

ORIGINAL ARTICLE



ctDNA-guided adjuvant treatment after radical-intent treatment of metastatic spread from colorectal cancer—the first interim results from the OPTIMISE study

Louise Bach Callesen^a , Torben Frøstrup Hansen^b, Rikke Fredslund Andersen^c, Niels Pallisgaard^d, Stine Kramer^e, Sven Schlönder^f, Søren Rafael Rafaelsen^g, Anders Kindberg Boysen^a, Lars Henrik Jensen^b , Anders Jakobsen^b and Karen-Lise Garm Spindler^a 

^aDepartment of Oncology, Aarhus University Hospital, Aarhus, Denmark; ^bDepartment of Oncology, Vejle Hospital, University Hospital of Southern Denmark, Vejle, Denmark; ^cDepartment of Biochemistry and Immunology, Vejle Hospital, University Hospital of Southern Denmark, Vejle, Denmark; ^dDepartment of Pathology, Zealand University Hospital, Næstved, Denmark; ^eDepartment of Nuclear Medicine & PET-Centre, Aarhus University Hospital, Aarhus, Denmark; ^fDepartment of Radiology, Aarhus University Hospital, Aarhus, Denmark; ^gDepartment of Radiology, Vejle Hospital, University Hospital of Southern Denmark, Vejle, Denmark

ABSTRACT

Background: Patients with detectable ctDNA after radical-intent treatment of metastatic spread from colorectal cancer (mCRC) have a very high risk of recurrence, which may be prevented with intensified adjuvant chemotherapy (aCTh). In the OPTIMISE study, we investigate ctDNA-guided aCTh after radical-intent treatment of mCRC. Here we present results from the preplanned interim analysis.

Material and methods: The study is an open-label 1:1 randomized clinical trial comparing ctDNA-guided aCTh against standard of care (SOC), with a run-in phase investigating feasibility measures. Key inclusion criteria; radical-intent treatment for mCRC and clinically eligible for triple-agent chemotherapy. Patients underwent a PET-CT scan before randomization. ctDNA analyses of plasma samples were done by ddPCR, detecting CRC-specific mutations and methylation of the *NPY* gene. In the ctDNA-guided arm, ctDNA positivity led to an escalation strategy with triple-agent chemotherapy, and conversely ctDNA negativity led to a de-escalation strategy by shared-decision making. Patients randomized to the standard arm were treated according to SOC. Feasibility measures for the run-in phase were; the inclusion of 30 patients over 12 months in two Danish hospitals, compliance with randomization >80%, rate of PET-CT-positive findings <20%, and eligibility for triple-agent chemotherapy >80%.

Results: Thirty-two patients were included. The rate of PET-CT-positive cases was 22% ($n = 7/32$). Ninety-seven percent of the patients were randomized. Fourteen patients were randomly assigned to SOC and sixteen to ctDNA-guided adjuvant treatment and follow-up. All analyses of baseline plasma samples in the ctDNA-guided arm passed the quality control, and 19% were ctDNA positive. The median time to result was three working days. All ctDNA-positive patients were eligible for triple-agent chemotherapy.

Conclusion: The study was proven to be feasible and continues in the planned large-scale phase II trial. Results from the OPTIMISE study will potentially optimize the adjuvant treatment of patients undergoing radical-intent treatment of mCRC, thereby improving survival and reducing chemotherapy-related toxicity.

ARTICLE HISTORY

Received 9 May 2023
Accepted 10 September 2023

KEYWORDS

Oligometastatic; colorectal adenocarcinoma; circulating tumor DNA; adjuvant treatment; randomized clinical trial

Survival from metastatic colorectal cancer (mCRC) has improved over the last two decades, with radical-intent local treatment as one of the drivers [1]. However, there is a high risk of relapse despite using adjuvant chemotherapy [2, 3]. The current recommendations are based mainly on two studies without a convincing effect of adjuvant treatment [4]. Today's standard of care (SOC) is unable to deliver a personalized approach.

Detectable ctDNA, i.e. minimal residual disease (MRD), in a plasma sample after radical-intent treatment of metastatic spread from colorectal cancer (CRC) is a very poor prognostic factor [5]. Across all stages of CRC, patients with circulating tumor DNA (ctDNA) after radical-intent treatment can become

ctDNA negative during adjuvant chemotherapy, and this conversion is associated with improved outcomes [6]. Furthermore, in a pilot study at our institution, we reported a relapse of ctDNA after the termination of SOC adjuvant chemotherapy, which could indicate a need for more intensified adjuvant chemotherapy in ctDNA-positive patients [7]. In low-risk primary colon cancer, ctDNA-guided adjuvant treatment was evaluated in a randomized clinical trial [8]. The results confirmed that the ctDNA-guided approach led to the use of less adjuvant chemotherapy without compromising survival.

