IMPROVE-IT2: Implementing non-invasive circulating tumor DNA analysis to optimize the operative and postoperative treatment for patients with colorectal cancer – Intervention Trial 2

NCT04084249

June 21st, 2024

Ethical approvals and amendments

Type	Changes – brief description	Date	Status
Main protocol	-	27-08-2019	Approved
Amendment 1	Adjustments of protocol to comply with SPIRIT guidelines	06-09-2019	Approved
Amendment 2	Adjustments of the following: - Inclusion criteria - Timing of QoL questionnaire, - Timing of patient inclusion - Randomization strategy - Evaluation of primary objective Addition of the following: - "European Quality of Life" questionnaire - Description of the targeted quality of PET/CT scans - A second opinion radiology committee - Description of quality of life and health economy analyses	02-12-2019	Approved
Amendment 3	Clarification of how approached patients that decide not to participate, are registered in the trial database for end-of-trial demographic comparison of the approached and the enrolled patient populations.	10-01-2020	Approved
Amendment 4	Extension of the study recruitment period due to COVID-19	06-11-2020	Approved
Amendment 5	Addition of a secondary objective regarding ctDNA growth rates, and 2 extra blood samples for ctDNA-positive patients in the experimental arm. Increased sample size (n = 310) due to newly published data showing that contemporary recurrence rates of high-risk stage II and stage III patients were lower than expected when the sample-size calculation was initially conducted.	13-06-2022	Approved
Amendment 6	Adjustments of protocol procedures regarding the 36-month blood sample.	06-08-2022	Approved
Amendment 7	Increased the needed sample size to n = 340, due to a competing study leading to a larger than anticipated drop-out rate.	29-11-2022	Approved
Amendment 8	Addition of finger-prick blood spot sample collection for ctDNA-positive patients in the experimental arm.	29-01-2024	Approved
Amendment 9	Aligning the objectives and endpoints with the published statistical analysis plan.	03-07-2024	Approved

IMPROVE-IT2

Implementing non-invasive circulating tumor DNA analysis to optimize the operative and postoperative treatment for patients with colorectal cancer – Intervention Trial 2

Primary Investigator, Clinical	Associate Professor, Specialty Registrar Kåre Andersson Gotschalck Department of Surgery Regional Hospital, Horsens Sundvej 30 DK8700 Horsens, Denmark +45 26497569 kaarsune@rm.dk
Sponsor and Primary Investigator, Molecular Biology	Professor Claus Lindbjerg Andersen Department of Molecular Medicine Aarhus University Hospital Palle Juul-Jensens Boulevard 99 DK8200 Aarhus N, Denmark +45 7845 5319 +45 2980 4321 cla@clin.au.dk

Trial Office

IMPROVE trial office

Department of Molecular Medicine, Aarhus University Hospital,

Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark

Phone: +45 7845 5325

E-mail: demuth@clin.au.dk

Protocol signature page

Primary Investigators and Sponsor

Trial title:

IMPROVE-IT2 trial: Implementing non-invasive circulating tumor DNA analysis to optimize the operative and postoperative treatment for patients with colorectal cancer – Intervention Trial 2

Primary Investigator, Clinical

Dr Kåre Andersson Gotschalck	Signature	Date

Sponsor and Primary Investigator, Molecular Biology

Professor Claus Lindbjerg Andersen	Signature	Date

Investigator signature page

Investigator

Trial title:

IMPROVE-IT2 trial: Implementing non-invasive circulating tumor DNA analysis to optimize the operative and postoperative treatment for patients with colorectal cancer – Intervention Trial 2

"I agree to the terms of this trial protocol. I will conduct the study in accordance with the procedures specified in the protocol, the ethical principles in the latest version of the Declaration of Helsinki, Good Clinical Practice and applicable regulatory requirements".

Center	Investigator name	Signature	Date

Trial administrative structure

This is an investigator-initiated study. Associate Professor, Specialty Registrar Kåre Andersson Gotschalck is the clinical coordinating investigator. Professor Claus Lindbjerg Andersen is the sponsor and the coordinator of the molecular analyses. The decision-making body of the study is the steering committee (SC), which will have representation from all participating departments. Professor Claus Lindbjerg Andersen is chair of the SC. The steering committee oversees the progress and funding of the trial, and determines the strategy for the execution of the trial.

It is the role of the sponsor to ensure funding to conduct the trial.

The organizations and foundations supporting the trial have no influence on design, execution or reporting from the trial.

It is the role of the clinical coordinating investigator and the sponsor to define the trial design and to ensure that the trial data is collected, analyzed and published. It is the role of the clinical coordinating investigator to oversee clinical part of the trial and to ensure that all trial personnel gets the necessary training to conduct their tasks in the trial. It is the task of the clinical coordinating investigator in collaboration with the coordinating center and sponsor solve the problems that occur during trial.

It is the role of the clinical coordinating investigator and sponsor to ensure that the trial at all times is conducted in agreement with the Danish legislation.

The role of the coordinating center is to ensure establishment of the necessary trial infrastructure, including databases, trial guidelines, and trial documents. The coordinating center houses the Clinical Trial Manager who's task it is to monitor the trial sites and the study database, and verify the quality of the data collected.

It is the task of the Sponsor to ensure that audits and inspections of the trial sites are conducted to assure the quality of trial processes, procedures and data.

Surgical departments in Denmark with a defined high volume of colorectal resections can participate in the study and will be invited to do so.

A list of participating study sites can be obtained from the clinical trial manager.

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1 Introduction

In Denmark, colorectal cancer (CRC) represents a significant health burden with ~5,000 new cases and ~2000 CRC related deaths in 2018[1]. Death of CRC is primarily associated to distant metastases, occurring at the time of diagnosis (synchronous) or appearing later in the course of the disease (metachronous).

In Denmark, we have introduced a screening program to improve survival by reducing the number of patients diagnosed with synchronous metastases, but poor survival due to disease recurrence remain a major problem. If postoperative residual disease could be detected early then survival could be improved.

Metachronous metastases are most frequently found in the liver and the lungs and if resected with curative intent, the 5-year relative survival is ~50-60%.[2–5] However, if the metastases are detected too late for intended curative treatment and the patients instead receive palliative chemotherapy or best-supportive care, then less than 10% are alive after 5 years.[2–4,6–8] The ability to resect, as well as the survival after resection, are negatively influenced by increasing numbers of metastatic sites and the size of the metastases.[2,3,5] Thus, early detection of recurrences when tumor burden is low is of paramount value, not only to increase the rate of curative resections, but also to increase survival after resection. Local treatment of metastases (e.g. radiofrequency ablation) that are small, but non-resectable or potentially resectable in patients ineligible for major surgery, have yielded 5-year survival rates up to 40-60%.[9–12] This implies that also patients considered ineligible to curative resection could benefit from early recurrence detection.

At time of diagnosis, the majority of patients (75%) present with non-metastatic disease (stage I-III), for which standard of care is curative surgery.[13] The 5-year relative survival rate for all stage I-III is 80 %, but drops to 65 % for stage III alone.[13] Overall 15-20 % of patients experience recurrence after curative surgery for stage I-III CRC with 70-90% detected within 3 years of primary surgery.[14–21] Stage III comprises only 37 % of stage I-III patients[13], but account for 70 % of recurrences.[22] Due to the high recurrence risk, adjuvant chemotherapy is standard of care for stage III patients[23] but still ~33% of patients relapse (DK, ~360 stage III recurrences/year[24]).[16,18,25] Extending duration of adjuvant chemotherapy beyond the current 6 months offers no further reduction in recurrence.[26–29] Even 3 months of oxaliplatin-based chemotherapy is sufficient in stage III patients with low-risk pathological features.[26–29] Consequently, the only alternative option after adjuvant chemotherapy is surveillance to enable detection of recurrence, when residual disease has grown to a detectable size, but at an early stage that allow curative treatment.[30–32] Hence, stage III patients represent the optimal focus point for investigation of novel surveillance strategies.

With current surveillance, only ~15% of recurrences are eligible to curative resection or local treatment[33,34] and the 5-year survival rate for metachronous metastasis is just 9%.[33] In Denmark, the national guideline recommends CT-scans of thorax and abdomen 12 and 36 months postoperatively, and colonoscopy every 5 years until age 75.[35] Individual centers may offer a slightly more intensive CT-scan surveillance. However, similar to previous studies[17,36], increasing intensity of CT-based surveillance for stage II-III did not increase survival in a large Danish randomized controlled trial.[37] More patients do receive intended curative surgery for recurrence with intensified surveillance,[17] but as more than 80% of patients with stage II-III patients do not relapse, this probably dilutes the impact on survival.[37] What is needed are methods to identify the ~30 % of stage III patients with microscopic residual disease after adjuvant chemotherapy who can benefit from high-intensity surveillance.[38]

