CLINICAL STUDY PROTOCOL

CfDNA in Hereditary And High-risk Malignancies (CHARM) 2: Evaluating the Performance of a cfDNA Blood Test for Early Cancer Detection

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Protocol Version # 10
Date: 22/April/2025

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List of Abbreviations/ Terminology *(in alphabetical order)*

ACMG - American College of Medical Genetics
CCMG - Canadian College of Medical Genetics
cfDNA – Cell-free Deoxyribonucleic Acids
cfMeDIP-seq – Cell-free Methylated DNA Immunoprecipitation and high-throughput sequencing
DNA – Deoxyribonucleic Acid
DOB - Date of Birth
DTA - Data Transfer Agreement
HBOC - Hereditary Breast and Ovarian Cancer
HCS - Hereditary Cancer Syndrome
HDGC – Hereditary Diffuse Gastric Cancer
EPR – Electronic Patient Records
FCS - Familial Cancer Syndrome
FFPE - Formalin-Fixed Paraffin-Embedded
LB-Seq – Liquid Biopsy Sequencing
LIBERATE – LIquid Biopsy Evaluation and Repository Development AT PrincEss Margaret
LFS – Li-Fraumeni Syndrome
Lynch/LS – Lynch Syndrome
MOHCCN - Marathon of Hope Cancer Centre Network
MTA - Material Transfer Agreement
MRN - Medical Record Number
NF1 – Neurofibromatosis Type 1
NPV - Negative Predictive Value
OICR - Ontario Institute for Cancer Research
PHI - Personal Health Information
PPV - Positive Predictive Value
SOP – Standard Operating Procedure
sWGS – Shallow Whole Genome Sequencing
TIDHI - Toronto Immune Digestive Health Institute
TP – Targeted Panel

Protocol Summary

CfDNA in Hereditary And High-risk Malignancies (CHARM) 2: Evaluating the Performance of a cfDNA Blood Test for Early Cancer Detection

Randomized Control Study

Sample Size
Total = 1000 patients with Hereditary Cancer Syndromes (HCS)
N = 500 (experimental arm)
N = 500 (control arm)

If other sites join our study, the appropriate MTAs and DTAs will be established, and samples collected at other institutions (who have institutional REB approval for the study) will be sent to UHN/OICR for analysis. Other sites may include: BC Cancer Agency, Mount Sinai Hospital, SickKids Hospital, Women's College Hospital, Sunnybrook Hospital, McGill Research Institute, Jewish General Hospital, IWK Health Centre, Eastern Health, Alberta University.

We will recruit up to 1000 patients at UHN.

Study Population
Our study focuses on patients with a known Hereditary Cancer Syndrome (HCS).

Accrual Period
January 2024 – December 2024

Study Design
Through the CHARM Consortium (www.charmconsortium.ca), we have shown that cell-free DNA (cfDNA) profiling can enable more frequent cancer surveillance from readily accessible blood collections and can detect cancer at the same time or earlier than clinical screening approaches. We are now conducting a prospective, multi-center, randomized control trial of cfDNA testing of 1,000 HCS carriers to 1) compare cancer detection rates with and without cfDNA testing, 2) assess cancer stage shift and clinical impact reducing mortality and morbidity cancers, and 3) assess impact of access to cfDNA results on patients' quality of life and psychological distress.

Study Duration
January 2024 – December 2031

Study Agent/ Intervention/ Procedure
cfDNA sequencing (on a blood sample), tri-annually for 4 years.

Primary Objective

Decrease the time to cancer detection and disease management in HCS carriers receiving cfDNA sequencing results compared to controls.

Secondary Objective
Use cfDNA sequencing to detect cancers that have low detection rates with standard of care surveillance methods.

Assess patient-reported outcomes and perceptions about cfDNA testing, including empowerment, distress, uncertainty, and anxiety.

Endpoints of the Study
The endpoints of this study are: 1) to assess the performance of the cfDNA assay for cancer detection in patients with HCS, including assay sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV), 2) to assess the time to cancer diagnosis using cfDNA sequencing compared to controls, 3) to assess the detection rate of cancer with no standard of care screening available, using cfDNA sequencing, 4) assess patient reported outcomes using validated scales administered before, during, and after the study.

1.0 General Information

1.1 **Study Title**

CfDNA in Hereditary And High-risk Malignancies (CHARM) 2: Evaluating the Performance of a cfDNA Blood Test for Early Cancer Detection, January, 2024

1.2 **Investigators**

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Dr. Patrick Veit-Haibach, Radiologist, Toronto General Hospital
Dr. Tracy Stockley, Clinical Laboratory Geneticist, Toronto General Hospital

2.0 Background Information

2.1 Background and Rationale

Early cancer detection is the surest path to survival, even cure. As cancer progresses, genetic changes continue to amass, thereby making late-stage cancers more aggressive, harder to eradicate, and more lethal. People who are affected by a hereditary cancer syndrome (HCS) have this lived experience, as they are born with a genetic change that puts them at high risk for developing multiple cancers throughout their lifetime. Over 200,000 Canadians harbor mutations in one of the >100 genes that cause an HCS, some facing a 100% lifetime risk of developing cancer [1,2]. To find these cancers in time for effective treatment, all of these carriers undergo highly specific medical imaging and physical exams on a biannual or annual basis, often travelling 100s of kilometers at significant personal stress, expense, and time investment. Sadly, many of these patients receive late-stage cancer diagnoses, necessitating immediate and often radical surgeries, radiation treatments, or enrollment in experimental clinical trials.

People who carry an HCS genetic variant describe themselves as "ticking cancer time bombs" as they await the accumulation of mutations during their lifetime that trigger cancer development[3]. Depending on cancer type, individuals may be able to enroll in publicly-funded annual surveillance programs that are cancer-type specific and entail an array of comprehensive clinical exams and imaging modalities, such as full- body MRI, colonoscopies and mammograms. In HCS that predispose cancers for which early detection has not yet been possible, prophylactic surgeries are recommended, such as removal of breasts, ovaries, and fallopian tubes for carriers of Hereditary Breast and Ovarian Cancer (HBOC), removal of uterus and ovaries in Lynch Syndrome (LS), and gastrectomy for carriers of Hereditary Diffuse Gastric Cancer (HDGC) [4,5,6,]. These life-altering surgeries place a heavy psychological and physical burden on patients, particularly those of childbearing age who face uncertain timing of growing a family versus potential development of a cancer.

Canadians with HCS face multiple challenges, primary among them being inequitable access to clinical screening programs. An HCS patient's children and other first-degree relatives each have a 50% chance of inheriting the same causal gene variant; timely screening and intervention can save their lives. Unfortunately, follow-up of at-risk family members is often inadequate or omitted entirely, in part due to complex referral criteria and provincial differences in screening recommendations. Additionally, people living in rural, remote and/or northern areas of Canada are isolated from the specialized genetics and oncology clinics that are typically only available in urban locations. Therefore, a paradigm shift is needed from the current fragmented approach to individual HCS towards a new, forward-thinking approach that utilizes a single, highly quantitative platform effective across all types of hereditary cancers.

A promising new technology for early cancer detection involves sequencing of the cell-free DNA (cfDNA), shed into the circulation by mostly healthy cells. Cancer cells can also shed DNA into the circulation, which can be distinguished from those shed by healthy cells. As access to high-risk screening is not available to all, cfDNA analysis from a simple blood test may provide a more accessible alternative method [7,8,9]. A blood test is minimally invasive and would require minimal infrastructure to deliver.

Our community, known as the CHARM Consortium (cfDNA in Hereditary And High-Risk Malignancies), was founded in 2018 to address HCS knowledge and delivery gaps through evaluation of blood-based cfDNA testing. Comprised of 8 genetics clinics from 5 Canadian provinces, CHARM is a patient-engaged biobanking and clinical data annotation service, where blood and available tumour samples are collected from HCS carriers at annual screening visits. Analysis of these samples has led to new advancements in cfDNA sequencing that we propose to test with this prospective trial, by evaluating the detection rate of cfDNA sequencing and the clinical benefit gained by following up on detection of early cancer using cfDNA. Specifically, we have demonstrated the use of three technologies to detect early cancer in HCS through analysis of cfDNA in blood plasma: 1) the CHARM gene panel, a targeted sequencing approach that provides in-depth mutational data on a core set of genes frequently mutated in FCS tumours and offered clinically by our program, 2) cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq), which can detect the specific organ from which the cancer-triggering DNA changes arise, and 3) shallow whole genome sequencing (sWGS), which detects accumulated mutations, copy number alterations and DNA fragmentation patterns that occur secondary to the inherited mutation. These results have suggested that integration of mutation, copy number, and functional (methylation or fragmentation) aspects of cfDNA provide the greatest sensitivity for early HCS detection.

In this study, we will enroll 1,000 patients from across Canada's HCS screening programs. Half of the patients will be randomized to have cfDNA analysis with results returned to managing physicians who will follow-up any abnormal cfDNA findings with appropriate clinical imaging and heightened surveillance. At the end of this study, the rate of cancer detection using cfDNA-informed surveillance will be compared with the rate of detection in the cohort of patients who did not receive the result of the cfDNA test but were managed according to current conventional screening programs.