We hypothesize that patients with detectable ctDNA after radical-intent treatment of mCRC have MRD and,

consequently, a very high risk of recurrence, which can be reduced with intensified adjuvant chemotherapy. Patients without detectable ctDNA have undergone successful radical treatment and may not benefit from adjuvant chemotherapy. Hence, ctDNA-guided adjuvant treatment may improve disease-free survival and minimize chemotherapy-related toxicity.

To the best of our knowledge, OPTIMISE is the first randomized trial to investigate the clinical utility of ctDNA-guided adjuvant treatment and follow-up in patients who have undergone radical-intent treatment of metastatic spread from CRC. Hence, there were significant reservations when planning the study. Due to the lack of precedent, the number of possible candidates who would consent to inclusion in the study was unknown. Furthermore, no studies report the fraction of PET-CT-positive cases after radical-intent treatment of mCRC nor on treatment options in patients with a positive PET-CT scan. In the literature, the rate of ctDNA-positive cases varies from 14 to 67% in patients after radical-intent treatment of mCRC. However, this is based solely on observational studies, with varying markers of ctDNA, analytical methods, and time-point for sampling [6, 7, 9–21].

Hence, it was of utmost importance to test the feasibility before running a larger-scale multicenter study. Consequently, we have initiated a run-in phase into the planned phase II, multicenter, randomized controlled trial. Here we present the preplanned interim analyses of the feasibility parameters.

Material and methods

The trial was initiated by investigators based at the Department of Oncology, Aarhus University Hospital, and the Department of Oncology, Vejle Hospital. The study protocol, available in [Supplementary Appendix 1](#), was approved by the Central Region Committees in Health Research Ethics (1-10-72-249-20) and Danish Medicines Agency (EudraCT No: 2020-004524-41), and it was prospectively registered with ClinicalTrials.gov (NCT04680260). Written and oral informed consent was obtained from all patients. The study complied with the Declaration of Helsinki. The study protocol has previously been published [22].

Study design

The trial is an open-label randomized phase II study investigating ctDNA-guided adjuvant treatment compared to SOC after radical-intent treatment of metastatic spread from CRC. Patients are randomly assigned in a 1:1 ratio to SOC and ctDNA-guided adjuvant treatment and follow-up. The study design is consistent for both the run-in phase and the planned large-scale phase II study.

Patients

Patients who had undergone radical-intent treatment for metastatic spread from CRC were included at the

Department of Oncology, Aarhus University Hospital (Aarhus, Denmark) and the Department of Oncology, Vejle Hospital (Vejle, Denmark). The key inclusion criteria were radical intended treatment for metastatic spread from CRC, no evidence of further disease based on pre-treatment work-up according to SOC, age at least 18 years, Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2, eligibility for triple chemotherapy, written and verbally informed consent. The key exclusion criteria comprised Neuropathy National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade > 1, significant other cancer disease within 5 years of inclusion, intolerance or allergy to 5-Fluorouracil (5-FU), leucovorin, irinotecan, oxaliplatin or capecitabine.

Radiological evaluation

All included patients underwent a PET-CT scan before randomization to exclude metabolically detectable metastatic lesions or other unforeseen concurrent diseases. Patients underwent standardized follow-ups with CT scans of the chest, abdomen, and pelvis planned for every three months after local treatment for the first year, then every four months for the second year, and hereafter once a year for the last 3 years up to 5 years of follow-up.

Blood sampling and analyses

The first plasma specimen was obtained for ctDNA analysis after randomization no earlier than 2–3 weeks post-local treatment. In patients treated with stereotactic body radiation therapy, a later time point of 4 weeks was chosen to allow for the deferred tumor reduction after radiotherapy. Hereafter, plasma specimens were obtained prospectively at each response evaluation. In the experimental arm, 4 × 10 ml blood samples were collected in Streck tubes and sent to the laboratories the same day for analyses. ctDNA results were made available to the treating clinician within four working days after obtaining the plasma specimen. In the standard arm, 3 × 10 ml blood samples were collected in ethylenediamine tetra-acetic acid (EDTA) tubes.

Plasma was isolated by double-centrifugation at room temperature; 2000g for 10 min and 10,000g for 10 min. Centrifugation was done within 2 h for the samples in EDTA tubes and within 4 days for the samples in Streck tubes (Streck, La Vista, NE). Plasma samples from patients in the standard arm were stored at –80 °C until further analyses at the end of the study. Plasma from patients in the experimental arm was isolated, and cysteine-rich polycomb-like protein 1 (CPP1) DNA fragments were added as an exogenous internal control [23].

