CLINICAL STUDY PROTOCOL

CHARM: CfDNA in Hereditary And High-risk Malignancies

Retrospective Study Protocol

Protocol Summary

CHARM: CfDNA in Hereditary and High-risk Malignancies – Retrospective Study
Clinical Study

Study Population
Our study focuses on patients with a known/suspected hereditary cancer syndrome

Primary Objective
To determine whether newly developed cfDNA analytical technologies can detect/monitor cancer in patients with a known or suspected hereditary cancer syndrome (HCS).

1.0 Background Information

1.1 **Background Summary**

Hereditary Cancer Syndromes

Across Canada, 5-10% of all cancers develop in individuals with a strong genetic predisposition to cancer. This predisposition is inherited within families and is caused by genetic abnormalities in genes that usually suppress cancer. Individuals affected by these hereditary cancer syndromes (HCS) undergo costly annua l screening using physical exams and imaging. Despite these best efforts, cancer is often not detected until it is at a late stage or in an organ that does not have an effective screening method. In these cases, patients incur high costs to the health care system to treat their advanced cancer and may still succumb to their disease. The key to preventing these tragedies is early detection of disease, ideally using a sensitive, quantitative technique that can screen all at-risk organs for cancer simultaneously, such as circulating tumour DNA (ctDNA). We will develop this method to detect early signs of cancer in 'previvors'; people with HCS that do not yet have a cancer diagnosis. This will enable prediction of cancer onset so that patients and their doctors can make decisions to treat or prevent the cancers. HCS patients will be recruited from across Canada to provide blood samples before and after cancer diagnosis. In parallel, we will develop a ctDNA-based test to detect early stage cancer and work with experts to determine the cost-effectiveness. In concert, consultation with patients and health care providers will occur to create recommendations for use within clinical care.

Oftentimes cancer develops when an important tumour suppressor gene becomes inactivated and can no longer produce the proper functional proteins to monitor, and limit, cellular growth. Inactivation of a tumour suppressor occurs through two hits, where one hit inactivates one allele and a second hit inactivates the remaining allele. For individuals with a HCS, the first-hit to the tumour suppressor is preexisting in the form of an inherited or *de novo* mutation. Depending on the gene and its essentiality to a given tissue, this first-hit can accelerate the onset of cancer by >30 years and amplifies the risk of developing multiple cancers throughout a lifetime. As first-hits are frequently inherited from parents, multiple family members can be at increased risk, as are future generations. These

"previvors" carry a first-hit in all of their tissues and are at risk of developing a second hit in a tissue which would result in cancer development. It is not unusual for numerous second hit events to happen, resulting in multiple cancer diagnoses. While each HCS follows a predictable two-hit model of cancer development, each syndrome has different cancer risks and affects different organ sites.

Of the >206,000 Canadians diagnosed with cancer every year, at least 5-10% are affected by a HCS. Individuals with HCS often have multiple family members affected with similar cancers, which frequently occur at a younger age. Some well-studied HCS for which established surveillance programs are available include the following:

A. Hereditary breast and ovarian cancer (HBOC), caused by germline BRCA1 and BRCA2 mutations: The carrier rate for HBOC in the general population is approximately 1/500. Mutations in *BRCA1* and *BRCA2* confer an increased lifetime risk of 40-90% for breast cancer, 10-60% risk for ovarian cancer, 20% risk for prostate cancer, 7-8% risk for male breast cancer, and 2-7% risk for pancreatic cancer. Patients with HBOC are eligible for participation in provincial programs such as the Cancer Care Ontario (CCO) High Risk Ontario Breast Screening Program (OBSP). HBOC patients are offered annual breast screening with mammography and breast MRI beginning at 25 years of age, as well as prophylactic mastectomy and oophorectomy that decreases breast and ovarian cancer risk and improves overall survival. While combination mammography and MRI has a high sensitivity for detection of breast cancer (93–100%), there is no current screening test for ovarian cancer. As a result, these aggressive tumours are often caught late with grim prognosis. This is an opportunity to significantly impact the 5-year survival rate of 17% for late stage ovarian cancer.

