**Perspective**

Current and new frontiers in hereditary cancer surveillance: Opportunities for liquid biopsy

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**Summary**

At least 5% of cancer diagnoses are attributed to a causal pathogenic or likely pathogenic germline genetic variant (hereditary cancer syndrome—HCS). These individuals are burdened with lifelong surveillance monitoring organs for a wide spectrum of cancers. This is associated with substantial uncertainty and anxiety in the time between screening tests and while the individuals are awaiting results. Cell-free DNA (cfDNA) sequencing has recently shown potential as a non-invasive strategy for monitoring cancer. There is an opportunity for high-yield cancer early detection in HCS. To assess clinical validity of cfDNA in individuals with HCS, representatives from eight genetics centers from across Canada founded the CHARM (cfDNA in Hereditary and High-Risk Malignancies) Consortium in 2017. In this perspective, we discuss operationalization of this consortium and early data emerging from the most common and well-characterized HCSs: hereditary breast and ovarian cancer, Lynch syndrome, Li-Fraumeni syndrome, and Neurofibromatosis type 1. We identify opportunities for the incorporation of cfDNA sequencing into surveillance protocols; these opportunities are backed by examples of earlier cancer detection efficacy in HCSs from the CHARM Consortium. We seek to establish a paradigm shift in early cancer surveillance in individuals with HCSs, away from highly centralized, regimented medical screening visits and toward more accessible, frequent, and proactive care for these high-risk individuals.

**Introduction to hereditary cancer syndromes**

Individuals with hereditary cancer syndromes (HCSs) are born with a pathogenic or likely pathogenic germline gene variant resulting in a predisposition to cancer development, often in multiple organs, throughout their lifetime. Five percent of all cancer diagnoses are estimated to have an underlying genetic cause. To date, more than 100 genes have been identified as being linked to HCSs; some of these result in a 100% lifetime cancer risk.1 Individuals with these conditions describe themselves as “ticking cancer time bombs,” as they await the accumulation of somatic gene alterations that will trigger cancer development.2 HCSs can affect multiple family members, both children and adults; the majority of HCSs are inherited in an autosomal-dominant fashion.1 The management of individuals with an HCS hinges on the identification of a causative germline pathogenic variant, leading to appropriate surveillance and treatment strategies.

Four of the most common and well-described HCSs are (1) hereditary breast and ovarian cancer (HBOC), (2) Lynch syndrome (previously called hereditary non-polyposis colorectal cancer syndrome), (3) Li-Fraumeni syndrome (LFS), and (4) neurofibromatosis type 1 (NF1). HBOC is caused by heterozygous pathogenic variants in either *BRCA1* or *BRCA2*, and individuals are at increased risk for developing various cancers, which can include female and male breast, ovarian, prostate, and pancreatic cancers.1 These cancers often exhibit genome alternations resulting from homology DNA-repair deficiency (HRD). Individuals with Lynch syndrome (caused by pathogenic variants in *MLH1*, *MSH2*, *MSH6*, or *PMS2* or by a deletion in the 3′ exons of *EPCAM*) are prone to cancers of the colorectum; endometrium; ovary; stomach; small bowel; ureter and/or kidney; urinary bladder; prostate; and brain. For individuals with Lynch syndrome, the specific mutated gene affects the associated risk per type of cancer.1 These cancers exhibit microsatellite...
instability reflective of DNA mismatch repair deficiency (MMRD). LFS, caused by a pathogenic variant in TP53, has been primarily associated with frequently occurring “core” cancers: soft tissue sarcomas, osteosarcomas, brain tumors, premenopausal breast cancer, and adenocortical carcinoma; however, the tumor spectrum has since widened to include hematologic malignancies and neuroblastoma, as well as lung, skin, gastrointestinal-tract, kidney, and thyroid cancers, among others.5–7 These cancers are driven by a wide range of molecular mechanisms, initiated in many cases by secondary loss of p53 activity.5 Children and adults with NF1 (pathogenic variants in NF1) may develop multi-system manifestations, including benign and malignant tumors of the central and peripheral nervous system, breast cancers, gastrointestinal stromal tumors (GIST), and pheochromocytomas.8 Individuals with NF1 often develop plexiform neurofibromas (pNFs), which require strict monitoring and are at risk of transforming into malignant peripheral nerve sheath tumors (MPNSTs), an aggressive sarcoma.9 There are currently no molecular tests predictive of transformation from pNFs to MPNSTs in individuals with NF1.

