Study protocol:

Circulating tumour DNA based decision for adjuvant treatment in colon cancer stage II evaluation (CIRCULATE)
AIO-KRK-0217

Investigator-initiated, multicentre, prospective, randomised, controlled study

Study groups:
Arbeitsgemeinschaft Internistische Onkologie (AIO, Germany)
Austrian Breast & Colorectal study group (ABCSG, Austria)
Swiss Group for Clinical Cancer Research (SAKK, Switzerland)

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Sponsor: Technische Universität Dresden, 01062 Dresden, Germany

Sponsor code: TUD-CIRC01-071
EudraCT number: 2018-003691-12
ClinicalTrials.gov: NCT04089631
Grant number: 01KG1817
(German Federal Ministry for Education and Research, German Aerospace Centre)

Version 2.0 - 11 Nov 2019
1 Content

1 Content ..................................................................................................................... 2

2 Abbreviations ........................................................................................................ 7

3 Protocol signatures .................................................................................................. 9

3.1 Protocol signature (Investigator) ......................................................................... 10

4 Protocol committee .................................................................................................. 11

4.1 Data safety monitoring board .............................................................................. 12

5 Study infrastructure ................................................................................................ 13

5.1 Study fax and study email .................................................................................... 13

5.2 Study management ............................................................................................... 13

5.3 Study data base, pharmacovigilance and monitoring ........................................... 13

5.4 Central laboratory for ctDNA analysis ................................................................ 13

5.5 Central pathology (AIO / Colopredict centres) ..................................................... 14

5.6 Central pathology (DKTK / NCT centres) ........................................................... 14

5.7 Financing of the study ........................................................................................ 14

6 Synopsis .................................................................................................................... 15

7 Visit overview .......................................................................................................... 21

8 Introduction .............................................................................................................. 23

8.1 Background .......................................................................................................... 23

8.1.1 Burden of disease ............................................................................................ 23

8.1.2 Adjuvant therapy in stage III ........................................................................... 23

8.1.3 Adjuvant therapy in stage II ............................................................................ 24

8.1.4 Circulating tumour (ct) DNA for minimal residual disease ............................... 25

8.2 Need for a trial ....................................................................................................... 26

8.3 Potential risks and benefit .................................................................................... 26

8.3.1 Screening of patients ....................................................................................... 26

8.3.2 Exclusion of patients with MSI-H tumours ..................................................... 27

8.3.3 Inclusion of patients with rectal cancer ........................................................... 27

8.3.4 No chemotherapy in the standard arm ............................................................ 27

8.3.5 Chemotherapy in ctDNA positive patients (experimental arm) ...................... 27

8.3.6 Chemotherapy toxicity in the experimental arm .............................................. 27

8.3.7 Randomisation of ctDNA negative patients .................................................... 28

8.3.8 Blinding of patients in the arm „Follow-up“ .................................................... 28

8.3.9 Plasma (ctDNA) samples during treatment and follow-up ............................. 28

8.3.10 Overall judgement ......................................................................................... 28

9 Study aims ............................................................................................................... 29
9.1 Primary aim ........................................................................................................ 29
9.2 Secondary aims .................................................................................................. 29
9.3 Translational aims ............................................................................................... 29

10 Description of the trial ...................................................................................... 30

10.1 Study design ..................................................................................................... 30
10.1.1 Screening phase .......................................................................................... 30
10.1.2 Randomised phase ...................................................................................... 30
10.2 Primary criterion ............................................................................................... 31
10.3 Secondary criteria ............................................................................................. 31
10.4 Translational endpoints: .................................................................................. 32
10.5 Patient number .................................................................................................. 32
10.5.1 Patient number for ctDNA positive patients ............................................. 32
10.5.2 Patient number for ctDNA negative patients ........................................... 33
10.5.3 Estimated prognosis according to treatment groups ......................... 34
10.5.4 Total number of patients in the study ......................................................... 35

10.6 Timelines ........................................................................................................... 35
10.7 Requirement on centres and investigators .................................................... 35
10.7.1 Study centres with treatment .................................................................. 35
10.7.2 Screening centres ....................................................................................... 35

11 Patient population ............................................................................................. 37

11.1 Inclusion criteria for screening ....................................................................... 37
11.2 Exclusion criteria for screening ....................................................................... 37
11.3 Inclusion criteria for randomised phase .......................................................... 38
11.4 Exclusion criteria for randomised phase .......................................................... 38

12 Study drug .......................................................................................................... 40

12.1 Treatment schedule ......................................................................................... 40
12.1.1 Capecitabine ............................................................................................... 40
12.1.2 Capecitabine, if combined with oxaliplatin ............................................. 40
12.2 Application ......................................................................................................... 40
12.2.1 Premedication ............................................................................................. 40
12.2.2 Start of the next cycle ................................................................................. 41
12.2.3 Dose of capecitabine in monotherapy ................................................... 41
12.2.4 Dose of capecitabine if combined with oxaliplatin .................................. 42
12.3 Dose modification ............................................................................................. 42
12.4 Concomitant medication ............................................................................... 43
12.5 Participation at other clinical trials ................................................................. 43
12.6 Compliance ......................................................................................................... 44
12.7 Description of the study drug ......................................................................... 44
12.7.1 Capecitabine ............................................................................................... 44
12.8 Toxicity and interactions .................................................................................. 44
12.8.1 Toxicities of capecitabine ....................................................................... 44
12.8.2 Interactions of capecitabine ..................................................................... 46
12.9 Preparation and labelling ............................................................................... 46
12.10 Storage and drug accountability ................................................................. 46
12.11 Blinding ....................................................................................................... 46

13 Study procedures .......................................................................................... 47
13.1 Screening phase ......................................................................................... 47
  13.1.1 Informed consent for screening ......................................................... 47
  13.1.2 Screening visit ................................................................................... 47
  13.1.3 Screening in platform screening trials .............................................. 48
13.2 Randomised phase ..................................................................................... 48
  13.2.1 Informed consent for the randomised phase .................................. 48
  13.2.2 Randomisation visit ......................................................................... 49
  13.2.3 Randomisation .................................................................................. 50
  13.2.4 Treatment visit ................................................................................. 50
  13.2.5 End of treatment (EOT) visit ............................................................ 50
  13.2.6 Follow-up visit .................................................................................. 51
  13.2.7 Long term follow up ........................................................................ 52
13.3 Assessment of efficacy ............................................................................. 52
13.4 Assessment of safety ................................................................................ 52
13.5 Central laboratory ...................................................................................... 53
13.6 Treatment after the trial .......................................................................... 53
13.7 Lost to follow-up ....................................................................................... 53
13.8 Termination of the trial for individual patients ....................................... 54
13.9 Early termination of the trial .................................................................. 54
13.10 Pregnancies and Contraception ............................................................ 54

14 Adverse events .............................................................................................. 56
14.1 Definitions .................................................................................................. 56
14.2 Documentation of AE's ........................................................................... 57
14.3 Documentation of SAE's ......................................................................... 57
14.4 Reporting of adverse events ..................................................................... 58
  14.4.1 Reporting Obligations of Study Site ................................................. 58
  14.4.2 Reporting Obligations of Sponsor ................................................... 58
14.5 Data Safety Monitoring Board (DSMB) .................................................. 60

15 Documentation .............................................................................................. 61
15.1 Patient list .................................................................................................. 61
15.2 Patient identification ................................................................................ 61
15.3 Case report forms ...................................................................................... 61
  15.3.1 Documentation of the screening ....................................................... 61
  15.3.2 Documentation of the randomised part ......................................... 62
  15.3.3 Source documents and background data ....................................... 62
15.4 Investigator's Files / Retention of documents ......................................... 63
15.5 Confidentiality of trial documents and patient records .......................... 63

16 Monitoring and audits .................................................................................. 64
17 Statistics and publication ............................................................................. 65
17.1 Sample size calculation ................................................................. 65
17.2 Patient registration ................................................................. 65
17.3 Randomisation stratification ......................................................... 65
17.4 Planned interim or sequential analysis ............................................ 66
17.5 Analysis populations and subgroups ............................................ 66
   17.5.1 Full analysis set (FAS) ...................................................... 66
   17.5.2 Per protocol analysis set (PPA) .......................................... 66
   17.5.3 Safety evaluation set (SES) ............................................... 66
   17.5.4 Subgroups ................................................................. 66
17.6 Data handling ........................................................................... 66
   17.6.1 Handling of missing data an outliers ..................................... 66
17.7 Variables for analysis ................................................................. 67
   17.7.1 Disposition of subjects ....................................................... 67
   17.7.2 Extent of exposure .......................................................... 67
   17.7.3 Primary endpoint: disease free survival ............................... 68
   17.7.4 Secondary endpoints ...................................................... 68
17.8 Statistical analysis methods .......................................................... 68
   17.8.1 General design of descriptive statistics .............................. 68
   17.8.2 Disposition of subjects ................................................. 68
   17.8.3 Evaluation of demographics and baseline characteristics ...... 68
   17.8.4 Primary endpoint: disease free survival ............................ 69
   17.8.5 Secondary endpoints .................................................... 69
   17.8.6 Sensitivity analysis of the primary variable ......................... 70
   17.8.7 Exploratory analyses ..................................................... 70
   17.8.8 Analysis of safety data .................................................. 71
17.9 Publications .............................................................................. 72

18 Translational research ................................................................. 73
   18.1 Conversion of ctDNA positive patients ................................... 73
   18.2 ctDNA level before recurrence .............................................. 73
   18.3 ctDNA level at time of enrolment and diagnosis ..................... 73
   18.4 Remaining material ............................................................ 73

19 Ethical and administrative aspects ................................................. 74
   19.1 Responsibility of the sponsor and the investigator .................. 74
   19.2 Patient information and Informed Consent .............................. 74
   19.3 Data Protection and Confidentiality ........................................ 75
   19.4 Insurance for trial participants ............................................. 76
   19.5 Study discontinuation ........................................................ 76
   19.6 Independent ethics committees and regulatory authorities ....... 77
      19.6.1 Approval of the study by the regulatory authority and EC .. 77
      19.6.2 Notification of the study .............................................. 77
      19.6.3 Report and documentation obligation ............................ 77
   19.7 Conditions for modifying the protocol ................................... 77

20 References .................................................................................. 78
Appendix: Sampling and shipment of ctDNA ................................................................. 81
Appendix: Shipment of the tumour block ................................................................. 82
Appendix: Central pathology ...................................................................................... 83
Appendix: ctDNA analysis ....................................................................................... 84
  24.1 Isolation of cfDNA from plasma ...................................................................... 84
  24.2 PCR and NGS-based detection of tumour specific single nucleotide variants (SNVs) 84
Appendix: Analysis of safety data ........................................................................... 86
Summary of Protocol Changes ............................................................................... 87
  26.1 Version 2.0 (11 Nov 2019) ............................................................................ 87
## 2 Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>5-FU</td>
<td>5-fluorouracil</td>
</tr>
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<td>AIO</td>
<td>Arbeitsgemeinschaft Internistische Onkologie (Working group Medical Oncology) of the Deutsche Krebsgesellschaft (German Cancer Society)</td>
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<tr>
<td>ABCSG</td>
<td>Austrian Breast and Colon Study group</td>
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<tr>
<td>AE</td>
<td>adverse event</td>
</tr>
<tr>
<td>ALAT</td>
<td>alanine aminotransferase</td>
</tr>
<tr>
<td>AMG</td>
<td>Arzneimittelgesetz (German, drug law)</td>
</tr>
<tr>
<td>AR</td>
<td>adverse reaction</td>
</tr>
<tr>
<td>ASAT</td>
<td>aspartate aminotransferase</td>
</tr>
<tr>
<td>ASA</td>
<td>acetylsalicylic acid (one trade name: aspirin)</td>
</tr>
<tr>
<td>BfArM</td>
<td>Bundesinstitut für Arzneimittel und Medizinprodukte (Federal institute for drugs and medical products, Germany)</td>
</tr>
<tr>
<td>BRAF</td>
<td>the gene B-Raf encoding the serine/threonine-protein kinase B-Raf</td>
</tr>
<tr>
<td>CA</td>
<td>competent authority</td>
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<tr>
<td>CapOx</td>
<td>Capecitabine and oxaliplatin</td>
</tr>
<tr>
<td>CDX2</td>
<td>a homebox protein, expressed in more differentiated colon cancer</td>
</tr>
<tr>
<td>CEA</td>
<td>carcinoembryonal antigen</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CRF</td>
<td>case report form</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>CTC</td>
<td>common toxicity criteria</td>
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<tr>
<td>ctDNA</td>
<td>circulating tumour DNA</td>
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<tr>
<td>DFS</td>
<td>Disease free survival</td>
</tr>
<tr>
<td>DLR</td>
<td>Deutsches Zentrum für Luft- und Raumfahrt (German Aerospace Centre, project agency for the research grant for this trial)</td>
</tr>
<tr>
<td>dMMR</td>
<td>mismatch repair deficiency</td>
</tr>
<tr>
<td>DNA</td>
<td>Desoxyribonucleic acid</td>
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<tr>
<td>DPD</td>
<td>dihydropyrimidine dehydrogenase</td>
</tr>
<tr>
<td>DSMB</td>
<td>Data safety and monitoring board</td>
</tr>
<tr>
<td>DSUR</td>
<td>Development Safety Update Report</td>
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<tr>
<td>EC</td>
<td>ethics committee</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiogram</td>
</tr>
<tr>
<td>eCRF</td>
<td>electronic case report form</td>
</tr>
<tr>
<td>EOT</td>
<td>End of treatment visit</td>
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<tr>
<td>ESMO</td>
<td>European Society for Medical Oncology</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>EudraCT</td>
<td>European Union Drug regulating authorities clinical trials, a register for clinical trials</td>
</tr>
<tr>
<td>FAS</td>
<td>full analysis set</td>
</tr>
<tr>
<td>FFPE</td>
<td>formalin fixed paraffin embedded (tissue)</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>Gpt</td>
<td>Gigaparticle</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonization</td>
</tr>
<tr>
<td>ILCO</td>
<td>Deutsche ILCO e.V. (ileum and colon, German patients organisation for patients with ileo- or colostoma or colon cancer)</td>
</tr>
<tr>
<td>ISF</td>
<td>investigator site file</td>
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<tr>
<td>KKS</td>
<td>Koordinierungszentrum für Klinische Studie (Coordination unit for clinical trials)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
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<tr>
<td>KRAS</td>
<td>the gene Kirsten RAt Sarcoma virus oncogene</td>
</tr>
<tr>
<td>LKP</td>
<td>Leiter der Klinischen Prüfung (Coordinating investigator the trial)</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
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<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MSI</td>
<td>microsatellite instability</td>
</tr>
<tr>
<td>MSI-H</td>
<td>microsatellite instability high</td>
</tr>
<tr>
<td>MSS</td>
<td>microsatellite stable</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute (USA)</td>
</tr>
<tr>
<td>NCT</td>
<td>National Center for Tumor diseases</td>
</tr>
<tr>
<td>NGS</td>
<td>next generation sequencing</td>
</tr>
<tr>
<td>NA</td>
<td>not applicable</td>
</tr>
<tr>
<td>ND</td>
<td>not done</td>
</tr>
<tr>
<td>NR</td>
<td>not reported</td>
</tr>
<tr>
<td>NRAS</td>
<td>the gene neuroblastoma RAS viral oncogene</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PPA</td>
<td>per protocol analysis set</td>
</tr>
<tr>
<td>PS</td>
<td>performance status</td>
</tr>
<tr>
<td>RFS</td>
<td>recurrence free survival</td>
</tr>
<tr>
<td>SAE</td>
<td>serious adverse event</td>
</tr>
<tr>
<td>SES</td>
<td>safety analysis set</td>
</tr>
<tr>
<td>SmPC</td>
<td>summary of product characteristics</td>
</tr>
<tr>
<td>SUSAR</td>
<td>serious unexpected adverse reaction</td>
</tr>
<tr>
<td>TP53</td>
<td></td>
</tr>
<tr>
<td>TU</td>
<td>Technical University</td>
</tr>
<tr>
<td>UAR</td>
<td>unexpected adverse reaction</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
3 Protocol signatures

The following persons confirm with their signature that they agree with the content of the clinical trial protocol CIRCULATE (TUD-CIRC01-071), version 2.0 (11 Nov 2019).