Minimally-invasive blood-based analysis of circulating tumor DNA (ctDNA) is an emerging tool that has this potential to identify microscopic residual disease.[39–41] It is based on the observation that tumors shed DNA fragments into the blood.[42] The half-life of ctDNA is less than two hours.[43] Consequently, cured patients will

not have ctDNA during follow-up, while patients with residual disease are likely to be ctDNA positive. Like for all quantification methods, there is a lower limit of detection for ctDNA analysis, and consequently ctDNA may not be detectable in patients with very low levels of residual disease e.g. after end of adjuvant chemotherapy. However, as the residual disease grows, ctDNA eventually becomes detectable if blood samples are analyzed longitudinally. In studies of patients not receiving adjuvant chemotherapy, we and others have shown that the risk of relapse in patients with postoperative ctDNA is close to 100%, while the risk of relapse in ctDNA negative patients is as low as 10%[39–41] (HR=37.66; 95% CI, 4.23 to 335.49; P<0.0001).[40,41,44] In a recent observational study, we analyzed blood samples collected longitudinally during surveillance after adjuvant chemotherapy.[45] ctDNA was identified in 13/58 patients of which 92% (12/13) relapsed. In multivariate analysis, ctDNA status was a stronger predictor of incipient relapse than other predictive factors such as UICC stage, lymphovascular invasion, micro-radical resection status, and carcinoembryonic antigen (CEA) (HR, 29.0; 95% CI, 6.4-130; *P*<0.001)(Figure 1B). Moreover, longitudinal ctDNA analysis detected relapse with an average lead time of 8.7 months compared to standard-of-care CT-imaging (Wilcoxon signed rank test; *P*<0.001) (Figure 1C). From first ctDNA detection, all plasma samples until radiological relapse were ctDNA-positive and revealed a 50-fold increase in the mean ctDNA level, indicating that the tumor burden increased dramatically while the patients awaited radiological detection of the relapse (Figure 1D). Hence, ctDNA surveillance may detect relapse sufficiently early to change patient management. Combining ctDNA and radiological assessments could result in earlier detection of recurrent disease and identify more patients eligible for curative treatment and generally enable early implementation of local and systemic therapies. This will likely improve treatment and survival, as one of the fundamental principles of oncology is that it is more effective to treat few and small lesions than multiple and large lesions.[46]

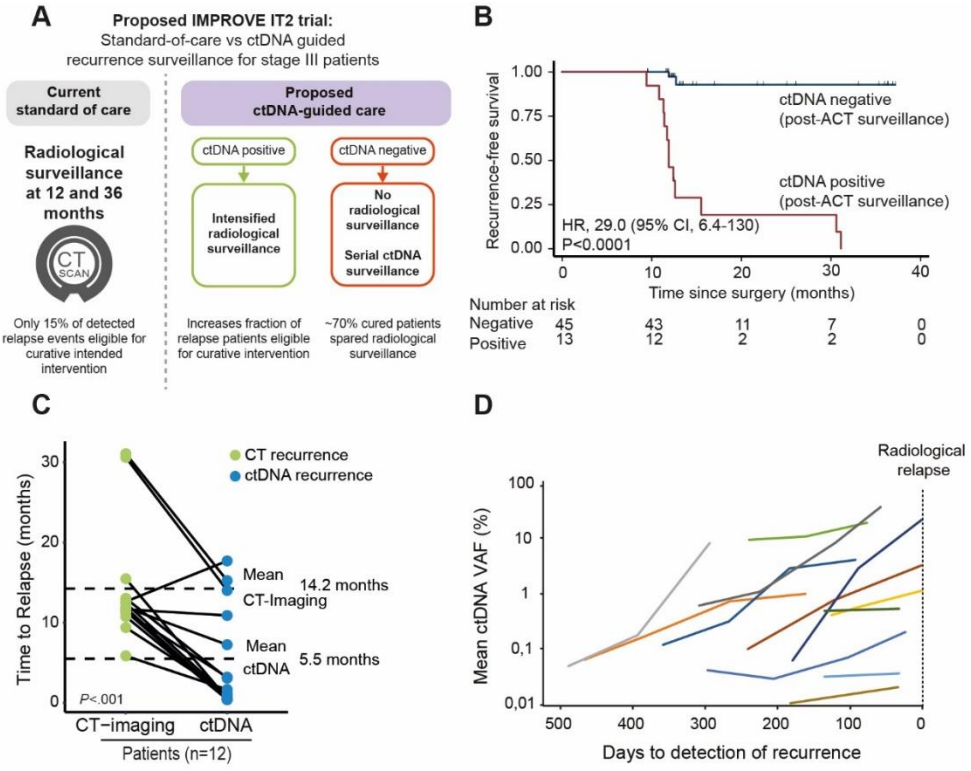

Figure 1 – Longitudinal ctDNA analysis after adjuvant chemotherapy enables early detection of relapse. (A) Proposed IMPROVE-IT2 trial comparing standard-of-care radiological surveillance to ctDNA guided surveillance. (B) Relapse risk after adjuvant chemotherapy stratified by post-adjuvant ctDNA status. Patients were scored ctDNA positive if they at any time-point post-adjuvant were positive

and negative if they at all post-adjuvant time-points were negative. (C) Comparison of time to relapse by ctDNA analysis and standard-of-care CT imaging. The average lead time of ctDNA was 8.7 months. (D) For all relapsing patients, the ctDNA levels in plasma increased over time from ctDNA detection to radiological detection.

2 Objectives

The overall objective of this study is to establish a randomized controlled trial investigating the benefit of ctDNA-guided postoperative surveillance for stage III and stage II high-risk colorectal cancer (CRC) patients compared to the current standard-of-care CT-scan surveillance (Figure 1A). Currently, only ~15% of recurrences are detected sufficiently early to enable curatively intended intervention. The central hypothesis of this study is that ctDNA-guided postoperative surveillance will lead to shorter time to recurrence detection, which will allow curatively intended intervention in time to improve overall survival.

2.1 Primary Objective

The primary objective is to investigate if ctDNA-guided post-operative surveillance to guide radiological assessments will result in a higher fraction of recurrence patients receiving curative intended or local metastasis-directed treatment for CRC recurrence as compared to current Danish surveillance strategy within 3 years after primary surgery.

2.2 Key Secondary Objectives - Oncological

2.2.1 Key Secondary Objective 1 (S1)

To investigate if ctDNA-guided surveillance is associated with a shorter time from randomization to clinical recurrence (TTCR) than standard-of-care surveillance.

2.2.2 Key Secondary Objective (S2)

To investigate if 3- and 5-year overall survival (OS) after surgery is non-inferior with ctDNA guided surveillance compared to current standard-of-care CT-based surveillance. To take immortal time, typically 4-8 months, from the time of surgery until randomization into account, overall survival will be estimated with two different starting points of follow-up: 1) from time of surgery and 2) from time of randomization. For both starting points, OS will be assessed at 3- and 5-years after surgery.

2.3 Key Secondary Objectives – Quality of Life

2.3.1 Key Secondary Objective 3 (S3)

To compare the quality of life (EORTC QLQ-C30 and EQ-5D-L5), fear of cancer recurrence inventory (FCRI), and impact of events scale for cancer (IES-C) for patients following ctDNA guided and the standard-of-care surveillance.

2.4 Secondary Objectives

2.4.1 Secondary Objective 4 (S4)

To assess the cost-effectiveness of ctDNA-guided and standard-of-care surveillance, to provide decision support for clinicians and other decision-makers.

2.4.2 Secondary Objective 5 (S5)

To assess, and compare, the protocol adherence rates (AR) for patients following ctDNA-guided and standard-of-care surveillance.

2.4.3 Secondary Objective 6 (S6)

To assess if the time to molecular recurrence (TTMR), i.e., detection of ctDNA- and/or elevated level of Carcinoembryonic antigen (CEA) are similar, for patients following ctDNA guided and patients following standard-of-care surveillance.

2.4.4 Secondary Objective 7 (S7)

To describe any changes in quality of life (EORTC QLQ-C30 and EQ-5D-L5), fear of cancer recurrence inventory (FCRI), and impact of events scale for cancer (IES-C) from time of positive ctDNA until time of clinically verified recurrence.

2.4.5 Secondary Objective 8 (S8)

To investigate if ctDNA growth rate analyses, performed on blood samples collected within a short interval (2-3 weeks apart), can stratify ctDNA-positive patients into groups of fast and slow-growing tumors.

2.4.6 Secondary Objective 9 (S9)

To compare the rate of clinical recurrence detection during the time intervals: randomization-12 months after surgery, 13-24 months after surgery, and 25-36 months after surgery for patients with ctDNA guided and standard-of-care surveillance.

2.4.7 Secondary Objective 10 (S10)

To compare the cumulative incidence of clinical recurrence at 12 months, 24 months, and 36 months after surgery for patients with ctDNA-guided and standard-of-care surveillance.

3 Investigational plan

3.1 Overall study design

The study will establish the randomized clinical trial IMPROVE-IT2 investigating the benefit of ctDNA guided surveillance for stage III and stage II high-risk CRC patients treated with adjuvant chemotherapy, compared to the current one-size fits all surveillance.

CRC patients treated with adjuvant chemotherapy will be randomized into two arms:

- The experimental arm: ctDNA analysis will be performed at months 4, 8, 12, 16, 20 and 24 post-operatively. ctDNA analysis terminates at month 24, because all relapse patients in our previous studies tested ctDNA positive no later than month 18 (Figure 1C). At time of first positive ctDNA, patients undergo a comprehensive radiological assessment with a whole-body PET/CT-scan. A colonoscopy will be performed if the PET/CT-scan is without evidence of recurrence. If the initial assessment is without evidence of recurrence, patients will be offered intensive radiological surveillance (PET/CT-scans every 3 months, until recurrence detection or 21 months has passed). Radiological follow-up is ended after 21 months, because the maximal lead time of ctDNA to CT-scan was 16.5 months in our previous prospective studies (Figure 1C). ctDNA negative patients will in addition to blood-sampling only receive an end-of-study CT-scan. This will be performed between months 36 and 37. A blood sample will be collected at the same time-point for molecular reference.

- The control arm: Patients will receive surveillance according to current Danish guidelines with CT-scans at months 12 and 36. Longitudinal blood samples will be collected at the same time-points as in the ctDNA surveillance arm but will not be analyzed until the end of the trial. They serve to enable post-trial comparison of oncological outcomes for the two arms stratified for ctDNA-status.

