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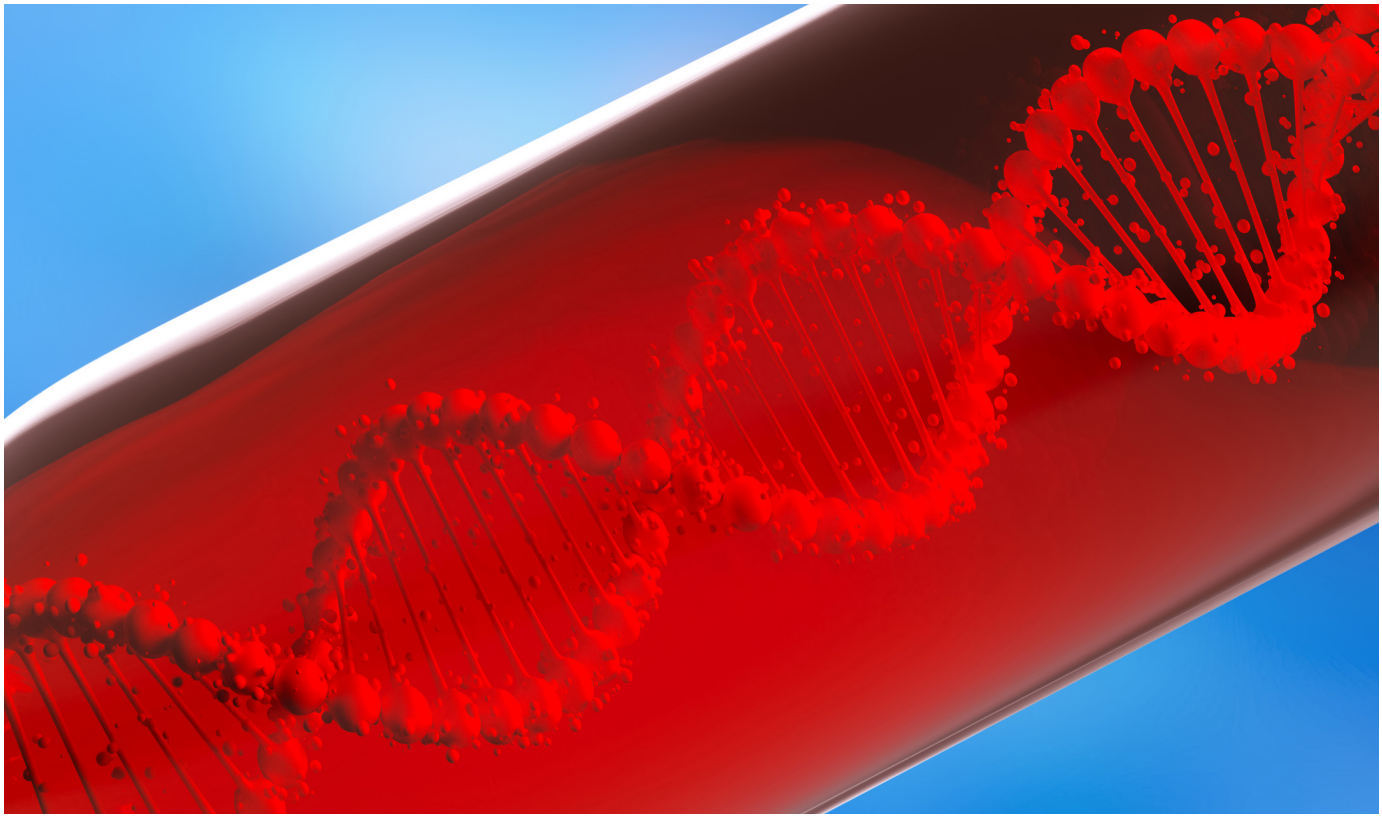
An Overview of Liquid Biopsy for Screening and Early Detection of Cancer



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Methods

CADTH Horizon Scanning bulletins present an overview of the technology and available evidence. They are not systematic reviews and do not involve critical appraisal, nor do they include a detailed summary of study findings. Therefore, they are not intended to provide recommendations for or against a particular technology.

Literature Search Strategy

A limited literature search was conducted by an information specialist on key resources including PubMed, MEDLINE and Embase via OVID, Scopus, the Cochrane Library, the University of York Centre for Reviews and Dissemination databases, the websites of Canadian and major international health technology agencies, as well as a focused Internet search. The search strategy was comprised of both controlled vocabulary, such as the National Library of Medicine's MeSH (Medical Subject Headings), and keywords. The main search concept was liquid biopsy. No filters were applied to limit the retrieval by study type. Where possible, retrieval was limited to the human population. The search was also limited to English language documents published between January 1, 2017 and July 9, 2019. Internet links were provided, where available.