2.4 Study Population

The study population will include patients with a confirmed genetic diagnosis of HBOC, LS, NF1, LFS, PALB2 and HDGC, and who are receiving standard-of-care clinical assessment for cancer by a managing physician under a provincial screening program or cancer surveillance protocol.

3.0 Study Objectives and Hypothesis

3.1 **Study Objectives**

3.1.1 Primary Objective

Our primary study objective is to establish whether blood plasma cfDNA testing can detect cancers at the same time as, or earlier, than conventional standard-of-care screening tests for carriers of HCS. We will also assess the time to disease management in carriers receiving cfDNA sequencing results compared to controls.

3.1.2 Secondary Objectives

Our secondary objective is to establish whether cfDNA testing increases the frequency of cancers detected, in particular cancer types with limited detection rates using standard-of-care protocols (e.g., pancreatic cancer, endometrial cancer).

We will also assess patient and provider perceptions about cfDNA testing, and patient-reported outcomes including empowerment, distress, uncertainty, and anxiety.

3.2 **Study hypothesis**

We hypothesize that testing of cfDNA more frequently than the current imaged-based screening protocols will result in detection of more cancers at earlier stages and improve medical management and quality of life for carriers with HCS.

We also hypothesize that a programmatic approach to cfDNA testing will decrease distress, uncertainty, anxiety and increase quality of life and empowerment among carriers with HCS.

4.0 Study Design

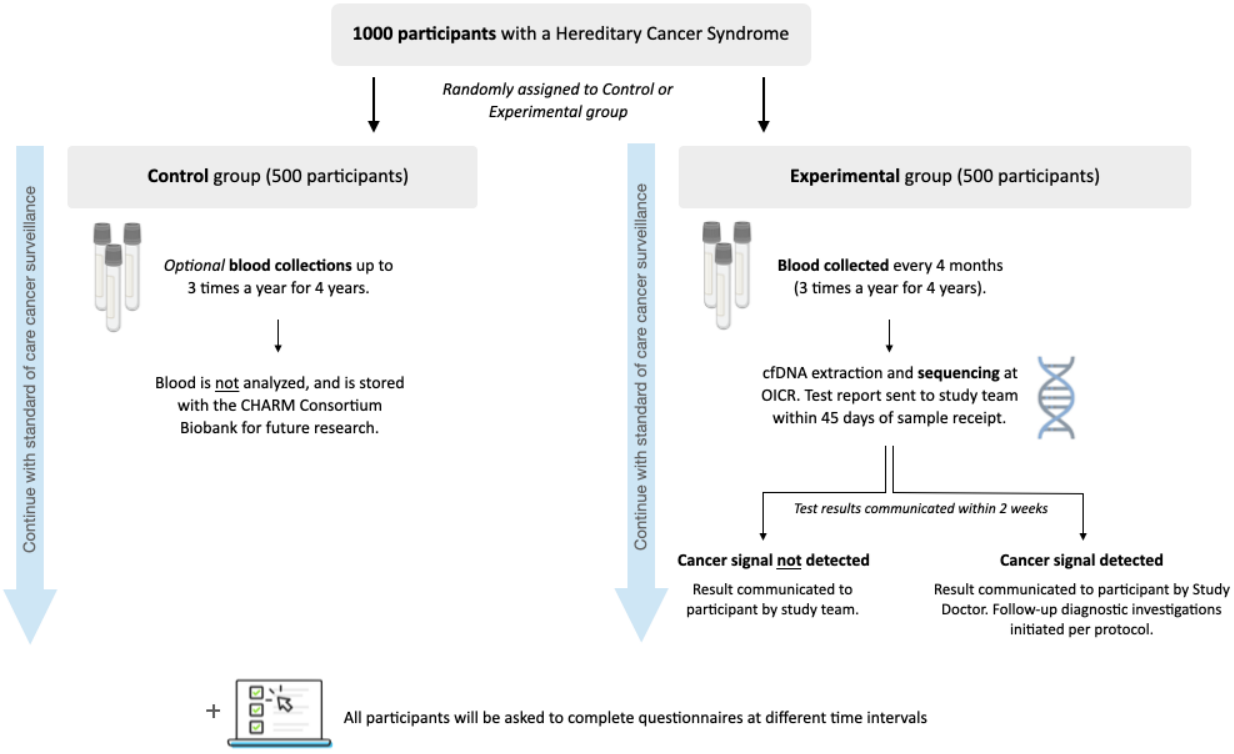
4.1 **Overall Study Design**

We are conducting a randomized control trial to assess the use of cfDNA analysis in cancer surveillance protocols for patients with HCS. We will recruit 1,000 patients across Canada with a known/suspected genetic diagnosis of one of the following HCS: HBOC, LS, NF1, LFS, PALB2, or HDGC that are receiving standard-of-care clinical assessment for cancer by a managing physician under a provincial screening program or cancer surveillance protocol.

The study design is illustrated in Figure 1. At study enrollment, participants will be randomized to a control cohort (n=500) or an experimental cohort (n=500), stratifying for genetic HCS background. All participants in the experimental cohort will provide blood samples tri-annually (every four months) for four years, either at the study hospital, or at a local blood laboratory (e.g., LifeLabs or Dynacare). Whenever possible, patients will have research blood collected at the same time as routine blood collections for clinical purposes, to avoid additional venipunctures. Participants randomized to the control cohort will have the option to donate samples to the CHARM Consortium Biobank, for use in future research. Participants in the control cohort can donate between 1-3 blood samples a year, for the duration of the study.

Participants in both the control and experimental cohorts will continue to receive standard-of-care cancer surveillance according to current guidelines, as they were prior to study enrollment. Blood samples from the experimental cohort will be subject to a

Figure 1. Study Design.



cfDNA sequencing assay, and results will be communicated to participants by the study team. Participants who receive a "positive" cfDNA assay result will be offered follow-up diagnostic procedures to confirm or rule out the presence of a malignancy. The diagnostic investigations are outlined in "Cohort Specific Diagnostic Workflows", appended to this protocol. If an abnormality is identified by a diagnostic investigation done in follow-up to a "positive" cfDNA assay result, additional investigations (e.g., imaging or biopsy) will be performed as part of the participants clinical care, and will be guided by the participant's clinical team, or study investigator. All participants will be followed by the study team for a minimum of 5 years, from their first blood collection. To assess the efficacy of the cfDNA assay, we will compare cfDNA assay results to the results of the participants standard of care cancer surveillance results.

Lastly, we will use questionnaires and semi-structured interviews to explore participants' experiences with cfDNA testing, to understand perceptions of the clinical utility of cfDNA tests for HCS management and identify factors facilitating or impeding the uptake of this test in HCS management and patient decision making.

4.2 **Biospecimen Collection**

Blood samples may be collected at UHN or in a community laboratory (e.g. LifeLabs or Dynacare). Repeat blood draws may be requested if technical issues with samples preclude sample processing or reporting (e.g., if blood is hemolyzed or collected in the wrong tube). Biospecimen labels will contain a unique study ID, and will not contain any identifying information (including name, DOB, MRN). Participants will be reminded to complete their blood collections by the study team either via phone or email, depending on participant preferences documented at the time of enrollment. We will tell participants that email is not secure, and therefore if they have any questions, they should not send sensitive information via email. Instead, they should email a member of the study team and arrange a phone call.

Hospital Blood Collection

Blood samples may be collected at UHN by either a study team member who has phlebotomy certification, or at the hospital blood collection laboratory. 30mL of blood (3x10mL Streck tubes) will be collected each time. Participants will have research blood work done at the hospital if they have an in-person clinical appointment around the same time as their next scheduled research blood collection, or if they express a preference for blood collection at the hospital over an external laboratory.

Blood collection at off-site blood laboratory

Blood samples may be collected at an external laboratory (LifeLabs or Dynacare). We will not disclose patient names or identifying information directly to Dynacare or LifeLabs. We will either contact patients over phone to obtain verbal consent for phlebotomy using Dynacare/LifeLabs, contact them via email (if they have previously consented to email communication), or speak to the patient during their in-person appointment. Patients will be provided with requisitions for external phlebotomy, which will include PI billing information and a study ID.

If we speak to the participant in-person, we will provide them with the requisition form and tell them they have to directly contact LifeLabs to arrange blood work. If the patient does not live near a LifeLabs location, and would prefer to go to Dynacare, we will provide the patient with a blood collection kit to bring to Dynacare. The kit will include the Dynacare requisition, the blood collection tubes, and packaging/materials to mail the kit back to the study team. The blood collection kit will not contain any personal identifying information.

