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To cite this article: Jesper Nors, Tenna Vesterman Henriksen, Kåre Andersson Gotschalck, Therese Juul, Jes Søgaard, Lene Hjerrild Iversen & Claus Lindbjerg Andersen (2020) IMPROVE-IT2: implementing noninvasive circulating tumor DNA analysis to optimize the operative and postoperative treatment for patients with colorectal cancer – intervention trial 2. Study protocol, Acta Oncologica, 59:3, 336-341, DOI: 10.1080/0284186X.2019.1711170

To link to this article: https://doi.org/10.1080/0284186X.2019.1711170
LETTER TO THE EDITOR

IMPROVE-IT2: implementing noninvasive circulating tumor DNA analysis to optimize the operative and postoperative treatment for patients with colorectal cancer – intervention trial 2. Study protocol

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ARTICLE HISTORY
Received 18 September 2019; accepted 26 December 2019

Background

Death of colorectal cancer (CRC) is primarily associated to distant metastases, present at the time of diagnosis or appearing later following a cancer-free period. At time of diagnosis, 75% of patients present with non-metastatic disease, for which standard of care is curative surgery [1]. Following curative surgery 15–20% of patients experience recurrence of which 70–90% are detected within 3 years of surgery [2–9]. Due to the high recurrence risk, adjuvant chemotherapy is standard of care for high risk UICC stage II and UICC stage III patients [10], but still ~33% experience recurrence [4,6,11]. Extending duration of adjuvant chemotherapy offers no further reduction in recurrence rates [12–15]. Consequently, the only alternative option after adjuvant chemotherapy is surveillance aimed at detecting recurrence sufficiently early to allow efficient treatment [16–18].

Current surveillance strategy in Denmark consists of computed tomography (CT) scans of thorax and abdomen at postoperative months 12 and 36, and colonoscopy every fifth year until age 75 years. Increasing intensity of CT-based surveillance for stage II-III has not been found to improve survival [5,19,20]. However, more patients receive curative intended surgery for recurrence with intense surveillance [5].

Methods to identify patients with microscopic residual disease after adjuvant chemotherapy are highly needed [21]. Minimally-invasive blood-based analysis of circulating tumor DNA (ctDNA) has this potential, since patients with positive ctDNA following adjuvant chemotherapy possess a risk of recurrence of close to 100%, and ctDNA negative patients a risk as low as 10% [22–24]. Also, longitudinal ctDNA analysis detect relapse with an average lead time of ~9 months compared to standard-of-care CT imaging [22,23].

Rationale

The hypothesis of this study is that ctDNA guided postoperative surveillance combining ctDNA analysis and radiological assessments will result in a higher fraction of patients with recurrent disease receiving intended curative or local metastasis-directed treatment as compared to current Danish surveillance strategy.

Aim and objectives

The aim of this study is to conduct a randomized controlled trial investigating the benefit of ctDNA guided postoperative surveillance for UICC stage II high-risk and UICC stage III CRC patients compared to the current standard-of-care CT scan surveillance. The main objective is to investigate if ctDNA guided surveillance increases the proportion of patients receiving curative intended resection or local metastasis directed treatment (Fraction receiving Curative Intervention, FCI). Secondary objectives include investigation of quality of life (QoL), overall survival, cost-effectiveness and time to recurrence detection.

Material and methods

Study design

IMPROVE-IT2 is a randomized multicenter trial comparing the outcomes of ctDNA guided post-operative surveillance and standard-of-care CT scan surveillance. The trial design is a parallel group study with 1:1 allocation. The trial profile is illustrated as a flow chart in Figure 1. The full protocol (see Supplementary file) is consistent with current Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines [25,26].
Figure 1. Trial profile. ctDNA: circulating tumor DNA; PET/CT: Fluo-Deoxy-Glucose Positron Emissions Tomography scan; FCRI: fear of cancer recurrence inventory; IES-C: Impact of Event Scale - Cancer; Mos: months; N.a.: nothing abnormal; QNR: questionnaires. A colonoscopy is performed in case of ctDNA+ blood sample and a PET-CT scan with no evidence or suspicion of residual disease or recurrence. Blood samples from the control group are not analyzed until end of study, but serve to enable post-trial comparison of oncological outcomes for the two groups stratified for ctDNA-status. 4 month blood sample is taken in case of 3 months adjuvant chemotherapy regime. In case of 6 months adjuvant chemotherapy the 8 month blood sample will serve as the first blood sample. QNR: At baseline and months 12, 18, 24 and 36 months patients complete the QoL questionnaires including EORTC QLQ-C30, EQ-5D-5L, FCRI, and IES-C. Further, ctDNA positive patients will complete the QNR questionnaires before each PET-CT scan.
Study population and eligibility criteria