B. Lynch syndrome (LS), caused by germline mutations in the mismatch repair genes (MLH1, EPCAM, MSH2, MSH6, PMS2)**:** LS follows a similar trend, wherein patients have a lifetime risk of 50-80% for colorectal cancer, 25-60% for endometrial cancer, 6-13% for gastric cancer, 4-12% for ovarian cancer, and 1.4-4% for hepatobiliary cancer, among other cancers. Outside of biennial colonoscopy to detect adenomas and early colorectal cancer, there is no evidence that any screening tool works to detect other cancers that may arise in these patients. As a result, patients with LS have an up to 60% lifetime risk of developing life threatening endometrial, gastric or ovarian cancer with no available screening test.

C. Li-Fraumeni syndrome (LFS), caused by TP53 mutations: For Li-Fraumeni syndrome, where the lifetime cancer risk approaches 100% in *TP53* carriers (93% in females, 75% in males), an intensive surveillance protocol is implemented immediately after birth or determination of *TP53* mutation status. This includes annual MRIs (whole body, brain and breast), biennial colonoscopy, semi-annual abdominal ultrasound and bloodwork.

D. Neurofibromatosis Type 1 (NF1), caused by loss-of-function mutations in the NF1 gene*:* NF1 represents the most common hereditary cancer syndrome in children (~1/3000). Children and adults with NF1 are predisposed to extensive tumorigenesis, including benign and malignant tumours that can develop in multiple areas of the body. These neoplasms

often appear at relatively young ages and include tumours of the nervous system (central and peripheral), breast cancers, gastrointestinal stromal tumours (GIST), and pheochromocytomas, among others. Although the disease is fully penetrant, there is marked inter- and intra-familial variation in clinical presentation. In particular, the emergence of cancer is highly unpredictable and afflicts both pediatric and adult NF1 patients. Guidelines for NF1 management do not recommend routine cancer surveillance with imaging, and many tumours are detected upon the presence of symptoms- often at a late stage. As such, cancer remains the leading cause of mortality in this patient population (with an excess of deaths in individuals < 40 years old). Indeed, NF1 individuals live an average of 15 years less than their unaffected counterparts due to their cancer risk.

In addition to the cancer predispositions described above, many other HCS suffer from challenges with effective screening, resulting in some patients diagnosed with late stage cancers. Thus, an improvement in screening for high-risk individuals is warranted.

Cell-free DNA

Fragments of DNA released by dividing cells into the bloodstream have long been known to be present in the blood plasma of patients with a variety of medical conditions, this is referred to as cell-free DNA (cfDNA). In the cancer field, fragmented DNA derived from a tumour cell is referred to as circulating tumour DNA (ctDNA) and may be a component of cfDNA. Detection of ctDNA has a variety of applications including cancer diagnosis, response and follow-up, and monitoring of tumour evolution and residual disease. cfDNA analysis can 1: enable detection of early stage cancers through broad mutational profiling and 2: establish the tissue of origin of tumour-derived DNA through cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP). Using cfDNA technology holds promise in improving early disease detection in individuals with HCS.

Over the past decade, cell-free DNA (cfDNA) has emerged as a useful biomarker for cancer diagnosis, response and follow-up (Wan et al. 2017; Polivka, Pesta, and Janku 2015). However, cfDNA-based assays have not been regularly used in clinical care due to inadequate detection limits (Merker et al. 2018; Burgener et al. 2017). We wish to evaluate whether novel cfDNA profiling technologies with high sensitivity can improve cancer care for patients with a known/suspected HCS.

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 tumor-specific mutations are also identified, these can likewise be used to monitor disease progression via cfDNA analysis. We wish to test whether profiling cfDNA from HCS patients can be used to detect cancer. This can be accomplished using four newly-developed cfDNA technologies:

1. To analyze cfDNA mutations, we will use a cfDNA sequencing assay, termed *Liquid Biopsy Sequencing* (LB-Seq), that uses hybrid-capture and ultra-deep sequencing to achieve >20,000X coverage. Exon-level copy number alterations and

somatic mutations can be detected using several algorithms(Kis et al. 2017). This can be used to interrogate the sequence of genes involved in cancer pathogenesis (e.g. *TP53*) or to profile immune cells (T/B-cell receptor sequencing).
2. To analyze cfDNA mutations, we will also use 30x plasma whole genome sequencing (pWGS). Exon-level and intron-level mutations can be detected.
3. To detect large copy number changes in cfDNA, we plan to use shallow whole genome sequencing (sWGS) to profile the entire cfDNA genome at very low sequencing depth.
4. Since HCS patients develop cancer in different organs, we plan to use *cell-free Methylated DNA Immunoprecipitation and high-throughput sequencing* (cfMeDIP-seq) or Enzymatic Methyl Sequencing (EM-Seq) to reveal the tumor's tissue-of-origin. This involves surveying the epigenetic marks of tumour-derived cfDNA (Shen et al. 2018).