If we communicate over the phone, following the study SOPs, we will obtain verbal consent from participants to send non-identifying documents to them over email. The external blood requisitions will not contain any personal identifying information. Upon request, we can mail the external blood lab requisition to a participant. If the participant does not live near a LifeLabs, we will mail out a blood collection kit to their home and instruct the participant to bring the kit to Dynacare. Participants will be notified that they will have to directly contact Dynacare or LifeLabs to arrange their blood work and take their requisition form or blood collection kit.

As per LifeLabs storage retention SOP, patient information will be kept as a scanned copy for 7 years. Patient names will not be collected, however, a scanned copy of the requisition form will be retained for tracking purposes. Participants will be verbally informed about this and should they not feel comfortable with this information being provided to Dynacare/LifeLabs, they will not be able to use their services. As per Ontario Dynacare's storage retention SOP, patient information will be kept as a hard copy for 10 years.

Tumour sample collection

If a malignancy is identified in a participant, we may request a tissue sample from the surgery or core biopsy and use it to determine the genetic concordance between tumours and matched blood samples. In addition, for participants with a previous history of cancers, we may request a tissue sample related to the previous malignancy from pathology archives, which will help the researchers to distinguish the mutational signatures associated with the new cancer diagnosis from signals from the other cancers.

This part of the study is optional. Some of the tissue-studies may be funded by the Terry Fox Marathon of Hope Cancer Network (MOHCCN) program. If a participant provides consent for data sharing in controlled access databases, we may share de-identified data (sequencing + clinical data) with the MOHCCN program. This de-identified data is uploaded to an access-restricted database. Only MOHCCN members, who sign joinder agreements and specify how data will be used, can access this data. Furthermore, data cannot be downloaded from the database. If a participant does not provide consent for data sharing in controlled-access databases, the CHARM study team may still sequencing tumour samples (if a participant has provided consent for tumour testing), however data generated from sequencing and associated health data will not be shared with the MOHCCN program. All samples will be collected under this research study. There will not be additional sample collection for MOHCCN only.

Participants will also have the option to donate any leftover tissue sample to the CHARM Consortium Biobank.

A fresh frozen or formalin-fixed paraffin-embedded (FFPE) tissue sample from a surgery or core biopsy may be requested by the study team. Unstained tissue sections for immunohistochemistry may also be requested (up to 15 unstained sections at 7 microns thickness plus 2 hemotoxylin and eosin (H&E)-stained slides).

4.3 **Biospecimen Processing**

Control Cohort: de-identified blood samples will be transferred to the CHARM Consortium Biobank (CAPCR 23-5772), housed within UHN or OICR for processing into plasma and buffy aliquots and for long storage. Samples will be processed by either a staff member of Dr. Trevor Pugh's laboratory, or the OICR Tissue Portal team. Genetic material will be extracted from some samples prior to storage, to better facilitate storage of a large

volume of samples. Blood samples collected from the control cohort may be used for study validation purposes, continued blood test development, or for other future research studies.

Experimental Cohort: de-identified blood samples will be processed (into buffy and plasma components) by either a staff member of Dr. Trevor Pugh's laboratory, or the OICR Tissue Portal team. Plasma cfDNA extraction will be completed by the Tissue Portal team, and extracted cfDNA will be submitted to OICR Genomics for sequencing. A buffy coat sample from a single timepoint will also be submitted to OICR Genomics for genomic sequencing. Remaining buffy coat samples, and any left over extracted plasma cfDNA will be stored at OICR until study completion, and then destroyed, unless the patient provides consent to donate leftover samples to the CHARM Consortium Biobank.

Tumour samples: if available and if the patient provides consent to this optional part of the study, tumour samples will be submitted to OICR for nucleic acid extraction and genomic sequencing. With patient consent, unused tumor samples will be stored in the CHARM Consortium Biobank.

4.4 **Sequencing Assays**

Since the molecular events driving hereditary cancers are known (i.e. the "first hit" is the inherited mutation and a "second hit" somatic mutation is acquired in the remaining wild-type allele), these "second hits" should be detectable in plasma cfDNA. If additional tumour-specific mutations are also identified, these can likewise be used to monitor disease progression via cfDNA analysis. OICR will perform the following tests on samples collected from the experimental cohort:

1. Shallow Whole Genome Sequencing (sWGS) to detect large copy number changes in cfDNA.
2. A targeted panel (the REVOLVE panel), which uses hybrid-capture and ultra-deep sequencing to achieve >20,000X coverage, to detect exon-level copy number alterations and somatic mutations. This can be used to interrogate the sequence of genes involved in cancer pathogenesis (e.g. *TP53*).
3. Methylation analysis with Cell-free Methylated DNA Immunoprecipitation and high-throughput sequencing (cfMeDIP-seq) or Enzymatic methyl sequencing (EM-seq) to reveal the tumour's tissue-of-origin. This involves surveying the epigenetic marks of tumour-derived cfDNA.

sWGS and the REVOLVE panel are clinically validated tests at OICR and will be done as first line tests on all experimental cohort samples. OICR bioinformaticians will analyze the sequencing results and generate a clinical report. Methylation studies (cfMeDIP-seq or EM-seq) will be performed on remaining cfDNA only following a positive sWGS and REVOLVE panel test result. These methylation studies are not clinically validated at OICR, and the generated sequencing data will be transferred to Trevor Pugh's laboratory at UHN for analysis.

The sequencing technology used to analyze blood samples (outlined above) may change over the course of the study (e.g., if a more sensitive test becomes clinically available at OICR, for example plasma 30x WGS).

If available, tumour tissue along with a matched buffy coat sample will undergo genomic studies at OICR, including whole-genome and whole-transcriptome sequencing. With a participants consent for data sharing, these tissue studies may be funded by the Terry Fox Marathon of Hope Cancer Centre Network (MOHCCN) program, and therefore genomic data generated from samples and de-identified health data (demographic factors, cancer risk factors, cancer diagnosis and treatment details) will be shared with the MOHCCN program. The study team will also submit unstained tissue sections for immunohistochemistry.

Any tumour sample (or extracted tumour genetic material) leftover after sequencing studies are completed will be stored with the CHARM Consortium Biobank (if consent by participant is provided) or will be returned to the study team, and destroyed.

4.5 **Clinical Reporting**

Blood test requisitions will be completed online through the OICR Genomics Requisition Portal by an authorized study team member. The following PHI will be recorded on the requisition form: participant name, date of birth, sex. The OICR requestion will also capture the participant's germline pathogenic variant in their respective HCS gene. The OICR Genomics Requisition Portal retains all PHI in an access-restricted database accessible only to requisitioners (i.e., study team members who will submit the requisition) and ACMG/CCMG licensed Geneticists who will sign out each report. The ACMG/CCMG licensed Geneticists will be part of the study team and will be internal to UHN. No OICR staff member will have access to the PHI submitted to the Requisition Portal.

We aim for the clinical report to be returned to the study investigator within 45 days of sample receipt by OICR, barring holidays and unforeseen circumstances. Clinical reports will be returned electronically, via OICR's online reporting system. Only the study investigator and designated team member will have access to the reporting system. The study team member will log into the OICR Genomics Requisition System and view/download a copy of the clinical report. The clinical report will contain PHI (patient name, DOB) and will be stored by the study team in a password-protected folder on a secure UHN network (UHN Sharepoint), only accessible by delegated study personnel. Per UHN Research Quality Integration's feedback, study clinical reports will be uploaded to EPIC.

4.6 **Return of Results and Follow-up Investigations**

Results will be communicated to the participant by the study team member as soon as possible, and reasonable attempts will be made by the study team to return results within 2 weeks from the date of report receipt by the study team.

At the time of study enrollment, participants may be provided with an information sheet explaining a "negative" and "positive" test result (please refer to Result Information Sheet). Participants will not be routinely provided a copy of the genetic testing report. However, upon participant request, a copy of the report can be provided. A physical copy (sent by mail, or provided at the time of an in person appointment) or electronic copies (sent via email) may be provided upon request.

Return of "Negative" cfDNA assay test result

A "negative" or "cancer signal not detected" cfDNA assay results will be communicated to participants by the Study Doctor or designated member of the study team via phone. Following result disclosure, the study team member will remind the participant that the cfDNA assay result does not confirm the absence of cancer, and will reinforce the importance of continuing with standard-of-care cancer screening. If after 3 phone call attempts the study team is not able to get in contact with the participant, they will email the participant the result (please see CHARM2 Negative Result Email). A participant will only be emailed if they have already consented to email communication at the time of enrollment.

Return of "Positive" cfDNA assay test result

A "positive" or "cancer signal detected" cfDNA assay results will be communicated to participants by the Study Doctor via phone call or in-person at the time of a clinical appointment. If the Study Doctor is not able to get in contact with the participant by phone, and if they do not have an upcoming in person clinical visit, the study team may email the participant to let them know their result is now available and to arrange a time for a phone call to review the result. Following result disclosure, the Study Doctor will remind the participant that the cfDNA assay result does not confirm the presence of cancer, and that follow-up diagnostic investigations are needed. The site Study Doctor will initiate the work up for the "positive" result.

