate disease-free survival in patients with high-risk rectal cancer by treating these patients with adjuvant chemotherapy in case of detectable ctDNA after surgery.

Main trial endpoints

The primary endpoint of the study will be disease-free survival in the intention-to-treat population, calculated from the date of surgery to the date of recurrence or death from any cause of the patient, whichever occurs first.

Secondary trial endpoints

Secondary outcomes will be disease-free survival, carried out as per protocol analysis to analyse pure treatment effect. In addition, overall survival will be calculated measured from the date of surgery to the date of death from any cause. Quality of life will be assessed in both groups by obtaining already completed questionnaires provided by the PLCRC cohort study to compare the effect of adjuvant chemotherapy on quality of life. The robustness of ctDNA as biomarker will be analysed by comparing the disease-free survival of patients with detectable ctDNA who are not treated adjuvant chemotherapy (control group) with patients with undetectable ctDNA. The clearance of ctDNA of the patients who received adjuvant chemotherapy in the experimental group will be compared with the patients in the control group. Lastly, the co-occurrence of ctDNA in peripheral blood at the timing of detection of recurrent disease on imaging will be studied.

Trial design

The proposed study is conducted within the prospective Dutch ColoRectal Cancer (PLCRC) cohort and follows the trial within cohort (TwiCs) design, i.e. a randomised controlled trial within a prospective cohort.(11)

Trial population

Patients ≥ 18 years of age with primary resected rectal cancer that meet the inclusion criteria, participating in the PLCRC cohort with informed consent for randomisation and considered physically fit for adjuvant chemotherapy are eligible.

Interventions

Patients with detectable ctDNA after surgery and randomised to the experimental group will be offered adjuvant chemotherapy within 8 weeks of surgery and no longer than 12 weeks after surgery. Adjuvant chemotherapy consists of 6 cycles of 5FU/folinic acid and oxaliplatin (FOLFOX) every 2 weeks, or 4 cycles of capecitabine and oxaliplatin (CAPOX). Duration of treatment will be 3 months (12 weeks).

Ethical considerations relating to the clinical trial including the expected benefit to the individual subject or group of patients represented by the trial subjects as well as the nature and extent of burden and risks

In current clinical practice there is no indication for adjuvant chemotherapy for patients after surgery for primary rectal cancer. Therefore all participating patients have no indication for adjuvant chemotherapy. Patients randomised to the experimental group will be offered adjuvant chemotherapy to reduce recurrence. According to routine clinical care, patients receiving adjuvant chemotherapy will undergo blood withdrawals and visit their treating physician before every cycle of chemotherapy. The combination chemotherapy schedule of CAPOX and FOLFOX is commonly administered in the adjuvant setting in current practice for colorectal cancer, therefore the risks and toxicity of the used adjuvant chemotherapy are well-known. The majority of side-effects are manageable and transient. The risk of the withdrawal of extra tubes of blood during regular blood withdrawal in all study participants is negligible. The benefit for participants enrolled in this trial is the potential chance to reduce their risk of recurrence with adjuvant chemotherapy.

3. INTRODUCTION AND RATIONALE

3.1 Therapeutic condition and current treatment status

Rectal cancer is a worldwide cause of cancer related mortality.(1) The incidence in the Netherlands is approximately 3500 patients per year.(2) The introduction of combined neoadjuvant (chemo)radiotherapy and total mesorectal excision (TME) significantly reduced the recurrence rate, but recurrence rates remain high.(3-5) Approximately 30% of the patients treated with curative intent for rectal cancer will develop recurrent disease, locally and distant.(4, 12, 13) Recurrences are likely to derive from micro metastases (minimal residual disease). Minimal residual disease is undetectable by imaging techniques but still present after surgery. Adjuvant chemotherapy can be beneficial for patients at high risk for recurrence by eliminating minimal residual disease.

The evidence for adjuvant chemotherapy in rectal cancer is sparse and there is no international consensus. There are few randomised controlled trials available regarding the effect of adjuvant chemotherapy for rectal cancer. The EORTC-22921-(14), I-CNR-RT-(15), PROCTOR-SCRIPT-(16) and CHRONICLE-trial(17), showed no benefit of adjuvant chemotherapy on overall and disease-free survival, but the QUASAR-trial(18) did. Meta-analyses also presented contradicting results. A Cochrane review(19) displayed an overall beneficial effect of adjuvant chemotherapy on overall and disease-free survival, however meta-analyses by Bujko(6) and Breugom(7) demonstrated no improvement. International efforts to improve overall survival outcomes of patients with rectal cancer, for example by the use of more intensified neoadjuvant and adjuvant treatment, have so far been to no avail.(8, 20) Toxicity of systematic chemotherapy remains an important downside of implementing these strategies as standard of care.

The recent years total neo-adjuvant treatment (TNT) has become of interest, which comprises the administration of both systemic chemotherapy and chemoradiotherapy before surgical resection. The Dutch RAPIDO study has investigated short course radiotherapy with subsequent doublet systematic chemotherapy (CAPOX/FOLFOX4) in comparison to standard treatment with chemoradiation in patients with locally advanced rectal cancer.(8) This study demonstrated a benefit of the RAPIDO treatment protocol with a 3-year disease related treatment failure improvement of 6.7%. However, the 3-year overall survival was similar between both groups and patients receiving the RAPIDO treatment protocol experienced more grade 3-4 adverse events (48% vs 25%). The modest improvement of disease-free survival with the RAPIDO treatment regime implies a significant number of patients being overtreated using an intensified treatment regimen, at the cost of increased toxicity without a certain overall survival benefit.

The PRODIGE-23 trial, which compared neo-adjuvant treatment using mFOLFIRINOX followed by chemoradiotherapy to chemoradiotherapy alone in patients with LARC, did demonstrate an overall survival benefit favoring the group who received TNT.(9) However, the overall survival curve shows an early effect on overall survival around 4 months with more or less parallel lines from that moment on. Based on the early studies on the effect of chemotherapy in non-metastatic disease, an effect of chemotherapy on overall survival is expected in the long term around 18 months.(21) Therefore, the observed overall survival benefit in the PRODIGE-23 trial is unlikely the result of the studied intervention and must be explained by other causes.

Patient selection for (neo)adjuvant chemotherapy for rectal cancer remains a major clinical dilemma. In countries where adjuvant chemotherapy is standard of care, selection is currently based on TNM staging, overtreating numerous patients as a consequence. Biomarkers to identify patients at high risk to recur are urgently needed to offer a more tailored treatment regime, hereby reducing the chance of unnecessary toxicity and impaired quality of life.

3.2 Clinical trial rationale

Circulating tumour DNA (ctDNA) in peripheral blood samples is a potential biomarker to identify patients at high risk for recurrence as these patients may particularly benefit from adjuvant chemotherapy.(22-24) Identification of patients at high risk with ctDNA and treatment of these patients with adjuvant chemotherapy could lower their recurrence rate and improve disease-related overall survival.

Circulating tumour DNA

CtDNA is a promising biomarker that is considered to be an important diagnostic tool for the detection of minimal residual disease.(22-24) CtDNA is part of the total amount of small fragments of DNA in the blood, called cell-free DNA. These fragments are shed into the bloodstream from dying cells during cellular turnover or other forms of cell death. The utility of ctDNA has already been suggested to aid in clinical decision-making in metastatic colorectal cancer. For example, resistance to monoclonal anti-EGFR antibodies such as cetuximab and panitumumab due to EGFR and downstream KRAS mutations, can be detected by ctDNA analysis as early as 10 months before radiologic disease progression is observed.(25-28) The detection limit of ctDNA analysis methods to detect tumour DNA in the total amount of cell-free DNA is below 0.05%, while still preserving a specificity of >99.99%.(23, 29)

The detection and use of ctDNA in patients with non-metastatic colorectal cancer have already been investigated. In colon cancer, the detection of ctDNA after surgery was strongly associated with recurrence of disease, with recurrence rates up to 79% within 2 years after surgery for patients with detectable ctDNA, compared to only 10% in patients without detectable ctDNA.(30) More importantly, although only shown in small sample sizes, the administration of adjuvant chemotherapy was able to change ctDNA from positive to negative.

In a recently published study with 159 patients with locally advanced rectal cancer (LARC) ctDNA status stratified cases into very high and low risk for recurrence groups.(31) Prior to neoadjuvant (chemo)radiotherapy, 122 (77%) patients had detectable ctDNA. After surgery, 19 patients (12%) had detectable ctDNA of which 58% recurred during follow-up (median 24 months). In contrast, recurrence occurred in only 8.6% of the patients with a negative ctDNA status (hazard ratio 13, 95%CI 5.5-31, p<0.001). The prognostic value of detectable ctDNA for recurrence was even stronger in patients with a high pathological stage (ypT3-4 and ypN1-2), demonstrated by recurrence rates up to 89% after 2 years.

Khakoo et al. investigated the role of ctDNA in patients with LARC and similarly showed that all 3 patients with detectable ctDNA after surgery had recurrent disease compared with none of the 20 patients with undetectable ctDNA (P = 0.001).(32) Other recently published studies among patients with LARC show promising results regarding the prognostic value and the applicability of ctDNA for the prediction of treatment response after neoadjuvant chemoradiation.(33, 34) Fur-

thermore, data from a recent trial in stage II colon cancer suggest that ctDNA positive patients appear to derive considerable benefit from adjuvant treatment in terms of recurrence-free survival, and that a ctDNA-guided treatment approach can reduce the number of patients who received adjuvant therapy whilst not altering the risk of recurrence.(35)

Collectively, these studies have consistently shown the high potential of ctDNA as future biomarker for detecting minimal residual disease after surgery. It can be concluded from these results that postoperative ctDNA analysis stratifies patients into very high and low risk groups for recurrence, independent of conventional clinicopathological risk factors. Unfortunately, the use of ctDNA is not yet implemented in daily practice. The potential benefit of adjuvant chemotherapy in patients with detectable ctDNA after surgery should be established.

*Note: a systematic review performed by the REACT research team, including a more comprehensive overview of ctDNA studies in the field of rectal cancer, can be found in the **REACT** literature review.*

Rationale
Use of ctDNA in clinical practice is not yet incorporated in clinical guidelines. Further research, validation and standardization needs to be done to enable the clinical application of ctDNA for diagnosis and monitoring of cancer. ctDNA in peripheral blood samples is a potential biomarker to identify patients at high risk for recurrence after curative surgery for rectal cancer and these high risk patients will possibly benefit from adjuvant chemotherapy.

The aim of this study is to reduce the recurrence rate and improve overall survival in patients with rectal cancer, by treating patients with adjuvant chemotherapy in case of detectable ctDNA after surgery. Our hypothesis is that adjuvant chemotherapy in high-risk rectal cancer patients with detectable ctDNA after surgery will lead to approximately 25% lower recurrence rate within two years compared to the current standard of care in patients with detectable ctDNA in the control group. With this phase III TwiCs study we want to identify a group of patients at high risk for recurrence after resection of the primary tumour based on detectable ctDNA and to show a beneficial effect of adjuvant chemotherapy in this high risk group of patients on recurrence rate and overall survival. We expect to present a clinically relevant decrease in recurrence rate in ctDNA positive patients treated with adjuvant chemotherapy as compared to patients with detectable ctDNA who will only receive follow-up according to the current guideline. We expect that this will translate into improved overall survival.

