RESEARCH Protocol

Feasibility of circulating tumour DNA (ctDNA) analysis using automated capillary blood sampling

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	**Adverse Event**
CEA	**Carcinoembryonic Antigen**
CI	**Confidence Interval**
CRC	**Colorectal Cancer**
CRLM	**Colorectal Liver Metastasis**
CT	**Computed Tomography**
cfDNA	**Cell-free DNA**
ctDNA	**Circulating tumour DNA**
dPCR	**Digital polymerase chain reaction**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation**
HIPEC	**Hyperthermic Intraperitoneal chemotherapy and cytoreductive surgery**
IC	**Informed Consent**
iMCQ	**iMTA Medical Consumption Questionnaire**
LoQ	**Limit of Quantification**
MRI	**Magnetic Resonance Imaging**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
NGS	**Next Generation Sequencing**
(S)AE	**(Serious) Adverse Event**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
TAP	**Touch Activated Phlebotomy device**
UAVG	**Implementation of the General Data Protection Regulation (in Dutch: Uitvoeringswet 'Algemene Verordening Gegevensbescherming')**
US	**UltraSound**
VAS	**Visual Analogue Scale**
WGA	**Whole-Genome Amplification**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

Rationale: Aging of the general population results in an increasing number of patients who need to be cared for by a decreasing number of health care professionals. To ensure sustainable and patient-centred health care in the near future, we need to rebalance hospital-based and home-based care. Following surgery of a primary tumour or metastases thereof, patients are offered blood-based follow-up in the hospital, which is necessary to detect recurrence but represents a major burden on the current health care system. The more recently discovered, highly specific circulating tumour-derived DNA fragments (ctDNA) have high potential as a new biomarker and clinical implementation of ctDNA in CRC patient care is expected in the future. However, feasibility of ctDNA detection in blood collected through automated capillary sampling had not yet been investigated.

Objective: We aim to determine the feasibility of ctDNA analysis of blood obtained using automated capillary sampling.

Study design: This proof-of-concept study aims to determine technical feasibility of ctDNA detection in small volumes of capillary blood in 35 patients. Venous blood from the same patients will be used as reference

Study population: All study subjects need to be 21 years or older and provide informed consent to participate in the study. Patients with growing colorectal liver metastases (CRLM) can be included.

Main study endpoint: The primary outcome measure of the study is the agreement between ctDNA measurements in venous versus capillary blood to determine the feasibility of ctDNA analysis through automated capillary blood sampling.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: There is no specific benefit associated with participation for the study subjects. Potential risks associated with participation are mild discomfort and/or temporary superficial hematomas of the skin following blood collection.

1. INTRODUCTION AND RATIONALE

Aging of the general population results in an increasing number of patients who need to be cared for by a decreasing number of health care professionals. To ensure sustainable and patient-centred health care in the near future, we need to rebalance hospital-based and home-based care. In oncological care, currently a large number of cancer survivors receives follow-up in the hospital setting for several years. For example, in colorectal cancer (CRC), which represents the second most common type of cancer in the Netherlands (>12.000 new patients in 2022), around 90% of patients can be treated with surgery.(1, 2) Still, about 30–40% of patients will eventually develop metastatic disease, with the liver being the most common site.(3) Approximately 25% of patients with colorectal liver metastases (CRLM) undergo treatment with curative intent through local therapies, again followed by blood-based follow up in the hospital setting.(4, 5) Following surgery of the primary tumour or metastases thereof, all these patients are offered blood-based follow-up in the hospital, which is necessary to detect recurrence but represents a major burden on the current health care system. Based on the foregoing there is a clear unmet need for reliable blood-based surveillance from home, which will not only enable more sustainable surveillance strategies but at the same time will improve both patient satisfaction and quality of life.