Study Selection

One author screened the literature search results and reviewed the full text of all potentially relevant studies. Studies were considered for inclusion if the review included a liquid biopsy that could be utilized for screening or the early detection of cancer. The final selection focused primarily on existing evidence syntheses including literature reviews and/or systematic reviews. Grey literature was included when it provided additional information to that available in the published studies.

Peer Review

A draft version of this bulletin was reviewed by one clinical expert. The manufacturers of the technologies were also given the opportunity to comment on an earlier draft. Feedback received from the manufacturers who submitted input was considered in the final review.

Summary

- Currently, population-based cancer screening is limited to select cancers (e.g., cervical cancer). Cancer diagnoses are confirmed using tissue biopsies in solid tumours.[1] However, these biopsies are invasive; associated with multiple complications;[2] and are limited by the anatomical location of the tumour,[3] the evolution of the genetic make-up of malignant neoplasms over time,[4] and potential variability between the primary tumour site and the metastatic sites.[2,5]
- Liquid biopsies are minimally invasive tests requiring a small sample of blood or urine that are capable of detecting cancer cells or genetic material that primary tumours release into body fluids (e.g., blood and urine) for solid tumour cancers.[1]
- Liquid biopsies have the potential to address key areas related to diagnosis, prognosis and therapeutics; monitoring the spread of cancer to other parts of the body; determining what genetic changes or mutations a tumour has; determining what treatments might work best for specific patients; and determining if treatments are working.[6,7]
- Liquid biopsies may also have the potential to be used for cancer screening; however, prior to implementation more evidence is needed regarding analytical and clinical validation of assays, prospective studies to better demonstrate the clinical utility of liquid biopsies, and assessment of whether liquid biopsies offer improved diagnostic outcomes and/or cost savings when compared with current standard-of-care practices.

Background

Cancer is the leading cause of death in Canada.[8] Approximately one in two Canadians are expected to be diagnosed with cancer at some point during their lifetimes, and the four most common cancers (lung, colorectal, breast, and prostate cancer) account for more than half of all cancer diagnoses and deaths in Canada. A 2018 population-based cost study found that the cost of cancer care increased from $2.9 billion in 2005 to $7.5 billion in 2012, demonstrating a substantial economic burden in Canada.[9] At present, the available options for population-based cancer screening are limited to select cancers and include fecal occult blood testing (colorectal cancer); pap testing and/or screening for human papilloma virus (cervical cancer); and mammography (breast cancer). However, effective screening tests for many other cancers, including pancreatic and liver cancers, are lacking.[10] Improved screening and early detection techniques for a variety of cancers could have a large impact on the stage at which a diagnosis is made;[6] when detected early enough, current therapies may allow the successful treatment of many patients, while metastatic disease remains incurable with very few exceptions.[7] As noted by the Canadian Cancer Society, the early detection of cancer is crucial for several reasons, as the cancer can usually be treated with less aggressive treatment, treatment is more effective, and survival rates tend to be higher.[1] When caught early enough, the tumour may be able to be removed by surgery rather than requiring treatment with chemotherapy or radiation.[1]

Currently, tissue biopsy is the primary method of making a definite cancer diagnosis in solid tumours; however, its application is limited in early, non-symptomatic cancers.[1] Tissue biopsies are typically taken from the primary tumour and, depending on the anatomical location of the tumour, there may be difficulties regarding accessibility.[3] For example, considerable challenges exist when obtaining a biopsy from a tumour located mid-lung via a transthoracic biopsy, in which a needle is passed through the chest and between two ribs. Furthermore, a tissue biopsy reflects the molecular composition of the tumour at the time the sample is taken; however, malignant neoplasms and their genetic make-up evolve continuously,[4] allowing cancers to adapt to changing environments, survive treatments, and spread, resulting in potential variability between the primary tumour site and the metastatic sites.[2,8] Utilizing repeated conventional biopsies to map tumour evolution is an invasive, painful, and

impractical approach that may lead to multiple complications (e.g., bleeding and/or the dislodging of cancer cells from the tumour, thus allowing them to spread elsewhere, also known as cancer cell seeding).[2] Furthermore, tumours that are not comprised of homogenous cells — referred to as intra-tumour heterogeneity — create sampling limitations that can result in misdiagnosis when using conventional surgical biopsy.[2,11]