Preliminary results of our own research
The departments of Medical Oncology, Surgery and Pathology of the Erasmus MC closely collaborate and have pioneered and gained expertise in the pre-analytical conditions and optimised isolation and molecular analyses of ctDNA. This resulted in multiple publications and the diagnostic implementation of NGS Oncomine cell-free DNA (cfDNA) assays (Thermo Fisher Scientific) for cancer patients since 2016.(28, 36-44) In a research setting, there is ample experience with breast cancer samples (Everolimus Biomarker study), colorectal cancer (CRC-IMPACT) and colorectal liver metastases samples (MIRACLE-study) that have been sequenced by the Department of Pathology using Oncomine assays. For example, the KWF funded MIRACLE-study aims to establish whether determination of cell-free DNA (cfDNA) in patients with isolated colorectal liver metastases undergoing hepatic resection can discriminate between patients showing a recurrence within 1 year

from those who do not. The presence of cfDNA in peripheral blood is analysed before and/or after hepatic resection to determine the association between ctDNA-based detection of MRD and clinical outcome. Currently, the MIRACLE study has reached full accrual of 240 participants and first results were presented at ASCO 2024 (45).

We briefly summarised the main findings of our completed projects:

- We showed that next generation sequencing (NGS) and digital polymerase chain reaction (PCR) analyses are both tools to detect ctDNA by identification of tumour-specific mutations in circulating free DNA from blood of metastatic breast and colorectal cancer patients.(28, 36, 37, 41)
- We optimised pre-analytical variables such as the blood collection, demonstrating that ctDNA levels remain stable over time when blood is collected in BCT and CellSave preservative tubes.(39) Blood collected in standard EDTA tubes, however, will result in a decrease in the fraction of ctDNA over time as shown indicated by lower variant allele frequencies at 96-h samples compared to 1-h samples.
- We pioneered different NGS approaches (38, 46, 47) to characterize mutations and measure accurately and sensitively ctDNA fractions. These include the standard customised Ampliseq panels (Thermo Fisher Scientific) (38, 47) the OnTarget assay to enrich for mutant alleles by synchronous coefficient of drag alteration technology (Boreal Genomics, Vancouver, Canada)(46), and recently the Oncomine cfDNA assays with unique molecule identifiers (UMIs) (Thermo Fisher Scientific).(28) The UMIs help to remove false positives due to library preparation and sequencing artefacts, resulting in more sensitive detection of mutations than standard NGS workflows. This enables us to detect ctDNA fractions as low as 0.1% in 20ng of circulating free DNA.
- We did a comparison study on expression of cfDNA in terms of variant allele frequency versus number of mutant molecules.(44)
- We demonstrated the feasibility of the Oncomine cfDNA colon assay in a cohort of metastatic colorectal cancer patients receiving anti-EGFR (cetuximab) monotherapy.(28) We showed that mutations related to therapy resistance emerge in ctDNA during treatment with anti-EGFR monoclonal antibodies. In addition, as described above, in the MIRACLE cohort we used the Oncomine cfDNA colon assay to pre-operatively determine the mutations of interest for follow up analyses post-surgically. In this cohort we observed a clear difference in recurrence-free survival between MRD-positive and MRD-negative patients (figure 1).
- Together with the Department of Pathology, Oncomine cfDNA assays and digital PCR analyses are now used in a routine diagnostic setting in the Erasmus MC for lung cancer patients.

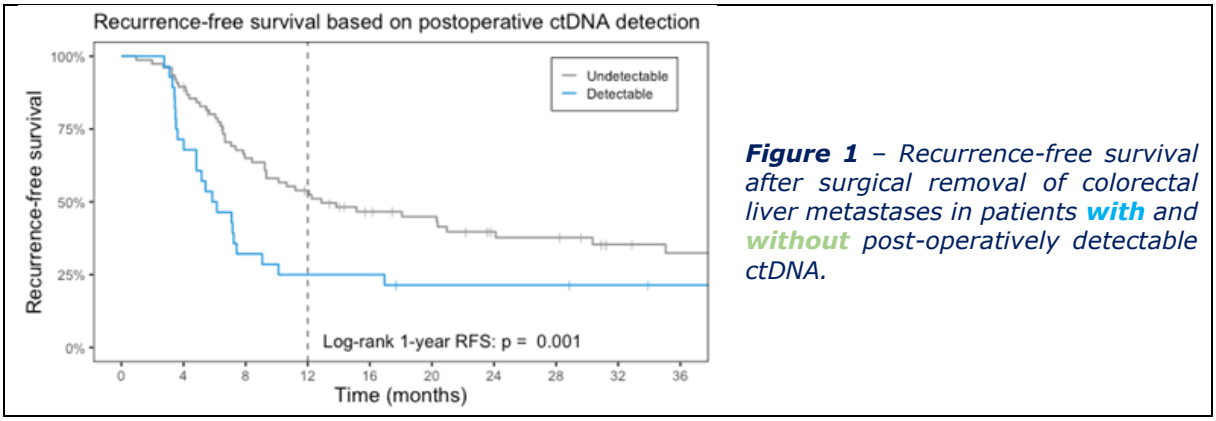

Figure 1 *– Recurrence-free survival after surgical removal of colorectal liver metastases in patients **with** and **without** post-operatively detectable ctDNA.*

3.3 Mechanism of action, Drug class

A summary of all products and its mechanisms of actions can be found in chapter 5 the SPC of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil.

3.4 Rationale for Dose Regimen/Dose Justification

Description and justification of route of administration and dose is provided in chapter 8, which are all according to the standard of care as prescribed in the Dutch Guidelines.

4. STRUCTURED RISK ANALYSIS

4.1 Potential issues of concern

4.1.1 Level of knowledge about mechanism of action

The most concerning medication that is administered to patients allocated to the intervention arm in this study is the chemotherapeutic agent oxaliplatin. Oxaliplatin is a third generation diaminocyclohexane (DACH) platinum compound that forms mainly intrastrand links between two adjacent guanine residues or a guanine and an adenine, disrupting DNA replication and transcription.(48) The details underlying the effects of oxaliplatin remain poorly understood. A summary of all products and its mechanisms of actions can be found in chapter 5 the SPC of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil.

4.1.2 Previous exposure of human beings

Several clinical studies have investigated the safety CAPOX/FOLFOX, and thousands of patients have been treated with this combination treatment in the investigated populations.(17, 49) This regimen is consider safe and is registered in the Netherlands for patients with colorectal cancer in the adjuvant setting. A summary of all products can be found in the SPC of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil.

4.1.3 Induction of the mechanism in animals and/or *ex-vivo*

A summary of findings regarding the primary or secondary mechanisms of each interventional product can be found in the SPC of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil.

4.1.4 Selectivity of the mechanism

A summary of findings regarding mechanism to target tissue of each interventional product can be found in the SPC of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil.

4.1.5 Analysis of potential effect

An analysis of the potential effect of each interventional product can be found in the SPC of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil

4.1.6 Pharmacokinetic considerations

Pharmacokinetic parameters can be found in chapter 5 of the SPC of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil.

4.1.7 Predictability of effect

The clinical effect of CAPOX/FOLFOX is largely investigated, and the side-effects are well-known (see 4.4 of the SPC of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil). Physicians treating patients in this study are informed of these effects, and are considered to be predictable.

4.1.8 Interaction with other products

See paragraph 4.5 of the SPC of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil.

4.1.9 Managing of effects

Most side effects of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil are manageable. See 4.9 of the SPC of capecitabine, oxaliplatin, leucovorin and 5-fluorouracil for a detailed description of antidotes, antagonists and countermeasures.

4.1.10 Study population

The study population consists of patients with colorectal cancer that are eligible for chemotherapy, at the discretion of the treating physician. This means that all subjects will be screened for eligibility by a medical oncologist. The use of CAPOX/FOLFOX is well investigated in this population, and the side-effects are well-known. No pregnant women will be included in this study.

4.2 Overall synthesis of the direct risks for the research subjects

In the experimental group, patients will be treated with adjuvant CAPOX chemotherapy or with adjuvant FOLFOX chemotherapy. The combination chemotherapy schedule of CAPOX and FOLFOX are both registered products, and are commonly administered in the adjuvant setting in current practice for colorectal cancer. Therefore, the risks and toxicity of the used adjuvant chemotherapy are well-known. The mechanism of action is well-understood, its side-effects can be managed well, and the potential effect has been proven, for instance in palliative setting and in the adjuvant setting of stage II and III colon cancer. Adjuvant chemotherapy for rectal cancer is standard of care in several countries, and although not recommended in the Dutch guidelines, population based studies from the Netherlands reveal that adjuvant chemotherapy is administered up to 29% of the patients with stage III rectal cancer.(50) All subjects in this trial will be adults. The extra potential risks of adjuvant chemotherapy are acceptable in our opinion as we hypothesise that a significant improvement in disease-free survival and overall survival can be obtained. Based on the guideline by the NFU (Dutch Federation of University Medical Centers) about quality insurance in human research (“Kwaliteitsborging van mensgebonden onderzoek”) we qualify the risk of this study as ‘slight risk (research involving a medical product)’ (moderate chance of slight damage). The most bothering toxicity of this regimen is sensory neuropathy, due to oxaliplatin. This neuropathy can be irreversible. Currently, we are not able to predict the chance of neuropathy upfront, and there are no effective drugs to prevent or treat this neuropathy. Trials to resolve this problem are ongoing. One year after treatment grade 2 neuropathy is 4.6% and grade 3 neuropathy is 1.3%.(51) Severe and possible lethal toxicity of fluoropyrimidines is partially prevented by upfront testing for DPD-deficiency. Treatment-related mortality is <0,5%. The risk of the withdrawal of extra tubes of blood during regular blood withdrawal in all study participants is negligible. The obvious benefit for participants enrolled in this trial is the potential chance to reduce their risk of recurrence with adjuvant chemotherapy. Subsequently, this will also increase their chance of achieving improved overall survival and quality of life. Thus, the investigators consider the extra

potential risk for patients in the experimental arm to be acceptable, hypothesising that a significant improvement in outcomes can be obtained with additional chemotherapy in the specific patient population.

5. OBJECTIVES AND ENDPOINTS

Objective(s)	**Endpoint(s)**
Primary objective(s)	**Endpoint for the primary objective(s)**
• To investigate whether the disease-free survival in patients with rectal cancer who have detectable ctDNA after primary tumour resection, can be improved by administration of adjuvant chemotherapy.	• Primary endpoint of the study will be disease-free survival, calculated from the date of surgery to the date of progression (recurrence) or death from any cause of the patient, whichever occurs first. Patients who did not experienced a recurrence and who are still alive at the end of the study will be censored at the date of last contact.
Secondary objective(s), if applicable	**Endpoint(s) for secondary objective(s), if applicable**
• To investigate whether overall survival of rectal cancer patients with detectable ctDNA after surgery can be improved with adjuvant chemotherapy.	• Overall survival will be calculated from the date of surgery to the date of death from any cause of the patient. Patients who are still alive at the end of the study will be censored at the date of last contact.
• To compare all (eligible) patients in the control group to patients in the experimental group who actually started the adjuvant chemotherapy by a per protocol analysis (for pure treatment effect) for disease-free and overall survival.	• In addition to intention-to-treat analysis to estimate the effect size, also a per-protocol analysis (for pure treatment effect) will be carried out for disease-free survival and overall survival. This analysis will compare all (eligible) patients in the control group with those patients in the experimental group who actually started the adjuvant chemotherapy.
• To compare the effect of adjuvant chemotherapy on quality of life.	• Quality of life will be assessed in both groups by obtaining questionnaires already provided by the PLCRC cohort study. This to compare the effect of adjuvant chemotherapy on quality of life. In the whole study period of 4 years there are 8 quality of life measurements. Quality of life is assessed with the following questionnaires: EQ-5D-5L, QLQ-C30 and QLQ-CR29.

