Prospective study protocol	
Protocol number	1
Document version	1.0
Study Title	Prospective data collection for early dynamic screening for colorectal cancer via novel protein biomarkers reflecting biological initiation mechanisms
Novel solution	New technology – DIOPTRA – for cancer screening and early detection
Organisation responsible for the study	Name, address
Local representative, if applicable	Name, address
Lead Principal Investigator (I)	Name, address
Coordinating investigator (CI)	Name, address

Confidentiality Statement

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_____ _____

(position, name, surname) (signature)

Ethical principles Statement

The information contained in this document and in related documents, which are prepared for this study, is in accordance with the recognised ethical principles (that have their origin in the Declaration of Helsinki) for medical research involving humans, and the principles of good clinical practice, as well as with the applicable regional or national regulatory requirements, any additional requirements imposed by the EC or regulatory authority.

_____ _____

(position, name, surname) (signature)

Preconditions to start prospective study Statement

The prospective study shall not begin until the required approval/favourable opinion from the EC and regulatory authority have been obtained.

_____ _____

(position, name, surname) (signature)

Approval sheet

_____ _____

(position) (name, surname)

_____ _____

(signature) (date)

Following persons are planned to be involved in the study:

Role	Organisation responsible for the study (S) / Principal Investigator (Clinical site) (I)/ Coordinating investigator (Clinical site) (CI)	Name	Title
Author			
Leadership			
Study team			
Study team			
Study team			
Study team			
Study team			
Quality			
Regulatory affair			
Medical writer			
Study Manager			

Contents

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1.1. DOCUMENT HISTORY

Revision	Date of enactment	Change author	Change description
1.0			Document is created

1. ABBREVIATIONS AND ACRONYMS

AI	Artificial Intelligence
AE	Adverse event - any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device and whether anticipated or unanticipated
AGSAVVAS	Geniko Antikarkiniko Ogkologiko Nosokomeio Athinon O Agios Savvas
BLOCKS	Blocks Health and Social Care EOOD
BURGOS	Fundacion Burgos Por La Investigacion De La Salud
CHUL	Centre Hospitalier Universitaire De Liege
CRC	Colorectal Cancer
FU	Follow up
GOC	Linac-Pet Scan Opco Limited
NKUA	National and Kapodistrian University of Athens
PSD	Prospective Study Design
RM-RRH	Region Midtjylland
SOP	Standard operating procedure
UKCM	Univerzitetni Klinicni Center Maribor

2. SYNOPSIS

Title	Prospective data collection for early dynamic screening for colorectal cancer via novel protein biomarkers reflecting biological initiation mechanisms
DIOPTRA screening system	AI-based solution for CRC early diagnosis and screening. The main means will be the biological sampling and analysis using in vitro diagnostics.
Purpose	The purpose of this study is the clinical refinement and validation of the DIOPTRA screening system.
Prospective study design (PSD)	Prospective, cohort, multi-center study.
PSD Primary objective	To validate the diagnostic sensitivity and specificity for CRC detection of the DIOPTRA screening system using clinical diagnosis as reference (colonoscopy).
PSD Secondary objectives	Secondary objectives include: 1. Validation of the clinical performance of the DIOPTRA screening system for the detection of advanced adenomas. 2. Refinement of the DIOPTRA screening system. 3. Evaluation of the effectiveness of behavioural suggestions to reduce CRC risk. 4. Assessment of cost-effectiveness of DIOPTRA system.
PSD Primary endpoint	Acceptable diagnostic specificity and sensitivity for CRC detection and for the detection of healthy and non-advanced adenoma groups, respectively.
PSD Secondary endpoints	Secondary endpoints include: 1. Acceptable diagnostic sensitivity for the detection of advanced adenomas. 2. Improvement of the performance metrics of the DIOPTRA screening system using the prospective data for refinement. 3. Statistically significant differences in risk factors and protein biomarker concentrations for individuals who have implemented the behavioural suggestions. 4. Significant reduction of the estimated DIOPTRA screening system costs compared to screening colonoscopy.
Duration of the study	35 months
Duration of study follow-up	1 year after follow up enrollment.
Subject population	Individuals that visit the clinical sites for a colonoscopy.
Number of subjects	At least 1612 participants are estimated to be recruited in 8 clinical sites (BLOCKS, CHUL, RM-RRH, UKCM, BURGOS, NKUA, GOC, AGSAVVAS) Based on sample size calculations, at least N=403 participants from each group are required to evaluate the primary and secondary endpoints of the study (A total of 1612 participants). Given the low CRC incidence rate, a much larger number is expected to participate in the study until the required numbers are recruited.
Number of Sites	8 clinical sites:

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	1) BLOCKS 2) CHUL 3) RM-RRH 4) UKCM 5) BURGOS 6) NKUA 7) GOC 8) AGSAVVAS
Prospective Study Procedures	The main study procedures (observational) are the following: 1. Enrollment of participants in the study once written informed consent is obtained and subject eligibility is confirmed. 2. Blood sample collection (serum & plasma). 3. Colonoscopy & clinical diagnosis according to each clinical site's standards. 4. Collection of DIOPTRA data. 5. End of study. During enrollment, participants will be given the option to potentially participate in the DIOPTRA follow up study. The procedures of the follow up study are: 1. Enrollment in the follow up study after subject eligibility is confirmed. 2. Download of the DIOPTRA mobile app for implementation of steps 3-5 below. 3. Answer questionnaire. 4. Receive behavioural suggestions. 5. Periodic data update. 6. Follow up blood collection and risk assessment. 7. End of follow-up study
Study financing	This study is part of the DIOPTRA European Project funded within the research and innovation program of the Horizon Europe under Grant Agreement N° 101096649.
Person paying compensation for costs and time incurred in participating in the study, procedure, and conditions for calculation and payment of compensation	No compensation is provided*. *The compensation for the 1 (one) year FU of the validation study will depend on each site's policy. As participants will be called back for blood sampling and re-assessment as part of a non-prescribed visit, certain sites may require that compensation should be provided for the travel to and back from the hospital.

3. BACKGROUND & RATIONALE

BACKGROUND

Incidence & Survival Rates. Colorectal cancer [1], [2] (CRC) is the third most common cancer in men and the second in women, accounting for 10% of all tumours worldwide. It ranks second in cancer-related deaths with 9.4%, only below lung cancer. About 1.9 million new cases were diagnosed in 2020, translating into 0.9 million deaths, while incidence is projected to rise significantly over the next decade, with 3.2 million new diagnoses annually by 2040. In affected EU individuals, 5-year survival ranges from 28.5% to 57% in men and from 30.9% to 60% in women, with pooled estimates in 23 countries of 46.8% and 48.4%, respectively. Moreover, CRC is among the five most likely to metastasise cancers. Upon initial diagnosis, 22% of cases are metastatic, while about 70% of patients will eventually develop metastatic relapse [3].

Existing Standard & Screening Impact. Screening methods consisting of endoscopic tests (e.g., colonoscopy) and non-invasive alternatives such as the faecal immunochemical test (FIT) have been put into action [1]. Studies have compared mortality rates for symptom-detected vs. screening-detected CRC, stating the considerable impact of screening via quantified reduction estimates surpassing 30% for screening-based detections [5]. Particularly, the 5-year survival rate can reach 90% for stage I diagnosis, being less than 15% for advanced stages [6]. Therefore, routine screening is key for reducing mortality and declining incidence rates since CRC is now considered as a highly preventable disease with a considerably wide temporal development window [7]. Namely, the transitional path from normal mucosa to pre-malignant growth and then to malignant lesion might spread over 15 to 20 years, with scientists seeking means for earlier, cost-effective, and less taxing detection of premalignant states.

Pressing Conditions. In determining the CRC risk status, factors such as age, BMI, diet, smoking habits, and family history [4] have been pinpointed by researchers and clinicians alike. Despite the long-assumed CRC preventability based on modifiable risk factors, awareness and knowledge exploitation remain extremely low. Overall, taxing procedures, citizen reluctance, poor awareness, and screening accessibility are hindering participation, forcing researchers into the survey of accessible, non-invasive biomarkers that bear the potential to render cancer screening less burdensome and more accessible to citizens.

Liquid Biopsy CRC Biomarkers. Liquid biopsy appears as a promising new tool for non-invasive, quick and safe assessment [5]. Among all liquid biopsy products, blood-derived proteins seem to constitute the most cost-effective solution judging by resources, sensitivity, and research maturity. On this premise, a vast protein pool has been tested, albeit evidence lacks comparative validation, perplexing standardisation margins. Research must highlight a small biomarker subset that can be feasibly exploited for population-based screening and sustainably covered by health insurance bodies.

Artificial Intelligence for Cancer Screening. AI has been widely employed in biomarker evaluation, from drug development to pathology and oncology [11]. However, despite the AI advances in CRC risk and progression assessment, the medical community is still sceptical and reluctant to trust the outcomes of machine learning. This is mainly due to the depth and confusing architecture of most neural network approaches, which are regarded as “black boxes” [12]. Explainable artificial intelligence (XAI) is gradually becoming a prerequisite for clinicians and policymakers, seeking to instil accountability and medical transparency into AI-assisted decisions for launching trustworthy clinical applications [13].

Risk Factor Analysis. Numerous studies have investigated the association of CRC incidence with demographic, behavioural, and environmental risk factors, including age, sex, and lifestyle. Age comprises the main factor assessed by current guidelines, formulating at-risk groups for recommended screening [14]. Clinical practice has shown that these thresholds are gradually decreasing, a fact under study by the medical community. Several lifestyle-related factors have been identified, which are modifiable through suitable behavioural screening and personalised interventions.

RATIONALE FOR THE PROSPECTIVE STUDY

DIOPTRA aims for an accessible and less taxing screening to attain a wider population outreach by exploiting blood-based biomarkers. Although several researchers have tried to assess this, the limitations

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in the number of participants and number of proteins studied hinder a generalised framework for early CRC screening and prevention. The same applies in AI-enabled CRC risk assessment, where clinical validation of established systems [2] is absent. DIOPTRA focuses on fulfilling the role of biomarker identification and risk factor stratification via validation on 8 different sites, utilising a large number of patients/ healthy citizens. As such, the evidence produced will not only be based on expert opinion but from vigorous validation procedures on the retrospective and prospective data (level of evidence B). The study's retrospective part hypothesised that a set of predictive variables is associated with the risk of developing CRC. Based on this study, risk factors will be identified to investigate their association with CRC and predict the early risk of CRC. Data from electronic health records will be used and analysed to isolate variables defined as risk factors based on four groups. Various methodologies, including statistical analysis and machine learning techniques, will be used to investigate the impact of each factor on CRC. More importantly, by employing cutting-edge *in vitro* protein analysis in (paired with the blood samples collected) biopsies, the molecular mechanism of CRC development will be uncovered, providing additional evidence needed and thus establishing a robust and efficient framework for early screening (level of evidence A).

4. STUDY DESIGN

The study is going to be a prospective, cohort, multi-center study with a partial follow-up of one year. It is not envisaged to change the recruitment process throughout the duration of the project. An equal sample size will be required for all 4 groups: healthy, non-advanced adenomas, advanced adenomas and CRC cases. For follow-up study, only the first two groups will be enrolled. Initial data obtained will be used for the algorithm training followed by validation of the pilot.

STUDY HYPOTHESIS

The main study hypothesis is that the DIOPTRA screening system has adequate clinical performance for the early diagnosis of CRC and advanced adenomas. An additional hypothesis is that the DIOPTRA system can accurately characterise the risk of an individual developing CRC. Finally, we hypothesise that the DIOPTRA behavioural suggestions, when applied, can significantly lower the risk of developing CRC. To evaluate these hypotheses, we will use multiplex protein biomarker measurements, along with demographic, behavioural, and clinical data from participants belonging to the DIOPTRA study groups to test and refine the DIOPTRA AI models.

TYPE OF INTERVENTION

Biological samples will be collected via a minimally invasive method. According to each clinical site's standards and pre-existing practice, enrolled individuals will undergo a screening colonoscopy, while blood will be drawn (prior to the colonoscopy) for the purposes of the study. All biological data will be used for *in vitro* protein-based analysis, allowing the construction of preliminary decision algorithms and AI analysis models.

SITES OF THE PROSPECTIVE STUDY

Research center No 1: ……..
Address: ……..
Tel: ……; Fax: …… ; e-mail: …….
Title of the department (s): ……..
Tel: ……..; Fax: ……. ; e-mail: …….. .

Research center No 2: ……..
Address: ……..
Tel: ……; Fax: …… ; e-mail: …….

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Title of the department (s): ……..
Tel: ……..; Fax: ……. ; e-mail: …….. .

Research center No 3: ……..
Address: ……..
Tel: ……; Fax: …… ; e-mail: …….
Title of the department (s): ……..
Tel: ……..; Fax: ……. ; e-mail: …….. .

Research center No 4: ……..
Address: ……..
Tel: ……; Fax: …… ; e-mail: …….
Title of the department (s): ……..
Tel: ……..; Fax: ……. ; e-mail: …….. .

Research center No 5: ……..
Address: ……..
Tel: ……; Fax: …… ; e-mail: …….
Title of the department (s): ……..
Tel: ……..; Fax: ……. ; e-mail: …….. .

Research center No 6: ……..
Address: ……..
Tel: ……; Fax: …… ; e-mail: …….
Title of the department (s): ……..
Tel: ……..; Fax: ……. ; e-mail: …….. .

Research center No 7: ……..
Address: ……..
Tel: ……; Fax: …… ; e-mail: …….
Title of the department (s): ……..
Tel: ……..; Fax: ……. ; e-mail: …….. .

Research center No 8: ……..
Address: ……..
Tel: ……; Fax: …… ; e-mail: …….
Title of the department (s): ……..
Tel: ……..; Fax: ……. ; e-mail: …….. .

5. OBJECTIVES

The purpose of this study is the clinical refinement and validation of the DIOPTRA screening system.
Primary objective: to validate the diagnostic sensitivity and specificity for CRC detection of the DIOPTRA screening system using clinical diagnosis as reference (colonoscopy).

Secondary objectives:

1. Validation of the clinical performance of the DIOPTRA screening system for the detection of advanced adenomas.
2. Refinement of the DIOPTRA screening system.
3. Evaluation of the effectiveness of behavioural suggestions to reduce CRC risk.