Methylation analyses

Tumor-specific methylated DNA was defined as DNA with methylation of the *NPY* gene promoter [24]. Circulating free DNA (cfDNA) was purified from 4 ml of plasma on the QIAasympphony SP instrument using QIAasympphony DSP

circulating DNA kit (Qiagen, Hilden, Germany) or the Maxwell purification instrument using the Maxwell RSC ccfDNA LV plasma kit according to the manufacturer's instructions. Before concentrating the purified DNA, 30 µl of the 200 µl was saved for quality assurance tests. The purified DNA was concentrated to 20 µl on Amicon Ultra Centrifugal filter units (Millipore, Burlington, MA, USA) and was bisulfite converted in a 150 µl reaction with the EZ DNA Methylation lightning spin-column kit (Zymo Research, Irvine, CA, USA) and eluted in 15 µl. The converted DNA was analyzed with a methylation-specific assay and a control assay (Albumin gene) using the BioRad Droplet Digital PCR (ddPCR) system QX200 (BioRad, Hercules, CA, USA). The Albumin/*NPY* duplex analysis was performed in two wells with 5 µl converted DNA per well in 20 µl reactions. ddPCR supermix for probes (no deoxyuridine triphosphate) and Albumin/*NPY* assays were applied [24]. Droplets were generated on the QX200 automated droplet generator from BioRad. PCR was completed on the Veriti PCR device (Thermo Fisher Scientific, Waltham, MA, USA). The QX200 droplet reader from BioRad counted droplets. Data analysis was performed with QuantaSoft by BioRad version 1.7.4.

Water and a pool of DNA from the whole blood of non-cancer individuals were used as negative controls, and universal human methylated control DNA (Zymo Research) and EpiTect control DNA (Qiagen) were used as positive controls. Negative controls and universal human methylated control DNA were bisulfite converted together with the samples. The methylation analysis has a limit of blank of 0.03% and a limit of detection of 0.04%.

Mutation analyses

cfDNA was purified from 4 ml plasma on a Chemagic 360 robot (PerkinElmer, Waltham, MA, USA) using a 1304 cfDNA purification kit (PerkinElmer) according to the manufacturer's instructions and eluted in 100 µl. DNA was analyzed by eight multiplex ddPCR assays using the BioRad ddPCR system QX200 (BioRad, Hercules, CA, USA). Seven multiplex ddPCR assays covering 33 mutations in the *KRAS* G12D, G12V, G12A, G12C, G12R, G12S, G13D; *KRAS* Q61K, Q61L, Q61R, Q61H (c.183A>T and c.183A>C); *KRAS* K117N (c.351A>T and c.351AC); *KRAS* A146T, A146V and A146P; *NRAS* G12A, G12C, G12D, G12S, G12V, G13D, G13R, G13V; *NRAS* Q61K, Q61L, Q61R Q61H (c.183A>T and c.183A>C); and *BRAF* V600E. The eighth multiplex ddPCR reaction served as a sample quality control analysis for *CPP1* spike-in (sample DNA recovery) and lymphocyte DNA contamination [23]. Five microliters of DNA eluate were used for each of the eight multiplex ddPCR reactions. Droplets were generated on the QX200 automated droplet generator from BioRad. PCR was completed on the Veriti PCR device (Thermo Fisher Scientific, Waltham, MA, USA). The QX200 droplet reader from BioRad counted droplets. Data analysis was performed with QuantaSoft by BioRad version 1.7.4. In case of a positive finding, a singleplex assay was performed to find the specific mutation. The mutational analysis has a limit of blank of 0.025–0.28% (depending on the mutation analyzed) and a

limit of detection of 0.05–0.3% (depending on the mutation analyzed).

Quality assurance

Three parameters were evaluated for quality assurance of analysis results (Supplementary Appendix 2). Firstly, a potential loss of DNA during purification and analysis was evaluated using a *CPP1* assay, as previously published [23]. Secondly, potential contamination of the purified DNA with DNA from white blood cells was evaluated using an immunoglobulin gene-specific assay (PBC) [23]. Thirdly, to control cfDNA amount and sample fragmentation, an in-house multiplex ddPCR reaction was performed, amplifying a 65 bp and 250 bp fragment of the *EMC7* gene [25]. All controls were included in each round of ddPCR.

Interpretation of blood sample analyses

If either the mutation analysis, the methylation analysis, or both analyses were positive, the ctDNA status was evaluated as positive. If both mutation and methylation analyses were negative, the ctDNA status was negative (Table 1).

Treatment

Patients randomized to the standard arm were offered adjuvant chemotherapy according to SOC. Plasma specimens will be analyzed retrospectively at the end of the study to evaluate ctDNA status for comparison.