Upon completion of this study, we will have determined whether these assays can be implemented to monitor HCS malignancies.

1.2. Rationale for the study

There is a significant need for novel tests that can identify cancer at early stages, when curative interventions are still available. This is particularly true for patients with HCS, who are at high risk of developing multiple malignancies in their lifetime. In recent years, cfDNA has emerged as a promising biomarker of cancer. By leveraging recent advances in sequencing technology, we wish to develop a minimally invasive cfDNA-based assay that can improve tumor detection in HCS individuals and guide subsequent management decisions. In addition, to assess whether widespread adoption of these assays is feasible, we will identify factors that promote (or detract) from their future clinical implementation.

2.0 Study Objectives and Hypothesis

2.1 Study objectives

To improve early cancer detection, we will: 1) build a HCS plasma and tumour biobank; 2) develop a cfDNA test for HCS and profile previvors to enable early cancer detection; and 3) gather preferences of patients and providers and perform a cost-effectiveness analysis with recommendations for implementation of cfDNA testing into health care systems.

2.2 Study hypothesis

We hypothesize that cfDNA sequencing and epigenetic profiling can be used to detect cancer in HCS patients.

3.0 Study Design

A HCS plasma and tumour biobank will be developed with specimens collected from both with and without a previous or current cancer diagnosis. Plasma and buffy coat will be collected from all individuals at least once per year and tumour tissue will be banked or requested from individuals with previous or active cancer, when available.

3.1 Biological Sample Collection

Patients will be asked to provide blood samples (where applicable):

- Baseline (time of enrollment)
- Clinic visits (typically every 6-12 months)
- Around the time of imaging or procedures
- Before treatment starts
- After the first dose of chemotherapy
- End of treatment
- Recurrence/Progression

Blood samples will be collected at UHN or at an off-site blood lab. We will be offering mobile phlebotomy using commercial blood laboratories (e.g., Dynacare and LifeLabs). The optimal location for blood collection will be determined based on the patient's preference.

Patients will have up to 30mL of study blood collected in 3 x 10mL Streck tubes (or other tubes if deemed appropriate). Wherever possible, patients will have research blood samples collected for the study at the same time as routine blood collection for clinical purposes, to avoid additional venipunctures. Patients who choose to have study blood drawn at an external laboratory will be given a study kit containing three 10mL Streck tubes (if not provided by the external laboratory), a blood draw requisition, and a shipment label for return of blood samples.

After blood draw, the blood will be processed (centrifuged with plasma and buffy coat separation). Samples will be stored in -80oC or liquid nitrogen until requested by the study team.

If available, an archival formalin-fixed paraffin-embedded (FFPE) tissue sample (up to 15 unstained sections at 7 microns thickness plus 2 hematoxylin and eosin (H&E)-stained slides) or fresh frozen tissue from a prior biopsy or surgery may be requested during the study and used for our research. Additional archival tissue samples may be requested and banked for future research. During their participation in the study, patients who have a surgery, biopsy, or other procedure as part of their care *outside* of this study's specified investigations will have an additional sample of tissue, blood, or fluid collected for research in this study if it is feasible and safe, as judged by the staff surgeon or radiologist performing the procedure. Standard operating procedures will be followed for the collection and processing of surgical specimens and core biopsies, both fresh frozen and

formalin-fixed paraffin embedded. Surgical tissue selection will be performed by trained personnel.

Germline and tumour tissue will be profiled using whole genome sequencing, T/B-cell Receptor sequencing, whole genome bisulphite (i.e. methylation) sequencing, and targeted gene panel sequencing to establish HCS tumor mutational signatures. This will allow us to investigate whether tumor-specific mutations are detectable in cfDNA. LB-Seq, T/B-cell Receptor sequencing, sWGS, and cfMeDIP-seq will then be employed to (a) determine if tumour-derived genomic alterations are detectable in cfDNA, and (b) test whether a malignancy's tissue-of-origin can be inferred from cfDNA epigenetic marks. To assess whether these technologies can be used to monitor 'previvors' (i.e. HCS patients that are pre-symptomatic), we plan to analyze cfDNA signals in serially collected samples from different stages in the HCS lifecycle (i.e. before and after tumors have developed, as well as during treatment).