Quality of life (QoL) will be monitored in all patients, independent of randomization, by use of the validated Danish version of the European Organization for Research and Treatment of Cancer Quality of Life questionnaire, Core 30 (EORTC QLQ-C30 ver. 3.0), Fear of Cancer Recurrence Inventory (FCRI), Impact of Events Scale for Cancer (IES-C) and European Quality of Life – 5 Dimensions (EQ-5D-5L).

Further, ctDNA positive patients (in the experimental arm) will be asked to complete the QLQ-C30, FCRI, IES-C and EQ-5D-5L questionnaires before each PET-CT scan. Patients will be their own controls. It is an explorative, longitudinal study.

3.2 Study timetable

Start inclusion: January 2020

End inclusion: December 2023

End data collection: December 2028

Primary endpoint reached, ready to publish: December 2025

Follow-up: 5 years

Last patient last visit: December 2026

3.3 Setting

The randomized trial "IMPROVE-IT2" is an independent sub-study of the ongoing prospective multicenter ctDNA study IMPROVE (ClinicalTrials.gov: NCT03637686, Committees on Biomedical Research Ethics in the Central Region of Denmark j. no. 1-10-72-3-18). IMPROVE-IT2 is registered with ClinicalTrial.gov: NCT04084249. During 2018-2020, IMPROVE will recruit 1,800 stage I-III CRC patients at the following major surgical departments in Denmark (Aalborg, Herning, Viborg, Randers, Aarhus, Horsens, Odense, Svendborg, Herlev, Bispebjerg, Roskilde, Slagelse og Køge). Part II of the IMPROVE study is an observational trial involving longitudinal ctDNA analysis of stage III and stage II high-risk patients. However, because of the convincing evidence supporting the clinical potential of ctDNA for early relapse detection, recently provided by us [40,45,47] and others[41,48–51], we have established IMPROVE-IT2 with the intention to progress from the observational to interventional setting. While IMPROVE-IT2 is recruiting, we will invite stage III and stage II high-risks patients treated with adjuvant chemotherapy to participate in IMPROVE-IT2, rather than IMPROVE part II.

3.4 Sample size

Assessment of the sample size needed to meet the Primary Objective (see 2.1)

Based on the first 6 months of enrollment in IMPROVE, it is expected that 385 stage III patients receiving adjuvant therapy will be approached in the recruitment period for IMPROVE IT2. Our current recruitment rate to the IMPROVE longitudinal blood sample study is 66%. Meaning that 254 patients are likely to participate (127 in each arm). The recurrence rate of this patient population is 33% (n=84). Hence, with a 1:1 randomization we expect 42 recurrences in each arm. Primary endpoint of the study is the fraction of patients with recurrent, metastatic disease receiving curative-intent resection or local treatment. With standard-of-care surveillance, approximately 15% of relapse events are eligible to curative-intent treatment. Assuming this fraction can be increased by a factor of 3 (to 45%) in the experimental arm. In order to achieve a power of 80% to detect the difference, at a 5% significance level, a sample size providing 35 recurrence patients in each arm is needed, which is below the number available, even when assuming a 10% drop-out rate.

UPDATED SAMPLE SIZE (MAY 2022)

Our new unpublished study on updated recurrence rates has disclosed, that the recurrence rates in more recent years have decreased to approximately 25 % (compared to 33 % in the above calculation). This means that our power calculation no longer applies. Instead, we will be aiming at recruiting 310 patients (155 in each arm). With an estimated recurrence rate of 25 % (n = 78), we expect to have 39 recurrences in each arm. Even with a drop-out rate of 10 % this will be enough to reach the sufficient power (35 recurrences in each arm, see above). We have recruited 82 patients in 2020, 88 patients in 2021 and up to now (May) we have recruited 50 patients in 2022, making a total of 220 patients. Recruiting another 90 patients will estimated take 1 year, meaning that it is feasible to reach the new aimed sample size by prolonging the recruitment period with 1 year. Furthermore, we are observing a drop-out rate of approximately 10 % which fits the estimated rate in the power calculation.

UPDATED SAMPLE SIZE (NOVEMBER 2022)

We are experiencing a larger drop-out rate than expected. Currently, we have a drop-out rate of 14 %, compared to the 10 % that we were expecting. To compensate for this, we need to update our power calculation. We want to increase the aimed number of recruited patients to 340. With an expected recurrence rate of 25 % (n = 85), this will lead to approximately 42 recurrences in each arm. Even with a dropout rate of 15 %, we will be able to reach sufficient power (at least 35 recurrences in each arm, see above). We are currently recruiting approximately 10 patients each month and have a total of 272 patients in the study, meaning that we will be able to reach the new aimed recruitment within the planned study period.

Assessment of the sample size needed to meet the secondary objective S3 related to Quality of Life (See 2.2.2)

A mean global health-status/QoL difference of 10 points is defined to be the threshold of clinical relevance.[52,53] Assuming a mean global health-status/QoL score of 73 with a standard deviation of 23[53], then with no difference between experimental and control arm, 91 patients in each arm are required to show with a power of 90% that the lower limit of a one-sided 95% confidence interval will be above the non-inferiority limit of -10.

3.5 Inclusion of patients

The patients eligible for the IMPROVE-IT2 study will be identified at the treating surgical department, and must meet the criteria described in the below section.

3.5.1 IMPROVE-IT2 patient criteria

3.5.1.1 Inclusion criteria

- Participation in IMPROVE (Committees on Biomedical Research Ethics in the Central Region of Denmark j. no. 1-10-72-3-18)
- Colon or rectal cancer, tumor stage III (pT1-4N1-2,cM0) or stage II high-risk (pT4N0,cM0 and pT3N0, cM0 with either of the risk factors (<12 lymph nodes, anastomosis leakage, emergency surgery, signet ring adenocarcinoma) described in the national guideline for adjuvant therapy to stage II cancer)
- Has received curative-intent resection and is a candidate for adjuvant chemotherapy (3 or 6 months regime)

3.5.1.2 Exclusion criteria

- Not treated with adjuvant chemotherapy (patients who receive incomplete treatment are NOT excluded, as the analysis is based on intention to treat)

- Synchronous colorectal and non-colorectal cancer diagnosed per-operatively (except skin cancer other than melanoma)
- Other cancers (excluding colorectal cancer or skin cancer other than melanoma) within 3 years from screening
- Patients who are unlikely to comply with the protocol, inability to return for subsequent visits, unable to have blood samples drawn and/or otherwise considered by the Investigator to be unlikely to complete the study

If a patient is excluded during the trial, it is the responsibility of the treating department to make sure that the patient is reenrolled in a surveillance program in accordance with national standards and guidelines.

3.5.2 Time of inclusion and allocation procedure

Two-stage procedure:

1. Day 14 post-OP visit (Baseline): (when patients are informed about the pathology of the resected tumor).

Patients with stage III or high-risk stage II CRC, who are referred to an oncological department for adjuvant chemotherapy, are informed about the IMPROVE-IT2 trial and informed consent will be obtained. The signed consent form can be collected both at the Day 14 and Month 4 visits.

2. Post-OP month 4: (Time of first blood-sample in IMPROVE-IT2):

Patients, who have not received any adjuvant chemotherapy, are excluded from IMPROVE-IT2.

Patients, who have received adjuvant chemotherapy, will be allocated to either ctDNA guided surveillance (experimental arm) or standard-of-care surveillance (control arm) using a concealed web-based randomization service managed by Aarhus University (REDCap) (See section 3.6).

Recruitment of patients already participating in IMPROVE part II:

Patients, who at the time of initiation of the IMPROVE-IT2 trial already are included in the ongoing observational IMPROVE part II surveillance study (See the protocol associated with j. no. 1-10-72-3-18 the Committees on Biomedical Research Ethics in the Central Region of Denmark), can be included in IMPROVE-IT2 before completion of adjuvant chemotherapy.

3.5.3 Procedures for patient recruitment and informed consent

Patients, who meet the inclusion criteria are approached in person at the treating surgical department before completion of adjuvant therapy. Here the patients are given written information about the project and it is arranged when verbal information about the project will be given. The patients are encouraged to bring an assessor to this session. The oral information is given by trained health care professionals (either the surgeon or specially trained project nurses). Oral information is given in a closed room to ensure that the conversation can take place undisturbed. Besides information about the project, the patients are also informed about their rights as participants in a research project. Furthermore, as the project involves genomic sequencing, which may disclose genetic variants predisposing to specific diseases, the participants are also offered genetic counseling before making their decision to participate. After the oral information has been given, the patients are allowed at least 24 hours to consider their decision to participate before they are approached in order to obtain written informed consent. Informed consent has to be obtained prior to commencement of any study-related procedure.

The signed and dated consent forms are stored in a locked room at the trial office and are available for audit and inspection at any time.

The written participant information contains contact information to the person being primary responsible for the project, and to the persons being responsible at the participating departments. The information clearly states that these people can be contacted in case of further questions. It also mentions that the participant at any time may withdraw his or her consent, without this having any impact on the relationship to the department, or on current or future treatment. It also mentions that study involves a biobank, and that residual biological material as well as clinical and sequencing data will be transferred to the Colorectal Cancer Research Biobank at Aarhus University Hospital for future research. The research biobank is approved by the Danish Data Protection Agency (j. no. 1-16-02-27-10).

3.5.4 Patient information and consent

The patient information text and consent form is available as Appendix A.

3.6 Randomization

Randomization will be performed at each clinical site using a concealed centralized web-based randomization service managed by Aarhus University (REDCap).