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date, signature

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[Signature]
date, signature

Michael Kramer
Trial statistician

[Signature]
date, signature
3.1 Protocol signature (Investigator)

The following persons confirm with their signature that they have read and understood the trial protocol and that they agree with the content of the clinical trial protocol CIRCULATE (TUD-CIRC01-071), version 2.0 (11 Nov 2019).

They confirm that they will ensure that all patients enrolled at their trial site are treated, followed up and documented according to the above mentioned protocol.

Furthermore, they confirm that persons dependent on the sponsor or on the investigator are not enrolled into the CIRCULATE trial.

____________________________ ____________________________
Investigator (full name)           date, signature

____________________________ ____________________________
Deputy (full name)                date, signature

____________________________ ____________________________
Deputy2 (full name)               date, signature
if applicable

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Institution
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Melanie Frömmrich – tel.: +49 351 458 4428; Melanie.Froemmrich@ukdd.de
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Tel: +49 234 302 4800
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Tel.: +49 351 458 3004
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5.7 Financing of the study
The study is financed by a grant of the German Federal Ministry for Education and Research with the responsible project agency of the German Aerospace Centre (DLR): 01KG1817
### 6 Synopsis

<table>
<thead>
<tr>
<th>Title</th>
<th>Circulating tumour DNA based decision for adjuvant treatment in colon cancer stage II evaluation (CIRCULATE) AIO-KRK-0217</th>
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<td>Sponsor</td>
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<td>Study codes:</td>
<td>Sponsor code: TUD-CIRC01-071 EudraCT number: 2018-003691-12 Arbeitsgemeinschaft Internistische Onkologie (AIO): AIO-KRK-0217</td>
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<td>Study type</td>
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<tr>
<td>Objectives</td>
<td>The study evaluates the adjuvant therapy in patients with colon cancer UICC stage II. Primary: To compare the disease free survival (DFS) in patients who are positive for ctDNA (ctDNApos) after the resection of the primary tumour with vs. without adjuvant chemotherapy Secondary: a) to compare the overall survival in colon cancer patients stage II in ctDNA positive patients with and without chemotherapy b) to determine the disease free survival in ctDNA negative patients c) to determine the overall survival of ctDNA negative patients d) to compare the disease free and overall survival in patients without adjuvant therapy according to their ctDNA status e) to compare the site of metastases according to the way of metastases (heamato- vs lymphogenic vs. local/peritoneal) and ctDNA status f) to determine the chemotherapy safety. Translational aims: g) to determine the rate and time of conversion to ctDNA negativity during chemotherapy h) ctDNA level before recurrence i) to determine the DFS according to the ctDNA level j) to explore further molecular tissue and plasma marker that are prognostic for recurrence or predictive for the</td>
</tr>
</tbody>
</table>
chemotherapy effect such as CDX2 in the tissue, immune infiltrating cells, immune related polymorphisms

**Interventions**

Patients with resected colon cancer stage II are screened. The paraffin embedded tumour block is tested for microsatellite instability (MSI) and by panel sequencing for frequent tumour mutations (i.e. KRAS, NRAS, BRAF, PIK3CA, TP53...). A patient specific mutation will be analysed in postoperative plasma samples by ultra-deep sequencing to determine the presence of the patient specific mutation (TP53, KRAS...).

This screening should preferably be initiated before the patient is discharged from the surgical ward, but can be initiated five weeks after resection at the latest. It can alternatively be performed within another screening study protocol, if the identical procedures are performed and the patient had agreed into the transfer of the data.

Three to eight weeks after resection, baseline visit for the randomised phase is performed (i.e. at the Oncology Department). At this time, the patient signs a second informed consent for the randomised part of the study and are the inclusion / exclusion criteria for the randomised part checked including whether the patient would be fit for a chemotherapy.

Patients who are positive for postoperative ctDNA (ctDNApos) and not microsatellite instable are randomized (2:1) to adjuvant chemotherapy with capecitabine or to follow up. Patients negative for postoperative ctDNA (ctDNAneg) are randomized (1:4) to follow-up within CIRCULATE or to routine follow up outside the trial protocol.

Experimental intervention:
- Capecitabine in ctDNApos patients (additional oxaliplatin allowed) and follow-up within the trial

Standard intervention: Follow-up (no chemotherapy)

Follow-up per patient: 5 years

Duration of intervention per patient:
- 6 months capecitabine, in combination with oxaliplatin
- 3 to 6 months capecitabine

**Inclusion criteria for screening**

A patient who meets all of the following criteria may be included in the screening:

1) Resected colon cancer stage II, OR
   Resected rectal cancer stage II, if there was no indication for radiotherapy (i.e. due to the localisation in the upper third of the rectum), so that the treatment follows the recommendations for colon cancer.
   Patients, in whom the tumour stage is not yet know, can be enrolled into the screening.

2) Signed informed consent for the screening phase
### Exclusion criteria for screening

A patient who meets any of the following criteria will be excluded from screening:

1. Patients with known microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR)
2. Known clinical high risk situation if it is regarded as certain indication for an adjuvant chemotherapy
3. Patients, who have an obvious contra-indication for adjuvant chemotherapy (i.e. due to the performance status, comorbidity, active second cancer or age)  
   It should be considered that patients with an age of more than 75 years frequently not fulfil criteria for adjuvant chemotherapy.
4. R1- or R2- status.  
   (Patients with [still] unknown R-status can be screened)
5. Patients, in whom the randomisation or chemotherapy is unfeasible due to logistic reasons (travel distance, compliance)
6. Age < 18 years
7. Pregnant or breast feeding patients

### Inclusion criteria for randomised phase

A patient who meets all of the following criteria may be included in the randomised phase of the study:

1. Resected colon cancer stage II, 
   OR  
   Resected rectal cancer stage II, if there was no indication for radiotherapy (i.e. due to the localisation in the upper third of the rectum), so that the treatment follows the recommendations for colon cancer.
2. Known microsatellite or mismatch repair status
3. Confirmation, that the ctDNA result is available
4. Signed second informed consent (for the randomised phase)

### Exclusion criteria for randomised phase

A patient who meets any of the following criteria will be excluded from the randomised phase of the study:

1. Patients with microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR)
2. Known clinical high risk situation if it is regarded as certain indication for an adjuvant chemotherapy
3. R1- or R2- status, or unknown R-status (Rx)
4. Number of investigated lymph nodes < 10
5. WHO performance status ≥ 2
6. Colon or rectal cancer with UICC stage III or IV
<p>| | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
</table>
| 7) | Second cancer, except  
|   | a. simultaneous or metachronous colon or rectal cancer with UICC stage ≤ I,  
|   | b. curatively treated basal cell carcinoma or squamous cell carcinoma of the skin and in-situ cervical carcinoma  
|   | c. tumours with a disease free survival of more than five years  
| 8) | Contraindications for chemotherapy, especially:  
|   | a. Leukocytes < 3,0 Gpt/l  
|   | b. Neutrophil granulocytes < 1,5 Gpt/l  
|   | c. Thrombocytes < 100 Gpt/l  
|   | d. ALAT or ASAT > 3 x ULN  
|   | e. Creatinine clearance (calculated according Cockcroft-Gault) < 30 ml/min  
| 9) | Comorbidities relevantly interfering with the prognosis of the patients, i.e.:  
|   | a. heart insufficiency NYHA III/IV  
|   | b. relevant coronary heart disease,  
|   | c. Diabetes mellitus with late sequelae  
| 10) | Organ, stem cell or bone marrow transplantation  
| 11) | Known hypersensitivity to capecitabine  
|   | In case of known hypersensitivity to oxaliplatin, the patients can participate, but not receive oxaliplatin  
| 12) | Medication with brivudine, sorivudine or analogues in the last four weeks before planned treatment start  
| 13) | Known dihydropyrimidine dehydrogenase (DPD)-deficiency  
| 14) | Acute infections  
| 15) | Known HIV- infections, known active hepatitis B or C- infection  
| 16) | Participation at another interventional study for medical treatment during the last four weeks before randomisation  
| 17) | Neoadjuvant therapy before resection  
| 18) | Patients, in whom the randomisation or chemotherapy is unfeasible due to logistic reasons (travel distance, compliance)  
| 19) | Age < 18 years  
| 20) | Pregnant or breast feeding patients
21) Women of childbearing potential and men with partner with childbearing potential who are not willing to take appropriate precautions to avoid pregnancy with a highly effective method in case they are randomised to “chemotherapy”

### Endpoints and analyses

**Primary endpoint:**

Disease free survival of ctDNA positive patients randomised to “chemotherapy” vs. “follow-up”, measured from randomisation to any recurrence, metastasis, second colorectal or non colorectal cancer and death from any cause. The primary endpoint will be tested in all randomised ctDNA positive patients and be evaluated by a stratified log rank test.

**Secondary endpoints:**

- a) Overall survival in ctDNApos patients with adjuvant therapy vs follow-up, measured from randomisation to death from any cause, in all randomised ctDNA positive patients and be evaluated by a stratified log rank test.
- b) Disease free survival in ctDNAneg patients randomised to follow up (rate of patients disease free and alive 3 years after randomisation according to Kaplan-Meier estimation with 95% CI, intention-to-treat analysis)
  - Any recurrence, metastasis, second colorectal or non-colorectal cancer and death from any cause is regarded as event
- c) Overall survival in ctDNAneg patients randomised to “follow up” (rate of patients alive after 5 years after randomisation according to Kaplan-Meier estimation with 95% CI)
- d) Disease free and overall survival of ctDNApos vs. ctDNAneg patients randomized to „follow-up” (measured from randomisation to the event in an intention-to-treat analysis by stratified log rank test).
  - Any recurrence, metastasis, second colorectal or non-colorectal cancer and death from any cause are regarded as event for DFS. Death of any cause will be regarded as event for overall survival.
- e) Site of metastases (lymph node vs. peritoneal/local recurrence vs other) in ctDNApos vs. ctDNAneg patients who have a recurrence / metastases
- f) Frequency of adverse events from start of chemotherapy until 30 days after chemotherapy (descriptive analysis for patients randomised to “chemotherapy” who have received at least one dose of chemotherapy).

**Translational endpoints:**

- a) Rate of patients in which ctDNA becomes non-measurable during or after chemotherapy (measured in ctDNApos patients receiving chemotherapy) and time to the first negative sample
- b) ctDNA level before recurrence
<table>
<thead>
<tr>
<th>Analysis of the primary endpoint</th>
<th>Stratified log rank test in the intention to treat population with a global one-sided alpha error of 0.025 after 154 events (approximately 5 years after study start), one interims analysis after 60% of the events (approximately 38 months after study start).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient numbers</td>
<td><strong>ctDNApos patients to be randomised:</strong> n=231 (number of patients for primary endpoint)</td>
</tr>
<tr>
<td></td>
<td>- patients randomised to chemotherapy: n=154</td>
</tr>
<tr>
<td></td>
<td>- patients randomised to follow-up: n=77</td>
</tr>
<tr>
<td></td>
<td><strong>ctDNAneg patients to be randomised:</strong> n≈2079</td>
</tr>
<tr>
<td></td>
<td>- patients randomised to follow-up: n=416</td>
</tr>
<tr>
<td></td>
<td>- patients randomised to off-study: n=1663</td>
</tr>
<tr>
<td>MSS patients screened for randomisation:</td>
<td>n=2880</td>
</tr>
<tr>
<td>MSI-H and MSS patients stage II:</td>
<td>n=3609</td>
</tr>
<tr>
<td>Patients entering screening:</td>
<td>n=4812</td>
</tr>
<tr>
<td>Analysis for secondary endpoint DFS in ctDNAneg patients:</td>
<td>n ≈ 416</td>
</tr>
<tr>
<td>Time lines</td>
<td><strong>First patient in (FPI):</strong> December 2019</td>
</tr>
<tr>
<td></td>
<td><strong>Last patient in (LPI):</strong> February 2023</td>
</tr>
<tr>
<td></td>
<td><strong>Last patient last visit:</strong> October 2023</td>
</tr>
<tr>
<td></td>
<td><strong>Follow-up until:</strong> March 2025</td>
</tr>
<tr>
<td>Study drug</td>
<td>Capecitabine, i.e. Xeloda</td>
</tr>
</tbody>
</table>
## 7 Visit overview

### Table 1: Schedule of visits

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Time point</th>
<th>Screening visit</th>
<th>Randomisation visit&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Treatment visits&lt;sup&gt;b&lt;/sup&gt;</th>
<th>End of treatment (EOT)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Follow-up visits&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Long term follow-up&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 days to 5 weeks after resection</td>
<td>3-8 weeks after resection</td>
<td>every 3 weeks</td>
<td>30 days after last treatment</td>
<td>every 6 months</td>
<td>year 1-5</td>
<td>every year, year 5-10</td>
</tr>
</tbody>
</table>

### Screening phase

- Informed consent for screening: x
- Eligibility criteria for screening: x
- Shipment of tumour block: x
- 2 plasma samples for ctDNA, shipment: x

### Randomised phase<sup>a</sup>

- Informed consent: x
- Eligibility criteria for randomisation: x
- Demographic data/comorbidity: x
- Height, weight, performance status: x
- Tumour history<sup>1</sup>: x
- Safety laboratory<sup>2</sup>: x x x
- Discussion fertility, preservation<sup>3</sup>: x x<sup>4</sup>
- Pregnancy test<sup>5</sup>: x x x
- Randomisation: x
- Study drug<sup>6</sup>: x
- Documentation of compliance<sup>7</sup>: x x
- Adverse events: x x
- Thoracic / abdominal imaging<sup>8</sup>: x x
- CEA: x x
- ctDNA samples, shipment<sup>9</sup>: x x x x
- Survival status<sup>10</sup>: x x

<sup>a</sup> for patients in stage II, only – and not for patients with microsatellite instability or delayed recovery from resection

<sup>b</sup> for patients randomised to „chemotherapy‟, only

<sup>c</sup>
5 for patients randomised to “chemotherapy” or “follow-up”, only
1 including CEA before resection
2 Blood count including haemoglobin, leukocytes, thrombocytes, neutrophils, creatinine, bilirubin, ALAT, ASAT
3 For patients with planned oxaliplatin and planned fatherhood / pregnancy, especially
4 Before the first cycle
5 in women, except after hysterectomy or postmenopausal (no menses for ≥ 12 months without an alternative medical cause)
6 6 months capecitabine, 3 or 6 months capecitabine/oxaliplatin allowed
7 patient diary
8 Abdominal ultrasound; CT or MRI allowed if local standard or medical indication, only
9 for translational research, only
10 Telephone contact with patient or general practitioner, no physical visit
8 Introduction

8.1 Background

8.1.1 Burden of disease

With an estimated number of 446,000 new patients in Europe and 60,580 new patients registered in Germany, colorectal cancer is the second most common cancer in Europe and in Germany. It leads to death in 214,000 patients in Europe (2012) – accounting for 13% of all cancer deaths – and is one of the two most common causes for cancer deaths in Europe¹ and in Germany (25,262 deaths in 2013).² Patients with colon cancer represent approx. 68% of all colorectal cancer patients, 36% of colon cancer patients are diagnosed at stage II³ (estimate for Germany: 15,000 per year).² Death after recurrence following stage II colon cancer contributes approx. 16% to the mortality from colon cancer.³ This would result in Germany in approximately 2,800 deaths per year – similar to all deaths from melanoma,² and approximately 23,000 deaths / year in Europe.