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4. Assessment of cost effectiveness of DIOPTRA system.

6. ENDPOINTS

Primary endpoint. Acceptable diagnostic specificity and sensitivity for CRC detection and for the detection of healthy and non-advanced adenoma groups, respectively.

Secondary endpoints include:

1. Acceptable diagnostic sensitivity for the detection of advanced adenomas.
2. Improvement of the performance metrics of the DIOPTRA screening system using the prospective data for refinement.
3. Statistically significant differences in risk factors and protein biomarker concentrations for individuals who have implemented the behavioural suggestions.
4. Significant reduction of the estimated DIOPTRA screening system costs compared to screening colonoscopy.

7. STUDY POPULATION

Prospective study will cover at least 1600 participants to be recruited across all the study's 8 clinical sites (BLOCKS, CHUL, RM-RRH, UKCM, BURGOS, NKUA, GOC, AGSAVVAS). Study population will cover participants that visit the clinical sites for a colonoscopy. Participants will be split into the following groups following the histopathological analysis of index lesions identified during colonoscopy:

- Healthy: no neoplastic findings after a colonoscopy;
- Non-advanced adenomas;
- Advanced adenomas. Under ESGE 2020 guidelines, the following adenoma should be classified as advanced adenomas: at least 1 adenoma ≥ 10 mm or with high-grade dysplasia or with high % of villous growth pattern, or any serrated polyp ≥ 10 mm or with dysplasia;
- Colorectal cancer CRC stage I, II, and III.

Gender distribution in the incidence of CRC will be taken into account in this study, including as similar rates of male and female participants as possible. However, it must be taken into account that males are 25% more prone to develop CRC in comparison to females, which could lead to a greater number of male participants in the study.

Inclusion criteria for prospective data collection and pilot evaluation:

- Any indication for total colonoscopy (including routine screening and presence of symptoms/ FIT positive).
- Age between 18-80 years at the moment of recruitment (see above)
- Absence of significant comorbidities (ASA IV)
- Ability to provide valid (written informed) consent

Inclusion criteria for the follow up study patients who will use the DIOPTRA mobile application:

- Presenting the 4 inclusion criteria here above.
- Patients willing to use the DIOPTRA application regularly.
- Level of digital literacy allowing to manage mobile terminals (smartphones, smartphone apps, tablets).
- Good coverage of internet connection at home.
- Availability of a smartphone/ tablet (in order to be able to use the app).

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- Belonging to the healthy or non-advanced adenoma groups

Exclusion criteria.
Persons belonging to the vulnerable group will not be included in the clinical study.
Other exclusion criteria for the prospective study:

- Age under 18 y/o or above 80 y/o
- Comorbidities ASA IV
- Recent major abdominal surgery (colectomy) or radiation prior to the recruitment
- Inflammatory bowel diseases
- Polyposis syndrome
- Pregnancy or suspicion of pregnancy
- Colorectal cancer history
- Not able to understand the study and provide valid consent

Exclusion criteria for the follow-up study:

- Classification in the CRC or advanced adenoma groups
- Non-availability of a smartphone/tablet or inability to use a mobile app (e.g., due to low digital literacy)

8. SAMPLE SIZE

To evaluate the endpoints of the study (diagnostic sensitivity and specificity of DIOPTRA), the exact binomial test will be used, with the NULL hypothesis $H_0: p \leqq p_0$, where p_0 is the pre-specified lower bound of the endpoint and p is the observed endpoint in the sample. The pre-specified lower bounds of the endpoints were selected based on the decision memo (CAG-00454N) from the Centers for Medicare & Medicaid Services (CMS) to cover a blood-based biomarker test as an appropriate colorectal cancer screening test.
In terms of the primary endpoint (diagnostic sensitivity for CRC detection), with the following assumptions:

- Required power = 0.8
- Confidence level = 0.05
- Lower bound of sensitivity = 0.74
- DIOPTRA sensitivity hypothesis = 0.8
- Safety factor = 1.33 (Taking into account the removal of participants due to poor sample/data quality and small deviations in the sensitivity hypothesis)

The required sample size is N = 403 participants in the CRC group. Using the same sample size for each of the healthy and non-advanced adenoma groups, along with the following assumptions:

- N = 403
- Confidence level = 0.05
- Specificity lower bound = 0.9
- DIOPTRA specificity hypothesis = 0.94

The statistical power of the exact binomial test for specificity is 0.91 (per group). The power is satisfactory to reject the NULL hypothesis.
Assuming the same sample size N = 403 for the advanced adenoma group and the following assumptions:

- N = 403
- Confidence level = 0.05
- Advanced adenoma sensitivity lower bound = 0.42
- DIOPTRA advanced adenoma sensitivity hypothesis = 0.5

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The statistical power of the exact binomial test for the sensitivity of advanced adenoma detection is 0.94. The power is satisfactory to reject the NULL hypothesis.
The confidence intervals for the diagnostic performance metrics of the study, using the calculated sample sizes, are shown in the table below.
Given the low incidence rate in advanced adenomas and CRC groups, a much larger number of individuals is expected to participate in the study until the required numbers are recruited. As the enrolment of participant will be before the colonoscopy procedure, one's diagnosis cannot be verified at the moment of enrollment. This will lead to a much larger number of healthy and non-advanced adenomas cases, which could be beneficial in the refinement of DIOPTRA screening system and also in the development of DIOPTRA mobile application's risk assessment model in healthy and non-advanced adenomas population.

Table 1. Diagnostic performance confidence intervals

Endpoint	DIOPTRA hypothesis	95% CI
Sensitivity for CRC detection	0.8	[0.76,0.84]
Diagnostic specificity (healthy & non-advanced adenomas)	0.94	[0.91,0.96]
Sensitivity for advanced adenoma detection	0.5	[0.45,0.55]

Participants who agree to enrol in the follow up DIOPTRA study will be split into two groups. The Case group will receive behavioural suggestions via the app to lower their CRC risk score, while the Control group will not. Each of these two groups will be subdivided into two groups, which will contain patients with healthy and non-advanced adenomas, will be included. The multiplex protein biomarker readouts at the initial visit and follow-up will be compared using the t-test for paired samples. The NULL hypothesis of the statistical test is that for each DIOPTRA study group, there is no difference in the mean of protein biomarkers measured from blood samples at the initial and follow-up stages. With the following assumptions:

- Normal distribution of biomarker readouts,
- Cohen's d = 0.4 (moderate effect size),
- Significance level = 0.05,
- Power = 0.8,
- Two-sided comparison,
- Dropout rate = 50%;

The required sample size to reject the NULL hypothesis is N = 78 (N = 52 before taking the dropout rate into account) for each DIOPTRA study group in each of the Case and Control groups. In total, N = 312 participants are required.

Additionally, for the comparison of the CRC risk score (expressed as a percentage) between the initial and follow-up stages, using the following assumptions:

- Effect size h = 0.4 (moderate to large),
- Significance level = 0.05;

The calculated sample size (N = 52) results in a statistical power of 0.89 to detect moderate to large differences in the CRC risk score after the implementation of DIOPTRA behavioural modification suggestions.

9. STUDY PROCEDURES

9.1 Overview

For the main observational study, the study procedures are the following:
Enrollment of participants in the study once written informed consent is obtained and subject eligibility is confirmed.

1. Blood sample collection (serum & plasma).
2. Colonoscopy & clinical diagnosis according to each clinical site's standards.
3. Collection of DIOPTRA data.
4. End of study.

During enrollment, participants will be given the option to be potentially contacted to participate in the DIOPTRA follow-up study. The procedures of the follow-up study are as follows:

1. Enrollment in the follow-up study after subject eligibility is confirmed.
2. Download the DIOPTRA mobile app for the implementation of steps 3-5 below.
3. Answer the questionnaire.
4. Receive behavioural suggestions.
5. Periodic data update.
6. Follow-up blood collection and risk assessment.
7. End of follow-up study.

9.2 Enrollment

Individuals that visit the hospital sites with an invitation for a total colonoscopy, including routine screening or due to symptoms, will be invited to participate in the DIOPTRA study. Subjects are considered enrolled participants once written informed consent is obtained, and subject eligibility is confirmed according to the inclusion and exclusion criteria.

9.3 Blood sample collection

Approximately 20 mL of peripheral blood will be collected from each participant. The blood sample collection, management, and storage will be performed according to the SOP: "Sample Collection & Management" provided by Protavio (Annex No. 3).

9.4 Colonoscopy and diagnosis

Each participant will undergo a colonoscopy procedure following the blood sample collection. The colonoscopy should be completed within 30 days of enrollment. The procedure and preparation will be performed according to each site's clinical standards. During the procedure, the study personnel must fill out the "Colonoscopy and Sample Collection Case Form" provided in Annex No.4. This form contains information regarding the quality of the colonoscopy (preparation and procedure) and the collected blood samples. Participants with inadequate bowel preparation (i.e. Boston Bowel Preparation Scale overall score <6 or score in any colon segment <2) or incomplete colonoscopy due to technical factors (including but not limited to redundant or tortuous colon, marked diverticular disease, fixation of colonic loops, adhesions due to previous surgery) or due to intolerance, resulting in an incomplete procedure, will be excluded from the study. However, participants in whom colonoscopy cannot be

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completed due to obstructive colorectal cancer will be included in the study. During the colonoscopy, index lesions will be biopsied and sent for diagnostic analysis according to each site's clinical standards. The study personnel will be responsible to gather the diagnostic data from the biopsies, following the analysis, and match it to the participant's records and forms. The diagnostic results will be used to assign the participants into the DIOPTRA study groups.

9.5 Collection of DIOPTRA data

Demographic, lifestyle and behavioural data corresponding to potential risk factors for CRC will be collected during the study via the DIOPTRA behavioural questionnaire. Additionally, medical data, personal and family history, along with symptoms will be collected during the study via the "Medical Information / History Case Form" (Annex No 5). All collected DIOPTRA data during the study will be uploaded by the study personnel to the DIOPTRA prospective platform.

9.6 End of Study

Participants will be considered completed from the main observational study when they have provided all DIOPTRA data and completed their colonoscopy procedure or at the point of subject withdrawal.
The study will be initiated on M1 after the clinical study preparation and ethics approvals. An overview of the study schedule is provided in Table 2, followed by all clinical sites.

Table 2. Overview of the study schedule

Description	**Timing**
Study initiation	After ethical approval
Enrollment of participants in the study	Following informed consent and eligibility confirmation
Blood sample collection (serum & plasma)	Following enrollment.
Colonoscopy & clinical diagnosis according to each clinical site's standards	Colonoscopy, no later than 30 days of blood draw. Diagnosis timing: approximately 3 months after the procedure
Collection of DIOPTRA data	From study initiation until end of study.
Follow-up study	Initiation of Follow-up study approximately after 18 months of initial ethical approval.
Follow-up study: 1. Participant recruitment 2. Behavioural data collection 3. Biological sample collection 4. Possible participant self-reported symptoms module integration in the mobile app 5. Data analysis	1 year after follow-up enrollment

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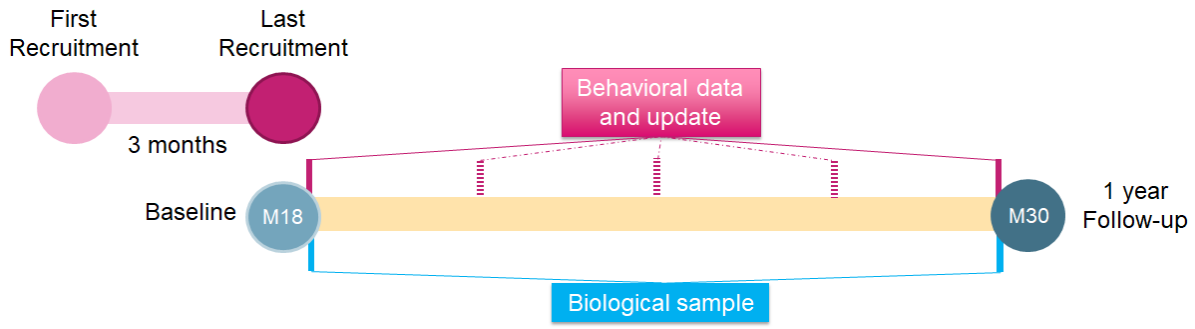
9.7. Follow-up study timing and procedures

Based on recruitment rate estimated on each clinical site during retrospective study, the maximum duration of follow-up study recruitment has been estimated to 3 months. The follow-up visit is at 1 year with consideration of mobile app development progression and project lifespan. (*Scheme 1*)

Scheme 1. Follow-up study timeline



9.7.1. Participant recruitment (M18)

Table 3. Participant recruitment from each clinical site

Participants/clinical site	Healthy	Non-advanced Adenomas
Case (Suggestions)	10	10
Control (No suggestions)	10	10

Prospective cohort enrollment will include participants that correspond to inclusion and exclusion criteria for the follow-up study. After consent signature, individuals will be split into two groups. The Case group will receive behavioural modification suggestions via the DIOPTRA mobile app, while the Control group will not. In each group, participants belong to two of the DIOPTRA study subgroups: healthy and non-advanced adenoma. In total, 320 participants from the same 8 clinical sites will be recruited. Randomisation will be performed to ensure against bias, using appropriate randomisation methods such as block randomisation and adaptive randomisation.

9.7.2. Behavioural data collection (M18, M30 and in-between)

All participants will answer the baseline behavioural data questionnaire in the DIOPTRA mobile app at the moment of recruitment or at the first moment that the participant will be available to answer the questionnaire after the recruitment. A modified follow-up questionnaire will be answered at 1-year follow-up at M12. Depending on the results of retrospective data analysis, adaptive questions will be updated and asked by a mobile app (more details will be generated in the DIOPTRA requirement work package). Depending on the DIOPTRA system construction progress, a participant self-reported symptoms module could be integrated into the mobile app to allow participants to report mild symptoms that don't need medical attention.

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9.7.3. Biological sample collection (M18 and M30)

Blood samples will be collected from each participant at baseline M18 and at the end of the study M30. Samples will be processed and sent to Protavio for biomarker analysis. Details of sample processing could be found in *Annex 2*, with an additional Sample Collection Form in Annex 4.