Patients randomized to the ctDNA-guided strategy had plasma specimens analyzed prospectively. ctDNA positivity led to escalated adjuvant treatment (4 months of FOLFOXIRI followed by 2 months of 5-FU monotherapy). ctDNA negativity opened the option of observation without further treatment. The advice to observation, single-agent chemotherapy with fluoropyrimidine, or combination chemotherapy with fluoropyrimidine and oxaliplatin was based on shared decision-making.

Endpoints and assessments

Feasibility measures:

- Inclusion of 30 patients over 12 months in two clinical centers
- Compliance with randomization >80%
- Rate of PET-CT positive cases at inclusion <20%

Table 1. Overview of conclusion on ctDNA status based on molecular analyses.

ctDNA status	Results of molecular analyses
ctDNA positive	+ Detection of CRC mutation + Detection of <i>NPY</i> methylated DNA + Detection of CRC mutation – Detection of <i>NPY</i> methylated DNA – Detection of CRC mutation + Detection of <i>NPY</i> methylated DNA
ctDNA negative	– Detection of CRC mutation – Detection of <i>NPY</i> methylated DNA

CRC, colorectal cancer.

Table 2. Patient characteristics.

Number of patients	32 (100)
Age, years, median (range)	61 (35–76)
Sex	
Male	22 (69%)
Female	10 (31%)
Performance status (PS)	
0	15 (47%)
1	11 (34%)
2	1 (3%)
Missing	5 (16%)
Histology	
Adenocarcinoma	31 (97%)
Mucinous adenocarcinoma	1 (3%)
Tissue mutation status	
<i>RAS/BRAF</i> wild type	10 (31%)
<i>RAS/BRAF</i> mutation	9 (28%)
Missing	13 (41%)
MSI status	
MSS	32 (100%)
MSI	0 (0%)
Stage at primary diagnosis	
I	7 (22%)
II	3 (9%)
III	7 (22%)
IV	15 (47%)
Time since primary diagnosis, months, median (range)	14.6 (1.7–115.7)
Time since initial diagnosis of metastatic disease, months, median (range)	5.9 (0.4–59.3)
Number of previous local treatments before randomization	
1	16 (50%)
2	5 (16%)
3	6 (19%)
≥4	3 (9%)
Not randomized	2 (6%)
Local treatment immediately before randomization	
Liver	25 (78%)
Resection	16 ^a
RFA	6
SBRT	2
MWA	1
Lung	4 (13%)
Resection	3
RFA	0
SBRT	0
MWA	1
Other	1 (3%)
Not randomized	2 (6%)

Values are expressed as *n* (%) unless otherwise specified.

MSI, microsatellite instability; MSS, microsatellite stability; RFA, radiofrequency ablation; SBRT, stereotactic body radiation therapy; MWA, microwave ablation.

^aTwo patients were treated with RFA during liver resection.

- ctDNA positivity rate at inclusion >20%
- Eligibility for triple-agent chemotherapy >80%

The overall primary endpoint is the rate of patients free from recurrent CRC 2 years after inclusion. Secondary endpoints include toxicity, molecular biological response to therapy, molecular biological disease-free survival 1 year from inclusion, time to recurrence, overall survival, patient quality of life, and cost-effectiveness. Here we report the results from the 12-month interim analyses of the feasibility measures. Outcomes for primary and secondary endpoints are not reported here.

Statistical analyses

The test turn-around time was calculated from the sample acquisition date to the results report date. Categorized

variables were expressed as counts and proportions, and continuous variables as medians, values, and ranges. Statistical analyses were performed using STATA/IC17.0 (StataCorp LLC).

Results

A total of 32 patients were enrolled between October 2021 and January 2023, corresponding to an average enrollment period of 12 months per center. The baseline characteristics of the included patients are presented in Table 2. The included patients had a median age of 61 years, and 69% were male. The vast majority had an ECOG PS of 0 (47%) or 1 (34%).