8.1.2 Adjuvant therapy in stage III

A postoperative – adjuvant – chemotherapy results in patients with resected primary tumour with locoregional lymph nodes metastases, but no distant metastases (UICC stage III) in a better overall survival. In the IMPACT meta analysis, the disease free survival was improved from 44% (without adjuvant therapy) to 62% with adjuvant 5-FU- based chemotherapy (hazard ratio 0.55 [95% CI: 0.44 – 0.70]. The 3 year overall survival increased from 64% to 76% (hazard ratio 0.70 [95% CI: 0.53 – 0.92]).⁴ Further improvement was achieved by adding oxaliplatin to the chemotherapy.⁵,⁶ In the MOSAIC study, the 6 year survival of colon cancer patients was improved from 69% to 73% (hazard ratio 0.80 [95% CI: 0.65 – 0.97]).⁵ It seems to be possible to substitute the infusional 5-FU by the oral prodrug capecitabine and to maintain the efficacy of in monotherapy⁷ or in combination with oxaliplatin.⁸ The common duration of adjuvant treatment of 6 months was questioned by a recent pre-planned meta-analysis of six studies with more than 13,000 patients, in which formally no non-inferiority was demonstrated for the shorter treatment duration. Remarkably, the difference in the 3 year disease free survival was 0.9 per cent points between six and three months treatment, only (75,5% vs. 74,6%, Hazard Ratio 1,07, 95% CI 1,00 – 1,15). Furthermore, a statistically significant interaction could be demonstrated between the fluoropyrimidine regimen (capecitabine or 5-FU) and treatment efficacy with a proven non-inferiority for 3 months capecitabine / oxaliplatin vs. the 6 months treatment with the same regimen. A non-significant interaction was detected for the tumour stage. In more than 7400 patients with a stage T1-3N1, the hazard ratio was 1.01 and the confidence interval did not cross the pre-defined boundaries for non-inferiority [95% CI
0.90 – 1.12]. There is a consensus to shorten the adjuvant therapy at least for these patients with “good” prognosis.

For elderly patients (age ≥70 age), an adjuvant therapy with oxaliplatin is – in contrast to the situation in younger patients – not associated with a survival benefit but rather with deleterious effect on overall survival (hazard ratio for overall survival with or without oxaliplatin 1.04 [95% CI 0.85 – 1.27]). There are limited data only, to shorten the adjuvant therapy in patients treated with a fluoropyrimidine monotherapy. A continuous 5-FU infusion had demonstrated a trend to a better survival than the former standard of 6 months bolus-5-FU.10

8.1.3 Adjuvant therapy in stage II

In contrast to stage III, there is no agreement whether to treat patients with locally advanced tumours (T3-4) without lymph node metastases (UICC II) with adjuvant therapy. A meta-analysis published in 2004 described a trend towards a better overall survival (hazard ratio 0.87 [95% CI: 0.75 – 1.01]).11 The later published QUASAR study demonstrate in 1073 patients with stage II tumours a similar trend to a better survival with adjuvant chemotherapy (hazard ratio 0.82 [95% CI 0.63 – 1.08]).12 The difference in the 5 year survival did not exceed 2 to 3 per cent points and is not regarded as sufficient for a general recommendation towards an adjuvant chemotherapy.

For patients in UICC stage II, who have – based on T4- tumour stage, an emergency resection or intraoperatively tumour perforation – a higher risk for recurrence, an adjuvant therapy is frequently recommended, even in absence of clear data supporting this recommendation.

An additional treatment with oxaliplatin was not associated with an improved overall survival in stage II patients (hazard ratio 1.004 [95% CI 0.744 – 1.354]) and was in patients with „low risk stage II“ rather detrimental (hazard ratio 1.168 [95% CI 0.730 – 1.870]), while there was a slight trend towards a better survival in the above mentioned group of patients with a clinical/pathological high risk stage II colon cancer treated with oxaliplatin (hazard ratio 0.895 [95% CI 0.606 – 1.323]).6

According to the German S3 guideline, an adjuvant therapy should be „considered“ in stage II – and then (according to the risk) be given as monotherapy.13 The ESMO guideline state that “adjuvant therapy should not be routinely recommended for unselected patients. In high-risk patients who present at least one of the […] clinical high-risk features […], adjuvant therapy could be considered in clinical practice.”14

In patients with a microsatellite unstable tumour (MSI-H), a negative effect of a an adjuvant, 5-FU based therapy was reported.15 Therefore, patients with MSI-H tumours are not randomised in this study.

Other prognostic tests as PIK3CA- mutations (and the use of ASA)16 or CDX217 as criterion are currently not validated for decision making towards or against an adjuvant therapy. The recurrence score based on gene expression analysis correlates with the prognosis of stage II patients,18,19 but is – due to the limited difference in high and low risk patients not recommended in current guidelines.13 Thus, a method that identifies patients with high risk is urgently needed.
8.1.4 Circulating tumour (ct) DNA for minimal residual disease

Treatment according to minimal residual disease (MRD) or according to molecular response is in different hematologic tumour diseases a well-established principle, i.e. in acute lymphoblastic leukaemia or acute or chronic myeloid leukaemia.\(^{20}\)

With the increasing sensitivity of next generation sequencing (NGS)- techniques, circulating tumour DNA (ctDNA) can be measured in the plasma, i.e. tumour specific TP53-, KRAS-, NRAS- or BRAF- mutations. In a study published 2015, KRAS mutations could be found in 106 patients with a sensitivity of 98% and a specificity of 92% (compared to the tumour tissue).\(^{21}\) The amount of ctDNA correlates with tumour stage and survival.\(^{22}\) In addition, a correlation with tumour progression can be demonstrated in metastatic colorectal carcinoma.\(^{23}\)

A recently published observational study demonstrated a highly prognostic effect of ctDNA for recurrence or metastases. In this trial, the mutations known from a mutational panel analysis of the paraffin embedded primary tumour were measured in postoperative plasma samples of stage II patients. Out of 230 patients not treated with chemotherapy, ctDNA was detected in 8% of the patients. The estimated recurrence free survival in ctDNA positive patients not

![Figure 1: Prognosis of patients with colon cancer stage II according to postoperative ctDNA\(^{40}\)](image-url)

(A) Recurrence free survival (RFS) according to ctDNA, (B) RFS according to clinical risk factors, (C) and (D) RFS according to ctDNA in patients with low and high clinical risk.
treated with chemotherapy was zero compared to 90% in ctDNA neg patients (Figure 1, hazard ratio 18; 95% CI 7.9-40, P=2.6x10^{-12}). In ctDNA pos patients treated with chemotherapy, ctDNA became negative during adjuvant therapy in all patients.24 A similarly high prognostic effect was reported after resection of rectal cancer,25 was reproduced by another group for colorectal cancer26 and was known from patients with breast cancer.27

In patients with colon cancer stage III, who were postoperatively ctDNA positive (and received adjuvant chemotherapy), the RFS after three years was 47%. Patients, who became ctDNA negative during adjuvant chemotherapy, had a RFS of 59%, patients remaining ctDNA positive despite adjuvant therapy had a RFS after 2 years of 33%.28

8.2 Need for a trial

Adjuvant chemotherapy has been demonstrated to improve the overall survival in patients resected for the primary tumour with regional lymph node metastases (stage III).4 In patients with stage II colon cancer, adjuvant treatment is controversially discussed and is not a generally accepted treatment standard due to a borderline improvement of overall survival if all stage II patients would receive chemotherapy after resection.29,30 The risk stratification criteria according to clinical / pathological risk factors or the recurrence scores are not prospectively evaluated as predictive tools for the selection of patients for adjuvant treatment and do not provide a convincing separation of patients with high and low risk for recurrence.

Thus, a better risk stratification and consecutive selection of patients who actually benefit from adjuvant chemotherapy is needed. ctDNA can reliably be measured in the plasma of patients, is highly specific and prognostic for recurrences.

The CIRCULATE study investigates the treatment efficacy in patients who are at higher risk according to the ctDNA in postoperative blood samples of patients with stage II colon cancer. The generated knowledge has the potential to improve the treatment of colon cancer patients in stage II and can be a model for similar treatment situations as after resection of rectal cancer, after resection of liver metastases or potentially for patients with colon cancer stage III.

8.3 Potential risks and benefit

8.3.1 Screening of patients

During screening, a tumour block is shipped, that is prepared routinely for pathological diagnostics. This block is transferred to one of the central pathology laboratories.

In addition, a blood sample of less than 20 ml blood is sampled in two special tubes. Mostly, this sampling can be performed at the time of a routine blood test so that mostly an additional venous puncture is not necessary.

Furthermore, the patients asked to have an appointment at an oncologist approximately four weeks after resection to discuss the option for adjuvant treatment and the potential enrolment into the randomised part of the study. The only physical risk for the patients consists in the potential venous puncture and is similar to many observational studies.
8.3.2 Exclusion of patients with MSI-H tumours

Patients with resected microsatellite instable (MSI-H) colon cancer have a relatively good prognosis. For patients with resected stage II, MSI-H colon cancer had a worse prognosis if treated with adjuvant 5-FU. There is a high correlation with mismatch repair (MMR) deficient tumours. Therefore, patients with MSI-H or deficient MMR tumours are excluded from the study.

8.3.3 Inclusion of patients with rectal cancer

For patients with the primary tumour in the upper part of the rectum, there is currently no indication for an adjuvant or neoadjuvant radiotherapy. It is generally recommended to treat these patients like colon cancer patients. This applies also for patients in whom based on the preoperative MRI no radiation therapy was administered if they do not qualify for postoperative radiotherapy.

8.3.4 No chemotherapy in the standard arm

In stage II colon cancer, there is no general recommendation towards an adjuvant chemotherapy. According to the German S3 guideline, an adjuvant chemotherapy should be considered in patients with high clinical risk. Patients, in whom with this background a certain indication for an adjuvant therapy is seen by the investigator and/or the physician, are excluded from the trial.

8.3.5 Chemotherapy in ctDNA positive patients (experimental arm)

Adjuvant chemotherapy in stage II is – with the relatively favourable risk – mostly a fluoropyrimidine monotherapy. Due to the relatively high risk of ctDNA positive patients, that is higher than those of stage II patients, adding oxaliplatin is allowed. So that the therapy reflects the standard treatment for stage III patients – even more with the background to shorten the adjuvant therapy to three months.

In stage III, the effect of any adjuvant chemotherapy (studies comparing 5-FU with no adjuvant therapy) was substantially higher than those of adding oxaliplatin. Thus, a relevant influence on the study effect is not expected and adding oxaliplatin at the discretion of the investigator. Because the choosing oxaliplatin as additional treatment might be dependent on the performance status and risk of the patients, the plan to add / not to add oxaliplatin will be one stratification factor.

Patients being ctDNA positive will receive adjuvant therapy within the study with a probability of 67%. This will apply to approximately 7% of all patients randomised in the study.

8.3.6 Chemotherapy toxicity in the experimental arm

There are extensive safety data on adjuvant therapy with capecitabine monotherapy (i.e. 995 patients for the registration trial, 938 patients for the registration trial combination with oxaliplatin in the adjuvant setting, and recently 5071 patients with the combination treated in the trials of the IDEA initiative. Additional safety data are available from patients treated for metastatic disease. With the given low patient number of 154 patients, it is unlikely that new safety signals regarding chemotherapy toxicity can be detected.
8.3.7 Randomisation of ctDNA negative patients

cDNA negative patients will be randomised to follow-up within the study or to be off-study after randomisation, because the prognosis of the ctDNA negative patients can be estimated sufficiently to achieve the study aim.

With this randomisation, the principle of data economy and of cost efficiency is respected.

8.3.8 Blinding of patients in the arm „Follow-up“

The ctDNA results are blinded to prevent a bias due to non-compliance to the randomisation result and cross-over to the active arm in ctDNA positive patients.

Patients in the arm „Follow-up“ have a prognosis that is similar to patients in stage II in whom the ctDNA test was not performed. Because ctDNA would not be tested outside the clinical trial, the patients do not have a disadvantage. In contrast, it can be assumed that the actual high risk patients receive with higher probability adjuvant therapy within this trial compared to a standard approach in routine clinical practice.

The recommended follow-up is close to the German guidelines for colon cancer. The centres are allowed to increase the frequency of follow-up visits if it is local standard and applied to all patients in both arms.

8.3.9 Plasma (ctDNA) samples during treatment and follow-up

At the visits, venous punctures are routinely performed – for the safety laboratory and the CEA so that additional venous punctures are typically not necessary.

The total amount of blood for ctDNA during all follow-up visits is approximately 120 ml (5 years), for patients in the chemotherapy arm additional 120 ml (6 months).

8.3.10 Overall judgement

Overall, patients with colon cancer stage II have an uncertain indication for chemotherapy. Therefore, currently are some patients treated with chemotherapy. The current selection for chemotherapy bases on with regard to the predictive effect not sufficiently evaluated parameters and according to the preference of the patient (and physician).

The additional intervention in the trial consists in a chemotherapy in 2/3 of the patients who have probably a higher risk of recurrence and in the follow-up of patients including the necessary diagnostics. In the vast majority of patients, the screening visit and the randomisation visit (at time of his first appointment at the oncologist) will be performed, only.
9 Study aims

9.1 Primary aim
The primary aim of the CIRCULATE study is to compare the disease free survival in patients who are positive for postoperative circulating tumour DNA with vs. without capecitabine.

9.2 Secondary aims
Secondary aims of the CIRCULATE study are,
   a) to compare the overall survival in colon cancer patients stage II in ctDNA positive patients with and without capecitabine
   b) to determine the disease free survival in ctDNA negative patients
   c) to determine the overall survival of ctDNA negative patients
   d) to compare the disease free and overall survival in patients without adjuvant therapy with capecitabine according to their ctDNA status
   e) to compare the site of metastases according to the way of metastases (heamato- vs lymphogenic vs. local/peritoneal) and ctDNA status
   f) to determine the capecitabine safety.

9.3 Translational aims
   g) to determine the rate and time of conversion to ctDNA negativity during chemotherapy with capecitabine
   h) ctDNA level before recurrence
   i) to determine the DFS according to the ctDNA level
   j) to explore further molecular tissue and plasma marker that are prognostic for recurrence or predictive for the chemotherapy effect such as CDX2 in the tissue, immune infiltrating cells, immune related polymorphisms
10 Description of the trial

10.1 Study design

CIRCULATE is an investigator-initiated, multicentre, prospective, randomised, controlled trial.

10.1.1 Screening phase

Patients with colon cancer (or rectal cancer, if a radiation is not indicated i.e. due to the tumour localisation) are postoperatively screened for this trial.

For this purpose, they sign an informed consent for screening. The FFPE tumour block is shipped to one of the central pathological laboratories and is analysed for microsatellite instability and by panel analysis for frequent mutations in the colorectal cancer. A plasma sample is sent in parallel to the central laboratory for ctDNA. The screening is preferably performed before the patient is discharged from the surgical department and at the latest 5 weeks after resection to allow sufficient time for the analysis.

The patient-specific tumour mutations known from the panel analysis are measured in the patient's plasma by ultra deep sequencing. The results of the analysis – positive for circulating tumour DNA (ctDNApos) or negative for circulating tumour DNA (ctDNAneg) – is not communicated to the patient or the investigator.

The screening may be performed in specific screening platform trials if they fulfil the criteria mentioned in chapter 11.

10.1.2 Randomised phase

Four to eight weeks after resection, the patient presents at an investigator that is experienced with chemotherapy (i.e. Medical Oncologist) and consent for the randomised part of the study with a second informed consent form. If this baseline visit confirms that there are no contraindications to chemotherapy and if no other exclusion criteria exist, the patient is randomised:

- ctDNApos patients are randomised (2:1) in “chemotherapy” (with capecitabine) or “follow-up”,
- ctDNAneg patients are randomised (1:4) in “follow-up" or “off study” which means that the follow-up will be organised within the routine clinical practice.

The result of the ctDNA will not be communicated to the patients and investigators, so that patients in the arm „follow-up“ remain blinded to the ctDNA result. Due to the randomisation ratio, the prognosis of these patients is similar to those in stage II without any ctDNA analysis and differs only slightly from patients not enrolled into a clinical trial.
Patients in the arm “chemotherapy” receive adjuvant therapy with 6 months capecitabine. The investigator can decide to add oxaliplatin and to shorten the adjuvant chemotherapy to 3 months if oxaliplatin is added.

Patients in the arms “chemotherapy” and “follow-up” are followed with the same methods and time point within the study.

Patients in the arm “off study” are recommended to be follow up according to the guidelines for stage II in the routine practice.