To complement the data generated through the CHARM study, we will access external datasets. These data may include methylation arrays, bisulphite sequencing, whole genome sequencing, and targeted panel. The datasets may be publicly available or accessible through databases that require approval such as EGA, dbGaP, or ICGC. In addition, we may access data derived from companies, once contracts/agreements are executed.

Standard operating procedures will be followed for the labeling and tracking of specimens (fresh frozen, FFPE) and blood samples. Samples will be processed and stored along with tumor tissue or tissue derivatives in a biorepository. Each specimen will be uniquely identified by a study ID and kept for up to 25 years.

3.2 Clinical Data Collection

All patients enrolled in this study will have the following essential clinical variables collected from their medical records:

1. Demographic information (sex, truncated date of birth- year and month, ethnicity, smoking status)
2. Basic medical information (medical history, current medications)
3. Cancer history and treatment (FIGO stage, TNM, size, histology, grade, date of diagnosis, tumour site, treatment type/dose/duration and treatment response- including survival)
4. Genetic diagnosis and date
5. Family history of cancer
6. Anthropometric measures (height, weight, BMI)
7. Standard molecular pathology information (microsatellite status, ER/PR/HER2 status, MMR/IHC status on tumours)
8. Date of blood sample and tumour tissue collection, if applicable
9. Site of tumour sample collection and indication or whether site is primary or metastatic, if applicable

10. Survival status (date of last contact, vital status, date of death)
11. Imaging results and date (mammography, breast MRI, breast ultrasound, transvaginal ultrasound)
12. Screening information and date (modality, frequency, CA-125 result, colonoscopy result)

Participants will be followed over the course of the study and any updates to medical history and screening results will be collected. Response to any relevant treatment, survival status and cause of death will be collected after the course of the study, if relevant. Follow up may include a review of medical records and further contact with study participants.

This data will be stored in a password protected database. Personal identifying information is required to access data from hospital records and to contact participants to determine the outcome of their treatment. However, to protect patient confidentiality, study program personnel will remove all personal identifiers from clinical data and assign a study ID number to each subject. Researchers, data entry staff, and statisticians will only receive clinical data that is identified by study ID. Only the Principal Investigators and designated study coordinators will be able to link the clinical data to the personal identifying information.

3.3 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 patient and his/her blood relatives, patients are asked to indicate whether they wish to be informed of such incidental germline findings. Alternatively, patients may indicate that they do not want to be told of clinically relevant incidental germline findings. For patients who wish to be informed, a study Principal Investigator or the treating physician will contact the patient (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. Patients will be offered the opportunity for rapid referral to a local medical genetics clinic, 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.

3.4 Data Sharing

In the past decade, large consortia have developed to allow different centres to send limited de-identified clinical data associated with molecular profiling. These strategies allow larger numbers of groups to combine data to increase the power to identify important profiling associations with outcomes. Examples include the US NIH-funded TCGA (The Cancer Genome Atlas), the International Cancer Genome Consortium, the Global Alliance of Genomics and Health to Enable Responsible Sharing of Genomic and Clinical Data,

multiple germline consortia for disease sites (such as BEACON, ILCCO, and the Head and Neck Cancer GWAS Consortia), and the American Association for Cancer Research (AACR) Genomics Evidence Neoplasia Information Exchange (GENIE) project. All participants will be asked to provide consent for sample and data sharing with other cancer 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).

3.5 External Collaborators

There is a potential that some samples may be analyzed by an external commercial entity or collaborator. Materials that may be sent include, but are not limited to, plasma, buffy coat, cfDNA, and/or tumor FFPE samples as required by the commercial entity to do their analysis. Analysis is dependent on the commercial entity and can include, but not limited to, tumor exome sequencing and/or various types of cfDNA assays. The study plan for any analysis with a third party will align with the objectives of the separate primary research study protocol in which sample analysis is described.

De-identified clinical data (which will meet the UHN de-identification policy, would be provided to the collaborator on the scientific requirements for the analysis) may be provided to the party to aid in analysis if it is a collaborative partnership. The exact type of data to be sent will be specific to fulfill the study objectives as outlined in the separate primary research study protocol. Data can include, but is not limited to, demographic information such as age, sex and histology of cancer, and outcome and survivorship status. This data would be used to correlate with the sequencing data. The collaborator's rights to use the de-identified clinical data, outside of the study, will be limited (any rights to use data that is PHI will need to have additional REB review).