The study flow is presented in Figure 1. All included patients underwent a PET-CT scan before randomization, and the rate of PET-CT-positive cases was 22% (*n* = 7/32). Five of

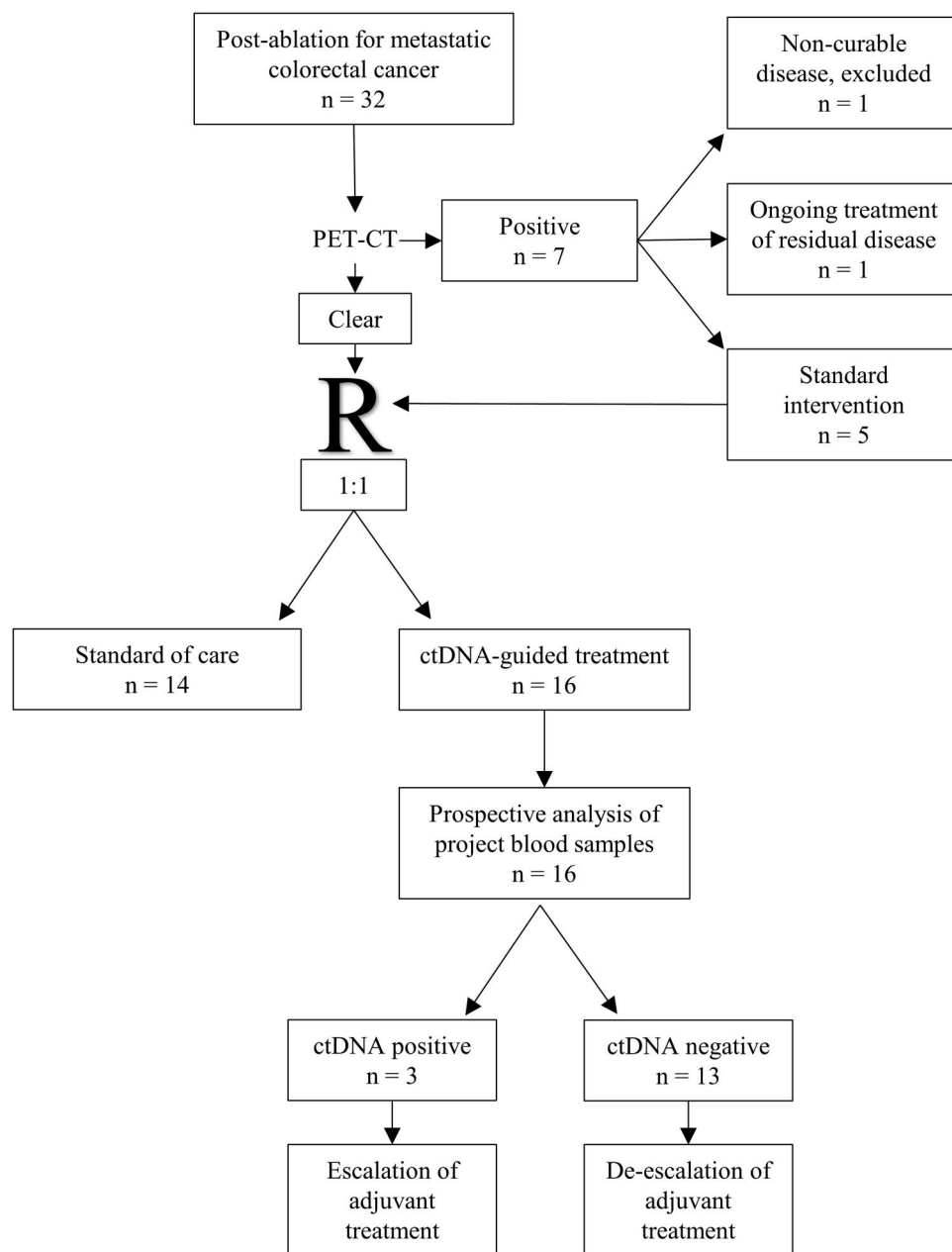


Figure 1. Study flow.

the PET-CT positive cases underwent curative intended treatment and hereafter randomization. One patient awaits randomization, and another was not randomized due to disseminated incurable disease on the PET-CT scan. In total, 97% of the patients were randomized ($n=30/31$). Fourteen patients were randomly assigned to SOC and 16 to ctDNA-guided adjuvant treatment and follow-up.

Plasma specimens from patients in the standard arm will not be analyzed until the end of the study. All analyses of the baseline plasma sample in the ctDNA-guided arm passed the quality control process. The median test turn-around time was three working days (range: 2–4 working days, $n=16$). Of the 16 patients in the ctDNA-guided arm, 19% ($n=3/16$) had a ctDNA-positive baseline plasma sample. All ctDNA-positive patients were eligible for and treated with triple-agent chemotherapy.

Discussion

Since the OPTIMISE study is the first investigation in the field, it was important to test feasibility early. Designing a prospective study solely based on retrospective data is associated with significant uncertainties. Within this relatively new, complex area of research there are numerous unknowns, i.e. logistics, biology, the role of imaging, and patients' and clinicians' approaches to this revised understanding of cancer. The early assessment of feasibility serves two purposes. Firstly, it is essential to make the necessary adjustments early on in order to create a valid study. Secondly, we want to share our experiences, opening the possibility for other groups to take advantage of the design in similar studies.

