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PII: S0163-7258(19)30210-4

DOI: https://doi.org/10.1016/j.pharmthera.2019.107458

Reference: JPT 107458

To appear in: *Pharmacology and Therapeutics*

Please cite this article as: A. Campos-Carrillo, J.N. Weitzel, P. Sahoo, et al., Circulating tumor DNA as an early cancer detection tool, *Pharmacology and Therapeutics*(2019), https://doi.org/10.1016/j.pharmthera.2019.107458

P&T #23517

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Abstract:

Circulating tumor DNA holds substantial promise as an early detection biomarker, particularly for cancers that do not have currently accepted screening methodologies, such as ovarian, pancreatic, and gastric cancers. Many features intrinsic to ctDNA analysis may be leveraged to enhance its use as an early cancer detection biomarker: including ctDNA fragment lengths, DNA copy number variations, and associated patient phenotypic information. Furthermore, ctDNA testing may be synergistically used with other multi-omic biomarkers to enhance early detection. For instance, assays may incorporate early detection proteins (i.e., CA-125), epigenetic markers, circulating tumor RNA, nucleosomes, exosomes, and associated immune markers. Many companies are currently competing to develop a marketable early cancer detection test that leverages ctDNA. Although some hurdles (like early stage disease assay accuracy, high implementation costs, confounding from clonal hematopoiesis, and lack of clinical utility studies) need to be addressed before integration into healthcare, ctDNA assays hold substantial potential as an early cancer screening test.

Keywords: circulating tumor DNA, early detection, cell-free DNA, cancer screening, cancer detection

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Abbreviations: cfDNA, cell free DNA; ctDNA, circulating tumor DNA; NIPT, noninvasive prenatal testing

1. Introduction

There is a great need to develop accurate tests for the early detection of cancer as therapy is more likely to be successful when tumors are small, less complex, and non- metastatic. Circulating tumor DNA (ctDNA) is emerging as one of the most promising biomarkers for early cancer detection, since tumors secrete ctDNA into the bloodstream before they are visible on imaging, signs of disease are detected, or both (Fiala, Kulasingam, & Diamandis, 2018). ctDNA is released into the bloodstream during tumor apoptosis or necrosis and by circulating tumor cells. ctDNA comprises only a fraction of total cell-free DNA (cfDNA) as cfDNA also includes DNA sloughed off from non-tumor cells. The detection and analysis of ctDNA, or "liquid biopsy," has shown excellent correlations with solid tumor molecular pathology. For example, ctDNA levels are higher in patients at advanced stages of breast, colorectal, pancreatic and gastro-esophageal cancer than in early stage patients (Cheng, Su, & Qian, 2016).

Cell-free DNA was originally identified in blood in 1948 (Sorenson, et al., 1994) but was not used to detect tumor-specific mutations until the mid-1990s (Mandel, et al., 1948). A substantial portion of ctDNA technology was developed through work to advance noninvasive prenatal testing (NIPT) in prenatal diagnostics. Initially, NIPT sequencing was based primarily on whole chromosome aneuploidies, with a focus on chromosomes 13, 18, 21, X, and Y (Zhao, et al., 2015). NIPT was also developed to detect microdeletion and duplication syndromes (Zhao, et al., 2015). As NIPT became more widespread, it was noted that some women had genetic abnormalities (often copy number variations [CNVs]) in their plasma that did not relate to fetal abnormalities. Some of these abnormalities were determined to originate from undiagnosed maternal cancer (Amant, et al., 2015; Bianchi, et al., 2015), highlighting the rationale and clinical significance of ctDNA as an early diagnostic platform. At the same time, as the sequencing of plasma DNA advanced, mutation-specific testing for cancer diagnostics was also being developed.