Incomplete precision cancer therapy options are available for HCSs

The phenotypic manifestations of individuals with an HCS seldom fall within the scope of a single medical specialist, and individuals who harbor a pathogenic variant often undergo lifelong surveillance to improve identification in early disease states and treatment strategies. Many surveillance guidelines exist for HBOC, Lynch syndrome, LFS and NF1; however, guidelines are variable between jurisdictions and working groups, including the National Comprehensive Cancer Network,10,11 the American Association for Cancer Research,9,12 the European Reference Network GENTURIS,13,14 the European Society for Medical Oncology,15 and the UKCGG Consensus Group.16

The most common surveillance strategies are diagnostic imaging and endoscopy of the organs at highest cancer risk for each HCS; however, this does not protect against unexpected and rare tumors. For individuals with a BRCA1/2 pathogenic variant, dynamic contrast-enhanced breast MRI has been identified as the most sensitive screening modality, in comparison to mammography or ultrasound.17–19 Although guidelines for Lynch syndrome include recommendations for colonoscopy, periodic upper endoscopy exams and endometrial biopsy, these modalities are not very effective, and early cancer detection rates are low for gastric, duodenal, small-bowel, urothelial, and endometrial cancers.9,20 As a result of the high lifetime risk of developing cancer for those with a TP53 pathogenic variant, intensive surveillance with frequent diagnostic imaging from birth (or time of diagnosis) via the “Toronto protocol” have demonstrated improved clinical outcomes.21,22 Whole-body MRI is recommended from an early age; however, this process has been described as time consuming and uncomfortable and has led to increased anxiety in family members.23 The emergence of malignant disease in NF1 is highly unpredictable,9 meaning that although guidelines for NF1 management have been published for both children24 and adults,25 surveillance between regions is variable.

Men with hereditary cancer are less likely to undertake health-seeking behaviors, including genetic testing, when compared to their female counterparts, in part due to the notion of their masculinity.26 For example, BRCA1/2 germ-line pathogenic variants are more commonly associated with breast cancer and the female gender, which might prevent men from seeking medical advice.27 In addition, the majority of studies focus on the psychosocial impact of BRCA1/2 variants in women, as a result of their greater risk of developing cancer, and limited attention is given to men undergoing the same diagnosis.

Once detected, the cancer management of each HCS is tailored to the defective gene, and many precision cancer treatment options are based on genotype. This includes poly-ADP-ribose polymerase (PARP)-inhibitors for cancers with homologous repair deficiency in HBOC,28,29 MEK (mitogen-activated extracellular signal-regulated kinase) inhibitors in NF1,30,31 immune checkpoint inhibition in individuals with colon cancer and Lynch syndrome,32 and avoidance, when feasible, of radiation in those with LFS33 and 5-fluorouracil-based chemotherapy in those with Lynch syndrome.34

Most organs have a screening modality recommended in clinical care, and many techniques are successfully used to identify cancer. However, not all at-risk organs have effective screening modalities—for example, ovarian cancer in HBOC. Although CA-125 (cancer antigen 125) is currently the best-characterized biomarker used for diagnosis and management, it lacks the sensitivity and specificity needed for routine screening purposes.35 For at-risk organs lacking an effective early cancer modality, aggressive tumors are often identified at later stages, leading to high mortality rates.36,37 As a prevention of primary manifestations for those with a BRCA1/2 or TP53 pathogenic variant, bilateral mastectomy is an option to prevent breast cancer; prophylactic salpingo-oophorectomy after childbearing is offered as a way to reduce the chances of ovarian cancer in HBOC.33 Risk-reducing salpingo-oophorectomy in a meta-analysis of women with BRCA1/2 pathogenic variants reported an approximate 80% reduction in ovarian cancer mortality and a reduction in all-cause mortality.38 Although some current screening methods work well, they are reliant on adherence of those with an HCS, and there are many barriers affecting compliance to current surveillance protocols.39

Issues with current hereditary-cancer-syndrome surveillance protocols

Access to screening programs is a challenge for individuals with HCSs because of the reliance on centralized clinical
screening expertise and equipment that requires individuals to travel hundreds or thousands of kilometers annually (or more frequently) for screening. This access barrier is amplified in remote communities, especially in geographically large countries that are systematically under-served with a lack of local screening programs. A lack of medical HCS awareness, complex referral criteria, and regional and institutional differences in screening recommendations can lead to fragmented care across the country, even within the same family. Individuals with LFS reported surveillance-related logistical issues, including time commitment, costs associated with traveling to the central location multiple times a year, and cost and/or coverage of insurance.