Automated capillary sampling

As blood sampling is and will remain a valuable tool in screening and follow-up, home-based blood sampling could give rise to more sustainable surveillance strategies with a decrease in the need for trained phlebotomists and an improvement in patient satisfaction and quality of life. Advancements in less invasive sampling options make it feasible for patients to conduct home-based blood sampling. Successful implementation of blood sampling methods relies on several factors, including their reliability, safety of use and patient satisfaction. Recently, an Erasmus Medical Center- initiated multicentre study determined the feasibility, reliability, and satisfaction of blood CEA measurements at home comparing automated and lancet capillary sampling.(6) In three Dutch hospitals, 102 participants were recruited between the 25th of March 2022 and the 2nd of March 2023. In total, 20 healthy volunteers, 60 patients after (metastatic) colorectal cancer treatment, and 22 patients with an elevated CEA were included. Herein patients conducted lancet capillary sampling (finger prick) and automated capillary sampling with the TAP® device (Touch Activated Phlebotomy). To assess reliability, blood samples collected under consistent pre-analytical conditions were analysed, comparing venipuncture with lancet capillary sampling and automated capillary sampling. Patient satisfaction of (automated) capillary sampling was evaluated in terms of patient reported outcome measures (PROMs) on pain, burden, ease of use, and preference. For the overall CEA concentration range, the

Passing-Bablok regression analysis of the TAP® showed comparable results when compared to venipuncture with a relative bias of +8.5 and +13.3% for TAP® vs. venipuncture and lancet vs. venipuncture, respectively. Focusing on the lower range of CEA concentration in which patients during follow-up will primarily be, considerable differences in the regression analysis emerged. While in the lower concentration range the relative bias between TAP® and venipuncture remains more or less the same (+8.0%), the relative bias of the lancet method vs. venipuncture increases to +114%. In addition, the TAP® method was rated as the least painful and least burdensome blood collection method. Results from the CASA study show homebased capillary blood collection using the TAP® automated device is feasible, painless, and reliable.(6)

New biomarkers in CRC and CRLM

The more recently discovered, highly specific circulating tumour-derived DNA fragments (ctDNA) were not evaluated in the CASA study, even though clinical implementation of ctDNA in CRC patient care is expected in the future.(7, 8) Recent literature has shown that ctDNA is a prognostic marker in the setting of primary CRC and ctDNA-guided treatment of stage II CRC reduced the use of adjuvant chemotherapy without compromising recurrence-free survival.(7) Additionally, a novel trial set to be initiated shortly to investigate disease-free survival in patients with high-risk rectal cancer (MEC-2022-0755, NL82006.078.22). Participants with detectable ctDNA after surgery will be randomised between adjuvant chemotherapy versus no adjuvant chemotherapy. A clinically relevant decrease in recurrence rate is expected in ctDNA positive patients treated with adjuvant chemotherapy as compared to patients with detectable ctDNA who will only receive follow-up according to the current Dutch guideline. The research group expects that this will translate into improved overall survival. Recent findings have confirmed that ctDNA is not only beneficial in adjuvant treatment decision making, but also in oncological follow-up. Ogaard and authors prospectively collected a total of 499 serial plasma samples from 96 patients undergoing CRLM resection and found that detectable ctDNA at the time of an inconclusive CT scan during follow-up predicted recurrence with a positive predictive value of 100%.(9) These results suggest that ctDNA could play a pivotal role during inconclusive imaging during the follow-up for recurrence detection. Additionally, several promising studies have been conducted in the setting of CRLM in which an association was shown between detectable ctDNA after treatment and oncological outcomes.(9-11) This latter association was confirmed in our own Erasmus MC-initiated MIRACLE study in which we used the Oncomine™ Colon cfDNA Assay targeting >240 mutational hotspots in 14 CRC-involved genes and targeted dPCR (digital polymerase chain reaction) assays to detect ctDNA following surgical removal of CRLM in 240 patients (MEC-2015-289, manuscript in preparation). Patients with remaining ctDNA-positivity after surgery had a significantly worse disease-free survival and this

association was independent from other clinical parameters (multivariable logistic regression model: Odds ratio 2.86; p = 0.04). In addition, a recent meta-analysis showed that ctDNA positivity had the highest specificity to detect recurrence in CRC patients (0.95; 95%CI 0.91–0.97), underlining the potential of ctDNA during follow up of CRC patients after surgery.(12)

Despite the low available blood volume obtained by capillary collection and the low amount of circulating DNA fragments in capillary blood, recent literature suggests it is possible to detect tumour-specific mutations and chromosomal copy number alterations in these samples.(13, 14) To provide solid proof-of-concept we will focus on follow-up in colorectal cancer patients in this proposal, since our departments already have experience with both ctDNA analyses in venous blood of these patients and capillary blood collection as described in above in the CASA study. Additionally, CRC patients have higher levels of mutated DNA in their bloodstream compared to other solid organ malignancies, making them the more ideal group to test this hypothesis in.(15-17) Research has shown that ctDNA detection is also influenced by the stage of the disease. ctDNA has been shown to be detectable in approximately 46% of patients with stage I disease, 73% of patients with stages II–III, and 90% of patients with metastatic CRC.(15, 18) Therefore, we expect ctDNA contents to be higher in patients with metastatic CRC. Lastly, patients with lung-only and peritoneum-only metastases have been shown to exhibit significantly lower ctDNA levels than those with liver-only metastases and, thus, this pilot study will focus on patients with a history of CRC and newly diagnosed with metastatic disease in the form of at least CRLM.(19)