ctDNA is now a frequently utilized, clinically accepted alternative to tissue biopsies for tumor genotyping and guiding treatment decisions in patients with metastatic cancer (Cheng, Su, & Qian, 2016; Kuderer, et al., 2017; Aggarwal, et al., 2019). Although it holds significant promise as a biomarker for early cancer detection, the need for further research and development remains. There are two broad approaches for ctDNA analysis. One approach is tumor-guided analysis of plasma DNA i.e. to start with prior knowledge of mutations specific to each patient's tumor. This is frequently done by first evaluating a biopsy or tumor specimen, choosing markers to probe (i.e., to indicate the presence of mutations), and evaluating those markers in the plasma (McDonald, et al., 2019). Another approach is tumor-independent analysis and assumes no prior knowledge of mutations in the original tumor. In some cases, such analysis is guided by prior knowledge of common mutations routinely found in cancer subtypes such as mutations in the *KRAS2*, *CDKN2A*, and *TP53* genes for pancreatic cancer. To develop an early detection test for previously undiagnosed cancer, the second method would need to be employed, and would require the use of a very broad panel of the most frequently mutated genes for the relevant malignancy. The purpose of this review is to describe the current development and utility of methods to identify ctDNA for the early detection of solid tumors, without *a priori* knowledge of a particular cancer's mutational profile.

2. Current uses of cell-free DNA in cancer diagnostics

2.1 Disease diagnosis and tracking

Unlike DNA extracted from traditional tissue biopsy, which evaluates only a small section of the tumor, ctDNA offers an unbiased representation of all mutations from across the patient's entire tumor burden

(Murtaza, et al., 2015; De Mattos-Arruda, et al., 2019). Despite the pathologic discrepancies found between tissue and ctDNA based mutation detection (Kuderer et al., 2017), ctDNA is frequently used to inform clinical decision making in late-stage disease diagnostic settings, particularly when biopsies are difficult to obtain or in situations where the primary location of the cancer is unknown (Cheng, Su, & Qian, 2016).

ctDNA can also be used for detecting metastases, monitoring treatment efficacy, and determining the best therapeutic approach to minimize therapy costs and side effects (Fiala, Kulasingam, & Diamandis, 2018). In contrast to tissue biopsy, which is invasive and thus may not easily be used to monitor tumor development over time, ctDNA may be better able to monitor resistance to current treatment. For instance, *KRAS* mutations in gastrointestinal cancer patients may indicate resistance, and changes in the variant allele fractions of *PIK3C* mutations in breast cancer patients may indicate relapse (Heizer, Haque, Roberts, & Speicher, 2019).

2.2 Minimal residual disease identification

After surgical resection, it is difficult to determine which patients have achieved remission as compared to those who have minimal residual disease. Studies have shown that the presence of ctDNA after surgery, which can be detected in plasma months earlier than recurrent tumors can be identified by imaging, is highly correlated with risk of relapse (Fiala, Kulasingam, & Diamandis, 2018; Diaz & Bardelli, 2014). Furthermore, ctDNA has a half-life of about 2 hours, which is shorter than that of other cancer protein biomarkers, so it provides a more accurate picture of current tumor burden (Cheng, Su, & Qian, 2016; Dawson, et al., 2013).

In addition, ctDNA has been shown to be a reliable residual disease biomarker after neoadjuvant chemotherapy in non-metastatic breast cancer (McDonald, et al., 2019). Using a personalized tumor-derived somatic mutation profile of 6 to 115 DNA variants per patient, McDonald et al., detected ctDNA in pre-treatment plasma samples from 32 of 32 patients (100%). After completion of neoadjuvant therapy, plasma concentrations of ctDNA were lower in those who achieved pathological complete response compared to those with residual disease ($p = 0.0057$, AUC = 0.83) (McDonald, et al., 2019).

3. Clinical areas that may uniquely benefit from early cancer detection using cell-free DNA.

Novel early detection strategies targeting ctDNA may prove beneficial particularly for cancers with limited screening options. Cohen et al. (2018) developed an early cancer detection test termed "CancerSEEK" with a major goal of targeting difficult-to-screen cancers. Using a 61-amplicon panel they analyzed blood samples to detect common mutations in several subtypes of solid tumors including breast, colorectal, esophagus, liver, lung, ovary, pancreas, and stomach, for which they did not have any prior knowledge of tumor-specific mutations. The detection rate was further enhanced by an additional set of eight common protein cancer biomarkers: cancer antigen 125 (CA-125), carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA 19-9), hepatocyte growth factor (HGF), myeloperoxidase (MPO), osteopontin (OPN), prolactin (PRL), and tissue inhibitor of metalloproteinases 1 (TIMP-1) (Cohen, et al., 2018). Their test showed 99% specificity with only 7 false positives out of 812 healthy controls, with sensitivities ranging from 33% for breast cancers to 98% for ovarian cancers. Overall, the authors identified cancer-specific profiles that could be used for the early detection (stage I-III) of over 82% of the cancers evaluated (Cohen, et al., 2018). A major finding of the study was that rates of early detection were 69% or better in some of the hardest to detect cancers, such as ovarian, hepatic,

pancreatic, esophageal, and gastric cancers, which currently have no guideline-recommended screening options.