Although individuals with an HCS generally see the benefit in surveillance, the techniques are often burdensome or unpleasant. Those with Lynch syndrome have described surveillance as overwhelming; scheduling appointments and waiting for test results is physically exhausting, time consuming, and burdensome. Some individuals with LFS describe their surveillance as “aggressive screening” and are non-compliant because they believe that screening is not useful, because they believe their lack of symptoms means there is no need for screening, because they fear tumor detection, or because the process is too time consuming.

A recurring issue reported by individuals with HCS is the lack of knowledge by medical professionals and a lack of follow-up care/coordinated approach, resulting in individuals being their own advocates and responsible for managing their own surveillance and care. There are inconsistencies between health care providers, as some are not as well-informed about HCS, not all accept risk-reducing surgeries as an option, and physicians interpret and adapt management guidelines, particularly related to frequency and age of surveillance. Furthermore, individuals may deem available cancer prevention options as insufficient.

For some HCS, surveillance protocols are lacking, and surgery is proposed as the most effective option. For those with Lynch syndrome, prophylactic gynecological surgery is the most cost-effective method (in terms of incremental cost-effective ratios, years gained, and quality-adjusted life-years gained) for endometrial cancer when this option is compared to transvaginal sonography, CA-125, or endometrial biopsy. Quality adjustment or quality-adjusted life-years (QALY) is a measure of disease burden used for assessing the value of health outcomes. Furthermore, researchers used a cohort-level Markov simulation model to examine MLH1, MSH2, MSH6, and PMS2 and the natural history of gynecologic cancer and found optimal strategies (e.g., ages of risk-reducing hysterectomy and bilateral salpingo-oophorectomy) related to cancer risk, and cost effectiveness differed by gene. Similarly, Markov modeling with simulations and probabilistic sensitivity analyses for women with a BRCA1/2 pathogenic variant identified prophylactic bilateral salpingo-oophorectomy alone (with quality adjustment), and prophylactic oophorectomy with mastectomy (without quality adjustment) as the most cost-effective methods. However, oophorectomy might lead to hormone imbalances and infertility, as well induce surgical menopause that increases the risk of cardiovascular disease, osteoporosis, reduced libido, vaginal dryness, and vasomotor symptoms.

Similar to those undergoing cancer screening in the general population, individuals with HCS may experience “scanxiety,” a colloquial term used to describe the anxiety, discomfort, and nervousness that they experience before having their cancer scans and while awaiting their results. Although distress is not higher in individuals with cancer risks that are not typically associated with effective surveillance (pancreatic, ovarian), it is increased in females, those who have previously had a cancer diagnosis, and those with a first-degree relative who died from cancer. Risk-reducing surgery is higher and/or earlier in women who experience guilt and fear that their children may inherit the same deleterious BRCA variant, those without children or who have at least one daughter, and women who have first-degree relatives who have had breast or ovarian cancer or who have young children.

Creation of the CHARM consortium

With the aim of improving HCS care, including surveillance and cancer early detection, we assembled the CHARM (cfDNA in Hereditary and High-Risk Malignancies) Consortium in 2017 (Figure S1A). Because of the rarity of HCS and the geographical size of Canada, multiple centers are engaged, including BC Cancer (covering all of British Columbia and Yukon); University Health Network, Sinai Health System, Women’s College Hospital and Unity Health Toronto in Ontario; McGill University Health Center and Jewish General Hospital in Quebec; IWK Health Center in Nova Scotia (with genetic services covering New Brunswick and Prince Edward Island); and Eastern Health in Newfoundland. The CHARM Consortium collects and analyzes longitudinal plasma samples, tumor tissue, genomic and epigenomic data, clinical data, and data on health services and preferences from participants harboring a genetic variant associated with an HCS (Figure S1B). Written informed participant consent was obtained for the CHARM study under LIBERATE (liquid biopsy evaluation and repository development at the Princess Margaret). Data were de-identified prior to analysis. The use of participant data for CHARM was approved by the Ontario Cancer Research Ethics Board and the Institutional Research Ethics Approval board at the University Health Network (18–5692).