Randomization 1:1 into two groups

1. ctDNA guided surveillance without routine CT-scans (experimental arm)
2. Standard-of-care surveillance with routine CT-scans (control arm)

Using a stratified block randomization algorithm, with block size 4. Stratification is based on three variables:

1. Location of primary tumor: right-sided colon incl. right flexure, transverse/left-sided colon, rectum
2. Tumor stage: pT3-4, pN0 and pT1-3, pN1 versus pT4, pN1 and pT1-4, pN2
3. Standard-of-care surveillance program intensity: sites following the national recommendation with 12 and 36 months CT-scans versus sites with more intensive surveillance)

We stratify for variables 1 and 2 with the aim to balance the recurrence rate in the two groups. The variables were chosen as it was assessed that they were most significantly associated with risk, location and prognosis of recurrence

We stratify for variable 3 with the aim to balance patients from sites with high (or low) intensity standard-of-care surveillance between the groups. This is important as most recruitment sites have low intensity standard-of-care surveillance and we want to avoid unbalanced randomization of patients from high-intensity sites.

3.7 Quality of Life baseline consideration:

The QoL of the individual patients may vary considerably after completion of adjuvant therapy. A very poor QoL of single individuals could endure for duration of the follow-up irrespectively of which group they are allocated to. To avoid that potential unbalances in patient QoL outlier distributions affect the QoL comparison between the surveillance groups, it is important to have a common baseline starting point for the QoL assessments. This will enable evaluation of differences in QoL between groups, by making the QoL measurements in each patient relative to the patient's own baseline value.

Baseline QoL is measured after end of adjuvant chemotherapy and before the patient and project personnel is informed about the allocated surveillance program. Thereby, we ensure the baseline assessment is unbiased.

3.8 Study flow

The time-schedule and procedures for inclusion and data collection at baseline (Day14) and during follow-up are described below and outlined in flow charts below. The specific procedures regarding blood sampling, handling and analysis will be described separately.

Flowchart: Control arm and experimental arm (ctDNA negative)

	Day14 (Baseline)	Mo3	Mo4	Mo7	Mo8	Mo12	Mo16	Mo18	Mo20	Mo24	Mo36	Mo36-Mo38
Patient inclusion												
Patient exclusion		If ACT was not initiated										
Informed consent												
Registration of baseline data												
Adjuvant chemotherapy (ACT) data registration	Record intention to administer ACT	Record if ACT was initiated or not, and the planned duration	If 3 mo ACT, record end date	If 6 mo ACT, record end date								
Randomization												
CT scan						Control arm only						
QoL questionnaires (QLQ-C30, FCRI, IES-C, EQ-5D-5L)			If 6 mo ACT; send out at mo7									
Blood sampling			If 3 mo ACT; draw blood after ACT end		If 6 mo ACT, draw blood after ACT end							

Flowchart: Experimental arm (ctDNA positive)

	Every 3 months until radiological recurrence or up to 21 months	Mo12	Mo36	Mo36-Mo38

CT scan			If no recurrence was detected during 21 months of PET-CT	
PET-CT scan				
Colonoscopy	Performed if the first PET/CT-scan is without evidence of recurrence			
QoL questionnaires (QLQ-C30, FCRI, IES-C, EQ-5D-5L)QoL questionnaires (FCRI, IES-C, and EQ-5D-5L)				
Blood sampling			If no recurrence was detected during 21 months of PET-CT	

Post OP day 14 visit (inclusion visit, Baseline)

- Patients full filling the inclusion criterias are informed about IMPROVE-IT2 and asked to consent to participation
- Patients informed about the pathology report
- Decision about referral to oncology department for adjuvant chemotherapy according to standard guidelines and patient's preferences
- Inform patients that IMPROVE-IT2 randomization will be conducted after adjuvant treatment has commenced.
- Inform patients if they do not receive adjuvant treatment, they will be excluded from IMPROVE-IT2, and instead offered participation in the observational study IMROVE part II surveillance.
- Inform patients that they as part of the trial will be asked to fill in questionnaires about their quality of life before start in the study (after end of adjuvant treatment), and again at 12, 18, and 24 months and after end of surveillance. Inform patients that access to the QoL questionaires will be sent to them by digital mail (e-boks).
- Record basic information on all patients invited to participate (**CRF Invitation**)
    - CPR number on all invited patients
    - whether they accepted participation or not
    - whether the patients accepted or declined to receive information about incidental genomic findings with potential health consequences
- Record baseline patient information (**CRF IMPROVE-IT2 BASELINE)**
    - WHO performance status
    - Height, weight and Body Mass Index (BMI)
    - Smoking and alcohol use
    - Imaging used for pre-operative work-out
    - If complete colonoscopy or Computed Tomography (CT) colon has been made or is scheduled post-operatively
    - diagnosis of other cancers within 3 years from screening
- Record intention to administer adjuvant chemotherapy (**CRF IMPROVE-IT2 ADJ1)**
- Record surgery and pathology data in the CRF (**CRF IMPROVE-IT2 PATHOLOGY**)

3.8.1 Post OP month 3 (Status of adjuvant therapy, EXCLUSION of patients NOT treated with adjuvant therapy, RANDOMIZATION of patients treated with adjuvant therapy, send QoL to patients expected to end adjuvant therapy before month 4)

- Record in information about adjuvant chemotherapy (**CRF IMPROVE-IT2 ADJ2**)
  - If adjuvant therapy was initiated (yes or no)
  - Adjuvant therapy start date
  - Planned regimen
  - Planned duration: 3 months or 6 months
- For patients who received adjuvant chemotherapy:
  - Check the patient file if adjuvant therapy has ended or not. Record the end date or the expected end date (**CRF IMPROVE-IT2 ADJ3).**
  - Call the patient to
    - Confirm status of adjuvant chemotherapy, the end date or expected end date.
    - Decide with the patient the date for the 4 month visit and blood sample collection.
      - For patients with adjuvant therapy “end date” or “expected end date” before month 4 (plus 14 days) make sure the visit is scheduled after the end of chemotherapy (at the earliest 10 days after).
    - For patients were adjuvant therapy has been, or is expected to end, before the 4 month visit:
      - Inform that a link to an online QoL questionnaire will be sent by digital mail (e-boks) and that this has to be completed after end of adjuvant chemotherapy and before the 4 month visit. The project personnel, shall ask if the patient has an digital e-boks. If not, then a paper version of the questionnaire shall be sent by “Quick” mail.
      - Complete the **CRF IMPROVE-IT2 QOL-TRIGGER**, which will trigger REDCap to send a questionnaire consisting of EORTC QLQ-C30, FCRI, IES-C and EQ-5D-5L to the patients.
    - For patients whose adjuvant therapy regimen is NOT expected to end before the 4 month visit:
      - The 4 month visit is only to collect blood
      - NOTE: These patients will be approached in month 7 to record the end date of adjuvant therapy and distribute the QoL questionnaires.
- For patients NOT treated with adjuvant therapy:
  - Call the patient to: 1) inform that the patient is EXCLUDED from IMPROVE-IT2 (complete **CRF IMPROVE-IT2 END OF STUDY**) and 2) inform about the very similar, but observational, study IMPROVE part II and ask the patient to consent to participate in this.
- Patients TREATED adjuvant chemotherapy are RANDOMIZED to either ctDNA guided surveillance (experimental arm) or standard surveillance (control arm). Randomization is performed in REDCap after the 3 month phone call to the patient. Randomization result is not revealed to patient nor project personnel at this time point.
  - Patients whose adjuvant therapy has ended before month 4 are informed at the 4 month visit.
  - Patients whose adjuvant therapy ends after month 4 are informed at the 8 month visit.

For the post OP month 4 visit, the handling of patients randomized to both the experimental and control arm are described together. Thereafter, the two arms are described separately.

3.8.2 Post OP month 4 visit (collection of a blood sample, and information about randomization)

- Register status of adjuvant therapy (**CRF IMPROVE-IT2 ADJ3**)
    - Register if adjuvant therapy has ended or not
    - If adjuvant therapy has ended, record end date
    - If adjuvant therapy has not ended, then check if expected end date should be updated
- Patients who are still receiving adjuvant therapy are informed that they receive the QoL-questionnaire and randomization result after end of adjuvant therapy.
- For patients who were sent the QoL questionnaire at post-OP month 3 (section 3.7.1) the project personnel will check if the QoL questionnaire has been completed. If not the project personnel will assist the patients in doing so on site.
- Randomization result: Will first be reveal to the personnel and patient at completion of the QoL-questionnaire. Only patients whose adjuvant therapy has ended are informed about their randomization allocation and the associated follow-up plan.
- Blood sampling is performed on all patients.
- For patients in the experimental arm where adjuvant therapy has ended, the blood is analyzed for ctDNA immediately. The result is registered in the IMPROVE-IT2 REDCap database by Department of Molecular Medicine
    - **ctDNA negative** result: patients will be informed about the result by secure digital mail via E-boks, and informed that they will be contacted again in due time before Post OP month 8 visit.
    - **ctDNA positive** result: patients will be informed about the result at a visit in the outpatient clinic and at the same time informed about the subsequent follow-up (See section 3.8.5.2.2).
- For all other patients the blood will be stored and analyzed at end of study (patients in the control arm and patients in the experimental arm still receiving adjuvant therapy).

3.8.3 Post OP month 7 (Status of adjuvant therapy for all patients who were still in active treatment at the month 4 visit)

- Check the patient file to record the end date or the expected end date (**CRF IMPROVE-IT2 ADJ3).**
- Call the patient to
    - Confirm status of adjuvant chemotherapy, the end date or expected end date.
    - Decide with the patient the date for the 8 month visit.
        - Make sure the visit is scheduled after the end of chemotherapy (at the earliest 10 days after).
    - Inform that a link to an online QoL questionnaire will be sent by digital mail (e-boks) and that this has to be completed after end of adjuvant chemotherapy and before the 8 month visit. The project personnel, shall ask if the patient has an digital e-boks. If not, then a paper version of the questionnaire shall be sent by “Quick” mail.
    - Complete the **CRF IMPROVE-IT2 QOL-TRIGGER**, which will trigger REDCap to send a questionnaire consisting of EORTC QLQ-C30, FCRI, IES-C and EQ-5D-5L to the patients.