**Figure 2: Study scheme**

1. Patients with still unknown tumour stage can be enrolled into screening
2. Preferably before discharge from surgical ward
3. The screening can be performed within other screening platform trials
4. The result of the ctDNA test is not communicated.
5. Stratification: pT3 vs pT4, emergency resection, planned oxaliplatin treatment
6. Capecitabine based, 6 months. Oxaliplatin as investigators choice (CapOx: 3 or 6 months)

10.2 Primary criterion

The primary criterion of the study is the disease free survival of ctDNA positive patients randomised to “chemotherapy” (with capecitabine) vs. “follow-up”, measured from randomisation to any recurrence, metastasis, second colorectal or non colorectal cancer and death from any cause.

The primary endpoint will be tested in all randomised ctDNA positive patients and be evaluated by a stratified log rank test.

10.3 Secondary criteria

Secondary criteria are:
a) Overall survival in ctDNApos patients with adjuvant therapy vs follow-up, measured from randomisation to death from any cause, in all randomised ctDNA positive patients and be evaluated by a stratified log rank test.

b) Disease free survival in ctDNAneg patients randomised to follow up (rate of patients disease free and alive 3 years after randomisation according to Kaplan-Meier estimation with 95% CI, intention-to-treat analysis)

Any recurrence, metastasis, second colorectal or non-colorectal cancer and death from any cause is regarded as event

c) Overall survival in ctDNAneg patients randomised to “follow up” (rate of patients alive after 5 years after randomisation according to Kaplan-Meier estimation with 95% CI)

d) Disease free and overall survival of ctDNApos vs. ctDNAneg patients randomized to „follow-up“ (measured from randomisation to the event in an intention-to-treat analysis by stratified log rank test).

Any recurrence, metastasis, second colorectal or non-colorectal cancer and death from any cause are regarded as event for DFS. Death of any cause will be regarded as event for overall survival.

e) Site of metastases (lymph node vs. peritoneal/local recurrence vs other) in ctDNApos vs. ctDNAneg patients who have a recurrence / metastases

f) Frequency of adverse events from start of chemotherapy until 30 days after chemotherapy (descriptive analysis for patients randomised to “chemotherapy” who have received at least one dose of chemotherapy).

10.4 Translational endpoints:

g) Rate of patients in which ctDNA becomes non-measurable during or after chemotherapy (measured in ctDNApos patients receiving chemotherapy) and time to the first negative sample

h) ctDNA level before recurrence

i) DFS according to ctDNA

j) Correlation of further molecular tissue and plasma marker to the risk of recurrence or metastases or the effect of chemotherapy (exploratory analysis).

10.5 Patient number

10.5.1 Patient number for ctDNA positive patients

The sample size was calculated for the comparison of DFS in ctDNA positive patients by study arm. The primary efficacy analysis will be done with a stratified log-rank test. The critical values and the test characteristics of the group sequential test design were calculated for the O’Brien and Fleming design, an one-sided alpha = 0.025, a power (1-beta) of 0.80 and an allocation ratio of 2:1 (chemotherapy arm : standard arm). For ctDNA positive patients, the assumed event rates at 3 years were 0.75 (standard arm) and 0.575 (chemotherapy arm; hazard ratio 0.617), corresponding to a 25% DFS rate in the standard arm and 42.5% DFS rate in the chemotherapy arm. In order to show superiority of chemotherapy over standard under these conditions,
154 events have to be analysed. This number of events is expected to occur after randomisation of 231 patients (154 patients to chemotherapy and 77 patients to observation) during 36 months of accrual and a follow up period of 24 months, allowing for a dropout rate of 2% at 3 years. The calculation accounts for one interim analysis after 60% of planned events (38 months).

10.5.2 Patient number for ctDNA negative patients

Given the necessary number of 231 ctDNA positive patients and assuming that 90% of patients will be ctDNA negative, in total 2310 patients have to be randomized. The 2079 ctDNA negative patients will be randomized at a ratio of 1:4 to observation within the study (n=416) or to go off study (n=1663, Figure 3). The number of ctDNA negative patients randomised to follow-up in this clinical trial will be sufficient to estimate the relapse incidence within tight margins. Exemplarily, in simulation studies for a 3-year cumulative incidence of relapse of 10%, the 95% confidence interval ranged from 6.7% to 13.7% (3 year disease free survival 86.3 – 93.3%). This precise estimate of the risk of relapse might be sufficient to align follow-up assessments for future patients to standard procedures for patients with stage I colon cancer.
10.5.3  Estimated prognosis according to treatment groups

Based on the above-mentioned DFS of 25% for ctDNA positive patients without chemotherapy and 42.5% with chemotherapy, and the DFS of 90% for ctDNA negative patients, the following prognosis is estimated for the three arms:

a) Arm “chemotherapy”: 154 ctDNA positive patients are randomised to this arm, representing 7% of the randomised study population. The estimated 3 year DFS is 42.5%.

b) Arm „follow-up“: 77 ctDNA positive patients (estimated 3 y. DFS: 25%) and approximately 416 ctDNA negative patients (estimated 3 y. DFS: 90%) are randomised to this arm. The total number of approx. 493 patients reflects 21.3% of the randomised study population. The estimated 3 year disease free survival in this group is 80%, which is similar to the general population of stage II patients.

c) Arm „off study“: Approximately 1663 ctDNA negative patients are randomised into this arm and leave the study after randomisation.

Figure 3: Overview on the number of patients in the trial
10.5.4 Total number of patients in the study

The number of ctDNA positive patients is critical for this trial. Based on 231 ctDNA positive patients to be randomised and a rate of 10% ctDNA positive patients, approximately 2310 patients have to randomised in total.

With the assumption that 20% of the patients are microsatellite instable and that approximately additional 20% of the screened patients are not randomised due to delayed recovery from surgery, logistic reasons or no second informed consent, approximately 3609 stage II patients have to be screened. If 25% of screened patients have at randomisation no proven stage II cancer, up to approximately 4812 patients are to be screened.

10.6 Timelines

First patient screened (FPI): December 2019
Last patient randomised (LPI): February 2023
Last patient last visit (LPLV): October 2023
Events for interim analysis: December 2022
Interims analysis: June 2023
Events for full analysis: February 2025
Follow-up until March 2025
Data base cleaning: May 2025
Final analysis: August 2025
Trial closed: December 2025

10.7 Requirement on centres and investigators

10.7.1 Study centres with treatment

Study centres participating at the randomised part of the study have large experience in adjuvant treatment of colon cancer (in Germany: equivalent to certified colon cancer centres). If the unit is not part of a certified centre for colon cancer, gastrointestinal cancer or oncology centre according to the Deutsche Krebsgesellschaft, it should have similar experience and qualification.

In addition, the knowledge on good clinical practice including experience in clinical studies has to be proven.

10.7.2 Screening centres

Centres can participate at the screening, only – without the randomisation and chemotherapy. In these centres, patients will only participate for the screening phase of the trial (patient informed consent for the screening part, shipment of plasma for ctDNA and of the tumour block)
- similar to observational studies or registries with biobanking (Germany: studies according to Medical association code of conduct, Berufsohrnung).

The patient informed consent (for screening, only) will be obtained by a physician at the centre and blood / plasma will be shipped. All aspects of the Code of conduct and the data privacy have to be followed.

Patients screened at these centres will present at full study centres (with treatment) for randomisation, and sign there a second informed consent before the baseline visit and randomisation.
11 Patient population

11.1 Inclusion criteria for screening
A patient who meets all of the following criteria may be included in the screening:

1) Resected colon cancer stage II,
   OR
   Resected rectal cancer stage II, if there was no indication for radiotherapy (i.e. due to the localisation in the upper third of the rectum), so that the treatment follows the recommendations for colon cancer.
   Patients, in whom the tumour stage is not yet known, can be enrolled into the screening
2) Signed informed consent for the screening phase.

11.2 Exclusion criteria for screening
A patient who meets any of the following criteria will be excluded from screening:

1) Patients with known microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR)
2) Known clinical high risk situation if it is regarded as certain indication for an adjuvant chemotherapy
3) Patients, who have an obvious contra-indication for adjuvant chemotherapy (i.e. due to the performance status, comorbidity, active second cancer or age)
   It should be considered that patients with an age of more than 75 years frequently do not fulfil criteria for adjuvant chemotherapy.
4) R1- or R2- status.
   (Patients with [still] unknown R-status can be screened)
5) Patients, in whom the randomisation or chemotherapy in unfeasible due to logistic reasons (travel distance, compliance)
6) Age < 18 years
7) Pregnant or breast feeding patients
11.3 Inclusion criteria for randomised phase

A patient who meets all of the following criteria may be included in the randomised phase of the study:

1) Resected colon cancer stage II,
   OR
   Resected rectal cancer stage II, if there was no indication for radiotherapy (i.e. due to the localisation in the upper third of the rectum), so that the treatment follows the recommendations for colon cancer.

2) Known microsatellite status or mismatch repair status

3) Confirmation, that the ctDNA result is available

4) Signed second informed consent (for the randomised phase).

11.4 Exclusion criteria for randomised phase

A patient who meets any of the following criteria will be excluded from the randomised phase of the study:

1) Patients with microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR)

2) Known clinical high risk situation if it is regarded as certain indication for an adjuvant chemotherapy

3) R1- or R2- status, or unknown R- status (Rx)

4) Number of investigated lymph nodes < 10

5) WHO performance status ≥ 2

6) Colon or rectal cancer with UICC stage III or IV

7) Second cancer, except
   a. simultaneous or metachronous colon or rectal cancer with UICC stage ≤ I,
   b. curatively treated basal cell carcinoma or squamous cell carcinoma of the skin and in-situ cervical carcinoma
   c. tumours with a disease free survival of more than five years

8) Contra indications for chemotherapy, especially:
   a. Leukocytes < 3,0 Gpt/l
   b. Neutrophil granulocytes < 1,5 Gpt/l
   c. Thrombocytes < 100 Gpt/l
   d. ALAT or ASAT > 3 x ULN
   e. Creatinine clearance (calculated according Cockcroft-Gault) < 30 ml/min
9) Comorbidities relevantly interfering with the prognosis of the patients, i.e.:
   a. heart insufficiency NYHA III/IV
   b. relevant coronary heart disease,
   c. Diabetes mellitus with late sequelae
10) Organ, stem cell or bone marrow transplantation
11) Known hypersensitivity to capecitabine
    In case of known hypersensitivity to oxaliplatin, the patients can participate, but not receive oxaliplatin
12) Medication with brivudine, sorivudine or analogues in the last four weeks before planned treatment start
13) Known dihydropyrimidine dehydrogenase (DPD)-deficiency
14) Acute infections
15) Known HIV- infections, known active hepatitis B or C- infection
16) Participation at another interventional study for medical treatment during the last four weeks before randomisation
17) Neoadjuvant therapy before resection
18) Patients, in whom the randomisation or chemotherapy in unfeasible due to logistic reasons (travel distance, compliance)
19) Age < 18 years
20) Pregnant or breast feeding patients
21) Women of childbearing potential and men with partner with childbearing potential who are not willing to take appropriate precautions to avoid pregnancy with a highly effective method in case they are randomised to “chemotherapy”
12 Study drug

12.1 Treatment schedule

The study medication is capecitabine. The investigator may decide to add oxaliplatin as additional treatment. In this case, the capecitabine dose has to be adapted as outlined below. The dose should be reduced in case of toxicities. A treatment start with reduced dose for the first dose is not planned – except the adaption for renal function and the combination oxaliplatin as described below.

12.1.1 Capecitabine

Capecitabine 2 x 1250 mg/m², oral (d1-14), repeated at day 22 (- 2 ... + 6 days)
Patients with a GFR between 30 and 50 ml/min start with capecitabine dose of 2 x 1000 mg/m².
Treatment duration: 8 cycles (approx. 6 months); Patient diary for documentation of the drug compliance.

12.1.2 Capecitabine, if combined with oxaliplatin

If the investigation decides to add oxaliplatin, the following schedule should be used:
[Oxaliplatin 130 mg/m² i.v. (2 hours on d1)]
Capecitabine 2 x 1000 mg/m², oral (d1-14), repeated at day 22 (- 2 ... + 6 days)
Treatment duration: 4 or 8 cycles (approx. 3 or 6 months); Patient diary for documentation of the drug compliance.

12.2 Application

12.2.1 Premedication

For the oral treatment with capecitabine, no premedication is recommended. For additional i.v. chemotherapy, the guidelines for supportive therapy should be followed.
The schedule capecitabine in combination with oxaliplatin is a moderate emetogenic regimen. Thus, a prophylaxis with a 5-HT3-antagonist (i.e. granisetron, ondansetron) plus dexamethasone (day 1 – 3) is recommended.
The recommended premedication can be adapted according to the local standard and the symptoms of the patient.
12.2.2 Start of the next cycle

Before the start of the next cycle, the results of the blood count and the information on toxicities during the last chemotherapy cycle must be available.

The chemotherapy can be continued if all the following conditions apply:

- neutrophils ≥ 1,5 Gpt/l
- thrombocytes ≥ 100 Gpt/l
- non-haematological toxicities grade ≤ 1 according NCI-CTC.

The therapy can also be continued in case of

- polyneuropathy grade ≥ 2. In this situation, oxaliplatin has to be paused (except cold-induced neuropathy).
- neutrophils ≥ 1,0 Gpt/l and < 1,5 Gpt/l, if this is local standard and the patient has a good performance status.
- thrombocytes ≥ 75 Gpt/l and < 100 Gpt/l, if this is local standard and the patient has a good performance status.
- toxicities considered by the physician being unlikely to become serious or life-threatening, e.g. alopecia, altered taste, nail changes etc.

12.2.3 Dose of capecitabine in monotherapy

In monotherapy, the standard dose is 2 x 1250 mg/m².

Table 2: Capecitabine doses in monotherapy

<table>
<thead>
<tr>
<th>Body surface area (m²)</th>
<th>Standard dose 2x 1,250 mg/m²</th>
<th>number of tablets (twice daily)</th>
<th>Reduced dose (75 %) 2 x 950 mg/m²</th>
<th>Reduced dose (50 %) 2 x 625 mg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1,26</td>
<td>single dose (mg)</td>
<td>150 mg</td>
<td>500 mg</td>
<td>single dose (mg)</td>
</tr>
<tr>
<td>1,27 - 1,38</td>
<td>1.500</td>
<td>-</td>
<td>3</td>
<td>1.150</td>
</tr>
<tr>
<td>1,39 - 1,52</td>
<td>1.650</td>
<td>1</td>
<td>3</td>
<td>1.300</td>
</tr>
<tr>
<td>1,53 - 1,66</td>
<td>1.800</td>
<td>2</td>
<td>3</td>
<td>1.450</td>
</tr>
<tr>
<td>1,67 - 1,78</td>
<td>2.000</td>
<td>-</td>
<td>4</td>
<td>1.500</td>
</tr>
<tr>
<td>1,79 - 1,92</td>
<td>2.150</td>
<td>1</td>
<td>4</td>
<td>1.650</td>
</tr>
<tr>
<td>1,93 - 2,06</td>
<td>2.300</td>
<td>2</td>
<td>4</td>
<td>1.800</td>
</tr>
<tr>
<td>2,07 - 2,18</td>
<td>2.500</td>
<td>-</td>
<td>5</td>
<td>1.950</td>
</tr>
<tr>
<td>≥ 2,19</td>
<td>2.800</td>
<td>2</td>
<td>5</td>
<td>2.150</td>
</tr>
</tbody>
</table>
12.2.4  Dose of capecitabine if combined with oxaliplatin

If capecitabine is combined with oxaliplatin, the standard dose is $2 \times 1000 \text{ mg/m}^2$.