Detection of cancer through non-invasive techniques has been under investigation for more than two decades.[12] The development of liquid biopsies is one technique that is becoming increasingly popular.[12] Currently, liquid biopsies are largely utilized to gather information on tumours which have already been diagnosed in order to inform treatment options (i.e., personalized medicine) and prognosis. However, screening and early cancer detection is an evolving area of research. Liquid biopsy has the potential to become an instrumental non-invasive screening tool for solid tumour cancers that currently lack diagnostic screening tools, and particularly for cancers that are not typically diagnosed until advanced stages (e.g., lung and pancreatic cancers).[1] The purpose of this Horizon Scan is to provide an overview of the potential use of liquid biopsies for cancer screening.

Table 1: Glossary of Terms

Term	Definition
Assay	Investigative procedure utilized to determine the content or the quantity of something (e.g., protein, cells, DNA) in a sample.
cfDNA	Cell-free DNA (cfDNA) is DNA that is freely floating in the circulation outside of a cell. cfDNA typically arises from normal cell turnover, when dead cells are broken down and released into the bloodstream.
Clinical utility	Usefulness of a device or intervention in patient care. Clinical utility of a device or test may be related to its ability to properly detect and diagnose a disease (i.e., diagnostic accuracy).
CTCs	Circulating tumour cells (CTC) are cells that have detached from a tumour and circulate in the bloodstream. These are thought to be a mechanism for metastases.
ctDNA/ctRNA	Circulating tumour DNA (ctDNA) or circulating tumour ribonucleaic acid (ctRNA) is DNA or RNA that comes from cancer cells and tumours. Throughout the course of tumour cell growth (cell turnover), contents of dead cells, including small portions of DNA or RNA, are broken down and enter the bloodstream.
Digital PCR	Digital polymerase chain reaction (dPCR) is a technology used to measure or quantify DNA or RNA in a sample.
Exosomes	Vesicles that contain various protein, lipid, and genetic material (RNA, DNA) that are released or secreted from cells.
Liquid biopsy	A liquid sample (e.g., blood, plasma, urine) used to detect the presence of cancerous cytogenetic (cell) or cell-free nucleic acid (both DNA and RNA) materials. Liquid biopsy poses a potential minimally invasive alternative to traditional tissue sampling.

What is Liquid Biopsy?

Liquid biopsy is a minimally invasive test that has the capability to detect intact cancer cells or material that tumours and/or metastatic lesions release into body fluids (e.g., blood and urine). Tumour cells in circulation were first discovered in the late 19th century. The term "cell-free DNA (cfDNA)" refers to fragmented DNA (genetic material) found outside cells in the blood and was first reported by Mandel and Metais in 1948.[2,5] It includes circulating tumour cells (CTCs), circulating tumour DNA and circulating tumour ribonucleic acid (ctDNA, ctRNA), exosomes, proteins, antibodies, and tumour-educated platelets.[1,3,4]

Compared to a classic tissue biopsy, liquid biopsies require a small sampling of blood or a urine or stool sample. Liquid biopsies are less invasive and present minimal procedural risk to the patient, resulting in a potentially less expensive sample collection when compared with surgical biopsies. Furthermore, the frequency of liquid biopsies can be performed on a serial basis to closely monitor treatment effectiveness and/or tumour progression to better inform therapeutic decisions. Therefore, liquid biopsy technology has the potential to provide a more comprehensive understanding of disease and overcome the spatial limitations of a tissue biopsy taken from a single lesion within a single anatomic site.[5,13]

While the current report focuses on the application of liquid biopsies for cancer screening and early detection, several other applications of liquid biopsies in oncology have emerged and developed at a rapid rate over the last decade, most notably in the areas of diagnosis, prognosis, or therapeutic decision-making including:[2,3]

- investigations of cancers of unknown primary origin
- risk-stratification and tumour staging
- tumour genotyping and assessment of clonal evolution
- therapy selection/guiding precision therapy
- monitoring response to treatment
- detection of emergence of treatment resistance
- detection of minimal residual disease.

How It Works

There are several mechanisms by which liquid biopsies can be utilized as a screening and/or diagnostic tool for various types of cancer. The primary analytes of interest include cfDNA, ctDNA, ctRNA, CTCs, proteins, antibodies, and exosomes. The following describes the primary mechanisms of measurement.