Participants will be asked to provide an additional blood sample after a "positive" cfDNA result, which will be transferred to OICR and analyzed (in the same methods as outlined earlier). The results of this additional test may not be returned to the participant, and will primarily be used by the study team to correlate with the previous result, and to rule out the possibility of a technical error/lab mix-up. Additionally, some of the blood sample will be used for clinical testing (CBC, tumor markers etc), as indicated in the cohort specific follow-up diagnostic workflows (appended).

The follow-up diagnostic investigations for a "positive" cfDNA test result, for each HCS cohort, are outlined in "Cohort Specific Diagnostic Workflows", appended to this protocol. These workflows were developed together with clinicians who follow these patient populations. If the above diagnostic investigations do not identify a malignancy, and the participants next scheduled research blood draw result is still "positive", a PET-CT will be performed as a final step to assess cancer status.

For participants enrolled at UHN, diagnostic investigations will be completed at UHN, whenever possible, or may be arranged at an external site if not offered at UHN. A contract will be signed with any external site used (for example, the Toronto Immune Digestive Health Institute, or In-Common Laboratories). Participants will be informed that if a diagnostic investigation needs to be completed at a non-UHN site, identifying information (including name, date of birth, OHIP number and version code) will be shared with that site.

The Study Doctor may determine that an alternative workup plan is necessary (for example, if a participant underwent relevant diagnostic investigations completed as part of their standard-of-care surveillance shortly before their blood test, a particular investigation is not available or the wait time is too long, or if the patient has any allergies which prevent a particular investigation). Any alternative workup plan will be documented by the study team. A diagnosis of cancer will be documented based on pathologic confirmation, imaging confirmation (if pathologic not possible), or by other clinical confirmation deemed appropriate by the managing clinician.

Participants with a "positive" cfDNA assay result may also have methylation studies completed (either cfMeDIP or EM-seq) on their remaining cfDNA sample at OICR. The methylation tests are not clinically validated at OICR, and the sequencing data will be transferred from OICR back to Trevor Pugh's laboratory at UHN and analyzed by a team member. The methylation analysis is exploratory, and therefore the results of the methylation studies may not be returned to the study doctor, if no tissue-of-origin signal is identified. If a tissue-of-origin signal is identified, results may be returned to the study doctor will be correlated with the results of the clinical follow-up investigations completed above. If a tissue of cancer origin is identified using methylation studies, additional targeted diagnostic investigations will ensue, if not done so previously as part of the initial follow-up investigations. Currently, only a methylation signature for breast cancer has been well established. Other methylation signatures are in progress (brain, pancreas, ovary), and may become available over the course of the study.

The CHARM 2 study clinical review committee, composed of study investigators from different study sites, will jointly review challenging cases (e.g. if diagnostic investigations completed in follow-up to a positive cfDNA result are inconclusive). Patient clinical information (including test results, cancer history, images) will be shared with members of this committee, to aid in result interpretation and discussion of best next steps. All information shared will be de-identified. No information will be shared prior to execution of appropriate DTAs.

4.7 **Diagnosis of Cancer**

If a participant in the control or experimental group is diagnosed with cancer over the course of the study, the study team may collect blood samples at different timepoints before, during and after their cancer treatment. This blood collection is optional. These samples may be sequenced by the study team to better understand the performance of the cfDNA blood test. If a participant in the experimental group develops cancer, optional

research blood samples collected after the diagnosis may not be analyzed immediately by the study team, and results will not be returned to the participants. Any samples not used by the study team will be banked with the CHARM Biobank for use in future research (for example, to understand how a cfDNA blood test could be used to monitor response to treatment).

If a participant is diagnosed with cancer over the course of the study, patient-outcome questionnaires will no longer be collected alongside the optional blood collections.

4.8 **Collection of Clinical Data**

Clinical data will be collected from participants' medical record and the medical history and demographic questionnaires completed by participants following informed consent (please see "Demographics and Medical History Questionnaire"). Participants will be given the option to complete the questionnaire in person (e.g., if the consent process occurred in person), or electronically. If completed electronically, the questionnaire may be emailed to the participant, or the participant may complete the questionnaire via REDCap (a link will be emailed to them). If a patient is not able to complete the questionnaire electronically, a physical copy may be mailed to them with a return envelope. The questionnaires will record the patient's study ID only (name, DOB, MRN will not be recorded). Updates to questionnaire results may be collected by the study team over the course of the study.

If a participant receives care at more than one study site, their medical records from any of the other sites may also be looked at, regardless of which site they are enrolled at for this study. Sharing of any study records between sites for patients who have received care at multiple sites will only occur following execution of appropriate DTAs. Study team members may also request medical history information from participants directly, if not available in their medical records (e.g. by setting up a phone call). Additionally, study team members may ask a participant to complete a Release of Information (ROI) form (appended to this application), to request medical records from external hospitals/clinics.

The following clinical data will be captured over the course of this study for all participants:

- Demographic information (including date of birth, self-reported gender and sex assigned at birth, ethnicity).
- Genetic diagnosis and date.
- Past medical history, including but not limited to personal history of cancer, cancer risk factors, cancer screening history, pregnancy status, current medications, comorbidities.
- Cancer screening – results and dates of any cancer screening/diagnosis investigations done prior to, or during the study (including the diagnostic investigations done in follow-up to a positive cfDNA test result).

- For participants with a history of cancer or diagnosed with cancer during the study – pathology information, imaging and procedure results, treatment details.

- Family history of cancer.

Collection of the above clinical information, and subsequent comparison to the results of the cfDNA test, is necessary in establishing the sensitivity, specificity, and PPV and NPV of the cfDNA test. The above clinical information will be documented for all participants for up to 7 years from the date of study enrollment. Continued collection of clinical data after all sample collection for the purpose of the study has been completed will be necessary to establish function of cfDNA test (for example, clinical detection of cancer may occur after the 5-year period).

4.8 **Participant Questionnaires and Interviews**

4.8.1 Patient outcome questionnaires

All participants will be invited to complete patient-outcome questionnaires throughout the course of their participation in the study (appended to this application):

- At the time of study enrollment, before randomization into the control or experimental group, participants will be provided a patient-outcome questionnaire.
- Experimental group:
    - Participants in the experimental group will be asked to complete a patient-outcome questionnaire following each result disclosure by the study team (approximately 45 days following the blood collection).
- Control group:
    - Participants in the control group will be asked to complete the patient-outcome questionnaire up to 3 times a year.

The questionnaire will include validated measures focused on anxiety, cancer worry, disease uncertainty, empowerment, among other emotional outcomes. Questionnaires will be completed electronically via REDCap, and participants will be emailed a link to the questionnaire by the study team. If a participant is unable/not comfortable completing a questionnaire electronically, a paper copy may be mailed to them. Each questionnaire should take no longer than 20 minutes to complete. Questionnaires will record the participants Study ID. Questionnaires will be analyzed jointly with Dr. Yvonne Bombard at Unity Health. Demographic and health information (e.g. genetic diagnosis, cancer history) will be shared with Dr. Bombard's study team, to aid in the interpretation of the questionnaires. Appropriate DTAs will be established prior to the sharing of any data. Completion of the questionnaires will be encouraged by the study team, however will not be mandatory for continued study participation (i.e. if a participant does not complete a questionnaire, they will not be removed from the study).

As the questionnaire includes some questions that participants may find distressing, we will include an option at the end of the questionnaire for participants to indicate that they would like to speak with a member of the study team. Together with the study Patient Partners, the study team will put together a list of resources, that may be shared with participants if needed.

4.8.2 Interviews

Semi-structured interviews will be conducted to explore participants' experience with cfDNA testing, perceptions of the clinical utility of cfDNA tests for HCS management and to identify factors facilitating or impeding the uptake of this test in HCS management. The interview component of this study is optional, and patients preferences will be captured on the consent form.

All participants who receive a positive cfDNA test result and who have consented to being contacted for an interview, will be invited for an interview approximately 6-12 months following a positive result disclosure. Additional participants will be invited for an interview, from the pool of participants who have agreed to participate in the interview portion of the study. Any participant may partake in up to 3 interviews over the duration of the study. A participant can decline to participate in an interview at any time during the study. Each interview will take approximately one hour to complete and will be conducted via telephone or Zoom healthcare (participant preference).

All interviews will be audiotaped using a handheld audio recorder. Audio-recorded interviews will be sent off site for transcription by Rev.com for transcription via their secure file transfer system. All data at Rev.com is encrypted at rest and in transit (https://www.rev.com/security). Only de-identified interview recordings will be uploaded onto Rev.com. This de-identified data will be stored on Rev.com's USA servers during the transcription process. Rev.com does not own the audio recording and transcripts or any study data. After the transcription is complete and verified, the recordings and transcripts will be removed from the Rev.com site and server. The transcribed interviews will be managed and analysed using Dedoose (https://www.dedoose.com/), a web-based qualitative analysis software.