Table 3: Capecitabine doses if combined with oxaliplatin

<table>
<thead>
<tr>
<th>Body surface area (m$^2$)</th>
<th>Standard dose 2x 1.000 mg/m$^2$</th>
<th>number of tablets (twice daily)</th>
<th>Reduced dose (75%) 2x 750 mg/m$^2$</th>
<th>Reduced dose (50%) 2x 500 mg/m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>single dose (mg)</td>
<td>150 mg 500 mg</td>
<td>single dose (mg)</td>
<td>single dose (mg)</td>
</tr>
<tr>
<td>≤ 1.26</td>
<td>1.150</td>
<td>1 2</td>
<td>800</td>
<td>600</td>
</tr>
<tr>
<td>1.27 – 1.38</td>
<td>1.300</td>
<td>2 2</td>
<td>1.000</td>
<td>600</td>
</tr>
<tr>
<td>1.39 – 1.52</td>
<td>1.450</td>
<td>3 2</td>
<td>1.100</td>
<td>750</td>
</tr>
<tr>
<td>1.53 – 1.66</td>
<td>1.600</td>
<td>4 2</td>
<td>1.200</td>
<td>800</td>
</tr>
<tr>
<td>1.67 – 1.78</td>
<td>1.750</td>
<td>5 2</td>
<td>1.300</td>
<td>800</td>
</tr>
<tr>
<td>1.79 – 1.92</td>
<td>1.800</td>
<td>2 3</td>
<td>1.400</td>
<td>900</td>
</tr>
<tr>
<td>1.93 – 2.06</td>
<td>2.000</td>
<td>– 4</td>
<td>1.500</td>
<td>1.000</td>
</tr>
<tr>
<td>2.07 – 2.18</td>
<td>2.150</td>
<td>1 4</td>
<td>1.600</td>
<td>1.050</td>
</tr>
<tr>
<td>≥ 2.19</td>
<td>2.300</td>
<td>2 4</td>
<td>1.750</td>
<td>1.100</td>
</tr>
</tbody>
</table>

12.3  Dose modification

Doses are adjusted according to the local standard that should usually follow the summary of product characteristics (SmPC, Germany: Fachinformation).

Capecitabine should be held especially in case of mucositis / stomatitis, diarrhoea and hand-foot-syndrome grade >1 and be restarted when it had been resolved to grade ≤ 1.

In combination with oxaliplatin, capecitabine should not be interrupted or reduced for toxicities that attributed to oxaliplatin (especially polyneuropathy and allergic reactions).

Oxaliplatin is administered as investigators choice and should be reduced according to local standard. If there is no local standard, oxaliplatin should be reduced by 20%

- in case of oxaliplatin-related, non-haematological toxicity grade 3,
- in case of oxaliplatin-related, long lasting non-haematological toxicity grade 2,
- if the dose was delayed by at least one week due to oxaliplatin-induced toxicity.

The dose should usually not be reduced for cold-induced neuropathy without paraesthesia or hypaesthesia and not for toxicity that is attributed to capecitabine, only, as hand-foot-syndrome.

Capecitabine is continued if oxaliplatin is paused or discontinued due to neuropathy.
### Table 4: Dose reductions for capecitabine

<table>
<thead>
<tr>
<th>Toxicity grade</th>
<th>Dose changes within a treatment cycle</th>
<th>Dose adjustment for next cycle/dose (% of starting dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Maintain dose level</td>
<td>Maintain dose level</td>
</tr>
<tr>
<td>Grade 2</td>
<td>- first appearance</td>
<td>Interrupt until resolved to grade ≤ 1</td>
</tr>
<tr>
<td></td>
<td>- second appearance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- third appearance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- forth appearance</td>
<td>Discontinue treatment permanently</td>
</tr>
<tr>
<td>Grade 3</td>
<td>- first appearance</td>
<td>Interrupt until resolved to grade ≤ 1</td>
</tr>
<tr>
<td></td>
<td>- second appearance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- third appearance</td>
<td>Discontinue treatment permanently</td>
</tr>
<tr>
<td>Grade 4</td>
<td>- first appearance</td>
<td>Interrupt until resolved to grade ≤ 1 or discontinue treatment permanently</td>
</tr>
<tr>
<td></td>
<td>- second appearance</td>
<td>Discontinue treatment permanently</td>
</tr>
</tbody>
</table>

### 12.4 Concomitant medication

Hematologic growth factors are allowed according to local standard. Brivudine and sorivudine containing drugs are not allowed due to potentially lethal interactions with capecitabine.

For all patients, additional tumour directed therapies – especially chemotherapy, radiation and immunotherapy – is not allowed during the chemotherapy and the follow-up before recurrence or the appearance of metastases.

### 12.5 Participation at other clinical trials

The participation at other interventional tumour directed trials is not allowed during the chemotherapy or follow-up before recurrence or the appearance of metastases.

During follow-up, the patients may participate at trials with another focus, i.e. arterial hypertension.
12.6 Compliance
The patients have a patient diary per cycle in which the number of tablets and potential toxicities are documented. At the diary, the batch number and the remaining tablets are documented at the end of each cycle.

12.7 Description of the study drug

12.7.1 Capecitabine
Generic name: Capecitabine
Commercial name: i.e. Capecitabin Accord, Capecitabin AL, Capecitabin beta, Capecitabin cell pharma, Capecitabin Hexal, Capecitabin medac, Capecitabin Mylan, Capecitabin oncovis, Capecitabin Ribosepharm, Capecitabin Teva, Capecitabin Waverley, Capecitabin Zentiva, Capecitabin-PharOs, Ecsya, Xeloda (All product with local approval may used.)
Pharmaceutical form: Film coated tablets 500 mg, Film coated tablets 150 mg
Package size: 120 tablets (500 mg) or 60 tablets (150 mg)
Storage: < 25...30 °C, see SmPC
Capecitabine should be swallowed with water 30 minutes after meal.

12.8 Toxicity and interactions

12.8.1 Toxicities of capecitabine
The most commonly reported or clinically relevant toxicities are gastrointestinal disorders (especially diarrhoea, nausea, vomiting, abdominal pain, stomatitis), hand-foot syndrome (palmar-plantar erythrodysesthesia), fatigue, asthenia, anorexia, cardiotoxicity, increased renal dysfunction on those with pre-existing compromised renal function, and thrombosis/embolism.

The frequency of side effects in an adjuvant trial with 995 patients treated with capecitabine monotherapy for colon cancer is listed in Table 5, for 938 patients treated with the combination with oxaliplatin in the adjuvant setting in Table 6.
In addition, a Cochrane review reports on efficacy and toxicity of randomized trials in colorectal cancer that enrolled 3285 pts (capecitabine) and 3418 pts (5-FU).
In a recent publication describing 3 or 6 months adjuvant therapy of colon cancer, 5071 patients received capecitabine / oxaliplatin. Further extensive data are available from further studies conducted i.e. in colorectal, gastric, breast and pancreatic cancer.
### Table 5: Toxicities of adjuvant monotherapy with capecitabine (995 patients)

<table>
<thead>
<tr>
<th>Event</th>
<th>All grades</th>
<th>Grade 3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>46%</td>
<td>11%</td>
</tr>
<tr>
<td>Nausea / vomiting</td>
<td>36%</td>
<td>3%</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>22%</td>
<td>2%</td>
</tr>
<tr>
<td>Hand-foot-syndrome</td>
<td>60%</td>
<td>17%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>23%</td>
<td>1%</td>
</tr>
<tr>
<td>Abdominal pain.</td>
<td>10%</td>
<td>2%</td>
</tr>
<tr>
<td>Alopecia</td>
<td>6%</td>
<td>NA</td>
</tr>
<tr>
<td>Lethargy</td>
<td>10%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Anorexia</td>
<td>9%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>32%</td>
<td>2%</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>50%</td>
<td>20%</td>
</tr>
</tbody>
</table>

NA – not applicable

### Table 6: Toxicities of adjuvant combination therapy of capecitabine and oxaliplatin (938 patients)

<table>
<thead>
<tr>
<th>Event</th>
<th>All grades</th>
<th>Grade 3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory neuropathy</td>
<td>78%</td>
<td>11%</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>60%</td>
<td>19%</td>
</tr>
<tr>
<td>Nausea</td>
<td>66%</td>
<td>5%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>43%</td>
<td>6%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>35%</td>
<td>NR</td>
</tr>
<tr>
<td>Hand-foot-syndrome</td>
<td>29%</td>
<td>5%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>27%</td>
<td>9%</td>
</tr>
<tr>
<td>Anorexia</td>
<td>24%</td>
<td>NR</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>21%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>17%</td>
<td>2%</td>
</tr>
<tr>
<td>Alopecia</td>
<td>4%</td>
<td>NA</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>NR</td>
<td>5%</td>
</tr>
<tr>
<td>Dehydration</td>
<td>NR</td>
<td>3%</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>NR</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

NA – not applicable, NR – not reported
12.8.2 Interactions of capecitabine

**Sorivudin and analogues:** inhibition of dihydropyrimidine dehydrogenase resulting in increased and potentially lethal fluoropyrimidine toxicity

**Folinic acid:** increased capecitabine toxicity

**Oxaliplatin:** separate dosing schedule recommended

**Coumadin-derivative anticoagulants:** altered coagulation parameters and/or bleeding. Patients taking coumadin-derivative anticoagulants concomitantly with capecitabine should be monitored regularly for alterations in their coagulation parameters (INR) and the anticoagulant dose adjusted accordingly.

**Phenytoin:** increased phenytoin plasma concentrations

**Antacids:** small increase in plasma concentrations of capecitabine and one of its metabolites (5'-DFCR)

**Allopurinol:** possible decreased efficacy of 5-FU. Concomitant use of allopurinol with capecitabine should be avoided.

**Interferon alpha:** increased capecitabine toxicity (not allowed in the study)

**Radiation therapy:** increased capecitabine toxicity (not allowed in the study)

**Bevacizumab:** no known interaction (not allowed in the study)

12.9 Preparation and labelling

Commercial capecitabine is used for this trial. The manufacturer and the batch numbers are documented.

12.10 Storage and drug accountability

For the trial, commercial capecitabine is used that is approved for the use in the country of the patients.

At the start of each cycle, the patient receives a diary, on which the planned dosing is noted. The patient documents the actual doses. The manufacturer, the name of the medicinal product and the batch numbers are documented for each cycle.

At the end of the therapy, the remaining tablets are documented.

12.11 Blinding

The medication is not blinded.

The results of the ctDNA analysis are not communicated. Thus, patients randomised to “follow-up” remain blinded to the results of the ctDNA analysis. De-blinding is not planned because the ctDNA result would not have any consequence for the treatment outside of the trial.
13 Study procedures

13.1 Screening phase

13.1.1 Informed consent for screening

Every patient is informed before screening on the trial and comprehensively on all the procedures that are related with the screening phase by a qualified physician at the screening centres or an investigator/qualified and designated sub-investigator at the treatment centres. The consent is documented by the participant's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the participant.

The patient may consent that the remaining material is used for further translational research (see chapter 18).

The patients sign a second informed consent before randomisation.

13.1.2 Screening visit

**Time window:** between fifth postoperative day and end of the fifth postoperative week, preferred: between fifth postoperative day and before being discharged from surgical department

**Procedures**

1. review of inclusion and exclusion criteria for screening
2. shipment of the FFPE tumour block for central mutational analysis
3. sampling and shipment of 2 special tubes for ctDNA analysis
4. documentation:
   a. date of informed consent into screening
   b. consent into further translational research (optional)
   c. date of resection,
   d. tumour localisation, histology, pTNM/L/V/R status, grading, number of evaluated lymph nodes
   e. MSI-status (if known)
   f. gender, year of birth
   g. CEA before resection
   h. centre
   i. date blood sampling
   j. block-number
   k. centre where the randomisation visit is planned
The patients are identified by a unique patient number that consists one letter for the group / screening platform, four digits for the study centre where the patient was registered for screening and four digits for a running number at the centre. This part number remains unchanged if the patient changes to another centre i.e. from screening to randomisation, see chapter 17.2, page 65.

13.1.3 Screening in platform screening trials

Patients can participate at the randomised part of the study if the screening procedures were performed in an approved screening trial provided:

- the procedures were performed in the same time window with the same techniques
- at least the data as in chapter 13.1.2 (screening visit) were documented
- the patients consents to the transfer of the data from the screening platform into CIR-CULATE
- the screening platform was positively reviewed by the ethic committee(s), the informed consent for the screening platform includes the procedures and the documentation as in chapter 13.1.2 (screening visit) and that the patient can be informed on the participation in a clinical trial based on the test results.

13.2 Randomised phase

13.2.1 Informed consent for the randomised phase

Before participation at the randomised phase, the patient is comprehensively informed on the randomisation, the randomised phase of the study and the side effects of the potential chemotherapy as well as on all other procedures by the investigators or qualified and designated sub-investigator. The patient consents that the data generated at the screening phase can be used for the randomised phase of the trial.

For patients with childbearing potential, the contraception should be discussed (see chapter 13.10, page 54). The potential consequences of oxaliplatin on fertility should be discussed and the options regarding fertility preservation, especially in patients who are planned for and still planning pregnancy or fatherhood.

The consent to the trial is documented by the participant's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the participant.

The patient may consent that the remaining material is used for further translational research (see chapter 18).

For the randomised part, the number of the randomising centre and a three letter code for the randomisation are added to the existing number from screening see chapter 17.2, page 65.
13.2.2 Randomisation visit

**Time window:** between the end of the third week to the end of the eighth week after resection

1. review of inclusion and exclusion criteria for randomisation
2. sampling and shipment of 3 special tubes for ctDNA analysis
   (optional, for translational research)
3. cancer history, comorbidities, medication
4. laboratory: haemoglobin, leukocytes, neutrophils, thrombocytes, ALAT, ASAT, bilirubin, creatinine, CEA, pregnancy test*
   (laboratory value measured during the last two weeks as part of the clinical routine may be used)
5. exclusion of distant metastases by thoracic and abdominal imaging.†
   Imaging performed in the clinical routine ca be used if performed not longer than 8 weeks before randomisation.
6. physical examination including performance status, height, weight
7. judgement of the investigator that the patient might receive chemotherapy
8. discussion of contraception and – in patients potentially planned for oxaliplatin – fertility preservation.

   For patients with planned oxaliplatin treatment and planned pregnancy / fatherhood, an appointment at gynaecology or urology / andrology is made

9. documentation:
   a) date of informed consent into randomisation
   b) consent into further translational research (optional)
   c) tumour localisation, histology, pTNM/L/V/R status, grading, number of evaluated lymph nodes (if changed)
   d) height, weight
   e) laboratory: haemoglobin, leukocytes, neutrophils, thrombocytes, ALAT, ASAT, bilirubin, creatinine, CEA, pregnancy test
   f) imaging with date and modality (i.e. ultra sound, CT, MRI) and result (presence of tumour / metastases)
   g) WHO-PS
   h) cancer history
   i) comorbidities
   j) documentation, that the patient might receive chemotherapy
   k) centrum

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* women, only. Not necessary in postmenopausal women (no menses for ≥ 12 months without an alternative medical cause) and after hysterectomy
† chest x-ray and abdominal ultra sound or (if local standard or medically indicated) CT or MRI. Ultrasound is adequate if all parts of the abdomen are well evaluable. Abdominal CT is adequate if performed as contrast enhanced CT scan, only.
13.2.3 Randomisation

The randomisation is performed via the eCRF, when all parameters of the baseline visit are entered. The randomisation is stratified according:

- ctDNA positive vs ctDNA negative
- pT3 tumour vs. pT4 tumour
- emergency resection vs. planned resection
- planned chemotherapy with vs. without oxaliplatin

The patient is assigned to one of the groups “chemotherapy”, “follow-up” or “off-study”. The ctDNA result for patients (in the group follow-up”) will not be communicated.

13.2.4 Treatment visit

**During the treatment phase, a follow-up visit might be necessary.**

Treatment visits are performed in patients randomised to “chemotherapy”, only.

**Time point:** day 1 of each cycle

Allowed window: up to 5 days before the cycle (monotherapy, only). In combination with oxaliplatin up to 2 days before the cycle

Number of treatment visits: eight, in combination with oxaliplatin four or eight

Procedures

1. laboratory: blood count including haemoglobin, leukocytes, neutrophils, thrombocytes, bilirubin, creatinine; further laboratory according to local standard, pregnancy test*
2. sampling and shipment two special tubes for ctDNA analysis (optional, for translational research)
3. physical examination (if necessary)
4. adverse events (AE’s)
5. patient diary for capecitabine (handing out and collection of last cycle)
6. documentation of adverse events with
   a. maximal grade within the cycle and causality
   b. dose delay / reduction
   c. start date / end data, treatment and co-medication for all SAE’s
7. documentation of the capecitabine and oxaliplatin dose if applicable

Before the first cycle, the contraception is discussed. In patients with planned pregnancy / fatherhood who are planned for oxaliplatin, a consultation with a specialist for fertility preservation (gynaecologist or andrologist/urologist) should have performed.