Beyond CRC, ctDNA is likely to enable blood-based surveillance in cancer types currently lacking a reliable tumour marker in the blood, which could reduce the current burden on imaging departments in many hospitals. ctDNA provides a highly sensitive and specific biomarker for both residual disease and recurrence detection in CRC/CRLM patients following surgery with curative intent and could enable blood-based surveillance beyond CRC patients.

Impact & Study rationale

CEA is still the standard of care biomarker investigated in patients who have been treated for colorectal cancer. However, it remains a relatively non-specific serum biomarker that is elevated in various malignancies, but may also be elevated in the case of inflammation, other liver diseases, smoking, and so on. Furthermore, ctDNA has the potential to reflect comprehensive genomic information and overcome intratumour heterogeneity. The current project aims to determine the feasibility of ctDNA detection in small volumes of capillary blood to ensure this promising new biomarker can be implemented in future home-based surveillance strategies for cancer patients. Although this project proposes to use patients with colorectal liver metastases

(CRLM) for proof of principle to leverage experiences gained in the MIRACLE and CASA studies, results will be highly relevant for other cancers currently lacking reliable blood-based tumour markers as well. If this pilot study is successful in showing that the biomarker ctDNA can be determined equally trustworthy in capillary blood through an automated collection device as it could be measured through regular blood collection, a follow-up study could investigate the possibility of utilizing the capillary blood collection in the home setting to analyse ctDNA closer to home. Ultimately this will pave the way for homebased, patient-centred follow up after surgical removal of cancerous lesions irrespective of tumour type, which will reduce the burden on the health care system while improving patient satisfaction.

The adoption of home-based oncology care offers several societal and economic advantages, especially in the long-term. Firstly and most importantly, bringing this type of care closer to a patient's home has the advantage of patient empowerment and the potential to increase quality of life and satisfaction during oncological follow-up. Additionally, it has the capacity to make surveillance strategies more cost-effective. Follow-up after curative treatment of cancer consists of several years of surveillance and multiple blood and imaging tests. The outpatient clinic visits with blood testing account for a substantial part of the expenses in oncological follow-up. A previous study has already shown that the lancet sampling technique for chronic care patients reduces the overall societal costs per patient.(20) Novel, painless techniques like the TAP® (Touch Activated Phlebotomy) device continue to gain interest of manufacturers and, therefore, expenses are expected to drop in the future. Additional to reducing healthcare costs, home-based sampling will alleviate hospital pressure and reduce the demand on healthcare workers, particularly important in times of ever increasing healthcare demands. Finally, it provides benefits on an environmental level by reducing the need for patients to travel to healthcare facilities. For now,

2. OBJECTIVES

Primary Objective:

The primary objective of the study is to determine the technical feasibility of ctDNA detection in small volumes of capillary blood. This will be investigated through determining the agreement between ctDNA measurements in venous (standard blood collection) versus capillary blood through automated capillary blood sampling. We will consider a Kappa >0.75 as sufficient agreement.

Secondary Objective(s):

- Next to agreement, we will also determine the correlation between ctDNA levels in venous and capillary blood. We will consider a correlation coefficient of >0.5 as sufficient.

3. STUDY DESIGN

This proof-of-concept study aims to determine technical feasibility of ctDNA detection in small volumes of capillary blood in 35 patients. Venous blood from the same patients will be used as reference.

4. STUDY POPULATION

4.1 Population (base)

All study subjects need to be 21 years or older and provide informed consent to participate in the study. Patients with newly diagnosed/and or progressive colorectal liver metastases (CRLM) can be included.