9.7.4. Possible participant self-reported symptoms (M18-M30)

Depending on the DIOPTRA system construction progress, a participant self-reported symptoms module could be integrated into the mobile app to allow participants to report mild symptoms.

9.8 Data flow and data processing (prospective, follow-up)

9.8.1 DIOPTRA software components

The project's implementation includes the development and/or integration of the following software components:

1. **Anonymization Tool (EHR data)**

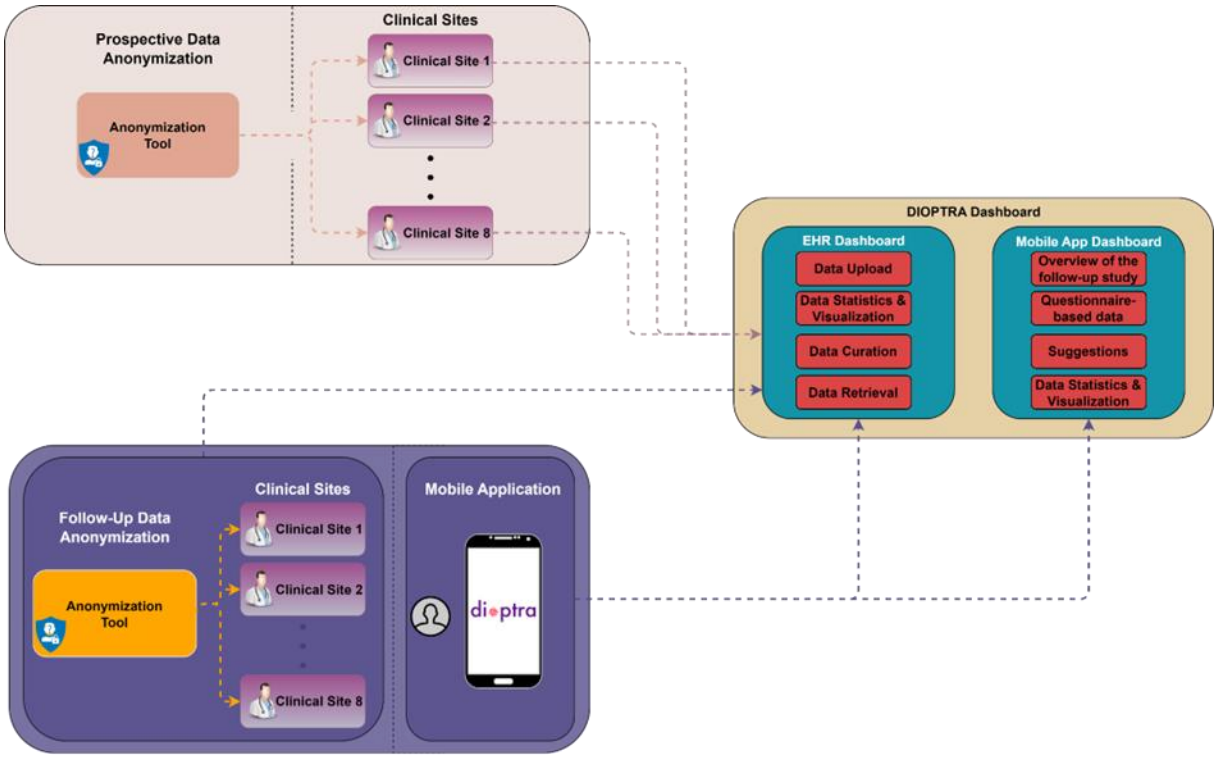
The Anonymization Tool provides an extra layer of privacy protection to already pseudonymized structured medical data. It will be used for EHR data prior to uploading to the Data Curation & Storage System

2. **Clinical Site Interface**

The clinical sites' DIOPTRA application provides functionality as follows:

EHR Dashboard (data from clinical variables lists) for uploading/downloading tabular retrospective and prospective data in various predefined formats and for providing information about the volume and quality of data uploaded from the clinical site. *Questionnaire Dashboard* providing an overview of follow-up study participants' data collected via the mobile app during the prospective studies and the option to download for further analysis depicted in Figure 1.

Figure 1. Data Curation & Storage Platform (including programming environment)



The anonymized retrospective and prospective data will be stored in the DIOPTRA centralised platform by utilising the ELK STACK. The ELK stack is the main data infrastructure responsible for data collection, curation, and storage, as well as advanced analysis-visualization (Kibana) and providing M2M data access endpoints for the various data-consuming applications. It offers advanced data storage and management services, such as: Heterogeneous Data Integration/Ingestion, Data Filtering/Harmonization, Annotation, Cataloguing and Management, (Meta)Data Storage and Distributed Data Lake, **S**ecurity, Data Protection, Secure Data Sharing, Customized Dashboards and diverse data visualizations, Federated Data Search, retrieval & Interoperable Access.

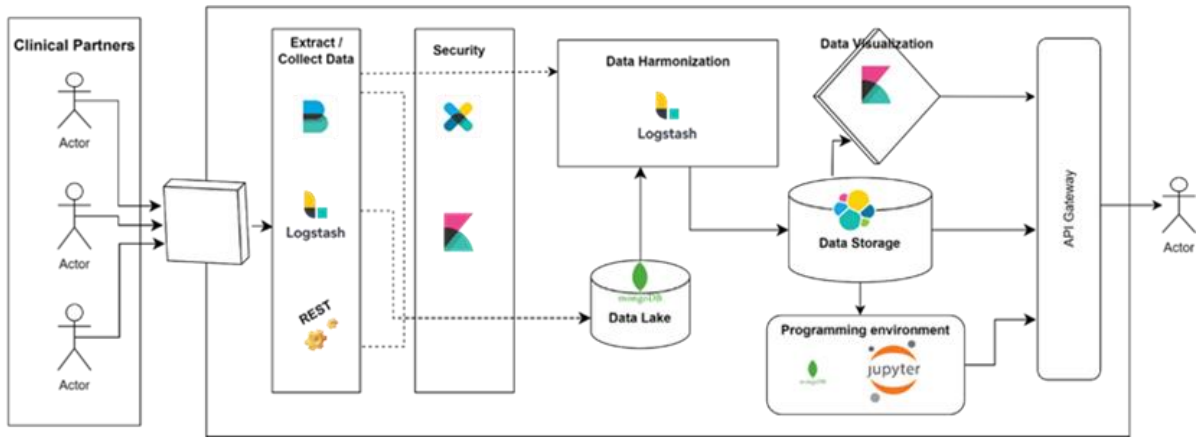
Elasticsearch comes up with Cross-cluster replication (CCR), a way to automatically synchronise indices from the primary cluster to a secondary remote cluster that can serve as backup. If the primary cluster fails, the secondary cluster can take over. Moreover, Elasticsearch provides snapshots as a backup of a running Elasticsearch cluster for data recovery stored in an off-cluster storage location called a snapshot repository.

Data curation techniques will be applied on the retrospective and prospective EHR data. These types of data will be stored in the centralised platform uploaded by each clinical partner. On ensuring overall interoperability and addressing heterogeneity and lack of shared semantics across sources, DIOPTRA will leverage widely adopted ontologies and standards, developing extensions to model relevant knowledge in the domains of the project for which no standards exist.

The Programming Environment provides access to a defined sub-dataset and tools that could be utilized for analysis and/or pattern recognition and model development.

A high-level scheme of the data management architecture is shown below *(Scheme 2).*

Sceme 2. Scheme of the data management architecture



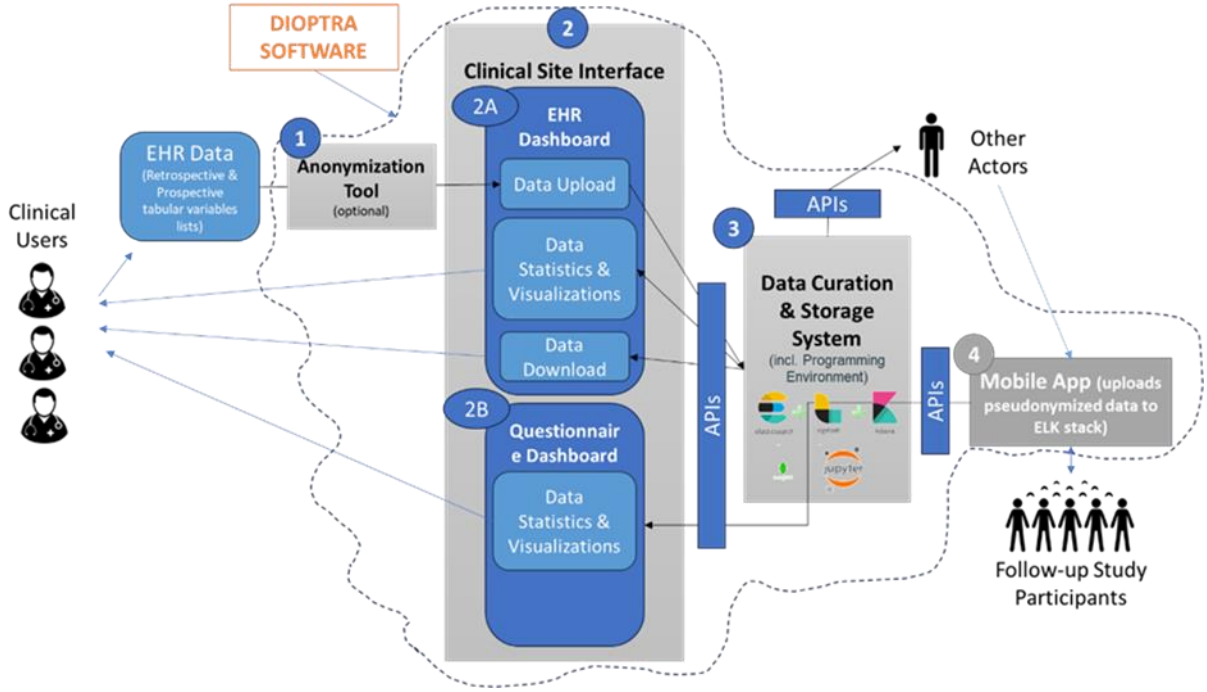
4. **Mobile App**

The mobile application, among other functionality (knowledge base, recommendations, etc.), collects and uploads questionnaire data from follow-up study participants. Each participant's credentials will consist of a unique participant ID and password. The participant must complete a questionnaire based on sociodemographic, lifestyle, diet, supplement consumption, and stress categories. Specific suggestions promoting a healthy lifestyle, encouraging the adoption of healthier eating habits, aligning with the direction of limiting alcohol consumption, promoting smoking cessation, and encouraging physical fitness will be triggered based on the user's responses. The pseudonymized questionnaire data along with the provided suggestions will be stored in the DIOPTRA central storage platform and will be available for review by the clinical users via the Questionnaire Dashboard

9.8.2 DIOPTRA software data flow

A conceptual scheme depicting data flow from and to the clinical sites' DIOPTRA application is shown in Scheme 3.

Scheme 3. Data flow from and to the clinical sites' DIOPTRA application



9.8.3 Hosting Infrastructure

For data storage, the infrastructure of GRNET will be used by the project. GRNET S.A. is a public sector technology company in Greece that has been operating since 1998 providing networking, cloud computing, HPC, data management services, and e-Infrastructures to academic and research institutions, educational bodies, and public sector agencies operating under the auspices of the Ministry of Digital Governance. In the context of the DIOPTRA Horizon project, GRNET will provide us with the following infrastructure and equipment, namely specific virtual machines (VMs):

- **VM1: Master Node , Logstash, Kibana, API Gateway**

  8 or 16 cores, 32 GB RAM, 500 GB disk (pref SSD)

- **VM2: Elastic Data nodes 1 & 2**

  8 cores, 16 GB RAM, 200 GB disk

- **VM3: Elastic Data nodes 3 & 4**

  8 cores, 16 GB RAM, 200 GB disk

- **VM4: Programming Environment & Interface**

  4 cores, 8 GB RAM, 100 GB disk

- **VM5: Staging Environment for all Services**

  8 cores, 16 GB RAM, 100 GB disk

- **OS: CentOS Linux**

9.9 Data analysis

Serum and plasma samples will be analysed to quantify the levels of the DIOPTRA protein biomarkers using multiplex proteomics. In terms of multiplex proteomics, the xMAP method will be used (Luminex Corp). Multiplex assays that utilise the xMAP technology rely on colour-coded microspheres (bead regions) to allow for the simultaneous detection of responses against multiple protein targets from the same sample. Each bead region is coated with an antibody that recognises and binds to a specific part of the protein. Mixtures of bead regions are used in a sandwich-type ELISA assay to provide absolute quantification of multiple proteins across the different conditions tested. These assays offer high multiplexability, sample throughput, quality of measurements, and specificity for the measurement of the identified biomarkers in serum and plasma. The multiplex biomarker readouts, along with the various behavioural, demographic, and clinical risk factors, will be used as input to validate and refine the DIOPTRA AI screening models.
In terms of AI screening models, several methodologies will be examined. Machine Learning algorithms like logistic regression, Support Vector Machines (SVM), Random Forests (RF) and Gradient Boosting (GB) can be trained on protein biomarkers data and risk factor information to classify participants as being at high risk of CRC. Feature selection techniques can help identify the most informative biomarkers and risk factors for efficient and accurate screening. On the other hand, Deep Learning models such as Convolutional Neural Networks (CNNs) and Recurrent Neural Networks (RNNs) will be employed. CNNs can analyse protein biomarker data, while RNNs can process sequential risk factor data to capture temporal patterns and dependencies. Hybrid models that combine deep learning with traditional ML algorithms can offer enhanced performance.
To evaluate the endpoints related to the clinical performance of the DIOPTRA screening system in terms of sensitivity and specificity, the confusion matrices between the reference method (colonoscopy & diagnosis) and the DIOPTRA system will be utilised. On this front, the confusion matrices (*Table 4*) for the different endpoints and models will be constructed. Diagnostic metrics will be calculated as Sensitivity = TP/(TP+FN) and Specificity = TN/(TN+FP). To compare the performance metrics to their respective lower bounds the exact binomial test will be utilised. Additionally, the confidence intervals will be calculated using the Clopper-Pearson exact method. The endpoints related to significant differences between categorical and numerical variables, i.e., the CRC risk factors and protein measurements following the behavioural suggestions, will be evaluated using: 1) chi-squared test, 2) ANOVA, 3) Generalised least squares for multi-level factors, 4) T-test and other statistical methods. Finally, the improvement in performance following the refinement of the DIOPTRA models using the prospective data will be evaluated using the exact binomial test and their respective confidence intervals.