Here we have presented the results of the preplanned interim analysis of feasibility parameters. Our results

complied with the feasibility measures but the rather small number of enrolled patients called for reservations due to uncertainty of the estimates.

The number of included patients was as anticipated. Over 12 months, the study included 32 patients at only two centers, confirming that a fair number of patients are eligible and consent to inclusion. It also holds promise of a potential benefit from an optimized, personalized adjuvant treatment and follow-up.

With the current knowledge regarding the high risk of recurrence in patients with MRD, the study design with randomization against SOC can be discussed. However, it is still unknown whether the increased risk of recurrence in patients with MRD can be reduced by adjuvant chemotherapy. Furthermore, randomization is of utmost importance to evaluate differences in treatment-related toxicity, quality of life, and cost-effectiveness. Residual disease detected on the pre-randomization PET-CT scan could be treated with radical intent in the vast majority of cases, and no patient withdrew their consent. Hence, compliance with randomization was very high, and the randomized study setup can continue.

The high rate of patients with residual disease detected on a PET-CT scan underlines the importance of enhanced imaging in high-risk patients, opening up the possibility of further local treatment and a better selection of patients suitable for intensified or less adjuvant treatment. Enhanced imaging and ctDNA analyses thus complement each other in the surveillance of patients treated with radical intent for metastatic spread from CRC.

The existing literature shows a major variance in the ctDNA positivity rate, which is expected when based on retrospective analyses from studies with significant inter-study heterogeneity. This emphasizes the importance of generating prospective data. Around 30–40% of patients with mCRC are expected not to harbor *RAS/BRAF* mutations, why the plasma mutation analyses were supplemented with a plasma methylation analysis detecting aberrant hypermethylation of the promotor region of the neuropeptide Y gene (*NPY*) [26]. Aberrant hypermethylation of the *NPY*-gene is highly frequent in CRC tumor tissue and is detected in 87–90% of all patients with mCRC [27–29]. The ctDNA positivity rate in the OPTIMISE study was consistent with the existing literature, and we observed a clinically relevant ctDNA detection rate.

In addition to the predefined feasibility parameters, we also evaluated the feasibility of the analytical setup. Firstly, a short time to result is mandatory in order to avoid unnecessary delay in starting adjuvant treatment. Secondly, many patients experience anxiety while waiting for results. The fast turn-around time for analyses is favorable and fits well into daily clinical practice. Importantly, the quality of analyses was adequate in all cases, and results were thus considered reliable. Our results support the choice of a tumor-agnostic approach with two ddPCR methods.

The results from this early assessment of feasibility give an indication that the study design is solid and can continue without revision. The inclusion of patients will continue in the preplanned run-in phase until a total of 100 patients are

included. The sample size calculations for a large-scale phase II, multicenter, randomized controlled trial will be re-evaluated based on results from the participants in the run-in phase. Hopefully, the results from the OPTIMISE study will optimize the adjuvant treatment of patients undergoing radical-intent treatment of metastatic spread from CRC, prolonging survival and reducing chemotherapy-related toxicity.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by DCCC ctDNA Research Center—The Danish Research Center for Circulating Tumor DNA Guided Cancer Management, Danish Cancer Society [Grant Number R257-A14700] and Danish Comprehensive Cancer Center; Danish Cancer Society [Grant Number R269-A15652]; and foundation of Central Denmark Region [Grant Number A1602]. The investigators have no financial relationship with the grant provider or financial interest in the project. No financial compensation is provided to patients included in the study. This funding source had no role in the design of this study, during its execution, analyses, interpretation of the data, or decision to submit results.

ORCID

Louise Bach Callesen  <http://orcid.org/0000-0003-2799-1492>

Lars Henrik Jensen  <http://orcid.org/0000-0002-0020-1537>

Karen-Lise Garm Spindler  <http://orcid.org/0000-0001-8781-3212>

Data availability statement

The datasets used and/or analyzed during the current study are not available since the study is ongoing