Liquid biopsy through cell-free DNA sequencing assesses cancer development

In recent years, the analysis of cell-free DNA (cfDNA) has emerged as a non-invasive strategy for monitoring disease.
cfDNA is thought to enter the circulation via several mechanisms, including cellular apoptosis, necrosis, autophagy, and necroptosis.\textsuperscript{55} Cell-free fetal DNA analysis is being routinely used for non-invasive prenatal testing and shows exceptionally high sensitivity and specificity for trisomy 21, as well as a high sensitivity for trisomy 18, sex-chromosome abnormalities, and trisomy 13.\textsuperscript{56} More recently, cfDNA has been explored in oncology practices.

In oncology, cfDNA profiling, often referred to as liquid biopsy, relies on the identification of highly specific DNA fragments (known as circulating tumor DNA [ctDNA]) released by cancer cells. The application of cfDNA sequencing in cancer has been explored in numerous studies examining methods of monitoring disease progression and predicting treatment response in sporadic cancers.\textsuperscript{57} One of the most common techniques is to employ a combination of targeted, ultra-deep sequencing ($\sim$1,000–20,000×)\textsuperscript{58,59} and sequencing error suppression (TS), which has been able to quantify mutant allelic fractions as low as 0.1\% across a variety of cancer types.\textsuperscript{60,61} However, targeted panels are often limited by their narrow scope, high amount of input material required, and the number of genomic equivalents available in a typical plasma sample. Using shallow whole-genome sequencing ($\sim$0.1–1×; sWGS), one can reliably detect tumor-associated chromosomal aberrations at a lower limit of 3\% of cfDNA, but detection is restricted to large chromosomal alterations ($>10\text{Mb}$).\textsuperscript{62,63} Although studies have shown success at employing TS and sWGS, they are often in the metastatic setting where ctDNA burden is high. Also, because both techniques rely on the identification of genetic alterations, both assays can be obfuscated by variants associated with clonal hematopoiesis of indeterminate potential.

Whereas TS relies on idealized scenarios where there is adequate input of cfDNA, sufficient number of genomic equivalents, and the presence of the target mutant molecule, pWGS instead relies on the probability of capturing any mutant molecule out of the 1,000s–10,000s of genome-wide mutations present within a tumor. Using a tumor-informed approach, current studies have shown effective detection of minimal residual disease with a limit of detection of between 0.1\% and 0.0001\% tumor fraction, dependent on mutation burden and sequencing depth.\textsuperscript{64} Recent advances using a tumor-naïve approach have also been effective in individuals with high-grade malignancies.\textsuperscript{65} Another advantage to genome-wide mutation profiling using pWGS is that the sampling of plasma is not affected by the spatial heterogeneity that is present in tumors and is known to affect surgical sampling. This suggests that the lower limit of detection via a tumor-naïve approach might be capable of even lower limits of detection than the current tumor-informed approaches.\textsuperscript{66}

One area of rapid development is cell-free fragmentomics, which leverages the unique fragmentation patterns of cfDNA to detect cancer-cell-associated signatures. In healthy cells, cfDNA fragments are typically 167 bp in length, which corresponds to the length of DNA that wraps around a nucleosome. Thus, the release of cfDNA fragments is a non-random process that is preserved at loci occupied by nucleosomes and is depleted at open chromatin regions. In addition, ctDNA is often more fragmented (shorter) than cfDNA derived from healthy cells. Utilizing these two concepts, studies have explored cancer detection through the analysis of fragment size,\textsuperscript{67} nucleosome positioning,\textsuperscript{68} open chromatin sites,\textsuperscript{69} inferred transcriptional activity,\textsuperscript{70} fragment end motifs,\textsuperscript{71} and fragment ratios.\textsuperscript{72} Another added benefit to fragmentomics is a wide breadth of analyses can be performed from one sequencing assay (whole-genome sequencing). Although most fragmentomic analyses can be performed at low sequencing depth ($\sim$1×; sWGS), the advantages of increased sequencing depth ($\sim$30×; pWGS) include both improved sensitivity enabled by locus-level resolution of fragmentation patterns and the potential for genome-wide mutation profiling. However, the advantages of increased sequencing depth and sensitivity should be weighed against the increased computational expertise and infrastructure required.