13.2.5 End of treatment (EOT) visit

Treatment visits are performed in patients randomised to “chemotherapy”, only.

* women, only. Not necessary in postmenopausal women (no menses for ≥ 12 months without an alternative medical cause) and after hysterectomy
Time point: 30 days after last chemotherapy dose

Procedures

1. laboratory: blood count including haemoglobin, leukocytes, neutrophils, thrombocytes, bilirubin, creatinine, further laboratory according to local standard, pregnancy test*

2. sampling and shipment two special tubes for ctDNA analysis (optional, for translational research)

3. physical examination (if necessary)

4. adverse events (AE’s)

5. collection of the patient diary for capecitabine

6. documentation of adverse events with
   (a) maximal grade within the cycle and causality
   (b) dose delay / reduction
   (c) start date / end data, treatment and co-medication for all SAE's

7. documentation of the capecitabine dose

8. documentation for the reason to discontinue chemotherapy (as planned, toxicity, patients withdrawal of informed consent, progression, death)

13.2.6 Follow-up visit

Follow-up visits are performed in patients randomised to “chemotherapy” or to “follow-up”.

Time points: 6, 12, 18, 24, 30, 36, 42, 48, 54 and 60 months after randomisation +/- 6 weeks. According to the local standard, the follow-up visits can be performed more frequently, if the same schedule is used in all patients in the trial.

1. laboratory: CEA. Further laboratory according to local standard.

2. sampling and shipment one special tubes for ctDNA analysis (optional, for translational research)

3. cancer history

4. thoracic and abdominal imaging.
   All parts of the abdomen have to be well evaluable if ultra sound is used†.

5. physical examination if necessary

6. documentation:
   (a) laboratory: CEA
   (b) imaging with date and modality (i.e. ultra sound, CT, MRI) and result (presence of tumour / metastases, metastatic site)
   (c) status of progression / date of progression, localisation of progression
   (d) survival status / date of death, reason of death

* women, only. Not necessary in postmenopausal women (no menses for ≥ 12 months without an alternative medical cause) and after hysterectomy
† chest x-ray and abdominal ultra sound or (if local standard or medically indicated) CT or MRI. Ultrasound is adequate if all parts of the abdomen are well evaluable. Abdominal CT is adequate if performed as contrast enhanced CT scan, only.
After progression, the survival status will be documented, only. A physical visit is not necessary. If the recurrence was not detected during a planned follow-up visit, plasma for ctDNA analysis should be sampled at the next feasible time point and be shipped (optional, translational research).

13.2.7 Long term follow up

Follow-up visits are performed in patients randomised to “chemotherapy” or to “follow-up”.

**Time points:** 1 x / year

1. telephone contact to the patient or general practitioner, no physical visit necessary
2. documentation:
   a. status of progression / date of progression, localisation of progression
   b. survival status / date of death, reason of death

13.3 Assessment of efficacy

Efficacy (disease free survival) is evaluated with the imaging described in chapter 13.2.6 (Follow-up visit).

Metastases and recurrences should follow the guidelines for new metastases according to RECIST 1.1:

- “the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumour...”
- “A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression.”
- “If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.”

If the suspected lesion defining the disease progression can retrospectively identified at the baseline imaging, the event will be set at time of randomisation.

An event for disease free survival is local recurrence, any metastasis, any second tumour and death from any cause. Increased CEA levels without imaging findings are not regarded as events.

An event for overall survival is any death independently from the cause.

13.4 Assessment of safety

Adverse events are classified according to NCI-CTC version 5.0 and can be accessed via the following link:
Adverse events are documented for patients randomised to „chemotherapy“ who have received at least one dose of chemotherapy.

13.5 Central laboratory

Mutations in the tumour (and if necessary MSI) are centrally assessed at the central pathology institutes

1. Institute for Pathology, University Hospital Carl Gustav Carus, Germany, and for patients screened in the AIO COLOPREDICT platform at the:
   2. Institute for Pathology, Ruhr University Bochum, Germany

The ctDNA analysis is performed at:

1. Haematological laboratory, Medical Department I, University Hospital Carl Gustav Carus, 01307 Dresden, Germany

ctDNA is measured according to the method published by Stasik et al. The details to the pathological techniques and the ctDNA analysis are described in the laboratory manual, the full addresses in chapter 5 (page 13).

The safety laboratory, the pregnancy test and CEA are locally analysed.

13.6 Treatment after the trial

Patients randomised to “off study” and patients randomised to “chemotherapy” or “follow-up” who prematurely discontinue participation at the trial are followed according to the national guidelines outside the clinical trial.

13.7 Lost to follow-up

If a patient does not return to the clinic for a planned study visit and/or if the site is unable to contact the participant,

- the site should attempt to contact the patient and reschedule the missed visit. If the patient is contacted, the participant should be counselled on the importance of maintaining the protocol-specified visit schedule.
- the investigator should make every effort to regain contact with the patient at each missed visit (eg, phone calls). These attempts to contact patients should be documented in the medical record.
13.8 Termination of the trial for individual patients

Patients randomised to “chemotherapy” or “follow-up” remain at the trial until the end of the follow-up period or death. Patient who experience disease progression, recurrence or a second cancer remain to the trial for survival follow-up.

Patient who discontinue study treatment (i.e. due to chemotherapy toxicity) or withdraw their informed for study treatment remain in the follow-up for the trial.

Patients may be excluded from further participation at the trial at the discretion of the investigator if suspects harm for the patients by further trial participation.

Furthermore, patients may at any time, without giving reasons withdraw their consent; they will no longer receive study treatment or be followed at scheduled protocol visits.

The reason for the study discontinuation is documented in the eCRF. All patients who discontinue the trial prematurely during chemotherapy are asked to participate at the EOT visit.

13.9 Early termination of the trial

The sponsor may terminate the trial early for relevant medical or administrative reasons, i.e.:

- if the recruitment into the trial does not allow a timely completion of the trial
- major problems with the quality of the data
- unforeseen circumstances at a trial site that do not allow to continue the trial
- early superiority or inferiority of one trial arm as defined by the interims analysis
- new risks and toxicities that required a new evaluation of the risk-/benefit ratio
- new scientific knowledge that does not allow the continuation of the trial

The study coordinator can in conjunction with the sponsor, the trial committee or the DSMB decide on the discontinuation of the study.

Patients who are at the time of termination on chemotherapy will be asked to undergo an EOT visit and may or may not continue the chemotherapy out of the study at the discretion of the investigator.

13.10 Pregnancies and Contraception

Pregnant and breast feeding woman are excluded from the participation at the trial.

During the trial and for the first six months after the trial, all patients should use highly effective methods for contraception, except

- woman who are postmenopausal (no menses for ≥ 12 months without an alternative medical cause), after hysterectomy or bilateral oophorectomy
- male patients whos is postmenopausal (no menses for ≥ 12 months without an alternative medical cause), had a hysterectomy or bilateral oophorectomy
- male and female patients who are sexually permanently abstinent during the trial period.

Highly effective methods include
- combined (oestrogen and progestogen containing) or progestogen-only hormonal contraception associated with inhibition of ovulation that may be oral, intravaginal or transdermal,
- progestogen-only hormonal contraception associated with inhibition of ovulation that may be oral, injectable or implantable,
- intrauterine devices (IUD) and intrauterine hormone-releasing systems (IUS),
- bilateral tubal occlusion
- vasectomised partners if a medical assessment was performed after surgery.

In case of diarrhoea, a second method of contraception should be used if the primary method is orally taken.

If a patient or the partner of a patient becomes pregnant during the first 6 months after the study, the further course of the pregnancy will be documented as well as the development of the child for the first year. Pregnancies and the non-normal development of the child are documented as SAE's.

The chemotherapy is not continued within the trial; the further treatment at discretion of the investigator and the treating physicians.
14 Adverse events

14.1 Definitions

An adverse event (AE) is defined in the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice as "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment." (ICH E6: section 1.2). An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

An adverse reaction (AR) is a response to a drug which is noxious and unintended and which is related to any dose administered. The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

An unexpected adverse reaction (UAR) is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. SMPC).

A serious adverse event (SAE) is any event that
- is fatal or
- life-threatening or
- results in persistent or significant disability/incapacity or
- requires inpatient hospitalization or prolongation of existing hospitalisation
- leads to a congenital anomaly/birth defect or
- medically important for other reasons.

Planned hospitalisation for chemotherapy and related procedures (i.e. port implantation) and tumour progression and death from colon cancer are no SAE's.

A suspected unexpected serious adverse reaction (SUSAR) is defined as an untoward and unintended response to a study drug, which is not listed is the applicable product information (see definition of ADR), and meets one of the following serious criteria: results in death, is life-threatening, requires hospitalisation or prolongation of an existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.
14.2 Documentation of AE's

There are extensive safety data for the adjuvant therapy with capecitabine available (Table 5, Table 6, chapter 12.8.1).

Adverse events are documented from the first dose of the study drug until the End of treatment (EOT) visit. They are classified and graded according the NCI-CTC version 5.0 that is accessible with the following link:


The adverse events are documented with the maximal grade per cycle.

A special focus for adverse documentation is on SAE's, toxicities leading to dose modifications or dose delays and the frequency of typical toxicities such as (leukopenia, neutropenia, fatigue, diarrhoea, mucositis / stomatitis, nausea/vomiting, hand-foot-syndrome, neuropathy).

The relationship to the study drug is described as „related“ (possible, probable or certain relationship), „not related“ (no or unlikely relationship) or „unknown / not assessable“.

The influence on the further treatment is described as “unchanged” or “dose delayed”, “dose modified” or “treatment discontinued”. For each AE and SAE, the outcome will be described (“recovered”, “improved”, “not recovered”, “recovered with sequelae”, “fatal”, “unknown”). For AE, a documentation in the next cycle or at the EOT visit with grade 0 will be defined as “recovered”.

All AE's have to be documented in the source data and in the eCRF within two weeks.

14.3 Documentation of SAE's

For SAE's, it will be documented:

- date/time of the AE that led to the SAE
- description of the SAE
- time when the investigators had knowledge from the SAE
- day of hospitalisation and discharge (if applicable)
- criterion that defined the SAE
- cause of death and result of the autopsy (if applicable)
- relationship to the study drug.

The investigator or a designated person reports SAE's within 24 hours after awareness to the sponsor via

Fax to +49 351 458 7291 or email: pharmakovigilanz@uniklinikum-dresden.de.

The SAE report should be as complete as possible and being updated by the investigator with a complete report as soon as new information is available. The investigator provides all additional information to the ethic committee(s), the health authorities and the sponsor that are necessary to fulfil their.
Overdosing and misuse of the study drug is reported as SAE as well as any death (except from tumour).

14.4 Reporting of adverse events

14.4.1 Reporting Obligations of Study Site

(According to § 12 (1) – (6) GCP-V, § 42 AMG)

14.4.1.1 Adverse events (AEs)

All AEs have to be documented in the eCRF as soon as possible, but at the latest within 2 weeks, see chapter 14.2.

14.4.1.2 Serious adverse events (SAEs)

The study site must report promptly any SAE or pregnancy which occurred to KKS Dresden, department of pharmacovigilance. The report has to be done by the investigator of the study site using the form Serious Adverse Report. For reporting of SAEs the same time frame applies as described previously (from first application of adjuvant chemotherapy to the end of treatment visit). However, pregnancy of female patients or female partners of mal participants must be reported up to 6 months after cessation of use.

KKS Dresden pharmacovigilance
Barbara Djawid
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14.4.1.3 Patient’s death

In case of a patient’s death study site will answer questions of the appropriate ethic committees, BfArM and the sponsor (or delegate) if requested.

14.4.2 Reporting Obligations of Sponsor

(According to § 13 (1) – (6) GCP-V, § 42 AMG)

14.4.2.1 Adverse events (AEs)

All reported AEs must be documented in detail by the sponsor (or the delegate) and submitted to the competent authorities on demand.

14.4.2.2 SUSARs

The sponsor (or the delegate) must report any known suspected case of an unexpected serious adverse reactions (SUSAR) immediately, at the latest within 15 days after becoming aware to
the ethic committees, competent authorities as well as all Principal Investigators who are involved in this clinical trial. The expedited reporting will be carried out by KKS Dresden.

Fatal and life-threatening SUSARs must be reported to the EC, BfArM and Principal Investigators of the study sites without delay, at the latest 7 calendar days after becoming aware of the minimum criteria for reporting. SUSARs that are not fatal or life threatening must be reported to the EC, BfArM and Principal Investigators of the study sites without delay at the latest within 15 calendar days (within a maximum of further 8 days) of becoming aware of the minimum criteria for reporting.

14.4.2.3 New review Risk/Benefit ratio

The sponsor (or the delegate) must report immediate, at the latest within 15 days after becoming aware to the EC and the BfArM about the facts, which will require a repeated review of the risk/benefit assessment of the study drug. Hereby include especially:

- Individual case of expected serious adverse reaction with an unexpected outcome
- Increase of the frequency expected serious side effects which are assessed as clinical relevant
- Suspected cases of serious Unexpected side effects which occur after the participants have already finished the study
- Events which are in relation to the study procedure or the development of the study drug, which could affect the patient’s safety

14.4.2.4 Development Safety Update Report

A comprehensive, thoughtful annual review and evaluation of pertinent safety information collected during the reporting period related to a drug under investigation, whether marketed or not.

The main objective of a DSUR is

- to assess whether the information obtained by the Sponsor during the reporting period is in accordance with previous drug safety knowledge
- to describe new safety issues that could have an impact on the protection of clinical trial subjects
- to summarise the current understanding and management of identified an potential risks
- to provide an update on the status of the clinical investigation/development program and study results.

14.4.2.5 Steps to protect any immediate hazard

If the safety of the participants is affected and the sponsor (or the delegate) as well as the Principal Coordinating Investigator take steps for protection of any immediate hazard, the Sponsor will inform the appropriate EC and CA about the interventions as well as the initiating circumstances.
14.5 Data Safety Monitoring Board (DSMB)

An independent data monitoring committee with two oncologists and a statistician will follow the progress of the clinical trial, evaluate the safety and primary efficacy parameters and will propose changes, ending or continuing of the trial to the sponsor. A separate DSMB charta will be developed and submitted to competent authority and EC.
15 Documentation

15.1 Patient list

All included patients have to be documented in a confidential patient identification list. This list contains the patient specific numbers together with date of screening or randomisation and the date of birth and the full name of the patient. Patient related data will be just transmitted in pseudonymised form. The identification list will stay at each centre.

In addition, the participation at the CIRCULATE study is documented in the source file.

Persons entering data to the CRF / eCRF are authorised by the investigator. This process is documented by a delegation list that contains the names, signatures, initials and the time of authorisation. The list is filed as original in the Investigator Site File (ISF) and as copy in the Trial Master File (TMF).

15.2 Patient identification

Patients will be coded by a Letter (study group), a four digit site number and a three-digit continuous number for the patients enrolled at the site. In order to avoid identification errors, the year of birth is added to the patient number.

15.3 Case report forms

For each patient enrolled, a (e)CRF is completed by the investigator or authorized delegate. This also applies to records for patients who fail to complete the study.

The electronic case report forms (eCRF) are specially created for CIRCULATE in a database with the study software MACRO4 for data handling. The data are backed up daily. The scope of the eCRF access and the associated authorizations are regulated by corresponding user roles.

The investigator ensures that the data generated at the study site are timely entered into the eCRF within two weeks, for the baseline visit: before randomisation by designated and authorised persons and the completeness of the data according to GCP-ICH.

Entered data are automatically checked for plausibility by programmed ranges and inconsistencies. In addition, manual queries can be generated that allow the investigators to answer on potential discrepancies. The study data base has an audit trail, so that all data corrections are tracked with the date, time and with the name of the person entering or correcting the data.

When the trial is closed and all relevant data have been entered as well as all queries have been clarified, the data base will be closed. Subsequent changes to the data can only be made with
the consent of the coordinating investigator. All processes regarding the database management are documented and filed in the TMF.

15.3.1 Documentation of the screening

The results of the screening visit are documented at a study-specific case report form. A copy is faxed to the central study fax. Further copies are used for shipment of the FFPE block and of the ctDNA samples.