Table 4. Confusion matrix

		DIOPTRA Predictions	
	Total Population (P+N)	Positive (PP)	Negative (PN)
Reference	Positive	True Positive (TP)	False Negative (FN)
	Negative	False Positive	True Negative (TN)

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		(FP)	

10.ETHICS & DATA MANAGEMENT

Participation in DIOPTRA Prospective Study will be done on the basis of an informed consent that will allow for the voluntary participation in the study and for the processing of personal information of the subjects.

Revision in Patient Information and Informed Consent Form. The organisation responsible for the study will inform the investigator whenever information becomes available that may be relevant to the subject's confirmed participation in the study. The investigator or his/her authorised designee should inform the subject in a timely manner.
The organisation responsible for the study will revise the written Informed Consent Form whenever new information becomes available that may be relevant to the subject's confirmed participation in the study. The revised information will be sent to the investigator for approval by the Bioethical Committee/ other regulatory authorities. After approval by the Bioethical Committee as applicable, a copy of this information must be provided to the participating subjects, and the informed consent process needs to be repeated.

Regulatory submission. No subjects will be enrolled in the study until all necessary approvals (e.g., by the Bioethical Committee of each DIOPTRA clinical partner and/or other competent authorities) have been obtained.

11.QUALITY CONTROL PROCEDURES

Data review and processing. Before study initiation, a representative of the study consortium will review the protocol with the local investigators and their team. During the study, the completeness of the collected records will be checked based on the accuracy of entries, the adherence to the protocol and to Good Clinical Practice, the progress of data collection, and to ensure that source documents for each patient are properly stored. Validation procedures within the system will continuously check for data discrepancies, and the Principal Investigator at each site must certify that the data entered are complete and accurate. Data management will be done according to the internal procedures of clinical investigators and the organisation responsible for the study. Related information will be made available on request. All collected data will be reviewed for completeness, correctness and consistency. In case of issues, queries will be sent to the clinical site to complete, correct or comment on the data.
Data collection. Each clinical site will handle data in accordance with the applicable EU and national laws and the respective internal policies. Each clinical site will, thus, ensure, among others, the accuracy, completeness, and timeliness of the data. Data which are derived from source documents must be consistent with the source documents, and discrepancies need to be justified in a documented rationale, signed and dated by the (principal) investigator and filed in the subject medical file. Any source documentation, as well as any imaging that is sent to the oganisation responsible for the study, should have all subject identifiers removed and replaced with the subject's study ID.

Monitoring procedures. Monitoring visits (physical or remote) may be conducted before, during, and at the closure of the study. The frequency and timing of monitoring visits shall be determined by the

organisation responsible for the study for each site based on the scope of collected data, study compliance, and findings from previous visits.
The monitoring strategy covers below mentioned actions (*Table 5*).

Table 5. Monitoring strategy

Actions	Parties involved	Methods to be used	Rationale for their use
Communication with stakeholders: 1) Clinical sites; 2) Bioethical Committee	Organisation responsible for the study and study team members, Bioethical Committee contact persons	Emails/ calls, visits (as appropriate to the specific issue(s) that trigger the communication with stakeholders).	Communication with stakeholders helps to ensure that the study conducts as planned (in full scope and related time frames) and that all changes are well managed.
Monitoring visits: interim visits. Not less than once per 3 months.	Organisation responsible for the study and study team members	Onsite/remote monitoring visits could be conducted.	Interim Monitoring Visits may be conducted throughout the study to verify that: ▪ The clinical site is conducting the study in accordance with applicable requirements, including the protocol, related procedures, and applicable regulatory requirements; ▪ Participant's safety, rights, and well-being are being protected; ▪ Recorded data are accurate, complete, and verifiable from source documentation.
Monitoring visits: For-cause visits (by request)	Organisation responsible for the study and study team members	These visits may involve either on-site monitoring or remote monitoring as appropriate to the specific issue(s) that trigger the visit.	For-cause visits will be conducted as applicable to address any unanticipated issues that arise in situations in which the site requires assistance. For-cause visits may be requested by the clinical site.
Monitoring visits: Close-out visit. Not later than 30 (thirty) working days after the clinical site approval	Organisation responsible for the study and study team members	The Close-Out Visit may be conducted either remotely or on-site.	A Close-Out Visit will be conducted to ensure that all study data and other study documentation are complete and accurate and that all study records have been reconciled.

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that the study is implemented.			

*Monitoring visits could be performed remotely.

Study deviations and clinical study protocol changes. The clinical site is not allowed to deviate from the Clinical Study Protocol except with prior approval and under emergency circumstances. All deviations shall be documented and explained, regardless of the reason for the deviation. The clinical site shall obtain documented approval from the organisation responsible for the clinical study, before implementation, for any change in or deviation from the Clinical Study Protocol. In case of study deviations that can affect the subject's rights, safety, and well-being or the scientific integrity of the clinical study, approval from the Bioethical Committee/ other regulatory authority must also be obtained before implementation.

Study suspension or early termination. The study may be terminated or suspended at the initiative of the investigators if any of the following reasons arise:

- Data Privacy Concerns: If there are concerns regarding patient privacy and data protection, it may lead to the suspension or termination of the protocol. This could occur if there are breaches in data security, unauthorised access to patient records, or non-compliance with data protection regulations.
- Legal or Regulatory Issues: If there are legal or regulatory violations related to the study, such as non-compliance with institutional policies, local regulations, or applicable laws, the protocol procedures may be suspended or terminated to address these issues.
- External Factors: External circumstances such as natural disasters, public health emergencies, or unforeseen events that disrupt the healthcare system or impede data access and retrieval from EHRs may necessitate the suspension or termination of the protocol procedures.

In this case, the clinical site must inform the Organisation responsible for the study of the reasons for the termination of the study, and the data collected prior to the termination of the study must be passed on to the organisation responsible for the study.
Any changes will be agreed in advance with the Bioethical Committee that authorised the study.

Study close out. Organisation responsible for the study will notify the site of the intention to close the study. Study close-out visits may be performed. During these visits, the monitors will ensure that the clinical site's regulatory files are up to date-and complete and that any outstanding issues from previous visits have been resolved. Organisation responsible for the study will notify and inform the site(s) that all requirements have been met with a study closure letter.
Organisation responsible for the study will notify the Bioethical Committee about the study closure by providing a Prospective Study report based on the Bioethical Committee/ other regulatory authority form.

12. BIBLIOGRAPHY

[1] Y. Xi and P. Xu, "Global colorectal cancer burden in 2020 and projections to 2040," Transl. Oncol., vol. 14, no. 10, p. 101174, Oct. 2021, doi: 10.1016/j.tranon.2023.101174
[2] G. Argilés et al., "Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†," Ann. Oncol., vol. 31, no. 10, pp. 1291–1305, Oct. 2020, doi: 10.1016/j.annonc.2020.06.022.
[3] M. Riihimäki, H. Thomsen, K. Sundquist, J. Sundquist, and K. Hemminki, "Clinical landscape of cancer metastases," Cancer Med., vol. 7, no. 11, pp. 5534–5542, Oct. 2018, doi: 10.1002/cam4.1697.
[4] D. E. O'Sullivan et al., "Combinations of modifiable lifestyle behaviours in relation to colorectal cancer risk in Alberta's Tomorrow Project," Sci. Rep., vol. 10, no. 1, Art. no. 1, Nov. 2020, doi: 10.1038/s41598-020- 76294-w.
[5] V. Vymetalkova, K. Cervena, L. Bartu, and P. Vodicka, "Circulating Cell-Free DNA and Colorectal Cancer: A Systematic Review," Int. J. Mol. Sci., vol. 19, no. 11, p. 3356, Oct. 2018, doi: 10.3390/ijms19113356
[6] O. Mazouji, A. Ouhajjou, R. Incitti, and H. Mansour, "Updates on Clinical Use of Liquid Biopsy in Colorectal Cancer Screening, Diagnosis, Follow-Up, and Treatment Guidance," Front. Cell Dev. Biol., vol. 9, 2021, Accessed: May. 17, 2023. [Online]. Available: https://www.frontiersin.org/article/10.3389/fcell.2021.660924
[7] A. Loktionov, "Biomarkers for detecting colorectal cancer non-invasively: DNA, RNA or proteins?," World J. Gastrointest. Oncol., vol. 12, no. 2, pp. 124–148, Feb. 2020, doi: 10.4251/wjgo.v12.i2.124.
[8] C. Pauli et al., "A Challenging Task: Identifying Patients with Cancer of Unknown Primary (CUP) According to ESMO Guidelines: The CUPISCO Trial Experience," The Oncologist, vol. 26, no. 5, pp. e769–e779, May 2021, doi: 10.1002/onco.13744.
[9] E. Rassy, T. Assi, and N. Pavlidis, "Exploring the biological hallmarks of cancer of unknown primary: where do we stand today?," Br. J. Cancer, vol. 122, no. 8, Art. no. 8, Apr. 2020, doi: 10.1038/s41416-019-0723-z.
[10] W. Zhao, M. Song, J. Zhang, M. Kuerban, and H. Wang, "Combined identification of long non-coding RNA CCAT1 and HOTAIR in serum as an effective screening for colorectal carcinoma," Int. J. Clin. Exp. Pathol., vol. 8, no. 11, pp. 14131–14140, Nov. 2015.
[11] R. Forghani, P. Savadjiev, A. Chatterjee, N. Muthukrishnan, C. Reinhold, and B. Forghani, "Radiomics and Artificial Intelligence for Biomarker and Prediction Model Development in Oncology," Comput. Struct. Biotechnol. J., vol. 17, pp. 995–1008, Jul. 2019, doi: 10.1016/j.csbj.2019.07.001.
[12] E. Tjoa and C. Guan, "A Survey on Explainable Artificial Intelligence (XAI): Toward Medical XAI," IEEE Trans. Neural Netw. Learn. Syst., vol. 32, no. 11, pp. 4793–4813, Nov. 2021, doi: 10.1109/TNNLS.2020.3027314.
[13] P. Sabol et al., "Explainable classifier for improving the accountability in decision-making for colorectal cancer diagnosis from histopathological images," J. Biomed. Inform., vol. 109, p. 103523, Sep. 2020, doi: 10.1016/j.jbi.2020.103523.
[14] F. Bénard, A. N. Barkun, M. Martel, and D. von Renteln, "Systematic review of colorectal cancer screening guidelines for average-risk adults: Summarizing the current global recommendations," World J. Gastroenterol., vol. 24, no. 1, pp. 124–138, Jan. 2018, doi: 10.3748/wjg.v24.i1.124.

13. ANNEXES

ANNEX No. 1.A INFORMED CONSENT FORM

The consent form below provides for the lawful participation of individuals in the prospective study and for the lawful processing of the respective personal data. To this end, the present consent form is largely based on the requirements set out under the General Data Protection Regulation (GDPR), as well as under other EU laws, such as the Medical Devices Regulation (MDR) and the ethical principles for medical research involving human subjects set forth under the Helsinki Declaration.

INFORMED CONSENT FORM

Title of study: ‘’Prospective study data collection for early dynamic screening for colorectal cancer via novel protein biomarkers reflecting biological initiation mechanisms’’
Protocol No.: 1
Organisation responsible for the study:
Address: Tel.: Email:
Representative of the organisation responsible for the prospective study: Local representative:
Clinical site:
Address: Tel.: Email:
Participant ID:

PURPOSE OF THIS DOCUMENT

By signing this document, you agree to participate in the prospective study. Read this document carefully, if you do not understand any word or statement, be sure to ask the researcher/other person authorised by the Organisation responsible for the study any questions you may have. You can talk to family, friends, or your doctor before making a decision.

GENERAL INTRODUCTION

If you have been invited to take part in this study, it is because you are about to undergo a colonoscopy as part of a colorectal cancer screening program.

1. Colorectal cancer

As the name suggests, a colorectal cancer develops in the colon, also known as the large intestine, or in its last part, the rectum. The starting point of a colorectal cancer is a protruding growth of tissue from the intestinal wall, called a polyp. Although in the majority of cases polyps are non-cancerous (benign), some are precancerous lesions and can give rise to a tumor. The evolution of precancerous polyps into tumors can take 10 to 15 years, as they go through several slow stages of transformation.

Colorectal cancer is the third most common cancer in men and the second in women, accounting for 10% of all cancers worldwide. It ranks second in terms of cancer-related mortality, just behind lung cancer.

2. Colorectal cancer screening

In [region or country*], a colorectal cancer screening program has been in operation since [year or exact time], for people aged between 50 and 74. This screening can be carried out at home, using a test based on the search for occult blood in a stool sample. Although this method is simple to perform, it only detects the presence or absence of blood in the stool — it can't determine what's causing the bleeding. If blood is detected through a fecal occult blood test, additional test may be needed to determine the source of the bleeding. Therefore, although more invasive, colonoscopy remains the most reliable method of screening for colorectal cancer in patients with positive fecal occult blood test, as it enables polyps and other lesions to be visualized and removed using an endoscope equipped with a camera.The risk of colorectal cancer following colonoscopy has been shown to be reduced by 70-90%. Early detection and removal of a pre-cancerous polyp prevents its progression to a cancer. In this way, colonoscopy saves many lives.

Nevertheless, although colorectal cancer is now considered an easily preventable disease thanks to screening, long waiting and preparation times for colonoscopy prevent the implementation of large-scale screening for systematic surveillance and follow-up.

Since the 1990s, there has been a gradual increase in the rate of colorectal cancer in adults under the age of 50. Although the reasons for this are still largely unknown, it has been suggested that environmental and behavioral changes influencing the microbiome along with familial predisposition are at the root of colorectal cancer in peop

le under 50. Colonoscopy reimbursement in your country if applicable.

The need to develop a large-scale, inexpensive and non-invasive method of early detection of colorectal cancer is therefore urgent.

*All the highlighted parts should be adjusted according to the individual circumstances of the clinical partners.