4.2 Inclusion criteria

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

- Age ≥ 21 years
- A history of histologically confirmed (metastatic) colorectal adenocarcinoma
- Currently diagnosed with (progressive) colorectal liver metastases (CRLM)
- Signed informed consent

4.3 Exclusion criteria

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

- Patients who are treated and having a response on chemotherapy, as this may have an effect on the investigated biomarker load
- Illiteracy and/or insufficient proficiency of the Dutch language
- Known medical history of superficial or deep skin infection after venipuncture or intravenous line that required antibiotic treatment and or hospital admittance
- Known medical history of immunodeficiency or current use of medical immunosuppressants
- Known medical history of blood-borne diseases such as, but not limited to, the human immunodeficiency virus, hepatitis and viral hemorrhagic fever

4.4 Sample size calculation

To determine agreement between 2 measurements using a discordance rate (alpha) of 5% and a tolerance probability (beta) of 80%, assuming all individual pairs (of venous and capillary blood samples) should agree with each other as previously demonstrated by Gyanchandani et al. (13), we need to include paired measurements of at least 32 patients (21). Similarly, to detect that a correlation coefficient of at least 0.5 significantly differs from 0 at an alpha of 5% with 80% power we need to include paired measurements of at least 29 patients. Therefore we propose to include 35 patients in the current study. For this proof of principle project, we propose to include patients with a history of histologically confirmed (metastatic) CRC and

currently diagnosed with colorectal liver metastases (CRLM), since we know from our previous work in the MIRACLE that ~65% of these patients will be positive for at least one of the tumour-specific mutations evaluated by our NGS panel in their venous blood(22).

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Capillary blood sampling will be carried out under supervision of the coordinating researchers using an automated blood sampling device at the surgical outpatient clinic by the patients themselves. The blood sampling kits with the TAP Sample Collection Device are provided by a third party (YourBio Health, 200 Boston Avenue, Suite 3700 Medford, MA 02155 USA, https://yourbiohealth.com/) which provides detailed instruction material including a high-quality video on the application with Dutch subtitles. This product is CE marked and fit for use in the adult population (>21 years, US). These kits have successfully been used in the past, with less than 1% of user failures. If blood-sampling fails, a new blood-sampling kit will be used.

As part of the standard care, patients who are newly diagnosed with CRLM will have to go through blood collections for standard bloodwork. At this time an extra blood tube will be filled with 10cc of venous blood. The ctDNA analysis of both samples will be performed in the medical oncology laboratory of the Erasmus MC to ensure high and consistent quality.

5.2 Use of co-intervention (if applicable)

Not applicable, as this is not an intervention study.

5.3 Escape medication (if applicable)

Not applicable, as this is not an intervention study.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

TAP® Sample Collection Device by YourBio Health (200 Boston Avenue, Suite 3700 Medford, MA 02155 USA, https://yourbiohealth.com/). This device has received CE approval by a notified body to be used on the European market. This Touch Activated Phlebotomy (TAP®) device enables virtually painless, remote blood collection of capillary blood. This provides automated sampling using the device on the upper arm of the patient.

6.2 Summary of findings from non-clinical studies

In 2018 Blicharz et al reported on the development of a microneedle-based device for the one-step painless collection of capillary blood samples.(23) The final developed and commercialised device, i.e. the TAP-device, utilises an array of 30 microneedles with 50µm thickness, 350µm width and 1000µm length to puncture the skin, after which in average 103.5 ± 19 µL of blood is collected in +/- 4 minutes using a pre-evacuated vacuum chamber.

6.3 Summary of findings from clinical studies

The performance of the TAP device compared to venipuncture for HbA1c determinations in a clinical setting was evaluated in 143 patients.(23) The mean bias of the TAP device assessed by Bland-Altman analysis compared to venipuncture was -0.1%, with corresponding 95% limits of agreement of -5.1 to 4.9%.

The primary objective of the CASA-I, performed within Erasmus MC, was to determine feasibility of CEA assessment at home using (automated) capillary sampling.(6) In this study, the use of a lancet and of a TAP® device were compared to blood sampling performed in hospital. In the complete dataset, both the TAP® and lancet methods exhibit excellent correlations, with high Pearson's r coefficients of 0.998 (95% CI 0.997–0.998) and 0.997 (95% CI 0.996–0.998), respectively. However, in the lower concentration range, correlation coefficients decrease. The TAP® method maintains a strong correlation with a Pearson's r of 0.765 (95% CI 0.684–0.827), while the lancet method shows a moderate correlation with a Pearson's r of 0.503 (95% CI 0.326–0.646).
After the initial collection, 86% of the patients preferred the TAP® method over venipuncture and lancet capillary sampling. The TAP® method was significantly less painful, as measured by a Visual Analogue Scale ($p<0.001$).(6)