• To compare the disease-free survival and overall survival of patients with detectable ctDNA but without receiving adjuvant chemotherapy with patients with undetectable ctDNA.	• The disease-free survival and overall survival of ctDNA negative patients will also be collected and analysed to confirm the robustness of post-operative ctDNA as biomarker in rectal cancer.
• To compare the clearance of ctDNA of the patients who received adjuvant chemotherapy in the experimental group with the patients in the control group.	• The results of the ctDNA analysis of the blood samples taken at the first follow-up of the randomised patients who were ctDNA positive after surgery will be compared between the patients who received adjuvant chemotherapy and those who did not.
• To investigate the co-occurrence of ctDNA in peripheral blood at the timing of detection of recurrent disease on imaging.	• Within the group of patients with detectable ctDNA after surgery, the proportion of patients with detectable ctDNA in the peripheral blood at the timing of detection of recurrent disease on imaging will be assessed.
Exploratory objective(s), if applicable	**Endpoint(s) for exploratory objective(s), if applicable**
• To determine the mutational profile and ctDNA levels in ctDNA-positive patients.	• The proportion of ctDNA-positive patients with specific mutational profiles. • Quantitative ctDNA levels
• To identify and analyse specific mutations detected in ctDNA and their correlation with clinical and pathological parameters, as well as time to recurrence.	• Correlations between ctDNA mutational profiles and clinical/pathological parameters and time to recurrence.
• To compare the mutational status of ctDNA with the mutational profile of available tumour tissue in patients, assessing concordance and potential discrepancies.	• Concordance rate (%) between mutations identified in ctDNA and matched tumour tissue.

6. STUDY PLAN AND DESIGN

6.1 Trial Design

Trial within cohorts (TwiCs) design

This study is designed as a randomised controlled trial within a prospective cohort of patients. The study design and randomisation progress is demonstrated in Figure 2.

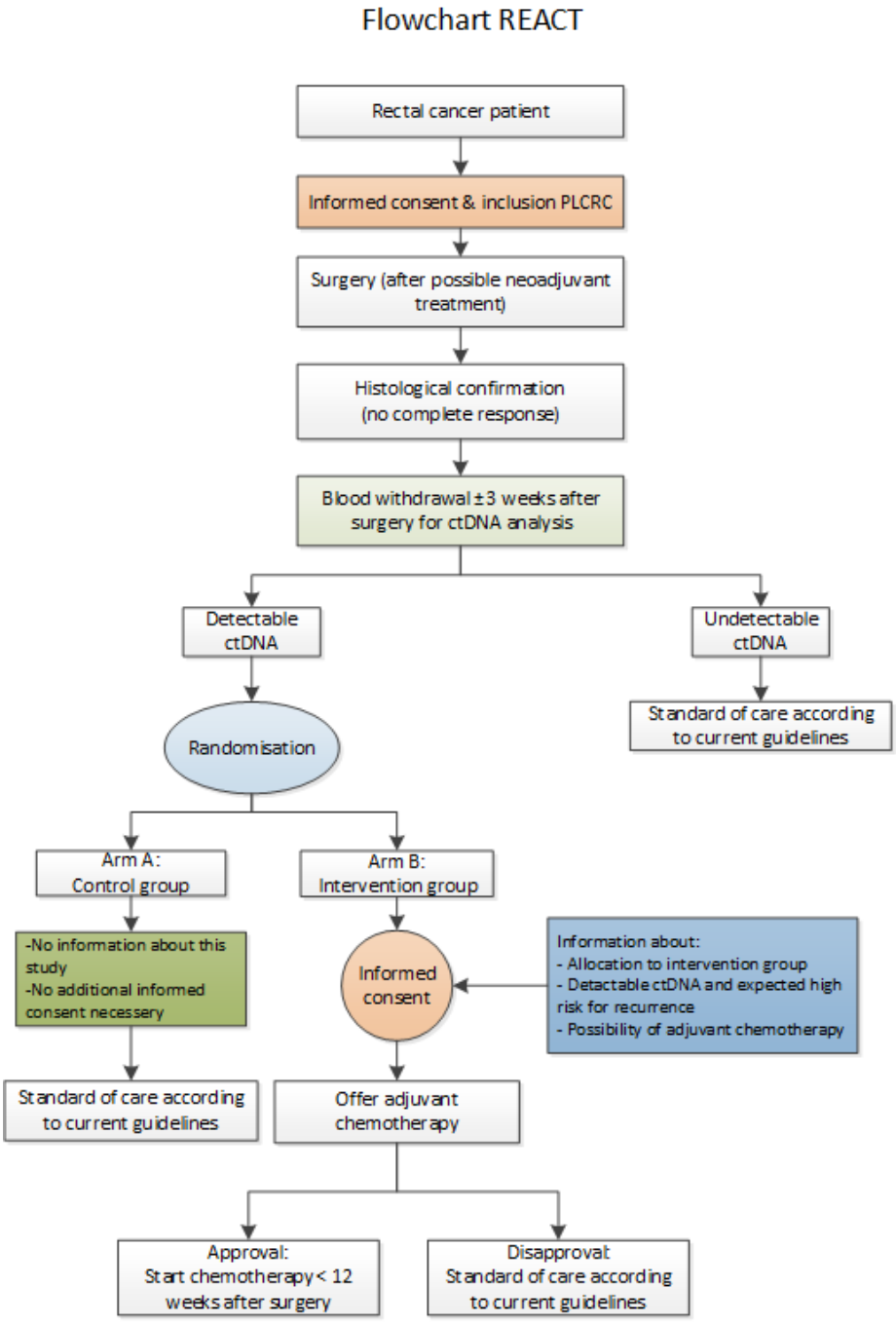

Figure 2. Flowchart of the REACT study in the trial within cohorts (TwiCs) design.

6.2 Number of Patients

With a detection rate of 15% for ctDNA after surgery in high-risk rectal cancer, 1373 patients' blood samples need to be analysed for ctDNA to include all patients with detectable ctDNA (n=206), 103 patients with detectable ctDNA for each group.

6.3 Overall study duration and follow-up

The anticipated overall study duration for each subject is 3 months (12 weeks), corresponding to the period of investigational treatment with adjuvant chemotherapy. Adjuvant chemotherapy consists of 6 cycles of 5FU/folinic acid and oxaliplatin (FOLFOX) every 2 weeks, or 4 cycles of capecitabine and oxaliplatin (CAPOX). Following study participation, postoperative follow-up will be conducted in accordance with the Dutch Colorectal Cancer Guidelines, which currently recommend monitoring for up to 5 years after rectal surgery.

6.4 Patient participation

The patient federation 'Stichting Darmkanker" was asked for assistance with the development of our study design and the informed consent form. Constructive meetings were held to ensure that all written information used in this study is understandable for patients with different educational levels. The patient federation supports our study design and will remain involved during further steps of this application. We have included a patient who had surgery and chemotherapy for rectal cancer in the past, to act as subject who has expertise by experience. Together with the other involved collaborators of Stichting Darmkanker, the principal investigators and the coordinating investigator, we have created an advisory group that will be involved from the beginning to end of the REACT study. During the trial, regular meetings and focus groups will be held with the advisory group and feedback will be provided. Where necessary, adjustments will be made. Also, the contact information from this advisory group will be included in the patient information letter (PIF) and the REACT protocol, so that this group can both support the participants of this trial, as well as provide feedback for the REACT study team.

7. STUDY POPULATION

7.1 Population

Patients included in PLCRC (MEC-2016-369), with high-risk rectal cancer who underwent surgery, fit to receive adjuvant chemotherapy (WHO performance score 0-1) will be selected for inclusion in this study. High-risk rectal cancer is defined as either; treated with neoadjuvant therapy; and/or clinical T4; and/or clinical N+. The aim is to include all patients within three years.

7.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Detectable ctDNA in the postoperative blood sample
- Age ≥ 18 years
- WHO performance score 0-1
- Informed consent for PLCRC with specific consent for additional blood withdrawals and offering of future experimental research
- Informed consent for the REACT trial.
- Histological confirmed rectal cancer; either treated with neoadjuvant (chemo)radiotherapy, and/or clinical/pathological T3/T4 and/or N+ in case no neoadjuvant therapy was administered.
- Eligible to receive treatment with combination adjuvant chemotherapy (CAPOX/FOLFOX) according to the treating physician.
- Mentally competent and able to read and understand Dutch language.

7.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Metastatic disease
- Another malignancy in previous 5 years, with the exception of treated carcinoma in situ or skin cancer other than melanoma
- Incomplete primary tumour resection (R1 or R2 resection)
- Contra-indication for fluoropyrimidines or oxaliplatin
- Neoadjuvant oxaliplatin based systemic treatment, e.g. treated with the RAPIDO regimen consisting of short course radiotherapy followed by 6 cycles of CAPOX or 9 cycles of FOLFOX prior to surgery
- Patients with a clinical complete response, who will not undergo surgery.
- Pregnant and lactating women
- History of psychiatric disability judged by the investigator to be clinically significant, precluding informed consent or interfering with compliance of the intervention group
- Serious concomitant systemic disorders that would compromise the safety of the patient or his/her ability to complete the study, at the discretion of the investigator
- Serious infections (uncontrolled or requiring treatment)
- Current or recent (within 28 days prior to randomisation) treatment with another investigational drug or participation in another study interfering with the primary endpoint.

7.4 Vulnerable populations and clinical trials in emergency situations

Not applicable

8. STUDY TREATMENTS

Treatment of patients in the experimental group:
Adjuvant chemotherapy is not a part of standard care in European guidelines, however in several countries it is the standard of care. The aim of this study is to use standard regimens of combined adjuvant chemotherapy, known from current treatment of colon cancer, only in high risk patients based on detectable ctDNA after surgery.
Adjuvant chemotherapy is initiated preferably within 8 weeks after surgery and no longer than 12 weeks after surgery. Adjuvant chemotherapy consists of 4 cycles CAPOX every 3 weeks or 6 cycles FOLFOX every 2 weeks.

The regimens and dosing schedules of CAPOX and FOLFOX are :

- CAPOX (4 cycles every 3 weeks)
  - Capecitabine: 1000 mg/m2 bid orally day 1-14
  - Oxaliplatin 130 mg/m2 iv. day 1 in 2 hours
- FOLFOX (6 cycles every 2 weeks)
  - 5FU: 400 mg/m2 iv. day 1, followed by continuous infusion 2400 mg/m2 for 46 h
  - Folinic acid: 400 mg/m2 day iv. day 1
  - Oxaliplatin: 85 mg/m2 iv. day 1

Note: Recent studies have demonstrated non-inferiority for three months versus six months of adjuvant chemotherapy in patients with colon cancer. (52-54) The adverse effects of oxaliplatin are well known in current practice. Currently this regiment is incorporated in the Dutch national guideline, therefore we choose for 3 months CAPOX or FOLFOX regimen of adjuvant chemotherapy.

8.1 Investigational Medicinal Product(s) (IMP(s))

8.1.1 Name and description of the IMP

In the present study, patients in the experimental group will receive adjuvant chemotherapy with CAPOX or FOLFOX. These are established chemotherapeutic regimens that are well characterized and known to be well tolerated by patients.

- Capecitabine (chemical name: L01BC06)
- Oxaliplatin (chemical name: L01XA03)
- 5-fluorouracil (chemical name: L01BC02)
- Folinic acid (chemical name: V03AF03)

All investigational products have a marketing authorisation and are used in the authorised form for the authorised indication.

8.1.2 Status of development of the IMP

A summary of findings can be found in paragraph 4.1 of the SPC of capecitabine, oxaliplatin, folinic acid and 5-fluorouracil.