Currently, 11 of 17 surgical centers in Denmark are participating. In total, the trial will include 250 patients resected for CRC and treated with adjuvant chemotherapy. The following eligibility criteria will be applied:

Inclusion criteria:

- 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, anastomotic leakage, emergency surgery, signet ring adenocarcinoma) described in the national guideline for adjuvant therapy to stage II cancer)
- Received intended curative resection and found eligible for adjuvant chemotherapy

Exclusion criteria:

- Not treated with adjuvant chemotherapy
- Synchronous colorectal and non-colorectal cancer diagnosed perioperatively (except skin cancer other than melanoma)
- Other cancers (excluding CRC or skin cancer other than melanoma) within 3 years from screening

Recruitment

Patients who meet the eligibility criteria are approached at the treating surgical department. The patients are given written project information and oral information by a trained health care professional. As the project involves genomic sequencing, the participants are offered genetic counseling before obtaining written informed consent. Informed consent is given on a voluntary basis and has to be obtained prior to commence of any study-related procedure. The consent may be withdrawn at any time without having any impact on current or future treatment.

Randomization

Randomization is performed using a predefined randomization module in the Research Electronic Data Capture (REDCap) database [27,28]. A block randomization is set up to stratify by tumor location, pN-stage and standard-of-care surveillance program intensity, as these factors are associated with risk, location and prognosis of recurrence.

Interventions

The experimental group:

ctDNA analysis will be performed every 4 months postoperatively (4, 8, 12, 16, 20 and 24). At time of first positive ctDNA, patients undergo a whole-body FDG-PET/CT scan for radiological assessment. If the initial assessment is without evidence of recurrence or another cancer, patients will be offered a colonoscopy once and high-intensive radiological surveillance with FDG-PET/CT scans every 3 months, until recurrence detection or 21 months have passed.

The control group:

Patients will undergo surveillance according to current Danish Guidelines with CT scans at months 12 and 36 postoperatively. Longitudinal blood samples will be collected at same time-points as in the experimental group but not analyzed until end of trial.

Both groups complete QoL questionnaires at baseline (prior to randomization and start of surveillance), and at 12, 18, 24 and 36 months. The questionnaire includes European Organization for Research and Treatment of Cancer Quality of Life questionnaire, Core 30 (EORTC QLQ-C30 ver. 3.0) [29], European Quality of Life – 5 Dimensions (EQ-5D-5L) [30,31], Fear of Cancer Recurrence Inventory (FCRI) [32], and Impact of Events Scale for Cancer (IES-C) [33]. Before every FDG-PET/CT scan in ctDNA positive patients, the patients also complete the questionnaires.

Trial status

Inclusion of patients is set to begin January 2020 and expected to end in December 2020. The primary endpoint will be reached and ready for publication in December 2023. The planned 5 years of follow-up will be complete and available for all patients by December 2025. The trial has been registered in the Clinical Trials database: NCT04084249.

tcDNA analysis

Circulating free DNA will be isolated from the longitudinally collected plasma samples using standard methods. DNA from the patients’ tumor will be whole exome sequenced to identify somatic mutations suitable for monitoring in blood plasma using droplet digital PCR (ddPCR). In cases where ddPCR is not possible, detection and quantification of ctDNA will be done using an optimized version of our recently developed ultra-sensitive targeted sequencing approach [34]. The targets include the regions of the genome that are most frequently mutated in CRC (Table 1).