3.8.4 Post OP month 8 visit

- For patients who were still in active treatment at the month 4 visit
    - Record end date of adjuvant chemotherapy for those with only expected end day at Post OP month 7 call. (update **CRF IMPROVE-IT2 ADJ3**)
    - The project personnel will check if the QoL questionnaire has been completed. If not the project personnel will assist the patients in doing so on site.
    - Randomization result: After the completion of the QoL questionnaire the patients and personnel are informed about the randomization allocation. The patients are informed about the associated follow-up plan.

- All patients: Now adjuvant therapy has ended for all patients, and they have all been informed about their randomization group and will receive follow-up accordingly
    - Blood sampling is performed on all patients.
    - For patients in the experimental arm the blood is analyzed for ctDNA immediately. The result is registered in the IMPROVE-IT2 REDCap database by Department of Molecular Medicine
        - **ctDNA negative** result: patients will be informed about the result by secure digital mail via E-boks, and informed that they will be contacted again in due time before Post OP month 12 visit.
        - **ctDNA positive** result: patients will be informed about the result at a visit in the outpatient clinic and at the same time informed about the subsequent follow-up (See section 3.8.5.2.2).
- For patients in the control arm the blood will be stored and analyzed at end of study.

3.8.5 Longitudinal post OP visits (mo12 to mo36)

3.8.5.1 For patients in the Control arm:

- Longitudinal PostOP blood sampling (mo12, mo16, mo20, mo24 and mo36)
- Follow-up: At months 12 and 36 project personnel records the results from the standard CT-scans performed at 12 and 36 months (**CRF CTSCAN 12mo, CRF CTSCAN 36mo**)
- Imaging because of suspicion of recurrence: At month 36 the hospital files are assessed for additional imaging events, and related pathology and treatment notes, performed due to suspicion of recurrence (**CRF-CTSCAN-Suspicion**).
- Quality of life at months 12, 18, 24 and 38: Before the blood collection visits at months 12 and 24, and at month 18 (between the blood collection visits at months 16 and 20), and again after end of the surveillance program (after the month 36 CT-scan and before month 38), a request to complete the online QoL questionnaire (EORTC QLQ-C30, FCRI, IES-C, and EQ-5D-5L) will be sent electronically to the patients via REDCap (**CRF IMPROVE-IT2 QOL-TRIGGER**). Patients without an e-boks will get the QoL questionnaires in paper form by regular mail and project personnel will register the results in REDCap manually.
- Mo12 and mo24 QoL questionnaire completion check: in relation to the subsequent blood collection visits (mo 12 and mo 24) the project personnel will check if the QoL questionnaire has been completed. If not, the project personnel will assist the patients in doing so on site. If this is not possible, the project personnel hand over paper versions and register the results in REDCap manually.

- Mo18 and mo38 QoL questionnaire completion check: As these time points are not related to hospital visits, project personnel will check if the QoL questionnaire has been completed 2 weeks after being submitted. If not the project personnel will trigger a digital reminder, which will be send to the patient (**CRF IMPROVE-IT2 QOL-TRIGGER**). Patients without e-boks, who has not completed the questionnaire, will be contacted by phone.

3.8.5.2 For patients in the Experimental arm

3.8.5.2.1 Experimental arm, ctDNA negative:

- Longitudinal PostOP blood sampling for ctDNA guided surveillance (mo12, mo16, mo20 & mo24)
  - blood is analyzed for ctDNA immediately. The result is registered in the IMPROVE-IT2 REDCap database by Department of Molecular Medicine.
  - **ctDNA negative** result: the patients are informed about the result by secure digital mail via E-boks. In due time, the patients are informed about the next post OP blood sampling visit
  - **ctDNA positive** result: See section 3.8.5.2.2
- End of study CT-scan and blood collection: after month 36 and before month 37, the patients are invited for a standard CT-scan. The result is recorded in the REDCap database (**CRF-CTSCAN36mo**). A blood sample will also be collected at this visit. The blood sample will be stored in the biobank and analysed at end of study.
- Quality of life at months 12, 18, 24 and 38: Before the blood collection visits at months 12 and 24, and at month 18 (between the blood collection visits at months 16 and 20), and again after end of the surveillance program (after the month 36 CT-scan and before month 38), a request to complete the online QoL questionnaire (EORTC QLQ-C30, FCRI, IES-C, and EQ-5D-5L) will be sent electronically to the patients via REDCap (**CRF IMPROVE-IT2 QOL-TRIGGER**). Patients without an e-boks will get the QoL questionnaire in paper form by regular mail and project personnel will register the results in REDCap manually.
- Mo12 and mo24 QoL questionnaire completion check: in relation to the subsequent blood collection visits (mo 12 and mo 24) the project personnel will check if the QoL questionnaire has been completed. If not, the project personnel will assist the patients in doing so on site. If this is not possible, the project personnel hand over paper versions and register the results in REDCap manually.
- Mo18 and mo38 QoL questionnaire completion check: As these time points are not related to hospital visits, project personnel will check if the QoL questionnaire has been completed 2 weeks after being submitted. If not the project personnel will trigger a digital reminder, which will be send to the patient (**CRF IMPROVE-IT2 QOL-TRIGGER**). Patients without e-boks, who has not completed the questionnaire, will be contacted by phone.
- Imaging because of suspicion of recurrence: At month 36 the hospital files are assessed for additional imaging events, and related pathology and treatment notes, performed due to suspicion of recurrence (**CRF-CTSCAN-Suspicion**).

3.8.5.2.2 Experimental arm, ctDNA positive:

- PET-CT follow-up plan: Patients will be informed about the positive ctDNA result, at a visit in the outpatient clinic and at the same time informed about the change in follow-up. The new follow-up entails PET-CT scans every third months until the residual disease has been detected, or 21 months has passed.
  - The first PET-CT scan shall be performed immediately after the outpatient visit.

- If the PET-CT scan is negative, the scan will be reviewed by a *second opinion board* to evaluate the technical quality and the finding (4.2.2).
- All PET-CT scans (positive or negative findings) will be discussed on the local site MDT-conference **(CRF IMPROVE-IT2 MDT)**.
  - If the local site MDT conference agrees that residual disease has been identified, the subsequent treatment will be at the discretion of the MDT.
    - The site must upload to the IMPROVE-IT2 REDCap database, a scan of the MDT records and, if relevant, other documents describing the final treatment decision.
  - If the MDT concludes that the finding is negative or inconclusive, then the patient will be invited for a new PET-CT scan every 3 months according to the PET-CT follow-up plan.
  - After the first negative PET-CT scan, the patient will be offered a colonoscopy.

- The results of PET-CT-scans are recorded in the IMPROVE IT2 REDCap database (**CRF PET 0mo, CRF PET 3-21mo**)

- Longitudinal blood sampling: blood is collected in relation to each PET-CT scan. These samples will not be analyzed before end of study.
- Quality of life: Before each PET-CT scan a questionnaire (EORTC QLQ-C3, FCRI, IES-C and EQ-5D-5L) will be sent to the patients electronically from REDCap (**CRF IMPROVE-IT2 QOL-TRIGGER**). Patients without an e-boks will get the QoL questionnaires in paper form by regular mail and project personnel will register the results in REDCap manually.
- At the hospital visits project personnel will check if the QoL questionnaires have been completed. If not, the project personnel will assist the patients in doing so on site. If this is not possible, the project personnel hand over paper versions and register the results in REDCap manually.
- The collection of QoL assessments stops, when recurrence has been diagnosed.
- At end of study (between months 36 and 38) all ctDNA positive patients in the experimental arm are requested to complete the online QoL questionnaire (EORTC QLQ-C30, FCRI, IES-C and EQ-5D-5L). This will be sent electronically to the patients from REDCap (**CRF IMPROVE-IT2 QOL-TRIGGER**). Patients without an e-boks will get the QoL questionnaire in paper form by regular mail and project personnel will register the results in REDCap manually. As this time point is not related to a hospital visit, project personnel will check if the QoL questionnaire has been completed 2 weeks after being submitted. If not the project personnel will trigger a digital reminder, which will be send to the patient (**CRF IMPROVE-IT2 QOL-TRIGGER**). Patients without e-boks, who has not completed the questionnaire, will be contacted by phone.
- End of study CT-scan: At month 36, patients who has completed 21 months of PET-CT follow-up without detection of recurrence are invited for a standard CT-scan. The result is recorded in the REDCap database (**CRF CTSCAN 36mo**).
- Imaging because of suspicion of recurrence: At month 36 the hospital files are assessed for additional imaging events, and related pathology and treatment notes, performed due to suspicion of recurrence (**CRF-CTSCAN-Suspicion**).

3.9 CT-scan standard

All CT-scans will be made in accordance with the national standard. This is the responsibility of the treating department.

3.10 PET-CT-scan technical standard

The quality of the PET-CT scans performed in the study should comply to the pre-specified minimum technical standard described in Appendix B. See also section 4.2.2, about the PET-CT second opinion board.