8.1.3 Description and justification of dosage and route of administration

Description and justification of route of administration and dose is provided above, which are all according to the standard of care as prescribed in the Dutch Guidelines.

8.2 Comparator IMP(s)

Not applicable

8.3 Placebo

Not applicable.

8.4 Auxiliary Medicinal Product(s) (AxMP(s))

Not applicable. No auxiliary medicinal products will be used in this study other than the investigational products as described above.

8.4.1 Name and description of the AxMP

Not applicable.

8.4.2 Statement on authorisation and justification unauthorised AxMP (if applicable)

Not applicable.

8.4.3 Description and justification of dosage and route of administration

Not applicable.

8.5 Additional considerations for trials involving a medical device

Not applicable

8.6 Additional considerations for trials involving an in-vitro diagnostic or companion diagnostic

The Oncomine™ Colon cfDNA Assay is performed in a laboratory for Molecular Diagnostics in the department of Pathology that is accredited according to ISO15189:2012 criteria of the Dutch Accreditation Council (RvA) and European IVD regulations (i.e. comply with the applicable General Safety and Performance Requirements as described in Annex I of the European Regulation EU 2017/746).

8.7 Preparation and labelling of the study treatment(s)

All investigational products have a marketing authorisation and are used in the authorised form for the authorised indication. Preparation and labelling will be according to local standard. No specific labelling is performed in this study.

9. OTHER TREATMENTS AND RESTRICTIONS

9.1 Concomitant therapy

9.1.1 Permitted medication(s)

During chemotherapeutic treatment patients will receive co-medication according to standard procedures of local site.

9.1.2 Prohibited medication(s)

At study initiation, patients should report their concomitant medications to their treating physician, if there are medications with interaction with CAPOX or FOLFOX the treating physician, if possible, can change or replace the concomitant medications of the patient. According to routine clinical practice, use of co-medication and escape medication to diminish side effects is allowed. Patients are recommended to contact their treating physician when adverse reactions occur according to routine clinical practice.

9.2 Lifestyle restrictions

9.2.1 Contraception measures

Women who are pregnant or breastfeeding are excluded from participation in this study. Additionally, women of childbearing potential are required to use highly effective contraception throughout the study period to prevent pregnancy. Male participants with female partners of childbearing potential must take measures to ensure that their partners do not become pregnant during the study. The investigator will provide detailed instructions regarding appropriate contraceptive methods, which should be discussed with the participant's partner.

Management of Pregnancy During the Study

If a female participant becomes pregnant during the study, she must immediately notify the investigator and discontinue participation. If the female partner of a male participant becomes pregnant during the study, the participant is encouraged to seek her consent to inform the investigator. Upon receiving consent, the investigator will arrange for additional monitoring of the pregnancy and may collect relevant information regarding the progress and outcome of the pregnancy from healthcare providers. Such monitoring and data collection will only occur if explicit consent is provided by the pregnant partner.

9.2.2 Other requirements

Not applicable

10. TRACEABLILITY, STORAGE, ACCOUNTABLILITY AND COMPLIANCE

10.1 Traceability and storage of the study treatment(s)?

Not applicable, since all investigational products are used from commercial stock in the authorised form for the authorised indication.

10.2 Accountability of the study treatment(s) and compliance

Not applicable.

11. STUDY ASSESSMENTS AND PROCEDURES

11.1 Screening procedure

Participating PLCRC centers can register patients for screening for the REACT study in the online Ldot software that is used by the PLCRC. If the patient is eligible for REACT and has given written informed consent for PLCRC, including informed consent for additional blood withdrawals and offering of future studies, the patient can be added to the REACT sub-study in Ldot. The REACT study team will then automatically be informed that a new patient has been added to the screenings list. At the same time, instructions about the timing and collection of the post-operative blood samples will be send out to the treatment team of the patient. Within 14-28 days after rectal surgery, blood collected in CellSave tubes will be send to the laboratory of the Medical Oncology department of Erasmus MC for ctDNA analysis.

11.2 Randomisation, blinding and treatment allocation

If patients have detectable ctDNA after surgery and are eligible for the REACT study, patients will be randomised 1:1 to the control group or intervention group within the database. Randomisation will be performed by the REACT research team using the LDOT software. The REACT research team from Erasmus MC Cancer Institute will receive the results from the randomisation and will inform the treating physician of the patient when included in the experimental group.

Control group: standard of care group
In this group, patients and their treating physician will not be informed about this study. Patients will not be informed about the randomisation to the control group, according to the TwiCs principle, for which patients already gave informed consent within the PLCRC study cohort. Patients and their treating physician will not be informed about the allocation in the control group and the results of the ctDNA analysis. Patients will not be offered adjuvant chemotherapy (= standard of care). All patients in the control group will receive standard of care and standard follow up, there are no consequences for this group.

Intervention group: adjuvant chemotherapy
In this group, patients will receive information about this study and randomisation to the intervention group by their treating physician. Patients will be informed about previous ctDNA analyses within the PLCRC study and the meaning of ctDNA. The finding of detectable ctDNA in their peripheral blood samples and the possible high risk on recurrence with detectable ctDNA will be discussed. Patients will be informed about the possibility to receive adjuvant chemotherapy and the potential beneficial effect of adjuvant chemotherapy will be explained. Patients who are interested in receiving adjuvant chemotherapy, will be referred to the medical oncology department. After extensive counselling by a medical oncologist, the patient will decide whether they want to receive adjuvant chemotherapy or not. If patients want to receive adjuvant chemotherapy, informed consent will be obtained and adjuvant chemotherapy (CAPOX or FOLFOX) will be administered.. After completing the adjuvant chemotherapy, patients will receive standard-of care follow-up.. If patients refuse adjuvant chemotherapy, they will receive standard follow-up by their own treating physician.

11.3 Study procedures and assessments

Prospective Dutch ColoRectal Cancer cohort
All patients with colorectal cancer can be included in the PLCRC cohort study (MEC-2016-369) in which clinical data, blood, tissue and Patient Reported Outcomes (PROMs) are registered from

diagnosis until death of stage I-IV colorectal cancer patients.(55) PLCRC was initiated as a multi-centre study in 2015. The aim of PLCRC is to improve outcomes of colorectal cancer patients by providing a research infrastructure.

Within PLCRC several observational and interventional sub-studies are currently being conducted, either according to the TwiCs design or not. From patients participating in the PLCRC cohort that meet the inclusion criteria, and fit for adjuvant chemotherapy (WHO performance score 0-1), who gave consent for additional blood withdrawal within the PLCRC study, blood samples will be collected within 14 – 28 days after surgery. These blood samples will be analysed for detectable ctDNA. Patients with undetectable ctDNA will not be randomised. In case of detectable ctDNA patients will be randomised in a 1:1 ratio to a control group (standard of care group consisting of follow-up) and an experimental group who will be offered adjuvant chemotherapy. In case of undetectable ctDNA or detectable ctDNA and allocation to the control group, test reports will not be reported to the patient and treating physician (see “Ethical considerations” (chapter 11)).

Patients that will undergo resection of rectal cancer with curative intent and meeting the inclusion criteria will undergo blood withdrawal for ctDNA analysis 2-4 weeks after surgery. The ctDNA analysis will take approximately 3 weeks. If there is detectable ctDNA after surgery and patients are eligible according to in- and exclusion criteria, these patients will be randomised according to the TwiCs design. For those patients with detectable ctDNA who are randomized to the control group, no additional informed consent is required; patients already gave informed consent to act as a control group with the PLCRC informed consent. They will receive standard of care and normal follow up. Patients with detectable ctDNA and randomised to the experimental group will be asked for written informed consent at the participating hospital after counselling about this study and the randomisation for the intervention. They are informed about the detected ctDNA and the associated high risk of recurrence. They are offered adjuvant chemotherapy with the aim to reduce the chance of disease recurrence. In this case, they will be counselled about adjuvant chemotherapy by a medical oncologist. Patients will decide whether to receive adjuvant chemotherapy or not. If they want to undergo adjuvant chemotherapy, this will be initiated as soon as possible, at a maximum of 12 weeks from surgery.

CtDNA analyses
Blood samples will be withdrawn at times of regular blood withdrawal. Patients have given informed consent in PLCRC for these additional blood withdrawals for research purposes with a maximum of 16 tubes per year (no more than 4 tubes at a time).
Timing of withdrawal of blood samples for baseline ctDNA testing are:
- Within 14-28 days after surgery (2 tubes of 10 mL)

Blood collected in CellSave tubes from patients of all PLCRC participating hospitals will be send to the Erasmus MC - Cancer Institute. The Medical Oncology liquid biopsy laboratory will process the blood within 96h after blood drawing to obtain plasma according to our standard guidelines.(39) It is expected that blood of about 5 patients will arrive on a weekly basis.

At week 1 work-day 5, circulating free DNA (cfDNA) will be isolated from 4 ml of plasma for all samples received in that week using the qiaAMP circulating nucleic acids kit (Qiagen) following the manufacturer’s instructions. In the second week, the obtained cfDNA will be quantified and at

least 10 ng will be used for the library preparation of the Oncomine cfDNA colon panel (ThermoFisher). This assay contains primers to sequence 49 amplicons for hotspot regions of 14 colon cancer relevant genes (*AKT1, APC, BRAF, CTNNB1, EGFR, ERBB2, FBX7, GNAS, KRAS, MAP2K1, NRAS, PIK3CA, SMAD4, and TP53*) to identify 236 hotspot mutations and indels. After quality control of the library preparations, these preps will be sequenced on a Ion Torrent S5 sequencer at high coverage (more than 20.000 reads depth).

At week 2 work-day 5, base calling, read mapping and variant calling are performed using the Torrent Suite (BaseCaller), tmap and torrent variant caller, (Thermofisher), with default settings for cfDNA.

In week 3, the reports will be evaluated and authorized by a team of ctDNA experts. We will retain variants which have a variant allele frequency (VAF) above the limit of detection based on the molecular coverage of the specific variant position (provided by torrent variant caller software).Most hotspot oncogenic mutations evaluated by our targeted panel are not suspect to clonal haematopoiesis of indeterminate potential (CHIP; i.e. the presence of a clonally expanded hematopoietic stem cell caused by a leukemogenic mutation in individuals without evidence malignancy). However when CHIP is suspected based on the list of canonical CHIP genes (PMID: 31768066) together with the observed VAF(), additional germline DNA analyses will be performed to determine whether the detected mutation originates from germline- or tumor DNA.

In case a blood sample contains ctDNA, identified by tumour-specific mutations, the laboratory staff will contact the Erasmus MC research team before the end of week 3, and they will handle the further actions of randomisation and, when allocated to experimental arm, inform the treating physician of the ctDNA detected patients

The additional blood samples of the ctDNA positive patients that will be collected at first follow-up after surgery and after recurrent disease will immediately be send to the Medical Oncology laboratory of Erasmus MC as well. Here they will be processed to plasma within 96 hours after blood withdrawal and then stored in the REACT biobank. After completion of the intervention part of this trial we will evaluate the presence and level of ctDNA at the time of the first return visit and at time of recurrence. For ctDNA analysis, the same steps will be followed as described above. If a targeted PCR is deemed feasible based on the baseline ctDNA analysis results, this will be performed instead of the Oncomine colon assay to reduce costs. We already have experience in this based on the MIRACLE study, which has provided us with several prepared assays for targeted PCR analysis.