Because of the inherent limitations of genomic-based ctDNA assays, there has been a rapid expansion and development of genome-wide analysis modalities that leverage cancer-associated signatures to detect cancer rather than relying on the detection of single-locus variants or copy-number detection. One emerging strategy is to profile the landscape of cell-free methylation. Methylation of DNA at CpG sites across the genome is an essential determinant of cell identity and is often conserved when cfDNA is released into the blood plasma. Several approaches for cell-free methylation profiling, including cell-free DNA immunoprecipitation (cfMeDIP), cell-free whole-genome and targeted bisulphite sequencing,\textsuperscript{73} enzymatic methyl-seq (EM-seq),\textsuperscript{74} and inference using fragmentation (FRAGMA), have been developed.\textsuperscript{75} Each method has intrinsic advantages and disadvantages. For example, the same limits of targeted mutation profiling are still prevalent in targeted methylation profiling. One method that has shown promise for cancer early detection is cfMeDIP. Studies have demonstrated not only sensitive detection of cancer (stage I/II) but also the ability to distinguish between cancer types.\textsuperscript{76} In one study, cfMeDIP-seq in combination with standard-of-care modalities was able to detect cancer up to four years before the diagnosis.\textsuperscript{77}

Historically, there were concerns that the blood-prostate and blood-brain barriers might limit the effectiveness of panel-based cfDNA assays for detecting prostate and brain tumors. However, several studies have now shown that the low performance was most likely due to technical, rather than biological, limitations. Several studies now show the efficacy of using liquid biopsy for brain tumors,\textsuperscript{67,78–82} and prostate cancers are routinely detected in the plasma of individuals.\textsuperscript{83,84} Because both methylome and fragmentome analyses are still developing and maturing, robust limits of detection have not been established for most assays. In most
Clinical utility of liquid biopsies in hereditary cancer

The positive predictive value (PPV) and negative predictive value (NPV) associated with liquid biopsy have been reported in previous studies, including our work in CHARM. This greatly varies depending on the assay and type of analysis. However, few studies have shown the utility of liquid biopsy in hereditary cancer. The identification of potential cell-free DNA-based and RNA-based biomarkers extracted from liquid biopsy might have a future role in the diagnosis of Lynch syndrome and in combination with, or as an alternative to, colonoscopy. However, these markers need to be confirmed and standardized, cost-efficient testing methods prior to clinical implementation need to be developed. A recent study reported that cfDNA multi-omic analysis distinguishes MPNST from plexiform neurofibromas with high sensitivity, and cfDNA levels in NF1 significantly correlate with MPNST tumor burden. By identifying distinctive pathways underlying MPNST pathogenesis, it this technique might allow clinicians to sub-classify MPNSTs to correlate with prognosis and develop personalized treatment plans.

Liquid biopsy is an especially attractive alternative to clinical screening programs for individuals with an HCS because it can be scheduled more frequently, provide personalized disease snapshots at regular time points, and allow for a more comprehensive and holistic picture of a tumor’s heterogeneity, as compared with tissue biopsies. One study provided average turnaround time for liquid biopsy results as nine days. A few health economic studies have evaluated the cost-effectiveness of liquid biopsies, with differing results. A modeling study in the Canadian health care system found that liquid biopsy in combination with tissue testing in advanced non-small cell lung cancer (NSCLC) resulted in incremental cost savings and a gain in QALY, as compared with tissue testing alone; these results were supported by another study that compared the cost-effectiveness of liquid biopsy and tissue biopsy in individuals with advanced NSCLC through a Markov model. A health technology assessment of liquid biopsy for individuals with advanced NSCLC through a systematic review in Canada found that liquid biopsy might be most effective as a triage test (followed by tissue biopsy), but because of the high cost of treatment, it may not be cost effective. A USA-based breast cancer microsimulation model estimated the benefits, harms, and costs of breast cancer early detection using liquid biopsies; the researchers used the model to establish threshold values for the use of novel screening tests and concluded that liquid biopsy might be best in addition to digital mammography, rather than as an alternative for mammography. However, a study in Colombia determined that a comprehensive ctDNA panel for HER2-positive breast cancer in addition to conventional treatment was more expensive and less effective than conventional treatment alone. Finally, a systematic review of liquid biopsy and tissue biopsy for treatment of localized prostate cancer indicate that although these tests might be cost effective, further clinical studies are needed for assessment of long-term outcomes. However, none of these studies of cost effectiveness were conducted in the context of HCSs.