In addition, the pathology reported is faxed to the central study fax for review. The patient identifiers are to be removed/deleted before transmission and replaced by the patient number and year of birth.

Entries are made using a document proof pen and are signed by the investigator. In case of corrections, the original data should remain readable. New entries have to be dated and signed. Data that are not available are marked with NA (not applicable) or ND (not done). Corrected CRFs are immediately faxed to the central study fax and are stored in the centre.

The data are centrally entered to the eCRF.

15.3.2 Documentation of the randomised part

The investigator will enter the data into the eCRF immediately after the visit and ensures that the data are complete, correct and reflect the data in the patient source files. The access data (esp. username and password) has to be kept secret. The investigator may authorize trained persons at his centre to enter data in the eCRF.

Corrections are made by the investigator or the authorised team members, only.

In addition to the eCRF, the patient diaries are designed in a way that allows central review of the drug accountability. They will be faxed to the central study fax. Patient identifiers beyond the patient number and the year of birth are removed before the form is transmitted.

Computerized and manual consistency checks will be performed on newly entered forms; queries will be issued in case of inconsistencies. Consistent forms will be validated by the data manager. Inconsistent forms will be kept “pending” until resolution of the inconsistencies.

15.3.3 Source documents and background data

The investigator ensures that all data in the (e)CRF are reflect entries in the source data patient hospital/clinic records (e.g. medical, pathology and laboratory reports, ECG, X-ray, etc.).

The investigator supplies the sponsor on request with any required background data from the study documentation or clinic records. This is particularly important when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.
15.4 Investigator’s Files / Retention of documents

The Investigator maintains adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified.

The Investigator’s Study File contains all essential documents as the protocol/amendments, patient information and informed consent form, ethics committee and regulatory authority approval, notification of the federal regulatory authority and competent regional authorities, if applicable, drug records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence etc. The investigator keeps these two categories of documents on file for at least 10 years (or more as legally required) after completion or discontinuation of the study. The documents are archived in a secure place and treated as confidential material. Should the investigator wish to assign the study records to another party or move them to another location, the sponsor is notified in advance. All documents must be archived in a secure place and treated as confidential material.

15.5 Confidentiality of trial documents and patient records

The investigator and the sponsor (or designee) must assure that according to the standards of the data protection law, all data obtained in the course of a clinical study must be treated with discretion in order to guarantee the rights of the patient’s privacy. Patient related documents must be submitted to the sponsor in a pseudonymous manner. The investigator should keep a patient identification log showing codes and names. The investigator will maintain documents not for submission to Sponsor, e.g., patients’ written consent forms, in strict confidence.
16 Monitoring and audits

Based on the review of the eCRF data and the central review of the pathology reports, and the patient diary a central monitoring is performed. A risk based on site monitoring is performed. Criteria increasing the risk at a centre are:

- (high) number of SAE's at the centre
- (low) plausibility of the eCRF entries
- (low) completeness of data entered into the eCRF
- (high) number of protocol deviations
- timeliness in which queries are answered (delayed)
- (high) number of patients enrolled at a centre.

On site monitoring will focus on the patient informed consent, inclusion criteria, chemotherapy intensity, the primary efficacy endpoint, SAE’s and the training of the investigators.

To ensure quality of data, study integrity, and compliance with the protocol and the various applicable regulations and guidelines, the sponsor/sponsor representative may conduct site visits to institutions participating to protocols.

The investigator, by accepting to participate to this protocol, agrees to co-operate fully with any quality assurance visit undertaken by third parties, including representatives from the sponsor and health authorities, as well as to allow direct access to documentation pertaining to the clinical trial (including eCRF, source documents, hospital subject charts and other study files) to these authorised individuals.

The investigator informs the sponsor representatives immediately in case a regulatory authority inspection is scheduled and provides the inspection report to the sponsor within 5 working days.
17 Statistics and publication

17.1 Sample size calculation
see chapter 10.5, Patient number

17.2 Patient registration

The patients are identified for the screening phase by a number that consists of one letter (coding for group or screening platform) and four digits for the study centre where the patient was registered for screening and four digits for a running number at the centre, i.e.: B2020 – 0004 [centre screening part] – [running no at the screening centre].

At time of data base entry, a unique, running three letter code (capital letters, only) is assigned with randomisation, starting with “AAA”.

For the randomised phase, the centre number of the randomising centre is added (one letter plus up to four digits), independently from the fact whether the patient changed the centre from screening to randomisation or not, see chapter 13.1.2, page 47.

The full number of the patients is i.e.:


After randomisation, the letter code alone allows a unique characterisation, and the remaining numbers serve as control letters.

17.3 Randomisation stratification

The randomisation is performed via the eCRF by computer based, central block randomisation, when all parameters of the baseline visit are entered that include the stratification parameters and the confirmation that the patient consented to randomisation. The randomisation is stratified according:

- pT3 tumour vs. pT4 tumour
- emergency resection vs. planned resection
- planned chemotherapy with vs. without oxaliplatin

The patients are assigned to one of the groups “chemotherapy“ and “follow-up“ (ctDNA positive patients) or “follow-up“ and “off-study“ (ctDNA negative patients). The ctDNA result for patients in the group follow-up“ will not be communicated.

Patients not receiving the planned therapy are not replaced.
17.4 Planned interim or sequential analysis

For the primary endpoint, an interim analysis is planned when 60% of the events are recorded (93 events, after approximately 38 months).

17.5 Analysis populations and subgroups

17.5.1 Full analysis set (FAS)

All randomised ctDNA positive patients will form the full analysis set. Patients not receiving the allocated treatment are not replaced and remain in the full analysis set according to the intention-to-treat principle.

17.5.2 Per protocol analysis set (PPA)

The per protocol analysis set will consist of all patients of the FAS who received at least one cycle of the study treatment in the chemotherapy arm and of all patients in the follow-up arm who did not receive any tumour directed therapy before the occurrence of a DFS event. Patients who received non-protocol, tumour directed therapy before or without a DFS event will be included in the PPA set, but the survival endpoints will be censored at the date of the last study visit before the premature end of study treatment.

17.5.3 Safety evaluation set (SES)

The safety evaluation set consists of all patients in the chemotherapy arm who received at least one dose of capecitabine or at least one dose of oxaliplatin.

17.5.4 Subgroups

- clinical high risk (pT4 or emergency resection) vs. low risk (pT3 and no emergency resection),
- planned use of oxaliplatin vs. no planned use of oxaliplatin,
- age,
- female vs. male gender,
- rectal vs. colon cancer,
- country,
- time from informed consent to screening until randomisation,
- CEA,
- thrombocytes at baseline

For all subgroups interaction with treatment efficacy and ctDNA positivity is tested.

17.6 Data handling

17.6.1 Handling of missing data an outliers

Numbers of missing and non-missing values will be listed for all analysis variables.
Patients who are lost to follow-up will be censored for the time-to-event endpoints at the time, when last information is known.

For stratified analyses the stratum information used for randomisation is used. If for any reason this information is missing it will be imputed using a regression approach. Sensitivity analyses will be conducted to assess the effect of imputation on the analysis of the primary endpoint.

All other analyses are planned to be conducted as complete-case-analyses.

17.7 Variables for analysis

17.7.1 Disposition of subjects

Disposition of subjects will be presented by means of:
- Number of patients screened
- Number of patients screened but not randomized
- Number of patients screened and randomized and fulfilling eligibility criteria (separately for ctDNApos and ctDNAneg patients)
- Number of patients screened and randomized not fulfilling eligibility criteria (separately for ctDNApos and ctDNAneg patients)
- Number of patients screened and randomized with premature termination of study treatment (for Chemo and Follow-up patients only)
- Number of patients screened and randomized but excluded from the primary analysis

Reasons for premature termination of study treatment and exclusion from primary analysis will be listed as well as eligibility criteria, which were not met at time of randomization.

17.7.2 Extent of exposure

The exposure of subjects to treatment will be presented by means of:
1. Number of administered treatment cycles
2. Number of treatment cycles with dose reductions (total number, median, interquartile range, minimum maximum)
3. Number of treatment cycles with interruptions
4. Cumulative dose of capecitabine
5. Cumulative dose of oxaliplatin

For 1 to 3 the total sum over all patients and the median, interquartile range, minimum and maximum numbers will be presented. For 2 and 3, the median, interquartile range, minimum and maximum will be presented.


17.7.3 **Primary endpoint: disease free survival**

Disease free survival is defined as the time from randomisation to any local recurrence, distant metastasis, second colorectal or non-colorectal tumour or death from any cause (see chapter 10.2, page 31). Patients for whom no event was observed will be censored at the date of their last visit when they were last known to be event-free.

17.7.4 **Secondary endpoints**

Overall survival is measured as time from randomisation to death from any cause. Patients for whom no event was observed will be censored on the date of their last visit when they were last known to be alive.

Time to recurrence is measured as time from randomisation to recurrence of colorectal cancer. Patients with second tumours or non-colorectal cancer deaths will be censored on date of the event. Patients for whom no event was observed will be censored on the date of their last visit when they were last known to be without recurrence. Deaths without recurrence will be censored on the date of death.

Cancer specific survival is measured as time from randomisation to death from colorectal cancer. Patients with non-colorectal cancer deaths will be censored on time of the event. Patients for whom no event was observed will be censored on the date of their last visit when they were last known to be alive.

17.8 **Statistical analysis methods**

17.8.1 **General design of descriptive statistics**

For continuous variables the number of non-missing observations, the number of missing observations, arithmetic mean, standard deviation, median, minimum, maximum, 25% quartile, and 75% quartile are given.

For categorical variables the number of non-missing observations, the number of missing observations, absolute number per category, and percentage per category based on non-missing observations are given.

17.8.2 **Disposition of subjects**

A patient flow diagram according to the ICH E3 guideline depicting the information defined in section 17.7.1. will be presented.

17.8.3 **Evaluation of demographics and baseline characteristics**

Demographics and baseline characteristics are presented summarized for:

- All ctDNApos patients
- ctDNApos patients randomized to chemotherapy
- ctDNApos patients randomized to follow-up
- All ctDNAneg patients
- ctDNAneg patients randomized to follow-up
Separate statistics will be presented for the full analysis set and the per protocol analysis set.

17.8.4 Primary endpoint: disease free survival

The primary efficacy endpoint (DFS) is planned to be analysed in an interim analysis, and if the study is continued after interim analysis, in a final analysis. The interim analysis will be performed when 60% of the events are recorded (93 events after approximately 38 months). The bound for rejection of the null hypothesis is 2.572 corresponding to a p-value of 0.0051. The estimated power to reject the null hypothesis with the interim analysis is 0.3493. To test the null hypothesis a stratified log-rank test will be used. Strata will be the same as described for stratified randomisation. The interim test will be one-sided with a significance level of 0.0051 so that the null hypothesis is rejected when the p-value is < 0.0051. The interim analysis is binding.

The final analysis will be performed when all planned 154 events are recorded (after approximately 60 months). The primary endpoint will be evaluated by a stratified log rank test. Strata will be the same as defined for randomisation and the interim analysis. The final test will be one-sided at a significance level of 0.0232 corresponding to a decision bound of 1.992 so that the null hypothesis is rejected if the p-value is < 0.0232. The overall significance level is 0.025 one-sided.

The test will be performed in the full analysis set. As sensitivity analysis the primary endpoint is also evaluated in the per-protocol-set.

Kaplan-Meier estimates of median survival with two-sided 95% confidence intervals as well as Kaplan-Meier estimates of 3-year and 5-year survival rates with two-sided 95% confidence intervals will be calculated for each group.

17.8.5 Secondary endpoints

For all time-to-event endpoints Kaplan-Meier estimates of median survival with two-sided 95% confidence intervals as well as Kaplan-Meier estimates of 3-year and 5-year survival rates with two-sided 95% confidence intervals will be calculated for each group.

Comparisons between groups will be performed with a stratified log-rank test. Strata will be the same as for the primary analysis. Significance level will be 0.025 one sided.

Following comparisons are planned:

- Overall survival of patients randomized to chemotherapy vs. patients randomized to follow-up in the group of ctDNApos patients
- Time to recurrence in patients randomized to chemotherapy vs. patients randomized to follow-up in the group of ctDNApos patients as sensitivity analysis
- Cancer specific survival of patients randomized to chemotherapy vs. patients randomized to follow-up in the group of ctDNApos patients as sensitivity analysis
- Disease-free survival of patients with ctDNA neg vs. ctDNA pos in the group of patients randomized to follow-up
- Overall survival of patients with ctDNA neg vs. ctDNA pos in the group of patients randomized to follow-up
- Time to recurrence in patients with ctDNA neg vs. ctDNA pos in the group of patients randomized to follow-up as sensitivity analysis
- Cancer specific survival of patients with ctDNA neg vs. ctDNA pos in the group of patients randomized to follow-up as sensitivity analysis

17.8.6 Sensitivity analysis of the primary variable

The primary endpoint variable DFS may be subject to bias from various sources of which the following are considered particularly critical:

- Deviations from the planned visits that might differ between the study arms and result in biased estimates of DFS times.
- Exact times of progression and metastases will usually not be known, because these events will be determined during study visits only. This may lead to an overestimation of DFS times.
- Non-protocol tumour directed therapies before a DFS event may result in overestimation of DFS times.

To address these issues the following sensitivity analyses are planned:

- An analysis of overall DFS events occurring at any time during the trial follow-up. Both treatment arms will be compared with a generalized linear model with complementary log-log (clog-log) link function. This analysis is intended to address the first two problems outlined above.
- An analysis of DFS, where the date of diagnosis of local recurrence, distant metastasis, second colorectal or non-colorectal tumour is substituted by the date of the next scheduled visit, if diagnosed outside a scheduled visit. This analysis is intended to reduce bias introduced by possible asymmetries of unscheduled staging visits between both arms. The comparison is conducted with the stratified log-rank test.
- An analysis of DFS, for which non-protocol tumour directed therapies are considered as events. The start date of the non-protocol tumour directed therapy will serve as event date. This analysis is intended to reduce bias introduced by the intervening non-protocol tumour directed therapies. The comparison is conducted with the stratified log-rank test.

17.8.7 Exploratory analyses

Multivariable Cox-regression models will be fitted to explore prognostic and predictive value of measured variables like molecular tissue and plasma markers and others on the variables defined in sections 17.7.3 and 17.7.4. These models will also always include the variables used for stratification.
17.8.8 Analysis of safety data

Safety data will be analysed in the safety evaluation set.

All adverse events that occurred at or after administration of the first dose of chemotherapy will be used for the safety analyses.

Following metrics will be calculated:

- Number and percentage of patients with at least one adverse event
- Absolute number of all adverse events
- Minimum, maximum and mean number of adverse events per patient
- Number and percentage of patients with at least one serious adverse event
- Absolute number of all serious adverse events
- Minimum, maximum and mean number of serious adverse events per patient
- Number and percentage of patients with at least one (serious) adverse event for which relatedness to study chemotherapy cannot be excluded
- Minimum, maximum and mean number of (serious) adverse events for which relatedness to the study chemotherapy cannot be excluded
- Absolute numbers and percentages of patients with defined adverse events will be tabulated by CTC AE grade
- Absolute number and percentages of patients with defined adverse events of grades 3 to 5 will be tabulated by CTC AE grade

Following parameters will be listed subject-wise:

- AEs with following detailed information:
  - description of AE
  - treatment cycle
  - date of first application chemotherapy
  - date of onset of AE
  - date of AE outcome
  - intensity
  - seriousness
  - relatedness to chemotherapy
  - actions taken
  - outcome

- SAEs will be listed in the same manner in a separate listing
  - All deaths will be listed with following detailed information:
    - Age
    - Gender
    - Weight
    - Height
    - Treatment cycle
    - AE
    - AE duration
    - AE severity
Incidences and intensities of all AEs and SAEs will be summarised on subject-level in tables as shown in the Appendix (see chapter 25).

All common adverse events (observed in at least 10% of all patients) and adverse events of CTC grading 3 - 5 will be summarised as shown in Table 7.

All adverse events of CTC grading 3 - 5 will be summarised as shown in Table 8.