Quality of life

Quality of life will be assessed in both groups by obtaining questionnaires already provided by the PLCRC cohort study. This to compare the effect of adjuvant chemotherapy on quality of life. Patients participating in PLCRC optionally receive validated quality of life questionnaires. These questionnaires are available for this study as well. We will assess and compare quality of life in the intervention group compared to the control group. Questionnaires are obtained within PLCRC (digital) at baseline, 3 months, 6 months, 1 year, 1.5 years, 2 years and yearly after. In the whole study period of 4 years there are 8 quality of life measurements. We expect a response rate of 80%, based on current results from the PLCRC study board. Quality of life is assessed with the following questionnaires: EQ-5D-5L, QLQ-C30 and QLQ-CR29.

Follow-up

Follow up starts from the day of surgery in both groups. Follow up for patients in the control and experimental group will be investigations according to Dutch national and participating hospital guidelines. Thus, follow-up for evaluation of recurrences will be within the same time intervals for both the control group and intervention group, despite adjuvant chemotherapy treatment in the intervention group. All follow-up is according to the Dutch national and hospital guidelines with blood sampling of tumour marker CEA and imaging by ultrasound or CT-thorax/abdomen.

Additional blood samples

At the first planned follow-up visit after rectal surgery according to the guidelines (approximately after 3-4 months), two additional CellSave tubes (10 mL) will be collected from all baseline ctDNA positive patients. In case a patient has received adjuvant chemotherapy, the blood withdrawal must take place at least 14 days after completion of chemotherapy. Furthermore, in case of recurrent disease another two CellSave tubes (10 mL) will be collected during the next scheduled blood withdrawal of all patients who had detectable ctDNA after surgery.

Importantly, in the PLCRC study patients can withdraw their informed consent for additional blood samples at any time. Therefore, informed consent will be checked before blood withdrawal and patients who no longer have informed consent will be excluded from these additional blood samples.

Data collection for PLCRC and REACT

The clinical data for PLCRC and (sub)studies are collected by data managers of the Netherlands Comprehensive Cancer Organization (IKNL). The data are registered in the Netherlands Cancer Registry (NCR) according to the standard protocols and definitions of IKNL. The observational data will prospectively be collected from medical charts and pathology reports.

All patients that underwent blood withdrawal for ctDNA analysis for the REACT study, will be registered in a study specific secured database with their unique pseudonymized REACT study number (REACT-SITE-XXXX) and PLCRC study number (PICNICXXXXXXXX). Data about timing and amount of blood withdrawals for ctDNA analysis will be recorded, as well as the results of the ctDNA analysis. From the patients in the intervention group, additional information about the timing and dosage of the adjuvant chemotherapy will be registered. No patient identifying data such as date of birth or date of surgery will be stored in the database. The REACT study team will manage the data management of the REACT database.

Halfway and at the end of the study, the clinical data of all patients that underwent blood withdrawal for ctDNA analysis for the REACT study will be extracted from the NCR after submitting a data request at IKNL. The clinical data will include all patient characteristics, tumour characteristics and oncological outcomes. The received dataset will be matched to the study specific dataset based on the unique PICNIC numbers and then used for the final analysis.

Survival data

The survival data of the Netherlands Cancer Registry will be used to ascertain the exact date that a certain patient has deceased and will be managed by the PLCRC. The Netherlands Cancer Registry routinely collects cancer survival data through linkage with the “Municipal Personal Records

Database" (Dutch: Basisregistratie personen), which is organized in a specific law (Wet Basisregistratie Personen, WBP).

Data extraction from the study database
Requested data can be extracted from the study databases of PLCRC and **REACT** by exporting data to Excel, SPSS and/or SAS. This extraction will contain data from all patients or, if requested, from a selection of patients. Patients participating in the **REACT** study are given an unique sub study patient number (**REACT** – SITE – study number). Data will only be exported coded (i.e. without any identifying information such as patient name, initials).

11.3.1 Efficacy assessments

The efficacy of the studied intervention of administration of adjuvant chemotherapy in ctDNA positive patients after rectal surgery is determined by the primary and secondary outcome measures of disease-free-survival and overall survival, respectively. In addition, the clearance of ctDNA of the patients who received adjuvant chemotherapy in the experimental group will be compared with the patients in the control group. The methods and timing of the analysis of these parameters can be found in chapter 14.

11.3.2 Safety assessments

Adverse events of established chemotherapeutic regimens (CAPOX, FOLFOX) after surgery in patients with colorectal cancer is well investigated. Therefore, no study specific safety assessments will be performed during the trial besides the standard of care procedures according to the Dutch Guidelines. The reporting of serious adverse events is further discussed in chapter 13.

12. STUDY DISCONTINUATION AND COMPLETION

12.1 Definition End of Trial

The end of the study is defined as the last included patient's last visit.

12.2 Criteria for temporary halt and early termination of the clinical trial

The Sponsor may decide to terminate the study prematurely based on the following criteria:

- There is evidence of an unacceptable risk for study patients in accordance with the principle investigator and/or study team (i.e. safety issue);
- There is reason to conclude that it will not be possible to collect the data necessary to reach the study objectives and it is therefore not ethical to continue enrolment of more patients; for example insufficient enrolment that cannot be improved.

The Sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of the decision to terminate the study. The Sponsor will provide information regarding the time lines of study termination and instructions regarding treatment and data collection of enrolled patients.

12.3 Discontinuation/withdrawal of individual subjects

Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

Specific criteria for withdrawal

Study treatment (adjuvant chemotherapy) will be terminated in case of severe adverse reactions, not manageable with standard interventions. These patients will be analysed according to the intention-to-treat principle. Patients in in the experimental group that did not started with adjuvant chemotherapy, will not be included in the per-protocol analysis.

Replacement of individual subjects after withdrawal

Subjects that are withdrawn from the study will not be replaced.

Follow-up of subjects withdrawn from treatment

Clinical data from patients that are withdrawn from treatment will be followed within the PLCRC cohort study. Data from patients that are lost to follow-up will be censored to the last date of data collection by data-managers. Subjects redrawn from treatment will receive standard-of-care treatment.

12.4 Arrangements for subjects after their participation in the clinical trial ended

Not applicable. Patients continue standard care according to Dutch guidelines.

13. SAFETY REPORTING

13.1 Definitions

13.1.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the experimental intervention (CAPOX or FOL-FOX).

13.1.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

13.1.3 Suspected unexpected serious adverse reactions (SUSARs)

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. The event must be serious;
2. There must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. The adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in the Summary of Product Characteristics (SPC).

13.2 Recording of AEs/SAEs/SUSARS

Chemotherapy is a standard of care in colorectal cancer patients, and AEs and SAEs are well-known. Adverse events of established chemotherapeutic regimens (CAPOX, FOLFOX) after surgery in patients with colorectal cancer is well investigated and will not be recorded. SAEs of patients allocated to the experimental group do not have to be reported immediately (<24 hours), but have to be reported in the study file and will be reported in the annual progress report. Only SAEs within 1 year after start of adjuvant chemotherapy have to be reported. Information on type of SAE and grade according the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 will be reported.(48)

The sponsor will report the SAEs to the European Medicines Agency (EMA) via the Eudravigilance database, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

All SUSARs should be reported in the electronic patient files as soon as possible. The local investigator should report all SUSARs within 24 hours to the electronic patient files using a SUSAR form. The REACT study team will evaluate whether the SAE indeed qualifies as a SUSAR and will report these in the (e)CRF and sub-study database. All SUSARs of patients allocated to the experimental group will be reported in the annual progress report. SUSARs of patients allocated to the experimental group do not have to be reported immediately because the toxicity and adverse effects of CAPOX and FOLFOX are well known and safety is not investigated in this study.

13.3 Reporting of AEs and SAEs

13.3.1 Reporting of SAEs by the investigator to the sponsor

SAEs of patients allocated to the experimental group do not have to be reported immediately (<24 hours) by the investigator to the sponsor, but have to be reported in the study file and will be reported in the annual progress report.

13.3.2 List of SAEs which do not require immediate reporting and procedure for reporting

SAEs of patients allocated to the experimental group do not have to be reported immediately (<24 hours) by the investigator to the sponsor, but have to be reported in the study file and will be reported in the annual progress report.

13.4 Follow-up of adverse events

SAEs of patients allocated to the experimental group need to be reported till the end of study within the Netherlands, as defined in the protocol. They will be recorded in the CRF. There is no active follow-up of SAEs by the central research team. AEs will not be reported. The treatment and follow-up of (S)AE will be according to the standard of care.

13.5 Reporting of SUSARs by the sponsor to EudraVigilance

The sponsor will report electronically and without delay to EudraVigilance all relevant information about any SUSAR (CTR: Article 42).

The period for the reporting of SUSARs by the sponsor to EudraVigilance will take account of the seriousness of the reaction and will be as follows:

- In the case of fatal or life-threatening SUSARs, as soon as possible and in any event not later than **7 days** after the sponsor became aware of the reaction (CTR: Article 42(2(a)));
- In the case of non-fatal or non-life-threatening SUSARs, not later than **15 days** after the sponsor became aware of the reaction (CTR: Article 42(2(b)));
- In the case of a SUSARs which was initially considered to be non-fatal or nonlife threatening but which turns out to be fatal or life-threatening, as soon as possible and in any event not later than **7 days** after the sponsor became aware of the reaction being fatal or life-threatening (CTR: Article 42(2(c))).

Where necessary to ensure timely reporting, the sponsor may, in accordance with section 2.4 of Annex III, submit an initial incomplete report followed up by a complete report (CTR: Article 42(2)).

13.6 Annual safety report

Regarding investigational medicinal products other than placebo, the sponsor shall submit annually through CTIS to all Member States concerned a report on the safety of each investigational medicinal product used in a clinical trial (CTR: Article 43).

13.7 Unblinding procedures for safety reporting

Not applicable

13.8 Temporary halt for reasons of subject safety

The sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will submit the notification through CTIS without undue delay of a temporary halt but not later than in 15 days of the date of the temporary halt. It shall include the reasons for such action and specify follow-up measures. The study will be suspended pending a further positive decision by the concerned member state (CTR: Article 38). The investigator will take care that all subjects are kept informed.

13.9 Urgent safety measures and other relevant safety reporting

Where an unexpected event is likely to seriously affect the benefit-risk balance, the sponsor and the investigator will take appropriate urgent safety measures to protect the subjects. In addition, the sponsor will notify the Member States concerned, through CTIS, of the event and the measures taken. That notification will be made without undue delay but no later than **7 days** from the date the measures have been taken (CTR: Article 54).

13.10 Data Safety Monitoring Board (DSMB)/Data Monitoring Committee (DMC)

A Data and Safety Monitoring Board will not be installed. In the present study, patients in the experimental group will be treated with established chemotherapeutic regimens (CAPOX, FOLFOX) that are well characterized and known for not harming patients. All subjects in this study will be adults. Based on previous data, it is not expected that the primary endpoint (disease-free survival) will be established with statistical significance prior to the date of last enrolment. Premature termination of the study for reasons of superiority (or futility) is therefore unexpected.

14. STATISTICAL ANALYSIS

14.1 Description of statistical methods

All main analyses will be according to the intention to treat principle, i.e. patients will be analysed according to the study group they were assigned to; this also holds for patients randomised to the experimental group but who refuse to receive adjuvant chemotherapy. However, patients initially randomised but considered ineligible afterwards based on information that should have been available before randomisation, will be excluded from all analyses. The main analysis addressing the primary endpoint will be done after 118 events, and is planned two years after the last included patient. Before this analysis, a statistical analysis plan will be prepared by the statistician in consultation with the principal investigator. Other analyses of the secondary endpoints and/or involving safety analyses could be performed when the needs arise and in discussion with the study coordinators. However, all other analyses except the primary analysis should be regarded as exploratory, and therefore only as hypothesis-generating. Analyses will be performed in R version 4.2.1 (https://www.r-project.org/).