MAIN OBJECTIVE OF THE STUDY

You are invited to participate in part of a project called DIOPTRA. DIOPTRA is a 4-year project funded by the European Commission under the Horizon Europe programme with project number 101096649 which aims to develop a routine blood test accessible to all ages, in order to identify people who would not otherwise be screened according to current European or national guidelines.

The previous part of the DIOPTRA project would have identified in around 200 participants a protein group whose quantity varies during a precancerous stage of colon cancer. By quantifying this group of proteins, this blood test will be able to identify those citizens who absolutely should undergo further screening by colonoscopy. To validate this method, you are invited to give a blood sample during your colonoscopy visit at Hospital's gastroenterology department. Once validated, this blood test has many advantages: it is almost non-invasive, inexpensive and could be well accepted by most of the population. As a result, DIOPTRA is positioning itself in the increasingly personalized medicine of the future, capable of adapting to the particularities of each individual.

OTHER OBJECTIVES OF THE STUDY

In addition to an early detection method for colon cancer, numerous scientific studies have identified parameters called "risk factors" which could be associated with the development of colorectal cancer, and their importance is not negligible. Suggestions for daily habits can be generated from these risk factors, and may be very useful in the prevention of colorectal cancer.

Another aim of the study is to validate some of the risk factors identified in the previous part of the project. To do this, we'll need to collect some information about you: socio-demographic data (age, sex, height, weight, occupation, education, standard of living, etc.), data and results of medical examinations (e.g. family history, colonoscopy diagnosis, medication, blood test results, etc.), behavioral information (cigarettes, alcohol) and nutritional habits, physical activity, etc.

The findings and the final DIOPTRA solution will be the subject of a study of healthcare performance indicators in view of widening screening eligibility thanks to an effective, minimally invasive and financially affordable method.

STUDY PROCEDURES

The study will be conducted only on the day of your colonoscopy visit:

Before inviting you to take part in this study, the healthcare professionals at Hospital will have consulted your medical file to ensure that you meet all the criteria for participation in this study.

During this initial phase, your participation will be limited to:

A. A donation of two blood samples of 10 ml each

B. On the day of your colonoscopy, answer questions about your socio-demographic, behavioural information, habits and physical activities etc, which will be asked by a health professional [adjust to local method].

C. Accept that part of your data will also be used anonymously for the study as mentioned above.

You will then undergo your colonoscopy as planned.

This study will include at least 1,600 participants from countries participating in DIOPTRA, of whom at least 200 will be enrolled at [clinical site].

In order to participate in this study, the medical staff will make sure that you:

- are between 18 and 80 years of age at the time of recruitment
- have a prescription for a total colonoscopy
- do not have a severe systemic abnormality
- are able to understand the study instructions and sign an informed consent form
- have not undergone major abdominal surgery (e.g. colectomy) or radiation treatment prior to colonoscopy
- are not pregnant
- have not been diagnosed with chronic inflammatory bowel disease (e.g. Crohn's disease or ulcerative colitis), polyposis syndrome or colorectal cancer

RISKS AND INCONVENIENCES

You will not experience any inconvenience by participating in this study. This study will not have any impact on the treatment you have been offered or the diagnostic and monitoring procedures of the usual medical practice in your clinical case. Blood sampling may (rarely) cause pain, bleeding, bruising, or localised infection at the blood sampling site. In addition, some people may feel dizzy or faint during the procedure.

You must be aware that any study or blood sampling may involve certain risks, same as with the standard treatment you receive. However, the researcher and all the members of the study team will do everything possible and necessary to ensure that these risks are kept to a minimum.

Given that the collection of blood samples will be done with a minimally invasive method and the study does not pose any additional risks, the study is not covered by the civil liability insurance of the clinical site and the organisation responsible for the study.

BENEFITS

You will not derive any direct benefit (medical, financial, or otherwise) from your participation in this study. The donation of samples of human body material is free of charge, and there will be no financial compensation if the research results in new medical treatments.

However, in general, early detection of CRC can significantly enhance survival rates and treatment outcomes. Medical specialists could offer personalised prevention plans to reduce CRC risk.

TERMINATION OF PARTICIPATION IN THIS STUDY

You participate in the study voluntarily, so you have the right to opt-out, and once you start, you can withdraw from it at any time without giving reasons and without any resulting detriment. If you are unable to decide on further access to the study due to your deteriorating health, this decision can be taken by your spouse or, if not, one of your parents, adult children, or another legal representative.

Your decision not to participate or to terminate your participation in the study will not affect the routine health care provided.

Your participation in the study will be automatically terminated if you no longer belong to the subject inclusion criteria or if you do not come to the scheduled visits or do not follow the investigators' instructions.

WILL YOU INCUR ANY COSTS IN PARTICIPATING IN THIS STUDY?

You will not incur any cost for participating in the study as your visits will be part of the routine healthcare service you have been offered by the responsible organisation. You will not be charged for any visits, consultations, examinations, or treatments specific to this study. Normal medical expenses (not related to the study), even if generated on the same day, will be billed to you (and/or your insurance company) as usual.

MANAGEMENT OF PERSONAL DATA

As part of your participation in the study, your personal data will also be processed. We ask your consent to collect, process, and store your personal data and your body material for the purpose of this study as described in detail in this section. This section also explains how you can exercise your data protection rights in accordance with the EU General Data Protection Regulation 2016/679 ('GDPR'), [*the national data protection law applicable to the organisation*] and other related laws and regulations.

Who is your Data Controller?

[*Organisation responsible for the study*] is the Data Controller of your personal data that will be processed for the study. The contact details can be found on the first page of this consent form.

The Data Controller has its own data protection officer ('DPO') who oversees compliance with the applicable data protection and privacy laws and functions as a point of contact for all privacy-related queries. If you have any queries regarding the protection of your personal data, you may directly contact DPO at [*email address or phone number of DPO*].

What personal data and body material do we process?

In this study, we collect, process, and store the following categories and types of your personal data; your name, gender, weight, height, date of birth, medical file number and information available in your medical file kept at the clinical site, including but not limited to colonoscopy results, genetic data, clinical diagnosis, prescribed medications, allergies and self-reported symptoms, your answers to the

questionnaire related to demographic, dietary, financial, lifestyle and behavioural data and your habits corresponding to potential risk factors for colorectal cancer and medical information including personal and family history along with symptoms that we collect during the study via the "Medical Information History Form". As for body material, we only collect, use and store a 20mL blood sample in two analysis tubes.

Who can access your personal data and body material?

*Your personal data will be accessed and processed only by the study team of [*organisation responsible for the study*].* After the anonymization of your personal data, the study team may share these anonymised data with other organisations participating in the DIOPTRA project for the purpose of the study. Your body material will be used by the study team in charge of managing your bold material in order to measure the concentration of a panel of protein biomarkers in your blood. The study team who uses your samples may also receive the personal data linked to the samples they need for their research. The study team has a duty of confidentiality with regard to the body materials and the personal data collected.

To verify the quality of the study or for regulatory compliance purposes, your data may be examined by third parties (for example, competent national and European authorities, including ethics committees, health authorities, and external auditors). In any event, this may only be done under the supervision of the study team in charge within [*organisation responsible for the study*] or the physician managing the human body material at the biobank, and/or by any other authorised persons bound by the obligation of professional secrecy and confidentiality.

Will your personal data be transferred to countries outside the European Union/European Economic Area?

No, your data will not be transferred to anyone outside the European Union/European Economic Area.

How do we protect your privacy?

To protect your personal data, your identity information will be replaced by a code, and together with your body material, your personal data will be stored in a pseudonymous (coded) form. We keep the key to the code in a safe place on the clinical site. When we process your data and body material, we always use only that code. In addition, all necessary measures are taken to protect the confidentiality and security of your encoded data, in accordance with the applicable legislation at the European and national levels.

For how long do we store your personal data and body material?

We aim to anonymise your personal data upon collection. However, your personal data that has not been anonymised will be stored in the local servers of the Data Controller, and your body materials will be stored in [*please indicate where body materials will be stored*]. Your personal data and body materials will be kept for the duration of the DIOPTRA project (maximum four (4) years starting from the time of the data collection), or the time may be required by other applicable laws to this study whichever comes later. If they are no longer needed for the purpose of the study or for compliance with other applicable laws, we will erase your personal data and destroy body samples before this date.

Whether we do automated decision-making or profiling

The study deploys DIOPTRA advanced Artificial Intelligence based cancer screening system, which is developed by the DIOPTRA project to analyse your personal data and information obtained from your body materials, to carry out colorectal cancer risk assessment, screening, and progression based on patients' profiles in general. However, any automated decision, including profiling generated by DIOPTRA's advanced Artificial Intelligence based cancer screening system, will not affect you nor have any impact on the healthcare service you receive from *[organisation responsible for the study]*.

What happens if there are coincidental findings?

It is possible that during the study, we discover something that is not directly relevant to the study but is important to your health or to the health of your family members. In that case, the study team, including your physician, will be informed. Under no circumstances can any coincidental findings be considered as results that can be used to make a medical diagnosis. The study team will, therefore, decide whether it is useful to communicate this information to you and whether to offer you, for instance, advice, request, complementary examinations, or treatments totally independent of the present study. This information may be of benefit to you in terms of your health, but in some cases, it may also cause you anxiety or other psychological difficulties.

What data protection rights do you have?

You have the right to have access to all study information concerning you and to request, if necessary, rectification, data portability and to restrict processing of your personal data. You have a right to withdraw your consent for the use of your personal data at any time. Please, inform the study team, if you wish to do so. Note that if you withdraw your consent, and the study team has already anonymised your data for the study, they are still allowed to use this anonymised information as it does not contain any personal data. The study team will destroy your body material and erase your personal data after you withdraw your consent. If, however, assessments with your body material have been carried out prior to the withdrawal of your consent, the study team may continue to use the results from such assessments, provided that such assessments do not contain your personal data. Do you want to know more about your rights when processing personal data? Visit [website].

CONTACT TO AUTHORITIES

For your rights as a study participant, you can apply to the Ethics Committee, which has given us a permit to conduct this study. To exercise your data protection rights, please directly contact [*the Data Protection Officer of the organisation responsible for the study*] [contact details, e.g., email, phone, etc., and website].

If you have any complaints about the processing of your personal data, we recommend that you first discuss them with the study team or directly contact the [*Data Protection Officer of the organisation responsible for the study*]. You can, also, submit a complaint to the national Data Protection Authority [Insert the full name and contact details of the national data protection authority in the country where the study is carried out].

CONSENT TO PARTICIPATE IN THE PROSPECTIVE STUDY

By signing this information and consent form, I hereby certify that:

- I have read this Informed Consent Form and have understood the information about the nature, objectives, benefits, implications, risks, and inconveniences of the study, the use of my body materials, its purpose, how it is carried out, and what is expected of me. I was given the opportunity to ask questions and received satisfactory answers. I have had enough time to calmly decide if I wanted to take part.

- I have filled in this informed consent form of my own free will and without being subject to any inappropriate pressure or influence by the researcher or by a member of the study team.

- I understand that participation in the study is voluntary. I also know that at any time I can withdraw from the study at any time without giving any reason [1].

- I understand that in order to withdraw my consent to participate in the study, I must inform the researcher / other person authorised by the clinical site identified below, in writing.
- I renounce any rights whatsoever over my body materials collected within the study and the results of the study to be carried out with these samples to the extent permitted by applicable law.
- I have been informed about the processing of my personal data for the purpose of this study, including types of personal data to be processed, the data controller, potential recipients of my personal data, data security measures, and my data protection rights, including my right to withdraw my consent to the processing at any time.
- I confirm that I have received a copy of the Informed Person Consent Form, signed by the researcher / other clinical site authorised person

To give your free and explicit consent, please tick yes or no in the table below:

I give my consent to participate in this study	Yes ☐	No☐
I give my explicit consent to the processing of my personal data, including special categories of personal data, for the purpose of this study, as stated herein.	Yes ☐	No☐
I agree to receive, via my referring physician, the information generated by the study or research on my body material samples of significant importance or potential interest for my state of health.	Yes ☐	No☐

Person (or other person with the right to give consent)										
								MMMM-mm-dd		_:_
name		surname		Representation basis		signature		Signing date		Signing time

I confirm that I have provided information about the study to the person mentioned above.

I confirm that the person (or other person entitled to give consent) has been given sufficient time to decide to participate in the study, taking into account the nature of the clinical study, as well as considering other circumstances that may influence the decision.

I encouraged the person (or other person with the right to consent) to ask questions and answered them.

Researcher / other person authorised by the clinical site.										
								MMMM-mm-dd		_:_
name		surname		duties in the study		signature		Signing date		Signing time

[1] If the consent to participate in the study is given by the person himself

ANNEX No. 1.B INFORMED CONSENT FORM

The consent form below provides for the lawful participation of individuals in the prospective follow-up study and for the lawful processing of the respective personal data. To this end, the present consent form is largely based on the requirements set out under the General Data Protection Regulation (GDPR), as well as under other EU laws, such as the Medical Devices Regulation (MDR) and the ethical principles for medical research involving human subjects set forth under the Helsinki Declaration.

INFORMED CONSENT FORM

Title of study: ''Prospective follow-up study data collection for early dynamic screening for colorectal cancer via novel protein biomarkers reflecting biological initiation mechanisms''
Protocol No.: 1
Organisation responsible for the study:
Address: Tel.: Email:
Representative of the organisation responsible for the prospective study: Local representative:
Clinical site:
Address: Tel.: Email:
Participant ID:

PURPOSE OF THIS DOCUMENT

By signing this document, you agree to participate in the prospective follow-up study. Read this document carefully, if you do not understand any word or statement, be sure to ask the researcher/other person authorised by the Organisation responsible for the study any questions you may have. You can talk to family, friends, or your doctor before making a decision.

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GENERAL INTRODUCTION

If you have been invited to take part in this study, it is because you are about to undergo a colonoscopy as part of a colorectal cancer screening program.

1. Colorectal cancer

As the name suggests, colorectal cancer develops in the colon, also known as the large intestine, or in its last part, the rectum. The starting point of colorectal cancer is a protruding growth of tissue from the intestinal wall, called a polyp. Although in the majority of cases polyps are non-cancerous (benign), some are precancerous lesions and can give rise to a tumor. The evolution of precancerous polyps into tumors can take 10 to 15 years, as they go through several slow stages of transformation.