Cell-free DNA is DNA thought to be released from a tumour cell during apoptosis (programmed cell death) or necrosis (cell death).[14] cfDNA may be freely detected in the circulation or within extracellular vesicles, called exosomes, and has been detected in both blood and other bodily fluids including urine, cerebrospinal fluid, pleural fluids around the lungs, and saliva.[14] cfDNA is evaluated for genetic and epigenetic DNA modifications to determine the genome or epigenome of the cell origin.[14]

Because cfDNA is a normal product of cell turnover that is detectable in healthy individuals, largely released from hematopoietic cells (cells that give rise blood cells), it is not exclusively linked to tumour cells.[14] However, mutations in the DNA of tumours are not an exact match to an individual's DNA and therefore can be highly specific markers for various cancers.[14] DNA released into the bloodstream from cancerous tumour cells during cell turnover have been termed "circulating tumour DNA" or ctDNA to indicate it's origin.[14] ctDNA molecules are shorter in length than cfDNA molecules, which may also be helpful in the differentiation of ctDNA from healthy cfDNA. Further, the half-life of cfDNA and ctDNA molecules in circulation is less than three hours; therefore, capturing ctDNA is considered to be a present or real-time measure of disease burden, as it does not accumulate over time (i.e., levels of ctDNA are related to severity of disease).[14]

Detection of ctDNA is quantified using various technologies to determine either the mutant allele concentration (which is defined as copies per millilitre), or the mutant allele fraction.[14] Currently, blood plasma is the ideal specimen for analysis of ctDNA.[13] However, tumour shedding is not constant and can vary depending on the cancer type, location(s), and vascularization. For example, some cancer types may have higher DNA content than what would be detected in the plasma and therefore the use of other conveniently obtained samples such as urine for bladder cancer, stool for colorectal cancers, and cervical smears for cervical cancer would be warranted.[14] One type of assay utilized to assess the concentration of ctDNA in a sample is digital polymerase chain reaction, or digital PCR. Digital PCR amplifies the concentration of mutant molecules of interest through hundreds, or even millions, of reactions so that small molecules that are difficult to detect normally can be identified and subsequently quantified.[14]

The concentration of ctDNA in the plasma is helpful for staging and prognosis, as it has been shown to correlate with tumour size and stage of disease.[14] It is important to note that ctDNA levels vary substantially between patients who have the same type and stage of cancer.[14] Variability of ctDNA concentration is related to numerous factors including poor tumour vascularization and histological differences that can influence the rate and type of cell death.[14] Variability aside, ctDNA concentration has been found to be a useful tool when determining prognosis, as increasing concentrations of ctDNA are correlated with poorer clinical outcomes at diagnosis and measured longitudinally throughout the course of treatment.[14] Although ctDNA concentrations are lower in the early stages of cancer, making them difficult to accurately detect, utilizing ctDNA for earlier diagnosis of disease is desirable in order to prevent metastatic spread and increase survival.

Analysis at the cellular level can be performed by measuring the quantity of CTCs in a blood sample that enter the circulation via passive shedding of the tumour.[15] CTCs can originate from either the primary tumour or from a metastatic site, making them potentially useful biomarkers of cancer to aid in early detection, staging, monitoring, and prognosis of cancer.[15] On-going research is underway to further develop the use of CTCs for various urologic cancers such as prostate, bladder, urothelial carcinoma, and renal (kidney) cancers.[15]

The Technologies

There are currently several liquid biopsies in development for oncology. These technologies, applications, and services are being developed for a variety of purposes, from research to screening and early detection, to assessing biomarkers to provide insights on prognosis and treatment selection. While some of these tests focus on specific cancers, others are being developed as screening tools for multiple solid tumour cancers. Table 2 summarizes the test, manufacturer, therapeutic area, technology use, and type of cancer signal detected.