3.11 Collection, handling, and biobanking of biological samples

3.11.1 Collection and handling of blood samples

Collection of blood samples is performed in collaboration with Regionernes Bio- og Genom Bank (RBGB). At each blood draw, a total of 80 mL blood is collected. Plasma, serum, and buffy coat (nucleated blood cells) are extracted from the blood. I addition to the 80 mL blood donation via syringe, ctDNA positive patients (see section 3.8.5.2.2) will be asked to donate a maximum of 24 droplets of blood (50-400 uL) to a blood spot, by pricking the finger with a lance. The blood spot sampling is optional.

Drawing, handling and storage of the blood is done in accordance to a standard operating procedure (SOP)(Appendix C). From the extracted plasma circulating-free-DNA will be extracted and analyzed for presence of tumor associated somatic mutations. The serum will be used for measurement of carcinoembryonic antigen (CEA), which is the only approved blood based biomarker for monitoring tumor burden. The CEA marker will be used as a reference for the performance of ctDNA as a marker of tumor burden. From the nucleated blood cells constitutional DNA will be extracted. This DNA will be used as a reference when identifying the tumor associated somatic mutations in the circulating free DNA (cfDNA) and in the tumor DNA. cfDNA will be extracted from blood spots and will be analyzed for the presence of ctDNA.

The tumor and normal mucosa tissue biopsies will be collected as part of the IMPROVE trial.

3.11.2 Research Biobank for the IMPROVE study:

All blood samples will be stored in a research biobank associated with the IMPROVE study (Danish Data Protection Agency approval j. no.: 1-16-02-968-17). Patients will only be included if they consent in writing to collection and biobanking of their blood, and clinical data.

3.11.3 Biobank for future research:

When the study is completed left-over biological materials, clinical data and sequencing data will be transferred to the “Colorectal Cancer biobank MOMA, AUH” with the aim to facilitate future research projects. The sequencing data will remain at GenomeDK (see 3.12.1.5), but the responsibility is transferred from the IMPROVE-IT2 study to the “Colorectal Cancer biobank MOMA, AUH”. The “Colorectal Cancer biobank MOMA, AUH” has been approved by the Danish Data Protection Agency 1-16-02-27-10. The biological materials, clinical and sequencing data will remain in the biobank, until the approval expires (currently 1 March 2030) or until the patients request their tissue and/or data removed. Patients may at any time request to have his or her samples and data removed from the “Colorectal Cancer biobank MOMA, AUH” and destroyed. The storage of tissue and data in the research biobank is done in accordance with the Danish Health Act and the Act on Processing of Personal Data. New projects based on the biobank will only be initiated after the pertinent permissions have been obtained from the relevant regulatory committees.

3.12 Molecular analysis

3.12.1 Detection and quantification of circulating tumor DNA

cfDNA will be isolated from the longitudinally collected plasma samples and blood spots using standard methods.

3.12.1.1 ctDNA detection by digital PCR

As part of the IMPROVE study, DNA from the patients' tumor and leucocytes has been whole exome sequenced. The IMPROVE-IT2 study relies on this data for ctDNA analysis. Provided that a suitable mutation can be identified based on the whole exome sequencing data, the presence of ctDNA in the patients plasma samples will be monitored using a digital droplet PCR (ddPCR) assay targeting the mutation. The Department of Molecular Medicine currently holds a collection of assays targeting more than 90 different mutations observed in CRC. Approximately 80% of colorectal tumors (patients) will have at least one of these mutations, and hence the plasma samples from these patients can be monitored by this approach.

3.12.1.2 ctDNA detection by ultra-deep targeted sequencing

In the cases where a ddPCR assay cannot be provided, detection and quantification of ctDNA will be done using an optimized version of our recently developed ultra-sensitive targeted duplex sequencing approach[54]. Using this approach, we will sequence more than 5,000 unique DNA molecules from each plasma sample. Thereby, detection of somatic mutations with allele frequencies down to ~0.1% is possible, i.e. detection of one tumor molecule among 1,000 wild type DNA molecules in plasma. The sequencing will be done using Illumina sequencers. Our approach is much more sensitive than conventional sequencing. Conventional sequencing does not allow detection of mutations constituting less than 5% of the DNA in the analyzed sample. The poor sensitivity is due to a high error-rate of conventional sequencing. This makes it impossible to distinguish actual low-frequency mutations from errors. Our improved method includes multiple steps aimed at reducing the error rate. Including the use of unique strand-specific molecular barcodes (UMIs) and redundant sequencing of each cfDNA fragment. Thereby we can distinguish polymerase induced errors from true mutations. Errors will occur only in a subset of the redundant reads, while genuine mutations are present in all.

Our ultra-deep targeted sequencing approach targets less than 2 million of the 3 billion bases in the human genome. The targets include the regions of the genome that are most frequently mutated in CRC (Table 1). Targeting these regions is sufficient to ensure that at least one somatic mutation will be identified in each CRC case and should therefore enable assessment of the ctDNA level in all included patients. The normal mucosa and CRC tumor tissue sequenced as part of the IMPROVE study serves as negative and positive references.

Gene	**Regions sequenced**
TP53	exons 5-8 (1792 bases)
KRAS	codons 12, 13, 61, 117, 146 (738 bases)
SMAD2	Selected frequently mutated regions (463 bases)
SMAD4	Selected frequently mutated regions (513 bases)
FAM123B	Selected frequently mutated regions (669 bases)
SOX9	Selected frequently mutated regions (693 bases)
APC	Selected frequently mutated regions (5477 bases)
TCF7L2	Selected frequently mutated regions (920 bases)

NRAS	codons 12, 13, 61 (405 bases)
BRAF	codon 600 and others nearby (200 bases)
FBXW7	Selected frequently mutated regions (868 bases)
PIK3CA	8 hotspot mutations (905 bases)

Table 1. CRC driver genes targeted by the CRC cfDNA sequencing panel

3.12.1.3 *ctDNA detection by low pass whole genome sequencing*

With the aim to develop a new platform for ctDNA detection, we will perform low pass (1-2x coverage) whole genome sequencing on plasma cfDNA from all patients. The sequencing will be done using Illumina sequencers. The data will be analyzed for a range of cancer associated features, including the cfDNA fragmentation patterns, copy number analysis mitochondrial DNA fraction[55]. We will investigate if these features can be used to discern the blood samples with and without ctDNA. As the golden standard, for the assessment we will use the digital PCR and ultra-deep targeted sequencing data and the clinical outcome of the patients.

3.12.1.4 Bioinformatic processing of sequencing data

Primary processing of sequencing data is done using Illumina Consensus Assessment of Sequence and Variation (CASAVA) software (v1.8), sequences are aligned against the human reference genome using the Burrows-Wheeler Alignment tool (BWA). Aligned data are processed using GATK best practice. Variant calling from whole exome sequencing data is done using MUTECT2. Variant calling from ultra-deep targeted sequencing data is done using a customized version of the R-package “deepsnv”. Analysis of processed and aligned low-pass whole genome sequencing data is done using custom build tools.

3.12.1.5 Storage of sequencing data

All sequencing data are processed and stored at GenomeDK. GenomeDK is a national high-performance computing facility for bioinformatics and life sciences managed by Center for Genome Analysis and Personalized Medicine, located at Aarhus University, Denmark. GenomeDK is used by Aarhus University Hospital and the Central Denmark Region for processing and storing sequencing data produced in the relation to the treatment of patients. IMPROVE-IT2 use the same security infrastructure and procedures as the Central Denmark Region. For duration of the storage see section 3.8.3.

3.12.2 Quantification of carcinoembryonic antigen (CEA)

Despite being an unspecific and insensitive marker of CRC, the blood-based protein biomarker CEA has been approved by both European and US regulatory authorities for surveillance after treatment for CRC. In this study CEA will be profiled in parallel to ctDNA and the performance of the two markers will be compared.

3.13 Collection of clinical Information

To enable identification recruitment of patients for IMPROVE-IT2, patients who have consented to participate in the IMPROVE study will be monitored to see if they full fill the IMPROVE-IT2 inclusion criteria (See 3.5.1.1). To enable assessment for potential biases between the patients accepting to participate and those who don’t the following information: CPR number, tumor UICC stage, tumor location, surgical center, age, and gender, of all patients asked to participate will be stored in the project database. Patients who consent to participate, further accept that a central element of the project involves comparison and correlation of circulating tumor DNA assessments (gathered from the blood samples) to treatment and clinical outcome. Therefore, participating patients accept that the principal investigator, sponsor, and representative of the sponsor have direct access to collect the necessary information regarding treatment and clinical outcome from hospital

records and health registries to complete the study. Specifically information about surgical intervention, pathology reports, oncological intervention, and radiological evaluations aiming at detecting disease recurrence or assessing changes in tumor burden (e.g. during surveillance and treatment) will be collected. Data will be collected for up to ten years after the last protocolled patient visit.

3.14 Definitions of predictor variables, co-variates and end-points

3.14.1 Primary endpoint

- Fraction of patients with relapse receiving intended curative resection or local treatment aiming at complete tumor destruction as defined by the Endpoint committee (3.14.4), within 3 years after surgery.