All audio recordings will be destroyed after they have been transcribed and verified for accuracy by the study team. Any identifying information such as names of individuals, institutions, or geographic locations will be removed from the audio recordings by the study team prior to sending the audio recorder for transcription. Each study participant will be assigned a unique identifier and will be referred to only by this code number in the transcript. The recording devices are password protected and will kept in a locked drawer in the study offices (at Unity Health) when not in use. Recording devices are only accessible by Unity Health study staff. Immediately after an interview has been completed, all recordings will be uploaded to secure servers at Unity Health or UHN, (identified only by study ID) subsequently the interview will be erased from the recorder. The recorder is not stored in the study offices with a recording left on it. When an interview is conducted it (whether over the phone or via Zoom healthcare) is always in

the possession of the study staff member and it is only used during the interview, which takes place in a private location.

4.9 **Randomization and Measures to Minimize Bias**

Cancer risks are different for the 5 HCS cohorts we will enroll in this study. Participants will be randomized into the control and experimental arms based on their HCS diagnosis, to achieve roughly the same number of participants from each HCS cohort in each arm of the study. To avoid over-stratification, we will not randomize participants based on other factors (such as age, prophylactic surgery, cancer history etc.). However, these other factors also influence cancer risks, and we will collect this information, to minimize bias in our subsequent analysis.

4.10 **Blinding Methods**

This study is not blinded.

4.11 **Endpoints**

The endpoints of this study are:

1. To assess the performance of the cfDNA assay for cancer detection in patients with HCS, including assay sensitivity, specificity, PPV, and NPV. The test performance endpoint is a diagnosis of cancer and will be assessed at multiple points during the study (at a minimum yearly). Cancer diagnosis will be confirmed by imaging, pathology, or other confirmation deemed appropriate by the treating physician.
2. To assess the time to cancer diagnosis using cfDNA sequencing compared to controls.
3. To assess the detection rate of cancer with no standard-of-care screening available (e.g., pancreatic cancer, endometrial cancer) using cfDNA sequencing.
4. To assess patient reported outcomes (including anxiety, empowerment, distress, risk perception) using validated scales administered before, during, and after the study.

4.12 **Study Duration and Participant Time Commitments**

The expected study duration for each participant will be approximately 5 years from the date of first sample collection (Table 1). We will do our best to minimize the number of on-site visits required by participants. Participants will have the option to provide research blood samples via their local community laboratory (e.g. LifeLabs or Dynacare). Result disclosure by the study team will be done over the phone, unless the participant is on-site for a clinical appointment, in which case results may be disclosed in person. Participants will be required to come into the hospital for diagnostic investigations done in follow-up to a “positive” cfDNA assay result.

Table 1. Participant requirements.

Activity	Year 1			Year 2			Year 3			Year 4			Year 5
Blood Collection[1]	x	x	x	x	x	x	x	x	x	x	x	x	
Test Results Returned to Participants (experimental cohort)	x	x	x	x	x	x	x	x	x	x	x	x	
Follow-up Diagnostic Investigations (experimental cohort)	Diagnostic investigations will ensue following a "positive" cfDNA assay result.												
Medical History and Demographic Questionnaire[2]	x												
Patient-Outcome Questionnaire	x	x	x	x	x	x	x	x	x	x	x	x	
Collection of clinical data by study team.	Clinical data will be collected by the study team for up to 7 years from the date of study enrollment.												
Interviews (optional)	Interviews will be conducted over the course of the study. They will begin at the end of year 1.												

[1] Tri-annual blood collection is only a study requirement for those randomized into the experimental cohort. Those in the control cohort have the option to provide up to 3 blood samples a year, [2] The medical history questionnaire will be completed at least once by all participants at the time of study enrolment. Participants may be asked to update the questionnaire over the course of the study.

4.13 **Risks and Benefits**

4.13.1 Potential Risks

Risks associated with sample collection

There is the potential for bleeding, bruising, discomfort, infections or pain at the needle site, or dizziness from the needle stick to take the blood samples.

There are no physical risks to the participant in releasing tumour samples for the purpose of this study since the tissue has already been obtained by a previous surgical/ biopsy procedure. In cases where tumour tissue is collected during a surgical procedure, the physical risks are those associated with the surgery being performed. Tumour tissue will only be collected after the specimen is removed from the patient's body and only if there is enough specimen available.

Risks associated with follow-up procedures

There are potential risks for study participants associated with the diagnostic evaluations following a "positive" cfDNA assay result. The diagnostic evaluations (procedure or imaging) will be deemed as appropriate by the Study Doctor and will reflect the current clinical standard-of-care surveillance guidelines for each respective HCS cohort. The potential risks associated with diagnostic evaluations will be explained to the participant during the consent processes, and again by their medical team prior to any diagnostic procedure or imaging. Potential risks associated with diagnostic evaluations include (but are not limited to):

- allergic reactions and respiratory problems, and in rare instances death from general anesthesia or sedatives that may be used with endoscopic procedures
- perforation of the colon, esophagus, or stomach by the colonoscope, sigmoidoscope, or endoscope
- prolonged or severe bleeding from endoscopy, colonoscopy, sigmoidoscopy, or laparoscopy injury
- infection from endoscopy, colonoscopy, sigmoidoscopy, or laparoscopy
- visceral injury and bleeding, injury to the bowel, or injury to the bladder from laparoscopy
- exposure to ionizing radiation from imaging procedures
- acute allergic or severe contrast reactions including acute renal failure, and in rare instances death from procedures using imaging contrast agents

If an abnormality is identified by a diagnostic procedure or imaging done in follow-up to a "positive" cfDNA assay result, additional investigations (e.g., imaging or biopsy) will be performed as part of the participants clinical care, and will be guided by the participant's clinical team, or study investigator. Any biopsies and/or surgeries performed following identification of an abnormality by a diagnostic procedure or imaging, are no longer considered a study-related procedure or a risk detailed in this protocol.

<u>Risks associated with the test result</u>

There is a potential risk of receiving a "false positive" test result (i.e., a "positive" signal is detected on the cfDNA assay, but no cancer is present (i.e., no cancer is detected on any of the follow-up diagnostic investigations). This could result in unnecessary procedures and imaging, which can cause both physical and psychological risks to the participant.

There is also a potential risk of receiving a "false negative" test result (i.e., a "negative" signal is detected on the cfDNA assay, but cancer is present). This may result in non-compliance with a participant's next standard-of-care cancer surveillance cycle, which

could cause a delay in a potential cancer diagnosis. During the consent process, and again at the time of cfDNA assay result disclosure, the study team will communicate to the participant that a "negative" cfDNA test result should not be interpreted as a definitive absence of cancer, and the cfDNA assay should not outweigh current clinical standard-of-care surveillance guidelines.

Risks associated with genetic testing

There is a risk that information gained from genetic research could be linked to the participant. To prevent this, all samples and data collected within this study will be identified by a unique study ID and all samples will also be tracked by a barcode. A list linking the study ID to participant information will only be made available to certain study team members, as permitted by REB. Samples will be held in a secure facility and will not be identified by the participant's name. The researchers believe the chance that someone will identify the participant is very small. Still, the risk may change in the future as people come up with new ways of tracing information and this will be communicated with the participant through the consent form.

4.13.2 Potential Benefits

Some participants will receive a "positive" cfDNA assay result, and the follow-up investigations may lead to a diagnosis of cancer, which can then be treated appropriately. The cancer diagnosis may happen earlier than if the participant were not involved in the study, and only partaking in standard-of-care surveillance.

This study will help evaluate the feasibility and acceptability of implementing cfDNA sequencing as part of standard-of-care surveillance for high risk patients. Should this study show benefits for detection of cancer in HCS patients comparable to current surveillance protocols, this may have broader implications for HCS families across Canada.

5.0 Selection of Subjects

This study will enroll 1000 participants across Canada. Up to 1000 will be enrolled at UHN. Participants will be recruited from the genetics, surgical, medical, and radiation oncology clinics. If other sites join our study, the appropriate MTAs and DTAs will be established, and samples collected at other institutions (who have institutional REB approval for the study) will be sent to UHN for analysis. We are still in the process of determining which other sites will be involved in our study. Other sites may include: BC Cancer Agency, Mount Sinai Hospital, SickKids Hospital, Women's College Hospital, Sunnybrook Hospital, McGill Research Institute, Jewish General Hospital, IWK Health Centre, Eastern Health. If the patient receives care at more than one of the above sites, their medical records from any of the other sites can also be looked at, regardless of which site they are enrolled at for this study.

5.1 **Subject inclusion criteria.**

Patients must fulfill the following criteria:

1. Patients with a confirmed diagnosis of HBOC, LS, NF1, LFS, PALB2, and HDGC (i.e., patients with an identified pathogenic variant in the respective cancer predisposition gene, or patients with uninformative genetic testing but with a family history suggestive of the cancer predisposition syndrome).
2. Patients must be receiving standard-of-care clinical assessment for cancer by a managing physician under a provincial screening program or cancer surveillance protocol.
3. All patients must have signed and dated an informed consent form for this study.