17.9 Publications

The final publication of the main trial results will be written by the coordinating investigator on the basis of the final analysis and published in a major scientific journal.

Authors of the final publication will include as first or last author the study coordinator and the coordinator of the ctDNA laboratory, as well as the coordinators for pathology, surgery and the AIO screening platform, the investigators or representatives of groups who have included more than 5% of the eligible patients in the trial, the trial statistician and further investigators according to their contribution to the trial. The final decision on the authorship and the order will be made by the steering committee based on the contribution to the trial. Further manuscripts will include the above mentioned authors. In case of collaborative publications with other groups (i.e. meta analyses), the authors will be selected according their contribution.

The final publication of associated translational research studies will be written by the coordinating investigator of the corresponding translational research study.

For publication of translational research results, co-authors will also include scientific collaborators who made substantial contribution to the research.

The above rules are applicable to publications involving any individual patient registered/randomized in the trial.

The investigators agree with the signature that they agree that their names, addresses and the participation at the trial can be published in relationship with the trial.
18 Translational research

To achieve a more profound understanding in the ctDNA based analysis and the development and diagnosis of colorectal cancer; additional investigations are made during and after the study. These investigations depend on the patient's consent to the translational research. There is no intention to communicate the individual results to the investigator or the patient.

18.1 Conversion of ctDNA positive patients

To determine the conversion rate and the time to conversion, a plasma sample is taken before each chemotherapy cycle during adjuvant therapy, at the EOT visit and at the follow-up visits until progression in ctDNA positive patients. The plasma is used to determine the ctDNA level during and after treatment. The rate of patients becoming ctDNA negative, the time to ctDNA negativity is calculated. These analyses may be performed during or after study.

18.2 ctDNA level before recurrence

In patients during follow-up, plasma samples are collected at the follow-up visits until disease recurrence / metastasis. The ctDNA level are determined during follow-up to determine the rate of ctDNA positivity at time of the clinical diagnosis of metastases/recurrence and to determine the mean time of when ctDNA was detected before the clinical diagnosis of metastases/recurrence.

18.3 ctDNA level at time of enrolment and diagnosis

The ctDNA level before resection, after resection (screening) and at randomisation are determined. The DFS is calculated according to the ctDNA level. In addition, the postoperative ctDNA positivity is calculated according the preoperative ctDNA level (if available).

18.4 Remaining material

Remaining material (tumour, buffy coat, plasma) is stored for further analyses related to the colorectal cancer, pharmacogenomics of the used drugs and immunologic analyses including polymorphisms. The material is stored in the biobank of the NCT biobank, Dresden, Germany, except material that was collected and analysed in screening platforms (and is covered by their informed consent). The steering committee will decide on the further use of the material for translational research.
19 Ethical and administrative aspects

19.1 Responsibility of the sponsor and the investigator

The sponsor of the clinical trial (Technische Universität Dresden) has the responsibility for the initiation, organisation and the financing of the clinical study according to the local regulations (Germany: AMG §4). The sponsor and the investigator ensures, that the clinical trial is conducted in agreement with the declaration of Helsinki that is available on the World Medical Association web site (http://www.wma.net), in accordance with the international Good Clinical Practice (ICH-GCP) standards and all local laws and regulations concerning clinical studies, for Germany the AMG and the GCP Verordnung.

The investigator accepts the requirements of the clinical study protocol. The investigator obligations are i.e.:

- understanding the properties of the investigational properties described in the Investigator's Brochure or in the product information,
- understanding and implementation of the trial protocol,
- to ensure that sufficient time and capacity are available to conduct the trial,
- correct collection and documentation of the data and appropriate reporting,
- to provide all required data for sponsor, monitoring or competent authorities for audits and / or inspections,
- to ensure that information about participants and any information received from the sponsor is treated confidentially by all persons involved in the trial,
- to declare if persons who may be dependent on the sponsor or the investigator are involved in the trial,
- to provide information on possible financial and other interests of the investigator with regard to the investigational medicinal products,

The investigator is responsible for the conduct of the clinical trial at the level of the study site (Germany: § 4 AMG)

19.2 Patient information and Informed Consent

It is the responsibility of the investigator to obtain written informed consent from each patient participating in this study, after adequate explanation of the aims, possible adverse event, procedures and possible risks, the mechanism of treatment allocation and the aspects of data protection – especially that the data are transferred to the sponsor using pseudonyms and that the medical records can be reviewed by authorised persons other than the treating physician, i.e. monitors, auditors, delegates of health authorities.
A written informed consent covering the referring part (screening, randomised part) of the study must be obtained before any study specific procedures are performed.

It is emphasized in the patient information sheet that participation is voluntary and that the patient is free to refuse further participation in the protocol without any impact on the subsequent care.

The informed consent form will be personally signed and dated by the patient and the investigator and must be kept on file by the investigator(s), and documented in the eCRF and the patients medical records. The investigator confirms obtaining the written informed consent to the sponsor. If new safety information results in significant changes in the risk/benefit assessment, the consent form is reviewed and updated if necessary. All patients (including those already being treated) will be informed of the new information and are asked for their written informed consent to continue in the study. If the family doctors are informed of their patients’ participation in the clinical study, this should be mentioned in the consent form.

19.3 Data Protection and Confidentiality

Recording, storage, disclosure, and analysis of personal data of the participants within this clinical trial are in accordance with legal requirements such as the EU privacy regulations, the German Federal law (Bundesdatenschutzgesetz) and the law of the state (Sächsisches Datenschutzgesetz). The requirement for this is the voluntary consent of the participating persons within the scope of the informed consent form prior to participation in the clinical trial.

The participants of the following within the information on this clinical trial:

1. Data are recorded electronically in eCRFs, will be handled confidentially, and only disclosed on to third parties without naming (pseudonymised):
   a. to the sponsor of the trial for the scientific analysis and assessment of adverse events
   b. the competent supervisory authorities (local and federal authorities), ethic committee and to the European database for checking the appropriate conduct of the clinical trial and for evaluating/collecting test results and undesirable events

2. If necessary for the review of the clinical trial, authorized representatives of the sponsor (monitoring, auditing) and/or the competent supervisory authority who are bound to confidentiality may inspect the personal data available at the trial site. For this purpose, the investigator is not bound to the obligation of medical confidentiality.

3. The consent to the recording and handling of personal data within the scope of this clinical trial is irrevocable. The participating person will be informed that he/she can discontinue participation in the clinical trial at any time - without giving reasons and without any subsequent consequences. In the event of revocation of the declaration of consent, the data stored up to this point in time will continue to be used without naming a name, if this is necessary to evaluate the effects of the investigational product and to ensure that the interests of the person concerned worthy of protection are not impaired.
19.4 Insurance for trial participants

On behalf of the sponsor, an insurance is contracted for all trial participants according to the country specific regulations (Germany: AMG § 40 Abs. 1 Nr. 8 and Abs. 3):

Insurance company: XL Insurance Company SE (AXA group), 8 St. Stephen’s Green, Dublin 2, Irland

German affiliation: Kranhaus 1, Im Zollhafen 18, 50678 Köln, Germany

Police number: DE00039998LI19A

Telephone: +49 89 63206 606

Email: neuschaden@axaxl.com

The insurance covers study related damages to the health of participants with a limit of 500 000 €/participant (Germany, Austria) or 1 million CHF (Switzerland) up to 10 million Euro (Germany) per study (Germany), 10 million CHF per study (Switzerland) or 5 million Euro per study (Austria). The insurance cover all potential damages that are directly or indirectly related to the study drug or interventions related to the study itself.

In order to maintain the insurance coverage, all participants have to follow the recommendations of the study personnel. Furthermore, the study participants are not allowed to undergo a medical treatment outside the study without previous agreement of the investigator except for emergencies. The study participants have to inform the investigator as soon as possible from a treatment for medical emergencies. The insurance company is to inform from a health damage potentially related to the clinical as early as possible. Furthermore, all necessary measures have to be made to clarify the background the consequences of the potential damage.

The study participant receives the insurance conditions with their patients informed consent.

19.5 Study discontinuation

The whole study may be discontinued at the discretion of the sponsor in the event of any of the following:

- Medical or ethical reasons affecting the continued performance of the study
- Difficulties in the recruitment of patients
- Occurrence of adverse events unknown to date in respect of their nature, severity, and duration or the unexpected incidence of known adverse events or other new medical aspects
19.6 Independent ethics committees and regulatory authorities

19.6.1 Approval of the study by the regulatory authority and EC

The sponsor submits the protocol and the necessary additional files to the applicable competent authorities and the ethics committees and contracts an indemnity insurance for the patients. The positive opinion of the responsible ethics committees and the approval of the authorities is a precondition to start the study at the sites.

19.6.2 Notification of the study

The sponsor is responsible to notify competent regional authority about the study and all investigators of the participating investigational sites, if applicable to local law.

19.6.3 Report and documentation obligation

The sponsor and the investigator are responsible to comply with the report and documentation obligations in accordance with local legal requirements, statutes and the European Clinical Trial Directive.

19.7 Conditions for modifying the protocol

Protocol modifications to the ongoing study are made via amendment. The sponsor is responsible to obtain independent approval for substantial amendments from the applicable competent authority and a positive opinion from the ethics committees in accordance with local legal requirements, statutes and the European Clinical Trial Directive. Approval must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects or when the changes are non-substantial and involve only logistical or administrative aspects of the trial (e.g. change of telephone numbers).
20 References


Appendix: Sampling and shipment of ctDNA

1) Blood samples are taken into two provided tubes

2) Fill the tubes until the marked level

3) Essential! Turn the tubes 5 x (to ensure that the blood mixes with the fluid in the tube)

4) Label the tubes with centre- and patient number

5) Put the tubes into the covering tubes

6) Ship the tubes with one copy of the screening form (during treatment: a copy of the shipment form) in the provided box to:

Universitätsklinikum Carl Gustav Carus
Medizinische Klinik I
Labor Molekulare Diagnostik / Prof. Thiede
Haus 65
Fetscherstr. 74
01307 Dresden

Abs.:
Appendix: Shipment of the tumour block

The screening form is faxed to: +49 351 45 888 7 666. The original form is filed in the local CRF.

A copy of the screening form is:

1) shipped with the ctDNA tubes
2) shipped with the tumour block
3) filed in the patient’s file.

The pathology report is faxed to: +49 351 45 888 7 666 for central review. The patient identifiers are removed an replaced by the patient and centre number before transmission.
23 Appendix: Central pathology

The formalin fixed paraffin embedded (FFPE) tumour block is shipped to the reference laboratory assigned to the centre. In addition, the pathology report is faxed for central review. The patient identifiers are to be removed/deleted before transmission and replaced by the patient number and year of birth.

At the central pathology laboratory, the tumour block is reviewed for the tumour cell content. Microsatellite instability is excluded by immunohistochemistry if the information is not available from the local pathologist.

The tumour tissue is macro-dissected and the DNA is extracted. By panel analysis, the patient individual mutation(s) are determined.

The results of the analysis are entered in the eCRF.

If the immunohistochemistry reveals microsatellite instability or when the central review or the updated information confirm a tumour stage different from UICC stage II, no further analysis is performed for potential randomisation.

The block is stored – depending on the informed consent of the patient – for later translational research.
24 Appendix: ctDNA analysis

24.1 Isolation of cfDNA from plasma

For separation of plasma, blood samples are centrifuged at 300 g for 20 min. Without disturbing the buffy coat, the plasma layer (supernatant) is carefully removed and transferred into a new 2 mL low-bind tube. To completely remove residual cells, plasma samples are then centrifuged at 5000 g for 10 min and either used for isolation of cfDNA directly or stored at -20 °C until cfDNA extraction.

For cfDNA extraction from plasma one of two different protocols is used: Zymo Quick cfDNA serum & plasma kit (spin-based, carrier RNA: no) (Zymo Research, Irvine, CA, USA) or the QIAamp Circulating NA Kit (vacuum-based, carrier RNA: yes) (Qiagen, Hilden, Germany). All extractions are performed using 3-5 mL of plasma according to manufacturer's protocols. For both kits cfDNA will be eluted into 35 μL ddH2O, quantified by a β-globin-specific qPCR in comparison to a serial dilution of a reference DNA with known quantity on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and stored at -20°C until downstream processing for NGS.

24.2 PCR and NGS-based detection of tumour specific single nucleotide variants (SNVs)

PCR to detect SNVs in ctDNA will be performed using 5–10 ng of plasma derived cfDNA (35-40 cycles) using the Q5® High-Fidelity polymerase (New England Biolabs, Beverly, MA, USA) according to the optimized conditions for NGS-based detection of low-level SNVs as described previously. Briefly, Fusion PCR primer for the preparation of amplicon libraries will be designed according to the manufacturer's recommendations (Fusion Method; Life Technologies) for all SNVs identified in the analysis of the tumour material of the screened patients. All PCR reactions were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). After a two-round purification process with Agencourt AMPure XP Reagent (Beckman Coulter, Krefeld, Germany) barcoded PCR products were quantified with a Qubit dsDNA HS Assay (Life Technologies) and sequenced unidirectional on an Ion Torrent S5xl semiconductor-based device (Life Technologies), according to manufacturer's protocols and as described by Stasik et al. Data will be analysed using the Torrent Suite Software version 3.2 and the Torrent Variant Caller (TVC, v.4.0) plugin with default settings and alignment to the hg19 human reference genome from the UCSC Genome Browser (http://genome.ucsc.edu/). According to false-positive rates of individual targets based on the analysis of wt-DNA, NGS-based ctDNA detection is conducted with a defined cut-off of 0.01%
or 0.1% VAF for certain transition substitutions at e.g. NRAS c35G>A/c.38G>A; KRAS c.40G>A/c.436 G>A and TP53 c.742C>T. To increase accuracy, triplicate reactions using three different barcodes will be used (barcode binning). VAFs below the predefined thresholds will be considered wild-type (wt), whereas all mutations present at levels >3 times above the background in all three reactions are considered ctDNA positive.
# Appendix: Analysis of safety data

<table>
<thead>
<tr>
<th>Table 7 Tabulation of all adverse events</th>
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<tbody>
<tr>
<td><strong>Adverse event</strong></td>
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<td>-------------------</td>
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<tr>
<td>Event 1, n (%)</td>
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<tr>
<td>Event 2, n (%)</td>
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<td>Event x, n (%)</td>
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<table>
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<tr>
<th>Table 8 Tabulation of adverse events of CTCAE grades 3 - 5</th>
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<tr>
<td><strong>Adverse event</strong></td>
</tr>
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<td>-------------------</td>
</tr>
<tr>
<td>Event 1, n (%)</td>
</tr>
<tr>
<td>Event 2, n (%)</td>
</tr>
<tr>
<td>Event x, n (%)</td>
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</tbody>
</table>
26 Summary of Protocol Changes

26.1 Version 2.0 (11 Nov 2019)

1. The pregnancy test for women with child-bearing potential before each treatment cycle and at end of treatment was added due to request of the German authority (BfArM) and the ethic committee. (Visit overview and chapter 13) The postmenopausal status was specified in each foot note.

2. The discussion of the potential consequences of oxaliplatin on fertility and the options regarding fertility preservation, especially for patients who are planned for and still planning pregnancy or fatherhood is described in the visit overview and in chapter 13. (Screening / patient informed consent, randomisation, first cycle) according to the request of the German authority (BfArM) and the ethic committee.

3. The chapter 13.10 (pregnancy and contraception) was modified and specified according to the request of the German authority (BfArM) and the ethic committee.

4. The changes in the DSMB are reflected in the protocol (Dr. Völp is added as statistician, Dr. Montemurro left the DSMB) at the administrative section and the chapter 14.5

5. The Swiss Group for Clinical Cancer Research was added as additional study group, including the coordinator for Switzerland

6. The per-protocol analysis was specified to reduce a potential bias (chapter 17.5.2)

7. The chapter “sensitivity analysis” (17.8.6) was added to describe the planned additional analyses

8. The chapter “protocol changes” (26) was added

9. Minor changes:
   a. registration number for clinicaltrials.gov added (title page)
   b. insurance: contact data added (chapter 19.4)
   c. composition of patient numbers (chapter 13.1.2 and 17.2)
   d. The timelines for the protocol were updated in order to reflect the delayed start.