14.2 Analysis sets

All patients who underwent blood withdrawal for ctDNA analysis for the REACT study who meet the in- and exclusion criteria, will be included in the statistical analyses.

14.3 Participant demographics and other baseline characteristics

Patient characteristics: age, sex, length, weight, cTNM, type of surgery, type of neoadjuvant treatment, pTNM, WHO performance status, type and duration of chemotherapy, dosing and dose adjustments of adjuvant chemotherapy, serious adverse events, extramural vascular invasion, mesorectal fascia involvement, lateral lymph node involvement.

14.4 Randomisation and blinding

Randomisation will be performed within the online Ldot software platform. Following eligibility verification by the REACT studyteam, patients with detectable ctDNA after rectal surgery will be randomized in a 1:1 ratio to either the control group or the experimental group. No stratification factors will be applied. Random block sizes of 4,6, or 8 will be used.

14.5 Sample size, trail power and level of significance used

In the Netherlands, rectal cancer is diagnosed in 3500 patients each year. The estimated proportion of patients with stage II or III rectal cancer is 65%. However, due to the nationwide bowel screening program which was implemented in the Netherlands in 2014, stage migration has been observed with detection of more early cancers, resulting in an estimated stage distribution of 50% for stage II-III during the inclusion period.

Of the patients with stage II-III disease, about 15% will have clinical complete response after neoadjuvant treatment and will not have surgery, therefore they cannot be screened. All other patients with meeting the inclusion criteria and detectable ctDNA post-surgery will be eligible for inclusion. The PLCRC study cohort will be used for inclusion of patients for the present study. Patients eligible for this study with detectable ctDNA will be randomised according to the TwiCs principle. For the control group no additional informed consent is required, only the experimental group needs

additional informed consent. With the use of the PLCRC cohort and its participating hospitals we expect to assure timely inclusion of 206 ctDNA positive patients, which are needed to assess the primary objective of this study.
Not all patients in the Netherlands will be treated with intentionally curative treatment with surgery in the participating PLCRC hospitals, and some patients treated in PLCRC hospitals do not participate in the PLCRC. The proportion of patients that are eligible for adjuvant chemotherapy, and are included in participating PLCRC centres, is set at 40%. With these figures, we estimate that 595 patients each year will be available to screen in 25 participating PLCRC hospitals. The prevalence of detectable ctDNA in these patients is 15%. Approximately 89.25 patients each year are expected to be eligible for inclusion and accrual will be completed somewhere between the third and fourth year of the study (accounting for start-up difficulties, we estimate that 70 patients each year will be included over the total inclusion period of three years).

Calculation: 3500*0.5*0.85*0.4*0.15=89.25 patients/year. 206/89.25 = 2.31 years.

With a detection rate of 15% for ctDNA after surgery in high-risk rectal cancer, 1373 patients' blood samples need to be analysed for ctDNA to include all patients with detectable ctDNA (n=206), 103 patients with detectable ctDNA for each group. The hypothesis of the REACT study is that adjuvant chemotherapy will improve disease-free survival of rectal cancer patients with detectable ctDNA after surgery and neoadjuvant treatment. Based on this hypothesis, patients with postoperative detectable ctDNA will be randomized to an experimental group receiving adjuvant chemotherapy, and a control group receiving standard of care (no additional treatment).

The largest study discussed in this literature review (Tie et al.) found that the 3-year disease-free survival in patients with detectable ctDNA after surgery is 33% (95% CI: 16% to 72%). (28) However, it has to be taken into account that 58% of these patients received adjuvant chemotherapy, and that the proportion of patients that would be disease-free is likely to be smaller if no additional treatment was administered. In the REACT population, patients with a pathological complete response will not be included, hereby excluding a group of patients with a relatively good prognosis.

Based on these figures and considerations, we expect that the 2-year disease-free survival in patients a) with detectable ctDNA after surgery and neoadjuvant treatment, b) without a complete response, and c) without any additional treatment is 30%. We aim to demonstrate a hazard ratio of 0.55 for adjuvant chemotherapy (pure treatment effect), but that this hazard ratio will be reduced to 0.64 when taken into account that 25% of patients in the intervention group will refuse experimental treatment or are not eligible for adjuvant chemotherapy due to comorbidity. This will translate into an improved disease-free survival in the adjuvant chemotherapy group from 30% to 55% (absolute risk increase of 25%).

For the detection of a hazard ratio of 0.64 in the experimental arm, 30% 2-year disease-free survival in the control group, and with two-sided significance level $\alpha = 0.05$ and 80% power, the number of patients needed to include is 206 in total (103 vs. 103).
Every hospital should screen approximately 14 patients per year and include 2-3 patients with detectable ctDNA per year for adjuvant chemotherapy.

14.6 Planned analysis

14.6.1 Analysis primary endpoint

The endpoint for the primary analysis will be disease free survival. The formal test for difference in disease-free survival between the two groups will be done with a Cox regression analysis with adjustment for patient-, pre-treatment- and tumour characteristics. The actuarial method of Kaplan and Meier will be used to estimate disease-free survival probabilities at appropriate time points, while the Greenwood estimate will be used to construct corresponding 95% confidence intervals (CIs). Kaplan-Meier curves will be generated to illustrate disease-free survival, for all patients as well as by group. The final analyses will not be performed until the required numbers of events (118) have been observed, and the data have been validated.

Primary endpoint of the study will be disease-free survival (DFS), calculated from the date of randomisation to the date of progression (recurrence) or death from any cause of the patient, whichever occurs first. Patients who did not experienced a recurrence and who are still alive at the end of the study will be censored at the date of last contact.

14.6.2 Analysis secondary endpoint(s)

In addition to intention-to-treat analysis to estimate the effect size, also a per protocol analysis (for pure treatment effect) will be carried out for disease-free survival and overall survival. This analysis will compare all (eligible) patients in the control group with those patients in the experimental group who actually started the adjuvant chemotherapy.
Secondary endpoint of the study will be overall survival, calculated from the date of surgery to the date death from any cause of the patient. Patients who are still alive at the end of the study will be censored at the date of last contact. The disease-free survival and overall survival of ctDNA negative patients will also be collected and analysed to confirm the robustness of post-operative ctDNA as biomarker in rectal cancer. Survival outcomes will be compared between patients who had undetectable ctDNA versus patients who had detectable ctDNA and did not undergo any additional therapy (control group of the randomised patients). Quality of life questionnaires are presented at different time points. Comparison between both groups will be calculated by a random effects regression model and will be based on the intention to treat principle. Repeated measurement analysis will be used to evaluate within and between group differences. The results of the ctDNA analysis of the blood samples taken at the first follow-up of the patients who were ctDNA positive after surgery will be compared between the patients who received adjuvant chemotherapy and those who did not. Within the group of patients with detectable ctDNA after surgery, the proportion of patients with detectable ctDNA in the peripheral blood at the timing of detection of recurrent disease on imaging will be assessed.

14.6.3 Analysis other study parameters/endpoints

In an exploratory analysis we will investigate the mutational profile and level of ctDNA of the ctDNA positive patients, the specific mutations that are demonstrated and possible correlations with clinical and pathological parameters and time to recurrence. In patients of whom tumour tissue is available, we will compare mutational status of ctDNA and tumour tissue.

14.7 Interim analysis

No interim analysis will be planned.

14.8 (Statistical) criteria for termination of the trial

While no interim analysis is planned, the trial may be terminated prematurely if certain unexpected circumstances arise that impede the successful completion of the study. Examples include inadequate patient screenings rates, changes in daily clinical practice due to newly published study results or other external factors that could compromise the ethical or scientific validity of the trial.

14.9 Procedure for accounting for missing, unused and spurious data

All the collected data are expected to be used, with exception of the data of patients that are considered ineligible afterwards based on information that should have been available at timing of ctDNA analysis.

14.10 Procedure for reporting any deviation(s) from the original statistical plan

Any deviation(s) from the original statistical plan will be described and justified in the protocol and/or in the final report, as appropriate.

15. ETHICAL CONSIDERATIONS

15.1 Declaration of Helsinki

The study will be conducted according to the principles of the Declaration of Helsinki (75th WMA General Assembly, Helsinki, Finland, October 2024) and in accordance with the Medical Research Involving Human Subjects Act (WMO) the Good Clinical Practice (GCP) guidelines, and this study protocol.

15.2 Recruitment and informed consent procedures

If patients have given written informed consent for PLCRC, and for withdrawal of additional blood samples and future (interventional) studies, after surgery and meeting the inclusion criteria, blood samples can be taken and analysed for the presence of ctDNA. Patients with detectable ctDNA can be randomised to the observational control group and the experimental group. Patients in the experimental group will be contacted by their treating physician (surgeon) that is included in the PLCRC study team. Patients will be asked to be contacted about the REACT study by one of the REACT investigators, for additional information about the REACT study. Patients in the control group will not be contacted, because they are anonymous for the study team and their treating physician.

Patients are free to refuse to undergo the experimental intervention at all times, when offered. None of the patients that participate in this project will be withheld treatments that have been proven to be effective in their disease subgroup, without prior obtaining explicit informed consent of the patient.

Information about REACT will be provided 2-4 weeks after ctDNA analysis in eligible patients. Patients will visit their treating physician (surgeon) at that time for a standard follow-up visit after surgery and when they fulfil criteria for REACT, they are randomised before this visit. The treating physician or research nurse will give information about REACT to patients randomised to the experimental group and about detectable ctDNA.

Patients who are interested in receiving adjuvant chemotherapy will be referred to the medical oncology department for chemotherapy counselling by a medical oncologist at the outpatient clinic. Additional information about REACT will be given by the REACT study team.
Patients will then decide based on the given counselling and information about ctDNA analysis, risk of recurrence and harms and benefits of adjuvant chemotherapy, if they want to receive adjuvant chemotherapy.
Patients will have time for consideration of participation and if they are willing to participate, they will sign informed consent for the REACT study and the schedule for adjuvant chemotherapy will be discussed with their treating physician. The formal written informed consent must be obtained before the start of adjuvant chemotherapy.

Trials within cohorts (TwiCs) design
In PLCRC (MEC-2016-369), patients are separately asked to give informed consent to be offered experimental interventions, and that their data can be used in comparative research. Although, at the time informed consent is signed, neither the patient nor the researcher knows for which (possibly running) studies this particular patient is or might become eligible. This is a situation of 'broad' consent (patients do not know for which research questions their data will be used at time of signing consent), most predominant in biobank studies. However, this approach within the TwiCs is specifically chosen to deliver the least (psychological or emotional) distress to the patient, since they are only informed if their data will be used for research questions and if they still have a choice if they want to undergo the offered experimental intervention or not. Thus, this broad consent approach could be explained to protect the patient from experiencing distress in case they enter the control group without receiving the opportunity to influence their treatment course or the way in which the data will be used. Nevertheless, all patients are well informed that they can withdraw from the study (including the broad consent) at any time without reasoning.

From the group of patients in the PLCRC cohort eligible for REACT the TwiCs randomly selects patients who will be offered the intervention. Patients from that same sub-cohort, who are not randomly selected, will undergo standard treatment (i.e. regular follow-up), without being notified about the fact that they were not (but could have been) randomly selected. All patients, randomly selected or not, are aware of the fact that they could have been invited for new treatments, and that their data are being used for evaluation of effectiveness of new interventions. The TwiCs is designed to prevent patients from receiving information about interventions they will not receive. We do acknowledge that some patients are therefore withheld from information about a treatment under study that some patients, comparable to themselves, will be offered to undergo, while they are not. This 'patient-centred' approach makes sure that informed consent for a particular intervention is sought only from those offered that intervention. Patients allocated to the control group are deliberately not informed about the presence of ctDNA, as this would entail an unnecessary burden for patients and their treating physicians. Furthermore, it might be uneasy for treating physicians to not offer any additional treatment, and could possibly result in drop-outs or even cross-over to the treatment group.