Additionally, inequities in service delivery and lack of access to specialist care in rural and remote communities are particularly acute in the HCS context. Liquid biopsy is a more accessible alternative for people who live in isolated areas and lack access to the screening programs associated with specialized genetics and oncology clinics, which are typically only available in urban locations. In Canada, where the CHARM consortium is located, there are often barriers to accessing this technology. We have taken great care to decrease geographical barriers by partnering with phlebotomy labs nationwide to encourage rural individuals to participate, and we have also partnered with HCS advocates. With the increase of telemedicine and the accessible nature of liquid biopsy, one of CHARM’s goals is to decrease health disparities in Canada.

Despite the development and advancement of ctDNA technologies, there remains a dearth of studies that explore either early detection or liquid biopsy within the HCS population despite the increased relative benefit gained from liquid biopsy for this population. This highlights a research gap where CHARM can contribute to the growing field of HCS cfDNA analysis. A carefully designed cfDNA technique could be leveraged for early detection of cancer in participants and at-risk family members across many HCSs.

Assessment of cfDNA in CHARM

As part of CHARM, we carried out a qualitative interpretive descriptive study to explore the clinical utility of liquid biopsy testing for early cancer detection in individuals with an HCS by interviewing 35 (28 female) health care professionals (HCPs) involved in HCS care and/or research across Canada. These HCPs describe the use of ctDNA as “transformative” and a “game-changer”; however, they are divided on its use in HCS management on the basis of the following issues: cfDNA’s clinical utility, its role in
cancer screening, and its level of invasiveness. Both groups express concern about participant burden around cancer screening and about decreased observance of scheduled screening as a result of false-negative results, leading to delayed diagnosis.

As part of the CHARM consortium, we also performed a qualitative interpretive description study by using telephone interviews with 30 adult individuals with an HCS (n = 19 women, age range 20s–70s, n = 25 White) to examine their perspective on the utility of ctDNA (unpublished data). Participants expressed enthusiasm for the potential of ctDNA to detect multiple cancers, detect cancers early, and personalize clinical care. Although participants acknowledged the limitations of ctDNA, such as additional anxiety while waiting for test results and risk of false positive or false negative results, they believed that the benefits overshadow the negatives.

Liquid-biopsy technologies used by the CHARM consortium

We assembled the CHARM consortium to make use of liquid-biopsy techniques in hereditary-cancer early detection. Enrolled participants donate blood samples annually alongside their physical exams and HCS-specific imaging protocols (e.g., whole-body MRI, mammogram, targeted ultrasound). Our protocols test blood plasma for the presence of occult tumor by using next-generation DNA sequencing techniques that identify genomic, fragmentomic, and epigenomic changes. To date, the CHARM consortium has tested three technologies to detect early cancer in individuals with HCS through analysis of cfDNA in blood plasma: (1) a targeted panel, (2) plasma WGS (pWGS), and (3) cfMeDIP-seq. To promote and develop international data-sharing standards and infrastructure, CHARM has developed strong partnerships with sister efforts in the USA (https://www.edisyn.org/) and UK (https://www.cancerresearchuk.org/). To aid in this, CHARM has also invested in developing data-sharing infrastructure. CHARM has established the Phenomic Liquid Biopsy Resource (PLBR) to share genome-wide fragment data, allowing for fragmentomic and methylation analysis to improve collaboration (https://fragmentomics.ca). We developed the mCORDER2 database for standardized clinical data abstraction using the Marathon of Hope Cancer Centers Network (MOHCCN) standard (https://mcoder2.ca/). CHARM is committed to abstracting clinical data to MOHCCN clinical-data standards to ensure consistency of cancer data available to clinicians and researchers. MOHCCN standards include elements from the International Cancer Genome Consortium Accelerating Research in Genomic Oncology (ICGC-ARGO), other unique MOHCCN elements, and the American Society of Clinical Oncology Minimal Common Oncology Data Elements (ASCO mCODE) data dictionaries, which define each data element that is collected.

Emerging liquid-biopsy data from the CHARM consortium

The CHARM gene panel is a targeted sequencing approach that provides in-depth mutational data on a core set of genes frequently mutated in HCS tumors; data are included for the exons of BRCA1, BRCA2, PALB2, TP53, APC, (3’UTR) EPCAM, MLH1, MSH2, MSH6, PMS2, and 173 microsatellite loci, as well as 44 SNPs and three sex-linked genes to confirm sample identity. Preliminary data from our analyses have shown that a targeted panel approach can be useful for detecting tumor-associated somatic TP53 variants up to five months preceding clinical diagnosis in individuals who are heterozygous for variants associated with LFS. Highlighting one individual (Figure 1), we detected a somatic TP53 variant 5 months prior to the diagnosis of lung cancer by imaging. The somatic TP53 variant was not detected at two subsequent timepoints after surgical resection.