6.4 Summary of known and potential risks and benefits

The TAP-device was compared to venipuncture on pain and dermal response in 481

subjects on whom blood sampling was performed using the TAP-device and in 162 subjects subjected to venipuncture.(23) Pain-scores were measured using a visual analogue scale ranging from 0 to 10. The range of pain scores reported for the TAP device and for venipuncture were 0–4 and 0–10, respectively. The mean pain scores for blood sampling with the TAP device and venipuncture were 0.4 (n = 481) and 1.5 (n = 162), respectively, with the TAP device being rated significantly less painful than venipuncture. Dermal response at the TAP device sampling sites was examined and scored immediately following the blood sample collection as well as 20 minutes later. The dermal response scale used ranged from 0–7, with 0 indicating no evidence of dermal irritation and 7 indicating a strong reaction spreading beyond the sampling site. The mean TAP device dermal response score across all subjects was 0.1 immediately after sampling (n = 481) and 0.6 just prior to discharge (n = 479). Similar dermal response scores were observed for the venipuncture sampling sites, with a mean of 0.1 immediately after sampling (n = 162) and 0.5 just prior to discharge (n = 152). In addition, no unresolved issues with the sampling sites were reported by the participants during a scheduled follow-up call five days after testing. Additionally, the Erasmus MC initiated CASA-I study revealed that 86% of the participants preferred the TAP® method after the first use over either venipuncture or lancet capillary sampling.(6) The TAP® method was significantly less painful, as measured by a Visual Analogue Scale ($p<0.001$). Based on these results, the TAP device does not appear to produce any clinically significant potential risks. A Dutch guide with user instructions and a comprehensive video with instructions for use are provided.

6.5 Description and justification of route of administration and dosage

Not applicable as this is not a study with an investigational medicinal product.

6.6 Dosages, dosage modifications and method of administration

Not applicable as this is not a study with an investigational medicinal product.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)
Not applicable, as this study does not involve a non-investigational product.

7.2 Summary of findings from non-clinical studies
Not applicable, as this study does not involve a non-investigational product.

7.3 Summary of findings from clinical studies
Not applicable, as this study does not involve a non-investigational product.

7.4 Summary of known and potential risks and benefits
Not applicable, as this study does not involve a non-investigational product.

7.5 Description and justification of route of administration and dosage
Not applicable, as this study does not involve a non-investigational product.

7.6 Dosages, dosage modifications and method of administration
Not applicable, as this study does not involve a non-investigational product.

7.7 Preparation and labelling of Non Investigational Medicinal Product
Not applicable, as this study does not involve a non-investigational product.

7.8 Drug accountability
Not applicable, as this study does not involve a non-investigational product.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary objective of the study is to determine the technical feasibility of ctDNA detection in small volumes of capillary blood. This will be investigated through determining the agreement between ctDNA measurements in venous (standard blood collection) versus capillary blood (TAP automatic blood collection device) to determine the feasibility of ctDNA analysis through automated capillary blood sampling. ctDNA in both samples will be detected using next generation sequencing (NGS). These results will be expressed in variant allele frequency (VAF; observed mutant molecules/observed wild-type molecules). When at least one of the observed VAFs is above the amplicon-specific limit-of-detection (which takes coverage into account) the sample will be scored ctDNA-positive and otherwise as ctDNA-negative. Subsequently we will determine the agreement between ctDNA-negative and positive calls between the paired venous and capillary samples. In previous literature a perfect agreement was observed between venous and capillary blood for tumour-specific mutations.(13) We therefore anticipate a Kappa of at least 0.75 to consider this pilot successful.

8.1.2. Secondary study parameters/endpoints

Next to agreement between the binary ctDNA-positive/negative score we will also determine the correlation between the observed variant allele frequencies (continuous scale) in venous and capillary blood. Due to variation in the total levels of cell-free DNA (tumour-derived and healthy cfDNA) between venous and capillary blood we do not expect perfect correlation. We deem a minimal correlation of 0.5 (moderate correlation) is needed to consider this pilot successful and have powered our study accordingly.

8.1.3. Other study parameters (if applicable)

The following information will be requested at registration:

- Sex
- Date of birth

- History of health
- (previous) Tumour and patients characteristics
- Contact information:
    - address
    - email
    - mobile phone-number
- Date written informed consent
- Eligibility criteria

8.2. Randomisation, blinding and treatment allocation

Randomisation and treatment allocation are not applicable, this study is not an intervention study. Data will not be blinded.