Table 2: Liquid Biopsy Technologies for Screening and Early Detection

Test, Manufacturer, Location	Therapeutic Area	Use	Type of Cancer Signal (CTCs, ctDNA, Exosomes)
Archer Reveal ctDNA 28[16] ArcherDX, Inc., Boulder, CO, US	Solid tumours	Research	circulating cfDNA/ cfDNA/ctDNA
BDX-XL2[17] Biodesix, Boulder, CO, US	Lung cancer	A pulmonary nodule classifier intended to identify low-to-moderate risk patients with a likely benign lung nodule	Proteomic analysis of two plasma proteins, LG3BP and C163A
CancerSEEK[18] Thrive Earlier Detection Corp., Cambridge, MA, US	Solid tumours	Early detection of multiple types of cancer, complementing other screening tools (including breast, colorectal, lung, esophagus, stomach cancers, and some cancers which currently lack effective screening tools such as ovarian, pancreatic, and liver cancers); identifying and locating an early-stage tumour with its respective tissue of origin	ctDNA
CellMax[19] CellMax Life, Sunnyvale, CA, US	Solid tumours	To detect pre-cancer and early-stage colorectal cancer; to reduce unnecessary biopsies in PSA grey zone patients suspicious of prostate cancer; to select immunotherapy and targeted therapy and to monitor treatment effectiveness and early recurrence in already diagnosed solid tumour cancers • CellMax-CRC Colorectal Cancer Early Detection Test (FirstSightCRC) • CellMax-Prostate Cancer Test • CellMax-LBx Liquid Biopsy	CTC, ctDNA
*Early*CDT—Lung[20] Oncimmune, Nottingham, UK	Lung Cancer	Pulmonary nodule risk assessment and lung cancer screening	This test detects 7 autoantibodies against tumour antigens in the blood
Epi proColon[21] Epigenomics AG, Berlin, Germany and Epigenomics Inc., San Diego, CA, and Germantown, MD, US	Colorectal cancer	To screen for CRC in adult patients with average risk for CRC, who have been offered and have a history of not completing conventional CRC screening	ctDNA

Test, Manufacturer, Location	Therapeutic Area	Use	Type of Cancer Signal (CTCs, ctDNA, Exosomes)
Epi proLung[22] Epigenomics AG, Berlin, Germany	Lung cancer	A complimentary test for screening lung cancer in patients with indeterminate findings	Free-circulating DNA
Freenome[23] Freenome Holdings, Inc., South San Francisco, CA, US	Colorectal cancer	Early cancer detection and therapy selection	cfDNA and cfRNA
GRAIL[24] GRAIL Inc., Menlo Park, CA, US	Multiple cancers, pan-cancer test	Early cancer detection determining the tissue of origin (solid tumours, lymphoma, and multiple myeloma)	cfNAs: ctDNA and ctRNA
IvyGene[25] Laboratory for Advanced Medicine, West Lafayette, IN, US	Multiple cancers	Early cancer detection, validated for breast, colon, liver, and lung cancers; the test is intended to be used in conjunction with other diagnostic and confirmatory tests • The IvyGene CORE test • The IvyGene Liver Test • IvyGene colorectal test • IvyGene breast test (to be available in 2019)	cfDNA
Lunar-1[26] Guardant Health, Inc., Redwood City, CA, US	Solid tumours	To monitor cancer recurrence in patients in remission, and to detect cancer residue; research use, only Initial focus on lung, breast, colorectal and ovarian cancers	ctDNA
Lunar-2[27] Guardant Health Inc., Redwood City, CA, US	Solid tumours	Cancer screening in high-risk population; research use only	ctDNA

CA = California; cfDNA = cell-free DNA; cfNAs = cell-free nucleic acids; CO= Colorado; CRC = colorectal cancer; CTC = circulating tumour cell; ctDNA = circulating tumour DNA; ctRNA = circulating tumour ribonucleic acid; IN = Indiana; MA = Massachusetts; MD = Maryland; NSCLC = non-small cell lung cancer; PSA = prostate-specific antigen.