5.2 **Subject exclusion criteria.**

1. Patients must not have a personal history of cancer diagnosed and treated within 3 years prior to the expected first sample collection date for this study. If a patient has a personal history of cancer, treatment must have been completed successfully at least 3 years prior to first study sample collection.
2. Patients diagnosed more than 3 years prior to the expected first sample collection date, but never been treated for the cancer.
3. Patients undergoing investigations for a clinical suspicion of cancer.
4. Patients who are not able to comply with the protocol (i.e., tri-annual blood sample collection if randomized into the experimental cohort).

5.3 **Withdrawal of Subjects**

If at any time a participant is found to be ineligible, as outlined in the inclusion and exclusion criteria, the participant will be removed from the study. In addition, if after obtaining informed consent the participant decides that they do not want to participate in the study, they may withdraw at any time at their own request. A participant may also be withdrawn from the study by the study team if not compliant with study requirements (e.g., completion of tri-annual blood collections if randomized into the experimental cohort).

If a participant is withdrawn from the study, the study team may continue to use the samples and corresponding data collected prior to withdrawal, unless the participant requests destruction of any samples not tested. Participant preferences will be documented by the study team. The study team will inform the participant that it is not possible to destroy samples and data which have already been processed and analyzed for research.

No study activities (including blood collections, questionnaires, data collection) will be completed for withdrawn participants. Any cfDNA test results received by the study team after a participant is withdrawn, will not be disclosed to the participant. Any diagnostic investigations following a positive cfDNA test result will not be initiated by the study investigator for withdrawn participants.

Withdrawn participants may be replaced by the study team, via the recruitment methods outlined above.

6.0 Study Intervention

All study participants will continue to receive standard-of-care cancer screening over the course of this study, as they have prior to study enrollment. Study participants randomized into the experimental arm will provide blood samples 3 times a year for 4 years. cfDNA sequencing will be performed on blood samples collected from participants in the experimental group. Results of the cfDNA sequencing (either "cancer signal detected" or "cancer signal not detected") will be communicated to the participant by a designated member of the study team. We expect up to approximately 5-10% of participants per year in the experimental arm will receive a "cancer signal detected" result, which will trigger follow-up diagnostic investigations to confirm if cancer is present or not (outlined in Appendix A: "Cohort Specific Diagnostic Workflows"). The type of follow-up diagnostic investigations will be consistent with standard-of-care recommendations.

7.0 Assessment of Efficacy and Safety

There is a possibility that the sequencing tests (sWGS, targeted panel, cfMeDIP) are less sensitive than standard-of-care screening. We will continuously monitor the concordance between cfDNA assay results and standard-of-care surveillance results. Confirmation of a cancer diagnosis will be supported by imaging confirmation, pathologic confirmation, or another form of clinical conformation deemed appropriate by the study investigator. Yearly, we will evaluate:

- the percentage of cancers found by standard-of-care screening missed by the cfDNA assay.

- the percentage of positive cfDNA results where no cancer is detected on any follow-up diagnostic investigations.

If we determine that one of the tests are consistently underperforming (e.g. if 50% of cancers found by standard of care screening are missed by cfDNA assay), we will consider discontinuing the underperforming assay, and replacing it with an alternative validated test (e.g. plasma WGS). If we change one or more of the assays over the course of the study, participants will be notified of this change verbally by a study team member. If no alternative test is available, we may decide to end the study early.

The Study Doctor will perform the following tasks to ensure the safety of subjects and the good conduct of the study, on a monthly basis:

- Review the study binder to ensure all documents and communications are up to date
- Review the Inclusion/Exclusion forms for completeness and compliance to study protocol
- Monitor the accrual rate and recruiting efforts
- Monitor the number of 'false positive' results, and the number of subsequent diagnostic investigations undergone by the participant
- Monitor participant compliance to routine cancer screening protocols

Study related adverse events will be documented and reported to the UHN REB, as per the standard institutional procedure.

8.0 Statistics

We will compare the cancer detection rate between control and experimental cohorts. Analysis will be stratified by the type of the tumours detected within each HCS, as well as across the entire cohort. Between the cohorts, we will first compare the cancers detected to ascertain whether cfDNA testing results in a more sensitive detection of clinically confirmed cancers, as well as calculate negative and positive predictive values of cfDNA findings as confirmed by clinical testing (MRI, ultrasound, biopsy, protein biomarkers, etc.). This analysis will also ascertain whether cfDNA is more or less effective at detecting specific cancer types compared to standard-of-care screening alone.

We will also conduct comparisons that compare categorical (e.g. cancer stage) and continuous time course data (e.g. time to symptom development, time to treatment, and time on treatment). The first comparison is a stage shift analysis, whereby we will assess whether cancers are being detected at earlier stages using cfDNA versus screening results from the same programs. Categorical variables of stages I-IV within each cancer type as defined by NCCN criteria will be compared using Pearson's $\chi 2$ test. Binary comparison of "early" and "late" stage across all cancer types will be performed using Fisher Exact test. The second comparison will be calculation of the time to first treatment relative to the date of the last annual clinical screening visit. This comparison of continuous variables using the t-test will establish whether triannual cfDNA testing accelerates off-cycle clinical follow-up and medical treatment compared to waiting for annual clinic visits. The third comparison will compare mortality and morbidity specifically for pancreas, esophagus, brain, lung, and stomach cancers. We will calculate the time between detection of a cancer and when patient manifest symptoms of cancer (morbidity 1), how long patients are afflicted with cancer symptoms after detection and during treatment (morbidity 2), and how long a patient undergoes treatment before succumbing to disease (mortality) or, in the case of effective treatments, the time until treatment is discontinued, or patients transition to the outpatient setting. Continuous variables will be compared by t-test.

Power analysis is based on the primary outcome of improved cancer detection at various diagnosis rate scenarios of the study cohort at the end of the study, range with current 5% detection rate for all HCS without cfDNA modeling, up to 10% should cfDNA improve

detection. Based on the power analysis, our study with 1,000 patients will achieve >97% statistical power to detect at least 2% increased diagnosis rate attributable to cfDNA testing with 2-sided significance level at 0.05.

Lastly, analysis of the survey data will be led by Dr. Yvonne Bombard at Unity Health and Dr. Holly Etchegary at Memorial University. DTAs will be established and signed by all institutions, prior to sharing of any data. Baseline adjusted mean differences between groups with 95% confidence intervals will be calculated for test-specific distress and uncertainty, generalized distress, anxiety, empowerment and quality of life; a regression framework for hypothesis-generating purposes will be used. Estimates of variance and correlation among emotional outcomes and empowerment, and quality of life and between groups will be determined. Proportion of cases and drop outs from rural/remote geographic regions across arms will be compared using a chi square test. P-values are set at 0.05 (two-tailed).

9.0 Direct Access to Source Data/Documents

Designated study team members at each study site (including the study investigator, research coordinators etc.) will have access to study related documents for participants enrolled at their respective site. If a participant has received care at more than one study site, source documents (e.g., results of cancer screening investigations) may be shared between sites. If a participant completes study-related investigations at an external hospital/clinic (e.g., diagnostic investigations following a positive cfDNA assay result), results will be requested by the study team.

10.0 Quality Control and Quality Assurance Procedures

Study coordinators will regularly complete clinical data collection and upload de-identified data to a secure UHN-housed study database. The study database will also collect information about the status of study-related tasks (including blood collections, diagnostic investigations following a positive cfDNA result). The study database will only be accessible to designated study staff. The UHN CHARM study manager will conduct quarterly audits of the database, to track recruitment progress, the completeness of clinical data collection, and participant compliance with study-related tasks.

11.0 Ethics

11.1 **How potential study participants will be identified**

Multiple resources and strategies will be utilized to identify eligible participants at UHN.

a. We will review several databases and programs, including the Balwani Familial Cancer Clinic PROGENY clinical database, Ontario Breast Screening Program, and High Risk Screening clinics. We will also use Pathways Healthcare

Scheduling (PHS) to access patients lists from all medical oncology, surgical oncology, radiation oncology, and genetics clinics to identify eligible patients. Electronic clinical charts will be reviewed by designated members of the CHARM study team to determine patient eligibility.

b. Patients who are enrolled in the CHARM 1 study at UHN or Sinai Health Systems, may be approached by the CHARM study team (if consent was provided for future research contact), or a member of the patient's circle of care (if consent was not provided for future research contact), to introduce the CHARM 2 study. Sinai Health Systems patients must have a UHN MRN to be enrolled in the study at UHN. If a patient enrolls into this CHARM 2 study, they will be unenrolled from the CHARM 1 study.

c. Patients who have previously consented to being contacted for research purposes will be identified using study records. If a patient has previously consented to being contacted about potentially participating in new research studies, a CHARM study member will contact the research group who initially obtained consent to ensure it is appropriate to contact the patient for recruitment. Once verbal or written confirmation is obtained, a CHARM member will contact the patient via an approved method to determine if they are interested in participating, and to assess their eligibility.