8.3.Study procedures

Assessment before inclusion:

– Full eligibility check based on in- and exclusion criteria

Study procedures

After informed consent has been obtained according to IHC-GCP guidelines, all subjects will be given the link to the online information video of the TAP® MICRO device. A meeting will be planned with the coordinating researcher at the department of surgical oncology to perform the blood collection using the automated capillary sampling device (TAP® MICRO). Patients can perform this blood withdrawal by themselves, and can receive help through the instructional video and researcher when needed. This meeting will be arranged according to the moment that a participant is asked to go to the blood collection point for standard of care preoperative laboratory assessment. At this moment a patient will already be in the hospital, so no extra trip to the hospital will be necessary. If another date and time suits a patient better, this will be arranged with the coordinating researcher. Additionally, during the standard of care venipuncture an additional blood tube will be collected.

All samples are then transported simultaneously to the medical oncology laboratory of the Erasmus MC for analysis where they are handed to a certified analyst. Hereafter all samples are analysed simultaneously using identical protocols. Plasma will be isolated from both TAP-collected capillary blood and venipuncture collected blood by two sequential centrifugation steps: (1) 1711 g for 10 min at room temperature and (2) 12 000 g for 10 min at room temperature. Plasma will be stored at −80 °C immediately after centrifugation until further analysis.(24) All obtained cfDNA will first be subjected to whole-genome amplification WGA)

using either the Ampli1™ WGA Kit or the picoPLEX WGA kit. Ampli-1 whole genome amplified cfDNA will be analysed by the dedicated Ampli1™ OncoSeek Panel, whereas picoPLEX-amplified cfDNA will be analysed by Oncomine cfDNA colon assay. All genes in the latter assay are also covered by the OncoSeek panel.

Return of results policy

As this biomarker is not yet part of standard of care, subjects will not receive the results of their measurement.

8.4. Withdrawal of individual research participants

Subjects can withdraw from the study at any time for any reason if they wish to do so without any consequences and reporting a reason. The investigator can decide to withdraw a subject from the study for urgent medical reasons (see 8.4.1).

8.4.1. Specific criteria for withdrawal

Specific criteria for withdrawal are:

- Palliative treatment
- Urgent medical reasons
- Death

8.5. Replacement of individual research participants after withdrawal

As this study proposes a small sample size, potential withdrawal needs to be considered. Therefore, patients who decide to withdraw from the study will be replaced by new patients inclusions until we have collected paired blood samples of a total of 35 patients.

8.6. Follow-up of research participants withdrawn from treatment

Follow-up of subjects withdrawn from treatment will be according to the standard follow-up protocol offered to patients during standard care. Reasons for withdrawal will be recorded.

8.7. Premature termination of the study

The study may be stopped prematurely if less than 10% of the inclusions has been met 12 months following inclusion of the first patient. This will be decided by the Sponsor/Principal Investigator.

9.0 SAFETY REPORTING

9.1. Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2. AEs, SAEs and SUSARs

9.2.1. Adverse events (AEs)

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

Our study population has a high risk of complications, including death, which are inherent to their vulnerable condition and these are considered unrelated to the intervention of this study. Because the patient population is expected to encounter a substantial amount of AEs not related to the experimental design but to the diagnosis, not all AE will be registered.

The following AEs will be registered:

- Experience of pain continued after two days after the blood collection.
- Continued, active bleeding 2 hours after the blood collection at the withdrawal site.

9.2.2. Serious adverse events (SAEs)

Due to the nature of minimally invasive blood sampling techniques serious adverse events will only be collected in the first 24 hours after the blood collection.

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

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;

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

The sponsor will report the SAEs through the web portal Research Portal to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.3. Follow-up of adverse events

All SAEs need to be reported until 30 days after end of treatment (for the SAE) of each subject, as defined in the protocol.

Follow up information on SAE's should be reported until recovery or until a stable situation has been reached. The final outcome of the SAE should be reported on a final SAE report.

9.4. Safety Committee

As no risky intervention or treatment will be performed, no Data Safety Monitoring Board or Safety Committee will be installed.

10. STATISTICAL ANALYSIS

All analyses will be performed using appropriate statistical software, such as R Project for Statistical Computing version 4.0.4. (https://www.r-project.org/) and SPSS version 21.0 (SPSS inc., Chicago, IL).