15.3 Benefits and risks assessment, group relatedness

It is expected that this study will contribute to more personalized treatment and improved survival of rectal cancer patients with detectable ctDNA postoperatively and improved quality of care.
The 103 patients in the experimental group that have detectable ctDNA after surgery are exposed to the risks of adjuvant chemotherapy. These risks are well-known from current clinical practice and patients will be counselled. The risk of additional blood withdrawals is negligible.

Minors will not be included in this study. Patients who are unable to understand the information and give informed consent will not be included in the study.

15.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

15.5 Compensation for subjects

Patients that have detectable ctDNA after surgery and that are randomised to the experimental group, will be offered adjuvant chemotherapy. By participation in this study, these patients are informed about their high risk of recurrence of disease and will be offered a treatment they would not have been offered in current clinical practice. The current data about the high recurrence rate in patients with detectable ctDNA will likely contribute to the preparedness of patients to receive adjuvant chemotherapy. No financial incentives are provided to participants of the study, but travel costs can be compensated

15.6 Compensation for investigators

Not applicable.

15.7 Other ethical considerations

Not applicable.

16. ADMINISTRATIVE ASPECTS, MONITORING AND CONFIDENTIALITY

The study will be conducted in compliance with the protocol, with Clinical Trials Regulation No 536/2014 and with the principles of good clinical practice.

16.1 Approval initial application and substantial modifications

The trial protocol, informed consent form, subject information leaflet, investigational medicinal product dossier, investigators brochure and any other documents required by the Regulation will be submitted for the regulatory approval before the clinical trial is started via CTIS.

The sponsor will also submit and obtain approval for substantial modifications to the original approved documents via CTIS.

A 'substantial modification' is defined in the CTR as any change to any aspect of the clinical trial which is made after notification of a decision referred to in Articles 8, 14, 19, 20 or 23 and which is likely to have a substantial impact on the safety or rights of the subjects or on the reliability and robustness of the data generated in the clinical trial.

16.2 Monitoring

Monitoring will be done following the requirements of the Netherlands Federation of University Medical Centres (NFU) based on the ICH Good Clinical Practice guidelines. The REACT study is classified as a medicinal study with negligible risk. For every site there will be an initiating visit, at least two on study visits (one per year) and a closing out visit. The timing of visits will depend on inclusion speed, amount of included subjects and previously observed deviations.

16.3 Recording, handling and storage of information

16.3.1 Handling of data and data protection

Individual patient information obtained as a result of this study is considered confidential and is handled conform the Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG (UAVG) . Disclosure to third parties is prohibited, except for authorities involved with monitoring and quality control of the clinical study such as monitors, auditors, members of the Medical Research Ethics Committee and the health care inspectorate. Patients confidentiality will be ensured by using identification numbers (for example: EMC-001, EMC-002, etc.). A subject identification code list is safeguarded by the local principal investigator in case data needs to be linked back to an individual subject. Research data that can be traced to individual persons can only be viewed by authorized personnel.
PLCRC data handling and storage will be conducted within the PLCRC study in UMC Utrecht, and supplemented with additional data by the IKNL Clinical Trial Centre. A separate REACT database (managed in Castor) is created for study-specific data, and will be filled in with the collected (pseudonymised) data from PLCRC and IKNL. The data will be kept for at least 25 years.

16.3.2 Source documents and case report forms (CRF)

Source documents for this study will include hospital records and procedure reports and data collection forms. These documents will be used to enter data on the CRFs. Data reported on the CRF that are derived from source documents must be consistent with the source documents or the discrepancies must be explained.

All documents will be stored safely in confidential conditions. On all study-specific documents other than the signed consent, the subject will be referred to by the study subject identification code.

16.3.3 Clinical trial master file and data archiving

The sponsor and the investigator shall keep a clinical trial master file. The clinical trial master file shall at all times contain the essential documents relating to the clinical trial which allow verification of the conduct of a clinical trial and the quality of the data generated (CTR: Article 57).

The sponsor and the investigator shall archive the content of the clinical trial master file for at least 25 years after the end of the clinical trial, unless other EU law requires archiving for a longer period. The medical files of subjects shall be archived in accordance with national law (CTR: Article 58).

The content of the clinical trial master file shall be archived in a way that ensures that it is readily available and accessible, upon request (CTR: Article 57).

16.3.4 Collection and storage of biological samples

Additional blood will be collected from consenting patients at the time of a regular blood draw for clinical practice. The blood samples will be coded with both the PLCRC PICNIC number and the REACT study-number. The PLCRC study team manages the patient information of all subjects and can identify the patient information of the biological sample if needed. Laboratory personnel will handle samples in a blinded fashion. Samples will be processed within 96 hours as described and stored at -80 degrees (plasma) or -30 degrees (cfDNA) until further analysis. All blood samples will be stored for 25 years, unless the patient explicitly declines consent for this in the patient informed consent form.

16.4 Audits and inspections and direct access to source data/documents

This trial may be subject to internal or external monitoring, auditing or inspections procedure to ensure adherence to GCP. Access to all trial-related documents including direct access to source data will be given at that time.

16.5 Reporting of serious breaches

The sponsor will notify the Member States concerned about a serious breach of the Regulation or of the version of the protocol applicable at the time of the breach through CTIS without undue delay but not later than **seven days** of becoming aware of that breach (CTR: Article 52).

16.6 Notification of the start and the end of the recruitment

The sponsor will notify within 15 days each Member State concerned of the start of a clinical trial in relation to that Member State through CTIS (CTR: Article 36(1)).

The sponsor will notify within 15 days each Member State concerned of the first visit of the first subject in relation to that Member State through CTIS (CTR: Article 36(2)).

The sponsor will notify within 15 days each Member State concerned of the end of the recruitment of subjects for a clinical trial in that Member State through the EU (CTR: Article 36(3)).

16.7 Temporary halt/(early) termination

The sponsor will notify within 15 days each Member State concerned of the end of a clinical trial in relation to that Member State through CTIS (CTR: Article 37(1)).
The sponsor will notify within 15 days each Member State concerned of the end of a clinical trial in all Member States concerned and in all third countries in which the clinical trial has been conducted through CTIS (CTR: Article 37(3)).

16.7.1 Temporary halt/early termination for reasons not affecting the benefit-risk balance

The sponsor will notify with 15 days each Member State concerned of a temporary halt of a clinical trial in all Member States concerned for reasons not affecting the benefit-risk balance through CTIS (CTR: Article 37(5)).

When a temporarily halted clinical trial for reasons not affecting the benefit-risk balance is resumed the sponsor will notify each Member State concerned through CTIS (CTR: Article 37(6)).

The sponsor will notify to the EU portal CTIS of early termination of the clinical trial for reasons not affecting the benefit-risk balance through CTIS. The reasons for such action and, when appropriate, follow-up measures for the subjects will be provided as well (CTR: Article 37(7)).

16.7.2 Temporary halt/early termination for reasons of subject safety

In accordance to article 38 of the CTR, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The temporary halt or early termination of a clinical trial for reasons of a change of the benefit-risk balance will be notified to the Member States concerned through the EU portal CTIS without undue delay but not later than in 15 days of the date of the temporary halt or early termination. It shall include the reasons for such action and specify follow-up measures. The restart of the clinical trial following a temporary halt as referred to in paragraph 1 shall be deemed to be a substantial modification subject to the authorisation procedure laid down in Chapter III of the CTR (CTR: Article 38).

16.8 Summary of the results

Within one year from the end of a clinical trial in all Member States concerned, the sponsor will submit to the EU database CTIS a summary of the results of the clinical trial. The content of the summary of the results is set out in CTR Annex IV. It shall be accompanied by a summary written in a manner that is understandable to laypersons. The content of the summary is set out in CTR Annex V (CTR: Article 37(4)).

16.9 Public disclosure and publication policy

This study will be registered before start of accrual in the WHO recognised ClinicalTrials.gov (https://clinicaltrials.gov/). Results will be reported according to the CONSORT guidelines. Publications resulting from this study will be submitted to peer-reviewed journals. The principle investigator and study coordinators will prepare the manuscript together with those who substantially contributed to the study, at the discretion of the principle investigator. Any publication, abstract or preservation based on patients included in this study must be approved by the primary investigator and the

study coordinators. This is applicable to any individual patient registered in the trial, or any subgroup of the trial patients. Such a publication cannot include any comparisons between the randomised groups or an analysis of any of the study end-points unless the final results of the trial have already been published. Within one year after the primary endpoint has been reached, final results of the study will be presented at (inter)national congresses and submitted to an international peer-reviewed journal.

17. REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: A Cancer Journal for Clinicians. 2020;70(1):7-30.
2. .www.iknl.nl/nkr-cijfers.
3. van Gijn W, Marijnen CA, Nagtegaal ID, Kranenbarg EM, Putter H, Wiggers T, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. Lancet Oncol. 2011;12(6):575-82.
4. Sauer R, Liersch T, Merkel S, Fietkau R, Hohenberger W, Hess C, et al. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: results of the German CAO/ARO/AIO-94 randomized phase III trial after a median follow-up of 11 years. J Clin Oncol. 2012;30(16):1926-33.
5. Ciseł B, Pietrzak L, Michalski W, Wyrwicz L, Rutkowski A, Kosakowska E, et al. Long-course preoperative chemoradiation versus 5 × 5 Gy and consolidation chemotherapy for clinical T4 and fixed clinical T3 rectal cancer: long-term results of the randomized Polish II study. Ann Oncol. 2019;30(8):1298-303.
6. Bujko K, Glynne-Jones R, Bujko M. Does adjuvant fluoropyrimidine-based chemotherapy provide a benefit for patients with resected rectal cancer who have already received neoadjuvant radiochemotherapy? A systematic review of randomised trials. Ann Oncol. 2010;21(9):1743-50.
7. Breugom AJ, Swets M, Bosset JF, Collette L, Sainato A, Cionini L, et al. Adjuvant chemotherapy after preoperative (chemo)radiotherapy and surgery for patients with rectal cancer: a systematic review and meta-analysis of individual patient data. Lancet Oncol. 2015;16(2):200-7.
8. Bahadoer RR, Dijkstra EA, van Etten B, Marijnen CAM, Putter H, Kranenbarg EM, et al. Short-course radiotherapy followed by chemotherapy before total mesorectal excision (TME) versus preoperative chemoradiotherapy, TME, and optional adjuvant chemotherapy in locally advanced rectal cancer (RAPIDO): a randomised, open-label, phase 3 trial. Lancet Oncol. 2021;22(1):29-42.
9. Conroy T, Castan F, Etienne PL, Rio E, Mesgouez-Nebout N, Evesque L, et al. Total neoadjuvant therapy with mFOLFIRINOX versus preoperative chemoradiotherapy in patients with locally advanced rectal cancer: long-term results of the UNICANCER-PRODIGE 23 trial. Annals of Oncology. 2024;35(10):873-81.
10. Socha J, Michalski W, Gerard JP, Bujko K. Does total neoadjuvant therapy improve overall survival in rectal cancer? Interpretation of the PRODIGE-23 and other studies. Annals of Oncology. 2024.
11. Burbach JP, Kurk SA, Coebergh van den Braak RR, Dik VK, May AM, Meijer GA, et al. Prospective Dutch colorectal cancer cohort: an infrastructure for long-term observational, prognostic, predictive and (randomized) intervention research. Acta Oncol. 2016;55(11):1273-80.
12. Engelen SM, Maas M, Lahaye MJ, Leijtens JW, van Berlo CL, Jansen RL, et al. Modern multidisciplinary treatment of rectal cancer based on staging with magnetic resonance imaging leads to excellent local control, but distant control remains a challenge. Eur J Cancer. 2013;49(10):2311-20.
13. Elferink MA, de Jong KP, Klaase JM, Siemerink EJ, de Wilt JH. Metachronous metastases from colorectal cancer: a population-based study in North-East Netherlands. Int J Colorectal Dis. 2015;30(2):205-12.
14. Bosset J-F, Calais G, Mineur L, Maingon P, Stojanovic-Rundic S, Bensadoun R-J, et al. Fluorouracil-based adjuvant chemotherapy after preoperative chemoradiotherapy in rectal cancer: long-term results of the EORTC 22921 randomised study. The Lancet Oncology.15(2):184-90.
15. Sainato A, Cernusco Luna Nunzia V, Valentini V, De Paoli A, Maurizi ER, Lupattelli M, et al. No benefit of adjuvant Fluorouracil Leucovorin chemotherapy after neoadjuvant chemoradiotherapy in locally advanced cancer of the rectum (LARC): Long term results of a randomized trial (I-CNR-RT). Radiother Oncol. 2014;113(2):223-9.
16. Breugom AJ, van Gijn W, Muller EW, Berglund A, van den Broek CB, Fokstuen T, et al. Adjuvant chemotherapy for rectal cancer patients treated with preoperative (chemo)radiotherapy and total mesorectal excision: a Dutch Colorectal Cancer Group (DCCG) randomized phase III trial. Ann Oncol. 2015;26(4):696-701.