References

- [1] Zeineddine FA, Zeineddine MA, Yousef A, et al. Survival improvement for patients with metastatic colorectal cancer over twenty years. *NPJ Precis Oncol.* 2023;7(1):16. doi: [10.1038/s41698-023-00353-4](https://doi.org/10.1038/s41698-023-00353-4).
- [2] Mise Y, Imamura H, Hashimoto T, et al. Cohort study of the survival benefit of resection for recurrent hepatic and/or pulmonary metastases after primary hepatectomy for colorectal metastases. *Ann Surg.* 2010;251(5):902–909. doi: [10.1097/SLA.0b013e3181c9868a](https://doi.org/10.1097/SLA.0b013e3181c9868a).
- [3] Serrano PE, Gu CS, Husien M, et al. Risk factors for survival following recurrence after first liver resection for colorectal cancer liver metastases. *J Surg Oncol.* 2019;120(8):1420–1426. doi: [10.1002/jso.25735](https://doi.org/10.1002/jso.25735).
- [4] Mitry E, Fields ALA, Bleiberg H, et al. Adjuvant chemotherapy after potentially curative resection of metastases from colorectal cancer: a pooled analysis of two randomized trials. *J Clin Oncol.* 2008;26(30):4906–4911. doi: [10.1200/JCO.2008.17.3781](https://doi.org/10.1200/JCO.2008.17.3781).
- [5] Callesen LB, Takacova T, Hamfjord J, et al. Circulating DNA in patients undergoing loco-regional treatment of colorectal cancer metastases: a systematic review and meta-analysis. *Ther Adv Med Oncol.* 2022;14:17588359221133171. doi: [10.1177/17588359221133171](https://doi.org/10.1177/17588359221133171).
- [6] Kotani D, Oki E, Nakamura Y, et al. Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer. *Nat Med.* 2023;29(1):127–134. doi: [10.1038/s41591-022-02115-4](https://doi.org/10.1038/s41591-022-02115-4).