10.1. Primary study parameter(s)

The primary outcome of the current proposal will be the agreement between ctDNA measurements in venous versus capillary blood to determine the feasibility of ctDNA analysis through automated capillary blood sampling. This agreement between measurements will be analysed through the interval approach proposed by Bland and Altman, which uses the limits of agreement together with a mean–difference graphic that plots the difference against the mean of the two measurements along with the 95% confidence limits of the difference, and Passing-Bablock regression analysis.(25) In addition to agreement this will also allow us to investigate the presence of any systematic differences (i.e. fixed bias) between capillary and venous blood measurements.

10.2. Secondary study parameter(s)

Additionally, we will also determine the correlation between ctDNA levels (VAF) in venous and capillary blood. Here, we will apply Spearman to determine the correlation between capillary and venous blood measurements.

10.3. Other study parameters

Other parameters will be utilised for baseline characteristics. Baseline characteristics will be compared using Fisher's exact test for discrete variables and the Kruskal–Wallis test for continuous variables. Continuous variables will be represented as median and IQR.

10.4. Interim analysis

Not applicable, as no interim analysis will be performed.

11. ETHICAL CONSIDERATIONS

11.1. Regulation statement

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki (64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013), in accordance with the Medical Research Involving Human Subjects Act (WMO) and the ICH-GCP Guidelines.

11.2. Recruitment and consent

Recruitment

Subjects will be recruited in the outpatient clinic of the Erasmus MC. The surgical oncology of the Erasmus MC performs multiple treatments for patients with colorectal cancer such as: surgical resection of primary colorectal tumour, surgical resection of (recurrent) colorectal liver metastases, resection or debulking of local recurrence of rectal carcinoma's and hyperthermic intraperitoneal chemotherapy and cytoreductive surgery (HIPEC). The treating physician will identify eligible patients and ask patients for their consent. Subsequently the trial coordinator will be informed

Consent

The trial coordinator will determine subject eligibility according to the in- and exclusion criteria. If a subject meets all the in- and exclusion criteria the trial coordinator will inform the patient of the study and include them according to good clinical practice guidelines (IHC-GCP). Additional information such as possible side effects will be given, and it will be made clear that refusal to participate will not influence further options for therapy. Written informed consent of patients is required before enrolment in the study and before any study related procedures take place.

Before informed consent may be obtained, the patient should have ample time and opportunity to inquire about details of the study and to decide whether or not to participate in the study. All questions about the study should be answered to the satisfaction of the patient. There is no set time limit for the patient to make a decision. The investigator should inform each participant if there is a specific reason why he/she must decide within a limited time frame, for example if a patient's condition necessitates start of treatment or if the trial is scheduled to close for enrolment.

The patient should be informed in a timely manner if new information becomes available that might be relevant to the patient's willingness to continue participation in the trial. The communication of this information should be documented.

The investigator shall provide a copy of the information sheet and the signed consent form to the patient and the signed original shall be maintained in the Investigator Site File.

11.3. Objection by minors or incapacitated subjects (if applicable)

Not applicable as no minors or incapacitated subjects are included.

11.4. Benefits and risks assessment, group relatedness

There is no specific benefit associated with participation for the study subjects. Potential risks associated with participation are low to mild discomfort and/or temporary superficial hematomas of the skin following venipuncture.

11.5. Compensation for injury

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

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

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

11.6. Incentives (if applicable)

Not applicable, as patients receive no incentive.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1. Handling and storage of data and documents

Individual patient information obtained as a result of this study is considered confidential and is handled conform the General Data Protection Regulation (GDPR) and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: Uitvoeringswet 'Algemene Verordening Gegevensbescherming' (UAVG)). Disclosure to third parties is prohibited, except for authorities involved with monitoring and quality control of the clinical study such as monitors, auditors and the health care inspectorate. Patient confidentiality will be ensured by using identification numbers (e.g. TAP-1, TAP-2, ...). A subject identification code list is safeguarded by the principle investigator in case data needs to be linked back to an individual subject. Individual patient information is stored in the web-based data management

Case Report Forms

Data will be collected on digital Case Report Forms (CRF) to document eligibility parameters and parameters necessary to evaluate the study endpoints. Data to be collected on the CRF are derived from the protocol.