The CHARM consortium is also exploring the integration of fragmentomic analyses for the cancer detection using both sWGS and pWGS. Cancer-associated fragmentomic signals can be detected at sensitivities of 5%–10% tumor fraction in the plasma of participants with an active cancer diagnosis (Figure 2). Given large enough cohorts, we anticipate establishing HCS-specific fragmentomic signatures that might be required for some HCSs that exhibit unique fragmentation patterns at baseline. Using deep pWGS, CHARM will explore the effectiveness of genome-wide mutation analysis compared to TS for the early detection of cancer. This strategy has the added benefit of increased resolution of fragmentomic analyses and eliminates the need for target enrichment for TS, which also requires high input material, thus conserving samples.
Lastly, using cfMeDIP-seq, the CHARM consortium is working on not only identifying whether a cancer is present but also inferring the tissue of origin of a cancer signal, a capability that is highly complementary to the detection capabilities of TS and WGS methods, which can also infer which tissue the cancer might be originating from. This would be highly beneficial in HCSs that affect multiple organs, such as LFS, and in detecting cancers that do not have effective screening methods, such as ovarian cancer.

The challenges of tumor detection using liquid biopsy is multifaceted. When one uses a genomic-alteration (mutation or copy-number) approach, it is difficult to detect tumors that are genomically stable and have few copy-number alterations. Conversely, when one uses a methylation or fragmentation approach, there are technical challenges associated with rare tumor types that have limited epigenetic datasets, which are required in building classifiers and signatures. One of the goals of CHARM is to be able to bank tumor tissues nationally, especially rare-syndrome-specific tumors, to profile and create signatures useful for cancer-type-specific liquid-biopsy analyses.

Conclusions

Individuals with an HCS consider themselves as a “ticking time bomb”; they are waiting for the development of cancer and are unsure when this event may occur. These individuals can experience uncertainty, stress, and anxiety as they undergo lifelong extensive surveillance, encompassing many health care providers and variable areas of expertise. Liquid biopsy is a non-invasive pan-cancer technology able to assess cancer-associated signatures and could serve as a tool for balancing psychosocial impacts that individuals with an HCS experience. The utility of this tool will need to be balanced against false-positive liquid-biopsy results where a cancer is not subsequently detected; such false-positive results could lead to undue anxiety. This will be the focus of subsequent psychosocial impact studies within the CHARM consortium.

CHARM consortium


Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2023.08.014.

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Author contributions


Declaration of interests

William D. Foulkes has research funding from AstraZeneca, Sophie Sun has a consulting and advisory relationship with Novartis, Bristol-Myers Squibb, Pfizer, Purdue, Takeda, and AstraZeneca. Kasmintan A. Schrader has a consulting and advisory relationship with and has received honoraria from AstraZeneca Canada and Pfizer and research funding from AstraZeneca Canada. Dean Regier has a consulting/advisory relationship with Roche Canada and AstraZeneca. Trevor J. Pugh has a consulting and advisory relationship with Chrysalis Biomedical Advisors and the Canadian Pension Plan Investment Board, is on the scientific advisory board for Illumina, has received honoraria from AstraZeneca, Merck, PACT Pharma, and SAGA Diagnostics, and has research funding from Roche (Genentech), the National Institutes of Health, and the US Department of Defense. Yvonne Bombard has ownership interests and intellectual property rights as an inventor and patent holder with Genetics Adviser. The other authors declare no competing interests.

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Supplemental information

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Figure S1. Participating centres and cumulative recruitment of CHARM participants from across Canada. A) Map of the provinces/territories where CHARM is active. B) Enrolled participants in CHARM separated by HCS since its founding in 2017 to December 2022. Other = BAP1, CDH1, CHEK2, DICER1, FANCC, FH, FLCN, MEN1, PALB2, PTEN, RAD51D, RET, SDHB/SDHD, STK11, VHL; LFS = Li-Fraumeni syndrome (TP53); NF1 = Neurofibromatosis type 1 (NF1); LS = Lynch syndrome (MLH1, MSH2, MSH6, PMS2, EPCAM); HBOC = hereditary breast and ovarian cancer (BRCA1, BRCA2).