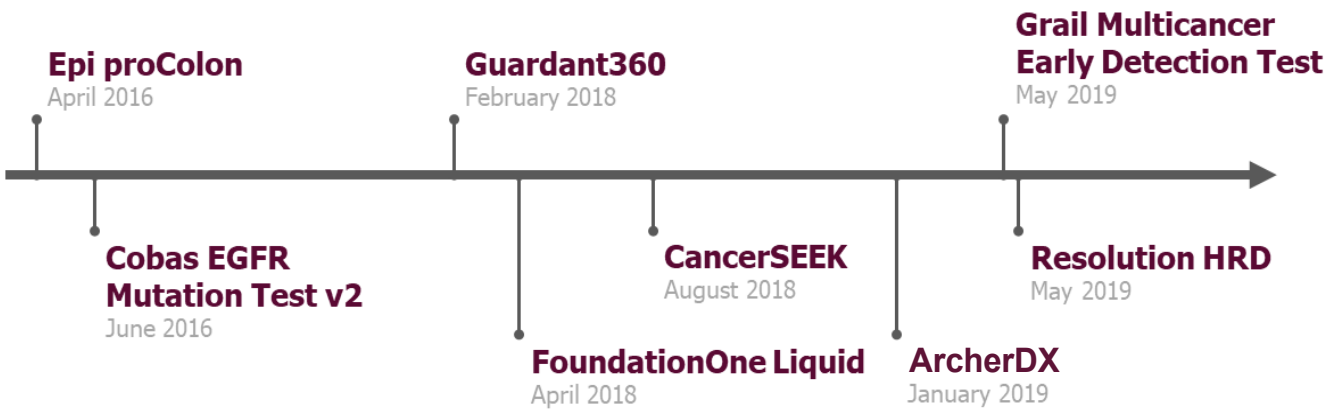
Regulatory Availability

Approved Liquid Biopsy Technologies

Currently, the CELLSEARCH CTC kit (Menarini Silicon Biosystems, Inc.) is the only liquid biopsy test that has been approved by Health Canada (March 9, 2010).[28] It is also the only CTC test that has obtained FDA 510(k) clearance for aiding in the monitoring of patients with metastatic breast, colorectal, or prostate cancer. In addition to its clearance in the US and approval in China, CELLSEARCH fulfills the requirements for CE marking in the European Union.[29]

It is important to note that liquid biopsy technology is in its early stages of development. The full utility of this technology for screening has yet to be realized. Currently, many of these devices are to be used as a supplement to traditional diagnostic procedures or as a companion diagnostic to guide therapeutic decision-making. Increased sensitivity is required when attempting to detect cancer in earlier stages because of low levels of circulating genetic or cellular material. Therefore, liquid biopsy technology used for the purposes of screening is relatively unrefined. Several liquid biopsies have received a breakthrough device designation (designed to provide patients with life-threating diseases and timely access to medical devices by speeding up the development, assessment, and review process)[30] from the FDA. An overview of the timelines for these liquid biopsies receiving FDA approval or breakthrough device designations is shown in Figure 1.

Figure 1: Timeline of FDA Approvals and Breakthrough Device Designations



Liquid Biopsies for Guiding Therapeutic Decision-Making or Companion Diagnostics

- The cobas EGFR Mutation Test v2 (Hoffman-La Roche Ltd.) was approved by the FDA on June 1, 2016 as a companion diagnostic test with Tarceva (erlotinib) for the first-line treatment of patients with metastatic non–small cell lung cancer (NSCLC).[31] The test received further approval as a companion diagnostic test with IRESSA (gefitinib) for the first-line treatment of patients with NSCLC on August 23, 2018.[32] The CobasEGFR Mutation Test v2 is used to guide therapy decisions in patients with NSCLC by employing a real-time PCR test to identify mutations for the epidermal growth factor receptor (EGFR) gene. This technology is approved as a companion diagnostic to guide therapeutic options by identifying patients who are candidates for targeted therapies.[33]
- Guardant360 (Guardant Health, Inc.), a liquid biopsy to inform treatment decisions for advanced cancer patients, received an expedited access pathway designation for breakthrough devices from the FDA on February 15, 2018.[34] While not used for screening, Guardant360 can be used to overcome challenges of traditional tissue biopsies in patients with NSCLC by utilizing liquid biopsies to inform treatment decisions before first-line treatment, as well as at disease progression.[35] In a study of patients with metastatic NSCLC, Guardant360 and tissue testing detected two times as many patients with targetable alterations versus tissue testing alone (82 patients versus 47 patients, respectively).[36] Guardant Health is also developing the LUNAR assay, which includes Lunar-1 for residual disease and recurrence detection, and LUNAR-2 for the early detection of cancer. The LUNAR assay is currently only available for research use.[37]
- FoundationOne Liquid (Foundation Medicine) was granted breakthrough device designation on April 28, 2018 as a new second-generation liquid biopsy test for patients with solid tumours. The company aims the test to be the first FDA-approved liquid biopsy assay to incorporate multiple companion diagnostics and multiple biomarkers to inform the use of targeted oncology therapies, including immunotherapies.[38] FoundationOne Liquid is a next-generation liquid biopsy (blood test) that uses ctDNA to assess solid tumours, and which can be used either as a complement to traditional tissue biopsy results or when a tissue biopsy is not ideal. The results of this liquid biopsy can be used to guide therapy selection among patients with advanced-stage cancers.[38]
- Resolution HRD (Resolution Bioscience) received FDA breakthrough device designation on May 30, 2019. The company plans to seek approval for the Resolution HRD assay as a companion diagnostic test in solid tumour cancers.[39] The Resolution HRD liquid biopsy assay is an in vitro diagnostic test used to detect sequent variation in genes related to homologous recombination deficiency.[39] The Resolution Bioscience ctDx platform has been validated to detect single nucleotide variants, indels, fusions, and copy number variation.[40] The company's liquid biopsy assays include ctDx-Lung for identifying targetable mutations for patients with NSCLC and the anaplastic lymphoma kinase (ALK) assay to identify ALK fusions and resistance.[41]