Colorectal cancer is the third most common cancer in men and the second in women, accounting for 10% of all cancers worldwide. It ranks second in terms of cancer-related mortality, just behind lung cancer.

2. Colorectal cancer screening

In [region or country], a colorectal cancer screening program has been in operation since [year or exact time], for people aged between 50 and 74. This screening can be carried out at home, using a test based on the search for occult blood in a stool sample. Although this method is simple to perform, it only detects the already symptomatic stage of the disease. Therefore, although more invasive, colonoscopy remains the most reliable method of screening for colorectal cancer, as it enables polyps and other lesions to be visualized and removed using an endoscope with a camera. The risk of colorectal cancer following colonoscopy has been shown to be reduced by 70-90%. Early detection and removal of a pre-cancerous polyp prevents its progression to a tumor. In this way, colonoscopy saves many lives.

Nevertheless, although colorectal cancer is now considered an easily preventable disease thanks to screening, long waiting and preparation times for colonoscopy prevent the implementation of large-scale screening for systematic surveillance and follow-up.

Since the 1990s, there has been a gradual increase in the rate of colorectal cancer in adults under the age of 50. Although the reasons for this are still unknown, it has been suggested that environmental and behavioral changes influencing the microbiome are at the root of colorectal cancer in people under 50. Colonoscopy reimbursement in your country if applicable.

The need to develop a large-scale, inexpensive, and non-invasive method of early detection of colorectal cancer is therefore urgent.

MAIN OBJECTIVE OF THE STUDY

You are invited to participate in part of a project called DIOPTRA. DIOPTRA is a 4-year project funded by the European Commission under the Horizon Europe programme with project number 101096649, which aims to develop a routine blood test accessible to all ages, in order to identify people who would not otherwise be screened according to current European or national guidelines.

The previous part of the DIOPTRA project would have identified in around 200 participants a protein group whose quantity varies during a precancerous stage of colon cancer. By quantifying this group of

proteins, this blood test will be able to identify those citizens who absolutely should undergo further screening by colonoscopy. To validate this method, you are invited to give a blood sample during your colonoscopy visit at Hospital's gastroenterology department. Once validated, this blood test has many advantages: it is almost non-invasive, inexpensive and could be well accepted by most of the population. As a result, DIOPTRA is positioning itself in the increasingly personalized medicine of the future, capable of adapting to the particularities of each individual.

OTHER OBJECTIVES OF THE STUDY

In addition to an early detection method for colon cancer, numerous scientific studies have identified parameters called "risk factors" which could be associated with the development of colorectal cancer, and their importance is not negligible. Suggestions for daily habits can be generated from these risk factors, and may be very useful in the prevention of colorectal cancer.

Another aim of the study is to validate some of the risk factors identified in the previous part of the project. To do this, we'll need to collect some information about you: socio-demographic data (age, sex, height, weight, occupation, education, standard of living, etc.), data and results of medical examinations (e.g. family history, colonoscopy diagnosis, medication, blood test results, etc.), behavioral information (cigarettes, alcohol) and nutritional habits, physical activity, etc. The DIOPTRA application would be created to help collect certain information, offer up-to-date personalized suggestions and raise awareness of early detection of colorectal cancer.

The findings and the final DIOPTRA solution will be the subject of a study of healthcare performance indicators in view of widening screening eligibility thanks to an effective, minimally invasive and financially affordable method.

STUDY PROCEDURES

The study will be conducted in two phases:

Phase I (day of the colonoscopy):

Before inviting you to take part in this study, the healthcare professionals at Hospital will have consulted your medical file to ensure that you meet all the criteria for participation in this study.

During this initial phase, your participation will be limited to:

A. A donation of two blood samples of 10 ml each

B. On the day of your colonoscopy, answer questions about your eating and exercise habits, which will be asked by a health professional[adjust to local method].

C. Accept that part of your data will also be used anonymously for the study as mentioned above.

You will then undergo your colonoscopy as planned.

This first part of the study will include at least 1,600 participants from countries participating in DIOPTRA, of whom at least 200 will be enrolled at [clinical site].

In order to participate in the first part of the study, the medical staff will make sure that you:

- are between 18 and 80 years of age at the time of recruitment
- have a prescription for a total colonoscopy
- do not have a severe systemic abnormality
- are able to understand the study instructions and sign an informed consent form
- have not undergone major abdominal surgery (e.g. colectomy) or radiation treatment prior to colonoscopy
- are not pregnant
- have not been diagnosed with chronic inflammatory bowel disease (e.g. Crohn's disease or ulcerative colitis), polyposis syndrome or colorectal cancer

Phase II:

You will participate in this phase:

A. Only if your colonoscopy shows no signs of colon cancer, which we hope you will.

B. If you have sufficient technological knowledge to manage smartphones and smartphone applications and agree to use the DIOPTRA application

C. Have good Internet connection coverage in your place of residence.

This second phase of the study will involve 320 participants, including 40 from Hospital.

After your hospital visit in Phase I, you will be contacted on a later date to confirm your participation in the second phase of the study. You will be provided with a mobile application called DIOPTRA that will contain information on:

· colorectal cancer occurrence and symptoms,
· local colorectal cancer screening guidelines,
· factors that may affect the risk of an individual developing colorectal cancer,
· lifestyle suggestions that are known to decrease the risk of developing colorectal cancer,
· DIOPTRA project

Its easy-to-use interface will help you in:

· recognising potential symptoms for you or your family members,
· learning current recommended screening guidelines,
· maintaining a healthy lifestyle that potentially reduce the risk of colorectal cancer.

Moreover, you will be able to use this application to get healthy lifestyle suggestions that reduce the risk of colorectal cancer that are tailored to your own needs and health status. To accomplish this, you will only need to fill a questionnaire within the application, which in turn will provide you with a personalised suggestion. On several occasions during the 12 months, you may be contacted by the application for some updated questions. You will be also offered the opportunity to be re-assessed after 12 months and check if you accept the suggestion and if a healthier lifestyle change has affected the risk of colorectal cancer. Therefore, you will be able to receive expert information on how your health has progressed, which will also be useful for you in the future.

Apart from using the application, you will be asked to return to the Hospital for a second visit, 12 months after your colonoscopy. During this second visit, you will be asked to donate two blood samples of 10 ml each that will be later used to validate developed blood test for early onset colorectal cancer detection.

Your participation in Phase II of the study ends once you complete the blood donation and answer all questionnaires in the application 12 months after your first visit.

RISKS AND INCONVENIENCES

You will not experience any inconvenience by participating in this study. This study will not have any impact on the treatment you have been offered or the diagnostic and monitoring procedures of the usual medical practice in your clinical case. Blood sampling may (rarely) cause pain, bleeding, bruising, or localised infection at the blood sampling site. In addition, some people may feel dizzy or faint during the procedure.

You must be aware that any study or blood sampling may involve certain risks, same as with the standard treatment you receive. However, the researcher and all the members of the study team will do everything possible and necessary to ensure that these risks are kept to a minimum.

Given that the collection of blood samples will be done with a minimally invasive method and the study does not pose any additional risks, the study is not covered by the civil liability insurance of the clinical site and the organisation responsible for the study.

BENEFITS

You will not derive any direct benefit (medical, financial, or otherwise) from your participation in this study. The donation of samples of human body material is free of charge, and there will be no financial compensation if the research results in new medical treatments.

However, in general, early detection of CRC can significantly enhance survival rates and treatment outcomes. Medical specialists could offer personalised prevention plans to reduce CRC risk.

TERMINATION OF PARTICIPATION IN THIS STUDY

You participate in the study voluntarily, so you have the right to opt-out, and once you start, you can withdraw from it at any time without giving reasons and without any resulting detriment. If you are

unable to decide on further access to the study due to your deteriorating health, this decision will be taken by your spouse or, if not, one of your parents, adult children, or another legal representative.

Your decision not to participate or to terminate your participation in the study will not affect the routine health care provided.

Your participation in the study will be automatically terminated if you no longer belong to the subject inclusion criteria or if you do not come to the scheduled visits or do not follow the investigators' instructions.

WILL YOU INCUR ANY COSTS IN PARTICIPATING IN THIS STUDY?

You will not incur any cost for participating in the study as your visits will be part of the routine healthcare service you have been offered by the responsible organisation. You will not be charged for any visits, consultations, examinations, or treatments specific to this study. Normal medical expenses (not related to the study), even if generated on the same day, will be billed to you (and/or your insurance company) as usual.

MANAGEMENT OF PERSONAL DATA

As part of your participation in the study, your personal data will also be processed. We ask your consent to collect, process, and store your personal data and your body material for the purpose of this study as described in detail in this section. This section also explains how you can exercise your data protection rights in accordance with the EU General Data Protection Regulation 2016/679 ('GDPR'), [*the national data protection law applicable to the organisation responsible for the study*] and other related laws and regulations.

Who is your Data Controller?

[*organisation responsible for the study*] is the Data Controller of your personal data that will be processed for the study. The contact details can be found on the first page of this consent form.

The Data Controller has its own data protection officer ('DPO') who oversees compliance with the applicable data protection and privacy laws and functions as a point of contact for all privacy-related queries. If you have any queries regarding the protection of your personal data, you may directly contact DPO at [*email address or phone number of DPO*].

What personal data and body material do we process?

In this project, we collect, process, and store the following categories and types of your personal data; your name, gender, weight, height, date of birth, your medical file number and information available in your medical file kept at the clinical site, including but not limited to colonoscopy results, genetic data, clinical diagnosis, prescribed medications, allergies, self-reported symptoms and other data related to your health, your answers to the questionnaires provided at the clinical site as well as in the mobile app related to demographic, dietary, financial, lifestyle and behavioural data corresponding to potential risk factors for colorectal cancer and medical information including personal and family history along with symptoms that we collect during the study via the "Medical Information History Form". As for body material, we only collect, use and store a 20mL blood sample in two analysis tubes in the first phase. If you also participate in the second phase of the study, we will also collect and analyse an additional 20mL blood sample.

Who can access your personal data and body material?

Your personal data will be accessed and processed only by the study team of [organisation responsible for the study]. After the anonymization of your personal data, the study team may share these anonymised data with other organisations participating in the DIOPTRA project for the purpose of the project. Your body material will be used by the study team in charge of managing your bold material in order to measure the concentration of a panel of protein biomarkers in your blood. The study team who uses your samples may also receive the personal data linked to the samples they need for their research. The study team has a duty of confidentiality with regard to the body materials and the personal data collected.

To verify the quality of the study or for regulatory compliance purposes, your data may be examined by third parties (for example, competent national and European authorities, including ethics committees, health authorities, and external auditors). In any event, this may only be done under the supervision of the study team in charge within [*organisation responsible for the study]* or the physician managing the human body material at the biobank, and/or by any other authorised persons bound by the obligation of professional secrecy and confidentiality.

Will your personal data be transferred to countries outside the European Union/European Economic Area?

No, your data will not be transferred to anyone outside the European Union/European Economic Area.

How do we protect your privacy?

To protect your personal data, your identity information will be replaced by a code. Together with your body material, your personal data will be stored in a pseudonymous (coded) form. We keep the key to the code in a safe place on the clinical site. When we process your data and body material, we always use only that code. In addition, all necessary measures are taken to protect the confidentiality and security of your encoded data, in accordance with the applicable legislation at the European and national levels.

For how long do we store your personal data and body material?

We aim to anonymise your personal data upon collection. However, your personal data that has not been anonymised will be stored in the local servers of the Data Controller, and your body materials will be stored in [*please indicate where body materials will be stored*]. Your personal data and body materials will be kept for the duration of the DIOPTRA project (maximum four (4) years starting from the time of the data collection), or the time may be required by other applicable laws to this study whichever comes later. If they are no longer needed for the purpose of the study or for compliance with other applicable laws, we will erase your personal data and destroy body samples before this date.

Whether we do automated decision-making or profiling

The study deploys DIOPTRA advanced Artificial Intelligence based cancer screening system, which is developed by the DIOPTRA project to analyze your personal data and information obtained from your body materials, to carry out colorectal cancer risk assessment, screening, and progression based on patients' profiles in general. However, any automated decision, including profiling generated by DIOPTRA's advanced Artificial Intelligence based cancer screening system, will affect you nor have any impact on the healthcare service you receive from *[organisation responsible for the study]*.

What happens if there are coincidental findings?

It is possible that during the study, we discover something that is not directly relevant to the study but is important to your health or to the health of your family members. In that case, the study team, including your physician, will be informed. Under no circumstances can any coincidental findings be considered as results that can be used to make a medical diagnosis. The physician will, therefore, decide whether it is useful to communicate this information to you and whether to offer you, for instance, advice, request, complementary examinations, or treatments totally independent of the present study.

This information may be of benefit to you in terms of your health, but in some cases, it may also cause you anxiety or other psychological difficulties.

What data protection rights do you have?

You have the right to have access to all study information concerning you and to request, if necessary, rectification, data portability and to restrict processing of your personal data. You have a right to withdraw your consent for the use of your personal data at any time. Please, inform the study team, if you wish to do so. Note that if you withdraw your consent, and the study team has already anonymised your data for the study, they are still allowed to use this anonymised information as it does not contain any personal data. The study team will destroy your body material and erase your personal data after you withdraw your consent. If, however, assessments with your body material have been carried out prior to the withdrawal of your consent, the study team may continue to use the results from such assessments, provided that such assessments do not contain your personal data. Do you want to know more about your rights when processing personal data? Visit [website].

CONTACT TO AUTHORITIES

For your rights as a study participant, you can apply to the Ethics Committee, which has given you a permit to conduct this study. To exercise your data protection rights, please directly contact [*Data Protection Officer of the organisation responsible for the study*] [contact details, e.g., email, phone, etc., and website].

If you have any complaints about the processing of your personal data, we recommend that you first discuss them with the study team or directly contact the [*Data Protection Officer of the organisation responsible for the study*]. You can, also, submit a complaint to the national Data Protection Authority [Insert the full name and contact details of the national data protection authority in the country where the study is carried out].