3.6 Risks and Benefits

<u>Potential Risks</u>

The only physical risk by taking part in this study is the potential for bleeding, bruising, discomfort, infections or pain at the needle site, or dizziness from the needlestick to take the blood samples. There are no physical risks to the participant in releasing archived tumor samples for the purpose of this study since the tissue has already been obtained by a previous biopsy/surgical 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.

The other risk to participants is the risk of re-identification. To prevent this, all samples and data collected within this study will be identified by a unique study ID (study code) and all samples will also be tracked by a barcode. Samples will be held in a secure facility and samples will not be identified by participant name.

Potential Benefits

There may be no direct benefit to participants in this study. The result of this study may or may not help a patient's physician determine whether a HCS patient has cancer, or how it is responding to treatment.

4.0 Selection of Subjects

4.1 Subject inclusion criteria.

Patients must fulfill the following criteria;
1. Patients that are high-risk for cancer (i.e. patients with an identified pathogenic variant in a cancer predisposition gene and/or a family history of cancer without a known aberration).
2. Patient must be ≥ 18 years old.
3. All patients must have signed and dated an informed consent form.

4.2 Subject exclusion criteria.

None

5.0 Ethics

5.1 Recruitment & consent process

Active HCS Patients: Eligible patients currently receiving care at UHN (e.g. cancer surveillance, cancer treatment, genetic counselling, etc.) will be approached by a CHARM study member during their clinical visit and recruited into the CHARM study. 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 the patient expresses interest in hearing more about our study a member of our team will approach the patient to obtain consent.

If a CHARM study member is unable to approach the interested patient in-person during their clinical visit, we will recruit the patient via telephone (please see recruitment script). All study-related telephone conversations will be received/initiated in a secure area to ensure the protection of confidential patient information. If patients express interest in participation over the phone, we will send them two copies of the consent form via mail or a secure file portal (i.e. email), along with a stamped self-addressed return envelope (if delivered by mail). After establishing a time for follow-up, CHARM study members will contact the individual via telephone to explain the study 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 form and send it to us via mail, or scan the signed consent and send it to us via email (CHARM@uhnresearch.ca). If they choose to send it via email, they will be reminded that this is not a secure way of sending information. The participant will retain the

remaining copy of the consent form for their own records. 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 with 4 weeks later if forms are incomplete or have not been returned, or if blood has not been collected.

Inactive HCS Patients: If we retrospectively identify eligible patients who (a) are not currently receiving care at UHN, or (b) have been transferred from UHN to another health care institution, we will send these patients a recruitment package (see below) 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 the CHARM study, as well as CHARM contact information so that patients can self-refer, if interested.

If the individual is not interested in participating, they will be asked to opt-out of future study-related communication by contacting study members via telephone or email no later than 4 weeks after receiving the invitation. If the individual expresses interest in our study via telephone, mail, or email, study members will contact the individual via telephone to explain the CHARM 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. The participant will retain the remaining copy of the consent form for their own records. 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.

Patients who previously consented to being contacted for research purposes: 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. If they are interested, they will be consented into CHARM during their next clinical visit at UHN, or via telephone (in the same manner as indicated above).

Regular reminders for consented patients: Participants will be advised that study members may contact them via telephone or email to remind them about their next blood donation, and/or to coordinate blood donation at UHN or an off-site blood collection lab.

5.2 Obtaining informed consent from research participants.

The Principal Investigator (PI), Co-Investigators (Co-I), or study team members will obtain consent for this minimal-risk, non-therapeutic study.

5.3 Data collection/confidentiality.

The CHARM consortium will utilize a variety of software systems for the storage, tracking and management of data derived from this study. The databases will be housed at UHN. Each site in the consortium will be able to upload clinical information remotely for their own patients. UHN will be able to view de-identified information from all participants (identified by a study ID); each site will be able to view information for their own participants.

5.4 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.

6.0 Data Handling and Record Keeping

6.1 Data Recording

Patient identifiers 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 a patient number. This number will be assigned to patients that meet inclusion criteria by study investigators.

6.2. 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 recorded. Patient data collected through LIBERATE will also be documented. In addition to clinical information, results of germline, tumor, and cfDNA genetic analysis will be collected.

6.3 Record Storage

Data collected through LIBERATE and or the CHARM study is stored in a secure UHN database, only accessible by delegated study personnel. All records and documents pertaining to the study will be retained by the study trial site at UHN for at least 25 years from the completion of the study.

7.0 References

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