Liquid Biopsies for Screening or Early Cancer Detection

- Epi proColon (Epigenomics AG) is the first and only blood test screening for colon cancer in average-risk patients older than 50 years. It received FDA approval on April 13, 2016. The test is also

available in Europe, China, and selected other countries.[21,42] Epi proColon 2.0 CE is a blood test that can be used as an alternative to conventional screening methods to allow for the early diagnosis of colorectal cancer. This test is based on a qualitative assay that detects methylated Septin9 DNA; in patients with positive results, these findings can be verified by colonoscopy or sigmoidoscopy.[43] In case-control studies, this technology was shown to discriminate between patients with colorectal cancer and healthy controls with high clinical sensitivity and specificity.[43] This test has been approved for people aged 50 or older with average risk for colorectal cancer, and provides another option for people who have a history of not completing screening (by flexible sigmoidoscopy, colonoscopy, or stool tests).[44] As noted by the company, this type of screening test may be more acceptable to patients and may increase screening participation among the eligible patients who currently do not participate in screening; currently in the European Union, one in seven patients undergo screening, despite counselling with a health care provider.[44]

- CancerSEEK (Thrive Earlier Detection Corp.) received FDA breakthrough device designation on August 8, 2018 for the detection of mutations and proteins associated with pancreatic and ovarian cancer.[10] CancerSEEK is a blood test that uses ctDNA and protein biomarkers to screen for eight types of cancer — five of which have no screening test (including ovarian, liver, stomach, pancreatic, and esophageal cancer).[45] CancerSEEK was tested in a study of 1,005 patients with diagnosed non-metastatic (stage I to III) cancer of the ovary, liver, stomach, pancreas, esophagus, colorectum, lung, or breast; these tests were positive in a median of 70% of the eight cancer types.[46] Although this technology is not ready for clinical uptake yet, larger studies of the test are currently being completed.
- ArcherDX's companion diagnostic assay for both liquid biopsy and tissue specimens was granted breakthrough device designation on January 8, 2019 to inform treatment selection of patients with advanced NSCLC.[47] ArcherDX has a number of assays, including the Archer Solid Tumor assay for analyzing solid cancer tumours. This includes the Archer REVEAL ctDNA 28 kit, which can be used to analyze plasma to identify cfDNA from 28 common gene mutations.[16]
- GRAIL multi-cancer early detection test (GRAIL, Inc.) was granted FDA breakthrough designation on May 13, 2019.[48] GRAIL is aiming to develop blood tests that can be used for the early detection of cancer. Its clinical research program includes three studies:
  - the Circulating Cell-free Genome Atlas (CCGA) Study, which enrolled individuals with and without cancer in order to characterize the landscape of genomic cancer signals in the blood[49,50]
  - the STRIVE Study, which enrolled a convenience sample of approximately 100,000 women in order to validate a blood test for the early detection of cancer[51,52]
  - and the SUMMIT Study, which will enrol approximately 50,000 participants aged 50 to 77 without cancer — half of whom who will have a high risk of lung and other cancers due to smoking history, and half without a high risk of lung or other cancers based on smoking history. This study is intended to evaluate a blood test for the detection of multiple cancers, including lung cancer.[53,54]

Who Might Benefit?

While the use of liquid biopsies for cancer screening is still a developing and evolving area, these technologies are increasingly being used for purposes such as determining what genetic changes or mutations a tumour has, informing treatment selection for patients (personalized oncology), monitoring whether treatments are working and if minimal residual disease is present after treatment, as well as monitoring if a cancer has spread to other parts of the body.[6,7] Liquid biopsies have the potential to become a cornerstone in oncology and there are a number of potential benefits across a range of applications.[7]

An area of potential benefit lies in the area of screening and early-stage detection of cancers.[7] While tissue biopsies are often used to diagnose late-stage disease, liquid biopsies may be able to detect genetic mutations at an earlier stage and thus allow for earlier treatment and better outcomes.[14] In cancers where historically late-stage diagnosis is the norm, liquid biopsies hold promise. For example, in lung cancer (where 49.6% of Canadians diagnosed in 2017 were stage IV) and colorectal cancer (where 29.1% of Canadians diagnosed in 2017 were stage III), earlier screening and detection could have a great impact on the Canadian cancer landscape.[6]