CONSENT TO PARTICIPATE IN THE PROSPECTIVE STUDY

By signing this information and consent form, I hereby certify that:

- I have read this Informed Consent Form and have understood the information about the nature, objectives, benefits, implications, risks, and inconveniences of the study, the use of my body materials, its purpose, how it is carried out, and what is expected of me. I was given the opportunity to ask questions and received satisfactory answers. I have had enough time to calmly decide if I wanted to take part.

- I have filled in this informed consent form of my own free will and without being subject to any inappropriate pressure or influence by the researcher or by a member of the study team.

- I understand that participation in the study is voluntary. I also know that at any time I can withdraw from the study at any time without giving any reason[1].

- I understand that in order to withdraw my consent to participate in the study, I must inform the researcher / other person authorised by the clinical site identified below, in writing.

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- I renounce any rights whatsoever over my body materials collected within the study and the results of the study to be carried out with these samples to the extent permitted by applicable law.
- I have been informed about the processing of my personal data for the purpose of this study, including types of personal data to be processed, the data controller, potential recipients of my personal data, data security measures, and my data protection rights, including my right to withdraw my consent to the processing at any time.
- I confirm that I have received a copy of the Informed Person Consent Form, signed by the researcher / other clinical site authorised person.

To give your free and explicit consent, please tick yes or no in the table below:

I give my consent to participate in this study	Yes ☐	No☐
I give my explicit consent to the processing of my personal data, including special categories of personal data, for the purpose of this study, as stated herein.	Yes ☐	No☐
I agree to be contacted by the study team to participate in the second phase of the study after my first colonoscopy visit.	Yes ☐	No☐
I agree to receive, via my referring physician, the information generated by the study or research on my body material samples of significant importance or potential interest for my state of health.	Yes ☐	No☐

Person (or other person with the right to give consent)										
								MMMM-mm-dd		_:_
name		surname		Representation basis		signature		Signing date		Signing time

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I confirm that I have provided information about the study to the person mentioned above.

I confirm that the person (or other person entitled to give consent) has been given sufficient time to decide to participate in the study, taking into account the nature of the clinical study, as well as considering other circumstances that may influence the decision.

I encouraged the person (or other person with the right to consent) to ask questions and answered them.

Researcher / other person authorised by the clinical site.										
								MMMM-mm-dd		_:_
name		surname		duties in the study		signature		Signing date		Signing time

[1] If the consent to participate in the study is given by the person himself

ANNEX No. 2. BEHAVIOURAL QUESTIONNAIRE

Sociodemographic (mark with an x)	
What is your age? (Write a number in years)	
Sex	☐ Male ☐ Female ☐ Not wish to answer
What is your country of birth?	
What is the country that you took the most part of your life?	
Weight (in kg)	
Height (in cm)	
Where have you usually resided for most of your life?	☐ Urban[1] ☐ Rural[2]
What is the highest level of education you have obtained?	☐ High school graduate ☐ Middle school graduate ☐ College or University degree ☐ Post-graduate degree
What is your monthly net income?	☐400€ to 1000€ ☐Less than 1000€ ☐1000€ to 2.000€ ☐2000€ to 3000€ ☐ More than 3000€
What is your occupation?	☐ Professional/Technical/Scientific ☐ Healthcare/Medical ☐ Managerial/Supervisory ☐ Education/Academic ☐ Business/Entrepreneur ☐ Arts/Creative ☐ Public Service/Government ☐ Retail/Hospitality ☐ Sales/Customer services ☐ Administrative/Clerical ☐ Skilled Trades/Manual Labor ☐ Student/not currently employed ☐ Retired ☐ Homemaker ☐ Unemployed ☐ Other

[1]This refers to areas characterized by higher population density and extensive human-built environments such as cities or towns. Urban areas typically have various amenities, services, and infrastructure.Typically, the population living in towns of 2,000 people or more, or in national and provincial capitals, is classified as urban.

[2]This pertains to areas with lower population density and less built-up infrastructure. Rural areas often have more open spaces, agricultural lands, and natural landscapes compared to urban areas.

[1]Professional occupations in natural and applied sciences, health, education, law and social, community and government services

[2]Management, business, finance, and administration occupations

[3]Sales and service occupations, including occupations related to the hospitality and tourism industries

[4]Administrative and office support occupations

[5]Occupations in manufacturing (e.g., metal, glass, chemicals, wood, pulp, textile), agriculture and natural resources (e.g., farming, fishing, forestry), construction, trades, transport, and equipment operation

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Lifestyle (mark with an x)	
Are you smoking?	☐ Yes ☐ No
On average, how many cigarette packs[1] do you smoke per day?	☐ Less than 1 ☐ 1 to 2 ☐ More than 2
If you are currently smoking, how long have you been smoking? (Write a number in years)	
If you have stopped smoking, how long have you been smoking in the past? (Write a number in years)	
Are you regularly exposed to secondhand smoke[2]?	☐ Yes ☐ No
How many days per week do you consume alcohol[3]?	☐ 0 to 1 ☐ 1 to 3 ☐ 4 to 6 ☐ 7
How many standard alcoholic drinks[3] do you consume per week?	☐ None ☐ 1 to 2 ☐ 3 to 4 ☐ 5 to 7 ☐ More than 7
How would you describe your current level of physical activity?	☐ Sedentary (less than 30 min of moderate physical activity per week) ☐ Little active (30 to 90 min of moderate physical activity per week) ☐ Moderately active (90 to 150 min of moderate physical activity per week) ☐ Active (150 min of moderate physical activity or more per week)
On average, how many minutes per day do you engage in physical activity?	☐ Less than 15 minutes ☐ 15 to 30 minutes ☐ 30 to 60 minutes ☐ More than 60 minutes
On average, how many days per week do you engage in physical activity? (Write in days)	☐ Less than 2 days ☐ 2 to 4 days ☐ More than 4 days
How long do your typical physical activity sessions last? (Write in minutes)	
What is your average daily sedentary time[4]?	☐ Less than 5 hours ☐ 5 to 10 hours ☐ More than 10 hours
How many hours per day do you engage in prolonged sitting[4]?	☐ Less than 2 hours ☐ More than 2 hours

[1]Suppose that 1 pack includes 20 cigarettes
[2]Daily exposure to the tobacco smoke of others at home, work, or public places
[3]Frequency of drinking alcoholic beverages (e.g., 354 ml can/bottle of beer, 118ml glass of wine, 44ml shot of hard liquor
[4]. Sedentary time refers to periods when an individual engages in very low physical activity or movement (sitting at work, at school, at home, in a car/bus/train, and during leisure time (e.g., watching TV, playing video games, using the computer, reading, socializing)). It includes any time spent in activities with minimal energy expenditure.

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[5]Prolonged sitting specifically refers to extended periods of sitting without breaks or movement. It highlights the negative effects of sitting for long stretches without interruptions or physical activity.

Diet (mark with an x)	
How often do you consume fruits[1] and vegetables[1] in your meals?	☐ Daily ☐ Several times a week[2] ☐ About once a week ☐ Rarely[3] ☐ Never
How often do you eat processed meat (sausages, bacon, etc.)?	☐ Daily ☐ Several times a week ☐ About once a week ☐ Rarely ☐ Never
How often do you include low-fat dairy products in your diet?	☐ Daily ☐ Several times a week ☐ About once a week ☐ Rarely ☐ Never
How often do you consume white meat, such as poultry or fish?	☐ Daily ☐ Several times a week ☐ About once a week ☐ Rarely ☐ Never
How often do you eat whole grains[4]?	☐ Daily ☐ Several times a week ☐ About once a week ☐ Rarely ☐ Never
How often do you consume sugary drinks[5]?	☐ Daily ☐ Several times a week ☐ About once a week ☐ Rarely ☐ Never
How often do you consume sugary desserts[6]?	☐ Daily ☐ Several times a week ☐ About once a week ☐ Rarely ☐ Never
How often do you eat fast food[7]?	☐ Daily ☐ Several times a week ☐ About once a week ☐ Rarely ☐ Never

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[1] Examples of fruit: fresh fruit, chopped, cooked or canned fruit, dried fruit, fruit juice. Examples of vegetables: raw leafy vegetables, cooked, canned, frozen, or chopped vegetables, vegetable juice.
[2] Several times a week: This means that you consume the item more than once in a week, but not every day. It indicates a frequency that is more than occasional but less than daily.
[3] Rarely: This means you consume the item infrequently, on special occasions, or very seldom. It indicates that the item is not a regular part of your diet.
[4] Whole grain is defined as: cooked brown rice or other cooked grain, cooked 100% whole-grain pasta, cooked hot cereal, such as oatmeal, uncooked whole grain pasta, brown rice or other grain, 100% whole grain bread, 100% whole grain muffin, 100% whole grain ready-to-eat cereal
[5]Sugary drinks such as soft drinks (excluding diet soda), vitamin drinks, energy drinks, and specialty coffee with syrup (e.g., mocha)
[6]Desserts containing sugar, such as candy, chocolate bars, cake, cookies, and ice cream
[7]Includes foods from fast food restaurants (e.g., burger, fries, taco), pizza, and instant meals (e.g., instant ramen noodles)

Supplements (mark with an x)	
How often do you consume omega 3 (including multivitamin)?	☐ Never[1] ☐ Rarely[2] ☐ Often[3]
Do you take a daily multivitamin supplement?	☐ Yes ☐ No
If not, which supplements do you consume?	☐ None of them ☐ Vitamin B6 ☐ Vitamin C ☐ Vitamin D ☐ Magnesium ☐ Calcium
How often do you consume these supplements (answer of previous question)?	☐ Never ☐ Rarely ☐ Often
How often do you consume probiotics[4]?	☐ Never ☐ Rarely ☐ Often
How often do you consume fiber supplements?	☐ Never ☐ Rarely ☐ Often
How often do you consume folic acid (females only)?	☐ Never ☐ Rarely ☐ Often
Please write the names of any other supplements you take.	

[1]Never: Indicates that the supplement is not consumed at all.
[2]Rarely: Indicates that the supplement is consumed infrequently or occasionally, but not on a regular basis.
[3]Often: Indicates that the supplement is consumed frequently or regularly as part of the dietary routine.
[4] Probiotics are a combination of live beneficial bacteria and/or yeasts.

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Stress-PSS4 (mark with an x)	
In the last 2 months, how often have you felt that you were unable to control the important things in your life?	☐ Never ☐ Almost Never ☐Sometimes ☐ Fairly Often ☐ Very Often
In the last 2 months, how often have you felt confident about your ability to handle your personal problems?	☐ Never ☐ Almost Never ☐Sometimes ☐ Fairly Often ☐ Very Often
In the last 2 months, how often have you felt nervous and stressed?	☐ Never ☐ Almost Never ☐Sometimes ☐ Fairly Often ☐ Very Often

ANNEX No. 3. SAMPLE COLLECTION & MANAGEMENT

1. SCOPE OF THE PROCEDURE

This SOP describes the processes for the collection of biological samples from study participants and the management of the collected samples from the Clinical Partners and Test facility. Specifically, it provides instructions for:

a. the collection, labeling, storage, and shipment of biological samples from the Clinical Partners to the Test Facility.
b. The receipt, inspection, handling, storage, recording, archiving, and disposal of biological samples by the Test Facility.

2. DEFINITIONS

- **Samples**: serum & plasma samples collected from subjects enrolled in the study.
- **Test Facility:** the partner that performs the biological analysis of samples. Protavio Ltd (former Protatonce Ltd) is the Test Facility for the DIOPTRA project.
- **Collection tubes**: serum/plasma tubes used for initial blood collection prior to centrifugation.
- **Transfer tubes:** 15mL centrifuge tubes used to transfer the upper liquid phase (serum or plasma samples) after centrifugation.
- **Storage tubes**: 2mL microcentrifuge screw-cap tubes that are used to aliquot and store samples.

3. EQUIPMENT / MATERIALS

3.1 EQUIPMENT

#	Description	Specifications	Recommended Cat No
1	Centrifuge	1300-1800 g (RCF) 18-25 °C For 16mm x 100mm tubes	N/A
2	Ultra-low Freezer	-80 °C or below	N/A
3	Pipette	Single channel 200-1000uL range	Rainin Pipet-Lite LTS Pipette L-1000XLS+, #17014382
4	Laminar flow hood (optional)	Class II A2 cabinet	N/A
5	Racks for collection/transfer/storage tubes	See tube specifications	VWR, # 211-0204 (for 2mL tubes)
6	Personal Protective equipment	Lab coat, gloves, etc	N/A

3.2 MATERIALS

#	Description	Specifications	Recommended Cat No
1	9-10mL Serum collection tubes	Plastic, 16x100mm, with clot activator (silica), **red cap color**, transparent	BD Vacutainer, #367896 Greiner Vacuette, #455092
2	9-10mL K2EDTA Plasma collection tubes	Plastic, 16x100mm, with K2EDTA additive, **purple/lavender cap color**, transparent	BD Vacutainer, #367525 Greiner Vacuette, #455045
3	15mL centrifuge tubes (transfer tubes)	nonpyrogenic and DNase-/RNase-free	Corning, #430791
4	2mL screw-cap microcentrifuge tubes (storage tubes)	nonpyrogenic and DNase-/RNase-free, non-sterile, freezable to -80 °C, can be centrifuged to 12,000×g, with silicone O-ring screw-caps	VWR, #525-0651 (tubes), #525-0653 (screw-caps)
5	Cryoboxes with dividers, 9x9 positions	133x133x50mm size, resistant to temperatures down to –140 °C, standard waterproof coating	VWR, #479-1417 (boxes), #479-1465 (dividers)

4. IDENTIFICATION

4.1 DIOPTRA PARTICIPANT ID

DIOPTA Participant IDs are aimed to differentiate participants and to ensure the anonymization of personal data during the submission of samples to the Test Facility performing the biological analysis.

The following identification system **should be followed** for a codification of participants:
Each ID will include the clinical site code followed by a 4-digit number that is unique to each participant and follows a continuous numbering starting from 0001. Continuous numbering is based on the date of the participant's inclusion in the study (date of signature of informed consent).