High-risk populations, in particular, may benefit from early cancer detection using ctDNA. For instance, a patient with a known germline mutation leading to cancer predisposition (e.g., a *BRCA1* mutation that causes breast and ovarian cancer susceptibility) may be evaluated using targeted ctDNA analyses to detect second hits in the gene (Slavin, et al., 2018). cfDNA evaluation can also identify germline mutations (similar to more standard peripheral leukocyte analyses) which may be used to assess an individual's risk for specific cancers, as well as their potential pharmacogenetically-mediated response to early therapeutic options if needed (Slavin et al., 2018).

4. Optimizing cell-free DNA for early cancer detection

The ability to use a single independent biomarker to predict clinical outcomes has a long history in clinical medicine, with many biomarkers showing significant promise but ultimately failing to demonstrate clinical utility (Burke,2016). In this light, the development of ctDNA as a stand-alone, clinically relevant biomarker for early cancer detection may prove challenging. However, there are some intrinsic qualities of ctDNA that may help enhance its ability to be used as an independent biomarker that go beyond the exploitation and evaluation of targeted tumor-specific mutations. In addition, evaluating patient risk profiles and integrating ctDNA assays with orthogonal multi-omic assays (major category examples shown below) have the potential to further enhance the utility of ctDNA as an early cancer detection tool (Figure 1).

4.1 Intrinsic assay features

DNA fragment length

Mouliere et al. (2018) argued that current methods to enhance early cancer detection using ctDNA have focused on genomic alterations, ignoring differences in the fragment lengths of ctDNA. They noted that in NIPT, circulating fetal DNA is shorter than circulating maternal DNA, and this difference in the size of the DNA fragments can be exploited to increase the sensitivity of NIPT. They hypothesized that differences in cell-free DNA fragment lengths could similarly be incorporated to increase the sensitivity of ctDNA detection. Specifically, they determined that ctDNA fragments are enriched between 90 and 150 base pairs. After developing a way to select cfDNA fragments of specific sizes, they found that the detection of ctDNA improved, with the ctDNA fraction having at least a two-fold median enrichment in more than 95% of cases (Mouliere, et al., 2018). More recent studies have evaluated differences in cell-free DNA fragmentation for direct detection of ctDNA in patients with early stage cancers (Cristiano, et al., 2019).

Copy number variation

Molparia et al. (2017) argued that the detection of tumor-derived copy number variation (CNV; i.e., large gains or losses of DNA sequence, including sequence encoding genes) has the potential to greatly enhance ctDNA-based screening for multiple cancer types compared to regular ctDNA targeted mutation analysis. This is because ctDNA comprises only a small fraction of cfDNA (<1%) during the early stages of cancer, which presents a challenge for the development of a high-sensitivity test based on a mutational assay alone. For example, a fraction of 0.1% ctDNA in cfDNA correlates with only 1–5 copies

of a ctDNA locus per 1 mL of blood, which is much lower than the error rate of standard next-generation sequencing (Molparia, Nichani, & Torkamani, 2017). Therefore, leveraging CNV analyses (both across the entire genome and at specific chromosomal loci) with targeted mutational testing may enhance detection of ctDNA.