Despite these potential benefits for cancer care and cancer patients, the implementation of liquid biopsies has a number of considerations and hurdles to overcome, such as the need for further validation,[55] increases in sensitivity and specificity, and the rare nature of cancer mutations.[6]

Technical and Feasibility Considerations

Although liquid biopsy has increasingly been adopted and explored for clinical care, careful analytic and clinical validation, and additional preclinical studies addressing the biology of liquid biopsy analytes are needed.[5]

Assays

Circulating tumour DNA can be measured across various scales ranging from single mutations to the analysis of the whole genome.[13,14] Targeting a known tumour mutation has shown to have greater sensitivity (a test that correctly identifies patients with the disease), whereas applying a broader genome approach can be less sensitive.[14] The type of method utilized can present different challenges when attempting to use it for purposes of screening or diagnosis. Using a targeted approach requires the detection of a known recurring variant or mutation specific for the disease of interest (e.g., identifying the *EGFR* mutation associated with non–small cell lung cancer, or the *BRCA1 and BRCA2* gene mutations in breast cancer).[13] As mentioned, this approach may be highly sensitive but only of value if the cancer mutation is known. Broad coverage assays examine a large set of genes and can be used for multiple tumour types.[13] Although this approach may be more cost-effective, the risk of false-positives increases with the number of genes and size of panel analyzed.[14] Therefore, at this point in time, ctDNA analysis is largely used to complement traditional detection methods of diagnosed tumours and not for screening or early detection.

An important limitation of these assays is that different ctDNA assays vary in performance. Due to the process of amplification, assays may have a different threshold for detection. This makes the comparison of different assays complicated, as they are not interchangeable.[13] This also highlights additional challenges associated with collection methods and sample preparation. Because analyses are conducted using a few millilitres of plasma containing a very small amount of genetic material, it is crucial that samples be handled with care during preparation to avoid contamination or damage in order to optimize the results.[14] Because of the variability in detection limits across assays, it has been suggested that laboratories would need to perform validation studies to demonstrate the reliability of their testing methods.[13] That optimal detection thresholds for different mutations have yet to be established is a major barrier.[13]

Clinical Utility

A key concern beyond testing validity for liquid biopsy technology is its translation into clinical practice, or clinical utility. Establishing clinical utility for appropriate decision-making will require evaluation in either prospective clinical trials or retrospective analysis of collected samples.[13] Each of these methodologies presents unique challenges. Although reliable, the use of evaluation of ctDNA utility in prospective clinical trials may have ethical considerations. For example, it is not uncommon to obtain positive mutation results with traditional tissue biopsy samples and negative ctDNA results in the plasma;[13] therefore, solely utilizing ctDNA as a diagnostic tool for treatment may not be appropriate. Similarly, any positive detection as a result of screening would need to be accompanied by a formal diagnosis. Additionally, patients included in clinical trials would need to be stratified utilizing a basket trial design in order to perform analyses based on mutation type rather than tumour histology.[14] Retrospective analysis of archived samples could provide great insight into levels of ctDNA prior to diagnosis. However, this would require that archived samples were handled and stored appropriately to be of high enough quality for analysis.[13]

Clinical utility at this time across various ctDNA platforms is mainly focused on the detection and monitoring of recurrent or resistant mutations rather than on screening.[14] Only limited technologies are approved for companion diagnostic applications for EGFR mutations in non-small cell lung cancer.[14]

Cost-Effectiveness

As liquid biopsy technology continues to develop, another essential consideration will be the cost-effectiveness of new liquid biopsy technologies and whether they offer improved diagnostic outcomes and/or cost savings when compared with current standard-of-care practices. Few studies have addressed the potential value of using liquid biopsy as a repeatable and non-invasive instrument for various types of cancers. The full potential of liquid biopsy has yet to be realized. Currently, it is difficult to determine if liquid biopsy is cost-effective as a screening or diagnostic tool, particularly as diagnostic capabilities are still undergoing research. Various considerations including type of cancer, staging, treatment course, pharmaceutical options, and prognosis differ across cancer types, limiting the ability to apply a broad evaluation of cost-effectiveness at this time.

Final Remarks

While liquid biopsies present as a new technology that may allow for the screening and earlier diagnosis of cancer, there are still a number of considerations before these technologies can be used in clinical practice and utilized for population-level screening. These include a need for prospective studies to demonstrate that these assays have both the sensitivity and specificity required to correctly identify people with cancer and people without cancer.[10]

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