Clinical Site Codes:

Clinical Site	Clinical Site Code
BLOCKS	CP09
CHUL	CP12
RM-RRH	CP15
UKCM	CP16
BURGOS	CP21
NKUA	CP01
GOC	CP23
AG.SAVVAS	CP24

An example of a DIOPTRA Participant ID is: CP24-0034

4.2 SAMPLE ID

Sample IDs are aimed to differentiate samples and aliquots coming from the same participant:
The following nomenclature is proposed for Sample IDs:
For serum samples:
DIOPTRA ID-S-N, where S stands for serum and N is the number of aliquot*.
Example: CP24-0034-S-1

For plasma samples:
DIOPTRA ID-P-N, where P stands for plasma and N is the number of aliquot*.
Example: CP24-0034-P-1

*Aliquot numbering is optional in case the clinical partner needs to catalog every tube collected in their database management system.

4.3 TUBE LABELING

Each storage tube should be clearly labeled either using digital labels or handwritten with permanent ink.
Each tube stored and shipped to the Test Facility should contain the following information:
- Sample ID
- Collection Date

5. PROCEDURE FOR SERUM & PLASMA COLLECTION

Serum and Plasma samples will be collected from each subject following a blood draw.
NOTES:
- *The blood draw should be performed before the colonoscopy.*
- *First draw blood for serum, then draw blood for plasma.*
- *Follow best practices to avoid hemolysis of samples.*

1. First, fill in the name and signature of the responsible of the clinical partner in the Sample Collection Form
2. Fill in the details of the participant and the sample collection date.

5.1 SERUM

1. Draw blood into one serum collection tube (red capped). Record the time of blood draw in the Collection Form.
2. Gently invert tube 5-6 times to mix blood with clot activator.
3. Place upright on a test rack and allow to sit for 30-60 min at 18-25ºC until clotting has occurred.
4. Centrifuge at 1,500-2,000 x g for 10 minutes at 18-25ºC. Record the time of initiation of centrifugation in the Collection Form.
5. Using a pipette, collect the upper liquid phase (serum) into a 15mL transfer tube taking care not to remove any of the clotted material.
6. Prepare 4 aliquots of 500µL using the 2mL storage tubes. Use correctly labelled tubes (see section 4.0 IDENTIFICATION). Record the number of aliquots prepared for DIOPTRA in the Collection Form.

Note: Left-over serum samples can be kept for internal biobanking by the clinical partner. Leftover samples can be handled according to clinical partner internal procedures.

7. Store serum aliquots in cryoboxes in an ultra-low freezer at -80ºC or below.

5.2 PLASMA

1. Draw blood into one K2EDTA collection tube (purple capped). Record the time of blood draw in the Collection Form.
2. Gently invert the EDTA tube 8-10 times immediately after the blood sample has been taken to avoid microclotting.
3. Centrifuge immediately (or within 1 hr from blood draw) at 1,500-2,000 x g for 10 minutes at 18-25ºC.
4. Using a pipette, collect the upper liquid phase (plasma) into a 15mL transfer tube taking care not to remove any of the middle and lower layers containing blood cells.
5. Prepare 4 aliquots of 500µL using the 2mL storage tubes. Use correctly labelled tubes (see section 4.0 IDENTIFICATION). Record the number of aliquots prepared for DIOPTRA in the Collection Form.

Note: Left-over plasma samples can be kept for internal biobanking by the clinical partner. Left-over samples can be handled according to clinical partner internal procedures.

6. Store plasma aliquots in cryoboxes in an ultra-low freezer at -80ºC or below.

5.3 COLONOSCOPY PROCEDURE

During the colonoscopy, the details related to the procedure and the quality of the procedure should be entered into the Sample Collection Form as presented in the form.

6. SHIPMENT TO TEST FACILITY

6.1 NUMBER OF ALIQUOTS TO BE SHIPPED

Each clinical partner should submit to the Test Facility:

- 2 x 500µL serum aliquots per patient &
- 2 x 500µL plasma aliquots per patient

Note: The remaining 2 aliquots of each type per patient should be kept by the Clinical Partner as reserved back-up material.

6.2 PACKAGING

The aliquots should be placed in cryoboxes and a map of the position of the aliquots corresponding to each patient ID in the box should be provided to the Test Facility by the Clinical Partner in excel format. Each cryobox should also be numerically labelled to avoid confusion during receipt.

6.3 PERIODICITY OF SHIPMENTS

Shipments should be arranged every 6 months by the clinical partner.

6.4 SHIPPING INSTRUCTIONS

1. Samples should be shipped in **dry ice** with **next-day courier delivery services**.

Note: Do NOT use FedEx as this courier does not deliver dry ice to Greece.

2. Use the following sample description:

UN3373 Biological Substance Cat B packed in Dry Ice, Class 9, UN1845 ….. kgs. Use for research purposes only.

3. Ensure cryoboxes are fully covered with dry ice during transport.
4. Arrange shipment between **Monday-Wednesday** to ensure that the package is delivered by the end of the week.

Shipping Address
Protavio Ltd
NCSR Demokritos
Lefkippos Technology Park, Bldg 27
Patriarchou Grigoriou E' & 27 Neapoleos Str.
15341, Ag. Paraskevi
Attiki
Greece

Contact Person:
Nikos Tsolakos
Email: nikos.tsolakos@protavio.com
Tel: +30 210 9610307

7. TEST FACILITY RECEIPT & TEMPORARY STORAGE

Biological Samples should be received by trained personnel, and the Biological Sample Receipt Form should be completed, dated and signed.

Trained personnel should provide a general description of received samples including:

a. Number of Biological Samples (boxes, tubes etc)
b. Quantity (volume) per tube (approximate)
c. Identification numbers
d. Shipping temperature

All relevant documentation that accompanies the shipment should be retained and handed in to the Study Director.

In case of deviations from the packing list (i.e. different number of boxes or vials received) or shipping temperature (i.e. shipment not in dry ice or samples appear defrosted due to lack of dry ice), these deviations should be recorded in the relevant Receipt form.

Upon receipt, biological samples should be immediately stored at -80^{o}C.

8. TEST FACILITY INSPECTION & STORAGE

Biological Samples should be inspected by the Study Director to ensure that the correct Biological Samples have been received, under the correct conditions and that they are uniquely identified and recorded.

The Study Director needs to perform the following activities:

- Verify the identity of Biological Samples. Verification should include ensuring that information on the container in which the test item is shipped and the labeling on the test item matches information recorded by the organization responsible for study on accompanying documentation and study protocols.

- Check that the types of samples, number of tubes and quantities are correct based on accompanying documentation and study protocols.
- Check the physical characteristics of the Biological Samples match the expected characteristics. Specifically, serum and plasma samples should be in liquid form (frozen) and appear yellow. Any deviations, e.g. hemolytic samples, samples received defrosted etc., should be recorded.
- Check transportation documents (including Biological Sample receipt form) for correct shipment conditions.

Observations including deviations should be recorded in the Biological Sample Receipt Form_Study Director.

Upon inspection, biological samples should be continuously stored at -80ºC. If samples are expected to be thawed multiple times, they should be further aliquoted in smaller volumes.

9. TEST FACILITY RECORDS

Biological Samples should be recorded in a Biological Sample Inventory. It is the responsibility of the Study Director and the Test facility to maintain, amend and archive this Inventory. The Inventory should contain at minimum the following information:

- Sample ID
- DIOPTRA Participant ID
- Sample Type
- Collection date
- Sender (Clinical Partner)
- Lab Reception Date
- Date of reception by Protavio
- No of tubes received
- Volume per tube
- Total volume received
- Visual Inspection results (normal, hemolytic, icteric or lipemic)
- Storage Temperature
- Storage Location
- Box ID
- Comments/Deviations from receipt process
- Comments/Deviations during sample collection (upon inspection of Sample Collection Form by the clinical partner)

10. CLINICAL CHARACTERIZATION

The clinical information of each participant will be collected in the Medical Information/History Case Form and will be used to categorize samples into the four groups of the clinical protocol. Collection of Medical Information per participant is performed by the Clinical Partners and is uploaded to the DIOPTRA prospective platform.

11. ARCHIVING

All documentation related to the receipt, storage and inventory of biological samples will be archived by the Test Facility.

The Test Facility will receive from each Clinical Partner two aliquots of serum and two aliquots of plasma samples per participant. Each Clinical Partner will retain two aliquots of serum and two aliquots of plasma samples per participant as back-up material for the DIOPTRA study along with any left-over sample volumes for internal biobanking.

DIOPTRA samples will be retained for the duration of the DIOPTRA project..

12. DISPOSAL

Biological samples will be disposed of at the end of the archival period and destroyed by incineration following internal disposal protocols and dedicated services for disposal of biological hazardous material.

ANNEX No. 4. COLONOSCOPY AND SAMPLE COLLECTION CASE FORM

SAMPLE COLLECTION FORM

DIOPTRA

(BASED ON SOP279-01.Sample Collection & Management)

CLINICAL PARTNER INFORMATION	
CLINICAL PARTNER	
COLLECTION REPSONSIBLE FULL NAME	
COLLECTION REPSONSIBLE SIGNATURE	

PARTICIPANT GENERAL INFORMATION	
PARTICIPANT FULL NAME	
DIOPTRA ID	
SAMPLE COLLECTION DATE	

COLONOSCOPY PROCEDURE INFORMATION	
Sedation Drug	
Sedation Drug Dose	
Use of CO_2	☐ YES ☐ NO
BBPS right colon (0-3)	☐ 0 ☐ 1 ☐ 2 ☐ 3
BBPS transversum (0-3)	☐ 0 ☐ 1 ☐ 2 ☐ 3
BBPS left colon (0-3)	☐ 0 ☐ 1 ☐ 2 ☐ 3
BBPS overall (0-9)	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9
Cecal intubation	☐ YES ☐ NO
Time to cecal intubation (min)	
Withdrawal time (min)	
Time required for interventions (min)	
Total Procedure Time (min)	
Gloucester Comfort Score (1-5)	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

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SERUM COLLECTION		
Collection Tube Lot #		
Collection Tube expiration date (YYYY/MM)		
Time of blood draw (HH:MM)		
Time centrifugation was initiated (HH:MM) No earlier than 30 min from blood draw		
Total Waiting Time from blood draw till centrifugation initiated (min) No longer than 60 min		
Number of storage tubes prepared (0.5 mL per tube)		
Left-over volume (mL) (if applicable)		
Storage Temperature (^{O}C)		
Visual Inspection	☐ Normal	☐ Hemolyzed (red colour)
	☐ Icteric (bright yellow)	☐ Lipemic (turbid)
Comments – Deviations from SOP Different Tube type, longer waiting times, low sample volumes, other centrifugation conditions etc		

PLASMA COLLECTION	
Collection Tube Lot #	
Collection Tube expiration date (YYYY/MM)	
Time of blood draw (HH:MM)	
Time centrifugation was initiated (HH:MM)	
Total Waiting Time from blood draw till centrifugation initiated (min) No longer than 60 min	
Number of storage tubes prepared (0.5 mL per tube)	
Left-over volume (mL)	

Funded by the European Union

Storage Temperature (^{O}C)		
Visual Inspection	☐ Normal	☐ Hemolyzed (red colour)
	☐ Icteric (bright yellow)	☐ Lipemic (turbid)
Comments – Deviations from SOP Different Tube type, longer waiting times, low sample volumes, other centrifugation conditions etc		

ANNEX No. 5. MEDICAL INFORMATION/HISTORY CASE FORM

MEDICAL INFORMATION/HISTORY CASE FORM

Family history (mark with an x)	
Do you have any family history of CRC?	☐ 1st degree ☐ 2nd degree ☐ No
What was the age of the youngest relative at diagnosis?	☐ Less than 50 years ☐ More than 50 years ☐ Unknown
What is the sex of the relative?	☐ Male ☐ Female

Personal History (mark with an x)	
Have you had a previous colonoscopy?	☐ Yes ☐ No
If yes, how many years ago?	
What were the findings of your last colonoscopy?	☐ Healthy ☐ Non advanced adenoma ☐ Advanced adenoma
Do you have Diabetes Type II?	☐ Yes ☐ No
If applicable, what is the potential measurement of your HbA1c[1] (glycated haemoglobin)?	☐ Less than 5.7% ☐ 5.7% to 6.4% ☐ More than 6.5%
Do you have hypertension (high blood pressure)?	☐ Yes ☐ No
Do you have dyslipidemia (high levels of fat – cholesterol and triglycerides in the blood)	☐ Yes ☐ No
Do you have cardiovascular disease?	☐ Yes ☐ No
Do you have chronic kidney disease?	☐ Yes ☐ No
Do you have any allergies or asthma?	☐ Allergy☐ Asthma ☐ No
If yes, what are you allergic to?	
At what age were you diagnosed with allergies?	☐ Less than 10 years ☐ 10 to 19 years ☐ More than 20 years

[1] Bennett, C. M., Guo, M., & Dharmage, S. C. (2007). HbA1c as a screening tool for detection of type 2 diabetes: a systematic review. *Diabetic medicine*, *24*(4), 333-343.

Medication (mark with an x)

Have you taken any medication within the last month?	☐ Antihypertensives ☐ Anticoagulants ☐ Aspirin ☐ NSAID ☐ Statin ☐ Insulin ☐ GLP-1 ☐ SGLT-2 ☐ Metformin ☐ Antiplatelet ☐ Corticosteroids ☐ DPP-4 ☐ Other ☐ No
If applicable, please specify any other medication you are currently taking.	

Symptoms (mark with an x)	
Are you experiencing abdominal pain?	☐ Yes ☐ No
Have you noticed a change in your defecation habits?	☐ Diarrhea ☐ Constipation ☐ No
Have you observed blood in the stool?	☐ Yes ☐ No
Are you experiencing bleeding from the rectum?	☐ Yes ☐ No
Are you experiencing symptoms such as gas, abdominal cramps and/or bloating?	☐ Yes ☐ No
Do you feel that your rectum is not completely empty after having a bowel movement?	☐ Yes ☐ No
Have you been diagnosed with anemia?	☐ Yes ☐ No

Female only	
How many pregnancies have you had?	☐ 0 ☐ 1 to 2 ☐ More than 3
At what age did you have your first pregnancy?	☐ Before 30 years old ☐ After 30 years old
Have you ever used oral contraceptive?	☐ Never ☐ Rarely ☐ Sometimes ☐ Fairly often ☐ Very often
What is your current menopausal status?	☐ Pre-menopausal ☐ Post-menopausal

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