Mathematics and modeling

Mathematical and computational modeling of ctDNA holds promise as a tool to advance the field of early cancer detection and enhance its use as a biomarker of disease progression. In recent years, some mathematical models have been developed that use ctDNA dynamics to make predictions of tumor volume (Sharon S. Hori & Gambhir, 2011; Lutz, Willmann, Cochran, Ray, & Gambhir, 2008). In particular, it has been reported that a higher concentration of ctDNA is associated with later stages of cancer in addition to greater tumor size (Abbosh et al., 2017; Bettegowda et al., 2014; Fiala & Diamandis 2018). Sun et al. showed that metastatic cell growth patterns were consistent with increased ctDNA concentration in blood and used a stochastic mathematical model to predict progression free survival in metastatic melanoma (Sun, Bao, & Shao, 2016). Hori et al. developed a mathematical model of ctDNA kinetics to examine 1) the role of ctDNA shedding from both tumor and healthy cells, 2) ctDNA entry into the vasculature, and 3) elimination of ctDNA from plasma (Sharon S. Hori & Gambhir, 2011; Sharon Seiko Hori, Lutz, Paulmurugan, & Gambhir, 2017). This model allows a distinction between aggressive versus non-aggressive tumors using blood biomarker sampling data alone. Overall, combining liquid biopsy findings with mathematical models of tumor growth and ctDNA kinetics may provide novel opportunities to enhance early detection strategies and tumor progression predictions.

Phenotypic variables

ctDNA algorithms could also incorporate demographic information to better interpret patient cancer risk profiles. For instance, because men are not at risk for ovarian cancer, ctDNA findings indicative of ovarian cancer in men may be quickly categorized as false positives or potentially due to another cancer. Likewise, ctDNA risk profiles indicative of embryologic tumors, such as Wilms tumors, would be unexpected in elderly patients. Additionally, as mentioned above, identifying germline mutations in cancer driver genes (e.g., germline mutations in *BRCA1*) may help focus ctDNA detection methodologies by looking for downstream second hits in the same gene or subsequent pathway errors.

4.2 Enhancing ctDNA analysis using multi-omics approaches

There are many ways in which ctDNA-based early cancer detection assays may be improved. Many of these strategies involve combining ctDNA detection strategies with the identification of other biomarkers or analytes that are thought to be associated with tumorigenesis. We hypothesize that leveraging the inherent specificity of somatic mutations in ctDNA together with other biomarkers that offer higher sensitivity may yield an effective cancer detection strategy. One possible framework may be to enrich for patients at high-risk of developing cancer using more permissive, higher sensitivity tests such as protein biomarkers. A follow-up higher specificity test such as analysis of tumor-specific mutations in ctDNA can then be performed in this selected population, to achieve greater effective diagnostic accuracy. Many emerging biomarkers have demonstrated synergistic potential with ctDNA for early cancer detection some of which are highlighted in the following sections

Proteins

As noted above, adding alternative biomarkers to increase the sensitivity and positive predictive value of ctDNA assays is an obvious way to develop tests that can detect multiple cancer types while maintaining low false positive rates. Proteins, such as those used in the CancerSEEK assay described above, have the longest history as potential early cancer detection biomarkers (Cohen, et al., 2018). As such, they were some of the first biomarkers to be incorporated into ctDNA assays (Cohen, et al., 2018).

Epigenetics

Genome-wide hypomethylation analysis may be a potentially cost-effective cancer biomarker. Specifically, Chan et al. (2013) used plasma hypomethylation (which can be detected at low sequencing depths and for a relatively low cost) to identify cancer that had not metastasized, achieving 74% and 94% sensitivity and specificity, respectively. Similarly, hypermethylation at certain CpG islands may serve as a useful biomarker. Hypermethylation can contribute to the silencing of tumor suppressors which are typically unmethylated in healthy patients (Dong & Ren, 2018). For example, hypermethylation of the promoter region of the *MLH1* gene occurs in around 15% of sporadic colorectal cancers. *MLH1* is a tumor suppressor that, when methylated (turned off), reduces the ability of cells to perform appropriate DNA mismatch repair functions. The loss of effective mismatch repair can lead to genomic mutations and downstream carcinogenesis (Crucianelli, et al., 2014).

RNA

Increasing evidence has found that tumors release large amounts of RNase-resistant RNA into the blood, which can be used for cancer diagnosis and prognosis (Kishikawa, et al., 2015). RNA has shown strong potential as an early cancer detection biomarker. Kishikawa et al. (2015) found that circulating RNA levels were found to be significantly higher in the plasma and serum of patients with cancer than in that of healthy controls (Kishikawa, et al., 2015). Some specific RNA biomarkers have been shown to be associated with the presence of cancer. For example, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), which is a long non-coding RNA, is upregulated in patients with breast cancer (Huang et al., 2016). Arun et al. (2016) also showed that tumor growth slowed, and metastasis was reduced when MALAT1 was knocked out by antisense oligonucleotides in mouse mammary carcinoma (Arun et al., 2016).