3.14.2 Secondary endpoints

- Time to clinical recurrence (TTCR)
- Overall survival at 3 and 5 years (3-year OS and 5-year OS)
- Time to molecular recurrence (TTMR) by either ctDNA or CEA
- Clinical recurrence rate at time-intervals: randomization-12 months, 13-24 months, and 25-36 months.
- Quality of Life (QoL) by use of EORTC QLQ-C30, version 3.0, using global health status and functional scales only (17 items)
- Fear of Cancer Recurrence Inventory (FCRI) (42 items)
- Impact of Events Scale Cancer (IES-C) (15 items)
- European Quality of life – 5 Dimensions (EQ-5D-5L) (5 items)
- Adherence rate (AR)
- Cost-effectiveness (CE)

3.14.3 Safety Endpoints

- Frequency and severity of adverse events (AE) in relation to the per protocol blood draws.
- Frequency of allergic reaction to contrast material in relation to PET/CT imaging

3.14.4 Definition of endpoints

- Definition of curative or palliative intended treatment: An *endpoint committee* appointed by the trial office will prospectively assess the local site multidisciplinary team (MDT) notes and medical records of all relapse patients (both arms) and decide if the planned treatment is curative intended or palliative. The decision will not be provided to the local sites or otherwise influence the actual treatment delivered.
- Overall survival from time of surgery is the outcome of primary interest and is calculated as the time from surgery to death from any cause. Due to immortal time from time of surgery until time of randomization (up till 8 months) an additional analysis will be performed calculating overall survival from time of randomization to death from any cause.
- Time to clinical recurrence is calculated from randomization until detection of loco-regional recurrence or distant metastases, or death from colorectal cancer.
-
- Time to molecular recurrence is calculated from randomization until detectable ctDNA with censoring at time of death or end of follow-up.
- Clinical recurrence rate is defined as total number of clinical recurrences detected divided by the total follow-up time during the specific time-interval.

- Cumulative incidence function of clinical recurrence is calculated treating clinical recurrence as event and death as competing event with censoring at end of follow-up.
- Quality of Life is assessed by the questionnaires: QLQ-C30 (Appendix D), fear of cancer recurrence inventory, FCRI (Appendix E), impact of events scale cancer, IES-C (Appendix F), European Quality of life – 5 Dimensions, EQ-5D-5L (Appendix G).
- Adherence rate assessed by the proportion of patients adhering 100% to the protocol. Protocol adherence is for both arms defined as attendance to all planned diagnostic surveillance according to the protocol and no extra planned surveillance (diagnostic examinations for clinical indications allowed).
- The cost-effectiveness analysis will be carried out from a health care perspective and the health outcome measure in the cost-effectiveness analysis will be the total quality adjusted life years per group.

3.15 Statistical analysis

The statistical analysis plan for IMPROVE-IT2 has been published on June 25th 2024 (DOI: 10.1101/2024.06.25.24309390).

3.15.1 Demographics and baseline data

All data will be presented using descriptive statistics. Continuous variables will be summarized using mean, standard deviation, median, minimum and maximum. Categorical variables will be summarized using the number and percentage of patients.

3.15.2 Analysis of OS, TTCR, QoL and CE

A missed blood sample will not automatically lead to exclusion from the trial. However, multiple missed samples with consequences for the analysis may lead to patient exclusion (see monitoring).

Data will be analyzed as intention-to-treat. Kaplan-Meier estimates will be used for the estimation of median times to recurrence (clinical and molecular), disease or death and their confidence intervals, stratified according follow-up intensity. Endpoints will be assessed using the log-rank test or a Cox regression model, with time to event (clinical or molecular recurrence or death) as response variable and intensity of follow-up as a factor.

Quality of life and fear of recurrence analysis: As indicated in secondary objective 1 (**Fejl! Henvisningskilde ikke fundet.**) we will assess the quality of life (QoL), fear of cancer recurrence (FCR), stress and anxiety of patients in the experimental and control arms. It is our hypothesis that there are no differences between the arms. The hypothesis will be assessed thrice during surveillance, at 12, 18 and 24 months, and after end of surveillance i.e. after the last scan and before month 38. The during surveillance assessments are done at two expected stress peaks (12 and 24 months - before a surveillance examination) and at an expected stress minimum (18 months – between two surveillance examinations).

For measurement of QoL, FCR, stress and anxiety we use the validated Danish versions of the European Organization for Research and Treatment of Cancer QLQ-C30 health-related QoL questionnaire[53], the Fear of Cancer Recurrence Inventory (FCRI)[56], the Impact of Events Scale Cancer (IES-C)[57], European Quality of life – 5 Dimensions (EQ-5D-5L)[58,59].

To have a baseline assessment of QoL, FCR, stress and anxiety all patients are asked to complete the questionnaires after completing adjuvant chemotherapy and before being informed of their randomization allocation and before starting surveillance.

All assessments of differences between the two arms will be relative to the baseline assessment.

We will also conduct an exploratory study of QoL, FCR, stress and anxiety of the patients in experimental arm that become ctDNA-positive and therefore receive high intensity PET-CT surveillance to identify the recurrence site (Secondary objective 7). These patients will be asked to complete the QoL questionnaires before each PET-CT. Changes in QoL, FCR, stress and anxiety will be longitudinally assessed (relative to baseline) from first PET-CT scan and until radiological recurrence.

QoL data collection will be monitored closely in order to minimize missing forms. In case of non-random missing forms and/or values (items), imputation of data will be handled according to EORTC's recommendations.

Cost-effectivenes analysis: QoL/Utility weights for the quality adjusted life years parameter will be EQ-5D-5L[58–60] and QLU-C10D based on EORTC QLQ-C30 for sensitivity analysis. As we shall apply a lifetime horizon for the analysis, parametric survival functions will be used to extrapolate disease-free and overall survival curves beyond five years. Central cost drivers are i) costs of blood tests and the ctDNA analyses, ii) CT scans and PET-CT scans, and iii) differential costs of recurrence treatments due to early versus late discovery. The cost of the ctDNA detection methods will be estimated by bottom up cost calculations, including costs of material, lab use, and personnel time. Depreciation of required equipment will also be performed and included to establish the cost price of ctDNA measurements. Medical resource use items, such as the costs of radiological imaging (PET-CT and CT scanning), treatment of recurrences (incl. surgery, radiofrequency ablation, chemotherapy), hospitalization, and non-molecular laboratory testing, will be extracted from the National Patient Register and if needed hospital records.

Two approaches to costing will be applied and compared. First, total hospital costs for all study participants will be calculated from the National Patient Register. To avoid bias from extreme high costs unrelated to the intervention, such costs will be defined as being 10x higher than median hospital cost. If patients with such costs are present in the study, a panel of medical experts will be consulted to agree whether or not the costs are unrelated to the intervention, and, if found unrelated, the patients' costs will then be excluded from the analysis. The second approach will calculate only cancer-related costs, as defined by having a cancer-associated ICD10 code in the primary diagnosis in the National Patient Register. Resource use will be linked to Danish tariffs. We will translate the cost-effectiveness assessments into business plans for hospitals and public finances to provide decision information for clinical and other decision makers. We shall also estimate productivity costs for labour market active patients but these are not included in the cost-effectiveness analysis.

Costs are reported as follows: 1) costs in each arm in four groups: i) surveillance costs, and ii) recurrence costs (both conditional on recurrence and unconditionally for either surveillance programs), iii) other post-surveillance health care costs, and iv) productivity costs; 2) in the cost-effectiveness analysis, i.e. in the ICER, cost components i-iii (but not iv) are added up; and 3) cost will be reported in intervals of one year i.e. 1st year costs, 2nd year costs, and 3rd year costs. All variables will be presented descriptively with point estimate and 95% confidence intervals.

To assess unintentional bias, the demographics and UICC stage of patients not willing to participate in the trial will be compared to the study population.

Statistics will be performed in close collaboration with professional health research statisticians and cost-effectiveness analysis will be performed by experts in health economics.

3.15.3 Analysis of Safety

AEs will be reported to the study coordinator. All AEs will be coded according to the standardized Medical Dictionary for Regulatory Activities (MedDRA). The coding will be performed by the study coordinator. AEs will be summarized by presenting their incidence of AEs, based on the numbers and percentages of patients with AEs.

4 Data Management

Data from the study will be collected from the medical records and from the Danish Colorectal Cancer Group database (DCCG database), Statistics Denmark (the BEF, BC, DREAM, FAIK, and CIV registries) and via questionnaires (e.g. QLQ-C30, FCRI, IES-C, and EQ-5D-5L). The information from the medical records will be entered into eCRF e.g. baseline information (including clinical TNM, WHO status), neoadjuvant treatment, pathology data, date of visits, adjuvant treatment, and radiological control visits. Other information will be imported to the study database from the DCCG database e.g. tumor characteristics.

Other external data e.g. laboratory data will be entered or transferred into the study database.

4.1 Quality Control

A quality control (QC) of data will be performed to ensure that data entry and verification have been performed correctly in accordance to pre-defined instructions. The QC will be performed before data is declared clean.

4.2 Data Quality Assurance

4.2.1 Case Report Forms (eCRFs)

The Monitors will review and verify the data collected in the eCRFs against the source documents during monitoring visits. The Monitors will address the discrepancies found in the data and will ensure that corrections to the data are properly made and documented by the site personnel. All corrections will be documented in an audit track.

4.2.2 PET/CT Second opinion board

A second opinion board will be formed by the participating nuclear medicine and radiology departments and consist of experts in gastrointestinal imaging. The task of the board is to review negative PET-CT scans for quality and content. The local site PI is obligated to make arrangements to ensure that the local PET-CT center provides the scans and the description to the board immediately following the initial description. The board is obligated to perform its second opinion review within 5 working days after having received the scans and forward the conclusion to the local site PI. If the quality of the scan does not comply with protocol standard (3.10), a new PET-CT scan must be provided by the local PET-CT center immediately. When the quality of the PET-CT scan is acceptable the second opinion board will provide their diagnostic conclusion; the local PI must record the second opinion conclusion in RedCap.

4.2.3 Monitoring

The trial site will be visited by the Clinical Trial Manager (Monitor) periodically at times agreed with the Investigator. It is the function of the Monitor to ascertain that all aspects of the protocol are complied with and that the conduct of the trial conforms to applicable regulatory requirements and established rules for Good Clinical Practice (GCP).