The inclusion CRF will be completed on site by the local investigator or an authorised staff member. The CRF and instructions for completing the CRF will be provided in Castor.

Filling of essential documents

Essential documents are those documents that permit evaluation of the conduct of a trial and the quality of the data produced. The essential documents may be subject to, and should be available for, audit by the Sponsor's auditor and inspection by the regulatory authority(/ies). The investigator should file all essential documents relevant to the conduct of the trial on site. The Sponsor will file all essential documents relevant to the overall conduct of the trial. Essential documents should be filed in such a manner that they are protected from accidental loss and can be easily retrieved for review.

Record retention

Essential documents should be retained for 15 years after the end of the trial (i.e. from date of last patient visit for this trial). They should be destroyed after this time.

12.2. Monitoring and Quality Assurance

Monitoring will be done in compliance with the NFU guidelines for low-risk studies.

12.3. Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4. Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5. Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient to complete all study procedures.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6. Public disclosure and publication policy

Publications resulting from this study will be submitted to peer-reviewed journals. The manuscript will be drafted following the STROBE guidelines. The principal investigator will prepare the manuscript together with the involved researchers. Any publication, abstract or preservation based on patients included in this study must be approved by the principle

investigators. This is applicable to any individual patient registered in the trial, or any subgroup of the trial patients.

This study will be registered in the Medisch-wetenschappelijk Onderzoek in Nederland (OMON) register.

13. STRUCTURED RISK ANALYSIS

13.1. Potential issues of concern

a. Level of knowledge about mechanism of action

The TAP® MICRO is a blood collection device with a detachable blood reservoir. Microneedles penetrate the epidermis which allows for interstitial blood collection via low pressure in the collection device.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

The TAP-device was compared to venipuncture on pain and dermal response in 481 subjects on whom blood sampling was performed using the TAP-device and in 162 subjects subjected to venipuncture (26). Pain-scores were measured using a visual analogue scale ranging from 0 to 10. The range of pain scores reported for the TAP device and for venipuncture were 0–4 and 0–10, respectively. The mean pain scores for blood sampling with the TAP device and venipuncture were 0.4 (n = 481) and 1.5 (n = 162), respectively, with the TAP device being rated significantly less painful than venipuncture. Dermal response at the TAP device sampling sites was examined and scored immediately following the blood sample collection as well as 20 minutes later. The dermal response scale used ranged from 0–7, with 0 indicating no evidence of dermal irritation and 7 indicating a strong reaction spreading beyond the sampling site. The mean TAP device dermal response score across all subjects was 0.1 immediately after sampling (n = 481) and 0.6 just prior to discharge (n = 479). Similar dermal response scores were observed for the venipuncture sampling sites, with a mean of 0.1 immediately after sampling (n = 162) and 0.5 just prior to discharge (n = 152). In addition, no unresolved issues with the sampling sites were reported by the participants during a scheduled follow-up call five days after testing. Based on these results, the TAP device does not appear to produce any clinically significant potential risks.

Additionally, the ErasmusMC initiated CASA-I study revealed that 86% of the participants preferred the TAP® MICRO method after the first use over venipuncture and lancet capillary sampling. The TAP® MICRO method was significantly less painful, as measured by a Visual Analogue Scale ($p<0.001$). Based on these results, the TAP device does not appear to produce any clinically significant potential risks. A Dutch guide with user instructions and a comprehensive video with instructions for use are provided.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Not applicable because there is no mechanism which can be induced in animals and/or in ex-vivo human cell material.

d. Selectivity of the mechanism to target tissue in animals and/or human beings
Not applicable because there is no mechanism to target tissue in animals and/or human beings.

e. Analysis of potential effect
Not applicable since there is no potential effect which can be analysed.

f. Pharmacokinetic considerations
Not applicable because there is no pharmacokinetic involved in this study.

g. Study population
The study population consists of patients with newly diagnosed (isolated) CRLM. Venipuncture is routinely used in the preoperative setting.

h. Interaction with other products
Not applicable because blood sampling does not have any interaction with other products.

i. Predictability of effect
Not applicable because there is no new biomarker used in this study.

j. Can effects be managed?
Possible side effects like hematoma and discomfort subside spontaneously without the need for medical intervention.

13.2. Synthesis

The blood sampling techniques used in this study (venipuncture and TAP® MICRO blood collection) are associated with minimal risks. No extra measures have been taken to minimise these risks. The potential associated risks are acceptable.

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