### Table 1. Colorectal cancer (CRC) driver genes targeted by the CRC ctDNA sequencing panel.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Regions sequenced</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>exons 5-8 (1792 bases)</td>
</tr>
<tr>
<td>KRAS</td>
<td>codons 12, 13, 61, 117, 146 (738 bases)</td>
</tr>
<tr>
<td>SMAD2</td>
<td>Selected frequently mutated regions (463 bases)</td>
</tr>
<tr>
<td>SMAD4</td>
<td>Selected frequently mutated regions (513 bases)</td>
</tr>
<tr>
<td>FAM123B</td>
<td>Selected frequently mutated regions (669 bases)</td>
</tr>
<tr>
<td>SOX9</td>
<td>Selected frequently mutated regions (693 bases)</td>
</tr>
<tr>
<td>APC</td>
<td>Selected frequently mutated regions (5477 bases)</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>Selected frequently mutated regions (920 bases)</td>
</tr>
<tr>
<td>NRAS</td>
<td>codons 12, 13, 61 (405 bases)</td>
</tr>
<tr>
<td>BRAF</td>
<td>codon 600 and others nearby (200 bases)</td>
</tr>
<tr>
<td>FBXW7</td>
<td>Selected frequently mutated regions (866 bases)</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>8 hotspot mutations (905 bases)</td>
</tr>
</tbody>
</table>
**Biobank**

Residual blood and tissue biopsies 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).

**Ethical considerations**

The risks and ethical concerns related to the project are limited. We will perform ultra-deep targeted sequencing of plasma DNA and leukocyte DNA to identify tumor specific alterations in the plasma DNA. The risk of accidentally finding a potential clinically relevant genomic variant related to inherited diseases is extremely low, and practically hypothetical.

Ionizing radiation is a known carcinogenic and increases the risk of cancer. ctDNA-positive patients in the experimental group receive intensified follow-up with ¹⁸F-FDG-PET combined with a diagnostic contrast-enhanced CT scan, ¹⁸F-FDG-PET/CT, resulting in a total radiation dose of app. 23.6 mSv pr. scan. We state that the potential benefit from identifying recurrence at an early stage with possible curative treatment, clearly outweighs the hypothetical risk (<3%) of a radiation-induced secondary cancer from participating in this study.

**Definition of endpoint**

The primary endpoint of this study is the fraction of patients with recurrence receiving intended curative or local metastasis-directed treatment (FCI) as defined and prospectively evaluated by a trial office appointed endpoint committee to ensure uniformity across study sites.

Secondary endpoints include:

- Overall survival at 3 and 5 years (3-yr OS and 5-yr OS)
- Time to clinical recurrence (TTCR)
- Time to molecular recurrence (TTMR)
- Quality of Life (QoL) by use of EORTC QLQ-C30, version 3.0
- Fear of Cancer Recurrence Inventory (FCRI)
- Impact of Events Scale Cancer (IES-C)
- Cost-effectiveness (CE)

**Data management**

This study complies with the Data Protection Act and the General Data Protection Regulation (GDPR). The project has been reported to the Central Denmark Region.

Data will be entered into electronic case report forms (eCRF) established using the Research Electronic Data Capture (REDCap) framework [27,28] at Aarhus University. A quality control of data will be performed to ensure that data entry and verification have been performed correctly in accordance to pre-defined instructions.

**Monitoring**

The trial sites will be visited by the Clinical Trial Manager (Monitor) periodically at times agreed with the investigator. The monitor has to ascertain protocol compliance and that the trial conduction conforms to applicable regulatory requirements and established rules for Good Clinical Practice.

The monitor will review and verify the data collected in the eCRFs against the source documents and address any discrepancies found in the data. All corrections will be documented in an audit trail.

**Statistical analysis and sample size**

All data will be presented using descriptive statistics and analyzed as intention-to-treat.

Kaplan-Meier estimates will be used to estimate median times to recurrence, disease or death, stratified according follow-up intensity. Endpoints will be assessed using the log-rank test or a Cox regression model, with time to event as response variable and intensity of follow-up as a factor.

QoL and fear of recurrence analysis will be done using the validated Danish versions of the EORTC QLQ-C30 health-related QoL questionnaire [29], the EQ-5D-5L [30,31], the FCRI [32], and the IES-C [33].