- [7] Boysen AK, Pallisgaard N, Andersen CSA, et al. Circulating tumor DNA as a marker of minimal residual disease following local treatment of metastases from colorectal cancer. *Acta Oncol.* 2020;59(12):1424–1429. doi: [10.1080/0284186X.2020.1806357](https://doi.org/10.1080/0284186X.2020.1806357).
- [8] Tie J, Cohen JD, Lahouel K, et al. Circulating tumor DNA analysis guiding adjuvant therapy in stage II Colon cancer. *N Engl J Med.* 2022;386(24):2261–2272. doi: [10.1056/NEJMoa2200075](https://doi.org/10.1056/NEJMoa2200075).
- [9] Nishioka Y, Chun YS, Overman MJ, et al. Effect of Co-mutation of RAS and TP53 on postoperative ctDNA detection and early recurrence after hepatectomy for colorectal liver metastases. *J Am Coll Surg.* 2022;234(4):474–483. doi: [10.1097/XCS.000000000000093](https://doi.org/10.1097/XCS.000000000000093).
- [10] Newhook TE, Overman MJ, Chun YS, et al. Prospective study of perioperative circulating tumor DNA dynamics in patients undergoing hepatectomy for colorectal liver metastases. *Ann Surg.* 2022;277(5):813–820. doi: [10.1097/SLA.0000000000005461](https://doi.org/10.1097/SLA.0000000000005461).
- [11] Lonardi S, Nimeiri H, Xu C, et al. Comprehensive genomic profiling (CGP)-informed personalized molecular residual disease (MRD) detection: an exploratory analysis from the PREDATOR study of metastatic colorectal cancer (mCRC) patients undergoing surgical resection. *Int J Mol Sci.* 2022;23:1–13.
- [12] Marmorino F, Prisciandaro M, Giordano M, et al. Circulating tumor DNA as a marker of minimal residual disease after radical resection of colorectal liver metastases. *J Clin Oncol Precis Oncol.* 2022;6:e2200244.
- [13] van 't Erve I, Medina JE, Leal A, et al. Metastatic colorectal cancer treatment response evaluation by ultra-deep sequencing of cell-free DNA and matched white blood cells. *Clin Cancer Res.* 2023;29(5):899–909. doi: [10.1158/1078-0432.CCR-22-2538](https://doi.org/10.1158/1078-0432.CCR-22-2538).
- [14] Mason MC, Tzeng C-WD, Tran Cao HS, et al. Preliminary analysis of liquid biopsy after hepatectomy for colorectal liver metastases. *J Am Coll Surg.* 2021;233(1):82–89. doi: [10.1016/j.jamcollsurg.2021.02.011](https://doi.org/10.1016/j.jamcollsurg.2021.02.011).
- [15] Tie J, Wang Y, Cohen J, et al. Circulating tumor DNA dynamics and recurrence risk in patients undergoing curative intent resection of colorectal cancer liver metastases: a prospective cohort study. *PLoS Med.* 2021;18(5):e1003620. doi: [10.1371/journal.pmed.1003620](https://doi.org/10.1371/journal.pmed.1003620).
- [16] Wang D-S, Yang H, Liu X-Y, et al. Dynamic monitoring of circulating tumor DNA to predict prognosis and efficacy of adjuvant chemotherapy after resection of colorectal liver metastases. *Theranostics.* 2021;11(14):7018–7028. doi: [10.7150/thno.59644](https://doi.org/10.7150/thno.59644).
- [17] Loupakis F, Sharma S, Derouazi M, et al. Detection of molecular residual disease using personalized circulating tumor DNA assay in patients with colorectal cancer undergoing resection of metastases. *J Clin Oncol Precis Oncol.* 2021;5:1166–1177. doi: [10.1200/PO.21.00101](https://doi.org/10.1200/PO.21.00101).
- [18] Bolhuis K, van 't Erve I, Mijns C, et al. Postoperative circulating tumour DNA is associated with pathologic response and recurrence-free survival after resection of colorectal cancer liver metastases. *EBioMedicine.* 2021;70:103498. doi: [10.1016/j.ebiom.2021.103498](https://doi.org/10.1016/j.ebiom.2021.103498).
- [19] Parikh AR, Van Seventer EE, Siravegna G, et al. Minimal residual disease detection using a plasma-only circulating tumor DNA assay in patients with colorectal cancer. *Clin Cancer Res.* 2021;27(20):5586–5594. doi: [10.1158/1078-0432.CCR-21-0410](https://doi.org/10.1158/1078-0432.CCR-21-0410).
- [20] Reinert T, Petersen LMS, Henriksen TV, et al. Circulating tumor DNA for prognosis assessment and postoperative management after curative-intent resection of colorectal liver metastases. *Int J Cancer.* 2022;150(9):1537–1548. doi: [10.1002/ijc.33924](https://doi.org/10.1002/ijc.33924).
- [21] Øgaard N, Reinert T, Henriksen TV, et al. Tumour-agnostic circulating tumour DNA analysis for improved recurrence surveillance after resection of colorectal liver metastases: a prospective cohort study. *Eur J Cancer.* 2022;163:163–176. doi: [10.1016/j.ejca.2021.12.026](https://doi.org/10.1016/j.ejca.2021.12.026).
- [22] Callesen LB, Hansen TF, Andersen RF, et al. OPTIMISE: optimisation of treatment selection and follow-up in oligometastatic colorectal cancer: a ctDNA-guided phase II randomised approach. Study protocol. *Acta Oncol.* 2022;61(9):1152–1156. doi: [10.1080/0284186X.2022.2116728](https://doi.org/10.1080/0284186X.2022.2116728).
- [23] Pallisgaard N, Spindler KLG, Andersen RF, et al. Controls to validate plasma samples for cell free DNA quantification. *Clin Chim Acta.* 2015;446:141–146. doi: [10.1016/j.cca.2015.04.015](https://doi.org/10.1016/j.cca.2015.04.015).
- [24] Roperch JP, Incitti R, Forbin S, et al. Aberrant methylation of NPY, PENK, and WIF1 as a promising marker for blood-based diagnosis of colorectal cancer. *BMC Cancer.* 2013;13(1):566. doi: [10.1186/1471-2407-13-566](https://doi.org/10.1186/1471-2407-13-566).
- [25] Lefèvre AC, Pallisgaard N, Kronborg C, et al. The clinical value of measuring circulating HPV DNA during chemo-radiotherapy in squamous cell carcinoma of the anus. *Cancers (Basel).* 2021;13(10):2451. doi: [10.3390/cancers13102451](https://doi.org/10.3390/cancers13102451).
- [26] Peeters M, Kafatos G, Taylor A, et al. Prevalence of RAS mutations and individual variation patterns among patients with metastatic colorectal cancer: a pooled analysis of randomised controlled trials. *Eur J Cancer.* 2015;51(13):1704–1713. doi: [10.1016/j.ejca.2015.05.017](https://doi.org/10.1016/j.ejca.2015.05.017).
- [27] Thomsen CB, Andersen RF, Lindebjerg J, et al. Correlation between tumor-specific mutated and methylated DNA in colorectal cancer. *J Clin Oncol Precis Oncol.* 2019;3:1–8. doi: [10.1200/PO.18.00162](https://doi.org/10.1200/PO.18.00162).
- [28] Thomsen CB, Hansen TF, Andersen RF, et al. Early identification of treatment benefit by methylated circulating tumor DNA in metastatic colorectal cancer. *Ther Adv Med Oncol.* 2020;12:1758835920918472. doi: [10.1177/1758835920918472](https://doi.org/10.1177/1758835920918472).
- [29] Raunkilde L, Hansen TF, Andersen RF, et al. NPY gene methylation in circulating tumor DNA as an early biomarker for treatment effect in metastatic colorectal cancer. *Cancers (Basel).* 2022;14(18):4459. doi: [10.3390/cancers14184459](https://doi.org/10.3390/cancers14184459).