d. We will offer participation to the relatives/friends of enrolled participants. If participants have relatives/friends who might be eligible and interested in joining our study, we will provide them with the study information sheet for dissemination (appended). This sheet will include the contact information for a study coordinator. If the individual is interested in enrolling in the study, they can contact the study coordinator who will facilitate enrolment. In addition, we may provide family members with the CHARM email (CHARM@uhnresearch.ca) and their family members may contact us at this email for further information.

e. Genetic counsellors, physicians and/or members of a patient's circle of care can introduce the patient to the study. A study information sheet (appended) may be provided to the clinical team, to share with the prospective participant. If a patient is interested, they can provide their clinical team member with verbal consent or written consent (using the "Study Contact Form", appended) for the study team to contact them. The health care provider will provide the study team with the patient's contact information or completed contact sheet. A study team member will then reach out to the patient. In addition, if the patient expresses interest, the health care provider can provide the patient with the study team email and the patient can directly contact us.

f. If patients known to a CHARM physician are eligible to participate, but do not have regular follow-up appointments scheduled at UHN, the treating physician will send the patient a mailed letter ("Mail Out Letter" appended) informing them of their eligibility for the study. If the patient is interested in participating or

learning more about the study, they will notify the physician to notify the CHARM team for contact or provide a self-referral.

g. A study information sheet (appended) will be posted throughout UHN. The sheet will contain a brief background of the study and contact details.

h. We will share the study information sheet and a study brochure (appended) with our Patient Advocates, who can share the study materials via their respective organization (e.g., through social media, newsletter etc.).

After a potential participants is identified by a member of their circle of care (for example, the Study Doctor), study team members who are not part of a patients circle of care (Study Manager and/or Study Coordinators listed as Study Personnel in the CAPCR submission) may review a potential participant's electronic medical records at UHN to confirm study eligibility prior to study introduction by a member of their circle of care. The study team member will use the Screening Eligibility Checklist (appended to this application) to confirm eligibility. The study team member will document Yes or No for each question on the checklist. No specific health information (e.g., details of a cancer diagnosis) will be documented by the study team prior to study enrollment. If a patient is deemed ineligible, or declines enrollment, the study eligibility checklist will be destroyed, and only the patient name and month/year of birth will be retained by the study team until the end of the enrollment period. We have requested an REB waiver to allow study team members who are not part of a patients circle of care to screen the medical records as described above, to determine participant eligibility.

Participants may also be identified at the Zane Cohen Centre for Digestive Disease at Mount Sinai Hospital. A member of a potential participant's circle of care (e.g. a Genetic Counsellor) at the Zane Cohen Centre can introduce the patient to the study. If a patient is interested, their clinical team member will complete the "Study Contact Form" and email the completed form to the CHARM2 study team. The CHARM2 team member will then contact the interested participant (as outlined below). All participants recruited through Mount Sinai Hospital will be enrolled into the CHARM2 study at UHN. No study procedures (other than recruitment) will be carried out at Mount Sinai Hospital. Participant recruitment will begin at Mount Sinai Hospital only after Research Ethics Approval is received by the Mount Sinai REB.

Participants may also be identified at Women's College Hospital, via the Hereditary Breast Clinic. A member of the potential participant's circle of care (e.g., a Genetic Counsellor), in the Hereditary Breast Clinic may introduce the study to the patient. Patients enrolled in other research studies via the Hereditary Breast Clinic, who consent to future research contact, may also be introduced to the study, by a member of the Women's College Hospital study team. If a patient is interested, they can self-refer to the CHARM2 study (e.g., via the CHARM website contact us page, or directly via the charm@uhn.ca email). The UHN approved study brochure may be shared with patients at Women's College Hospital, by a member of the patient's circle of care. No study procedures other than recruitment will be carried out at Women's College Hospital. All participants recruited at

Women's College Hospital will be enrolled into the CHARM2 study at UHN. Participant recruitment will begin at Women's College Hospital only after Research Ethics Approval is received by the Women's College Hospital REB.

11.2 **Method of patient contact**

Active UHN patients
Communication with participants may include multiple modalities, including: telephone, in-person, mail, email and virtual appointment. A study coordinator will contact eligible individuals via an approved method if the patient fulfills any of the following criteria: (a) the individual was introduced to the study by a member of their circle of care and provided verbal consent to being contacted, or filled out a contact form (appended)(b) the individual is a member of another study or database and previously consented to being re-contacted for other research purposes, (c) an individual heard about our study (e.g., from a family member/friend or from a posted/flyer) and initiated contact with a study member (via telephone or email) to express interest in participating.

We will also utilize the "OK to Contact" research participation feature in EPIC. Eligible patients, (identified via one of the methods listed above), who have their research contact preferences documented as "Yes" in EPIC, will be approached by the study team at the time of their scheduled visit or hospital admission at UHN, by phone (if the patient has a number listed in EPIC), or by email (if the patient's communication preferences for "research study invitations" in EPIC allows for email communication). If initiating contact with patients by phone, up to 3 contact attempts may be considered by the study team. Individuals who have their research contact preferences documented as "No", "No (organizational default)" or "undecided", may still be introduced to the study by a member of their circle of care. If the patient expresses interest in hearing more about our study a member of our team will approach the patient to obtain consent in person or via telephone. The following PHI will be reviewed to screen patients for eligibility: genetic test report (to confirm carrier status) most recent oncology-related clinic notes (to confirm cancer and treatment status). Patient name, DOB and contact information will also be collected by the study team, to facilitate initial contact. If a patient is contacted by the study team and declines participation, or if the study team is not able to contact the patient, then only patient name and month/year of birth will be retained by the study team.

For individuals who initiate contact with team members via email, we will send them an email message inviting them to contact a study coordinator via telephone, indicating that email is not a secure method of communication. We will only initiate contact with patients via methods they approve (as expressed verbally, in writing using contact/consent forms, or in EPIC's "research study invitations" preferences).

Active UHN patients who are not able to be approached by the study team in person (for example, if they do not have an upcoming clinical appointment) and do not have their research participation preferences in EPIC recorded as "Yes", may be sent a mailout

letter (as outlined below). All prospective participants sent a recruitment letter by mail will be within the circle of care of the Study Doctor.

Inactive UHN patients
If we identify eligible patients who are not currently receiving care at UHN and have not provided consent to be contacted by research staff in EPIC (i.e., the "OK to contact" feature), we will send them mailout letter via mail to inform them of their eligibility. If CHARM members have not heard from eligible participants 4 weeks after shipping, a CHARM study member will follow up via telephone (please see telephone recruitment script). Recruitment packages will include the following:

(1) A letter introducing study (see Mailout Letter)
(2) Study Brochure or Study Information Sheet.

If the individual expresses interest in our study via telephone or email, study members provide the prospective participant a copy of the consent form by mail or email, depending on participant preferences. The study team member will then contact the individual via telephone to explain the consent form and answer any questions. The individual will be told that they have unlimited time to decide if they wish to consent to the study. If the individual consents, they will be asked to sign one copy of the consent and send it back to study members via mail or email. If they choose to send it via email, they will be reminded that this is not a secure way of sending information. CHARM study members will then coordinate blood donation at UHN, or at an off-site blood collection lab. Follow-up contact will be made 4 weeks later if forms are incomplete or have not been returned, or if blood samples have not been collected.

Address information will be obtained from the Patient Electronic Medical Record. Individuals who are listed as expired/deceased will be removed from the mail-out list. A cut-off date of January 1, 2014 was selected to increase the likelihood that the recorded patient contact information is accurate.

11.3 **Patient consent**

Every individual consented into the study will be given unlimited time to review the consent form prior to signing. All participants will be provided with two consent forms: this research study consent form (CHARM 2 consent form) and the CHARM Consortium Biobank consent form. Consent will be carried out by the study manager, or study coordinators/analysts listed in the CAPCR submission. Participants will be able to consent into the study and biobank using multiple methods:

a) In-person consent: if CHARM study members are not already part of the patient's circle of care, we will request an introduction via a member of the patient's circle of care. If they express interest, a member of the study team will verbally review information contained in the consent form. The study member will provide the patient with an opportunity to seek clarification and emphasize that they have unlimited time to decide whether they wish to participate. If the patient chooses to participate in the study, they will be asked to provide a

signature on the consent form. Signed consent forms will be collected and retained by study members. An additional copy of the consent will be provided to each participant for their own records. If a CHARM study member is unable to consent the interested patient in-person during their clinical visit, they will recruit the patient via telephone (see below).

b) Virtual consent (telephone or MS teams): all study-related telephone or MS teams conversations will be received/initiated in a secure area to ensure the protection of confidential patient information. If an individual expressed interest in participating in our study, a study member will provide the individual with a copy of the consent form via email, if the individual provided verbal consent for email use, or via mail. If the consent form is sent by regular mail, a return envelope will be included. Consent for email communication will be collected verbally and documented by a study team member. Study team members will remind the patient that email communication is not secure. All participants will be provided the option for follow-up appointment via telephone or MS teams. If the prospective participant prefers MS teams, the study team will email the participant a meeting invite from the CHARM study UHN email address.