Preferably at the time of each monitoring visit, the Monitor will

- review the completed eCRFs to ascertain that items have been completed and that the data provided are accurate and obtained in the manner specified in the protocol.
- verify that the data in the eCRF and DCCG database are consistent with the clinical records or other relevant records (Source Data Verification) and that trial results are recorded completely and correctly.
- review the completeness of longitudinal blood sampling. In case of missing samples, the decision of letting the patient continue in the trial or be excluded will be at the discretion of the reviewing monitor.

For this purpose, the Monitor must be given direct access to clinical records, original laboratory data, etc., as far as these relate to the trial and without jeopardizing patient integrity. The Investigator and other relevant personnel should be available during the monitoring visit and should devote sufficient time.

The Monitor will continuously monitor the trial database and report on participant retention and completeness of follow-up collection. If problems or deviations are observed the monitor will inform the clinical coordinating investigator. Together they will formulate strategies to solve the problems. If necessary in collaboration with the steering committee.

Data collection on QoL will be monitored closely in order to minimize missing forms.

4.2.4 Training of staff

All Investigators and staff carrying out observations of primary or other major efficacy variables involved in the trial should provide a curriculum vitae. The Investigator will keep a list of all personnel involved in the trial together with their function and trial related duties delegated. He/she will ensure that appropriate trial related training is given to all of the staff, and that any new information of relevance to the performance of this trial is forwarded to the staff involved.

Before inclusion of patients, the Monitor will perform a trial initiation visit to inform and train relevant trial staff.

4.2.5 Audit and inspections

The trial site may be submitted to quality assurance audited by the Sponsor or someone appointed for this task by the Sponsor. The procedures of such a visit would be similar to those of a monitoring visit, and data already checked by the Monitor may be checked again. The Investigator and other relevant personnel must be available during the audit/inspection and must devote sufficient time.

4.2.6 Changes in the approved Trial Protocol

Any substantial change to the approved Final Trial Protocol will be documented in a written and numbered Protocol Amendment. Any proposed substantial change to the Final Trial Protocol must be discussed with and approved by the Sponsor before submitted to the relevant Regulatory Authority for approval.

5 Processing of personal data in the IMPROVE-IT2 study

This study complies with the Data Protection Act and the General Data Protection Regulation (GDPR).
The Health Act and the Helsinki II declaration are observed and will be complied with unconditionally. The project have been reported to the Central Denmark Region and is indexed in their internal record of ongoing research projects.

Throughout the study, all clinical data and samples will be labeled with non-personal identifiers. Information to identify individual patients will only be available to the study coordinator Prof. Claus Lindbjerg Andersen at the Department of Molecular Medicine (MOMA) and will only be used when collecting clinical information from the patient files or when a result is obtained indicating that a patient is qualified for randomization to receive higher follow-up intensity. The molecular and clinical data produced in the study will be stored on keyword protected and log-file operated servers operated by Aarhus University and Aarhus University Hospital.
After the study has been completed and in relation to publication of the study results, the study data (health data and genomic data) will be transferred to the European restricted access database "European Genome-Phenome Archive"(https://ega-archive.org/) in a non-personalized format (pseudonymized). This is done to enable sharing of the data for confirmation and future research purposes and is put forward by the bodies funding the study and the scientific journals in which the results will be published. Data sharing is controlled and only researchers, who through their institutions accept to comply with the European data protection regulations and who after application has been approved by the IMPROVE-IT2 steering committee, will be granted access.

6 Ethics and risk

6.1 Ethics committee approval

IMPROVE-IT2 has been approved by the Ethics Committee of the Central Denmark Region (j. no. 1-10-72-162-19)

6.2 Blood sampling

Patients will have blood samples drawn as part of this project. Per draw 80 ml of blood will be collected by syringe and ctDNA positive patients will in addition be asked to donate 50-400 uL blood via a blood spot. This volume is clinically irrelevant (<2% of the total blood volume). Blood is sampled from a vein in the arm or by finger prick for blood spots, and this can be associated with temporary discomfort. The needle can cause local pain, bruising, and temporary redness at the injection site, but overall blood sampling is associated with minimal risk for the patients. In order to limit the inconvenience as much as possible the samples will be drawn as part of the routine management, whenever this is possible. Taken together, the benefits of participating in the study clearly outweigh the risk associated with the extra blood sampling.

6.3 Genomic sequence analysis

In this study, we will perform ultra-deep targeted sequencing (~5000x coverage) of plasma DNA and leukocyte DNA to identify tumor specific alterations in the plasma DNA. We will disregard all similarities between the two samples i.e. all inherited mutations. Consequently, the risk of incidental finding a potential clinically relevant, genomic variant related to inherited diseases is extremely low, and practically hypothetical. We will also perform low pass whole genome sequencing (<2x coverage). This coverage is too low to allow identification of mutations. Instead, the data will be used to derive a range of cancer associated features such as cfDNA copy numbers, DNA fragmentation patterns, and the mitochondrial DNA fraction. These features we will be used in our assessment of the likelihood that the plasma contains tumor DNA.

If genetic variants with potential clinical relevance are identified in the process, their importance will be evaluated by an expert committee, appointed by Department of Molecular Medicine.

Patients are informed that they can decide to deny to receive information about incidental findings. If they do so, the decision must be indicated at the appropriate place on the consent form.

The committee is appointed when needed and the members will be chosen according to the potential disease. The committee will include a molecular biologist, specialized in genetic sequencing, and a medical doctor specialized in personalized medicine, a clinical geneticist specialized in inherited diseases, and a medical doctor specialized in the disease in question. If deemed relevant other specialists may be included.

This committee will assess if

1) The technological quality of the analysis is sufficient for a reliable result.
2) There is sufficient evidence in the literature for a clinical relevance (e.g. expected penetrance)
3) The sum of information justifies a relevant risk for a genetic disposition
4) The disease, according to current standards, can be treated or prevented

Based on the assessments the committee decides, whether or not the patient (and/or his family) should be informed (by written letter) that the research accidentally has resulted in a finding, with potential influence on his or hers health, and that further information and advice on the matter is offered to him/her and/or potentially affected family members. If accepted, this will be initiated.

Whether or not to provide feedback to relatives of deceased study participants or to study participants who, themselves, deny information about genetic issues, will be decided based on a medical perspective according to "DNVKs retningslinjer af 29. april 2013, sundhedslovens § 43, stk. 2, nr.2" and in "Autorisationsreglerne om lægers omhu og samvittighedsfuldhed".

6.4 ^{18}FDG-PET/CT scan

Patients becoming ctDNA positive in the experimental arm receive intensified follow-up with ^{18}FDG-PET combined with a diagnostic contrast-enhanced CT-scan, ^{18}FDG-PET/CT.

Intravenous access for injection of ^{18}FDG and CT-contrast is made under aseptic condition and may be associated with minor pain. Risk of infection at the puncture site is small.

CT-contrast may cause a warm body sensation or a taste of metal in the mouth. Allergic reactions such as a rash or asthmatic symptoms occur in a few percent of patients. The CT-contrast is similar to the contrast used in the current follow-up after treatment for colorectal cancer.

The total radiation dose of one ^{18}FDG/CT is app. 23.6 mSv (^{18}FDG 6 mSv and CT-scan x17.6 mSv). The exposure to a ctDNA positive patient who receive the maximum number of eight ^{18}FDG/CT is 188.8 mSv. In comparison, the background radiation in Denmark is 3 mSv per year. The radiation dose of the ^{18}FDG/CT does not reach a level causing risk of deterministic damages. There is no threshold dose below which it is certain that a stochastic effect cannot occur. Ionizing radiation is a known carcinogenic and increases the risk of cancer. The life-time risk of death of cancer in the general population is 25 %. The increased life-time risk of cancer in an adult from the background population exposed to a radiation dose similar to the maximum number of ^{18}FDG/CT in this protocol is less than 3 %. The increased risk of a radiation-induced secondary cancer in the population included in IMPROVE-IT2 is considerably less (<1%), as mean age at time of CRC is about 70 years and secondary cancer risk decreases substantially with increasing age of exposure. In contrast, the ctDNA positive patients among stage III CRC patients have an exceedingly high relapse risk approaching 100 %. The overall 5-year survival is only 9 % among relapsing patients. This is due to relapse discovered at an advanced stage with curative intended treatment in only 15 %. The potential of intensified follow-up in ctDNA positive patients identifying relapse, at an early stage, where curative treatment is still possible, clearly outweigh the risk of a radiation-induced secondary cancer.

7 Publication and reporting from the trial

The results of the IMPROVE-IT2 study are expected to be published in international scientific journals. The reporting will follow the CONSORT guidelines for reporting randomized controlled trials (http://www.consort-statement.org/). Positive, negative and non-definable findings will be published as soon as at it is considered professionally sound. Authorship will follow the Vancouver recommendations. If results unexpectedly cannot be published in journals other means on publication will be sought such via the partners´ institutional websites.

8 Economy

IMPROVE-IT2 is a researcher initiated study. The study (incl. experimental costs and salaries) is supported by research grants given to Claus Lindbjerg Andersen (3,900,000 DKK from Danish Cancer Society and 12,500,000 DKK from Innovation Fund Denmark). The research grants are paid to and administered by Aarhus University and Aarhus University Hospital. The investigators have no financial relation to the grant provider nor any financial interests in the project. The sponsor, the clinical coordinating investigator and the site investigators have no competing interests.

8.1 Remuneration to participants

There is no remuneration to the study participants.

8.2 Patient compensation scheme (patienterstatningen)

The study is covered by the Danish Patient compensation scheme (patienterstatningsordningen).

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