17. Glynne-Jones R, Counsell N, Quirke P, Mortensen N, Maraveyas A, Meadows HM, et al. Chronicle: results of a randomised phase III trial in locally advanced rectal cancer after neoadjuvant chemoradiation randomising postoperative adjuvant capecitabine plus oxaliplatin (XELOX) versus control. Ann Oncol. 2014;25(7):1356-62.
18. Quasar Collaborative G, Gray R, Barnwell J, McConkey C, Hills RK, Williams NS, Kerr DJ. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. Lancet. 2007;370(9604):2020-9.
19. Petersen SH, Harling H, Kirkeby LT, Wille-Jørgensen P, Mocellin S. Postoperative adjuvant chemotherapy in rectal cancer operated for cure. Cochrane Database of Systematic Reviews. 2012(3).
20. Conroy T, Bosset J-F, Etienne P-L, Rio E, François É, Mesgouez-Nebout N, et al. Neoadjuvant chemotherapy with FOLFIRINOX and preoperative chemoradiotherapy for patients with locally advanced rectal cancer (UNICANCER-PRODIGE 23): a multicentre, randomised, open-label, phase 3 trial. The Lancet Oncology. 2021;22(5):702-15.
21. Moertel CG, Fleming TR, Macdonald JS, Haller DG, Laurie JA, Goodman PJ, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med. 1990;322(6):352-8.
22. Diaz LA, Jr., Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol. 2014;32(6):579-86.
23. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies. Science Translational Medicine. 2014;6(224):224ra24-ra24.
24. Schøler LV, Reinert T, Ørntoft MW, Kassentoft CG, Árnadóttir SS, Vang S, et al. Clinical Implications of Monitoring Circulating Tumor DNA in Patients with Colorectal Cancer. Clin Cancer Res. 2017;23(18):5437-45.
25. Diaz LA, Jr., Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature. 2012;486(7404):537-40.
26. Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature. 2012;486(7404):532-6.
27. Siravegna G, Mussolin B, Buscarino M, Corti G, Cassingena A, Crisafulli G, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat Med. 2015;21(7):795-801.
28. van Helden EJ, Angus L, Menke-van der Houven van Oordt CW, Heideman DAM, Boon E, van Es SC, et al. RAS and BRAF mutations in cell-free DNA are predictive for outcome of cetuximab monotherapy in patients with tissue-tested RAS wild-type advanced colorectal cancer. Mol Oncol. 2019;13(11):2361-74.
29. Diaz LA, Bardelli A. Liquid Biopsies: Genotyping Circulating Tumor DNA. Journal of Clinical Oncology. 2014;32(6):579-86.
30. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med. 2016;8(346):346ra92.
31. Tie J, Cohen JD, Wang Y, Li L, Christie M, Simons K, et al. Serial circulating tumour DNA analysis during multimodality treatment of locally advanced rectal cancer: a prospective biomarker study. Gut. 2019;68(4):663-71.
32. Khakoo S, Carter PD, Brown G, Valeri N, Picchia S, Bali MA, et al. MRI Tumor Regression Grade and Circulating Tumor DNA as Complementary Tools to Assess Response and Guide Therapy Adaptation in Rectal Cancer. Clin Cancer Res. 2020;26(1):183-92.
33. Zhou J, Wang C, Lin G, Xiao Y, Jia W, Xiao G, et al. Serial Circulating Tumor DNA in Predicting and Monitoring the Effect of Neoadjuvant Chemoradiotherapy in Patients with Rectal Cancer: A Prospective Multicenter Study. Clinical Cancer Research. 2021;27(1):301-10.

34. Murahashi S, Akiyoshi T, Sano T, Fukunaga Y, Noda T, Ueno M, Zembutsu H. Serial circulating tumour DNA analysis for locally advanced rectal cancer treated with preoperative therapy: prediction of pathological response and postoperative recurrence. Br J Cancer. 2020;123(5):803-10.
35. Tie J, Cohen JD, Lahouel K, Lo SN, Wang Y, Kosmider S, et al. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. New England Journal of Medicine. 2022.
36. Beije N, Helmijr JC, Weerts MJA, Beaufort CM, Wiggin M, Marziali A, et al. Somatic mutation detection using various targeted detection assays in paired samples of circulating tumor DNA, primary tumor and metastases from patients undergoing resection of colorectal liver metastases. Mol Oncol. 2016;10(10):1575-84.
37. Beije N, Sieuwerts AM, Kraan J, Van NM, Onstenk W, Vitale SR, et al. Estrogen receptor mutations and splice variants determined in liquid biopsies from metastatic breast cancer patients. Mol Oncol. 2018;12(1):48-57.
38. Jansen MP, Martens JW, Helmijr JC, Beaufort CM, van Marion R, Krol NM, et al. Cell-free DNA mutations as biomarkers in breast cancer patients receiving tamoxifen. Oncotarget. 2016;7(28):43412-8.
39. van Dessel LF, Beije N, Helmijr JC, Vitale SR, Kraan J, Look MP, et al. Application of circulating tumor DNA in prospective clinical oncology trials - standardization of preanalytical conditions. Mol Oncol. 2017;11(3):295-304.
40. van Dessel LF, Vitale SR, Helmijr JCA, Wilting SM, van der Vlugt-Daane M, Oomen-de Hoop E, et al. High-throughput isolation of circulating tumor DNA: a comparison of automated platforms. Mol Oncol. 2019;13(2):392-402.
41. Kruger DT, Jansen M, Konings I, Dercksen WM, Jager A, Oulad Hadj J, et al. High ctDNA molecule numbers relate with poor outcome in advanced ER+, HER2- postmenopausal breast cancer patients treated with everolimus and exemestane. Mol Oncol. 2020;14(3):490-503.
42. Bos MK, Nasserinejad K, Jansen MPHM, Angus L, Atmodimedjo PN, de Jonge E, et al. Comparison of variant allele frequency and number of mutant molecules as units of measurement for circulating tumor DNA. Molecular oncology. 2021;15(1):57-66.
43. Bos MK, Angus L, Nasserinejad K, Jager A, Jansen M, Martens JWM, Sleijfer S. Whole exome sequencing of cell-free DNA - A systematic review and Bayesian individual patient data meta-analysis. Cancer Treat Rev. 2020;83:101951.
44. Bos MK, Nasserinejad K, Jansen M, Angus L, Atmodimedjo PN, de Jonge E, et al. Comparison of variant allele frequency and number of mutant molecules as units of measurement for circulating tumor DNA. Mol Oncol. 2021;15(1):57-66.
45. Lissa W, Maurice PHMJ, Jaco K, Corine MB, Ngoc MV, Eric JTB, et al. Circulating tumor cells and tumor DNA in patients with resectable colorectal liver metastases: The MIRACLE. Journal of Clinical Oncology. 2024;42(16_suppl):3011-.
46. Beije N, Helmijr JC, Weerts MJ, Beaufort CM, Wiggin M, Marziali A, et al. Somatic mutation detection using various targeted detection assays in paired samples of circulating tumor DNA, primary tumor and metastases from patients undergoing resection of colorectal liver metastases. Mol Oncol. 2016.
47. Weerts MJA, van Marion R, Helmijr JCA, Beaufort CM, Krol NMG, Trapman-Jansen A, et al. Somatic Tumor Mutations Detected by Targeted Next Generation Sequencing in Minute Amounts of Serum-Derived Cell-Free DNA. Sci Rep. 2017;7(1):2136.
48. Arango D, Wilson AJ, Shi Q, Corner GA, Arañes MJ, Nicholas C, et al. Molecular mechanisms of action and prediction of response to oxaliplatin in colorectal cancer cells. British Journal of Cancer. 2004;91(11):1931-46.
49. Breugom AJ, van Gijn W, Muller EW, Berglund Å, van den Broek CBM, Fokstuen T, et al. Adjuvant chemotherapy for rectal cancer patients treated with preoperative (chemo)radiotherapy and total mesorectal excision: a Dutch Colorectal Cancer Group (DCCG) randomized phase III trial. Ann Oncol. 2015;26(4):696-701.

50. Elferink MA, van Steenbergen LN, Krijnen P, Lemmens VE, Rutten HJ, Marijnen CA, et al. Marked improvements in survival of patients with rectal cancer in the Netherlands following changes in therapy, 1989-2006. Eur J Cancer. 2010;46(8):1421-9.
51. André T, de Gramont A, Vernerey D, Chibaudel B, Bonnetain F, Tijeras-Raballand A, et al. Adjuvant Fluorouracil, Leucovorin, and Oxaliplatin in Stage II to III Colon Cancer: Updated 10-Year Survival and Outcomes According to BRAF Mutation and Mismatch Repair Status of the MOSAIC Study. Journal of Clinical Oncology. 2015;33(35):4176-87.
52. Grothey A, Sobrero AF, Shields AF, Yoshino T, Paul J, Taieb J, et al. Duration of Adjuvant Chemotherapy for Stage III Colon Cancer. New England Journal of Medicine. 2018;378(13):1177-88.
53. André T, Meyerhardt J, Iveson T, Sobrero A, Yoshino T, Souglakos I, et al. Effect of duration of adjuvant chemotherapy for patients with stage III colon cancer (IDEA collaboration): final results from a prospective, pooled analysis of six randomised, phase 3 trials. Lancet Oncol. 2020;21(12):1620-9.
54. Iveson TJ, Kerr RS, Saunders MP, Cassidy J, Hollander NH, Tabernero J, et al. 3 versus 6 months of adjuvant oxaliplatin-fluoropyrimidine combination therapy for colorectal cancer (SCOT): an international, randomised, phase 3, non-inferiority trial. The Lancet Oncology. 2018;19(4):562-78.
55. Burbach JPM, Kurk SA, Coebergh van den Braak RRJ, Dik VK, May AM, Meijer GA, et al. Prospective Dutch colorectal cancer cohort: an infrastructure for long-term observational, prognostic, predictive and (randomized) intervention research. Acta Oncologica. 2016;55(11):1273-80.