Similarly, microRNAs (miRNAs) can be used as an early detection biomarker. miRNAs are often down- or up-regulated in cancerous cells compared to healthy cells. For example, oncogenic miRNAs like miR-21 play a role in the progression and metastasis of colon cancer: making them excellent biomarkers for early detection (Toiyama, et al., 2013; Hur, et al., 2015; Toiyama, et al., 2017; Nagaraju et al., 2016). The down-regulation of miRNA-141 was also found to be correlated with increased lymphatic invasion of pancreatic cancer cells (Nagaraju et al. 2016).

Nucleosomes

Nucleosomes, as well as post-translational modifications to nucleosomes, have the potential to be used to enhance ctDNA early detection assays. For example, McAnena et al. (2017), showed that H4K16ac levels were low or absent in most breast cancer tumors, whereas the loss of H4K20me3 was associated with reduced survival and increased invasiveness. This makes the two nucleosome components good biomarker candidates for early breast cancer detection and treatment response (McAnena, Brown, & Kerin, 2017).

Exosomes

Exosomes are microvesicles found in healthy and cancerous cell secretions. Tumor exosomes also appear to play a role in tumor growth, angiogenesis, and escape from immune surveillance (Abusamra et al., 2005; Liu et al., 2006). Exosomes, unlike the whole cell, are rich in transcripts specific to tumors. These tumor-specific transcripts could potentially be used to aid in cancer detection. For example, prostate-specific antigen (PSA) is routinely used to diagnose prostate cancer and predict responses to treatment. However, it has a low specificity leading to the need for additional predictive biomarkers for prostate cancer detection (Nilsson et al., 2009). Nilsson et al., (2009) analyzed RNA in urine exosomes to determine if the presence of known predictive biomarkers for prostate cancer could be detected and found two prostate cancer-specific biomarkers: *PCA-3* and *TMPRSS2: ERG*. In another example, analysis of miRNA in colorectal adenomas found that exosomal miR-21 correlated significantly with total adenoma number as well as adenoma size and that miR-21 indicated the presence of high-risk adenomas (Uratani, et al., 2016).

Autoantibodies

Autoantibodies target tumor-associated antigens and can be found in the sera of patients with cancer in the early stages of the disease (Tan, Low, Lim, & Chung, 2009). In some cases, autoantibodies have been found as early as five years before the onset of cancer (Fernandez Madrid, 2005). One of the benefits of using autoantibodies for early cancer detection is that they are more highly concentrated in the serum than autoantigens and are thus more easily detected. This discrepancy occurs because the immune system can amplify the production of autoantibodies in response to the presence of a single antigen (Tan, Low, Lim, & Chung, 2009).

Autoantibody signatures have been shown to potentially be able to distinguish between confined and disseminated disease (Wilson et al., 2018). For instance, IgA-reactive antigens were seen much more frequently in early-stage plasma samples as compared to late-stage plasma samples (Wilson et al., 2018).

5. The race to develop an early cancer detection test

An early cancer detection test could provide an immense, universal benefit to humankind. As such, the technology could be very lucrative, and many companies have raised significant venture capital hoping to be the first to complete early detection trials, publish results, and release a clinical test available to the average patient/consumer. For example, the billion-dollar biotechnology company GRAIL, Inc., (https://grail.com) has set out to develop a ctDNA-based multi-cancer screening test using advanced next generation sequencing approaches and machine learning (Aravanis, Lee, & Klausner, 2017). Ongoing studies are evaluating data from hundreds of thousands of patients to create a reference library for mutations found in the blood of cancer patients with the most common cancers (Aravanis, Lee, Klausner, 2017).