For cost-effectiveness analysis, QoL/Utility weights for the quality-adjusted life years parameter will be QLU-C10D based on EORTC QLC-C30.

Sample size calculations are based on our experience and preliminary data from the IMPROVE trial (ClinicalTrials.gov Identifier: NCT03637686). In total, 250 patients will be included in IMPROVE-IT2 (125 in each group). The expected recurrence rate is ~33% (n = 84), hence, with a 1:1 randomization, we expect 42 recurrences in each group. With standard-of-care surveillance, approximately 15% of recurrences are eligible for curative treatment. Assuming this fraction can be tripled, we will reach a power of 80% to detect a difference at a 5% significance level with 35 recurrence patients in each arm.

**Dissemination**

The results from IMPROVE-IT2 are planned for publication in peer-reviewed scientific journals.

**Discussion**

Metachronous metastases most frequently affect the liver and the lungs. If it is detected too late for curative resection, less than 10% of patients are alive after 5 years [35–40]. The ability to resect and the survival after resection are negatively affected by increasing numbers of metastatic sites and the size of the metastases [35,36,41]. With current surveillance, only ~15% of recurrences are eligible for curative resection or local treatment [42,43]. Thus, early detection of recurrences when tumor burden is low is paramount, not only to increase the rate of curative resections, but also to improve survival following resection.
The minimal-invasive blood-based analysis of ctDNA is based on the principle that tumors shed DNA fragments with a half-life of less than two hours [44] into the blood [45]. Consequently, patients with residual disease are likely to be ctDNA positive during follow-up. The plasma level of ctDNA may initially be below the lower detection level of the analysis, but we have shown that ctDNA undergoes a 5-fold increase from first ctDNA detection to radiological relapse detection with standard surveillance [46]. This underlines the importance of collecting blood samples longitudinally as the residual disease grows, resulting in ctDNA levels reaching a detectable level during the course of follow-up.

Longitudinal blood samples will be collected at the same time points in the control group as in the experimental group but not analyzed until end of trial. They serve to enable post-trial comparison of oncological outcomes for the two groups stratified for ctDNA-status. Besides the additional blood sampling, the control group undergo surveillance in complete accordance to national guidelines and, thus, represents current surveillance practice.

A potential negative effect of recurrence surveillance is increased fear of recurrence and reduced QoL. To enable comparison of the impact on QoL and fear of recurrence of the two surveillance programs, we will collect QoL and fear of recurrence information longitudinally during surveillance.

IMPROVE-IT2 is the first randomized controlled trial of its kind and has the potential to provide a tool for earlier detection of recurrent disease and to identify more patients eligible for curative treatment. IMPROVE-IT2 is a national study. Consequently, if the trial confirms that ctDNA-guided surveillance is superior to standard-of-care surveillance, and if the national health authorities decide to implement it, this can be done at a national level immediately.

Acknowledgments
The authors want to thank the IMPROVE-study-group and all participating surgical departments for their corporation in this study.

Ethics approval and consent to participate
This study is approved by the Central Denmark Region Committees on Health Research Ethics (j. no. 1-10-72-162-19). All participants are offered genetic counseling before obtaining written informed consent. Informed consent is given on a voluntary basis and is obtained prior to commencement of any study-related procedure. The consent may withdrawn at any time without consequence. This study will not present any personal data in any way and will not require consent for publication by any participant(s).

Author contributions
JN drafted this manuscript. KAG, LHI, TJ, JS and CLA designed the study. TVH drafted the full protocol. All authors reviewed and approved the final manuscript.

Disclosure statement
The author(s) have no affiliations or financial involvement with any organization or entity with a financial interest or financial conflict with the study subject or materials discussed in the manuscript.

Funding
IMPROVE-IT2 is a researcher initiated study and administered trial. The study (incl. experimental costs and salaries) is supported by research grants given to Claus Lindbjerg Andersen by Danish Cancer Society and 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 funding bodies have no influence on trial design and execution. There is no economic incentive to participate in IMPROVE-IT2 for either the participants or centers.

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Availability of data and materials
The datasets used and/or analyzed during the current study will be available from the corresponding author on reasonable request.

References