   After establishing a time for follow-up, CHARM study members will contact the individual (telephone or MS teams) to explain the study consent form and answer any questions. An approved telephone recruitment script will be used (appended). The individual will be told that they have unlimited time to decide if they wish to consent to the study. If the individual consents, they will be asked to sign one copy of the consent form and send it to us via mail, or scan the signed consent and send it to us via email (CHARM@uhnresearch.ca). The participant will retain the remaining copy of the consent form for their own records. Alternatively, consent may be done via RedCAP, in which case the study team member will email the patient a link to the online consent form. The CHARM study member will then coordinate blood donation at UHN, or at an off-site blood collection lab. Follow-up contact will be made up to 4 weeks later if forms are incomplete or have not been returned, or if blood has not been collected.

11.4 **Data sharing with MOHCCN**

Over the course of the CHARM2 study, the study team may share de-identified tumour sequencing and associated health data with MOHCCN. If a participant enrolled in CHARM2 provides consent for data sharing with other researchers via controlled-access databases, and tumour is sequenced as part of the CHARM2 study, the study team may share the de-identified tumour sequencing data along with relevant health data with the MOHCCN program.

The MOHCCN program uploads de-identified data to a secure controlled-access database. This database is accessible to researchers who are members of the MOHCCN, and who sign confidentiality agreements outlining how data may be used.

11.5 **Reminders for consented patients**

Participants will be advised that study members will contact them via telephone or email to remind them about tri-annual study blood draws, to complete the patient-outcome questionnaires, to collect necessary medical history information that is not available in their medical records, and to communicate the results of their cfDNA test. Participant preferences for telephone or email communication reminders will be collected verbally and documented by study staff at the time of enrollment.

11.6 **Sample Biobanking and Use of Remaining Samples**

All participants will be given the option to donate samples to the CHARM Consortium Biobank. As outlined in the biobank consent form, unused samples may be used in future REB-approved research studies by either the CHARM study team, or external collaborators. All future research will focus on early cancer detection, cancer monitoring and/or cancer treatment.

All samples will be stored in the CHARM Consortium Biobank (CAPCR 23-5772).The CHARM Consortium Biobank protocol documents how samples will be utilized for future research purposes.

11.7 **Confidentiality**

To protect patient confidentiality, study personnel will remove all personal identifiers from clinical data and assign a study ID number to each subject. Only the Study Doctor, designated study team members, and designated OICR genomics staff (i.e. the ACMG/CCMG licensed Geneticists who will sign out each clinical report) will be able to link the clinical data to the personal identifying information using the study ID. A master file linking the participant with the study ID will be kept in secure locations on secure computers. All other databases will include only a unique study ID number and no personal identifying information. The study ID will be recorded on the medical history and demographic questionnaires. Patient name, DOB or MRN will not be recorded.

Patient outcome questionnaires will be labelled with the study ID. The analysis of questionnaires will be done jointly with Dr. Yvonne Bombard at Unity Health. Limited medical information (including demographic information, genetic diagnosis, cancer history) will also be shared with Dr. Bombard, to aid in the interpretation of the questionnaires. If participants consent to the interview portion of the study, Dr. Bombard's team will contact participants directly to arrange the interviews. Participants will be informed in the consenting process that the above information will be shared with Dr. Bombard's team, and that they be contacted by Dr. Bombard's team to arrange an interview. No sharing of data will occur until Dr. Bombard receives REB approval

from Unity Health for this study, and appropriate DTAs are established between institutions.

This study may include multiple sites in Ontario (UHN, Mount Sinai Hospital, and Sunnybrook Hospital), and across Canada (BC Cancer, Jewish General Hospital, IWK Health, Eastern Health). We would like to share de-identified of clinical data (e.g. genetic diagnosis, results of diagnostic imaging) between sites, which will be important for joint review of difficult cases (e.g. if follow-up clinical imaging for a positive cfDNA test result is inconclusive). Only the Study Doctors at each site, and designated members of the study team will be able to access this information. Data sharing between sites will only begin once each site receives REB approval for the study, and after appropriate DTAs have been signed by all institutions. Participants will be informed in the consent process that de-identified clinical data may be shared between study sites for the above purposes.

The study will utilize a variety of software systems for the storage, tracking and management of data derived from this study. Data will be stored either on RedCAP, a secure database created by Trevor Pugh Lab's (e.g. OncoGrapher) housed on a UHN server, or on another secure server at UHN. Each site in this study will be able to upload de-identified clinical information for their own patients (identified only by a study ID). The UHN site will maintain a study database of all enrolled study subjects, while each site will maintain a study database of subjects they recruited at their site.

11.8 **Data Sharing**

Participants will be given the option to allow the study team to use their study data in future research aimed at further improvement to the cfDNA blood test, and/or future health care utilization research. Participants will also be given the option to allow the study team to share their de-identified data with other researchers through both open-access databases (publicly accessible database that contains limited clinical information and sample analyses) and controlled-access databases (more detailed clinical information and the results of prior and ongoing treatments, sample analyses; this is only accessible to collaborating researchers). For example, de-identified genomic data may be uploaded to the secure UHN cBioportal instance. cBioportal is a controlled-access database, which requires login credentials and limit users to specific datasets.

The CHARM Consortium steering committee composed of one individual from each participating institution has already been established and will govern use of the genomic and clinical data for secondary research studies. All secondary studies will require REB approval from the investigator's host institution.

With consent, unused samples collected in this study (e.g. from the control cohort, or left over samples from the experimental cohort) will be stored with the CHARM Consortium Biobank (CAPCR 23-5772). The use and sharing of these samples for future studies is outlined in the CHARM Consortium Biobank protocol.

11.9 **Incidental Findings**

Due to the nature of genomic sequencing, findings from this research (medically actionable variants) may have implications on the health of study participants or their family. In the event that a pathogenic germline variant is identified in a disease-relevant gene that may have implications for the health of the participant and his/her blood relatives, participants are asked to indicate whether they wish to be informed of such incidental germline findings. Alternatively, participants may indicate that they do not want to be told of clinically relevant incidental germline findings. For participants who wish to be informed, the study doctor or treating physician will contact the individual (or his/her specified delegate in the event that the patient is unwell or deceased), to inform him/her that a potentially clinically relevant incidental finding has been identified without disclosing the specific variant identified or its health implications. Participants will be offered the opportunity for rapid referral to a local medical genetics clinic (e.g. where the patient was first identified to have their HCS mutation), where a genetic counselor will review the medical, familial, and insurance considerations of disclosure of the incidental germline findings before the information is shared with the consenting patient or his/her delegate.

11.10 **Research Ethics Board approval**

No work will be conducted on this study until its approval by the UHN Research Ethics Board. No changes will be implemented until they have been approved by said Board.

12.0 Data Handling and Record Keeping

12.1 **Data Recording**

Patient identifiers (name, DOB, MRN) will be kept separate from their clinical information and will not be included in any publications. Clinical information recorded outside of a chart will be de-identified and referred to only by the study ID (e.g. CHM-001) This number will be assigned to participants that meet inclusion criteria by study investigators.

12.2. **Source Data**

Information regarding the patients' clinical history (cancer history, cancer treatment, medications, other medical conditions), their family history of cancer, and surveillance with respect to cancer will be collected from electronic/paper medical records, a study questionnaire (Demographics and Medical History Questionnaire), or from the patient directly. In addition to clinical information, results of germline, tumour, and cfDNA genetic analysis will be collected.

12.3 **Record Storage**

Data collected above will be stored in a secure UHN database, only accessible by delegated study personnel. Presently, multiple secure databases are being tested for data storage on a secure UHN Network, or a secure cloud approved by UHN privacy and security. Data will

be stored either on REDCap, a secure database created by Trevor Pugh Lab's (e.g. OncoGrapher), or on another secure server at UHN. All records and documents pertaining to the study will be retained by the study trial site at UHN for 10 years from the completion of the study.

Authorized study team members will upload de-identified clinical data into a secure database (e.g., REDCap or Oncographer). The database will only be accessible to designated study team members, as permitted by REB, and will require user login to access the data. Study IDs will be used to link data captured in the database to study participants. Physical and/or electronic copies of the signed consent forms and medical history questionnaires will be stored by the study site in a secure location (locked cabinet in a locked office at Princess Margret Cancer Centre) or on a secure network accessible only to the principal investigator and designated study team members.

De-identified raw genomic data generated from analyses performed during our study will be transferred from OICR to UHN, and stored on hospital servers and may be uploaded to the secure UHN cBioPortal instance. OICR is only providing a service and will not retain the results of testing.

14.0 References

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13.0 Supplemental Material

The following supplemental material is appended to this application:

Medical History and Demographics Questionnaire

Study Information Sheet

Study Contact Form

Mail Out Letter

Email Invitation

Telephone Recruitment Script

Study Brochure

Patient Outcome Questionnaire

Negative/Positive Result Information Letters

OICR Negative Report

CHARM Phase 2 Study Consent Form

CHARM 2 Optional Data Sharing Consent Form