Another company, Thrive Earlier Detection Corp. (https://thrivedetect.com), developed CancerSEEK (see above) and received $110 million in funding in 2019 to further develop and commercialize the technology to detect mutations and protein biomarkers associated with colon and pancreatic cancers (Cohen et al., 2018). In early 2019, CancerSEEK received Breakthrough Device Designation from the United States Food and Drug Administration, which is granted to devices that are more effective than others on the market for the treatment and diagnosis of life-threatening/debilitating diseases (Thrive Earlier Detection Press Release,

https://thrivedetect.com/press-release/thrive-launches/, 2019 ; United States Food and Drug Administration, https://www.fda.gov/medical-devices/how-study-and-market-your-device/breakthrough-devices-program, 2019). Many other large genomics companies are similarly developing an early ctDNA detection test, such as the planned LUNAR-2 assay from Guardant Health, Inc (https://guardanthealth.com/solutions/) and a multi-omics assay by Freenome, Inc (https://www.freenome.com/).

Currently, on ClinicalTrials.gov, there are over 30 active trials in various stages of recruitment evaluating plasma-based DNA methods for early cancer detection (see https://clinicaltrials.gov/). Some notable large trials include: 1) GRAIL, Inc.'s the "Circulating Cell-free Genome Atlas Study," the "STRIVE Study": a prospective cohort of 100,000 women enrolled at time of their screening mammogram, the "SUMMIT study": a United Kingdom-based prospective cohort study of 50,000 older adults without cancer at time of enrollment, and "Development of a blood test to improve the performance of breast cancer screening," 2) Guardant Health, Inc.'s "Biomarkers for the stratification in lung cancer" and "ECLIPSE study: Evaluation of the ctDNA LUNAR Test in an Average Patient Screening Episode," 3) Memorial Sloan Kettering's "A study of blood-based biomarkers for pancreas adenocarcinoma," and 4) Freenome Inc.'s "AI-EMERGE: Development and Validation of a Multi-analyte, Blood-based Colorectal Cancer Screening Test."

In the United States, any new diagnostic biomarker must move through a multitude of clinical phases of test development and, ultimately regulatory approval. Pepe MS, et al., 2001, proposed guidelines for early cancer detection biomarker development (Pepe, et al., 2001). The developmental phases include 1) preclinical exploratory studies, 2) clinical assay development and analytical validation, 3) retrospective longitudinal repository studies and clinical validation, and 4) clinical utility studies, including both prospective screening and cancer control studies (Pepe, et al., 2001). Concomitantly, laboratory developed biomarker tests must also be developed following proper institutional (i.e., institutional review boards) and governmental ((i.e., clinical laboratory improvement act (CLIA) and the Food and drug administration (FDA)) regulations. Some resources regarding the clinical development and regulation of diagnostic biomarkers include Mazzone PJ, et al., 2017 (Mazzone, et al., 2017) and https://www.fda.gov/science-research/about-science-research-fda/biomarkers-fda.

6. Potential hurdles

Some significant barriers stand in the way of developing ctDNA-based early cancer detection tests. As noted above, one major barrier is that an early detection test must be able to identify multiple cancer types without prior knowledge of any specific cancer mutations. Considering the current expense of ctDNA studies, it would not be cost-effective to evaluate all coding regions of all genes associated with cancer. Furthermore, the depth of sequencing required to detect true "needle in the haystack" mutations substantially drives up sequencing costs. Although the costs of sequencing will decrease over time, current approaches will likely reduce the cost of ctDNA testing by focusing on specific genes, parts of genes, or gene mutations rather than all known cancer-associated genes. Therefore, this method is less likely to detect uncommon cancers with unusual cancer profiles, for which tumor-specific mutations may be unknown.

A second major barrier is clonal hematopoiesis, which refers to the clonal expansion of abnormal blood cells in an individual (Jaiswal, et al., 2014). Clonal hematopoiesis has been shown to complicate ctDNA results (Hu, Y et al., 2018). For example, Hu et al. (2018) determined that in patients with non-small cell lung cancer the majority of *JAK2* mutations, and a proportion of *TP53* mutations, detected through cfDNA may be due to clonal hematopoiesis. Both cancer and clonal hematopoiesis increase with age, making it difficult to determine whether certain mutations are a result of the aging

process or if they are due to the presence of a malignancy. Furthermore, clonal hematopoiesis also increases as a result of cancer treatment and is likely to confound ctDNA results for individuals who have previously been treated for cancer and are being screened for a second malignancy (Jaiswal, et al., 2014; Weitzel, et al., 2018; Hu et al., 2018). Razavi P, et al., 2019, showed clonal hematopoiesis can be identified, and removed as a confounder from ctDNA detection, using matched cfDNA and white blood cell sequencing (Razavi, et al., 2019).

A third hurdle is that for early detection, precancerous lesions and early-stage cancers may not ultimately shed enough ctDNA due to lower disease burden, making early detection a challenge. Therefore, limitations in circulating ctDNA concentrations may necessitate utilization of a combination of various analytes to achieve required sensitivity and specificity for a robust early-detection assay.

Despite these limitations, the methods described above, including layering multi-omic technologies are expected to help overcome these hurdles and enhance early cancer detection.

7. Conclusion

ctDNA holds great promise as an early detection biomarker, particularly for diseases that do not have currently accepted screening options, such as ovarian, pancreatic, and gastric cancers. In conjunction with other non-DNA based biomarkers, ctDNA holds substantial potential to be a key component of early cancer detection assays. However, for ctDNA to be routinely used for early detection, more data are needed to establish its clinical utility. Until there is clear evidence of clinical utility, access to these technologies may be limited to their necessary evaluation in clinical trials.

Conflicts of interest statement

MM is an inventor on patent applications covering technologies described herein. SWG is a consultant to Grail, Inc. Laura Goetz holds an equity position in Q Bio, Inc. All other authors declare no conflicts of interest.

Acknowledgments

The research reported in this publication was supported by the National Cancer Institute (NCI) of the National Institutes of Health (NIH) under award numbers P30CA33572 and K08CA234394 (PI: T. Slavin), a TGEN-City of Hope Precision Medicine Pilot Award, and a City of Hope Cancer Control and Populations Sciences Pilot Award. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. We would also like to thank scientific editor Kerin Higa and research assistant Kevin Tsang.

Figure Legend

Figure 1. Synergistic inclusion of both intrinsic variables with multi-omic technologies may enhance circulating tumor DNA as a tool for early cancer detection. A patient with an undiagnosed lung cancer is shown. Phenotypic variables in this case could include age and smoking status to help inform the prior probability of lung adenocarcinoma.

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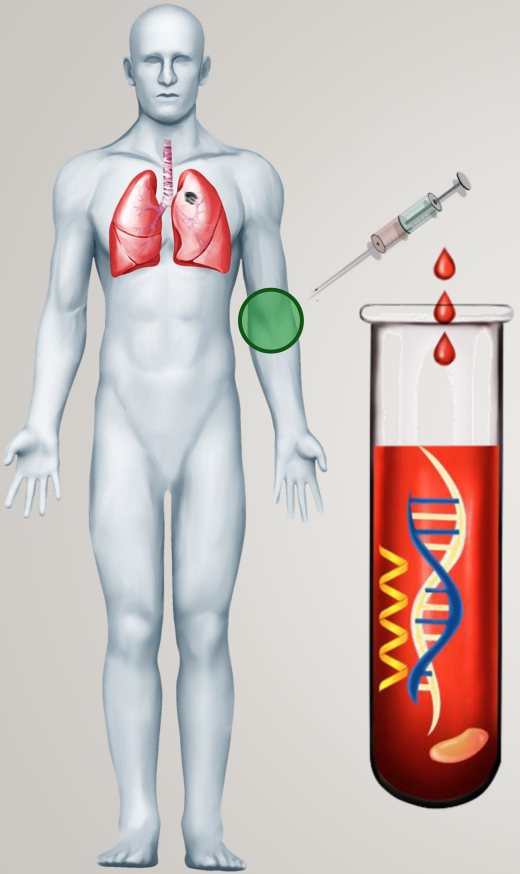

The use of intrinsic distinguishing variables

- Tumor specific mutations
- Phenotypic variables
- DNA Copy Number Variation
- Mathematics and modeling
- DNA Fragment Size

Layering in additional multi-omic technologies

- Proteins
- Nucleosomes
- Epigenetics
- Exosomes
- RNA
- Autoantibodies

Figure 1